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Research Article

Effects of arbuscular mycorrhizal fungi on the growth and metabolism of perennial ryegrass (*Lolium perenne*) under salt stress

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Abstract

Perennial ryegrass is considered an excellent cold-season turfgrass, and salinity is an important environmental factor that affects its growth. Many studies have shown that arbuscular mycorrhizal (AM) fungi can alleviate stress responses in plants, but there have been few reports on the promotion of turfgrass growth in saline-alkali land by AM fungi. We designed an experiment to investigate the effects of different saline conditions (0%, 0.05%, 0.1% salt, w/w) on the growth and physiological metabolism of perennial ryegrass. The results showed that under 0.05% salt stress, AM fungi increased the plant height by 25.9%, the aboveground and underground dry weight by 37.1% and 55.6%, respectively; and the content of chlorophyll a in the plants by 31.0%. Inoculation with AM fungi increased the net photosynthetic rate (A), transpiration rate (E), intercellular CO_2 concentration (Ci), and stomatal conductance (Gs) of perennial ryegrass, and the increase in their levels negatively correlated with increasing salt concentrations. Under 0.1% salt stress, the A, Ci, Gs, and E of perennial ryegrass inoculated with AM fungi increased by 24.6%, 23.2%, 139.1%, and 40.7%, respectively. However, inoculation with AM fungi reduced F_v/F_m and had no significant impacts on the SOD activity, MDA, proline (Pro) content, soluble sugar content, and soluble protein content of perennial ryegrass. In conclusion, AM fungi can promote the growth of perennial ryegrass under salt stress, enhance its photosynthetic capacity, and improve the ability of perennial ryegrass to resist salt stress.

Keywords: arbuscular mycorrhizal fungi; salt stress; *Lolium perenne*; photosynthesis; chlorophyll fluorescence

Introduction

Soil salinity significantly affects approximately 932 million hectares of land globally and reduces the productivity of roughly 100 million hectares in Asia alone (Rao *et al*., 2008). The area of salinized soil in China is 3,467 hectares, and it is increasing each year. Soil salinization has had a significant detrimental effect on the ecological environment and agricultural production. Salt stress is one of the main environmental factors that cause abiotic stress in plants. With its relatively high concentration in the soil, plant water loss occurs and the K^+/Na^+ ratio changes, thereby resulting in an increase in the contents of Na^+ and Cl^- and causing plant injury. Several studies have shown that the photosynthetic parameters, osmotic potential, and transpiration rate of

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plant leaves changed under salt stress (Sudhir and Murthy, 2004) The decrease in photosynthetic efficiency caused by salt stress mainly depends on the decrease of the intracellular CO_2 concentration, stomatal closure, non-somatic limitation (Pooja *et al*., 2010), and the reduction of photosynthetic pigment content (Shu *et al*., 2012). The study showed that the chlorophyll content of *Prunus sinensis* seedlings first increased and then decreased with the increase of salt stress, while that of the fast-growing white elm seedlings was lower than that of the control group. Sun *et al*., 2020 found that salt stress induced by a low concentration of NaCl (of 0.2% or less, w/w) in *Rhizoma atractylodis* did not exert any substantial effect on either chlorophyll content or chlorophyll fluorescence parameters, however, with the increase of the salt concentration, the researchers observed decreases in the chlorophyll content, light fluorescence, PSII effective photochemical quantum yield (F_v/F_m), PSII maximum quantum yield of photochemical reaction (F_v/F_m), PSII potential photochemical activity coefficient (F_v/F_0), photochemical quenching (qP), and apparent electron transport rate (ETR), as well as gradual increases in the initial fluorescence (F_s) and non-photochemical quenching (NPQ).

Salt stress can lead to an increased generation of reactive oxygen species (ROS), which is not conducive to the survival of plants under stress. ROS can promote membrane lipid peroxidation (Hu *et al*., 2011) and reduce photosynthesis (Steduto *et al*., 2000). The production and clearance of ROS are well-regulated under normal physiological conditions, and plants exhibit complex oxidative defense strategies under stress. Under saline-alkali stress, the plant can employ its antioxidant defense system, which is comprised of the enzymes superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Wang *et al*., 2008). Many studies have emphasized the close relationship between enhanced antioxidant enzyme activity and increased resistance to environmental stress (Bor *et al*., 2003; Upadhyaya *et al*., 2008).

Arbuscular Mycorrhizae (AM) fungus is a beneficial microorganism that is widely distributed in the soil. It can form a symbiosis with plants to regulate their metabolic activities in various ways, including enhancing the absorption of minerals in the soil, promoting growth and development, and enhancing salt tolerance (Yang *et al*., 2014). Numerous studies have shown that AM fungi can affect the physiological and biochemical processes of plants by infecting their roots and forming arbuscular mycorrhiza, thus alleviating the adverse effects of salt stress. One study showed that the fresh weight, dry weight, height, and chlorophyll content of tomato plants inoculated with AM fungi increased markedly under salt stress treatment (Abdelhameid *et al*., 2020). Wang *et al.* (2019) showed that AM fungi could improve the activity of PSII under salt stress. Other studies have shown that AM fungi can alleviate the inhibitory effect of salt stress on *Lilium brownii* var. *viridulum* Baker (Li *et al*., 2018), *Leymus chinensis* (Trin.) Tzvel (Wang *et al*., 2018), has been observed to significantly increase plant biomass. Evidently, AM fungi can alleviate the inhibition of salt stress on plant growth and play an important role in the survival of plants in high-salinity environments.

Perennial ryegrass (*Lolium perenne* L.) is a perennial cool-season turfgrass that belongs to Gramineae ryegrass. The plant is native to southwest Asia, southern Europe, north Africa, and other places, and has been widely cultivated all over the world for its strong adaptability to various environmental conditions. Many studies have confirmed that the inoculation of AM fungi can alleviate drought stress in perennial ryegrass (Li *et al*., 2019), improve the disease tolerance of perennial ryegrass (Guo *et al*., 2019), and even alleviate the harm caused by heavy metals to plants (Bahmani-Babanari *et al*., 2021). However, most of the studies that have investigated the effects of AM inoculation on the resistance of plants to salt stress have focused on crops, while there have been few studies focused on its ability to improve the growth and physiological metabolism of turfgrass under salt stress. Therefore, the objective of this study was to better understand the physiology and possible mitigation of NaCl stress within perennial ryegrass with the inoculation of AM fungi. The findings provide a theoretical basis for the application of perennial ryegrass in saline-alkali land greening.

Materials and Methods

Plant materials, culture medium, and inoculum

Perennial ryegrass seeds were purchased from Beijing Best grass Industries Co. and *Funneliformis mosseae* was isolated and preserved by the Institute of Mycorrhizal Biotechnology of Qingdao Agricultural University. The culture medium was peat: the surface soil (0-20 cm) was mixed in a volume ratio of 1:1 before being sterilized (121 °C, 2 h) for reserve use. Substrate containing the roots, hyphae, and spores of clover was used as the inoculum.

Experimental design

Two-factor control experiments were carried out in this study (inoculating fungi × salt treatment). Plants inoculated with *Funneliformis mosseae* and a negative control group were set up and marked as the A and N groups, respectively. Three salt concentrations (0%, 0.05%, and 0.1%, w/w) were used to cause varying degrees of salt stress, denoted S1, S2, and S3, respectively. A total of six treatments were set up; namely, AS1, AS2, AS3, NS1, NS2, and NS3, and each treatment included 9 biological repeats. Sterilized substrate was placed into 2/3 of an aseptic round plastic flowerpot (upper diameter 19 cm, bottom diameter 16 cm, height 20 cm). In the AS group, 800 g of inoculum and approximately 5000 inoculation units of *F. mosseae* were added. In the NS group, the same amount of sterilized inoculum was added and an additional 3-5 cm of substrate was covered on top of the existing substrate. Perennial ryegrass seeds were sterilized with 10% hydrogen peroxide for 10 minutes and then washed twice with sterile water. Following this, 1.0 g of perennial ryegrass seeds were evenly spread on the surface of the substrate and then covered with another 1 cm of substrate. And the weight of each pot of substrate finally reached 2.0 kg. After 10 days of germination, the perennial ryegrass was treated with different concentrations of salt (outlined above) to induce salt stress for 30 days. During the cultivation period, the light cycle was set as 12 h light/12 h dark and the temperature was maintained within the range of 20-27 °C to ensure good ventilation. The grass was watered every two days to prevent leakage and ensure the maintenance of stable salt concentrations.

Measurement of growth index and biomass

The height of each plant was measured before harvesting. Then, after removing any yellow leaves, the aboveground part of the perennial ryegrass was used to measure the fresh weight of each group. The roots were then washed with water, dried with filter paper, and their fresh weights were recorded. Following this, the leaves and roots were killed in an oven at 105 °C for 30 minutes and then dried to constant weight at 80 °C to obtain the dry weights of the perennial ryegrass groups.

Photosynthetic pigment content and chlorophyll fluorescence

Chlorophyll a (Chla), chlorophyll b (Chlb), and carotenoids (Car) were extracted from the perennial ryegrass leaves using absolute ethanol. Then, the absorbances of the extracts at 470 nm, 645 nm, and 663 nm were determined by UV-vis spectrophotometry. The calculation method was as follows:

Chlorophyll content (mg/g) = (chlorophyll concentration C × extract V × dilution times) / sample fresh weight

$Chla = 13.95 \times A_{663} - 6.88 \times A_{645}$

$Chlb = 24.96 \times A_{645} - 7.32 \times A_{663}$

$Car = (1000 \times A_{470} - 2.05 \times Chla - 114.8 \times Chlb) / 245$

The chlorophyll fluorescence parameters were measured using a PAM-2500 chlorophyll fluorescence meter. Two measuring points were selected from each pot. The initial fluorescence (F_0), maximum fluorescence

(F_m), and maximum photochemical efficiency (F_v/F_m) of perennial ryegrass leaves were measured after the samples underwent 30 min of adaptation to darkness.

Photosynthetic parameters

Photosynthesis parameters were measured from 8:30 am to 11:30 am under sunny and windless conditions. Two leaves were randomly selected from each pot and a CIRAS-3 portable photosynthetic apparatus was used to measure the photosynthetic parameters of the perennial ryegrass (net photosynthetic rate (A), transpiration rate (E), intercellular CO_2 concentration (Ci), stomatal conductance (Gs), water use efficiency (WUE), and saturation vapor pressure difference (VPD)).

Determination of physiological index

Fresh perennial ryegrass leaves were taken for index determination. During sampling, the leaves were wrapped in tin foil and stored in liquid nitrogen. Then the leaves were moved to a -80 °C freezer for storage. The activity of superoxide dismutase (SOD) was determined by the nitroblue tetrazolium color development method. The activity of peroxidase (POD) was determined by the guaiacol colorimetric method. The measurement of malondialdehyde (MDA) content was determined by the thiobarbituric acid coloration method. The soluble sugar content was determined by the anthrone chromogenic method. The soluble protein content was determined by the Coomassie bright blue G250 color development method.

Data analysis and statistics

Excel 2011 software was used to process and draw the data, and all data were analysed statistically by analysis of variance using the software package SPSS 16.0 (SPSS 16.0 for windows, USA) with the means separated using Duncan's multiple range test. $P<0.05$ was used to indicate significance. All figures were generated with Origin 8.0.

Results

Growth indicators of perennial ryegrass

Table 1 shows the impacts of salt stress and AS fungi inoculation on the growth indices of perennial ryegrass. The inoculation of AM fungi had a significant impact on the dry weight of the overground portion, the dry weight of the underground portion, and the total dry weight of perennial ryegrass ($P<0.001$). However, the combined interaction effect of salt × AM fungi had no significant impact on these indicators ($P>0.05$). The effects of salt treatment on the dry weight of the overground portion and the root/shoot ratio of perennial ryegrass were not significant ($P>0.05$). Inoculation with AM fungi prior to salt stress also had a significant impact on plant height ($P<0.001$) (Table 1).

The biomass of perennial ryegrass decreased with the increase in the salt concentration. Significance analysis showed that inoculation of AM fungi significantly increased both the aboveground and underground biomass of perennial ryegrass under salt stress (S2 and S3). The aboveground dry weight of perennial ryegrass was increased by 44.0% (S1), 37.1% (S2), and 30.5% (S3), while the underground dry weight was increased by 70.2% (S1), 55.6% (S2), and 62.9% (S3) (Figure 1).

Table 1. Effects of AMF on flowering duration (days) in snapdragon during the full-bloom period

Parameter	F factor and significance		
	AM fungi	Salt	AM fungi × Salt
Plant height	170.171***	15.316***	1.741NS
Above ground dry weight	30.183***	3.341NS	0.581NS
Underground dry weight	98.199***	4.665*	0.912NS
The total dry weight	149.957***	10.177**	1.745NS
Root/shoot ratio	5.405*	0.118NS	0.044NS

Note: * denotes significance level $P<0.05$, ** $P< 0.01$, *** $P<0.001$, and NS denotes $P > 0.05$, the same as below.

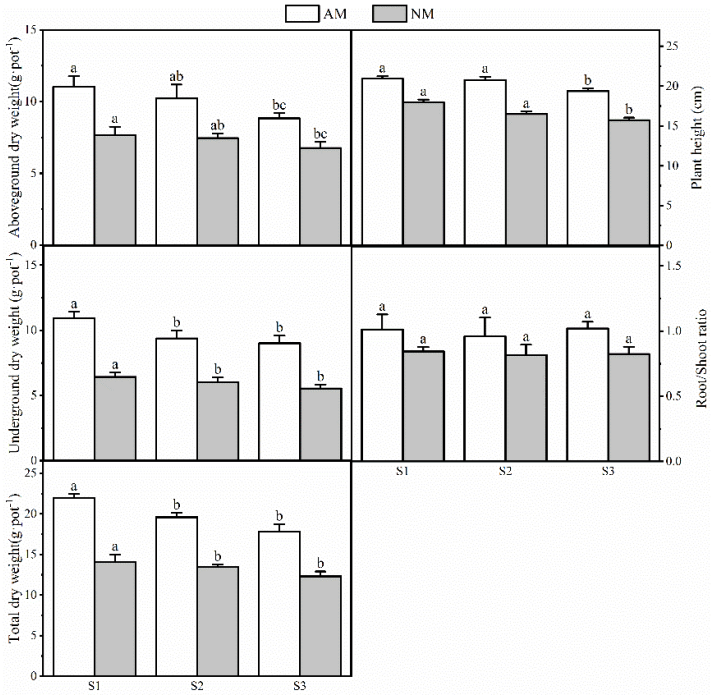

Figure 1. Effect of AM fungi and salt on the biomass of perennial ryegrass
Letters indicate significant differences (P < 0.05) according to Tukey's post hoc test and the error bars represent the standard error (SE).

The average height of perennial ryegrass in the AS groups (AM fungi + salt) was higher than that in the NS controls (no fungi + salt). The AS plant height increased by 16.7%, 25.9%, and 23.2%, for each salt condition (S1, S2, S3) compared to the controls, respectively. Both in the AS and NS groups, perennial ryegrass had the lowest plant heights under stress induced by the highest salt concentration (S3) (Figure 1). The root/shoot ratio of perennial ryegrass inoculated with AM fungi increased by 19.8%, 17.4%, and 23.6% under the respective salt treatments S1, S2, and S3 compared to the NS group, however, the differences between them were not significant (Figure 1).

Photosynthetic pigment content

Table 2 shows the effects of AM fungi on the photosynthetic pigment content of perennial ryegrass at different salt concentrations. The inoculation of AM fungi significantly impacted Chla content ($P<0.001$), carotenoid content ($P<0.01$), and Chlb content ($P<0.05$). Salt stress only played a minor role, showing insignificant ($P>0.05$) impacts on Chla content, Chlb content, and carotene content. In addition, there was no significant interaction ($P>0.05$) found between the salt concentration and the inoculation of AM fungi on Chla content, Chlb content, and carotene content (Table 2).

Table 2. Effects of salt stress and AM fungi on photosynthetic pigment content of perennial ryegrass

Parameter	F factor and significance		
	AM fungi	Salt	AM fungi × Salt
Chla	16.473***	1.139NS	0.658NS
Chlb	5.484*	3.089NS	1.261NS
Chl(a+b)	13.482**	1.705NS	0.861NS
Chla/b	10.214**	1.127NS	0.352NS
Car	15.409**	1.329NS	0.294NS

The contents of chlorophyll (Chl) and carotenoids (Car) in the AS group inoculated with AM fungi were higher than those in the NM group. The contents of Chla in the AS group were 37.3%, 31.0%, and 16.9% higher than those in the NS group under the S1, S2, and S3 conditions, respectively. The contents of Chlb and Car in the AS1 group were 26.0% and 37.9% higher than those in the NS1 group. There was no significant difference found between the AS and NS groups in either Chlb content or Car content under S2 and S3 salt stress (Figure 2).

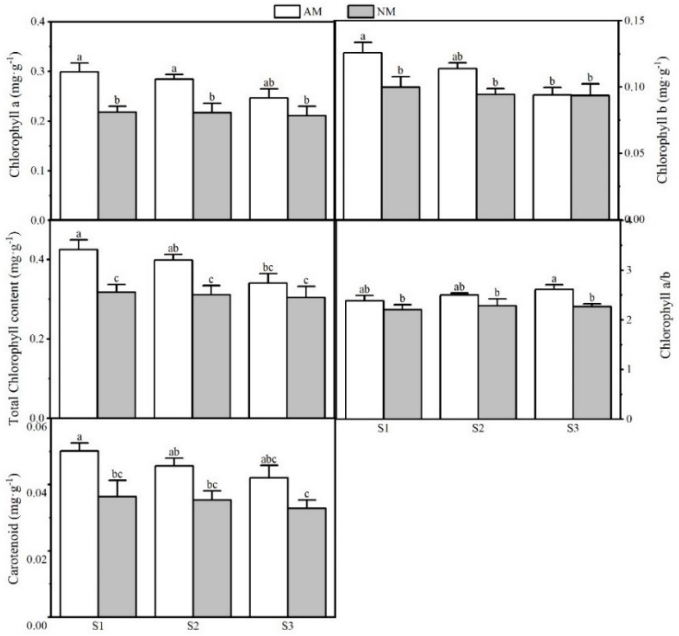

Figure 2. Effect of AM fungi and salt on the chlorophyll content of perennial ryegrass
Letters indicate significant differences (P < 0.05) according to Tukey's post hoc test and the error bars represent the standard error (SE).

Photosynthetic characteristics

Table 3 shows that the inoculation of AM fungi had a significant impact on the A, E, Gs, Ci, and VPD (P<0.001) in perennial ryegrass but not on WUE (P>0.05); salt stress had a significant impact on the A, E, Ci, WUE, and VPD (P<0.001), but not on Gs (P>0.05). In addition, there were significant interactions between the salt concentration and the inoculation of AM fungi on the E (P<0.05) and WUE (P<0.001), but not on the A, Gs, Ci, or VPD (Table 3).

Table 3. Effects of salt stress and AM fungi on photosynthetic characteristics of perennial ryegrass

Parameter	F factor and significance		
	AM fungi	Salt	AM fungi × Salt
A	57.287***	13.861***	2.01NS
E	86.188***	40.073***	3.423*
Gs	26.850***	2.251NS	0.247NS
Ci	230.806***	35.736***	0.192NS
WUE	2.105NS	152.314***	11.959***
VPD	58.628***	26.469***	0.534NS

The A of perennial ryegrass inoculated with AM fungi was higher than that without inoculation for all salt conditions: 37.5% higher in group AS1 than in group NS1, 45.4% higher in group AS2 than in group NS2, and 24.6% higher in group AS3 than NS3. Evidently, inoculation with AM fungi improved the A of perennial ryegrass (Figure 3).

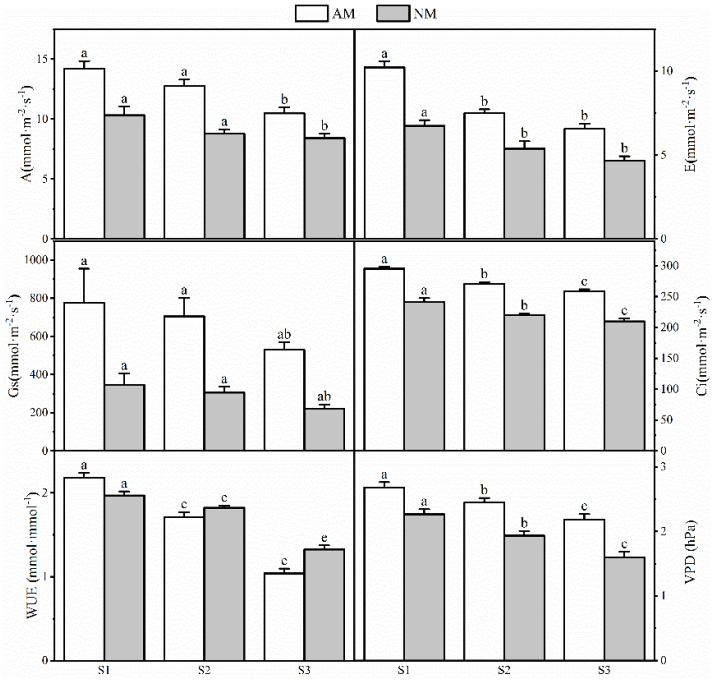

Figure 3. Effect of AM fungi and salt on the photosynthesis of perennial ryegrass
Letters indicate significant differences (P< 0.05) according to Tukey's post hoc test and the error bars represent the standard error (SE).

The E values of perennial ryegrass in the AS groups (AM fungi + salt) were significantly higher than those in the NS groups. The AS1, AS2, and AS3 groups had E values that were 37.5%, 39.3%, and 40.7% higher than those of NS1, NS2, and NS3 groups, respectively. The E of perennial ryegrass was significantly decreased under salt stress (Figure 3).

The Gs values of perennial ryegrass inoculated with AM fungi were significantly higher than those of the non-inoculated ryegrass: the values for the AS group were 123.9%, 130.3%, and 139.1% higher than those in the corresponding NS groups under the S1, S2, and S3 salt treatments, respectively. Therefore, regardless of which salt treatment was administered, the Gs of perennial ryegrass could be increased significantly by inoculation with AM fungi (Figure 3).

Perennial ryegrass inoculated with AM fungi had higher Ci values than non-inoculated ryegrass. The Ci of AS1 was 22.3% higher than that of NS1, that of AS2 was 23.0% higher than NS2, and that of AS3 was 23.2% higher than NS3. Inoculation with AM fungi therefore significantly improved the Ci of perennial ryegrass. Under the condition of no added salt (S1), the Ci values of perennial ryegrass were highest, and this was true for both the AS and NS groups. The AS groups with AM fungi inoculation showed a significantly decreased trend of Ci with increasing salt concentration, while the Ci difference between the NS2 group and NS3 group was not significant (Figure 3).

Perennial ryegrass inoculated with AM fungi in the AS1 group had higher WUE compared to the non-inoculated NS1 group (10.9% higher). However, in the salt-treated groups (S2 and S3), WUE was 6.2% lower in the AS2 group and 21.5% lower in the AS3 group compared to the NS2 and NS3 groups, respectively. Therefore, inoculation with AM fungi reduced the WUE of perennial ryegrass under salt stress. With higher degrees of salt stress, the WUE of the AS group inoculated with AM fungi showed a significant downward trend (Figure 3).

The VPD of perennial ryegrass was significantly higher in the AS groups at all salt levels compared to the NS groups. Both the AS and NS groups showed a significant downward trend with the increase in the degree of salt stress. The VPD of perennial ryegrass inoculated with AM fungi was 18.3%, 26.5%, and 36.2% higher than that of the NS1, NS2, and NS3 treatments, respectively (Figure 3).

Correlation between growth indicators and photosynthesis

Table 4 shows the correlation between the growth indicators and photosynthesis of perennial ryegrass. Significant correlations were detected between underground dry weight, total weight, and photosynthesis indexes (A, E, Gs, Ci, WUE, and VPD), and the strongest correlation was found between plant height and the Ci (0.886).

Table 4. Correlation between the growth indicators and photosynthesis of perennial ryegrass

	A	E	Gs	Ci	WUE	VPD
Plant height	0.700**	0.514*	0.671**	0.886**	0.337	0.746**
Above ground dry weight	0.599**	0.608**	0.397	0.794**	0.367	0.601**
Underground dry weight	0.706**	0.569**	0.513*	0.804**	0.426*	0.794**
The total dry weight	0.715**	0.640**	0.500*	0.871**	0.435*	0.768**
Root/shoot ratio	0.394	0.185	0.347	0.322	0.200	0.460*

According to Tukey's post hoc test, ** at the level of $P<0.01$ (double-tailed), the correlation was significant.

Chlorophyll fluorescence parameters

Table 5 shows the impact of AM fungi on the chlorophyll properties of perennial ryegrass under different salt concentrations. Both the inoculation of AM fungi and the interaction between added salt and AM fungi had no significant ($P>0.05$) impacts on F0, Fm, or F_v/F_m. The salt treatment had no insignificant impact on F_v/F_m ($P>0.05$) but did have a significant impact on F0 ($P<0.01$) and Fm ($P<0.001$).

Table 5. Effects of salt stress and AM fungi on chlorophyll characteristics of perennial ryegrass

Parameter	F factor and significance		
	AM fungi	Salt	AM fungi* Salt
F_0	1.028NS	5.428**	0.088NS
F_m	3.178NS	10.905***	0.021NS
F_v/F_m	2.729NS	0.671NS	0.353NS

There was no significant difference in the F0 values between the AS groups inoculated with AM fungi and the NS groups without AM fungi for all three salt concentrations. The F0 was significantly lower under the 0.1% salt treatment (S3) compared to the no salt treatment (S1) inoculated with AM fungi (Figure 4).

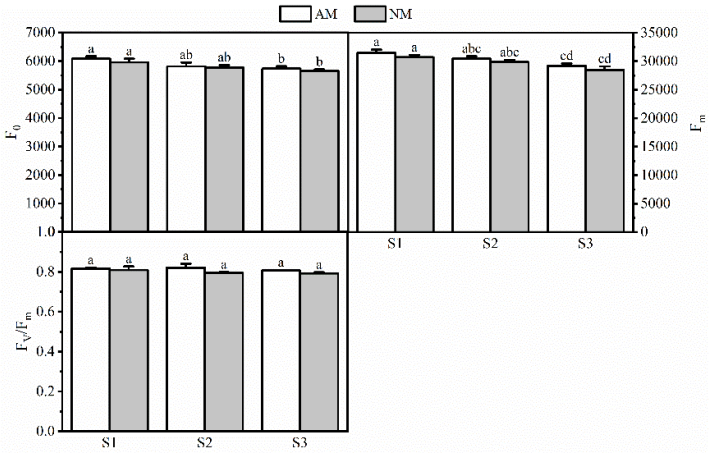

Figure 4. Effect of AM fungi and salt on the chlorophyll fluorescence of perennial ryegrass
Letters indicate significant differences (P < 0.05) according to Tukey's post hoc test and the error bars represent the standard error (SE).

The Fm values of the inoculated groups (AM) were not significantly different from those of the non-inoculated groups (NM). However, the Fm was significantly lower under a high degree of salt stress (S3) compared to the no-salt treatment (S1) (Figure 4).

The F_v/F_m values of the AS groups inoculated with AM fungi were not significantly different from those of the NS groups. The F_v/F_m of perennial ryegrass was also not significantly different between different salt concentrations (Figure 4).

Antioxidant enzyme activity

Table 6 shows the impact of salt stress and AM fungi inoculation on the physiological indexes of perennial ryegrass. The inoculation of AM fungi, treatment with salt, and the interaction of salt and AM fungi all had no significant (P>0.05) impacts on SOD activity, the MDA content, and the proline (Pro) content. Inoculation with AM fungi and the addition of salt both had significant impacts on POD activity (P<0.05), while the interaction of AM fungi and salt had no significant impact (P>0.05) (Table 6).

Table 6. Effects of salt stress and AM fungi on the physiological indexes of perennial ryegrass

Parameter	F factor and significance		
	AM fungi	Salt	AM fungi×Salt
SOD	0.598NS	2.396NS	0.002NS
POD	4.245*	4.461*	0.647NS
MDA	4.369NS	3.062NS	1.370NS
Pro	0.069NS	2.224NS	0.021NS

The SOD activity and Pro content of perennial ryegrass in the inoculated groups (AS) were not significantly different from the non-inoculated group (NM) across all salt concentrations. Further, there was no significant difference found between different salt concentrations (Figure 5).

The POD activity of perennial ryegrass inoculated with AM fungi (AS) was not significantly different from that of the non-inoculated groups (NS). The POD activity of the AS1 group was significantly higher than

that of the AS3 group, but there was no significant difference under the medium and high salt concentrations (S2 and S3) (Figure 5).

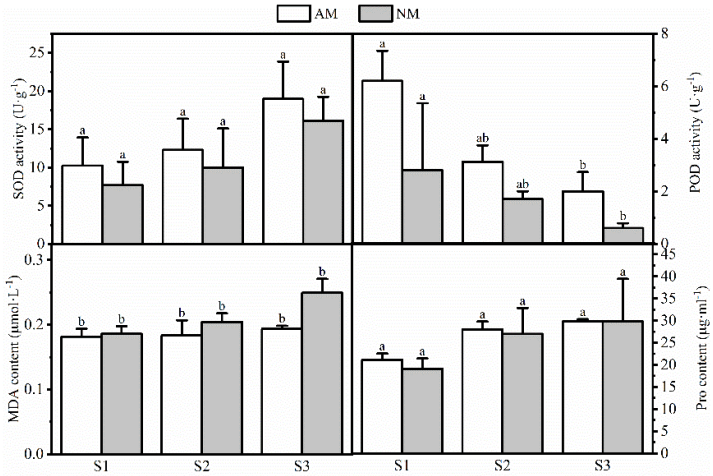

Figure 5. Effect of AM fungi and salt on the SOD, POD, MDA and Pro of perennial ryegrass
Letters indicate significant differences (P < 0.05) according to Tukey's post hoc test and the error bars represent the standard error (SE).

In the lower salt concentration (S1, S2), there was no significant difference in the MDA content between the inoculated groups and the non-inoculated groups. Under the highest salt concentration (S3), the MDA content of the inoculation group (AS3) was significantly lower than that of the non-inoculated group (NS3). The MDA content of perennial ryegrass in the AS3 group decreased by 63.1% compared to the NS3 group (Figure 5)

Soluble sugar and soluble protein content of perennial ryegrass

Table 7 shows the impacts of salt stress and AM fungi inoculation on the soluble sugar and soluble protein contents of perennial ryegrass. The results show that neither inoculation with AM fungi nor the interaction between AM fungi and salt had a significant impact on the soluble sugar and soluble protein contents (P>0.05). However, salt concentration did exhibit a significant impact on the soluble protein content and soluble sugar content (P<0.01) (Table 7).

Under no salt stress (S1), the soluble sugar content of perennial ryegrass in the inoculated group (AS1) was 55.8% higher than that in the non-inoculation group (NS1), while under salt stress (S2 and S3), there was no significant difference in the soluble sugar content between the inoculated group (AS) and the non-inoculated group (NS) (Figure 6).

Table 7. Effects of salt stress and AM fungi on the content of soluble sugar and soluble protein in perennial ryegrass

Parameter	F factor and significance		
	AM fungi	Salt	AM fungi×Salt
Soluble sugar	2.028NS	9.400**	1.733NS
Soluble protein	2.670NS	6.292**	0.448NS

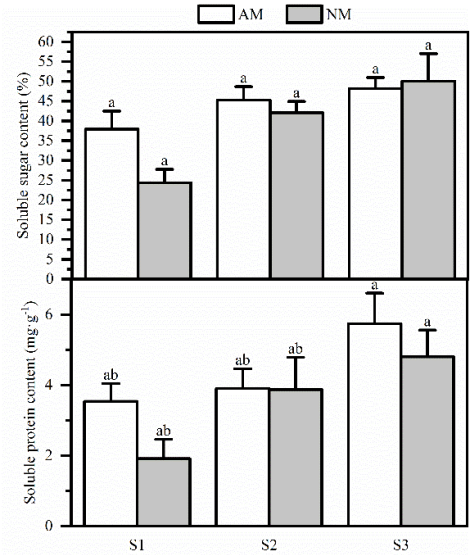

Figure 6. Effect of AM fungi and salt on the soluble sugars and soluble proteins of perennial ryegrass
Letters indicate significant differences ($P < 0.05$) according to Tukey's post hoc test and the error bars represent the standard error (SE).

It can be seen from Figure 6 that the different salt treatments caused no significant impact on soluble sugar content in the different AS groups. The soluble protein content in the NS1 group was significantly lower than that in the AS3 and NS3 groups (Figure 6).

Discussion

Endophytes have increasingly been described as plant mutualists, providing competitive advantages to their host plants, especially under stressful conditions (Malinowski and Belesky, 2000). In this study, perennial ryegrass was tested to determine whether the presence of the symbiotic fungus *F. mosseae* conferred the plant tolerance to NaCl. The total biomass of plants is often used as an indicator of a plant's salt tolerance (Gupta *et al*., 2021); thus, biomass was measured to assess the growth of perennial ryegrass in response to NaCl stress. For the indexes of plant height, above-ground dry weight, under-ground dry weight, and total dry weight of perennial ryegrass, significant increases were observed under NaCl stress following AM fungi inoculation. Inoculation with AM fungi has been found to be effective in increasing plant biomass against the negative effects of salt stress (Rudgers and Swafford, 2009; Meena *et al*., 2010). These results were confirmed by the present study, whereby an increased accumulated biomass and plant growth of perennial ryegrass was observed under NaCl stress following AM fungi inoculation. Saline-alkali stress can also lead to the loss of normal physiological function and damage root cell structures (Yang *et al*., 2008), thereby ultimately inhibiting the absorption of inorganic nutrients. In the current study, inoculation with AM fungi was shown to promote the accumulation of nutrients and increase the number of biological substances in ryegrass under salt stress.

Chlorophyll is an important substance for plant photosynthesis, and is hence used as an index to reflect both the photosynthetic capacity of leaves and the tolerance of plants to salt stress. Carotenoids serve as photo-protectants and function as safety valves to release excess energy before it can damage plant cells (Shumskaya and Wurtzel, 2013). One study found that under NaCl stress, the inoculation of AM fungi could significantly promote the growth of *Festuca arundinacea* and bluegrass, improve the relative content of chlorophyll in leaves, increase the salt tolerance coefficient, and enhance the visual quality of lawns (Yang *et al*., 2014). Similar results

were also found in this experiment, under both salt stress and salt-free stress, inoculation with AM fungi significantly increased the contents of chlorophyll in perennial ryegrass leaves.

Salt stress leads to severe reductions in photosynthesis and gas exchange parameters (Negrão *et al*., 2017). Under salt stress, due to the increase in osmotic stress and the decrease in solubility, the ability of plants to absorb water and nutrients is reduced, which leads to reductions in leaf swelling and photosynthesis (Liu *et al*., 2019). Stomatal conductance is one of the parameters used for evaluating the rate of photosynthesis (Santosh and Prianka, 2020). Under salt stress, the decrease in stomatal conductance is related to the closure of the stomata to minimize water loss and maintain cell swelling (Liang *et al*., 2019). The results of our study show that inoculation with AM fungi can significantly increase stomatal conductance in ryegrass. This increase may be related to an increase in the relative water content (Polcyn *et al*., 2019). Other studies have shown that inoculation with AM fungi can significantly increase the values of photosynthetic parameters in plants (Wu *et al*., 2010; Alinia *et al*., 2021). This finding is consistent with our results. Zhao (2015) found that plants could improve their photosynthetic capacity by boosting their accumulation of rubisco and increasing their soluble protein content.

Chlorophyll fluorescence parameters can be used to reflect the tolerance of plants to high and low temperatures, drought conditions, saline-alkali conditions, and other stressors. Salt stress can damage the photosynthetic structure of perennial ryegrass and lead to a decrease in photochemical reaction efficiency. One study showed that under salt stress, inoculation with AM fungi protected the photosynthetic apparatuses of rice plants by enhancing the actual quantum yield of PSII photochemistry and reducing the quantum yield of non-photochemical quenching ΦNPQ (Porcel *et al*., 2015). Conversely, our results show that inoculation with AM fungi on perennial ryegrass under salt stress yielded no significant impact on the chlorophyll fluorescence parameters. This discrepancy may be because salt stress destroyed the assimilation and regulation abilities of the photosynthetic apparatus. The mechanism underlying this may be that salinity initially reduces stomatal conductance and thus photosynthesis, thereby leaving PSII unaffected in the early stages of stress (Baker and Rosenqvist, 2004). The maximum quantum efficiency (F_v/F_m) of PSII is affected only under severe salt stress, because both absorption and capture efficiency are improved under salt stress (Shamshiri *et al*., 2016).

Antioxidant enzymes reduce the harmful effects of oxidative stress by scavenging ROS (Hashe *et al*., 2015). Studies have shown that following AM fungi inoculation, the SOD and POD contents of *Taraxacum mongolicum* (Gao *et al*., 2019) and *Cucumis melo* (Xu *et al*., 2017) were both higher than those without inoculation, while their MDA contents were reduced. AM fungi have the ability to increase the activity of a variety of antioxidant enzymes in plant cells in order to eliminate specific ROS and improve the antioxidant capacity. However, the ability of AM fungi to infect plants under chronic high-salt conditions was inhibited, and the activity of various enzymes was reduced, thereby leading to the insignificant overall effect of AM fungi in the study.

AM fungi can facilitate the ability of their host plants to rapidly absorb and accumulate osmotic-regulating substances, change their chemical composition and contents, reduce their lipid peroxidation level, directly or indirectly eliminate ROS, and participate in various antioxidant reactions (Xu *et al*., 2016). Osmolytes are also involved in quenching ROS, maintaining membrane integrity, and stabilizing enzymes. Studies that have inoculated *Triticum aestivum* (Talaat *et al*., 2014), *Suaeda glauca* (Bunge) (Ma *et al*., 2021), and *Leymus chinensis* (Wang *et al*., 2022) with different types of AM fungi jointly reveal that the fungi can promote the accumulation of osmotic substances in plants. A large number of mycelia are formed between AM fungi and the roots of their host plants, which expand the absorption range of roots and enhance their ability to absorb nutrients (Li *et al*., 2019). Soluble sugars can function as signaling molecules, under salt stress, AM fungi can promote the synthesis of soluble sugar and regulate its composition, thus improving the water conductivity of roots as well as improving the water absorption and transport capacity of plants. Studies have shown that AM fungi were able to maintain higher leaf water potential (LWP) and malondialdehyde (MDA) content, thereby inducing the accumulation of osmotic substances (sugar) and antioxidant enzymes (Ben-

Laouane *et al*., 2019). However, other studies have reported that inoculation with AM fungi had no positive effect on the accumulation of soluble sugars in some host plants (Porcel *et al*., 2012). The salt tolerance of plants is related to photosynthesis. When the salt concentration reaches a certain level, the chlorophyll enzyme activity in plants is enhanced, which in turn degrades photosynthetic pigments and inhibits the resynthesis of the pigment-protein complex. This ultimately weakens photosynthetic efficiency and reduces the production of soluble proteins and sugars (Bhattacharjya *et al*., 2018). Therefore, the processes of osmotic regulation, photosynthesis, and antioxidant reactions in symbionts are closely related to each other. At present, the mechanism of action employed by AM fungi on soluble sugars in host plants under salt stress is relatively complex and requires further study. AM fungi can promote the osmotic regulation of their host plants under salt stress, and soluble proteins are important substances that regulate osmosis. Wang *et al.* (2022) and Talaat *et al.* (2014) studied *Kalanchoe Blossfeldiana* and wheat (*Triticum aestivum*) to demonstrate that the inoculation of AM fungi could promote the accumulation of osmotic substances in the plants. However, the results of this experiment showed that inoculation with AM fungi had no significant effect on the soluble sugar content and soluble protein content of perennial ryegrass.

Conclusions

Based on these findings, AM fungi can effectively alleviate the negative effects of NaCl stress on the growth of perennial ryegrass. The contribution of AM fungi to plant NaCl tolerance was closely related to increases in the net photosynthetic rate, transpiration rate, intercellular CO_2 concentration, stomatal conductance, and photosynthetic pigment content. Perennial ryegrass inoculated with AM fungi showed significantly enhanced biomass accumulation. Our results reveal that the protective effect conferred to perennial ryegrass against NaCl stress following inoculation was not significantly related to the activity of endogenous antioxidant enzymes and osmotic adjustment substances, and this is worthy of further study. In conclusion, AM fungi inoculation can be used as an effective tool to alleviate NaCl stress in perennial ryegrass.

Authors' Contributions

W.L. and S.G. designed the experiment. Y.Z. and X.H. performed the experiment. W.L. participated in writing the manuscript. Y.Z. and W.L. participated in revising the manuscript.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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