Physiological Responses of Mycorrhizal Pinus sylvestris var. mongolica Seedlings to Drought Stress in Sandy Land: Postprint

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Date: 2021-12-12T00:00:00+00:00

Abstract

Ectomycorrhiza can effectively promote water absorption in forest trees, enhance drought resistance, and maintain forest ecosystem stability. Mongolian pine (Pinus sylvestris var. mongolica) in sandy lands is a typical ectomycorrhiza-dependent tree species. To compare and analyze the drought resistance of mycorrhizal Mongolian pine seedlings, we selected seedlings colonized by three important ectomycorrhizal fungi—Suillus granulatus (Sg), Tricholoma sp. (Ts), and Suillus luteus (Sl)—as experimental subjects. Through controlled indoor experiments, five water treatment gradients were established based on soil saturated water content: 80% (well-watered), 40% (moist), 20% (moderate water), 10% (mild drought), and 5% (severe drought), and physiological parameters of Mongolian pine seedlings under drought stress were measured. The results showed that: (1) The Sl treatment group primarily alleviated reactive oxygen species damage to cells by enhancing antioxidant enzyme activity, thereby resisting drought stress. Under mild drought, superoxide dismutase and peroxidase activities reached maximum values of 425.16 $U \cdot g^{-1}$ and 202.73 $U \cdot g^{-1}$, respectively. (2) The Sg treatment group both enhanced antioxidant enzyme activity to resist drought stress and alleviated drought effects by accumulating proline to regulate cell osmotic pressure. Under mild drought, superoxide dismutase activity and soluble sugar content reached maximum values of 397.01 $U \cdot g^{-1}$ and 199.50 $g \cdot mL^{-1}$, respectively. (3) The Ts treatment group primarily resisted drought stress by enhancing maximum photochemical efficiency. Under normal water conditions and mild drought stress, leaf maximum photochemical efficiency was significantly higher than that of the control group ($P<0.05$). When drought stress occurs, mycorrhizal seedlings can ensure normal physiological activities of Mongolian pine and resist drought stress through enhancing antioxidant enzyme activity, regulating osmotic substance content, and improving leaf photochemical efficiency. However, different ectomycorrhizal fungi employ distinct pathways to enhance drought tolerance in Mongolian pine. These results

can provide a theoretical basis for deepening understanding of ectomycorrhizal ecological functions and developing mycorrhizal afforestation technologies for Mongolian pine in sandy lands.

Full Text

Physiological Responses of Mycorrhizal *Pinus sylvestris* var. *mongolica* Seedlings to Drought Stress

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Abstract

Ectomycorrhiza can effectively promote water absorption in forest trees, enhance drought resistance, and maintain forest ecosystem stability. *Pinus sylvestris* var. *mongolica* is a typical ectomycorrhiza-dependent tree species. To compare and analyze the drought resistance of mycorrhizal *P. sylvestris* var. *mongolica* seedlings, we studied seedlings infected by three important ectomycorrhizal fungi: *Suillus granulatus* (Sg), *Tricholoma* sp. (Ts), and *Suillus luteus* (Sl). Through indoor controlled experiments, we established soil saturated water content gradients of 80% (wet), 40% (suitable water), and 5% (severe drought) as water treatment gradients, and measured physiological characteristic parameters of *P. sylvestris* var. *mongolica* seedlings under drought stress. Results showed: (1) The Sl treatment group mainly resisted drought stress by increasing antioxidant enzyme activity to reduce free oxygen damage to cells. Under mild drought, superoxide dismutase (SOD) and peroxidase activities reached maximum values of 202.73 U · g and 202.73 U · g, respectively. (2) The Sg treatment group both increased antioxidant enzyme activity to resist drought stress and alleviated the impact by accumulating proline to regulate cell osmotic pressure. Under mild drought, SOD activity and soluble sugar content reached 199.50 g · mL. (3) The Ts treatment group mainly resisted drought stress by increasing maximum photochemical efficiency. Under normal water content and mild drought stress, leaf maximum photochemical efficiency was significantly higher than the control group ($P<0.05$). When drought stress occurred, mycorrhizal seedlings could ensure normal physiological activities of *P. sylvestris*

var. *mongolica* and resist drought stress by improving antioxidant enzyme activity, regulating osmotic substance content, and increasing leaf photochemical efficiency, but different ectomycorrhizal fungi had different approaches to improving drought tolerance of *P. sylvestris* var. *mongolica*. The research results can provide theoretical basis for deeply understanding the ecological functions of ectomycorrhiza and developing mycorrhizal afforestation technology for *P. sylvestris* var. *mongolica*.

Keywords: *Pinus sylvestris* var. *mongolica*; ectomycorrhizal fungus; drought stress; antioxidant enzyme activity; osmotic regulation substance; photochemical efficiency

Introduction

Ectomycorrhiza is a symbiotic structure formed by various fungal hyphae and fine roots in soil, widely present in forest ecosystems[1]. In arid environments, ectomycorrhiza can effectively improve the ability of host plants to resist drought stress, thereby regulating and maintaining host plant health[2-3]. On one hand, ectomycorrhiza can directly regulate root microstructure through extraradical hyphae and hyphal mantles, improving water absorption capacity and efficiency of host plants, reducing root water loss, and ensuring normal growth and physiological metabolism[4-6]. On the other hand, ectomycorrhiza can indirectly improve water absorption capacity of host plants by enhancing reactive oxygen species scavenging system enzyme activity, phosphorus absorption capacity, and net photosynthetic rate, delaying the lethal time of drought stress[7].

Pinus plants are common hosts of ectomycorrhizal fungi, with over 2000 ectomycorrhizal fungi forming ectomycorrhiza on *Pinus* plant roots[8]. *Pinus sylvestris* var. *mongolica* is drought-resistant, cold-resistant, and barren-tolerant, and is an important tree species for windbreak, sand fixation, and timber in China' s "Three North" region[9]. Although *P. sylvestris* var. *mongolica* has strong adaptability and stress resistance, the site conditions in the "Three North" region are harsh, and *P. sylvestris* var. *mongolica* still suffers severe drought stress, especially for seedlings with relatively weaker adaptability and stress resistance[10]. Therefore, improving the drought resistance of *P. sylvestris* var. *mongolica* seedlings remains an important issue urgently needed to be solved in afforestation and management of *P. sylvestris* var. *mongolica*.

Pinus sylvestris var. *mongolica* is a typical ectomycorrhiza-dependent tree species[11], and mycorrhizal technology may be an important approach to improve the drought resistance of *P. sylvestris* var. *mongolica* seedlings. In recent years, mycorrhizal technology has been applied in *P. sylvestris* var. *mongolica* afforestation, but basic research on mycorrhizal technology remains very limited and weak due to the complex composition of ectomycorrhizal fungal communities[12]. Related studies on tree species have mostly focused on single ectomycorrhizal fungi and host plant phenotypes and physiological characteristics[5].

Our previous research found that through network construction and module screening, *Suillus granulatus*, *Tricholoma* sp., and *Suillus luteus* are important nodes maintaining the stability of the ectomycorrhizal fungal network associated with *P. sylvestris* var. *mongolica*, and their relative abundances have obvious quantitative advantages[12]. From the perspective of known ecological functions, *Suillus* is a common ectomycorrhizal fungal group in forest ecosystems that can promote soil water and phosphorus absorption, improve host plant physiological functions, and enhance host plant drought resistance[13-14]. *Tricholoma* species are richly diverse, and their ectomycorrhiza can affect plant hormone and volatile organic compound production, inhibit electrolyte leakage, and improve plant cell water absorption and retention capacity, helping host plants adapt to arid environments[15]. Therefore, *Suillus granulatus*, *Tricholoma* sp., and *Suillus luteus* are key ectomycorrhizal fungi closely related to the drought resistance of *P. sylvestris* var. *mongolica*.

This study used *P. sylvestris* var. *mongolica* as the research object, cultivated mycorrhizal seedlings through pot control experiments, and analyzed the physiological responses of *P. sylvestris* var. *mongolica* seedlings infected by three important ectomycorrhizal fungi to drought stress, aiming to deeply understand the ecological functions of ectomycorrhiza and provide theoretical support for developing mycorrhizal afforestation technology for *P. sylvestris* var. *mongolica*.

Materials and Methods

1.2.1 Mycorrhizal Cultivation of *P. sylvestris* var. *mongolica* Seedlings

The experimental soil was common aeolian sandy soil from northern sandy areas. The experimental strains were obtained through fruiting body tissue isolation and provided by the China Agricultural Microorganism Culture Collection Management Center. The research object was *P. sylvestris* var. *mongolica* seedlings after inoculation with the strains.

In a laminar flow hood, potato dextrose agar solid medium (PDA) was inoculated with *Suillus granulatus* (Sg), *Tricholoma* sp. (Ts), and *Suillus luteus* (Sl) strains. The liquid medium was sealed with sterile sealing film and cultured in the dark at 25°C for use in inoculating *P. sylvestris* var. *mongolica* seedlings.

After disinfecting *P. sylvestris* var. *mongolica* seeds and promoting germination, they were placed on the surface of sterilized aeolian sandy soil, covered with 0.5 cm of soil, and sprayed with sufficient distilled water. During the period, 0.1% carbendazim was sprayed 3 times to eliminate other fungal interference. Potting used rectangular plastic pots (25 cm × 20 cm × 13 cm) with drainage holes at the bottom, with approximately 20 seedlings planted per pot. The experiment was conducted indoors (average temperature 25.10°C, average relative humidity 50.25%). After 3 months, the three inoculants were broken into mycelial suspensions using a blender, sprayed evenly on the seedbed, while the blank control group was sprayed with sterile water. To prevent other contamination, different

inoculation treatment groups were spaced 50 cm apart, with $KMnO_4$ solution sprayed around for disinfection and separated by plastic plates. Cultivation continued for another 3 months to allow sufficient colonization by ectomycorrhizal fungi.

1.2.2 Drought Stress Experiment on Mycorrhizal Seedlings

After seedlings were cultivated until August, the saturated water content of the experimental aeolian sandy soil was measured as 23.12% using the drying method. Based on this, three drought gradients were established: soil saturated water content of 80% (wet), 40% (suitable water), and 5% (severe drought) as water treatment gradients. Each drought gradient included 3 ectomycorrhizal treatment groups and 1 blank control group, totaling 12 mycorrhizal seedling treatment groups. Physiological indicators of *P. sylvestris* var. *mongolica* seedlings after drought stress treatment were measured, with 5 samples randomly selected from each treatment for each indicator, and each sample was tested 3 times. Indicators included cell membrane structure damage degree (relative conductivity, malondialdehyde MDA), osmotic regulation substances (proline, soluble sugar), antioxidant enzyme activities (superoxide dismutase SOD, peroxidase POD), leaf water potential, and maximum photochemical efficiency Fv/Fm.

Relative conductivity was measured using a conductivity meter (DDS-11A, China). MDA content was determined using the thiobarbituric acid colorimetric method. Proline and soluble sugar contents were determined using the ninhydrin colorimetric method and anthrone colorimetric method, respectively. SOD and POD activities were determined using the nitroblue tetrazolium method and guaiacol method, respectively[16]. Leaf water potential and Fv/Fm were measured using a dew point potentiometer (WP4C, USA) and chlorophyll fluorescence imager (FluorCam). For Fv/Fm measurement, leaves were first fully dark-adapted for 20 min to obtain Fo (minimum fluorescence under dark adaptation), then exposed to saturating light pulse (intensity 0.8 s) to maximize chlorophyll fluorescence intensity and obtain Fm (maximum fluorescence under light response), with Fv/Fm calculated as (Fm-Fo)/Fm.

1.3 Data Processing and Analysis

Data processing used SPSS 19.0 software. Differences in physiological indicators of mycorrhizal *P. sylvestris* var. *mongolica* seedlings under different drought stress treatments were analyzed by one-way ANOVA with Duncan' s test, with significance level at $P<0.05$. Figures were plotted using Origin 2016 software.

Results

2.1 Cell Membrane Structure Damage Degree of Mycorrhizal *P. sylvestris* var. *mongolica* Seedlings

Mycorrhizal infection could reduce leaf relative conductivity to varying degrees. Both mycorrhizal and control treatment groups showed gradually increasing leaf relative conductivity with intensifying drought stress ($P<0.05$). The Sl treatment group relative conductivity increased by 48.89% from sufficient water to severe drought stress, while the control group increased by 42.59%. Under severe drought stress, compared with the control group, Sg, Ts, and Sl treatment groups decreased relative conductivity by 37.15%, 39.29%, and 14.19%, respectively. Among different treatment groups, under sufficient water and severe drought stress, Sg and Sl treatment group leaf relative conductivity was significantly lower than the control group ($P<0.05$). Under wet conditions, Ts treatment group relative conductivity decreased by 4.60% compared with the control group. Under suitable water and mild drought stress, Sg, Ts, and Sl treatment groups decreased relative conductivity by 11.16%, 13.26%, and 4.60% respectively compared with the control group, with no significant differences among the three treatment groups ($P>0.05$).

MDA content increased with drought stress ($P<0.05$). Under drought stress, MDA content in mycorrhizal seedlings decreased to varying degrees (Fig. 1). MDA content in Sg, Ts, and Sl treatment groups increased by 3.61 $\mathrm{mol \cdot g^{-1}}$, 2.97 $\mathrm{mol \cdot g^{-1}}$, and 2.73 $\mathrm{mol \cdot g^{-1}}$ respectively from sufficient water to severe drought stress, while the control group increased by 3.77 $\mathrm{mol \cdot g^{-1}}$. Among different treatment groups, under wet conditions, Sl treatment group had the lowest MDA content, decreasing by 31.41% compared with the control group ($P<0.05$). No significant differences were found among Sg, Ts, and control groups ($P>0.05$). No obvious differences were found among treatment groups at other water gradients.

[Figure 1: see original paper]

2.2 Osmotic Regulation Substance Content of Mycorrhizal *P. sylvestris* var. *mongolica* Seedlings

Both mycorrhizal and control treatment groups showed gradually increasing leaf proline content with intensifying drought stress ($P<0.05$). Under mild and severe stress conditions, no significant differences were found in leaf proline content between Sg, Ts, Sl, and control treatment groups ($P>0.05$). Leaf proline content in Sg, Ts, Sl, and control treatment groups increased by 422.69 $\mathrm{g \cdot g^{-1}}$, 351.58 $\mathrm{g \cdot g^{-1}}$, 351.11 $\mathrm{g \cdot g^{-1}}$, and 165.08 $\mathrm{g \cdot g^{-1}}$ respectively from sufficient water to severe drought stress. Among different treatment groups, control group leaf proline content was significantly lower than the three mycorrhizal treatment groups ($P<0.05$). No significant differences were found between Sg, Ts, Sl treatment group leaf proline content and the control group under sufficient water and wet conditions ($P>0.05$). Under suitable water and mild drought stress,

Sg and Sl treatment group proline content was significantly different from the control group ($P<0.05$), increasing by 88.26 $g \cdot g^{-1}$ and 26.24% respectively. Under severe drought stress, Ts treatment group proline content was the highest, increasing by 26.24% compared with the control group, with significant differences among Sg, Ts, and Sl treatment groups ($P<0.05$).

Both mycorrhizal and control treatment groups showed significantly increasing soluble sugar content from sufficient water to suitable water conditions with intensifying drought stress ($P<0.05$). Mycorrhizal seedlings could significantly increase leaf soluble sugar content ($P<0.05$), with no significant difference only under sufficient water conditions ($P>0.05$). Drought stress caused soluble sugar content in *P. sylvestris* var. *mongolica* seedlings to first increase then decrease (Fig. 2). The control group soluble sugar content began to decrease before reaching mild drought stress, but mycorrhizal seedling soluble sugar content decreased only under severe drought stress. Under wet drought stress, Sg, Ts, and Sl treatment group seedling soluble sugar content significantly increased ($P<0.05$), reaching maximum values of 213.15 $g \cdot mL^{-1}$, 199.50 $g \cdot mL^{-1}$, and 165.08 $g \cdot mL^{-1}$ respectively under mild drought stress, while the control group reached its maximum of 199.50 $g \cdot mL^{-1}$ under suitable water conditions.

[Figure 2: see original paper]

2.3 Antioxidant Enzyme Activity of Mycorrhizal *P. sylvestris* var. *mongolica* Seedlings

SOD activity in Sg, Ts, Sl, and control treatment groups first increased then decreased with intensifying drought stress, reaching maximum values under mild drought stress. SOD activity increased by 397.01 $U \cdot g^{-1}$, 315.24 $U \cdot g^{-1}$, 311.42 $U \cdot g^{-1}$, and 259.56 $U \cdot g^{-1}$ respectively from sufficient water to severe drought stress. Under sufficient water conditions, SOD activity was significantly lower than under other stress gradients ($P<0.05$). Among different treatment groups, under wet to severe drought stress, Sg, Ts, and Sl treatment group leaf SOD activity was higher than the control group ($P<0.05$), with no significant difference only under wet conditions ($P>0.05$). Under sufficient water conditions, Sg treatment group leaf SOD activity was significantly different from the control group ($P<0.05$), increasing by 17.58%.

POD activity in Sg, Ts, Sl, and control groups increased with drought stress, reaching the highest level under severe drought stress at 172.00 $U \cdot g^{-1}$, 202.73 $U \cdot g^{-1}$, 425.16 $U \cdot g^{-1}$, and 397.01 $U \cdot g^{-1}$ respectively. POD activity was significantly lower under sufficient water and wet conditions than under other water gradients ($P<0.05$). Sg, Ts, and Sl treatment group leaf POD activity was significantly lower under wet and suitable water conditions than under mild and severe stress ($P<0.05$). Among different treatment groups, under sufficient water and wet conditions, Sg, Ts, and Sl treatment group leaf POD activity was significantly higher than the control group ($P<0.05$). Under suitable water conditions, Sg and Sl treatment group leaf POD activity was significantly

different from the control group, increasing by 98.09%. Under mild and severe drought stress, Sg, Ts, and Sl treatment group leaf POD activity was significantly higher than the control group, with significant differences among Sg, Ts, and Sl treatment groups ($P<0.05$).

[Figure 3: see original paper]

2.4 Maximum Photochemical Efficiency and Leaf Water Potential of Mycorrhizal *P. sylvestris* var. *mongolica* Seedlings

Drought stress had significant effects on leaf Fv/Fm. Fv/Fm in Sg, Ts, Sl, and control treatment groups first increased then decreased, being significantly lower under severe drought stress than under other water gradients ($P<0.05$). Fv/Fm reached maximum values of 0.85, 0.84, 0.86, and 0.82 respectively under mild stress. Among different treatment groups, Sg, Ts, and Sl treatment group Fv/Fm was significantly higher than the control group under wet conditions ($P<0.05$), with no significant differences among Sg, Ts, and Sl treatment groups ($P>0.05$). No significant differences were found among treatment groups under sufficient water conditions ($P>0.05$). Under mild drought, Ts treatment group Fv/Fm was 14.29% higher than the control group, with no significant differences among Sg, Ts, and Sl treatment groups ($P>0.05$). No significant differences were found among treatment groups under severe stress ($P>0.05$).

Leaf water potential in Sg, Ts, Sl, and control treatment groups decreased with intensifying drought stress ($P<0.05$), decreasing by 0.21 MPa, 0.19 MPa, 0.21 MPa, and 0.21 MPa respectively. Mycorrhizal seedlings increased leaf water potential significantly, but showed no obvious difference from the control group under sufficient water conditions (Fig. 4). Among different treatment groups, Sg, Ts, and Sl treatment group leaf water potential was significantly higher than the control group under wet, mild drought, and severe drought conditions ($P<0.05$), with no significant differences among the three treatment groups ($P>0.05$). No significant differences were found among treatment groups under sufficient water conditions ($P>0.05$). Under mild drought stress, Ts treatment group leaf water potential was significantly different from Sg and Sl treatment groups ($P<0.05$).

[Figure 4: see original paper]

Discussion

3.1 Effects of Ectomycorrhiza on Cell Membrane Damage Degree of *P. sylvestris* var. *mongolica* Seedlings

Plant leaf relative conductivity can reflect cell membrane permeability. With the occurrence and intensification of drought stress, relative conductivity of *P. sylvestris* var. *mongolica* seedlings showed a gradual increasing trend, but all three mycorrhizal seedling groups were lower than the control group, indicating that mycorrhizal infection alleviated drought stress damage to leaf cell membranes, consistent with previous research results[17]. Ectomycorrhiza formation

improved plant cell membrane stability, alleviated the consequences of cytoplasm leakage caused by changes in cell membrane permeability, inhibited the increase in tissue fluid relative conductivity[18].

MDA is the final product of membrane lipid peroxidation, and its content can characterize the degree of injury in plants under stress[19]. Meanwhile, with intensified membrane lipid peroxidation, MDA content continuously increases. Under drought stress, MDA content in mycorrhizal seedlings was significantly lower than in the control group, consistent with previous research results[20]. After inoculation with ectomycorrhizal fungi, the colonized ectomycorrhiza inhibited MDA accumulation, slowed the membrane lipid peroxidation process, reduced cell membrane oxidative damage, decreased cell membrane permeability, and thus slowed the increase in relative conductivity. Differences in MDA content in plants under drought stress can reflect their drought resistance performance. In this study, the increase in MDA content in Sg, Ts, and Sl treatment groups was lower than in the control group, indicating they suffered less damage and had stronger drought resistance. Among them, the Sl treatment group had a relatively larger increase, while the Sg treatment group had a smaller increase, suggesting Sg was more effective than Sl in terms of MDA content.

3.2 Effects of Ectomycorrhiza on Osmotic Regulation Substance Content of *P. sylvestris* var. *mongolica* Seedlings

As a free osmotic regulation substance, proline can prevent cell water loss under drought conditions and maintain normal cell osmotic pressure[21]. Under drought stress, proline content in mycorrhizal seedlings continuously increased and was significantly higher than in the control group, consistent with previous research results[22]. When drought stress occurred, the Sg treatment group improved proline accumulation efficiency. The Ts treatment group had strong drought resistance under wet conditions, while the Sl treatment group had strong drought resistance under mild and severe drought stress. Differences exist in osmotic regulation methods among different tree species and plant hosts after ectomycorrhizal fungal infection. For example, proline content in *Cyclobalanopsis glauca* mycorrhizal seedlings was lower than in the control group under drought conditions, while soluble sugar was significantly higher than in the control group[23]. Ectomycorrhizal fungi improved plant water metabolism and osmotic regulation capacity by promoting proline synthesis, reducing the degree of plant damage[24]. Similar to proline, soluble sugar can be produced under drought stress to balance plant cell osmotic pressure and maintain cell stability. It includes glucose, trehalose, and other small molecules that can osmotically regulate in plant cells[25].

In this study, soluble sugar content first increased then decreased with intensifying drought stress. Control group soluble sugar content began to decrease before reaching mild drought stress, but mycorrhizal seedling soluble sugar content decreased only under severe drought stress. After drought stress intensified, soluble sugar participated in the osmotic regulation process, causing significant

decreases in plant soluble sugar content. Ectomycorrhizal fungal infection improved the ability of seedlings to produce soluble sugar, accelerating the accumulation of osmotic regulation substances, lowering cell water potential, promoting water entry into cells from outside, and helping plants maintain basic physiological activities under more severe drought stress. Under wet, mild drought, and severe drought conditions, Sg, Ts, and Sl treatment group soluble sugar content showed no significant differences, indicating that Sg, Ts, and Sl had similar effects in terms of soluble sugar content.

3.3 Effects of Ectomycorrhiza on Antioxidant Enzyme Activity of *P. sylvestris* var. *mongolica* Seedlings

SOD and POD are major enzymes that improve plant drought resistance under drought stress and are important protective enzymes in the plant cell membrane lipid peroxidation enzymatic detoxification system[26]. SOD widely exists in plant antioxidant systems, converting superoxide anion radicals to hydrogen peroxide, while POD converts hydrogen peroxide to water. Ectomycorrhizal fungal infection can increase SOD and POD activities in plants, accelerating the conversion of superoxide anion radicals to water when drought occurs, thereby improving plant reactive oxygen species scavenging capacity and making plants more adaptable to arid environments. With intensifying drought stress, plant leaf SOD and POD activities first increased then decreased, consistent with previous research results[27-28].

The stronger the enzyme activity, the better the scavenging effect on superoxide free radicals, ensuring material transport and energy regulation in plants. In this study, SOD activity in Sg, Ts, and Sl treatment groups was always higher than in the control group, and only significantly higher than Ts under mild drought. Different ectomycorrhizal fungal infections have different effects on improving plant antioxidant enzyme activity. Mycorrhizal seedlings inoculated with Sg showed significantly higher leaf POD activity than the control group, while POD activity differences were not significant between Ts and control. When soil water content was 10%-20%, both SOD and POD activities showed increasing trends, with Sg treatment group SOD activity significantly higher than Sl, but POD activity differences between Sl and Sg were not significant. The Sg treatment group improved plant drought resistance by increasing antioxidant enzyme activity in the reactive oxygen species scavenging system, helping plants cope with more severe drought stress. The Sl treatment group had more significant effects on improving antioxidant enzyme activity.

3.4 Effects of Ectomycorrhiza on Leaf Maximum Photochemical Efficiency and Water Potential of *P. sylvestris* var. *mongolica* Seedlings

Fv/Fm is a commonly used method to evaluate plant physiological status. Plant photosynthetic rate and Fv/Fm show decreasing trends with drought stress[29]. When water is deficient, leaf stomata close, stomatal conductance decreases, but ectomycorrhizal fungi can inhibit the decrease in stomatal conductance,

increase stomatal conductance, reduce resistance, and improve plant photosynthetic efficiency. Ectomycorrhizal fungal infection can improve plant Fv/Fm, and mycorrhizal seedlings of *Betula alnoides* showed significantly higher Fv/Fm than the control group[30]. In this study, control group Fv/Fm appeared before mild drought stress, while all three treatment groups appeared after mild drought stress, with Ts treatment group appearing under more severe drought stress. This phenomenon may be related to initial fluorescence and chlorophyll content.

As a reference indicator reflecting plant water status, leaf water potential can reflect the degree of drought stress suffered by plants—the more severe the drought stress, the lower the plant leaf water potential[31]. Ectomycorrhizal colonization enabled plant leaves to maintain better water status, slowing the occurrence of water stress in plants. In this study, control group leaf water potential was lower than the three treatment groups except under sufficient water conditions, and responded most strongly to intensified drought stress. Ectomycorrhizal fungal infection significantly improved seedling leaf water potential, with the most significant improvement effect on leaf water potential of *P. sylvestris* var. *mongolica* seedlings inoculated with Sl under natural drought stress[32]. Mycorrhizal seedlings showed no obvious difference from the control group under sufficient water conditions, indicating that water exchange in *P. sylvestris* var. *mongolica* seedlings had reached equilibrium at this time. Improving leaf water potential can reduce water loss from plant cells under drought conditions and enhance plant drought resistance.

Conclusion

Under the water treatment gradients set in this study, ectomycorrhiza significantly improved the drought resistance of *P. sylvestris* var. *mongolica*. The Sl treatment group mycorrhizal seedlings had higher antioxidant enzyme activity, proline content, and Fv/Fm than the control group. The Sg treatment group had higher proline content than other treatment groups, while the Ts treatment group had the highest Fv/Fm. With the help of ectomycorrhiza, *P. sylvestris* var. *mongolica* overall regulated its response to drought stress by avoiding reactive oxygen species damage, regulating the osmotic process, and improving photochemical efficiency.

Different ectomycorrhizal fungi improved the drought resistance of *P. sylvestris* var. *mongolica* through different pathways. The Sl treatment group improved antioxidant enzyme activity through the enzymatic scavenging mechanism in the reactive oxygen species scavenging system to resist damage caused by reactive oxygen species to cells, but the Sg treatment group had more significant effects on improving SOD activity. The Sg treatment group promoted osmotic regulation by accumulating proline content in plants, while the Ts treatment group improved the drought resistance of *P. sylvestris* var. *mongolica* by improving plant photochemical efficiency. Ectomycorrhizal fungi are beneficial for improving the drought resistance of *P. sylvestris* var. *mongolica*. The research

results are of great significance for deeply understanding the ecological functions of ectomycorrhiza and can provide theoretical basis for developing mycorrhizal afforestation technology for *P. sylvestris* var. *mongolica*.

References

[1] Chen Lulu, Feng Qiuhong, Sun Jianxin. Differentiation of ectomycorrhizal morphology in *Abies faxoniana* along an elevation gradient in a subalpine forest of western Sichuan Province, China[J]. Chinese Journal of Applied Ecology, 2020, 31(9): 2911-2922.

[2] Guo Mishan, Ding Guodong, Gao Guanglei, et al. Enhancement to host plant resistance in abiotic stress by ectomycorrhizae[J]. World Forestry Research, 2019, 32(5): 15-21.

[3] Van Nuland M E, Peay K G. Symbiotic niche mapping reveals functional specialization by two ectomycorrhizal fungi that expands the host plant niche[J]. Fungal Ecology, 2020, DOI: 10.1016/j.funeco.2020.100960.

[4] Zhao Peishan, Guo Mishan, Gao Guanglei, et al. Characteristics of community structure and functional group of fungi in roots of *Pinus sylvestris* var. *mongolica* in the Horqin Sandy Land[J]. Scientia Silvae Sinicae, 2020, 56(9): 87-96.

[5] Wang Jiayuan, Yin Xiaolin, Ren Yue, et al. Diversity characteristics of ectomycorrhizal fungi associated with *Pinus sylvestris* var. *mongolica* in the Mu Us sandy land[J]. Microbiology China, 2020, 47(11): 3856-3867.

[6] Ren Yue, Gao Guanglei, Ding Guodong, et al. Characteristics of organic carbon content of leaf litter-soil system in *Pinus sylvestris* var. *mongolica* plantations[J]. Journal of Beijing Forestry University, 2018, 40(7): 36-44.

[7] Wang Kai, Shen Chao, Song Lining, et al. Variations in C, N and P stoichiometry of *Pinus sylvestris* var. *mongolica* seedlings under continuous drought[J]. Chinese Journal of Ecology, 2020, 39(7): 2175-2184.

[8] Guo M S, Ding G D, Gao G L, et al. Community composition of ectomycorrhizal fungi associated with *Pinus sylvestris* var. *mongolica* plantations of various ages in the Horqin Sandy Land[J]. Forests, 2020, 11(3): 323.

[9] Zhao Min, Hao Wenying, Ning Xinzhe, et al. Screening of excellent ectomycorrhizal fungi-tree combinations for drought resistant *Pinus sylvestris* var. *mongolica*[J]. Bulletin of Botanical Research, 2020, 40(1): 133-140.

[10] Guo M S, Ding G D, Gao G L, et al. Drivers of ectomycorrhizal fungal community structure associated with *Pinus sylvestris* var. *mongolica* differ at regional vs. local spatial scales in the northern China[J]. Fungal Ecology, 2020, DOI: 10.1016/j.funeco.2020.100960.

[11] Wang Jugang, Zheng Rong, Bai Shulan, et al. Responses of ectomycorrhiza to drought stress[J]. Chinese Journal of Ecology, 2012, 31(6): 1571-1576.

[12] Guo Mishan, Gao Guanglei, Ding Guodong, et al. Diversity of ectomycorrhizal fungi associated with *Pinus sylvestris* var. *mongolica* in Hulunbuir Sandy Land[J]. Mycosystema, 2018, 37(9): 1133-1142.

[13] Chen Jie, Liu Xuefeng, Hao Xin, et al. Effect of *Suillus granulatus* inoculation on disease resistance of *Pinus sylvestris* var. *mongolica* to shoot blight[J]. Journal of Northeast Forestry University, 2019, 47(3): 94-99, 110.

[14] Anna Lena Z, Dennis K, Gila M, et al. Distribution of major and trace elements in the bolete mushroom *Suillus luteus* and the bioavailability of rare earth elements[J]. Chemical Geology, 2018, 483: 419-500.

[15] Abdulsalam O, Wagner K, Wirth S, et al. Phytohormones and volatile organic compounds, like geosmin, in the ectomycorrhiza of *Tricholoma vaccinum* and Norway spruce (*Picea abies*)[J]. Mycorrhiza, 2020, 1-16.

[16] Li Hesheng. Principles and Techniques of Plant Physiological and Biochemical Experiments[M]. Beijing: Higher Education Press, 2003: 156-261.

[17] Duan Na, Wang Jia, Liu Fang, et al. Research progress on drought resistance of plant[J]. Molecular Plant Breeding, 2018, 16(15): 5093-5099.

[18] Chen Jie, Xie Jing, Tang Ming. Effects of arbuscular mycorrhizal fungi on the growth and drought resistance of *Amorpha fruticosa* under water stress[J]. Journal of Beijing Forestry University, 2014, 36(6): 142-148.

[19] Zhang Dan, Ren Jie, Wang Huimei. Response of photosynthetic characteristics and antioxidant system in needles and bark chlorenchyma of Korean pine to drought stress and rehydration[J]. Chinese Journal of Ecology, 2016, 35(10): 2606-2614.

[20] Qi Jinyu, Yin Dachuan, Song Ruiqing. Effects of *Suillus luteus* on salt resistance of *Pinus sylvestris* var. *mongolica*[J]. Journal of Shenyang Agricultural University, 2019, 50(1): 108-113.

[21] Guo Y Y, Yu H Y, Yang M M, et al. Effect of drought stress on lipid peroxidation, osmotic adjustment and antioxidant enzyme activity of leaves and roots of *Lycium ruthenicum* Murr. seedling[J]. Russian Journal of Plant Physiology, 2018, 65(2): 244-250.

[22] Huang Haixia, Lian Zhuanhong, Wang Liang, et al. Response of osmotic regulation substances and antioxidant enzyme activity in leaves of *Gymnocarpos przewalskii* to drought[J]. Arid Zone Research, 2020, 37(1): 227-235.

[23] Zhang Zhongfeng, Zhang Jinchi, Huang Yuqing, et al. Effects of mycorrhizal fungi on the drought tolerance of *Cyclobalanopsis glauca* seedlings[J]. Acta Ecologica Sinica, 2016, 36(11): 3402-3410.

[24] Manoharan P T, Shanmugaiah V, Balasubramanian N, et al. Influence of AM fungi on the growth and physiological status of *Erythrina variegata* Linn. grown under different water stress conditions[J]. European Journal of Soil Biology, 2010, 46(2): 151-156.

[25] Yin D C, Song R Q, Qi J Y, et al. Ectomycorrhizal fungus enhances drought tolerance of *Pinus sylvestris* var. *mongolica* seedlings and improves soil condition[J]. Journal of Forestry Research, 2017, 29(6): 1775-1788.

[26] Xu Chao, Wu Xiaoqin. Drought resistance of *Pinus massoniana* seedlings inoculated with ectomycorrhizal fungi[J]. Journal of West China Forestry Science, 2012, 41(6): 43-47.

[27] Li Delu, Liu Shizeng, Kang Caizhou, et al. Effects of water stress on chlorophyll fluorescence characteristics of two *Picea* species[J]. Journal of Arid Land Resources and Environment, 2015, 29(6): 117-121.

[28] Su Zhihao, Zhou Xiaobing, Jiang Tao, et al. Effects of brassinolide on growth, physiology and photosynthetic characteristics of cured tobacco seedlings under drought stress[J]. Journal of Northwest A & F University (Natural Sciences Edition), 2020, 48(11): 33-41.

[29] Zou Hui, Wang Chunsheng, Zeng Jie. Effect of native mycorrhizal fungi on photosynthetic physiology of *Betula alnoides* clones seedlings[J]. Journal of Central South University of Forestry & Technology, 2019, 39(1): 1-7.

[30] Khajeddin S, Matinkhah S, Jafari Z. A drought resistance index to select drought resistant plant species based on leaf water potential measurements[J]. Journal of Arid Land, 2019, 11(4): 623-635.

[31] Zhang Wenquan, Yan Wei. Effect of ectomycorrhizae on the growth of *Pinus sylvestris* var. *mongolica* seedlings[J]. Acta Botanica Boreali Occidentalia Sinica, 2013, 33(5): 998-1003.

[32] Wang Lijun, Li Dong, Shen Hongxiaolong, et al. Physiological and biochemical characteristics and adaptability of *Tamarix taklamakanensis* in different ecological habitats in the Tarim Basin[J]. Arid Zone Research, 2021, 38(1): 198-206.

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