Research on soil enzymes and polysaccharides secreted by the roots of Salvia miltiorrhiza Bunge under abiotic stress

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Abstract

Root exudates serve as crucial mediators for the information exchange between plants and soil, which is an important evolutionary mechanism for plants to adapt to environmental changes. In this study, 15 different abiotic stress models were established using various stress factors, including drought (D), high temperature (T), nitrogen deficiency (N), and phosphorus deficiency (P) and their combinations. We investigated their effects on the seedling growth of Salvia miltiorrhiza Bunge and the activities of

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Abstract

Root exudates serve as crucial mediators for the information exchange between plants and soil, which is an important evolutionary mechanism for plants to adapt to environmental changes. In this study, 15 different abiotic stress models were established using various stress factors, including drought (D), high-temperature (T), nitrogen deficiency (N), and phosphorus deficiency (P) and their combinations. We investigated their effects on the seedling growth of *Salvia miltiorrhiza* Bunge and the activities of urease (S-UE), nitrite reductase (S-NiR), nitrate reductase (S-NR), phosphotransferase (S-PT), and catalase (S-CAT), as well as the contents of polysaccharides in the culture medium. The results showed that the growth of *S. miltiorrhiza* was inhibited under 15 stress conditions, among which 13 stress conditions could increase the root-shoot ratio. These 15 stress conditions significantly down-regulated the activity of S-NR, synergistic stresses of drought and nitrogen deficiency (DN) and synergistic stresses of high-temperature and nitrogen deficiency (TN) significantly up-regulated the activity of S-NIR (p <0.05). The N, D, T, synergistic stresses of drought and high-temperature (DT), DN, synergistic stresses of drought and phosphorus deficiency (DP), and synergistic stresses of high-temperature, nitrogen deficiency, and phosphorus deficiency (TNP) stresses conditions significantly increased the activity of S-UE (p <0.05). The activity of soil enzyme S-PT could be down-regulated under most stress conditions, but only D and T stresses could significantly up-regulate S-PT activity (p <0.05). The N, DN, and TN stresses conditions significantly reduced S-CAT activity. The content of total polysaccharides in soil was decreased under most stress conditions, and P, DT, and synergistic stresses of

drought, high-temperature, and phosphorus deficiency (DTP) stresses were significantly decreased (p <0.05). These results indicated that abiotic stress inhibited the growth of *S. miltiorrhiza* and altered the root secretion behavior. Plants respond to different abiotic stresses by regulating root secretions, including enzymes of the soil nitrogen cycle, phosphorus transport-related enzymes, and antioxidant enzymes. In conclusion, plants regulate the utilization of rhizosphere substances by regulating the intensity of soil enzymes and polysaccharides secreted by roots to cope with abiotic stress. At the same time, soil carbon sequestration is affected by the adverse environment, which restricts the input of organic matter into the soil.

KEYWORDS

abiotic stress, *Salvia miltiorrhiza* seedlings, soil enzymes, total polysaccharides, soil carbon sequestration

1 | INTRODUCTION

Root exudates are a complex mixture of biochemical compounds that are secreted actively or passively by plant roots. These compounds comprise diverse small molecules, such as amino acids and organic acids, and macromolecules, including polysaccharides, proteins, and biologically active enzymes (Chai and Schachtman, 2022). Biological and abiotic stress conditions alter the composition and quantity of plant root exudates, thereby altering the rhizosphere ecosystem and assisting plants in adapting to different stress environments (Chai and Schachtman, 2022, Calvo et al., 2017). During the process of plant growth, development and reproduction, about 21% of the net photosynthetic products of plants enter the soil as root exudates, and when plants adapt to different stress environments (Wang et al., 2021, Panchal et al., 2022, Pramanik and Phukan, 2020). Moreover, root exudates provide nutrients and energy to the rhizosphere microbiome, regulate the structure of microbial communities in soil, affect enzyme activities produced by microorganisms, and ultimately influence the decomposition, mineralization, and availability of organic compounds and soil nutrients (Lu et al., 2021). It can be seen that root exudates affect the action strength and direction of soil enzymes, and play a key role in the soil material cycle, energy conversion and plant carbon storage (Panchal et al., 2022). Especially, it plays an important role in the soil nitrogen and phosphorus cycle (Malek et al., 2021).

Soil enzymes are a complex mixture of both plant and microbial sources. Root secretion is not only an important source of soil enzymes but also the main factor driving microorganisms to secrete soil enzymes. Many enzymes play key roles in soil nitrogen cycling, such as Solid-Nitrate Reductase (S-NR), Solid-Nitrite reductase (S-NiR) and Solid-Urease (S-UE). Specifically, S-NR catalyzes the reduction of nitrate to nitrite, S-NiR catalyzes the reduction of nitrite to nitric oxide (Jian-guo and Wei-guo, 2018), and S-UE hydrolyzes urea to produce ammonia and carbonic acid (Fisher et al., 2017). The solid-phosphotransferase (S-PT) plays a crucial role in phosphorus uptake and utilization by catalyzing the transfer of phosphate groups from donors to acceptors (Wohlgemuth et al., 2017). Solid-Catalase (S-CAT) prevents the accumulation of toxic substances by promoting the degradation of hydrogen peroxide (Cusack et al., 2011). Additionally, root-secreted polysaccharides can enhance the growth of polysaccharide-utilizing microbial communities and stimulate the production of extracellular enzymes, thereby facilitating the decomposition of soil organic matter and nutrient cycling (Morcillo and Manzanera, 2021). Furthermore, these polysaccharides also serve as a significant source of organic matter in the soil (Pansu and Gautheyrou, 2006). Abiotic stresses such as drought (Staszel et al., 2022) and nitrogen (Jia et al., 2020) can change soil enzyme activities, thereby affecting soil-plant interactions. Nevertheless, root secretion plays an important role in plant response to environmental stress, especially soil enzymes secreted by roots and soil enzymes secreted by microorganisms driven by root exudates. However, reports on soil enzymes and polysaccharides in root exudates under sterile conditions less.

Global climate change is one of the important factors limiting crop yields (Yuan et al., 2009), mainly causing the loss of nutrients such as nitrogen and phosphorus in the soil, resulting in reduced crop yields (Bojko and Kabala, 2016, Widdig et al., 2020, Twining et al., 2022). High-temperature, drought, nitrogen deficiency, and phosphorus deficiency are common factors that limit crop yield. In nature, plants are often

under combined stress of multiple environmental factors, but how they affect plant growth and development is poorly understood (Zandalinas et al., 2021). There is also a lack of direct evidence that roots secrete soil enzymes and polysaccharides into the soil when plants respond to these adverse environments. Based on this, under sterile conditions, 15 stress models were designed with high-temperature (T), drought (D), nitrogen deficiency (N), phosphorus deficiency (P) and their combinations. After the stress culture of *S. miltiorrhiza*test-tube seedlings, the secretion of S-UE, S-NIR, S-NR, S-PT, and S-CAT activities and total polysaccharide content changes were measured. To understand the effects of *S. miltiorrhiza* on soil nitrogen, phosphorus cycling and soil polysaccharides in response to abiotic stress, explore the ecological strategy and role of plant root secretion response under abiotic stress.

2 | MATERIALS AND METHODS

2.1 | Study site and test materials

Tissue culture seedlings were selected from Chengdu University of Traditional Chinese Medicine by excising young leaves from the stem apex of the same plant and cultivating them into tissue culture seedlings after surface sterilization, tissue healing, bud clustering, and root induction in the laboratory (E: 103.81°, N: 30.68°).

The culture medium utilized was shell shale sourced from Ximeishan Village (E: 104.54°, N: 30.94°), Shiquan Township, Zhongjiang County, Sichuan Province. The medium was air-dried, passed through a 1-5 mm sieve, cleansed of impurities, sterilized at 180 for 3 h in a dry heating process, and cycled twice to eliminate microorganisms and their exudates. After cooling, it was packed in kraft paper and then sterilized again through dry heat at 120 for 3-4 h before use. The pH value of the shell shale was 7.78, with an organic matter content of 0.69 g/kg, alkaline nitrogen of 4.2 mg/kg, potassium of 3.79 mg/kg, and phosphorus of 2.23 mg/kg.

The culture device used was a circular polypropylene box with a top diameter of 17.2 cm, a bottom diameter of 11.8 cm, and a height of 9.7 cm. Each box was wrapped in kraft paper and sterilized at high-pressure steam of 0.1MPa and 121 for 30 min, and after cooling, filled with 600 g of culture medium.

2.2 | Experimental design

Four stress factors, including high-temperature (T), drought (D), nitrogen deficiency (N), and phosphorus deficiency (P), at 2 levels per factor, for 16 treatments, of which CK was the control group the treatment conditions for each group are shown in Table 1. As a full nutrient solution, Hoagland's solution was used (table S1). In the reference method (Torres-Rodriguez et al., 2021, Zhao et al., 2005), the phosphorus content is set to 0 mM for the phosphorus stress treatment, and the nitrogen content is set to 0 mM for the nitrogen deficiency stress treatment. The corresponding concentrations of KH_2PO_4, $Ca(NO_3)_2$, KNO_3, and NH_4NO_3 were adjusted to 0%, respectively. The lacking Ca and K in the deficit solutions were $CaCl_2$ and KCl, respectively, while the remaining components of Hoagland's solution remained unchanged. Based on ground temperature data from the *S. miltiorrhiza* cultivation base in Zhongjiang, Sichuan, the temperature was chosen with 25/20 (day/night) for the normal conditions and 42/35 (day/night) for the high-temperature stress condition. The water holding capacity (WHC) was 75% for normal moisture conditions and 30% for drought stress conditions (Liu et al., 2011).

TABLE 1 Design of each condition for Salvia seedlings

Group	Abbreviations	Watering nutrients	WHC	Incubation temperature (d
Control	CK	Full strength nutrient solution	75%	25/20
Drought	D	Full strength nutrient solution	30%	25/20
High-temperature	T	Full strength nutrient solution	75%	42/35
Nitrogen deficiency	N	Nitrogen-deficient nutrient solution	75%	25/20
Phosphorus deficiency	P	Phosphorus-deficient nutrient solution	75%	25/20

Note: The experiments were carried out strictly by the above conditions.

2.3 | Seedling cultivation

Under aseptic conditions, test tube seedlings with 3-5 uniform long roots weighing approximately 0.5 g were carefully extracted from the culture flasks, and any adhering agar was eliminated using sterile filter paper. The seedlings were weighed and transferred to culture boxes containing 600 g of shale pellets, 2 plants per box. A total of 96 boxes were planted. Each box was filled with 100 mL of sterile water and 5 mL of sterilized Hoagland's total nutrient solution at the seedlings' roots. Each box's total weight was recorded as a reference value for irrigation. Place on the culture rack in the tissue culture room for cultivation at 25/20 (day/night) and a light intensity of 3000 lx. Weigh and replace the water every 3 days to maintain 75% WHC of the culture medium, and water the roots of the seedlings with 5 mL of Hoagland's full nutrient solution each week. After 4 weeks of incubation, the plants were randomly divided into 16 groups of 6 boxes each and watered with the nutrient solution required for the different stress treatment groups. After 3 weeks of culture, observe the morphological characteristics of the plant's nitrogen and phosphorus deficiency stress and then perform other synergistic stress treatments. The natural precipitation method was used for drought treatment in the drought synergistic stress treatment group. After the WHC dropped to 30%, the 30% WHC was maintained until the end of the treatment. The plants were transferred to an incubator at 42/35 (day/night) under the same light conditions for the temperature stress treatment. All experiments were completed after 5 days of heat stress.

2.4 | Sample collection and determination

At the end of the culture experiments, the roots were washed with deionized water and blotted dry with absorbent paper, and the weights of the plants' above- and below-ground parts were measured separately. Two boxes of culture substrates in each group were randomly combined into one sample, mixed and sampled by quartering. Pass through a 40-mesh sieve after grinding to determine soil enzymes and polysaccharides. Enzymatic activities of S-UE, S-NiR, S-NR, and S-CAT were evaluated using cominbio kits (Suzhou Keming Biotechnology Co., Ltd.) following the manufacturer's guidelines, while mlbio kits (Shanghai Enzyme Biotechnology Co., Ltd.) were employed to measure the activity of S-PT enzymes. The determination of pentoses adopts the lichenol-hydrochloric acid method (Haoli et al., 2014), and the determination of hexoses adopts the anthrone-sulfuric acid method (wei-jie, 1999), and the sum of the two is calculated as the total polysaccharide content.

S-NiR activity was assessed using the cominbio kit's method. The amount of reducing 1 μmol NO_2- per g soil sample per day was regarded as an enzyme activity unit. In a 2 mL centrifuge tube, 0.02 g of air-dried soil sample was mixed with 40 μL each of sodium nitrite and glucose solutions. The mixture was thoroughly combined and reacted for one hour at 25. Subsequently, 40 μL of aluminum potassium alum solution was added, and the content was fully agitated for thirty seconds before centrifugation was facilitated at 10,000 rpm, at 4, for ten minutes. At this stage, 70μL of the supernatant was extracted, along with 1:1 p-amino benzene sulfonic acid-phosphoric acid solution and 140 μL of N-1-naphthalene ethylenediamine hydrochloride solution. The components were mixed well and evaluated for absorbance at 540 nm. Control tubes were supplied with distilled water instead of sodium nitrite solution, while blank tubes had no samples. S-NR activity was assessed using the cominbio kit's method, and the amount of 1 μmol NO_2- produced per gram of soil sample per day was regarded as one S-NR activity unit. Air-dried soil samples weighing 0.06 g were included in a 2 mL centrifuge tube containing 225 μL of KNO_3 solution and 75 μL of Reduced Coenzyme I (NADH) solution. Samples were then subjected to a 24-hour water bath at 37 before centrifugation at 8000g and 25 for 10 min. At this stage, 130 μL of the supernatant was collected and added to 85 μL each of p-amino benzene sulfonic acid solution and α-naphthylamine solution. The resulting mixture was agitated and left for color development at 25 for 20 min before centrifugation at 4000g for 10 min at 25. 200 μL of the supernatant was transferred to a 96-well plate and monitored for absorbance at 540 nm. Distilled water replaced the KNO_3 solution for the control tube, while the blank tubes omitted soil samples. The standard tube replaced the soil sample with 0.1 μmol/mL $NaNO_2$solution. S-UE activity was assessed using the cominbio kit's method, and 1 μg of NH_3-N produced per gram of soil sample per day was defined as

an enzyme activity unit. Air-dried soil samples weighing 0.06 g were placed in 2 mL centrifuge tubes after adding 20 μL of toluene and shaking, then placed at room temperature for 15 min. Afterward, 90 μL of urea and 190 μL of citric acid-potassium hydroxide solution were blended before a 24 h water bath at 37. Following this, centrifugation was carried out at 10,000g and 25 for 10 min. The supernatant was then diluted ten-fold, with 80 μL taken, and the mixture was coupled with 15 μL of phenol, methanol, acetone, absolute ethanol, and NaOH solutions. Next, 15 μL of sodium hypochlorite solution was added and mixed well before being placed at room temperature for 20 min. The absorbance of the resultant mixture was monitored at 578 nm after the inclusion of 90 μL of distilled water. Distilled water substituted urea in the control tube. S-CAT activity was assessed using the cominbio kit's method, and the degradation of 1 μmol H_2O_2 catalyzed per g of air-dried soil sample per day was defined as an enzyme activity unit. Air-dried soil samples weighing 0.03 g were included in a 2 mL centrifuge tube and supplemented with 260 μL of hydrogen peroxide. Samples were subjected to a 20 min incubation at 25 while agitated at 500r/min. Next, 10 μL of aluminum potassium alum was added, followed by centrifugation at 8000g and 25 for 5 min. 180 μL of the supernatant was collected and mixed with 20 μL of sulfuric acid solution before monitoring absorptivity at 240 nm. The control tube contained distilled water instead of hydrogen peroxide, while the blank tubes contained no soil samples. S-PT activity was assessed using the mlbio kit's method. To prepare the soil samples for testing, 0.1 g of soil was mixed with 0.9 mL of Phosphate buffer saline (PBS) before centrifugation at 4000r for 15 min. The resultant supernatant became the sample for examination. Next, 50 μL of the diluted standard and 40 μL of the sample diluent were added to the sample, which was then sealed with a sealing film and incubated at 37 for 30 min. Afterward, the liquid was discarded, followed by the introduction of wash solution, left for 30 s, and discarded five times. Subsequently, 50 μL of HRP enzyme-labeled reagent was included, and incubation and washing processes resumed before adding 50 μL of carbamide peroxide color reagent and 50 μL of color reagent. At this stage, color development was initiated, with 10 min allotted for completion in the dark at 37. Additionally, 50 μL of termination solution was infused before absorbance levels were measured at 450 nm. Blank wells that omitted samples and enzyme labeling reagents were also featured. Phosphotransferase markers (Figure. S1). The content determination method of the total polysaccharide in the soil adopts the method of pansu et al. (Pansu and Gautheyrou, 2006). Preparation of the test solution: weigh 2.5g of air-dried soil, put it in a 50mL Conical flask, then add 20 mL of 2.5M H_2SO_4, heat and reflux in a water bath at 100 for 20 min, let cool, filter, wash, and collect the filtrate. Put the residue in a 50mL Conical flask, add 1mL 13M H_2SO_4, and place it at room temperature for 16 h. Slowly add 25 mL of distilled water, let cool, heat at 100 for 5 h after airtight, let cool, filter, combine the filtrate with the above filtrate and dilute to 50mL to obtain the test solution. The total polysaccharide content was determined using the lichenol-hydrochloric acid method for pentose, while hexose was assessed using the anthrone-sulfuric acid method. Pentose and hexose markers, respectively (figure s3, figure s2). Finally, the sum of the two was calculated as the polysaccharide content.

2.5 | Data statistics

The data were analyzed using IBM SPSS 21.0 software for one-way analysis of variance, and the least significant difference method and Duncan's were used for multiple comparisons. Data presented here as a fully randomized design with three replicates expressed as mean ± standard deviation. Different lowercase letters in the table indicate a significant difference from the blank. They were graphing with GraphPad Prism 9 and Spearman correlation analysis with R (version 4.1.2).

3. | RESULTS

3.1 | Changes in growing *S. miltiorrhiza*seedlings

Abiotic stress is not conducive to the growth of *S. miltiorrhiza*seedlings, among which P, synergistic stresses of drought and nitrogen deficiency (DN), synergistic stresses of drought, nitrogen deficiency and phosphorus deficiency (DNP) and synergistic stresses of drought, high-temperature, nitrogen deficiency and phosphorus deficiency (DTNP) stresses reached a significant level (p <0.05). P, DN, synergistic stresses of drought, high-temperature and nitrogen deficiency (DTN), synergistic stresses of drought high-temperature and phosphorus deficiency (DTP), DNP, synergistic stresses of high-temperature, nitrogen deficiency and phosphorus

deficiency (TNP), and DTNP stresses significantly inhibited the fresh weight of aboveground parts of *S. miltiorrhiza* (p <0.05) (Figure 1). Drought or synergistic stresses of drought and high-temperature exacerbated the plant growth inhibition of nitrogen deficiency, but not significantly. (e.g., DN and DTN stresses) (Figure 1-a). Drought or high-temperature or synergistic stresses of drought and high-temperatures alleviated the plant growth inhibition of phosphorus deficiency, but not significantly. (e.g., DP, TP, and DTP stresses) (Figure 1-b). Synergistic stresses of drought and high-temperatures aggravated the plant growth inhibition of nitrogen and phosphorus deficiency (e.g., DTNP stresses) (p <0.05) (Figure 1-c). The synergistic stresses of drought and high-temperatures exacerbated the effects of single-factor inhibition, but not to a significant extent (e.g., DT stress) (Figure 1-d).

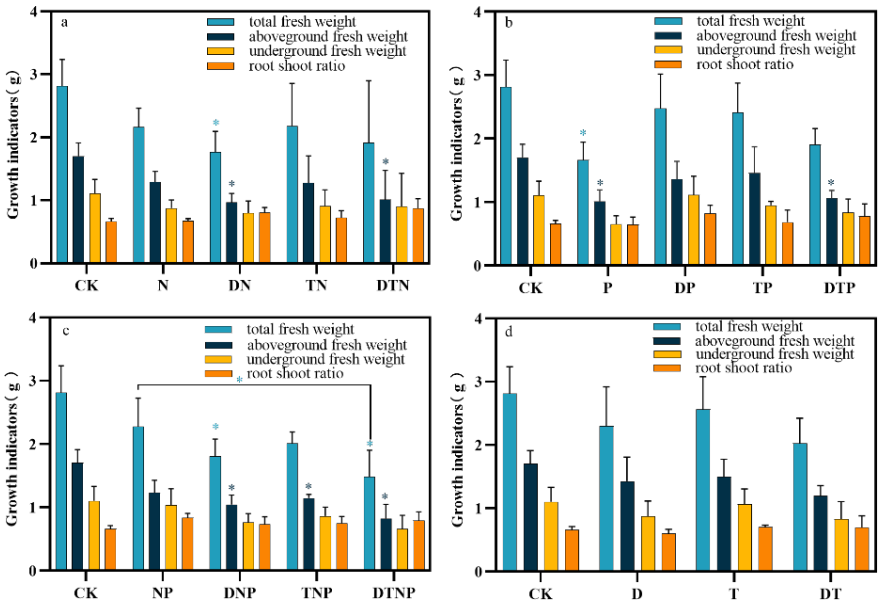

FIGURE 1 The growth changes of *S. miltiorrhiza* seedlings under abiotic stress. (a), (b), and (c) respectively represent the changes in the growth effects of the superposition of various environmental factors under the stress of nitrogen, phosphorus and nitrogen and phosphorus deficiency, and (d) represents the changes in the growth effects of the superposition of drought and high temperature. * indicating a significant difference to the CK treatment, p< 0.05.

3.2 | The activity of enzymes related to nitrogen metabolism in the medium

Abiotic stress can significantly down-regulated S-NR activity in the culture medium, with single factors N and T stresses having the strongest inhibitory effect, while the combined effect was most severe in synergistic stresses of high-temperature and nitrogen deficiency (TN), synergistic stresses of nitrogen and phosphorus deficiency (NP), and DTN stresses (p <0.05). DN and TN stress up-regulated S-NiR activity (p <0.05), but the other factors did not show statistical significance. Moreover, N, T, D, synergistic stresses of high-temperature and drought (DT), DN, synergistic stresses of drought and phosphorus deficiency (DP), and TNP stresses all elevated S-UE activity in the culture medium, while synergistic stresses of high-temperature and phosphorus deficiency (TP), TN, NP, and DTN stresses significantly down-regulated S-UE activity in the culture medium (p <0.05) (Figure 2). These findings indicate that under abiotic stress, plants reduce the secretion of S-NR enzyme from their root system, which curtails denitrification and prevents nitrate-nitrogen loss. In response to N, T, D, DT, DN, DP, and TNP stresses, plants secrete S-UE to enhance organic nitrogen utilization.

Drought or high-temperature stress can significantly up-regulate the effect of nitrogen deficiency on the release of S-NiR from roots (e.g., DN and TN stresses) (p <0.05). Drought stress can significantly alleviate

the effect of nitrogen deficiency on the release of S-NR from roots and simultaneously significantly promote the release of S-UE from roots (e.g., DN stress) (p <0.05). High-temperature or synergistic stresses of drought and high-temperature inhibit the effect of nitrogen deficit on the induction of S-UE release from roots (e.g., TN and DTN stresses) (p <0.05) (Figure 2-a). Moreover, drought stress can significantly enhance the effect of phosphorus deficiency on the release of S-UE from roots, while the high-temperature can alleviate the effect of phosphorus deficiency on the release of S-NR from roots and inhibit the release of S-UE from roots (e.g., DP and TP stresses) (p <0.05) (Figure 2-b). Furthermore, drought or high-temperature or the synergistic stresses of both can alleviate the effect of nitrogen and phosphorus deficiency on the inhibition of root secretion of S-UE and the synergistic stresses of high-temperature and drought can also significantly promote the release of S-NR from roots (e.g., DNP, TNP, and DTNP stresses) (p <0.05) (Figure 2-c). Finally, the synergistic stresses of drought and high-temperature can significantly reduce the effect of drought on the release of S-UE from roots (e.g., DT stress) (p <0.05) (Figure 2-d).

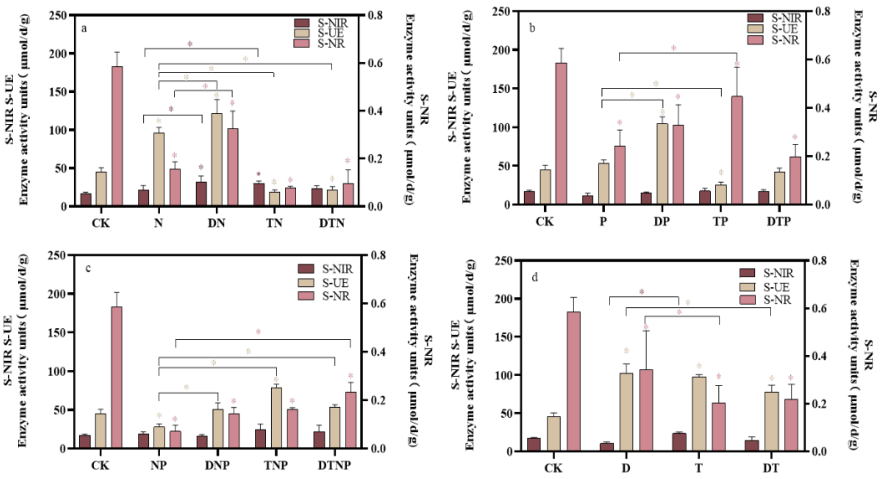

FIGURE 2 Roots in the culture medium secrete the activity of enzymes related to nitrogen metabolism. (a), (b), and (c) respectively represent the changes in nitrogen metabolism-related enzymes under the superimposed environmental factors of nitrogen, phosphorus and nitrogen-phosphorus deficit stresses. (d) indicates the changes in nitrogen metabolism-related enzymes under the superimposed drought and high temperature. * indicating a significant difference to the N, P, NP, D, and CK treatment, $p < 0.05$.

3.3 | The phosphotransferase activity in the medium

D and T stresses can up-regulate the activity of S-PT in the culture medium, whereas all stresses except N stress can reduce the activity of S-PT in the culture medium (p <0.05) (Figure 3). Drought or high-temperature or the synergistic stresses of drought and high-temperature can significantly reduce the effect of nitrogen deficiency on up-regulating S-PT activity (e.g., DN, TN, and DTN stresses) (p <0.05) (Figure 3-a). Moreover, drought can alleviate the effect of phosphorus deficiency on down-regulating S-PT activity, while the synergistic stresses of drought and high-temperature can aggravate the effect of phosphorus deficiency on down-regulating S-PT activity (e.g., DP and DTP stresses) (p <0.05) (Figure 3-b). Furthermore, drought or high-temperature or synergistic stresses of drought and high-temperature can alleviate the effect of nitrogen and phosphorus deficiency on the inhibition of S-PT activity (e.g., DNP, TNP, and DTNP stresses) (p <0.05) (Figure 3-c). Finally, synergistic stresses of drought and high-temperature inhibit the induction effect of a single factor on S-PT activity (e.g., DT stress) (p <0.05) (Figure 3-d). It shows that under D and T, plants secrete S-PT through the root system to improve the transport and utilization of phosphorus, but most stresses are not conducive to the transport and utilization of phosphorus.

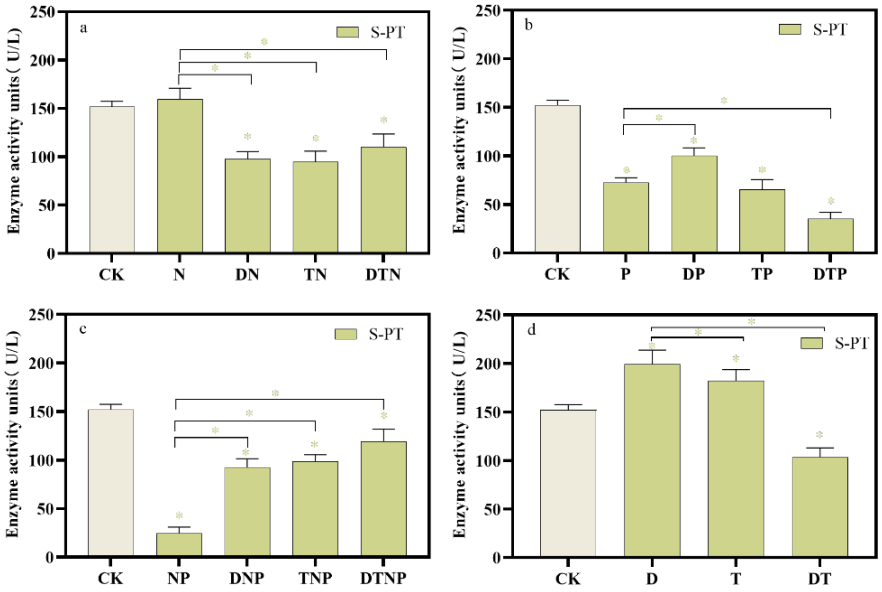

FIGURE 3 The root system secretes phosphotransferase activity in the culture medium. (a), (b), and (c) respectively represent the changes in phosphotransferases under the superimposed environmental factors of nitrogen, phosphorus and nitrogen-phosphorus deficit stress. (d) indicates the changes in phosphotransferase under the superposition of drought and high temperature. * indicating a significant difference to the N, P, NP, D, and CK treatment, $p < 0.05$.

3.4 | The Catalase activity in the medium

N, DN, and TN stress significantly down-regulated the activity of S-CAT in the culture medium (p <0.05), while the other groups do not show statistical significance. In addition, synergistic stresses of drought and high-temperature can significantly improve the effect of nitrogen deficiency in down-regulating S-CAT activity (e.g., DTN stress) (p <0.05) (Figure 4-a), but the other groups are not statistically different. It shows that N, DN, and TN stresses are not conducive to removing root peroxide.

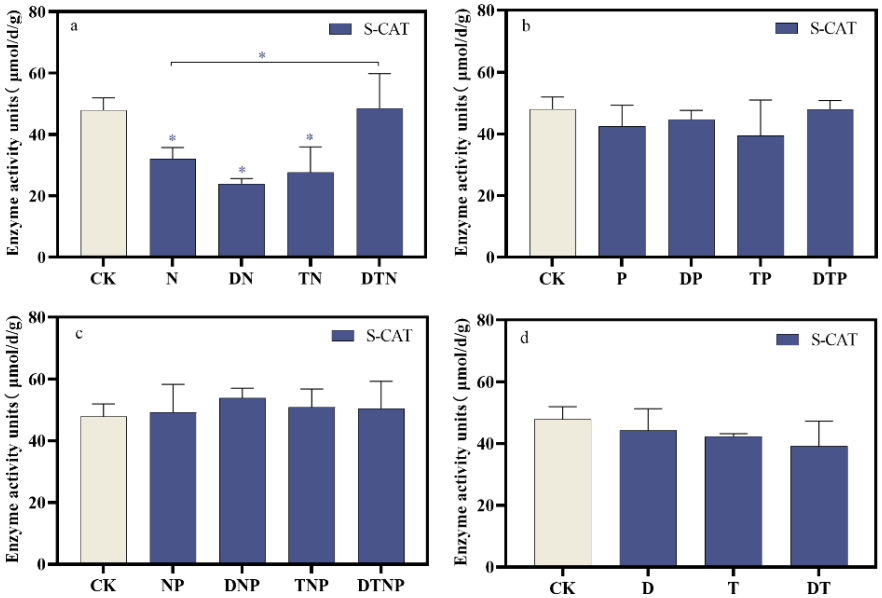

FIGURE 4 The root system secretes catalase activity in the culture medium. (a), (b), and (c) respectively represent the changes in catalase under the superimposed environmental factors of nitrogen, phosphorus and nitrogen-phosphorus deficit stress. (d) indicates the changes in catalase under the superimposed drought and high temperature. * indicating a significant difference to the N, P, NP, D, and CK treatment, $p < 0.05$.

3.5 | The polysaccharide content in the medium

P, DT, and DTP stresses can significantly reduce the polysaccharides content in the culture medium (p <0.05), and all groups except DP, TP, and DN stresses tend to reduce the content of polysaccharides in the culture medium (Figure 5). Additionally, drought or high-temperature can significantly alleviate the effects of the down-regulation of root polysaccharides secreted by phosphorus deficiency (e.g., DP and TP stresses) (p <0.05) (Figure 5-b). However, the synergistic stresses of drought and high-temperatures can exacerbate the suppressive effects of drought (e.g., DT stress) (p <0.05) (Figure 5-d). These results suggest that under abiotic stress, the root system of plants reduces the secretion of polysaccharides, thereby reducing the consumption of carbon and energy, but the effects of DP, TP, and DN stresses are not obvious.

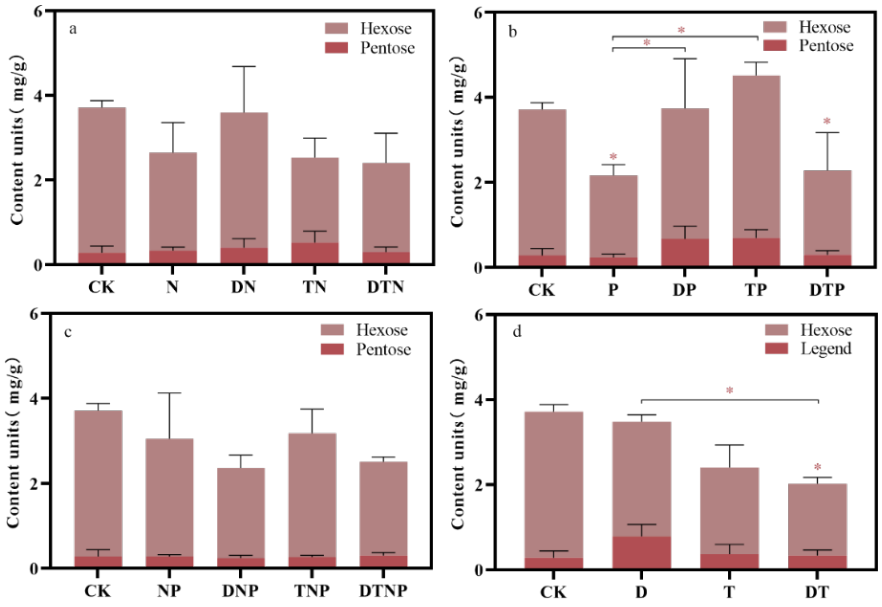

FIGURE 5 Content of total polysaccharides secreted by roots in culture media. (a), (b), and (c) respectively represent the changes in polysaccharide content under the superimposed environmental factors of nitrogen, phosphorus and nitrogen-phosphorus deficit stress. (d) indicates the changes in polysaccharide content under superimposed drought and high temperature. * indicating a significant difference to the N, P, NP, D, and CK treatment, $p < 0.05$.

3.6 | Correlation of soil enzyme activity and polysaccharide content with the seedling weight

The polysaccharide content in the culture medium showed a significant positive correlation with the fresh weight of *S. miltiorrhiza*seedlings and the fresh weight of their aboveground or underground parts (p <0.05). Additionally, S-NR and S-PT were found to have an extremely significant negative correlation with the root-shoot ratio (p <0.001), while S-NiR had a significant positive correlation with the same ratio (p <0.05). Furthermore, S-UE had a significant positive correlation with S-NR and S-PT, whereas S-NiR had a significant negative correlation with S-NR (p <0.05) (Figure 6). These findings suggest that the release of

polysaccharides from the root system into the environment is closely related to the growth and developmental status of the plant.

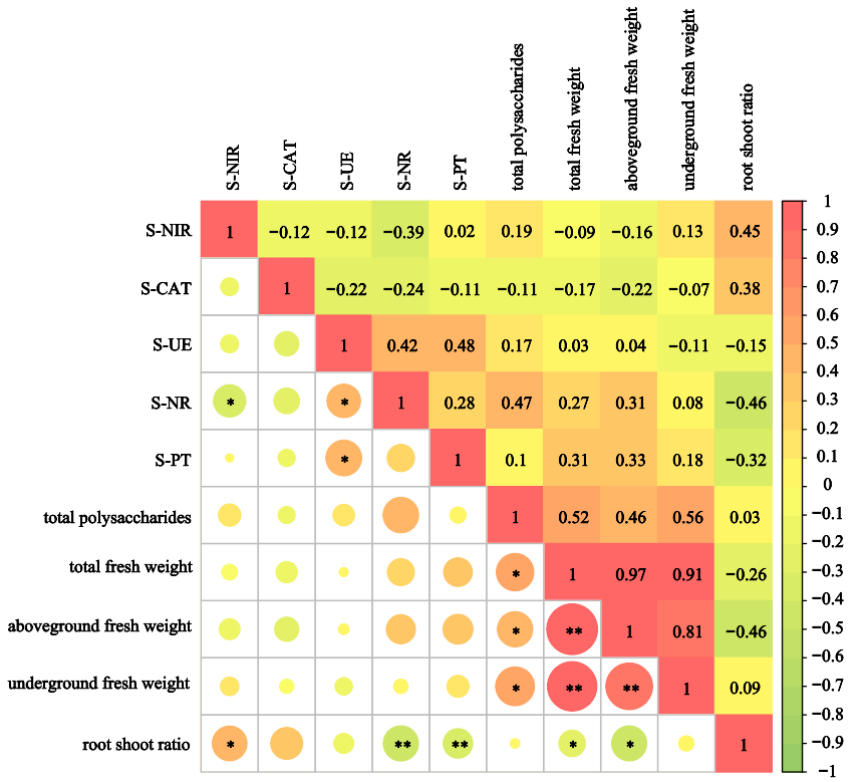

FIGURE 6 Correlation of soil enzyme activity and polysaccharide content with the seedling weight. The top right corner of the graph indicates correlation coefficients and the bottom left corner indicates significant differences in correlation. * indicates a significant correlation (p <0.05), ** indicates a strong significant correlation (p <0.01).

4 | DISCUSSION

4.1 | Root secretion and soil nitrate cycling and utilization

Plants require nitrogen to form new cells (Ke et al., 2020), and nitrogen deficiency is a limiting factor in crop productivity (Zhao et al., 2005). Soil nitrogen exists in organic and inorganic forms, with nitrate and ammonium being the plant-available types (Xu et al., 2012). Root secretion of soil nitrogen cycle enzymes influences nitrogen use (Dong et al., 2021). This experiment revealed that S-NR activity secreted by *S. miltiorrhiza* roots was significantly down-regulated under 15 different abiotic stress conditions. This suggests that under stress, *S. miltiorrhiza* can maintain the supply of nitrate-nitrogen in the root environment by down-regulating the activity of root-secreted S-NR and reducing its nitrate-nitrogen reduction ability (Coskun et al., 2017). This is consistent with nitrogen stress down-regulating NR secretion from maize roots (Qiang et al., 2021) and phosphorus stress down-regulating NR activity from cowpea roots (Qi bing-lin et al., 2010). The suggestion is that reducing nitrogen loss is a strategy for plants to cope with abiotic stress. However, the results of this study differ from the drought stress induced upregulation of NR activity in wheat roots (Hosseini et al., 2022) and the improvement of S-NR enzyme activity in soil by nutrient stress treatment (Meng et al., 2021). The involvement of microorganisms in root exudation activities, and the

increased activity of denitrifying bacteria, may contribute to the differences observed in nitrate reductase activity in the soil.

This experiment shows that *S. miltiorrhiza* often up-regulates the activity of root-secreted S-NiR under most stress conditions, which is consistent with the fact that drought stress significantly increases the activity of S-NiR in the rhizosphere of maize (Lin et al., 2021) but different from the fact that low nitrogen reduces the activity of NiR in the rhizosphere of tobacco (Xihuan et al., 2020). Notably, *S. miltiorrhiza* significantly increased the activity of S-NiR in both DN and TN stresses, indicating improved denitrification, which may be related to the high-temperature causing soil oxygen overflow (Guan hui-lin et al., 2010). It shows that after stress, plants can reduce the damage of nitrite salt in the rhizosphere environment by up-regulating the activity of S-NiR secreted from the root system.

S-UE plays a critical role in converting organic nitrogen in soil into ammonium nitrogen available for plants. This study shows that *S. miltiorrhiza* can significantly up-regulate the activity of S-UE secreted by roots under N, D, T, DT, DN, DP, and TNP stresses, consistent with the results that increasing temperature increases the activity of S-UE in soil (Bai et al., 2017). Moreover, nitrogen deficiency and its synergistic stress with drought can significantly up-regulate the activity of root-secreting S-UE, but the synergistic stress of high-temperature or synergistic stresses of drought and high-temperature can significantly down-regulate the activity of root-secreting S-UE (Figure 2-a) (e.g., DN, TN, and DTN stresses). In addition, nitrogen deficiency, phosphorus deficiency and their synergistic stress with drought or high-temperature or drought and high-temperature can significantly up-regulate the activity of root-secreting S-UE (Figure 2-c) (e.g., DNP, TNP, and DTNP stresses). These findings suggest that plants demonstrate unique responses to different synergistic stress. Under abiotic environmental stress, plants can reduce the reduction of nitrate nitrogen and accelerate the transformation of organic nitrogen by altering the activities of enzymes involved in root secretion and soil nitrogen cycling to maintain nitrogen available to plants and improve the reduction of nitrite to reduce its harmfulness, and then improve nitrogen conversion and use efficiency.

4.2 | Root secretion and soil phosphorus utilization

Phosphorus is one of the essential elements for plants (Arwenyo et al., 2022), and the distribution and transport of soil phosphorus affect plant growth and productivity (Xia et al., 2020, Yadav et al., 2020). S-PT is an enzyme that catalyzes the transfer of phosphate groups from donors to acceptors and is closely related to phosphorus uptake and transport. This study found that under D, T, and N stresses, *S. miltiorrhiza* up-regulates the activity of root-secreted S-PT, while other stresses down-regulate this activity significantly. In the context of nitrogen stress, synergistic stress of drought or synergistic stress of high-temperature, or synergistic stresses of drought and high-temperature, can significantly down-regulate the activity of root-secreted S-PT (Figure 3-a) (e.g., DN, TN, and DTN stresses). However, under nitrogen and phosphorus stress, synergistic stress of drought or synergistic stress of high-temperature or synergistic stresses of drought and high-temperature synergistic stress can significantly up-regulate the activity of root-secreted S-PT (Figure 3-c) (e.g., DNP, TNP, and DTNP stresses). This also supports the claim that plants respond uniquely to different synergistic stress. It is also suggested that in the absence of microbial participation, most stress environments are not conducive to phosphorus uptake and transport. Most stressful environments that promote phosphorus uptake and transport in plant roots may be directly related to rhizosphere microorganisms (Glaesner et al., 2016).

4.3 | Abiotic stress and inter-root antioxidant

The accumulation of active oxygen free radicals in the rhizosphere of plants causes oxidative damage to the cell membrane system of the root tip and leads to a series of physiological and biochemical changes in the root's absorptive function (Mittler et al., 2022). The results of this study showed that *S. miltiorrhiza* significantly down-regulated the activity of S-CAT secreted by the root under N, DN, and TN stresses. Under the synergistic stresses of three and four factors, *S. miltiorrhiza* has tended to up-regulate the activity of root-secreted S-CAT. This is similar to the effect of nitrogen inhibiting the secretion of enzymes in maize roots (Liang et al., 2016) but different from the effect of drought stress stimulating the secretion of antioxidant

substances in maize roots (Song et al., 2012). Overall, N, DN, and TN stresses can cause active oxygen hazards in the rhizosphere, affecting the uptake and transport of rhizosphere nutrients.

4.4 | Abiotic stress and soil polysaccharide composition

The saccharides secreted by plant roots are an important source of soil organic matter, microbial carbon, and energy, mainly hexoses and a small number of pentoses. This study shows that pentose content tends to increase under most stress conditions, but it is not synchronized with the change in hexose. Under N stress, the secretion of polysaccharides from *S. miltiorrhiza* roots was down-regulated, confirming that nitrogen stress significantly reduced the secretion of saccharides from maize roots (Carvalhais et al., 2010, Zhu et al., 2016). P, DTP, and DT stress significantly down-regulated polysaccharide secretion in *S. miltiorrhiza* roots, unlike phosphorus deficiency, which promoted sugar secretion in maize roots (Carvalhais et al., 2010), and drought stress, which promoted polysaccharide secretion in plant roots (Ulrich et al., 2022). The release of root exudates positively correlates with microbial growth (Bengtson et al., 2012), suggesting that this result and the literature results may be related to the lack of microbial involvement in rhizosphere activities. Further analysis shows that the content of polysaccharides secreted by roots is positively correlated with plant yield. Abiotic stress is not conducive to the accumulation of plant biomass and may also affect the distribution of plant polysaccharides, resulting in most energy substances, such as carbon source synthetic sugars, being supplied to plants, reducing the roots secrete polysaccharides that are not conducive to carbon sequestration in the soil. Thus, unfavorable environment affects plant growth and also affects the carbon stored in the soil by the plants.

5 | CONCLUSIONS

When *S. miltiorrhiza* is subjected to different abiotic stress conditions, changes occur in the soil enzyme activity that is secreted by its root system, altering the soil nitrogen cycle, phosphorus transport and utilization, and the antioxidant capacity of the rhizosphere. Plants obtain nutrients and reduce oxidative damage to roots by altering the levels of soil enzymes secreted by roots. Unfavorable environments can directly affect plant growth and development, affecting the amount of organic matter that plants put into the soil, thereby affecting soil carbon storage.

AUTHOR CONTRIBUTIONS

Yong Qin : Conducting experiments, Formal analysis, Writing - original draft. **Xiaoyu Li** : Data collection. **Yanhong Wu** : Conducting experiments, Paper check. **Hai Wang** : Content is critically revised. Paper check. **Guiqi Han** : Content is critically revised. Paper check. **Zhuyun Yan** : Experimental design, Methodology, Writing - review & editing, Supervision.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data is available in the main text and the supplementary materials.

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