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Research Article

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Study on the regulation of manganese oxide-mediated salt stress alleviation on the root yield and quality of *Glycyrrhiza glabra*

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Abstract

Background: To address the decline in yield and the deterioration of medicinal quality in cultivated *Glycyrrhiza glabra* L. under salinized soil conditions , this study subjected *G. glabra* seedlings to 160 mM NaCl stress. Different concentrations of manganese oxide (MnO) solution were applied via rhizosphere irrigation to investigate the regulatory effect of exogenous MnO in alleviating salt stress injury and to determine the optimal application concentration, thereby providing a scientific basis for the high-quality cultivation of *G. glabra* in salinized soils.

Methods: The results showed that exogenous application of MnO effectively alleviated salt-induced damage in a markedly concentration-dependent manner. Among the tested concentrations, the 20 $mg \cdot L^{-1}$ MnO treatment produced the optimal effect: compared with the NaCl stress group, the shoot and root biomass increased significantly by 150% and 60%, respectively; root morphology was markedly improved, and photosynthetic efficiency was maintained at a higher level; Antioxidant enzyme activities

(SOD, POD, and CAT) were significantly enhanced, while cell membrane damage was substantially reduced. More importantly, while alleviating salt stress, the treatment significantly promoted the accumulation of key bioactive constituents in the roots, including glycyrrhizic acid, liquiritin, glabridin, liquiritigenin, and isoliquiritigenin. Compared with the NaCl stress group, the concentrations of these five compounds of the 20 mg·L^{-1} MnO treatment increased by 13.03%, 29.32%, 137.43%, 158.73%, and 73.51%, respectively. And its the average contents of these five compounds per plant increased by 83%, 118%, 279%, 262%, and 233%, respectively, compared to the NaCl stress group.

Conclusions: Principal component analysis and correlation analysis further confirmed that MnO at an appropriate concentration can systematically regulate the physiological and metabolic network of *G. glabra* by coordinating the growth-defense trade-off.

Keywords: *Glycyrrhiza glabra* L.; Salt stress; Micronutrients; Salt tolerance; Yield and quality; Glabridin

1. Introduction

Glycyrrhiza glabra L., a species of the family Fabaceae, derives its medicinal value from the dried roots and rhizomes. It is mainly distributed in Europe, Central Asia, and the Xinjiang region of China, and is one of the medicinal plants officially recorded in the 2025 edition of the *Pharmacopoeia of the People's Republic of China*[1], the principal active constituents in its roots include triterpenoid saponins such as glycyrrhizic acid and glycyrrhetinic acid, as well as flavonoids including liquiritin, liquiritigenin, isoliquiritigenin, and glabridin. These compounds confer a broad spectrum of pharmacological activities, including immunomodulation, antioxidation, antiviral, anti-inflammatory, and antimicrobial effects[2,3]. Notably, glabridin is a unique constituent specific to the roots of the licorice and is recognized as the most effective plant-derived skin-whitening agent, earning it the designation "gold standard for skin" whitening. Consequently, this compound has been extensively incorporated into cosmetic products.

Over the past five decades, the demand for *G. glabra* and its derived products has grown steadily (at an annual rate of 8.7%), overexploitation and habitat destruction have caused the plant to plummet drastically, pushing the species to the brink of exhaustion[4]. Cultivation-based substitution not only contributes to the conservation of its natural populations but also represents a viable approach to effectively alleviating the imbalance between supply and demand, thereby serving as an effective measure for achieving its sustainable utilization[5,6]. However, many regions in Xinjiang are confronted with severe soil salinization, and seedlings of *G. glabra* are relatively sensitive to soil salinity. Consequently, cultivating *G. glabra* in salinized soils often results in early seedling mortality and reduced yields[7]. Therefore, enhancing the salt tolerance of this plant at the seedling stage constitutes one of the key challenges for improving both the quality and yield of cultivated *G. glabra* in salinized soils.

In recent years, the application of exogenous substances has emerged as a research hotspot for enhancing crop stress resistance[8-11]. Manganese (Mn), as an essential element for plant growth, plays a critical role in crop growth and development[12,13]. At appropriate levels, manganese can enhance nitrogen metabolism in crops, thereby promoting both vegetative and reproductive growth, and ultimately improving their yield and quality[14-16]. However, studies concerning the physiological regulation of manganese on *G. glabra* under salt stress, as well as its effects on the yield and quality of the medicinal material, have yet to be reported. Therefore, the objective of the present study is to elucidate the effects of manganese oxide on alleviating salt-induced damage in *G. glabra* and on enhancing both its the yield and medicinal quality. Furthermore, this study aims to explore the underlying physiological mechanisms by which manganese enhances salt tolerance of the licorice through the modulation of antioxidant systems, osmotic balance, and secondary metabolism. Our results will offer theoretical support for optimizing manganese fertilization strategies to improve *G. glabra* cultivation on marginal saline lands.

2. Materials and methods

2.1 Experimental Materials

The experiment was conducted during 2024-2025 at the campus of Shihezi University (44°18′35″ N, 86°03′27″ E), which is characterized by a temperate continental climate. Seeds of *G. glabra* were provided by the Licorice Research Institute of Shihezi University. Analytical-grade sodium chloride

(NaCl) and manganese oxide (MnO) used in this experiment were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China).

2.2 Experimental Methods

Uniformly sized seeds of *G. glabra* were selected and soaked in 98% sulfuric acid at room temperature for 30 min, then rinsed under running water until no sulfuric acid residue remained on the seed surface, and were finally soaked in distilled water for 12 h. Subsequently, the seeds were evenly sown in plastic pots (upper diameter 25 cm, lower diameter 16 cm, height 25 cm). Sandy soil was used as the cultivation substrate, composed of river sand and loam at a ratio of 3:7. Each pot was filled with 8 kg of sandy soil, which had the following properties: total nitragen, total phosphorus, and total potassium contents of 0.315 $g \cdot kg^{-1}$, 0.131 $g \cdot kg^{-1}$, and 5.47 $g \cdot kg^{-1}$, respectively; available nitrogen, available phosphorus, and available potassium contents of 52.59 $mg \cdot kg^{-1}$, 5.23 $mg \cdot kg^{-1}$, and 50.04 $mg \cdot kg^{-1}$, respectively; and an organic matter content of 6.64 $mg \cdot kg^{-1}$. Ten seeds were sown per pot at a depth of 1 cm. When the seedlings developed the fourth true leaf, fertilizer was applied according to the method described by Dong et al[17]. Soil water content was regulated using the gravimetric method and maintained at 80% of the maximum water-holding capacity throughout the growth period. All plastic pots were placed in an open area, and to minimize the influence of light and other environmental factors on plant growth, the positions of the pots were randomly rotated on a weekly basis.

When the *G. glabra* seedlings developed their sixth true leaf, only six uniformly growing seedlings were retained per pot, and salt stress treatment was initiated. A 160 mM NaCl solution was applied to each pot in three split applications, administered once every two weeks, with each pot receiving 500 mL per application. Seven days after the first salt treatment, rhizosphere irrigation with manganese oxide (MnO) suspensions at different concentrations (0, 10, 20, 40, and 80 $mg \cdot L^{-1}$) was initiated. The MnO suspensions were applied once every 7 days, with 500 mL per pot per application, for a total of eight applications. Each treatment had three replicates. Sampling was conducted on the 7th day after the final MnO application. Morphological and physiological parameters were measured for each treatment group, and upon completion of all measurements, the remaining plants were harvested for biomass determination. Detailed information on the treatments is presented in Table **1**.

Table 1. Experimental treatments

Treatments abbreviations	Salt concentration （mM）	MnO concentration （mg·L^{-1}）
NaCl	160	0
NaCl+M10	160	10
NaCl+M20	160	20
NaCl+M40	160	40
NaCl+M80	160	80

2.3 Measurement of Plant Growth Parameters

Morphological Parameters of the Root System

Three individual plants were randomly selected from each treatment group, and their roots were gently rinsed under running water to remove the sandy soil on their surface. Subsequently, the roots were scanned using a digital scanner (Expression 11000XL; Epson, Suwa City, Japan). The scanned images were analyzed with the WinRHIZO root analysis system (WinRHIZO Pro2012b; Regent Instruments Inc., Quebec City, QC, Canada) to measure total root length (TRL), total root surface area (TRSA), and total root volume (TRV).

Biomass

After the *G. glabra* were harvested, the roots, stems, and leaves were separately placed into paper bags and oven-dried at 75 ℃ to a constant weight. The dry weights of the roots, stems, and leaves were measured, and their mean values were calculated and recorded as root biomass (R-biomass), stem biomass (S-biomass), and leaf biomass (L-biomass), respectively.

Photosynthetic Gas Exchange Parameters

On the 7th day after the final Mn treatment, 6plants of each treatment were selected between 9:00 a.m. and 11:00 a.m. Beijing time, the gas exchange parameters of the third fully expanded leaves from the top of the plants were measured using a Li-6400 portable photosynthesis measurement system (Li-Cor Inc., Lincoln, NE, USA). Measurements were taken for photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), and intercellular CO_2 concentration (Ci). During the measurements, photosynthetically active photon flux density (PPFD) was provided by a Q-Beam light source (blue-red

dual color diode) and was set at 1200 μmol·m^{-2}·s^{-1}. Leaf temperature, ambient water vapor pressure, and CO_2 concentration were maintained at 25.5 ± 1.0 °C, 1.30 ± 0.15 kPa, and 400 ppm, respectively.

2.4 Leaf Chlorophyll Content

The relative chlorophyll content (SPAD) of the third fully expanded leaves was measured using a portable chlorophyll meter (SPAD 502 Plus, Konica Minolta, Japan). Measurements were performed three times for each treatment group, and the mean values were calculated.

2.5 Antioxidant Enzyme Activities

The activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in the leaves were determined using commercial assay kits (SOD-BC0175, POD-BC0090, CAT-BC0200, GR-BC1165; Solarbio Science & Technology Co., Ltd., Beijing, China) according to the manufacturer's instructions. Each treatment group was measured in triplicate, and the mean values were calculated.

2.6 Membrane Permeability and Lipid Peroxidation Indicators

The relative electrical conductivity (REC) of the leaves was determined using the electrical conductivity method. Fresh leaf samples (1.00 g) were accurately weighed and placed into 15 mL centrifuge tubes containing 10 mL of deionized water, and then soaked at room temperature for 12 h. The electrical conductivity of the solution was subsequently measured using a conductivity meter (Bante 540; Bante Instruments, Shanghai, China) and was recorded as E1. Thereafter, the centrifuge tubes were heated in a 100 °C water bath for 30 min. After cooling to room temperature, the electrical conductivity of the solution was measured again and was recorded as E2. The relative electrical conductivity was calculated using the following formula:

$$REC=E1/E2\times100$$

The contents of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and superoxide anion (O_2^-) in the leaves were determined using commercial assay kits (MDA-BC0025, H_2O_2-BC3595, O_2^--BC1295; Solarbio Science & Technology Co., Ltd., Beijing, China), strictly following the manufacturer's instructions. Each treatment group was measured in triplicate, and the mean values were calculated.

2.7 Bioactive Substance Contents

The soluble sugar (S-sugar) content in the leaves was determined using a commercial assay kit (BC0035; Solarbio Science & Technology Co., Ltd., Beijing, China). The soluble protein (S-protein) content was measured using a plant-specific ELISA kit (JM-110029P1; Jiangsu Jingmei Biotechnology Co., Ltd., Yancheng, China). The proline (Pro) content was determined using the acid-ninhydrin method. The absorbance of the reaction solutions was measured at 520 nm using a multifunctional microplate reader. Each treatment group was performed in triplicate, and the mean values were calculated.

2.8 Secondary Metabolite Content

Standard reference compounds of glycyrrhizic acid, liquiritin, glabridin, liquiritigenin, and isoliquiritigenin (2.0 mg each) were separately weighed and transferred into a 10 mL volumetric flask, then diluted to volume with methanol to obtain a mixed standard stock solution at a concentration of 200 μg·mL^{-1}. The stock solution was subsequently diluted with methanol to prepare a series of mixed standard solutions at concentrations of 1, 10, 100, 500, 1000, 5000, and 10000 ng·mL^{-1}. The analytes were determined using ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Calibration curves were constructed by plotting the peak areas of the quantitative ions (Y-axis) against the corresponding concentrations (X-axis, ng·mL^{-1}), and the regression equations were derived. The results demonstrated that the correlation coefficients (R^2) for all these compounds were greater than 0.99 within their respective linear ranges, indicating an excellent linear relationship between concentration and peak area. The mass spectrometry conditions, regression equations, and correlation coefficients are presented in Table **2**.

Table 2. Optimized mass spectrometry conditions and regression equations for 5 compounds.

Compounds	Parent Ion (m·z^{-1})	Daughter Ion (m·z^{-1})	Ionization Mode	Retention Time (min)	Regression Equation	Correlation Coefficients R^2	Linear Over (ng·mL^{-1})
Glycyrrhizic acid	821.2	350.8* 112.9	ESI−	4.81	Y=401.136x+10.3513	0.9986	1.2-1031.9
Liquiritin	416.9	254.9* 134.9	ESI−	1.91	Y=1324.91x+237.424	0.9995	1-992
Glabridin	322.9	134.9* 200.9	ESI−	2.64	Y=1246.54x-84.3157	0.9987	1.2-1028.3
Liquiritigenin	257.1	136.9* 146.9	ESI+	3.23	Y=2297.08x+5659.22	0.9959	0.8-484.3
Isoliquiritigenin	245.9	136.9* 146.9	ESI+	4.46	Y=41600.1x+269157	0.9998	0.9-247.8

Note: * Stands for quantifier ions.

The dried roots of *G. glabra* were ground into powder and passed through an 80-mesh sieve. Root powder (0.500 g) from each treatment group was accurately weighed and transferred into a 10 mL centrifuge tube, followed by the addition of 5 mL of chromatography-grade methanol. Extraction was performed at room temperature using an ultrasonic extractor (KQ-300E; Kunshan Ultrasonic Instrument Co., Ltd., Kunshan, China) for 2 h. The extract was centrifuged at 12,000 rpm for 15 min, and the supernatant was filtered through a 0.25 μm microporous membrane filter. The filtrate was analyzed using an Acquity UPLC system coupled with a Waters XEVO TQS triple quadrupole mass spectrometer to obtain the chromatographic peak areas of glycyrrhizic acid, liquiritin, glabridin, liquiritigenin, and isoliquiritigenin in the sample solutions[18]. The concentrations of these five compounds were calculated based on the regression equations described above. Each sample was measured in triplicate, and the mean values were taken.

The average content ($mg \cdot plant^{-1}$) of glycyrrhizic acid, liquiritin, glabridin, liquiritigenin, and isoliquiritigenin per plant was calculated by multiplying the concentration of each compound by the mean root biomass per plant.

2.9 Data Analysis

Statistical analyses were performed using SPSS 20.0 (IBM Corp., New York, USA). One-way analysis of variance (ANOVA) combined with the least significant difference (LSD) test and Duncan's multiple range test were employed to evaluate the differences among treatment groups. Statistical significance was set at $P < 0.05$. All data are expressed as the mean ± standard deviation (SD). Principal component analysis (PCA) was conducted based on the measured parameters. Figures were generated using OriginPro 2022b (Electronic Arts Inc., New York, USA).

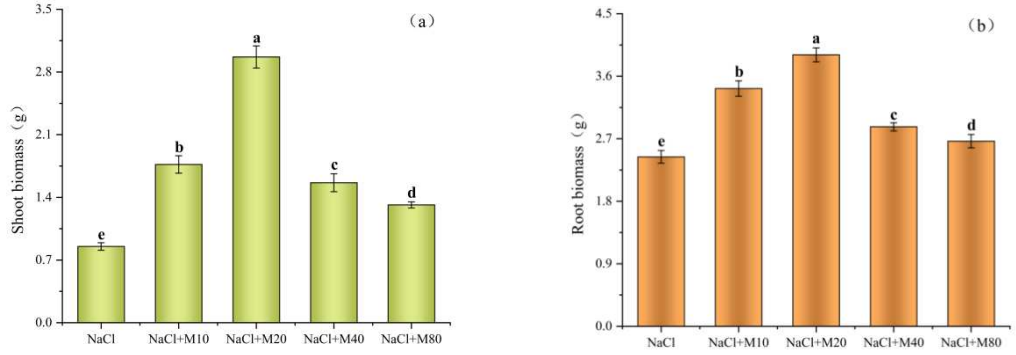
3. Results

3.1 Effects of manganese oxide on the growth of *G. glabra* seedlings.

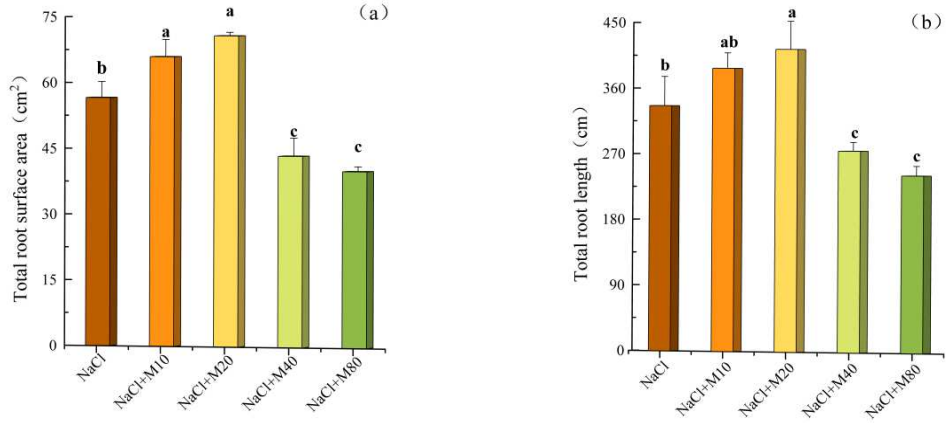
Exogenous application of manganese oxide significantly improved the growth of *G. glabra* seedlings under NaCl stress, as reflected by marked increases in both shoot and root biomass. Among the

192 treatments, the NaCl+M20 treatment group exhibited the most pronounced effect, with shoot biomass
193 and root biomass increased by 150% and 60%, respectively, compared with the NaCl treatment group
194 (Figure 1, $P < 0.05$).

195 Exogenous application of manganese oxide significantly affected root system development in *G.*
196 *glabra* seedlings. The NaCl+M10 and NaCl+M20 treatments significantly increased the total root length,
197 total root surface area, and total root volume of *G. glabra*, with the NaCl+M20 treatment exhibiting the
198 most pronounced promotive effect. Compared with the NaCl stress treatment, the total root surface area,
199 total root length, and total root volume of seedlings in the NaCl+M20 treatment increased by 25.22%,
200 23.24%, and 27.76%, respectively. In contrast, the NaCl+M40 and NaCl+M80 treatments significantly
201 reduced the total root length, total root surface area, and total root volume of *G. glabra*, with the
202 NaCl+M80 treatment showing the strongest inhibitory effect(Figure 2).



203 Figure 1. Effects of manganese oxide on biomass of *G. glabra* seedlings under salt stress.
204 (a) Shoot biomass;(b) Root biomass.
205 （Different lowercase letters denote significant differences among treatments at the $P < 0.05$ level;
206 the same below.）
207



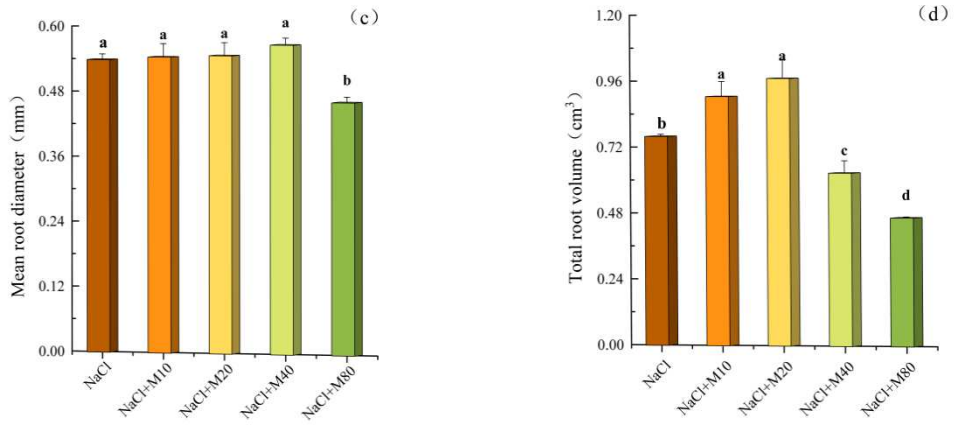
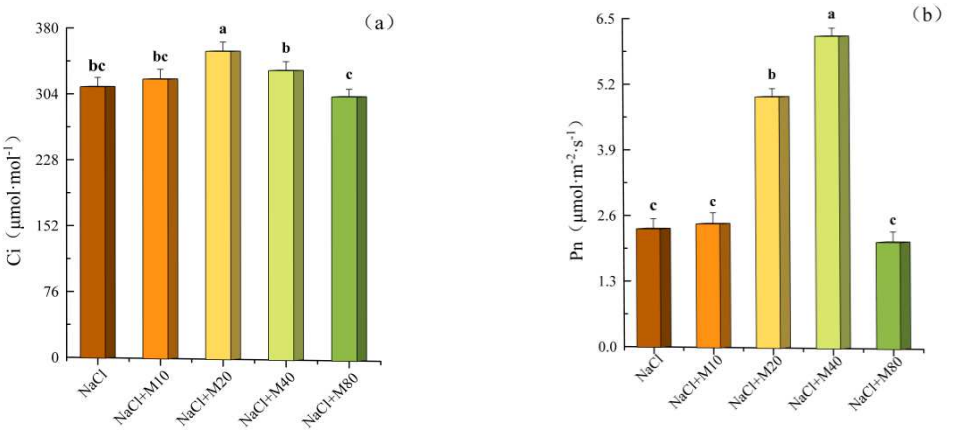


Figure 2. Effects of manganese oxide on root morphological parameters of *G. glabra* seedlings under salt stress.

(a) Total root surface area; (b) Total root length; (c) Mean root diameter; (d) Total root volume.

3.2 Effects of manganese oxide on photosynthetic gas exchange parameters and relative chlorophyll content.

NaCl treatment largely reduced the photosynthetic gas exchange performance and chlorophyll content in leaves. Exogenous application of manganese oxide significantly alleviated these inhibitory effects, with the NaCl+M10, NaCl+M20, and NaCl+M40 treatments all significantly increasing Ci, Pn, Gs, Tr, and SPAD values. Notably, the NaCl+M40 treatment exhibited the most pronounced enhancement effects of Pn, Tr, Gs, and SPAD, which increased by 162%, 97.6%, 123.8%, and 69.1%, respectively, compared with the NaCl treatment (Figure 3, $P < 0.05$).



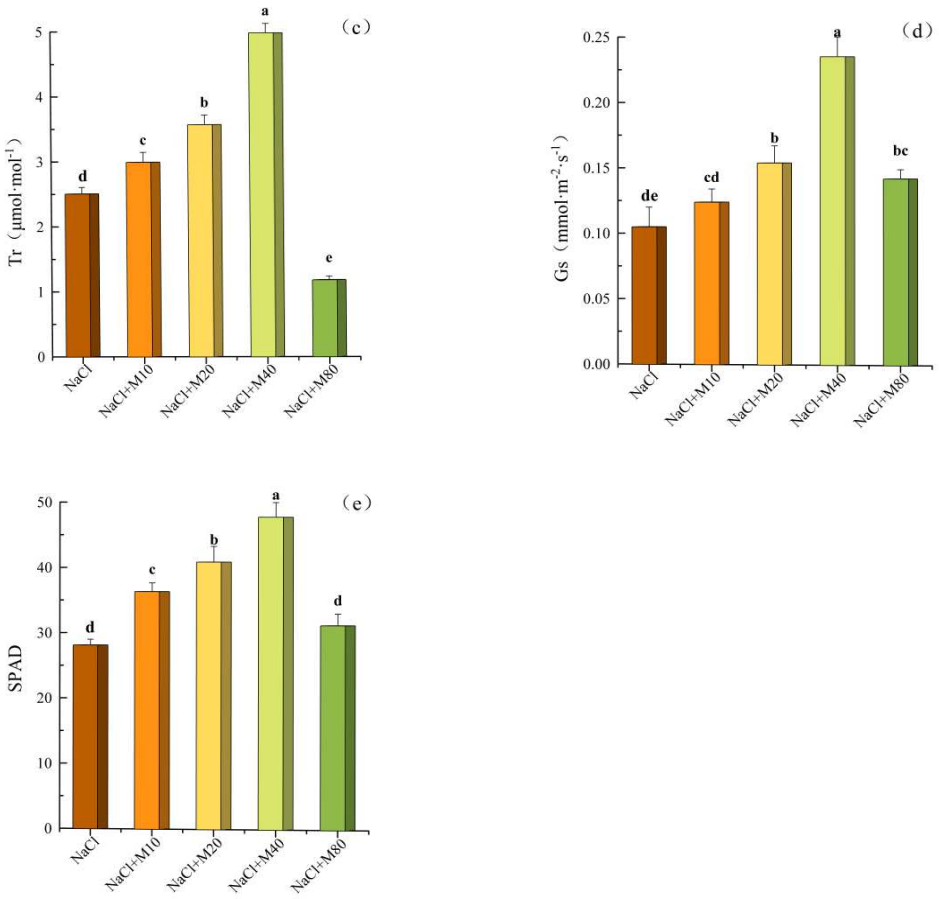


Figure 3. Effects of manganese oxide on photosynthetic gas exchange parameters and relative chlorophyll content of *G. glabra* under salt stress. (a) Intercellular CO_2 concentration; (b) Net photosynthetic rate; (c) Transpiration rate; (d) Stomatal conductance; (e) Relative chlorophyll content.

3.3 Effects of manganese oxide on the antioxidant enzyme system.

Exogenous application of manganese oxide significantly enhanced the activities of antioxidant enzymes in *G. glabra* seedlings under NaCl stress. Among the treatments, the NaCl+M40 treatment group exhibited the highest SOD and CAT activities, which increased by 189.67% and 159.79%, respectively, compared with the NaCl treatment. The NaCl+M20 treatment showed the highest POD activity, which increased by 44.78% compared with the NaCl treatment (Figure 4, $P < 0.05$).

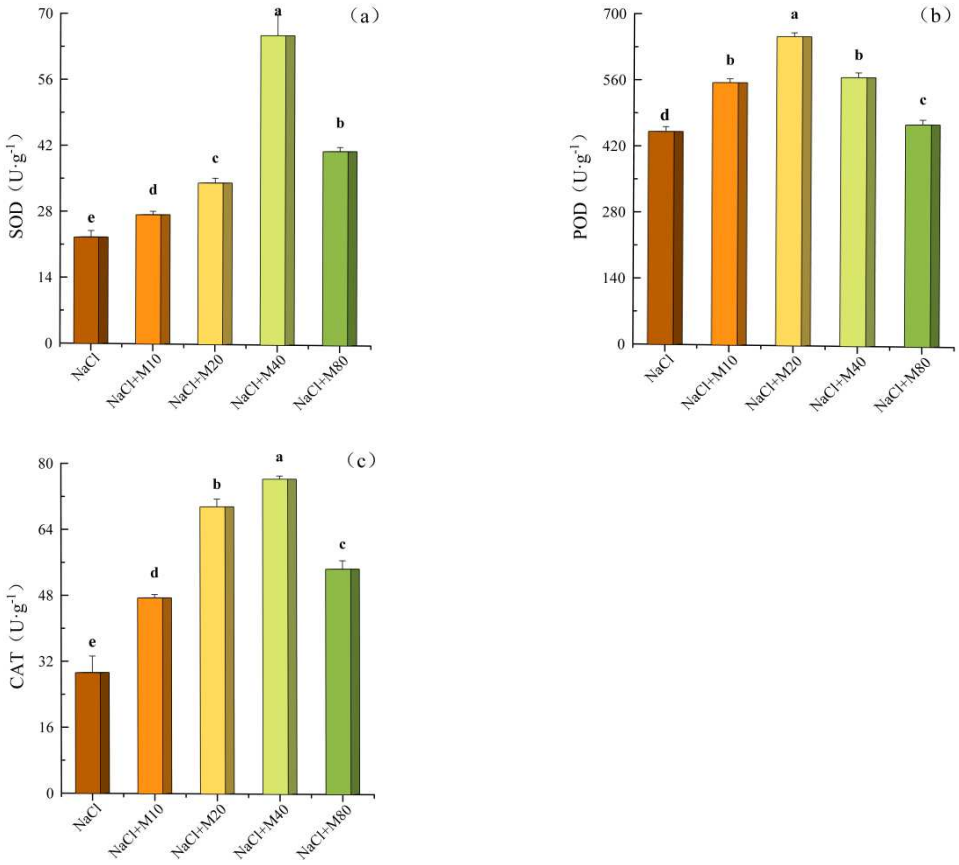


Figure 4. Effects of manganese oxide on antioxidant enzyme activities in leaves of *G. glabra* seedlings under salt stress (a) Superoxide dismutase activity; (b) Catalase activity; (c) Peroxidase activity.

3.4 Effects of manganese oxide on membrane permeability and lipid peroxidation.

The results demonstrated that exogenous application of manganese oxide significantly reduced the reactive oxygen species (ROS) levels in *G. glabra* under NaCl stress conditions. Specifically, the NaCl+M20 treatment group (supplemented with 20 mg·L^{-1} manganese oxide) exhibited the lowest H_2O_2 content, which decreased by 51.75% compared with the NaCl treatment group, while the NaCl+M40 treatment group (supplemented with 40 mg·L^{-1} manganese oxide) showed the lowest O_2^- content, which decreased by 41.26% compared with the NaCl treatment. The NaCl+M20 treatment group also exhibited the lowest MDA content and relative electrolyte leakage, which were reduced by 23.41% and 25.36%, respectively, compared with the NaCl treatment. Collectively, these findings indicate that manganese oxide can significantly alleviate the oxidative damage induced by NaCl stress and contribute to the maintenance of cell membrane stability. NaCl stress induced a significant accumulation of reactive oxygen species (ROS) in *G. glabra*, and exogenous application of manganese oxide effectively mitigated

the oxidative stress, with the NaCl+M20 treatment group showing the most pronounced alleviation effect (Figure 5, $P < 0.05$).

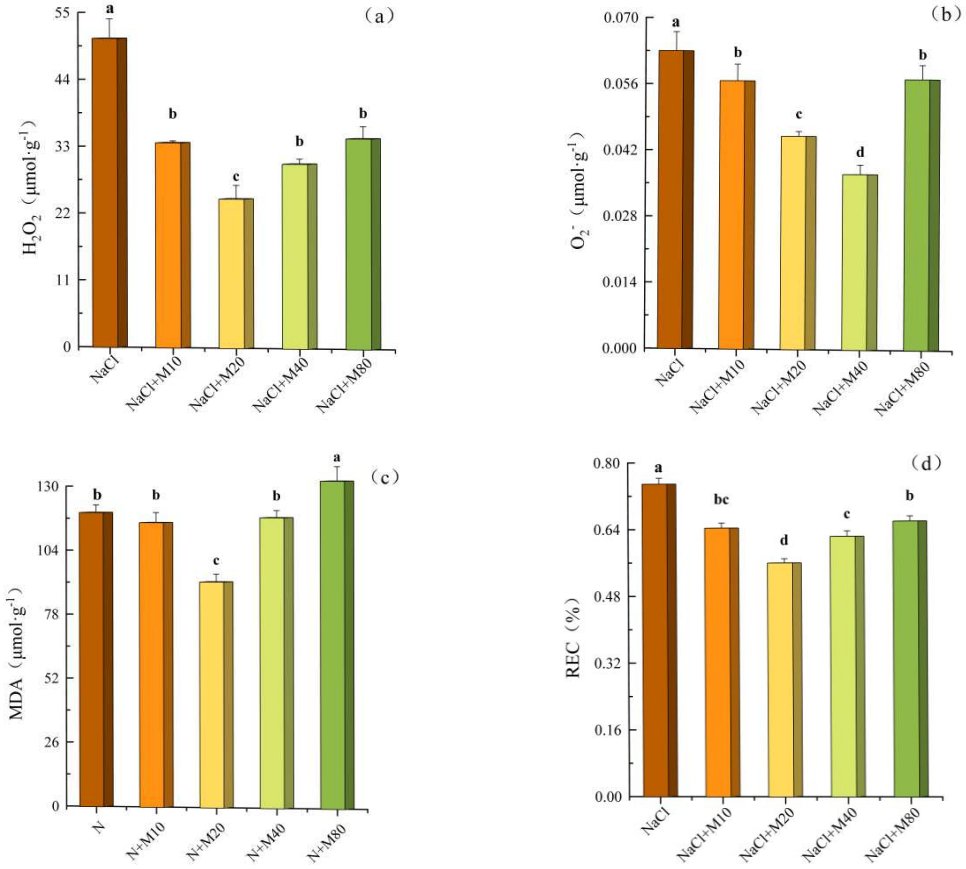


Figure 5. Effects of manganese oxide on membrane lipid peroxidation in leaves of *G. glabra* seedlings under salt stress (a) Hydrogen peroxide content; (b) Superoxide anion content; (c) Malondialdehyde content; (d) Relative electrical conductivity.

3.5 Effects of Manganese Oxide on the Accumulation of Osmoregulatory Substances

Application of manganese oxide significantly affected the contents of osmoregulatory substances in the leaves of *G. glabra* seedlings under NaCl stress, as evidenced by significantly elevated levels of soluble proteins, soluble sugars, and proline. Specifically, the soluble protein content in the NaCl+M20 treatment group increased by 27.31% compared with the NaCl treatment group, while the soluble sugar and proline contents in the NaCl+M40 treatment group increased by 118.75% and 113.43%, respectively, compared with the NaCl treatment (Figure 6, $P < 0.05$).

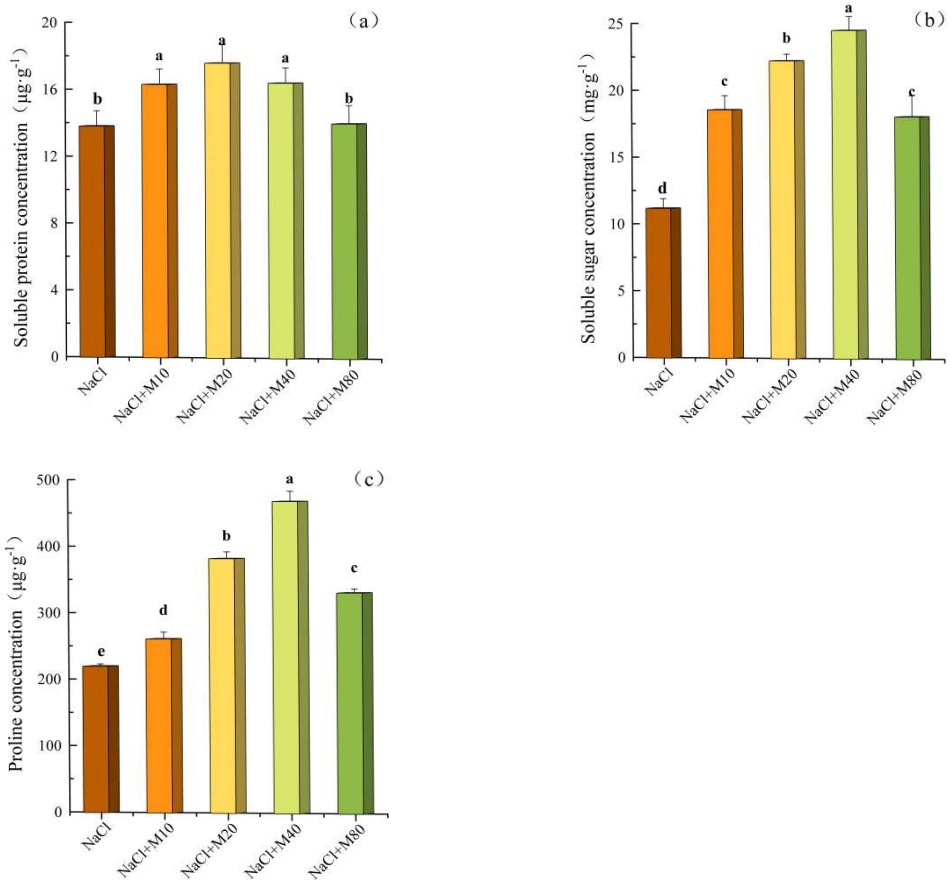


Figure 6. Effects of manganese oxide on the contents of osmotic adjustment substances in leaves of *G. glabra* seedlings under salt stress (a) Soluble protein concentration; (b) Soluble sugar concentration; (c) Proline concentration.

3.6 Principal Component Analysis (PCA)

Figure 7 illustrates the distributional differences among five sample groups (NaCl treatment, NaCl+M10, NaCl+M20, NaCl+M40, and NaCl+M80 manganese oxide treatments) along with the correlations and contributions of 20 physiological and growth indicators. Principal component 1 (PC1) and principal component 2 (PC2) explained 49.4% and 26.9% of the total variance, respectively, cumulatively accounting for 76.3% of the total information. This indicates that the first two principal components sufficiently capture the comprehensive differences among various indicators of across different treatments.

With increasing manganese oxide concentration, the treatment groups exhibited a clear gradient distribution along the PC1 axis from negative to positive values. The low-concentration manganese oxide treatment (NaCl+M10) and the NaCl treatment were located near the negative semi-axis of PC1. The medium-to-high concentration manganese oxide treatments (NaCl+M20 and NaCl+M40) were

distributed along the positive semi-axis of PC1 and were completely separated from the low-concentration treatment groups. The distribution area of the NaCl+M80 treatment group partially overlapped with that of the NaCl+M40 group, but shifted closer to the negative direction of PC1 overall. This distribution pattern indicates that NaCl stress and manganese oxide treatments significantly altered the physiological and growth characteristics of *G. glabra* in a pronounced concentration-dependent manner, with the medium-concentration manganese oxide treatments (NaCl+M20 and NaCl+M40) exhibiting the most prominent ameliorative effects.

Photosynthetic parameters (Pn, Tr, Ci, Gs, and SPAD), antioxidant enzyme activities (SOD, POD, and CAT), osmoregulatory substances (soluble protein, soluble sugar, and proline), and root growth indicators (ARD, TRL, TRV, and root biomass) exhibited relatively high levels in the medium-concentration manganese oxide treatment groups (NaCl+M20 and NaCl+M40), suggesting that PC1 primarily reflects the promotive effects of manganese oxide treatment on photosynthetic efficiency, antioxidant capacity, and osmotic regulation. In contrast, oxidative damage indicators (O_2^-, H_2O_2, REC, and MDA) were present at higher levels in the low-concentration treatment groups, indicating that the negative direction of PC1 represents the degree of oxidative stress and membrane damage.

The NaCl+M20 treatment was predominantly distributed in the high-value region of PC1 (> 3) and the intermediate region of PC2 (near 0), achieving the highest PC1 score among all treatments. The NaCl+M40 treatment was distributed in the intermediate region of PC1 (0-3) and the low-value region of PC2 (0 to −4), with its overall PC1 score being markedly lower than that of NaCl+M20. Given that PC1 explained 48.7% of the total variance and represents the core dimension of dominant differences-encompassing positive growth-promoting and quality-enhancing indicators such as photosynthesis, antioxidant enzyme activities, and osmoregulatory substances-the NaCl+M20 treatment demonstrated superior performance along this core dimension. Its higher score on the core principal component PC1 indicates a more pronounced comprehensive effect in promoting growth and enhancing quality. Although the NaCl+M40 treatment also exhibited positive effects, its overall performance was weaker than that of NaCl+M20, and its closer proximity to the negative direction of PC2 (toward the MDA arrow) suggests a trend of rebounding oxidative damage.

Taken together, the PCA results clearly elucidate the regulatory effects of different concentrations of exogenously applied manganese oxide on the physiological and growth indicators of *G. glabra* under

NaCl stress, demonstrating that the NaCl+M20 treatment with medium-concentration manganese oxide can maximally enhance the photosynthetic efficiency and stress resistance of *G. glabra* while reducing oxidative stress. These findings provide an important scientific basis for the rational application of manganese fertilizer in *G. glabra* cultivation.

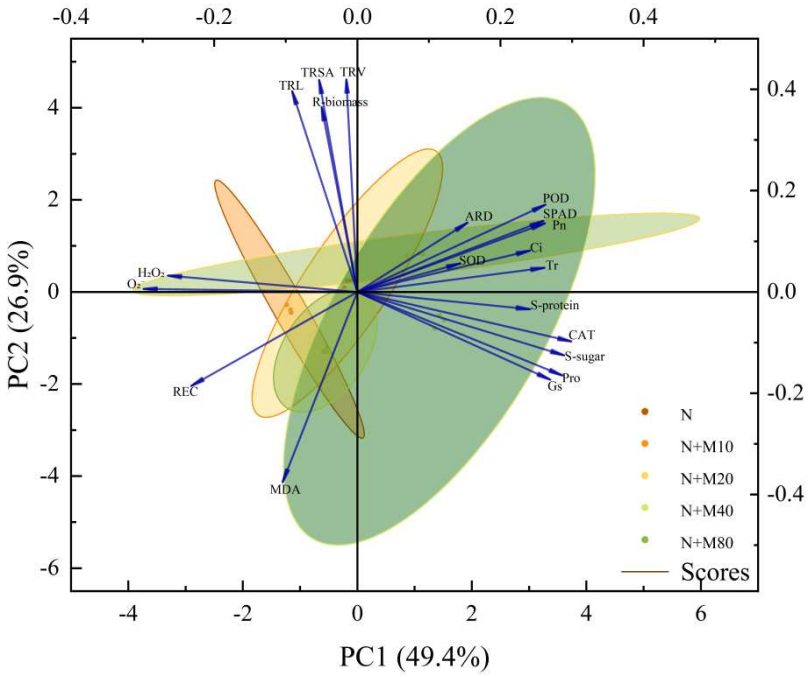


Figure 7. Principal component analysis of seedling growth and physiological indices in *G.glabra* seedlings under salt stress as affected by manganese oxide

3.7 Pearson correlation analysis

To elucidate the intrinsic relationships among plant growth, photosynthetic, and stress-resistance physiological indicators, Pearson correlation analysis was performed on 20 traits measured from *G. glabra* seedlings under salt stress, and a heatmap was constructed to visualize the linear correlations among multiple physiological indicators (Figure 8). In the heatmap, the color gradient represents the magnitude of the correlation coefficient (r), ranging from dark brown ($r = 1.0$, strong positive correlation) to dark green ($r = -1.0$, strong negative correlation), with asterisks denoting statistical significance (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$).

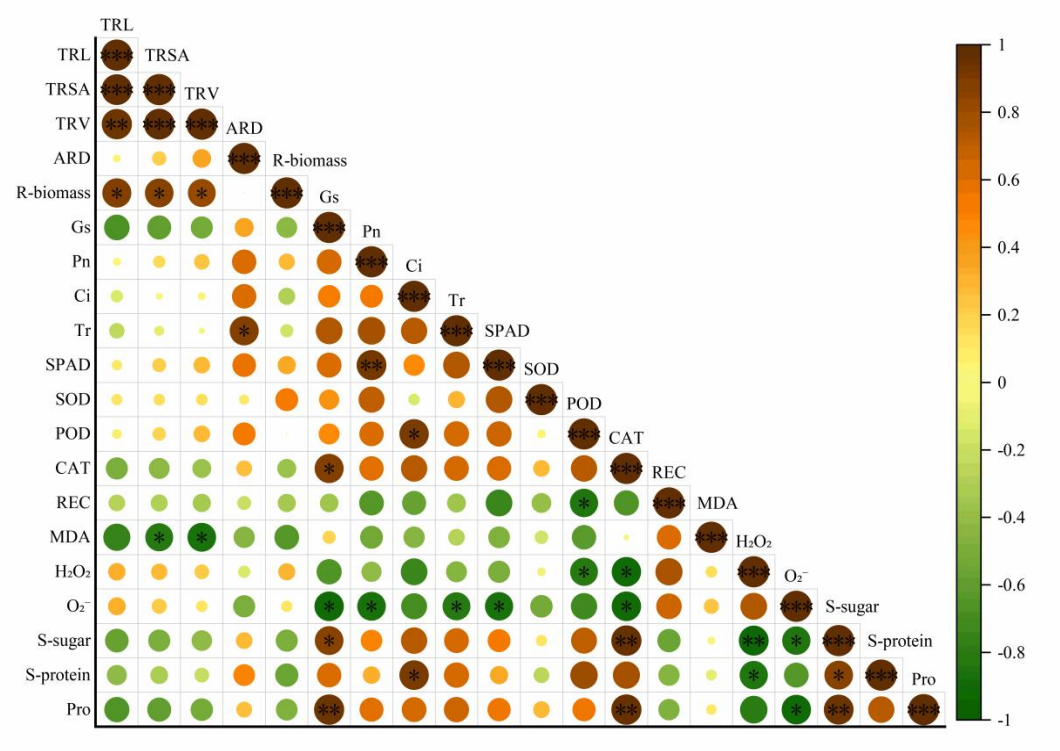
The root growth indicators exhibited an extremely strong synergistic relationship: total root length (TRL), total root surface area (TRSA), total root volume (TRV), and root biomass (R-biomass) all showed highly significant positive pairwise correlations (r = 0.83-0.97, P < 0.001), indicating not only a high degree of consistency between root morphological expansion and biomass accumulation, but also that the overall development of the root system is the core factor driving the enhancement of root biomass. The correlations between average root diameter (ARD) and other root indicators were weaker than those among TRL, TRSA, and TRV, suggesting that root elongation, rather than increased root thickness, is the primary driver of biomass accumulation.

Net photosynthetic rate (Pn) exhibited highly significant positive correlations with stomatal conductance (Gs) and transpiration rate (Tr) (r = 0.76-0.91, P < 0.01), as well as a significant positive correlation with chlorophyll content (SPAD) (r = 0.72, P < 0.01), indicating that stomatal regulation and chlorophyll levels jointly determine photosynthetic efficiency, and that increased stomatal opening simultaneously promotes CO_2 uptake and water transpiration. The correlation between intercellular CO_2 concentration (Ci) and Pn was weak and even partially negative, reflecting the characteristic pattern of "enhanced photosynthetic utilization," whereby intercellular CO_2 is rapidly consumed as the photosynthetic rate increases, leading to a decline in its concentration. Photosynthetic indicators (Pn, Gs, SPAD) were significantly positively correlated with root biomass (R-biomass), demonstrating that the accumulation of photosynthetic products promotes root growth.

The stress-resistance physiological indicators displayed pronounced functional cooperativity: the activities of antioxidant enzymes (SOD, POD, CAT) showed highly significant positive pairwise correlations (r = 0.74-0.89, P < 0.01); membrane damage indicators (MDA, REC) and reactive oxygen species (H_2O_2, O_2^-) contents were also highly significantly positively correlated (r = 0.78-0.93, P < 0.001); and osmoregulatory substances (soluble sugar, soluble protein, proline) likewise exhibited highly synchronized changes (r = 0.71-0.87, P < 0.01). These results indicate that the antioxidant system, membrane damage response, and osmotic regulation system respond to environmental stress in a coordinated and integrated manner, collectively maintaining cellular homeostasis.

Root growth indicators (TRL, TRSA, R-biomass) and photosynthetic indicators (Pn, SPAD) were significantly negative correlated oxidative damage indicators (MDA, H_2O_2, O_2^-) and antioxidant enzyme activities parameters (r = −0.65 to −0.92, P < 0.01). This result clearly demonstrates that when plant

resources are preferentially allocated to root growth and photosynthetic carbon fixation, oxidative stress remains low; conversely, when stress responses are activated, a substantial amount of resources is diverted to the synthesis of antioxidant enzymes and osmoregulatory substances, thereby inhibiting growth and photosynthetic processes (Figure 8).



(*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$)

Figure 8. Correlation analysis between 20 physiological and growth indices of *G. glabra* seedlings under treatment of salt and manganese oxide

3.8 Effect of Manganese Oxide on the Accumulation of Secondary Metabolites

PCA demonstrated that the moderate concentration of manganese treatment (NaCl+M20) markedly enhanced photosynthetic efficiency and stress tolerance of *G. glabra*, reduced its oxidative stress, and exhibited the most pronounced alleviation of NaCl-induced stress among all treatments. We subsequently quantified the concentrations of glycyrrhizic acid, liquiritin, glabridin, liquiritigenin, and isoliquiritigenin in the roots of seedlings of the NaCl and the NaCl+M20 treatments.

Under NaCl stress, exogenous application of 20 $mg \cdot L^{-1}$ MnO (NaCl+M20) significantly promoted the accumulation of glycyrrhizic acid, liquiritin, glabridin, liquiritigenin, and isoliquiritigenin in *G.*

glabra roots. Compared with the NaCl stress group, the concentrations of these five compounds increased by 13.03%, 29.32%, 137.43%, 158.73%, and 73.51%, respectively (Table 3). More strikingly, the per-plant contents of these five compounds were 83%, 118%, 279%, 262%, and 233% higher, respectively, than those in the NaCl stress group (Table 4).

Table 3. Effects of manganese oxide on the concentration of five active components in the root system of *G. glabra* seedlings under salt stress.

Treatments	Concentrations of Active Components（$\mu g \cdot g^{-1}$）				
	Glycyrrhizic Acid	Liquiritin	Glabridin	Liquiritigenin	Isoliquiritigenin
NaCl	364.07 ± 9.45 b	48.33 ± 3.70 b	400.32 ± 31.96 b	46.50 ± 5.27 b	15.25 ± 1.40 b
NaCl+M20	411.53 ± 15.57 a	62.5 ± 8.75 a	950.46 ± 54.48 a	120.31 ± 3.96 a	26.46 ± 1.35 a

Means followed by different lowercase letters differ significantly, (Mean ± SD, $P < 0.05$).

Table 4. Effects of manganese oxide on the five active ingredient content per plant in the root system of *G. glabra* seedlings under salt stress.

Treatments	Active Ingredient Content Per Plant ($mg \cdot Plant^{-1}$)				
	Glycyrrhizic Acid	Liquiritin	Glabridin	Liquiritigenin	Isoliquiritigenin
NaCl	0.88 ± 0.02 b	0.11 ± 0.01 b	0.98 ± 0.07 b	0.13 ± 0.01 b	0.03 ± 0.01 b
NaCl+M20	1.61 ± 0.06 a	0.24 ± 00.03 a	3.71 ± 0.21 a	0.47 ± 0.02 a	0.1 ± 0.01 a

Means followed by different lowercase letters differ significantly, (Mean ± SD, $P < 0.05$).

4. Discussion

Our results demonstrate that exogenous application of MnO at an appropriate concentration effectively alleviates the growth inhibition induced by 160 mM NaCl stress of *G. glabra* seedlings by improving root architecture, maintaining photosynthetic efficiency, activating the antioxidant system, and enhancing osmotic adjustment capacity, while also significantly promoting the accumulation of key secondary metabolites, including glycyrrhizic acid, liquiritin, and glabridin, in roots. We found 20 $mg \cdot L^{-1}$ as the optimal application concentration, providing a scientific basis for stress-resilient cultivation and quality improvement of *G. glabra* in salinized soil.

Xinjiang province stands out as the epicenter of soil salinization within China, characterized by an exceptional diversity of saline soil types that has prompted its characterization as a "world museum of salinized soils." Seedlings of many plant species demonstrate pronounced vulnerability to saline conditions, rendering the early growth phase a pivotal window for screening salt-tolerant genotypes[19].

G. glabra relies on its roots as the economic organ, which are rich in various bioactive constituents, including liquiritin, glycyrrhizic acid, glabridin, and isoliquiritigenin[20]. Root growth and secondary metabolites' content are therefore directly tied to economic returns. Our results showed that exogenous MnO application increased total root length, surface area, and total root volume of *G. glabra*, significantly improving root architecture and promoting root biomass accumulation. This, in turn, provided stronger structural support and more efficient water and mineral nutrient acquisition for aboveground development[20]. Manganese serves as a cofactor in indole-3-acetic acid (IAA) biosynthesis, directly influencing IAA synthesis and accumulation[21]. Legumes depend on adequate IAA levels for nodule formation, and changes in plant manganese status directly affect IAA oxidase activity, thereby impacting nodulation and nitrogen fixation[22,23]. Manganese fertilization was shown to significantly enhance the nitrogen-fixing capacity of soybean (*Glycine max*) root nodules, strengthen nitrogen metabolism, and promote legume growth[24]. Moderate level of manganese supplementation markedly increased soybean root vitality and reduced malondialdehyde (MDA) content in leaves[25]. Manganese also influenced ammonia transformation and protein synthesis; $MnSO_4$ treatment significantly increased height of soybean by 21.30% relative to the control[26,27].

Plant cultivation faces formidable challenges from saline conditions, which rank among the most pervasive non-biotic stresses restricting crop development globally, with arid and semi-arid regions bearing the brunt[28]. The escalating extent of soil salinization constitutes a formidable ecological threat to agricultural sustainability. As a organ directly contacted with saline soil, the root system exhibited large-scale programmed cell death in root tip meristematic cells within 24 h under salt stress[29]. Salt stress significantly influences malondialdehyde (MDA) content, osmolyte levels, and the activities of antioxidant enzymes primarily catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) in plants. Malondialdehyde (MDA), generated as a consequence of lipid peroxidation cascades triggered by environmental adversity, has been widely adopted as a biomarker for assessing the extent of oxidative injury in plants, with elevated MDA levels typically correlating with reduced stress resilience[30]. In our study, NaCl-induced MDA accumulation was counteracted by MnO application, suggesting that manganese supplementation helps preserve membrane integrity by mitigating oxidative degradation. Under salt stress, Plants deploy a suite of enzymatic defenses-including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT)-to neutralize reactive oxygen species and safeguard cellular components from oxidative degradation[31]. SOD catalyzes the dismutation of superoxide radicals,

initiating a detoxification cascade that serves as the primary buffer against salt-triggered redox homeostasis disruption. SOD initiates the detoxification cascade by converting superoxide radicals, its activity is a key indicator of plant stress tolerance[32]. Proline (Pro) accumulation helps maintain cellular osmotic potential under salt stress, and its content is also an important indicator of stress resistance[33]. Increased soluble sugar content ensures normal metabolic activities within the plant[32,34]. Protective enzyme activities are positively correlated with antioxidant stress tolerance in plants; under salt stress, halophytes exhibit higher SOD, POD, and CAT activities than non-halophytes, enabling more effective ROS scavenging and prevention of membrane lipid peroxidation, while POD and CAT provide complementary scavenging routes for the resulting hydrogen peroxide. The magnitude of this enzymatic response correlates with the degree of salinity adaptation. Salinity adaptation in plants frequently entails the buildup of osmoprotectants to sustain cell turgor alongside the activation of enzymatic antioxidant systems to quench excess radicals, working in concert to lessen salt-induced physiological harm[35,36]. Manganese, as an activator of multiple antioxidant and photosynthesis-related enzymes, enhances ROS scavenging capacity by increasing SOD, POD, and CAT activities, thereby maintaining chlorophyll content and net photosynthetic rate while promoting root growth and biomass accumulation. Over the course of long-term evolution, plants have developed an efficient defense system against environmental stress, comprising both antioxidant enzymes (SOD, POD, CAT) and non-enzymatic components[37]. Through this coordinated mechanism, plants are able to dampen the buildup of reactive oxygen species, thus conferring protection against salt-triggered oxidative deterioration[38,39].

160 mM NaCl stress significantly reduced the activities of SOD, POD, and CAT in plant leaves, indicating severe oxidative stress[40]. Under stress conditions, lipid peroxidation is accelerated and membrane integrity is compromised; excessive ROS oxidize fatty acids in biological membranes, generating MDA a key biomarker for evaluating the severity of oxidative damage[41]. Lipid peroxidation compromises membrane integrity by elevating permeability and undermining structural stability, ultimately resulting in the efflux of cytoplasmic contents. The coordinated elevation of leaf soluble sugars, proteins, and proline under NaCl treatment in our study points to a integrated osmoprotective response. Rather than acting in isolation, these osmolytes appear to function synergistically, collectively stabilizing macromolecular structures and maintaining hydration status, while simultaneously supporting the energetic demands of stress adaptation. The observed positive associations between antioxidant capacity and osmoprotectant accumulation, coupled with their inverse relationships with markers of

membrane damage, collectively underscore that the integrated operation of osmoprotective and antioxidative mechanisms forms the physiological cornerstone of salt tolerance in *G. glabra*. Although these osmolytes help stabilize cellular osmotic pressure, their synthesis may consume substantial energy and carbon resources, thereby limiting the allocation of photosynthates to vegetative growth, a common trade-off strategy in plant responses to osmotic stress[42].

Salt stress is one of the primary abiotic factors limiting plant photosynthetic efficiency. It significantly reduced the net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), and intercellular CO_2 concentration (Ci) in plant leaves. Saline conditions constrain leaf gas exchange dynamics through dual mechanisms: reduction of chlorophyll pigments and constriction of stomatal apertures[43]. Stomatal closure, although an adaptive water-conservation mechanism, inadvertently limits CO_2 entry into leaf mesophyll, thereby constraining photosynthetic carbon assimilation[44,45]. Chlorophyll is a critical pigment in plant photosynthesis, playing a vital role in light absorption, energy transfer, and electron transport[46]. Under saline conditions, excessive reactive oxygen species accumulation inflicts harm on the photosynthetic apparatus by compromising chloroplast membrane integrity and accelerating the breakdown of photosynthetic pigments, thereby suppressing overall photosynthetic performance. This may represent another reason for the decline in photosynthetic performance, growth inhibition, and reduced biomass accumulation in plant under NaCl stress[47]. Exogenous application of appropriate MnO concentrations significantly increased the SPAD value, net photosynthetic rate, stomatal conductance, and transpiration rate in *G. glabra*. This result is consistent with the findings reported by Huang et al. in maize[48]. The underlying mechanism may involve manganese-mediated alleviation of salt stress-induced damage to the photosynthetic apparatus by regulating the antioxidant system and maintaining chloroplast structure; through protecting photosynthetic pigments and improving stomatal function, manganese enables *G. glabra* to sustain carbon assimilation efficiency at a relatively high level.

Under complex and variable environmental conditions, plants synthesize diverse secondary metabolites, some of which possess significant application value in the cosmetics, pharmaceutical, and food industries. *G. glabra* roots are rich in various bioactive constituents and are widely used in the pharmaceutical, food, health product, and cosmetics sectors[49]. In particular, glabridin is a flavonoid with remarkable skin-whitening, antioxidant, and anti-inflammatory activities, earning it the title of

"skin-whitening gold"; however, its naturally low content limits its broader application in the cosmetics industry[50]. In addition to glabridin, *G. glabra* roots also contain glycyrrhizic acid, liquiritin, liquiritigenin, isoliquiritigenin, and other secondary metabolites, which possess a range of pharmacological activities including anti-inflammatory, antiviral, antioxidant, antitumor, and antimicrobial effects[3,51]. This study demonstrated that the 20 $mg \cdot L^{-1}$ MnO treatment substantially increased the concentrations and per-plant contents of glycyrrhizic acid, liquiritin, glabridin, liquiritigenin, and isoliquiritigenin in *G. glabra* roots. The underlying mechanism may be that, following the alleviation of salt stress, enhanced photosynthetic carbon fixation, improved nitrogen metabolism, and adequate energy and reducing power supply collectively channel more carbon skeletons toward the triterpenoid saponin and flavonoid biosynthetic pathways, achieving synergistic gains in stress tolerance enhancement, growth recovery, and medicinal quality improvement.

5. Conclusions

This study investigated the regulatory effects of exogenous manganese oxide (MnO) application on individual growth, physiological characteristics, and root secondary metabolite accumulation in *G. glabra* seedlings under salt stress, with the aim of identifying the optimal application concentration and elucidating the underlying mechanisms of salt injury alleviation. The main conclusions are as follows:

NaCl stress induced excessive accumulation of reactive oxygen species (ROS), aggravated membrane lipid peroxidation, and disrupted the osmotic regulation system in plant, thereby inhibiting root development, reducing biomass, photosynthetic capacity, and chlorophyll content. Exogenous application of MnO at an appropriate concentration effectively alleviated salt stress-induced damage, with the 20 $mg \cdot L^{-1}$ MnO treatment yielding the most pronounced ameliorative effects. Specifically, this dosage significantly promoted root elongation and increased root surface area and root volume in *G. glabra*, optimized root architecture, and maintained efficient carbon assimilation by enhancing net photosynthetic rate and chlorophyll content. It also activated the SOD, POD, and CAT antioxidant enzyme systems, reduced H_2O_2, O_2^-, and MDA contents as well as electrolyte leakage, thereby mitigating oxidative damage. Concurrently, it enhanced osmotic adjustment capacity by increasing soluble sugar, soluble protein, and proline contents. Beyond alleviating salt injury, MnO treatment simultaneously boosted osmotic balance and secondary metabolite biosynthesis, suggesting a dual benefit of improving both stress resilience and medicinal quality. Principal component analysis and

correlation analysis further confirmed that an appropriate concentration of MnO can systematically regulate the physiological and metabolic networks of *G. glabra* by coordinating the growth-defense trade-off.

In conclusion, 20 $mg \cdot L^{-1}$ MnO can serve as an exogenous micronutrient to effectively enhance salt tolerance, promote growth, and improve medicinal quality in *G. glabra* seedlings, providing a scientific basis for high-quality and efficient cultivation of *G. glabra* on salinized lands in Xinjiang.

Declarations

Abbreviations: Not applicable.

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: B.T. conducted the experiments, collected and analyzed the data, prepared the figures, and drafted the manuscript. T.G. is responsible for assisting with experiments and organizing data. M.M. conceived and supervised the study, acquired funding, and revised the manuscript. All authors read and approved the final manuscript.

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