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Arbuscular Mycorrhizal Fungi Promote *Gleditsia sinensis* Lam. Root Growth under Salt Stress by Regulating Nutrient Uptake and Physiology

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Abstract: Towards the improvement of plant productivity in saline–alkali soils, the application of arbuscular mycorrhizal fungi (AMF) is an intensive topic of research. For this study, three inoculation treatments, namely, autoclaved AMF inocula (CK), *Funneliformis mosseae* (FM), and *Corymbiglomus tortuosum* (CT), and four NaCl levels, namely, 0, 50, 100, and 150 mM were established to investigate the growth and physiological responses of mycorrhizal *Gleditsia sinensis* Lam. root systems to increase salinity through root dry weight, morphology, nutrient content, and physiology, and soil nutrient content. As NaCl levels increased, root dry weight, morphology, and nutrient content under the CK treatment exhibited a downward trend, while FM and CT treatments weakened this trend and significantly improved root dry weight and morphology, which increased by more than 200%. Under high NaCl levels, root activity under the FM treatment was significantly higher than that under the CK, with an average increase of 120.86%. In contrast to the activity of nitrate reductase, niacinamide adenine dinucleotide oxidase activity under CK was significantly less than that in FM and CT treatments. Moreover, inoculation with AMF significantly affected soil alkali-hydrolyzable nitrogen (AN), total nitrogen (TN), and phosphorus (TP), while NaCl had no significant impact on soil nutrients. Further, both soil salinity and mycorrhizal colonization rate had significant direct effects on root growth. However, soil salinity primarily influenced root growth through indirect effects on root nitrogen content, while mycorrhizal colonization rate indirectly impacted root nitrate reductase activity, and root nitrogen and phosphorus content. Our results suggested that the use of suitable AMF (e.g., *Funneliformis mosseae*) might effectively improve the currently unfavorable situation of economic tree species production on land with saline soils, which may greatly optimize the utility of these areas.

Keywords: saline–alkali soil; *Funneliformis mosseae*; *Gleditsia sinensis*; root growth; soil environment

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1. Introduction

Soil salinity is one of the major challenges that hinders environmental sustainability on a global scale [1]. Recently, the rapid expansion of the global economy has led to a sharp increase in demand for land [2]; thus, saline–alkali land is increasingly valued as a land reserve resource. However, excessive soil salinity limits the development and utilization potential of plants, as it is difficult to protect and revegetate plants on saline–alkali land [3]. Although we initially developed coastal shelterbelts, the selection of tree species played a significant role in ecological protection [4,5], where the economic supply capacity of

saline–alkali land was only slightly engaged. Compared with traditional halophytes, the roots of economical tree species have weak tolerance against salt; thus, increasing salinity has serious deleterious effects on their growth. It is urgent to enhance the productivity of economic tree species on saline–alkali lands through the improvement of root growth.

Roots maintain plant growth via mechanical support, nutrient extraction, gas exchange, and the recruitment of beneficial soil microorganisms [6]. This assists plants in resisting various biotic and abiotic stresses such as salinity, acidity, drought, and pests [7]. Moreover, the soil environment strongly impacts the growth, density, and distribution of roots [8], while excessive soil salinity can have various harmful effects on them. These detrimental effects include physiological drought caused by poor water penetration, cytotoxicity induced through the accumulation of sodium and chloride ions, and nutrient imbalances caused by reduced nutrient absorption [3,9], which can inhibit root growth and even cause plant death. Recently, the use of plant growth-promoting microorganisms that alleviate salt stress to improve plant tolerance and productivity in saline–alkali soils has emerged as a new and intensive area of research [10–13].

As a critical component of terrestrial ecosystems [14], arbuscular mycorrhizal fungi (AMF) can form symbiotic relationships with >80% of land plants [15]. Numerous studies confirmed that mycorrhizal association enhances the salt tolerance and productivity of plants in saline–alkali soils by augmenting the absorption of nutrients and accumulation of dry matter [5]. Further, it regulates antioxidant enzyme activities [4] and assists in osmotic adjustments [16] while improving photosynthesis and water utilization [17]. However, the effects of mycorrhizal association are primarily determined by plant–AMF symbiosis and environmental factors [18]. Associations among the growth, nutrient absorption, and physiological and biochemical processes of different AMF on specific host plants under salt stress still require further exploration. This contributes to the more effective application of AMF to agricultural and forestry production in saline–alkali areas. Research into the use of AMF to improve plant salt tolerance has mostly been concentrated on agriculture rather than forestry [14,19,20], where investigations into woody plants for forestry are even rarer [4,5,21]. As valuable tree species, *Gleditsia Linn* and *Gleditsia sinensis* Lam. are extensively distributed across Asia, the Americas, and Africa [22]. In addition to providing vegetable gum, pod powder, and wood, *G. sinensis* also have important ecological benefits by serving as shelterbelts [23]. *G. sinensis* has attracted broad attention due to its excellent drought resistance, low soil requirements, and salt tolerance of up to 3‰ [24,25]. However, the use of AMF to improve plant tolerance and productivity under salt stress toward the development of coastal shelterbelts remains poorly understood. Although our earlier research confirmed that AMF enhanced the growth of *G. sinensis* [26], there remains a lack of studies on the advantages of mycorrhizal association at various salinity levels using different strains. Even rarer is research on the effects of different AMF strains on the root growth and soil environment of *G. sinensis* under salt stress.

To investigate the impact of various NaCl levels and different AMF species on the root systems of *G. sinensis*, we established simulated salt damage tests using potted plants. Our objective was to illustrate the effects of increasing levels of NaCl and different AMF species on the root systems of *G. sinensis*, which encompassed root dry weight, morphology, nutrient content, and physiology, and soil nutrient content. According to previous investigations, we propose the following hypotheses: (1) salinity inhibits the root growth of *G. sinensis* where the degree of inhibition increases with higher NaCl levels; (2) this inhibition can be alleviated with the inoculation of AMF; (3) AMF promotes root growth by regulating root nutrient uptake and physiology under salt stress.

2. Materials and Methods

2.1. Experimental Design

For this study, *G. sinensis* was employed as the research species, and a completely random block design included two factors: AMF treatment and salt treatment. The AMF treatment comprised three inoculation entities (autoclaved AMF inocula (CK), *Funneliformis*

mosseae (FM), and *Corymbiglomus tortuosum* (CT)). The salt treatment comprised four levels of NaCl (0, 50, 100, and 150 mM). Each treatment contained 12 biological replicates for a total of 3 × 4 × 12 = 144 pots.

2.2. Plant Seeds, AMF Inoculant, and Soil

G. sinensis seeds were provided by the Jiangsu Forestry Station with a thousand seed weight of 182.25 g. To break dormancy, seeds were etched with concentrated sulfuric acid for 10 min under constant stirring. When the seeds turned dark red, they were extracted and rinsed with distilled water several times until the pH value of the residual water on the surface of the seeds was ~7. Seeds were then poured into warm water at 30 °C for 2 days till they were fully swollen with water. Expanded seeds were screened out and buried in sterilized wet sand, placed in an incubator at 28 °C for cultivation under dark conditions, and germinated within 3–5 days.

Inoculated strains (*F. mosseae* and *C. tortuosum*) were purchased from the Institute of Plant Nutrition and Resources at the Beijing Academy of Agriculture and Forestry Sciences. For the propagation of the AMF inoculant, maize and clover were used as host plants, and yellow sand was employed as the propagation substrate, and they were cultured in an artificial climate chamber for three months. Hoagland nutrient solution containing 25% phosphorus was added to each plant weekly, and water was added every three days. The ambient conditions of the artificial climate chamber were as follows: temperature of 22–25 °C, relative humidity of 60%–80%, light period of 14/10 h, and light intensity of 800 mol/(m^2·s). The harvested yellow sand, colonized root segments, hypha, and spores (>7/g) were utilized as the inoculation agent.

In order to maintain the consistency of the substrate in each pot, the nursery substrate employed for potting was comprised of topsoil, yellow sand, and vermiculite mixed at a 1:1:1 volume ratio. The topsoil was collected from the 5–30 cm soil layer of the Xiashu Forest Farm. The physical and chemical properties of the topsoil were as follows: total carbon content (1.55%); total nitrogen content (0.03%); total phosphorus content (570.48 mg/kg); total potassium content (15.18 g/kg); available phosphorus content (10.00 mg/kg); available potassium content (101.39 mg/kg); electric conductivity (0.23 mS/cm); and pH (7.15).

The topsoil and yellow sand were screened to 2 mm and then mixed with vermiculite in a high-pressure steam sterilization pot at 121 °C under 1.4 MPa for 2 h. The autoclaved nursery substrate was placed in a greenhouse for at least 1 week to remove the odor. Pot dimensions were 27 × 21 × 19 cm^3, and pots were soaked in a 0.3% $KMnO_4$ solution for 3 h and then rinsed with distilled water prior to use.

2.3. Inoculation and Salt Stress Treatment

The pot experiment was conducted in a greenhouse at the Xiashu Forest Farm of Nanjing Forestry University. The indoor environmental conditions of the greenhouse were as follows: temperature, 18–35 °C; relative humidity, 40%–80%; light period, 14/10 h; and light intensity, 700–1000 mol/(m^2·s). In March 2018, germinated *G. sinensis* seeds with bud lengths of ~5 cm were selected for inoculation. Each pot was inoculated with one germinated *G. sinensis* seed, and the culture substrate was ~2.5 kg and contained 80 g of AMF inoculant; an 80 g AMF inoculant inactivated via an autoclaved pot was used as the control. When inoculating *G. sinensis* seeds, the AMF inoculant was applied to the surface layer of the culture substrate <5 cm, and seed buds remained exposed; after that, each pot was watered. Germinated seedlings were cultivated until June 2018, watered regularly, and irrigated with 300 mL of Hoagland nutrient solution with 25% phosphorus in each pot every month.

Following the confirmation of AMF colonization status in *G. sinensis* roots and verifying that AMF had formed a stable symbiotic relationship with *G. sinensis* roots, salt stress treatments commenced at the end of June 2018. The three inoculation treatments (CK, FM, and CT) were irrigated with NaCl concentrations of 0, 50, 100, and 150 mM, and the salt was gradually added to avoid salt shock reactions. Seedlings in each pot were irrigated

with 300 mL of the NaCl solutions at corresponding concentrations every week until the end of August 2018, during which water was also added to prevent drought stress. Each pot was irrigated each month with 300 mL of a Hoagland nutrient solution containing 25% phosphorus. After the addition of different salt concentrations, the pH of 0 mM NaCl level was 7.64 ± 0.09 m and electric conductivity (EC) was 0.71 ± 0.07 mS/cm; pH of the 50 mM NaCl level was 7.67 ± 0.18, and EC was 4.67 ± 0.57 mS/cm; pH of the 100 mM NaCl level was 7.68 ± 0.22, and EC was 6.35 ± 0.99 mS/cm; and pH of the 150 mM NaCl level was 7.63 ± 0.25, with an EC of 8.69 ± 1.09 mS/cm. Plants were harvested in September 2018, and three saplings from each of the inoculation treatments under each NaCl level were selected for the determination of each index.

2.4. Determination of Mycorrhizal Colonization Status

Fine roots were cut into 1 cm long segments, soaked in 10% (*w*/*v*) KOH, incubated in a water bath (90 °C) for 1 h, stained with basic H_2O_2 (containing 30 mL of 10% (*v*/*v*) H_2O_2, 3 mL of concentrated NH_4OH, and 60 mL of water) for 25 min, soaked in 1% (*w*/*v*) HCl for three min, stained with 0.05% (*w*/*v*) of a Trypan Blue solution, and placed in a water bath (90 °C) for 30 min [27]. Subsequently, roots were soaked in lactic acid:glycerol (1:1) to eliminate any excess Trypan Blue solution, and examined for the presence of AMF structures under a semiautomatic digital microscope (Leica DM5000B, Wetzlar, Germany) at 100 × 400 magnification [28]. The mycorrhizal colonization rate was calculated using the following equation: mycorrhizal colonization rate (%) = number of infected root segments/total number of segments × 100%.

2.5. Determination of Root Dry Weight and Morphology

The roots of one seedling were rinsed with deionized water and scanned using an Epson Expression 12000XL scanner (Seiko Epson, Suwa, Japan). Images were analyzed using WinRHIZO (WinRHIZO Pro2016) to determine root length, root surface area, and root tip number [29]. Once the root samples had been rinsed, they were dried at 105 °C for 30 min and at 70 °C for 48 h to a constant weight, and then weighed [26].

2.6. Determination of Root Nitrogen and Phosphorus

Dried roots were separately ground and sifted through a 0.5 mm sieve, after which 50 mg samples were used to determine nitrogen concentration via an elemental analyzer (Vario MACRO cube; Elementar Trading Shanghai, Shanghai, China). Next, 0.2 g samples were digested in an acidic mixture ($HClO_4$:HNO_3, 1:5) and diluted with double-distilled water to quantify the concentrations of phosphorus using the ammonium molybdate blue method with a spectrophotometer (UV 2700, SHIMADZU, Tokyo, Japan) [26].

2.7. Determination of Root Enzyme Activities

Root enzyme activity was measured using the triphenyl tetrazolium chloride (TTC) method as described in [4]. Dehydrogenase activities indicated those of the roots. To determine dehydrogenase activity, fine roots were soaked in 10 mL of a mixed solution that included 0.4% TTC and a phosphate buffer and kept in the dark at 37 °C for 2 h. Subsequently, 1 mol/L of H_2SO_4 was added to stop the reaction; afterwards, roots were ground with 10 mL ethyl acetate, and the absorbance of the solution was obtained at 485 nm.

Nitrate reductase (NR) activity was determined according to a previous technique [30]. The frozen root was homogenized in a chilled mortar and pestle with 100 mM of a potassium phosphate buffer (pH 7.4). The extract was incubated in a reaction mixture that contained a 100 mM potassium phosphate buffer (pH 7.4), 10 mM EDTA, 0.15 mM NADH, and 0.1 M KNO_3 at 30 °C for 30 min. The reaction was stopped using 100 µL of 1 M zinc acetate. The absorbance of the supernatant was determined at 540 nm following the diazotation of nitrite ions with 5.8 mM sulfanilamide and 0.8 mMN-(1-naphthyl)-ethylenediamine-dihydrochloride (NNEDD).

Glutathione reductase (GR) activity was determined following a previous procedure [31], and the root was homogenized to a powder in liquid nitrogen. The powder was then used for the extraction of soluble proteins in 5 mL of 50 mM Tris-HCl (pH 7.0) on ice. After centrifugation, the supernatant was used for later determination. GR activity was measured by the dropout value at 340 nm with a reaction mixture of 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM GSSG, 0.4 mM NADPH, and enzyme extract.

Niacinamide adenine dinucleotide oxidase (NOX) activity was determined according to an earlier method [32]. NOX activity was revealed in an assay mixture containing 100 mM sodium acetate (pH 6.5), 1 mM $MnCl_2$, 0.5 mM p-coumaric acid, 0.2 mM NADH, and enzyme extract. The reaction was monitored by following the decrease in absorbance at 340 nm with an extinction coefficient of 6.22 mM^{-1} cm^{-1}.

2.8. Determination of Soil Nitrogen and Phosphorus

The total nitrogen content of the soil was quantified using an elemental analyzer, and alkali-hydrolyzable nitrogen content was found using an alkali-hydrolyzable diffusion method [33]. Total phosphorus and available phosphorus content was obtained via molybdenum antimony antispectrophotometry [34].

2.9. Statistical Analyses

Differences in mycorrhizal colonization rate, root dry weight, morphology, nutrient content, and physiology, and soil nutrient content between various salinity treatments and different inoculation treatments were analyzed using one-way ANOVA, followed by a Duncan test. Two-way ANOVA was employed to test the main effects on and interactions between NaCl and AMF levels, and mycorrhizal colonization rate, root dry weight, morphology, nutrient content, and physiology, and soil nutrient content (SPSS 26.0 Inc., Chicago, IL, USA).

Redundant analysis (RDA) was utilized to reveal the relationships between NaCl levels, and mycorrhizal colonization rate, root dry weight, morphology, nutrient content, and physiology, and soil nutrient content, which were visualized using a Canoco 5.0 (Microcomputer Power, Ithaca, NY, USA). An AMOS 22.0 (SPSS Inc., Chicago, IL, USA) was employed for structural equation modelling construction to test whether NaCl level and mycorrhizal colonization rate directly or indirectly impacted root dry weight and tip number through the modification of root nutrient content and activity, NR activity, and available soil nutrient content.

3. Results

3.1. Mycorrhizal Colonization Status

The AMF colonization status profiles of the three treatments are shown in Figure 1. Hyphae, vesicles, spores, and arbuscules were observed as a result of FM and CT treatments; however, they were not found in CK (Figure 1A–C). Under the four NaCl levels, the mycorrhizal colonization rate of the FM treatment was significantly higher than that of the CT treatment ($p < 0.05$), with increases of 21.61%, 38.16%, 13.79%, and 15.56%. Furthermore, different NaCl levels slightly impacted mycorrhizal colonization rate under the FM treatment. However, compared with the three other NaCl levels, 50 mM significantly reduced the mycorrhizal colonization rate of CT ($p < 0.05$) (Figure 1D).

3.2. Root Dry Weight and Morphology

Regardless of AMF inoculation, as NaCl level increased, root dry weight, length, surface area, and tip number showed a downward trend. When the NaCl level reached 150 mM, all indicators were significantly lower than 0 mM ($p < 0.05$) (Figure 2). For each NaCl level, root dry weight, length, surface area, and tip number under FM and CT treatments were significantly higher than those of CK ($p < 0.05$) (Figure 2). Further, when NaCl level reached 100 mM, root length and tip number under FM treatment were significantly higher than those under the CT treatment ($p < 0.05$), with an increase of 12.26%

and 14.85%, respectively (Figure 2B,D). When NaCl level reached 150 mM, root dry weight under FM treatment was significantly higher than that of CT ($p < 0.05$), with an increase of 46.69% (Figure 2A).

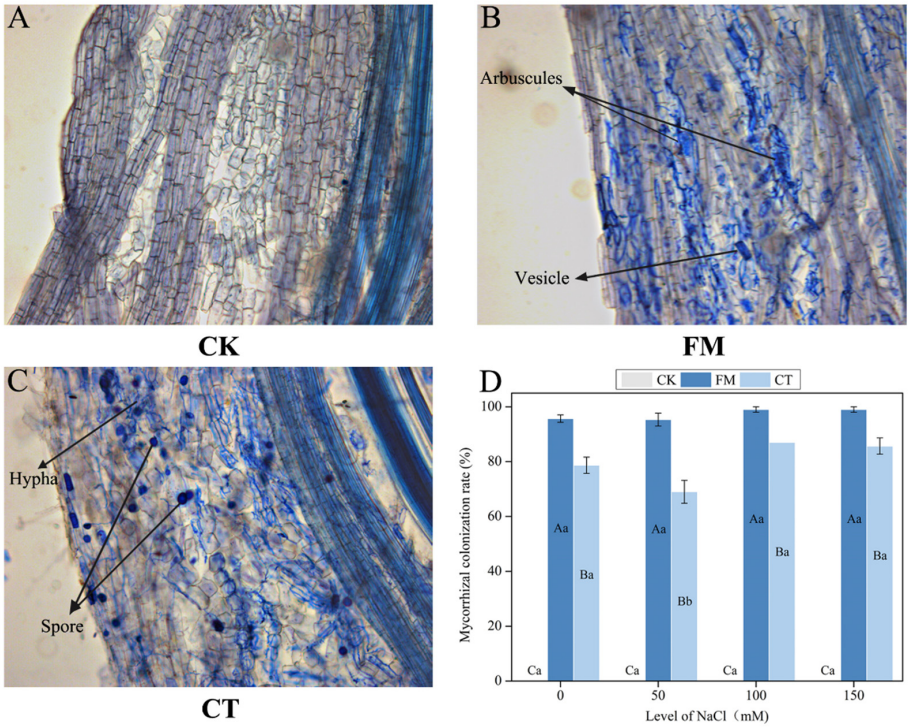

Figure 1. Variations in mycorrhizal colonization status between treatments. (**A**) mycorrhizal colonization statu of CK treatment; (**B**) mycorrhizal colonization statu of FM treatment; (**C**) mycorrhizal colonization statu of CT treatment; (**D**) mycorrhizal colonization rate of different treatments. Different uppercase letters indicate significant differences between various inoculation treatments under same salt levels ($p < 0.05$). Different lowercase letters indicate significant differences between various salt levels under inoculation treatments ($p < 0.05$). Error bars refer to standard errors.

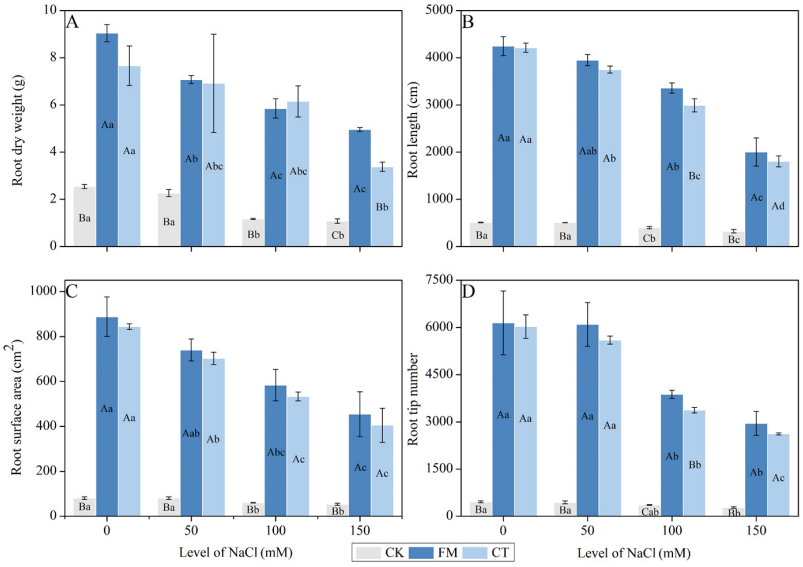

Figure 2. Variations in the root dry weight and morphologies between treatments. (**A**) root dry weight of different treatments; (**B**) root length of different treatments; (**C**) root surface area of different treatments; (**D**) root tip number of different treatments. The meanings of the letters and error bars are consistent with Figure 1.

3.3. Root Nitrogen and Phosphorus Content

As the NaCl levels increased, both the root nitrogen and phosphorus content under the CK treatment initially increased and then decreased; however, this trend was weakened under the FM and CT treatments (Figure 3A,B). At NaCl levels of 0 mM and 50 mM, the root nitrogen contents under the FM and CT treatments were significantly lower than that of the CK ($p < 0.05$). When the NaCl level reached 100 mM, the root nitrogen content under the CT treatment was significantly lower than that of the CK and FM treatments ($p < 0.05$). However, when the NaCl level reached 150 mM, there were no significant differences between the CK, FM, and CT treatments (Figure 3A).

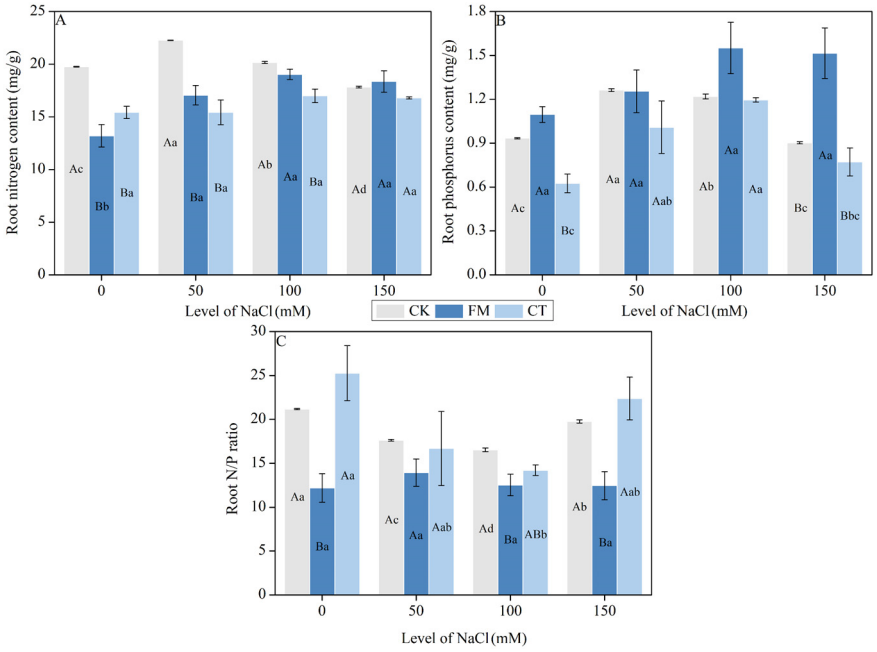

Figure 3. Variations in root nitrogen, phosphorus content, and N/P ratios between treatments. (**A**) root nitrogen content of different treatments; (**B**) root phosphorus content of different treatments; (**C**) root N/P ratio of different treatments. Letter and error bar meanings are consistent with Figure 1.

Moreover, when NaCl level was 0 mM, root phosphorus content under the CT treatment was significantly lower than that of CK and FM treatments ($p < 0.05$). When NaCl level was increased to 150 mM, root phosphorus content under FM treatment was significantly higher than that of CK and CT treatments ($p < 0.05$), with increases of 68.04% and 96.60%, respectively (Figure 3B). For the root N/P ratio under increasing NaCl levels, the N/P ratio under CK and CT treatments showed an initial increase and then a decrease. Except for 100 mM salinity, N/P ratios under FM treatment at other NaCl levels were significantly lower than that of the CK treatment ($p < 0.05$) (Figure 3C).

3.4. Root Physiology

At higher NaCl levels, root activity under CK treatment continued to decrease. It was only when the NaCl level was higher than 100 mM that root activity under CT treatment exhibited a significant decline ($p < 0.05$). However, regardless of how much the NaCl level changed, its effect on root activity under FM treatment was not significant. When NaCl level was higher than or equal to 50 mM, root activity under the FM treatment was significantly higher than that of CK ($p < 0.05$), with increases of 85.08%, 131.49%, and 170.01% (Figure 4A).

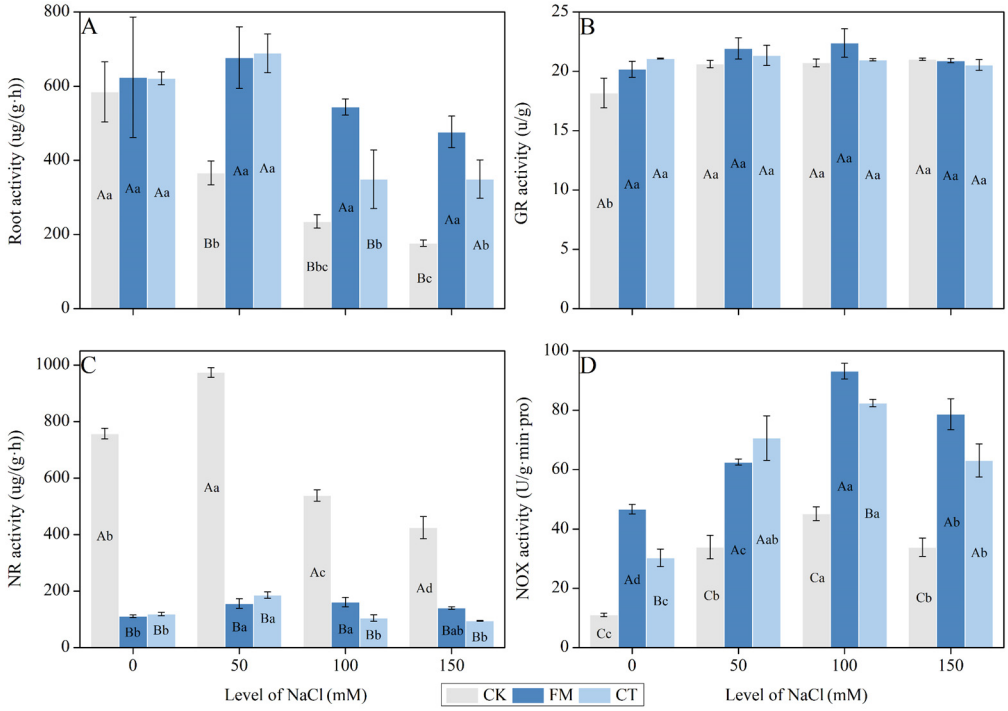

Figure 4. Variations in root physiological characteristics between treatments. (**A**) root activity of different treatments; (**B**) root glutathione reductase activity of different treatments; (**C**) root nitrate reductase activity of different treatments; (**D**) root niacinamide adenine dinucleotide oxidase activity of different treatments. Meanings of letters and error bars are consistent with Figure 1. GR, glutathione reductase; NR, nitrate reductase; NOX, niacinamide adenine dinucleotide oxidase.

Among the four NaCl levels, there were no major differences in GR activity between the FM and CT treatments, although salt stress significantly increased GR activity under CK treatment ($p < 0.05$) (Figure 4B). Regardless of AMF inoculation status, with higher NaCl levels, both NR and NOX activities exhibited increasing and then decreasing trends. Nevertheless, in contrast to NR, NOX activities under FM and CT treatments were significantly higher than that of CK ($p < 0.05$) (Figure 4C,D).

3.5. Soil Nitrogen and Phosphorus Content

When NaCl level was <150 mM, soil alkali-hydrolyzable nitrogen (AN) under CT treatment was significantly lower than that of CK treatment, with a decrease of more than 50%. It was only when the NaCl levels were 0 and 100 mM that soil AN was significantly reduced under FM treatment compared with the CK treatment ($p < 0.05$), with decreases of 40.91% and 41.67%, respectively (Figure 5A). For soil TN, when NaCl level was 100 mM, there was a difference between CT and CK treatments ($p < 0.05$), whereas for the other NaCl levels, there were no significant differences between treatments (Figure 5C). When NaCl level was 0 mM, CT treatment significantly increased soil available phosphorus (AP) compared with the CK treatment ($p < 0.05$), with an increase of 27.10% (Figure 5B). Further, when NaCl level was 150 mM, CT treatment significantly increased soil total phosphorous (TP) in contrast to CK and FM treatments ($p < 0.05$), with increases of 20.16% and 16.41%, respectively (Figure 5D).

3.6. Two-Way Analysis of Variance Results

NaCl, AMF, and their interactions significantly impacted mycorrhizal colonization rate, root length, surface area, tip number, nitrogen content, and NR and NOX activities, while NaCl and AMF were significant factors that affected the root dry weight, phosphorus content, activity, and N/P ratio. Moreover, AMF significantly affected soil AN, TN, and TP,

while only GR activity was significantly impacted by NaCl. Furthermore, the influences of NaCl and AMF, and their interactions on soil AP were not significant (Table 1).

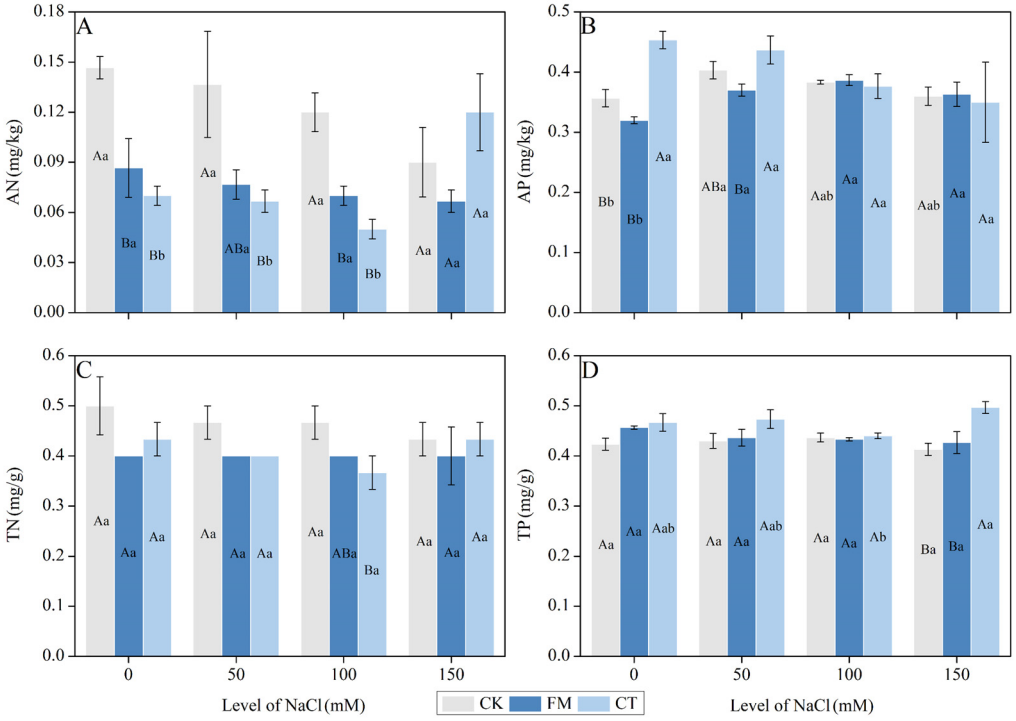

Figure 5. Variations in soil nitrogen and phosphorus contents between treatments. (**A**) soil alkali-hydrolyzable nitrogen content under different treatments; (**B**) soil available phosphorus content under different treatments; (**C**) soil total nitrogen content under different treatments; (**D**) soil total phosphorus content under different treatments. Meanings of letters and error bars are consistent with Figure 1. AN, soil alkali-hydrolyzable nitrogen; TN, soil total nitrogen; AP, soil available phosphorus; TP, soil total phosphorus.

Table 1. Two-way analysis of variance on indices of *G. sinensis* Lam. root system and soil.

Parameters	NaCl	AMF	NaCl × AMF
MCR	0.000 ***	0.000 ***	0.002 **
RDW	0.000 ***	0.000 ***	0.296NS
RL	0.000 ***	0.000 ***	0.000 ***
RS	0.000 ***	0.000 ***	0.007 **
RTN	0.000 ***	0.000 ***	0.001 **
RNC	0.001 **	0.000 ***	0.000 ***
RPC	0.000 ***	0.000 ***	0.100NS
N/P	0.015 *	0.000 ***	0.085NS
TTC	0.000 ***	0.000 ***	0.210NS
GR	0.035 *	0.050NS	0.244NS
NR	0.000 ***	0.000 ***	0.000 ***
NOX	0.000 ***	0.000 ***	0.015 *
AN	0.409NS	0.000 ***	0.024 *
AP	0.167NS	0.047NS	0.070NS
TN	0.667NS	0.018 *	0.782NS
TP	0.707NS	0.000 ***	0.112NS

Note: main impacts and interactivity of NaCl and AMF were assessed by two-way ANOVA. NS, no significance; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

3.7. Redundant Analysis Results

Using root nutrient content, root physiological characteristics, and soil nutrient content as explanatory variables, redundant analysis was performed on root dry weight and mor-

phologies. Among them, Axis 1 explained 96.81% of variation, whereas Axis 2 accounted for 0.46%. As depicted in Figure 6, high root surface area, length, tip number, and dry weight were found at the left side of the coordinate axis, which were positively correlated with mycorrhizal colonization rate, root activity, NOX activity, GR activity, root phosphorus content, and soil TP and AP. However, they were negatively correlated with root nitrogen content, soil AN and TN, NR activity, root N/P ratio, and NaCl level.

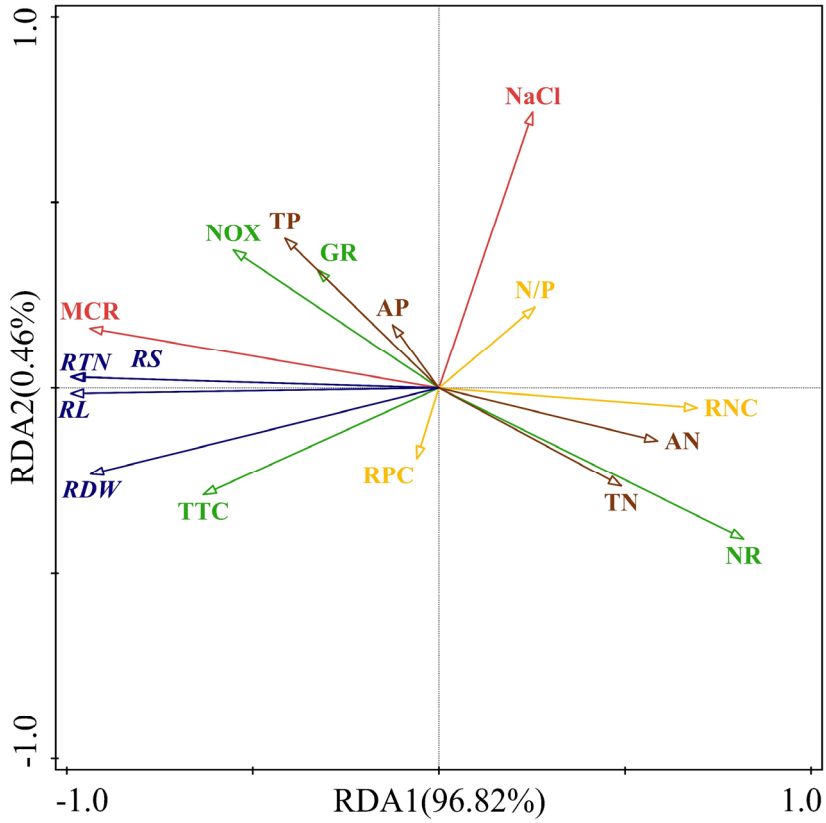

Figure 6. Redundant analysis of NaCl levels, mycorrhizal colonization rate, root morphologies, root nutrient contents, root physiological properties, and soil nutrient contents. Angles and lengths of arrows indicate direction and strength of the relationship of the above indicators. Abbreviations: NaCl, NaCl level; MCR, mycorrhizal colonization rate; AN, soil alkali−hydrolyzable nitrogen; TN, soil total nitrogen; AP, soil available phosphorus; TP, soil total phosphorus; NR, nitrate reductase activity; TTC, root activity; NOX, niacinamide adenine dinucleotide oxidase activity; GR, glutathione reductase activity; RNC, root nitrogen content; RPC, root phosphorus content; N/P, root nitrogen to phosphorus ratio; RDW, root dry weight; RL, root length; RS, root surface area; RTN, root tip number.

3.8. Structural Equation Model Results

The structural equation modelling of *G. sinensis* was estimated using Amos (Figure 7) by extracting root dry weight and root tip number to characterize root growth; soil AN and AP to differentiate the active soil nutrient content; root nitrogen and phosphorus content to characterize root nutrient content; and root and NR activities to describe root physiology. The χ^2 test indicated that, in Model A, $\chi^2 = 6.082$, df = 5, and $p = 0.298$ (>0.05). The goodness-of-fit index was 0.968 (>0.900), and the approximate root mean square error was 0.079 (<0.080).

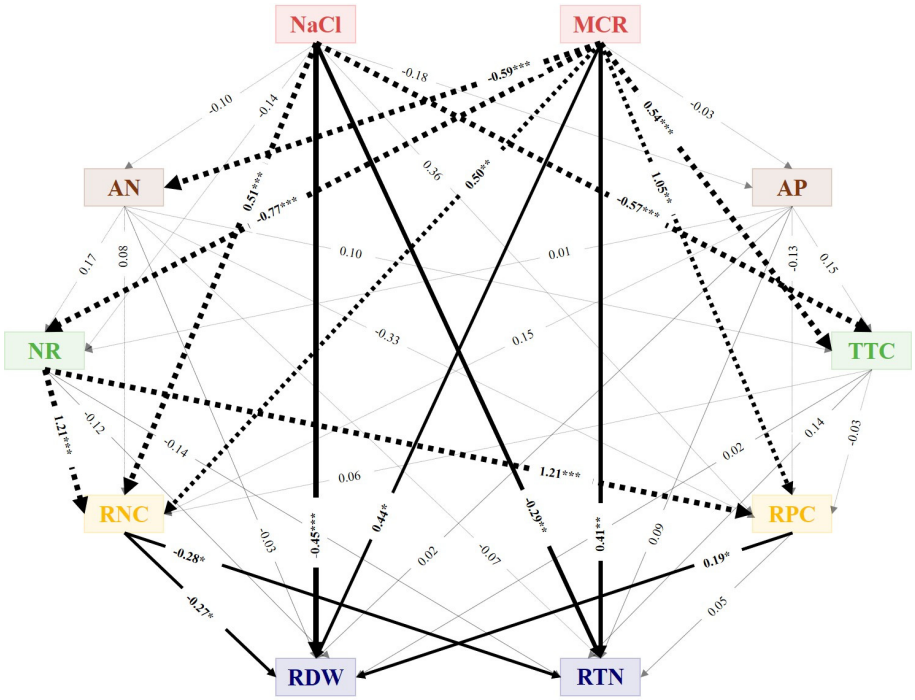

Figure 7. Structural equation model of effects of NaCl level and mycorrhizal colonization rate on root dry weight and tip number. Numbers on lines are standardized path coefficients. Widths of lines indicate strength of causal influence. Solid lines represent direct effects on root dry weight and tip number; dotted lines represent effects on soil nutrient contents, root physiologies, and nutrient contents. Abbreviations: NaCl, NaCl level; MCR, mycorrhizal colonization rate; AN, soil alkali−hydrolyzable nitrogen; AP, soil available phosphorus; NR, nitrate reductase activity; TTC, root activity; RNC, root nitrogen content; RPC, root phosphorus content; RDW, root dry weight; RTN, root tip number. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Results revealed that the direct effects of NaCl levels on root dry weight (−0.45, $p < 0.001$) and root tip number (−0.29, $p < 0.01$) were significant, while the mycorrhizal colonization rate had a substantial direct effect on root dry weight (0.44, $p < 0.05$) and root tip number (0.41, $p < 0.01$). Due to the indirect influences of induced active soil nutrients, and root nutrients and physiologies, standardized total effects of the NaCl levels on the root dry weight and tip number were −0.02 and −0.13, respectively. The total effects of mycorrhizal colonization rate on root dry weight and tip number were 0.33 and 0.40, respectively (Table 2).

Table 2. Direct, indirect, and total impacts of NaCl levels and mycorrhizal colonization rates on indices of *G. sinensis* root systems and soil.

		G. sinensis							
		AN	**AP**	**NR**	**TTC**	**RNC**	**RPC**	**RDW**	**RTN**
NaCl	SDE	−0.10	−0.18	−0.14	−0.57	0.51	0.36	−0.45	−0.29
	SIE	0.00	0.00	−0.02	−0.04	−0.26	−0.12	−0.02	−0.13
	STE	−0.10	−0.18	−0.16	−0.61	0.25	0.24	−0.47	−0.42
MCR	SDE	−0.59	−0.03	−0.77	0.54	0.50	1.05	0.44	0.41
	SIE	0.00	0.00	−0.10	−0.06	−1.09	−0.88	0.33	0.40
	STE	−0.59	−0.03	−0.88	0.47	−0.59	0.16	0.76	0.81

Note: standardized direct, indirect, and total effects of NaCl levels and mycorrhizal colonization rates on indices of *G. sinensis* root systems and rhizosphere soil. Abbreviations: NaCl, NaCl level; MCR, mycorrhizal colonization rate; AN, soil alkali-hydrolyzable nitrogen; AP, soil available phosphorus; NR, nitrate reductase activity; TTC, root activity; RNC, root nitrogen content; RPC, root phosphorus content; RDW, root dry weight; RTN, root tip number; SDE, standardized direct effects; SID, standardized indirect effects; STE, standardized total effects.

4. Discussion

4.1. Responses of Root Growth to AMF Inoculation under Salt Stress

Mycorrhizal colonization rate may be determined by combining various AMF strains and host plant species [35]. In this study, the mycorrhizal colonization rate of *F. mosseae* to *G. sinensis* was significantly higher than that of *C. tortuosum* at all salinity levels, which also confirmed the above conclusion. In general, salinity limits AMF hyphae growth and spore germination, thereby reducing the mycorrhizal colonization rate [36,37]. This was reflected by the significant decrease in the mycorrhizal colonization rate of *C. tortuosum* when salinity was 50 mM. An earlier study found that the environmental tolerances of different AMF strains were variable, and suitable soil environments and plants were also different [38]. The combination of *F. mosseae* and *G. sinensis* roots maintained a high mycorrhizal colonization rate at all salinity levels, exhibiting excellent host and soil environment adaptation in contrast to *C. tortuosum*.

Since root morphologies (e.g., root length, surface area, root tip number) and biomass are sensitive to environmental factors, they may be employed to reflect root growth conditions [39]. Consistent with our first hypothesis and previous studies [40,41], salt stress significantly reduced the root dry weight, length, surface area, and tip number of *G. sinensis*. Compared with nonmycorrhizal plants, the improved growth and biomass of mycorrhizal plants are important indicators of greater salt tolerance [42]. In this study, mycorrhizal seedlings, particularly those inoculated with *F. mosseae*, had greater root dry weight under various salinities, which was positively related to the optimization of the root morphology of the mycorrhizal seedlings. Since longer root lengths, larger absorption areas, and abundant root tips can absorb more water and nutrients to better support plants [43], mycorrhizal seedlings were more tolerant to salt than nonmycorrhizal seedlings were. The above results confirmed our second hypothesis.

4.2. Effects of Nutrient Uptake on Root Growth under Salt Stresss

Nitrogen is a critical component for all enzymes, while phosphorus is essential for rRNA synthesis. Therefore, changes in the concentrations of phosphorus and nitrogen can impact the allocation of nutrients to cell components, life history strategies, and physiological functions [44]. We found that AMF inoculation could significantly reduce root nitrogen content under low salinity (<50 mM), and this difference would gradually be weakened as salinity increased. When salinity reached 150 mM, the inoculation of *F. mosseae* significantly increased the phosphorus content of the roots. These results suggested that AMF inoculation could regulate the uptake of nitrogen and phosphorus in roots, improve the nutritional status of plants, and meet the needs for protein and chlorophyll synthesis while assisting plants in resisting the toxic effects of sodium ions under high salinity [45,46]. Biomass N/P ratio was primarily employed to assess whether nitrogen or phosphorus created any additional constraints. In general, an optimal N/P ratio by mass is considered to be in the range of 10–20 [47]. In this study, N/P ratios of all *G. sinensis* roots inoculated with *F. mosseae* at all salinity levels were within this range, which confirmed that *F. mosseae* inoculation could effectively regulate nutrient utilization under salt stress.

Redundancy analysis indicated that root dry weight and morphology were negatively correlated with soil TN and AN, and positively associated with TP and AP. Combined with root N/P ratio, we surmised that the main soil limiting nutrient for the growth of *G. sinensis* in this study was phosphorus [48]. It is generally believed that AMF inoculation can promote root nitrogen uptake [46]; however, in our results, mycorrhizal colonization rate and root dry weight were negatively correlated with root nitrogen content. By establishing a structural equation model, mycorrhizal colonization rate had a positive direct effect on root nitrogen content, but could reduce it through the indirect reduction in root NR activity, which explained the above issues.

4.3. Effects of Physiology on Root Growth under Salt Stresss

Numerous works (including our previous study) demonstrated that AMF can regulate superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, and other antioxidant enzymes under salt stress [24,49,50]. However, this paper explored changes in NOX and GR activities of mycorrhizal symbiosis under salt stress. NOX activity is primarily involved in the production of H_2O_2 as an early response to salt stress [51], which plays a critical role in many aspects of signal transduction and plant development, including plant defense, root hair development, and stomatal closure [52]. This investigation revealed that AMF inoculation significantly increased NOX activities at each salinity level, while GR activities were essentially unaffected. Combined with previous studies on NOX activities [53,54], and their positive correlations with root dry weight and morphology in our study, we speculated that AMF inoculation promoted the signal transmission and defense of *G. sinensis* to salt stress, and promoted root development by increasing NOX activity. However, GR activity was not the main enzyme in the active oxygen balance system of *G. sinensis* mycorrhizal seedlings under salt stress.

Root activity is an important physiological index for the evaluation of water and nutrient uptake [55]. Our experimental results revealed that *F. mosseae* inoculation at all salinity levels could significantly enhance *G. sinensis* root activities, which was consistent with results of previous tobacco studies and the structural equation model in our study [56]. Combining its positive correlation with root dry weight and morphology, AMF may promote plant growth by enhancing *G. sinensis* root activity under salt stress. Salinity typically affects nitrogen metabolism in plants by disrupting the activities of key enzymes [57]. In this investigation, with higher salinity, root NR activities under all treatments showed an initial increasing and then decreasing trend. The decrease was particularly strong when salinity reached 100 mM, which also confirmed the above conclusion. As the first enzyme in the nitrate assimilation pathway, NR activities regulate plant nitrogen content. This study revealed that root NR activity was significantly reduced by AMF inoculation at all salinity levels, which was different from the results of previous studies [58]. Combining its negative correlation with root dry weight and morphology, rhizosphere nitrogen eutrophication in the pot experiment may have had a negative effect on AMF colonization [59], where the AMF ensured a more suitable living environment through the regulation of root NR activities. In addition, a structural equation model indicated that both soil salinity and mycorrhizal colonization rate had significant direct effects on root dry weight and root tip number. However, soil salinity primarily impacted root growth by indirectly affecting root nitrogen content, while mycorrhizal colonization rate influenced root growth via its indirect effects on root NR activity, and root nitrogen and phosphorus content. The above results also support our third hypothesis.

Since salt in the soil of the eastern coastal areas of China is mostly NaCl, NaCl solution stress was used in the greenhouse to examine growth and physiological responses to AMF and salt stress in *G. sinensis* root systems. However, this soil also contains small quantities of other types of salts such as Na_2CO_3 and Na_2SO_4. It remains to be seen whether different salt types might also influence mycorrhizal association. To assess whether AMF should be applied to the coastal areas of East China, the effects of different types of salt on the efficacy of mycorrhizal association should be further explored. Additionally, the molecular mechanisms of AMF involved with enhancing the salt tolerance of *G. sinensis* should be elucidated through omics analysis.

5. Conclusions

Through the introduction of various AMF inoculations under different salt concentrations in pot experiments, AMF inoculation could greatly promote the growth of roots by regulating their nutrient uptakes and physiologies under salt stress. Compared with *C. tortuosum*, the inoculation effects of *F. mosseae* were superior, which was the recommended strain for the bioremediation of saline–alkali soils.

In summary, the use of suitable AMF might effectively improve the currently unfavorable situation of economic tree species production in saline soils, with great potential for optimized utility of these areas.

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Abbreviations

AMF: arbuscular mycorrhizal fungi; NaCl, NaCl level; MCR, mycorrhizal colonization rate; AN, soil alkali-hydrolyzable nitrogen; TN, soil total nitrogen; AP, soil available phosphorus; TP, soil total phosphorus; NR, nitrate reductase activity; TTC, root activity; NOX, niacinamide adenine dinucleotide oxidase activity; GR, glutathione reductase activity; RNC, root nitrogen content; RPC, root phosphorus content; N/P, root nitrogen to phosphorus ratio; RDW, root dry weight; RL, root length; RS, root surface area; RTN, root tip number; SDE, standardized direct effects; SID, standardized indirect effects; STE, standardized total effects.

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