Physiological mechanism of enhancing salinity tolerance of *Gleditsia sinensis* Lam. by arbuscular mycorrhizal fungi

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Jinping Wang  
nanjing forestry university

Bo Zhang  
university of california, davis

Jinchi Zhang  
Nanjing Forestry University

✉ zhangjc8811@gmail.com
Corresponding Author  
ORCiD: https://orcid.org/0000-0002-0517-7214

G. Geoff Wang  
Clemson University

Jie Lin  
Nanjing Forestry University

Xin Liu  
nanjing forestry university

Cuiyu Liu  
nanjing forestry university

Shilin Ma  
Nanjing forestry University

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Abstract

Background and Aims

The protective effects of arbuscular mycorrhizal fungi (AMF) on salt-stressed crop plants had been well studied. However, the physiological mechanism of AMF in mitigating adverse impact caused by salinity stress in different tissues of woody plants is not clear. *Gleditsia sinensis* Lam. is a valuable tree species with various pharmaceutical uses; however, high soil NaCl concentration limits its growth in saline soil including coastal areas. This study aimed to investigate the effects of AMF on *G. sinensis* salinity tolerance and reveal its underlying physiological mechanism.

Methods

A greenhouse experiment was performed. *G. sinensis* seedlings with and without AMF inoculation were subjected to four salinity levels (0, 50, 100, and 150 mM NaCl). After 2 months, the seedlings were harvested and analyzed for growth and biochemical parameters.

Results

High AMF colonization rates (over 95%) and high mycorrhizal dependency (over 75%) were observed across all NaCl levels, and AMF-inoculated plants presented significantly higher aboveground and below ground growth than non-inoculated plants. AMF effectively enhanced the salinity tolerance of *G. sinensis* seedlings by enhancing leaf gas exchanges inducing higher leaf net photosynthetic rates; improving peroxidase, catalase, and superoxide dismutase activities resulting in higher membrane stability indexes and lower malondialdehyde contents in leaves and roots; increasing P uptake and P/N ratio to mitigate P-limited biomass products; selectively absorbing less Na + and more Ca 2+ in their tissues to alleviate ion toxicity and maintain more favorable ion balances (e.g., K + /Na + ) in their tissues.

Conclusions

The results suggested the feasibility of using AMF to improve salinity tolerance as well as afforestation and rehabilitation of *G. sinensis* in coastal areas.

Introduction

Salinization of soil is a severe and common environmental problem, particularly in arid and semiarid
regions or low-lying coastal areas around the world (Porcel et al. 2012). One of the significant natural factors contributing to salinization of soils is oceanic salt deposition. Globally, salinization of soil, especially in the coastal areas, is increasing owing to the rising sea level and climate change, significantly affecting the multifunction, conservation, and rehabilitation of coastal ecosystems (Wilson 1999). Salinity stress restricts plant growth and development by inducing osmotic stress, oxidative stress, and ion toxicity, ultimately resulting in biomass production losses (Evelin et al. 2009). In the short term, accumulation of salt in the root zone causes decrease in osmotic potential, which leads to a decrease in water and nutrient availability. In the long-term, excessive uptake of Na$^+$ and Cl$^-$ cause nutrient imbalances and ion toxicity, which disrupt cell organelles, plasma membrane, and enzyme structures. Concurrently, over accumulation of reactive oxygen species (ROS) as a result of oxidative stress disrupts the normal metabolism of lipids, proteins, and nucleic acids (Muchate et al. 2016). Hence, improving salinity tolerance of plants is crucial for their survival and success of vegetation rehabilitation in coastal areas.

Many approaches have been developed to enhance salinity tolerance of plants via breeding, genetic engineering, microbial technology, etc. (Xu et al. 2008; Talaat and Shawky 2014). Among them, application of arbuscular mycorrhizal fungi (AMF) is considered to be an ecologically and economically feasible strategy. AMF are ubiquitous and ecologically important soil microorganisms that form mutualistic symbioses with the roots of more than 80% of terrestrial plant species (Fernández et al. 2011). They are widely distributed in various ecosystems, including coastal ecosystem, and play an important role in the establishment and survival of coastal dune plant communities (Rodríguez-Echeverría et al. 2008). Their external mycorrhizal hyphae act as root extension and help plants acquire water and nutrients. As the interface for the uptake and exchange of nutrients with host plants, arbuscules significantly increase nutrient absorption, thus enhancing plant growth. Besides, AMF vesicles are storage and vacuolated organelles that can absorb high concentrations of Na$^+$ and Cl$^-$, creating a dilution effect on the toxicity of these ions to plants (Augé 2001). Moreover, AMF regulate the physiological, biochemical, and molecular processes of plants, such as photosynthesis
pathway, ion balance, antioxidant system, osmoregulators, sodium compartmentalization, hormones, and aquaporins, ultimately helping plants to better cope with salinity stress (Porcel et al. 2012). The positive effects and underlying mechanisms of AMF involving in crop plant salt tolerance had been widely studied (Sharifi et al. 2007; Daei et al. 2009; Abdel-Fattah 2012; Evelin et al. 2012; Talaat and Shawky 2014; Garg and Pandey 2015; Porcel et al. 2015; Sarwat et al. 2016; Bulgarelli et al. 2017; Lin et al. 2017; Pollastri et al. 2018; Zhang et al. 2018), but few investigated woody plants subjected to salt stress. Recently, the positive effects of AMF had been reported on several salinity-stressed woody plants such as citrus aurantium L. (Khalil et al. 2011), Populus tomentosa Carrière (Lu et al. 2014), Elaeagnus angustifolia L. (Chang et al. 2018). However, the role of AMF in integrated physiological process in different tissues of woody plants subjected to salinity stress remains unclear. Especially, Gleditsia sinensis Lam. is a leguminous plant whose rhizobia symbiosis (nitrogen fixation) is more favorable for AMF functioning, but no researches had been made on the influences of AMF in salt tolerance of G. sinensis currently. Moreover, G. sinensis is a previous economically important tree species with multiple pharmaceutical values (Zhang et al. 2016). It is widely distributed in China and well adapted to many soil types. However, G. sinensis has very low salinity tolerance, withstanding only 0.3% salinity under greenhouse condition (Lei et al. 2008). As a result, its suitability as an afforestation and rehabilitation tree species in coastal areas is severely limited. To investigate the effects of AMF on improving salinity tolerance of woody plant species and reveal the underlying physiological mechanism, we performed a greenhouse experiment with G. sinensis. The membrane stability index, malondialdehyde contents, growth parameters including height growth, diameter growth, leaf area, dry biomass, and root morphology were measured to ascertain whether AMF could effectively enhance the salinity tolerance of G. sinensis seedlings. Besides, the chlorophyll contents, photosynthetic parameters, osmoregulators, antioxidant system, N, P contents and ion balance in different tissues were determined to analyze the physiological mechanism of AMF in alleviating salt-induced adverse effects on G. sinensis seedlings.

Materials And Methods

Experimental design

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The pot experiment was conducted in a glasshouse of Xiashu Forest Farm, Nanjing Forestry University, China, from March to October 2018. The experiment consisted of a completely randomized block design with two inoculation treatments: non-mycorrhizal control plants and plants inoculated with the model AMF, Funneliformis mosseae (C. Walker & A. Schüßler). Each inoculation treatment comprised 48 replicates, totaling to 96 pots (1 plant per pot). The 48 pots in each treatment were randomly divided into four groups (12 pots per group), and each group was subjected to one of the four NaCl concentrations (0, 50, 100, and 150 mM NaCl).

Plant material and soil
The seeds of G. sinensis were provided by Jiangsu forestry station. All the seeds were soaked in concentrated H$_2$SO$_4$ for 10 min until the color of the seeds turned crimson, and then washed with sterile distilled water until the pH of residual water on the surface of the seeds turned to about 7.0. After that, the seeds were soaked in warm water for 2 days. The inflated seeds were embedded in wet yellow sand (which was previously sterilized in an autoclave for 2 h at 0.14 MPa and 121 °C) and incubated in plant incubator under dark condition at 25 °C.

Loamy soil was collected from Xiashu Forest Farm of Nanjing Forestry University, China, sieved (2 mm), mixed with yellow sand (< 2 mm) and vermiculite (1:1:1, topsoil/sand/vermiculite, v/v/v), autoclaved at 0.14 MPa and 121 °C for 2 h, and used as nursery substrates. The soil mixture was tested for its physicochemical properties: total C, 1.55%; total N, 0.03%; total P, 570.48 mg·kg$^{-1}$; total K, 15.18 g·kg$^{-1}$; available P, 10.00 mg·kg$^{-1}$; available K, 101.39 mg·kg$^{-1}$; electrical conductivity, 0.23 mS·cm$^{-1}$ (soil:water ratio, 1:5); and pH, 7.15 (soil:water ratio, 1:5).

Inoculation treatment
F. mosseae (isolate number: BGC G201C) was obtained from the Beijing Academy of Agriculture and Forestry Science, China. The inoculum was bulked in an open-pot sterilized yellow sand culture together with maize and clover as trap plants. After 3 months, the aboveground was cleared, and the roots were chopped into small pieces and mixed with the sand of the culture pot. This sand-based inoculum, consisting of yellow sand, infected root fragments, and mycorrhizal spores (> 7 g$^{-1}$), were collected and used in this study. The uniform seedlings (5 cm in length) were transported to the pots
(1 seedling per pot). Before transportation, the pots were soaked in 0.3% KMnO$_4$ solution for 3 h and washed with tap water. About 2.5 kg of the autoclaved nursery substrates were dispensed into each pot and 80 g of sand-based inoculum were added 5 cm below the surface of the nursery substrates. The non-inoculated control pots contained the fungal inoculums filtration and the same dosage of sterilized inoculum to provide the same microbial community (except for AMF) with inoculated treatment.

**Growth conditions**

The seedlings were grown in the glasshouse under the following conditions: 18 °C night/30 °C day temperature, 50–80% relative humidity, and 14 h/10 h diurnal light/dark cycles with a photosynthetic photon flux density of about 700–1,000 µmol m$^{-2}$·s$^{-1}$. Water was supplied adequately during the entire period of the experiment to avoid any drought effects, and modified Hoagland’s nutrient solution containing only 25% P concentration (300 mL per pot every time) was irrigated every month. The seedlings were cultivated for about 4 months prior to salinization to allow adequate plant growth and symbiotic establishment. Subsequently, the four groups of non-mycorrhizal control and mycorrhizal treatments were respectively gradually supplemented with aqueous NaCl solution (300 mL per pot) at the concentrations of 0, 50, 100, and 150 mM NaCl every week for 2 months. In order to avoid salt shock, all three salt treatments (50, 100, and 150 mM NaCl treatments) were treated with 50 mM NaCl for the first week; salt treatment (50 mM NaCl treatment) were treated with 50 mM NaCl, and salt treatments (100 and 150 mM NaCl treatments) were treated with 100 mM NaCl for the second week; salt treatments (50, 100, and 150 mM NaCl treatments) were treated with 50, 100, and 150 mM NaCl, respectively, from week 3 onwards, the seedlings were harvested and analyzed for growth and biochemical parameters.

**Plant harvest and chemical analyses**

Before and after salt stress, the seedling height was measured using a steel ruler, and basal diameter was measured using calipers. After harvesting, the plants were rinsed with tap water, and separated into leaf, stem, and root. The leaf area and root system characteristics (root length, root surface area, and root tip number) were determined using a LA2400 Scanner (Expression 12000XL, EPSON, Long
Beach, CA, USA). The dry weights of plant tissues (leaf, stem, and root) were recorded after drying the plant tissues in an oven at 70 °C to a constant weight. The mycorrhizal dependency was calculated using the formula (Wang et al. 2018):

\[
\text{mycorrhizal dependency (\%) = \frac{(\text{dry weight biomass of inoculated seedlings} - \text{mean of dry weight biomass of non-inoculated seedlings})}{\text{dry weight biomass of inoculated seedlings}} \times 100%.
\]

The dried plant tissues were ground separately, sieved through a 0.5-mm sieve. 50 mg of each sample was weighed to determine the concentrations of N using an elemental analyzer (Vario MACRO cube, Elementar Trading Shanghai, Shanghai, China). 0.2 g of each sample was digested in 10 mL of acid mixture (HClO₄:HNO₃, 1:5), and diluted with double-distilled water. The concentrations of P were ascertained spectrophotometrically using ammonium molybdate blue method, the concentrations of K⁺, Ca²⁺, Mg²⁺, and Na⁺ were ascertained with an atomic absorption spectrophotometer (AA900T, Perkin Elmer, Norwalk, CA, USA) (Allen 1989), and the K⁺/Na⁺, Ca²⁺/Na⁺, and Mg²⁺/Na⁺ ratios in the tissues were calculated using K⁺, Ca²⁺, Mg²⁺, and Na⁺ data.

**Estimation of root mycorrhizal colonization**

For the quantification of mycorrhizal colonization, the washed fine roots were cut into 1-cm-long segments. The root segments were clarified with 10% (w/v) KOH at 90 °C for 1 h, stained with basic H₂O₂ (containing 30 mL of 10% (v/v) H₂O₂, 3 mL of concentrated NH₄OH, and 60 mL of water) for 25 min, soaked in 1% (w/v) HCl for 3 min, and stained with 0.05% (w/v) Trypan Blue solution as described by Philips and Hayman (1970). Subsequently, the root segments were soaked in lactic acid-glycerol (1:1) to eliminate excess Trypan Blue solution, and microscopically examined for AMF colonization based on the presence of arbuscules, vesicles, hyphae, and spores (Giovannetti and Mosse 1980).

**Determination of chlorophyll contents and photosynthetic parameters**

The chlorophyll contents (Chl) in leaves were determined according to Lichtenthaler (1987) with minor modification. Fresh mature leaves (0.1 g) of each plant were cut into small pieces and completely submerged in acetone solution (0.5 mL of pure acetone and 15 mL of 80% acetone). The samples were incubated at 35 °C under dark condition. After the leaf turned white in color, the samples were
diluted with 80% acetone to 25 mL. The absorbance of the extracts was determined using an ultraviolet spectrophotometer (UV 2700, Shimadzu) at 663, 645, and 470 nm, respectively.

Leaf gas exchange ($G_s$) was evaluated on the mature expanded leaf using an infrared gas analyzer (LI-6400, LI-COR, Lincoln, NE, USA) during the day between 09:30 and 11:30 am under the following condition: photosynthetically active radiation, 1000 µmol m$^{-2}$ s$^{-1}$; CO$_2$ concentration, 390 µmol mol$^{-1}$; leaf temperature, 25 °C; leaf humidity, 35-50%; and air flow rate, 0.5 dm$^3$ min$^{-1}$. Leaf net photosynthetic rate ($P_n$), intercellular CO$_2$ concentration ($C_i$), room CO$_2$ concentration ($CO_2R$) and transpiration rate ($T_r$) were simultaneously recorded, and leaf limiting value of stomata (Ls) was calculated using the formula: Ls = 1 - $C_i$/ $CO_2R$.

**Measurement of relative water content and membrane stability**

The leaf relative water content (RWC) was measured according to the previous method described by Wang et al. (2019) using the following formula: $RWC = (FW - DW) / (TW - DW) \times 100\%$, where FW is fresh weight, DW is dry weight, and TW is turgid weight obtained after the leaf was soaked for 24 h in deionized water. The membrane stability index (MSI) was estimated according to the method described by Talaat and Shawky (2014) using the formula: $MSI = (1 - C1/C2) \times 100\%$, where C1 is the electrical conductivity bridge after the leaves were heated at 40 °C for 30 min in a water bath and C2 is the electrical conductivity bridge after the leaves were boiled at 100 °C in a boiling water bath for 10 min.

**Determination of lipid peroxidation and proline content**

Lipid peroxidation in leaves and roots was estimated by measuring the concentration of malondialdehyde (MDA) as described by Hodges et al. (1999) with minor modification. The leaves and roots samples were homogenized and dissolved with quartz powders in 5% (w/v) trichloroacetic (TCA) solution under cold condition. The homogenate was centrifuged at 12,000 rpm for 10 min at 4 °C. The reaction mixture containing 2.0 mL of supernatant and 2.0 mL of 0.6% (w/v) thiobarbituric acid (TBA) was heated in a water bath at 95 °C for 30 min. Then, the boiled reaction mixture was immediately cooled in an ice bath and centrifuged at 3,000 rpm for 10 min. The absorbance of the supernatant was measured at 532, 600, and 450 nm, respectively. The concentration of MDA was calculated by
using the formula given by Hodges et al. (1999). The concentration of proline (Pro) generated was ascertained via ninhydrin reaction as described by Bates et al. (1973). The leaves and roots were cut into small pieces, completely submerged in 3% (w/v) sulfosalicylic acid solution, and heated in a water bath at 100 °C for 15 min. Then, 2 mL of the extract were added to 2 mL of glacial acetic acid and 2 mL of 2.5% ninhydrin solution, and heated in a water bath at 100 °C for 15 min. Subsequently, the reaction mixture was cooled down and 5 mL of methylbenzene were added to it and placed under dark condition. After the mixture completely separated into different layers, the absorbance of methylbenzene layer was measured at 520 nm.

**Soluble proteins and antioxidant enzymes assay**

Crude enzymes were extracted from the leaf and root samples homogenized in an ice bath with 50 mmol·L^{-1} sodium phosphate buffer (pH 7.0) containing 1% (w/v) PVP-40 (polyvinylpyrrolidone). The mixture was centrifuged at 12,000 rpm for 20 min at 4 °C, and the supernatant was collected for soluble proteins (SP) measurement and antioxidant enzymes analyses. The SP contents of leaves and roots were determined using the method of Coomassie Brilliant Blue G250 (Blakesley and Boezi 1977), and a commercial Bradford reagent (Sigma) and BSA (Merck) were employed as standard. Superoxide dismutase (SOD) activity was assayed using nitro blue tetrazolium (NBT) reduction test by measuring the ability of SOD to inhibit photochemical reduction of NBT (Giannopolitis and Ries 1977). A 50% inhibition of NBT reduction was considered as one unit of SOD activity at 560 nm. Peroxidase (POD) activity was assayed using guaiacol test and spectrophotometrically determined at 470 nm (Chance and Maehly 1955). Catalase (CAT) activity was ascertained by monitoring the decrease in the absorbance of H_2O_2 at 240 nm (Chance and Maehly 1955), and ascorbate peroxidase (APX) activity was determined by examining the decrease in the absorbance of ascorbate at 290 nm (Nakano and Asada 1981).

**Statistical analysis**

The data obtained were analyzed using SPSS19.0 (SPSS Inc., Chicago, IL, USA). Two-way ANOVA was used to determine the effects of NaCl levels, AMF inoculation, and their interactions. Multiple comparisons of means were performed by Tukey’s test (P ≤ 0.05). All the figures were derived using
Origin 8.5 (Origin Lab, Northampton, USA), and all data are presented as mean ± standard deviation of at least three plants.

Results

Mycorrhizal colonization, dependency and plant growth

No AMF structure was found in the roots of non-inoculated seedlings at all NaCl levels, whereas arbuscules, vesicles, and hyphae were observed in AMF-inoculated seedlings. The percentages of AMF colonization were very high (above 95%) across all NaCl levels (Fig. 1a). Mycorrhizal dependency was significantly influenced by salinity (Table S1), the values of mycorrhizal dependency were also very high (above 75%) across all NaCl levels, and significantly increased under high salinity conditions (100 and 150 mM NaCl) (Fig. 1b and Table S1).

Salinity and AMF inoculation had significant interactions on all growth parameters (height growth, basal diameter growth, leaf area, root length, root surface, root tip number, leaf biomass, stem biomass, and root biomass), except for leaf area and stem biomass (Table S1). In general, the growth parameters decreased with the increasing NaCl levels, and AMF inoculation significantly and positively influenced plant growth parameters, except leaf area, across all NaCl levels (Table 1 and Fig. 2).

Leaf chlorophyll contents and photosynthetic parameters

Salinity significantly decreased the chlorophyll contents (Table S1), with the reduced values reaching a significant level at 150 mM NaCl (Table S2). In contrast, AMF inoculation enhanced the photosynthesis pigments under salinity condition, presented significant increase at 150 mM NaCl, when compared with those in non-inoculated plants. Photosynthetic parameters ($P_n$, $G_s$, $T_r$ and $L_s$) significantly decreased by salinity stress, among the photosynthetic parameters, $P_n$, $G_s$ and $T_r$ were significantly enhanced by AMF under salinity conditions (Table S2).

Relative water contents, membrane stability and lipid peroxidation

Salinity significantly decreased the leaf RWC at 150 mM NaCl (Fig. 3a), whereas AMF inoculation had no significant positive effect on leaf RWC (Table S1 and Fig. 3a). Salinity had significant effects on leaf MSI and MDA contents in leaves and roots (Table S1). Salinity decreased the leaf MSI, with the reduced value reaching a significant level at 100 and 150 mM NaCl (Fig. 3b). However, AMF inoculation improved leaf MSI, especially at high NaCl levels, with increased values reaching 10.33%
(P < 0.05) and 9.49% at 100 and 150 mM NaCl, respectively. The MDA contents in leaves and roots increased as the NaCl levels increased, and were significantly higher at high NaCl levels (100 and 150 mM NaCl), when compared with no-salinity treatments (Fig. 3c and d). AMF inoculation decreased the MDA contents across all the NaCl levels, with decreased values reaching 11.69%, 16.00%, 18.44% (P < 0.05), and 28.44% in leaves and 12.83%, 16.15% (P < 0.05), 12.97% (P < 0.05), and 23.48% in roots at 0, 50, 100, and 150 mM NaCl, respectively, when compared with those in non-inoculated seedlings.

**Proline and soluble protein**

Salinity, AMF inoculation, and their interaction had significant effects on the Pro content in leaves, whereas only salinity significantly affected the Pro content in roots (Table S1). The Pro content in the leaves and roots of non-inoculated plants increased with the increasing NaCl levels, reaching significant values in leaves and roots at 100 and 150 mM NaCl, respectively (Table 2). In contrast, under salinity condition, AMF inoculation significantly reduced the Pro content in leaves, but produced insignificant increase in Pro content in roots (Table 2). Furthermore, salinity significantly increased the SP content in leaves (Table S1). However, AMF inoculation decreased the SP content in leaves and increased it in roots, especially at 150 mM NaCl (P < 0.05), when compared with non-inoculated plants (Table 2).

**Antioxidant enzymes activities**

Salinity significantly affected the antioxidant enzymes (POD, SOD, CAT, and APX) activities, whereas AMF inoculation only significantly affected POD, SOD, and CAT, and their interaction showed significant effects only on POD, SOD, and APX in roots (Table S1). The activities of POD and CAT reached the highest values at 100 mM NaCl, while the highest SOD activity was noted in the leaves, but not in roots (Table 2). The APX activities in leaves and roots increased with the increase in NaCl levels in non-inoculated plants, whereas such trend was not observed in AMF-inoculated plants. AMF inoculation significantly enhanced the activities of POD in leaves and roots under salinity conditions. AMF inoculation enhanced the activities of CAT, the increased values were 101.51% (P < 0.05), 124.36% (P < 0.05), 86.48%, and 88.38% (P < 0.05) in leaves and 82.08% (P < 0.05), 67.39% (P <
0.05), 15.32%, and 87.96% in roots at 0, 50, 100, and 150 mM NaCl, respectively. AMF inoculation enhanced the activities of SOD mainly in roots, and the enhanced values reached significant levels at 0, 50, 100 mM NaCl. The activities of APX were not significantly enhanced by AMF both in leaves and roots.

**N, P concentration and N/P ratio**

Salinity, AMF inoculation, and their interaction had significant effects on N, P concentrations and N/P ratios of plants (Table S1). While AMF inoculation did not increase the N concentrations in the tissues of plants, it significantly enhanced the P concentrations in stems and roots at 100 and 150 mM NaCl, when compared with those in non-inoculated plants (Fig. 4a-f). AMF decreased the N/P ratios in the tissues of plants under salinity condition, the decreased values of stems and roots reached significant levels across all NaCl levels (Fig. 4g-i).

**Ion concentration and ion balance**

Salinity, AMF inoculation, and their interaction had significant effects on the Na\(^+\) and Ca\(^{2+}\) content in the three plant tissues (leaf, stem, and root), K\(^+\) content in leaves, and Mg\(^{2+}\) content in stems (Table S1). While salinity significantly enhanced the concentrations of Na\(^+\), AMF inoculation significantly decreased the concentrations of Na\(^+\) in the three tissues, when compared with those in non-inoculated plants (Fig. 5a-c). The concentrations of K\(^+\) were not significantly influenced by AMF inoculation across all NaCl levels except for 0 mM in leaves (Fig. 5d-f). AMF inoculation increased the concentrations of Ca\(^{2+}\), especially at high NaCl levels (100 and 150 mM), and the increase was significantly higher in roots, when compared with that in non-inoculated plants (Fig. 5g-i). However, the concentrations of Mg\(^{2+}\) were lower, especially in stem, following AMF inoculation, when compared with those in non-inoculated plants (Fig. 5k).

Salinity had significant effects on K\(^+\)/Na\(^+\), Ca\(^{2+}\)/Na\(^+\), Mg\(^{2+}\)/Na\(^+\), and Ca\(^{2+}\)/Mg\(^{2+}\) ratios in the three plant tissues (Table S1). While salinity reduced the K\(^+\)/Na\(^+\), Ca\(^{2+}\)/Na\(^+\), and Mg\(^{2+}\)/Na\(^+\) ratios in the plant tissues, AMF inoculation enhanced these ionic ratios across all the NaCl levels, when compared with those in non-inoculated plants (Table S3). In particular, the values of K\(^+\)/Na\(^+\) ratios were
increased by 37.66% (P < 0.05), 28.57% (P < 0.05), 47.89% (P < 0.05) in leaves, 82.68% (P < 0.05), 415.75% (P < 0.05), 399.25% (P < 0.05) in stems, and 11.67%, 40.07% (P < 0.05), 52.15% (P < 0.05) in roots at 50, 100, 150 mM NaCl level after AMF inoculation (Table S3). The values of Ca^{2+}/Na^+ and Mg^{2+}/Na^+ ratios were also increased by AMF under salinity conditions. In total, the increased effects of AMF inoculation showed the following trend: Ca^{2+}/Na^+ ratios > K^+/Na^+ ratios > Mg^{2+}/Na^+ ratios.

Besides, AMF inoculation also had positive effects on Ca^{2+}/Mg^{2+} ratios, especially in stems and roots.

Discussion

Effects of AMF inoculation on plant growth and root morphology under salinity stress

The results of the present study showed that the AMF, F. mosseae, had more than 95% colonization rate on the roots of G. sinensis across all NaCl levels (Fig. 1a), confirming previous reports indicating that F. mosseae had high tolerance to various stress, including salinity stress, and produced positive effects on host plant growth (Lin et al. 2017; Zhang et al. 2018). It must be noted that the mycorrhizal effects on plant growth differed among plant species, because plants with possibly thick and less branched roots and few root hairs generally present higher mycorrhizal dependency (Yang et al. 2015). In the present study, G. sinensis seedlings with coarse root architecture were found to grow very slowly without AMF colonization, but grew much faster in the presence of AMF. Furthermore, AMF-inoculated seedlings showed considerably higher height growth, diameter growth, and biomass accumulation (Table 1 and Fig. 2), indicating that the growth of G. sinensis highly depended on AMF, the values of mycorrhizal dependency (above 75%) confirmed it (Fig. 1b). Similar positive effects of AMF have also been found on other leguminous plants such as fenugreek (Trigonella foenum-graecum L.) (Evelin et al. 2012), pigeonpea (Cajanus cajan L. Millsp) (Garg and Pandey 2015), and soybean (Glycine max L. Merrill) (Bulgarelli et al. 2017). These effects might be attributed to the enhancement of nutrients and water acquisition by external mycorrhizal hyphae (Abdel-Fattah 2012). In addition, AMF can not only enhance plant root growth, modify root morphology, and architecture (considerably higher root length, surface area, and tip number) (Yang et al. 2015), but can also improve plants nutrients and water uptake, ultimately contributing to the growth and biomass accumulation of
Effects of AMF inoculation on Chlorophyll content and photosynthesis under salinity stress

Chlorophyll content reflects plant photosynthetic ability and indicates the relative plant salt tolerance to some extent (Takai et al. 2010). The reduction in the chlorophyll content in the present study under salinity stress might be caused by the decrease in Mg and K absorption (because Na has an antagonistic effect on Mg and K absorption) (Daei et al. 2009) or suppression of specific enzymes responsible for the synthesis of chlorophyll content (Murkute et al. 2006). And, the increase in chlorophyll contents following AMF inoculation under salinity stress suggested that the chlorophyll synthesis was less affected by salinity stress in the presence of AMF (Table S2). The higher values of Mg$^{2+}$/Na$^{+}$ ratios in the tissues of AMF-inoculated plants further implied that AMF inoculation effectively suppressed the antagonistic effect of Na$^{+}$ on Mg$^{2+}$, thus increasing chlorophyll contents, consistent with the findings of previous studies (Giri et al. 2003; Hajiboland et al. 2010; Porcel et al. 2015). Moreover, the substances secreted by AMF, such as cytokines, could be beneficial for the development of chloroplast and enhancement of chlorophyll levels (Thanaa and Nawar 1994), resulting in higher $P_{n}$ values (Table S2). Besides, significant higher $G_{s}$ values were observed in mycorrhizal plants under salinity conditions, which was also beneficial to increased $P_{n}$ values. The higher $G_{s}$ values in mycorrhizal plants indicated AMF could mitigate salt-induced reduction in stomatal conductance, which might attribute to the increase in nutrient and water uptake caused by AMF (Zhu et al. 2010). AMF inoculation significantly enhanced the $T_{r}$ values, but significantly decreased $L_{s}$ under salinity conditions, similar results also be reported by many researchers (Talaat and Shawky 2014; Lin et al. 2017; Zhang et al. 2018).

Effects of AMF inoculation on osmotic adjustment under salinity stress

There are some physiological mechanisms related to the protective effects of AMF on plants under salt stress condition. First, the present study showed that AMF-inoculated G. sinensis seedlings exhibited higher Pro content in the roots, but significant lower Pro content in leaves (Table 2), which is consistent with that noted in mycorrhizal soybean (Sharifi et al. 2007). Similar results of higher
accumulation of Pro in AMF-inoculated plants have also been reported in previous studies (Evelin et al. 2013; Talaat and Shawky 2014). In particular, higher accumulation of Pro in roots has been found to be beneficial for maintaining osmotic balance between water-absorbing root cells and external media (Evelin et al. 2009), whereas low accumulation of Pro in the leaves of AMF-inoculated plants might suggest less injury because Pro is also considered as an indicator of salt-induced damage (Evelin et al. 2013). The increase in the Pro content in roots could be attributed to the reduction in oxidation of Pro to glutamate or induction of Pro biosynthesis enzymes (Stewart 1981). Besides, higher SP concentration especially at 150 mM NaCl was also noted in the roots of AMF-inoculated plants (Table 2), which could be ascribed to the higher Pro accumulation, because Pro plays an important function in the stabilization of proteins. SP help in osmotic adjustment and play an essential role in maintaining water and nutrient absorption and membrane stabilization (MSI) (Goudarzi and Pakniyat 2009). Thus, the higher Pro and SP concentrations in the roots of AMF-inoculated plants indicated higher efficiency of osmotic regulation system in these plants.

**Effects of AMF inoculation on antioxidant enzymes under salinity stress**

Under salt stress, ROS, such as superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), hydroxyl radical (OH), and singlet oxygen (1O$_2$), are generated in different cell compartments, including chloroplasts, mitochondria, and apoplastic space (Jithesh et al. 2006), and could disrupt the normal metabolism of lipids, proteins, and nucleic acids (Muchate et al. 2016). The higher SP contents in leaves and higher MDA contents in both leaves and roots with increasing NaCl levels suggested enhanced lipid peroxidation and protein oxidation under salinity condition, which is consistent with the findings of previous studies (Navarro et al. 2014; Talaat and Shawky 2014). To scavenge ROS, plants possess defense systems involving enzymes and antioxidants (Jiang and Zhang 2002). Among the antioxidant enzymes, SOD metabolizes O$_2^-$ to H$_2$O$_2$, which protects plant cells from damage, while CAT, POD, and APX directly convert H$_2$O$_2$ to H$_2$O and O$_2$. The results of the present study indicated that G. sinensis showed increased antioxidant enzymes activities to resist oxidative stress at a certain NaCl level (< 100 mM). However, these higher activities were not adequate to scavenge ROS, especially when the
NaCl level reached 100 mM; hence, the MDA content in the leaves of plants at 100 mM NaCl was significantly higher than that in plants under no salinity treatment (Fig. 3c). Many studies have reported higher antioxidant enzymes activities in AMF-inoculated plants, when compared with those in non-inoculated plants (Hajiboland et al. 2010; Lu et al. 2014). In the present study, higher activities of antioxidant enzymes (POD, CAT, and SOD) were observed in AMF-inoculated plants, especially in roots, when compared with those in non-inoculated plants. In addition, lower MDA contents in the AMF-inoculated plants indicated lower oxidative damage, especially at high NaCl levels, which might be partly owing to the higher Pro level in roots because Pro also play an important role in ROS detoxification (Muchate et al. 2016).

Effects of AMF inoculation on N and P contents under salinity stress
N and P elements are vital important for the growth of plant. Generally, the enhancement of P uptake is considered the most important salt stress tolerance mechanism in mycorrhizal plants (Bolan 1991; Liu et al. 2016), as the fungal hyphae of AMF function analogous to fine root hairs and acquire nutrients especially relatively immobile elements such as P (Koltai and Kapulnik 2010). Higher N concentrations in mycorrhizal plants compared with no-mycorrhizal plants had also been reported (Talaat and Shawky 2014; Wang et al. 2018). In the present study, AMF inoculation mainly enhanced the P concentration, but not the N concentration of plants at high NaCl level. In addition, lower N/P ratios in the tissues especially in stems and roots of mycorrhizal plants (Fig. 4g-i) provided support for that the enhancement of P uptake as one of the underlying mechanism by AMF which alleviate salt damage to plants. N/P ratio in the shoot biomass could be used to evaluate whether N or P is the limiting factor for plant biomass product, generally N/P ratios < 10 and > 20 correspond to N- and P-limited biomass production (Güsewell 2004). The N/P ratios in leaves and stems of no-inoculation plants were > 20 under salinity conditions indicated that G. sinensis was P-limited biomass product when exposed to salinity, the lower N/P ratios in mycorrhizal plants clearly showed that AMF inoculation could effectively allviate P-limited biomass product caused by salinity stress.

Effects of AMF inoculation on ion contents and ion balances under salinity stress
High Na$^+$ concentration in soil has been reported to inhibit the uptake of other nutrients such as K$^+$,
Ca$^{2+}$, Mg$^{2+}$, etc., resulting in nutrient imbalance and thus plant growth restriction (Parida and Das 2005). K$^+$ plays a key role in plant metabolism, including stomatal movement, protein synthesis, and enzymes activation (Khalil et al. 2011). In the present study, the K$^+$ content significantly decreased when the Na$^+$ content increased (Fig. 5a-f), because Na$^+$ ions compete with K$^+$ ions for binding sites essential for various cellular activities. It has been revealed that mycorrhizal colonization can enhance K$^+$ absorption under salinity condition (Giri et al. 2007; Evelin et al. 2012). The results of the present study showed no obvious difference in the K$^+$ content between AMF-inoculated and non-inoculated plants, but indicated obviously higher K$^+$/Na$^+$ ratios in the tissues of mycorrhizal plants at all NaCl levels (Table S3), which can be attributed to the lower Na$^+$ content, when compared with that in non-mycorrhizal plants.

The lower levels of Na$^+$ in AMF-inoculated plants have also been reported in many previous studies (Evelin et al. 2012; Lu et al. 2014; Pollastri et al. 2018). However, Allen and Cunningham (1983) indicated that AMF can occasionally enhance Na$^+$ uptake, suggesting that AMF could induce a buffering effect on the uptake of Na$^+$ causing higher Na$^+$ concentration in mycorrhizal plants at low salinity and lower Na$^+$ concentration at higher salinity. The strong enhancement of plant growth following AMF inoculation can also contribute to the decrease in Na$^+$ concentration in the tissues (Juniper and Abbott 1993; Al-Karaki 2006). A sustained high K$^+$/Na$^+$ ratio is considered to be one of the key indicators for the evaluation of salt tolerance in plants because it prevents disruption of various enzymatic processes and inhibition of protein synthesis (Maathuis and Amtmann 1999; Dasgan et al. 2002). The higher K$^+$/Na$^+$ ratios in the tissues of AMF-inoculated plants may be one of the primary reasons for the improvement in growth of G. sinensis seedlings under salinity condition, and similar findings have also been reported in previous studies (Hajiboland et al. 2010; Evelin et al. 2012).

Ca$^{2+}$ concentrations in the plant tissues increased under salt stress, which is beneficial for
transducing signal because Ca\(^{2+}\) acts as a second messenger. A higher Ca\(^{2+}\) concentration was also observed especially in roots of AMF-inoculated plants, consistent with the previous reports on mycorrhizal lettuce and tomato (Cantrell and Linderman 2001; Hajiboland et al. 2010). It has been indicated that high Ca\(^{2+}\) concentration in tissues can preserve the structural and functional integrity of membranes, stabilize cell wall structures, and regulate ion transport and selectivity (Munns 2002; Maathuis 2009). Hence, the higher MSI values and K\(^{+}\)/Na\(^{+}\) ratios could be partly attributed to the enhancement of Ca\(^{2+}\) content following AMF inoculation. Moreover, significant increment in Ca\(^{2+}\) concentration in the roots of mycorrhizal plants observed in the present study might account for the high AMF colonization at all NaCl levels, because high Ca\(^{2+}\) is known to enhance AMF colonization and sporulation (Jarstfer et al. 1998).

Mg\(^{2+}\) is essential for the biosynthesis of chlorophyll. Although salt stress restrains the uptake of Mg\(^{2+}\), the effect of AMF on Mg\(^{2+}\) concentration in plants is controversial. In a previous study, Evelin et al. (2012) demonstrated that the concentration of Mg\(^{2+}\) in mycorrhizal plants was higher in roots, but lower in shoot, when compared with that in non-mycorrhizal plants. However, Talaat and Shawky (2014) showed that the concentration of Mg\(^{2+}\) in leaves of wheat was significantly enhanced by AMF. The results of the present study indicated that the concentrations of Mg\(^{2+}\) in the leaves and roots of mycorrhizal plants were lower than those in non-mycorrhizal plants, which might be owing to the stronger competition by Ca\(^{2+}\). As Ca\(^{2+}\) has higher affinity to the binding sites of plasma membrane than Mg\(^{2+}\) (Marschner 1995), a higher Ca\(^{2+}\)/Mg\(^{2+}\) ratio was noted in mycorrhizal plants. However, a higher Mg\(^{2+}\)/Na\(^{+}\) ratio in mycorrhizal plants suggested that the function of Mg\(^{2+}\) was less suppressed by salinity in these plants.

Conclusions
We revealed that the AMF F. mosseae significantly enhanced the growth and biomass accumulation of G. sinensis seedlings both under normal and salinity conditions. The AMF inoculation alleviated salt-induced deleterious effects on G. sinensis seedlings growth by multiple ways. Enhanced Ca\(^{2+}\) uptake
by AMF might be beneficial to maintain high colonization under high salinity stress. Higher P/N ratio in AMF inoculation seedlings was one of important mechanisms for accumulating biomass under salinity conditions. In addition, AMF decreased Na\(^+\) absorption resulting in more favorable ion balances, and enhanced antioxidant enzymes (POD, CAT, and SOD) to scavenge ROS. Consequently, higher P\(_n\) and MSI in leaves, and lower Pro contents in leaves, lower MDA content in the tissues of mycorrhizal plants were noted, ultimately leading to better tolerance to salt stress. These findings clearly demonstrated the significant potential application of AMF in G. sinensis afforestation and rehabilitation in saline soil, including coastal areas.

### Abbreviations

- **AMF**: arbuscular mycorrhizal fungi
- **Chl**: leaf chlorophyll contents
- **G\(_s\)**: leaf gas exchange
- **P\(_n\)**: leaf net photosynthetic rate
- **T\(_r\)**: transpiration rate
- **Ls**: leaf limiting value of stomata
- **Pro**: proline content
- **SP**: soluble protein
- **MDA**: malondialdehyde
- **RWC**: leaf relative water content
- **MSI**: membrane stability index
- **POD**: peroxidase
- **CAT**: catalase
- **APX**: ascorbate peroxidase
- **SOD**: superoxide dismutase

### Additional Files

**Table S1** Result of two way ANOVA test for independent variables including salinity treatment, AMF inoculation and their interaction
Table S2 Effects of *F. mosseae* on chlorophyll contents and photosynthetic parameters of *G. sinensis* seedling at different NaCl levels

Declarations

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**Availability of data and materials**

All data generated or analyzed during this study are in this article (and its supplementary information files) or are available from the corresponding author on reasonable request.

**Authors’ contributions**

JZ and JW designed the experiments. JZ acquired the funding and administered the projects. JW, JL, JY, CL and SM conducted the experiments. GW, JW, BZ and XL interpreted data. JW wrote the manuscript. GW and BZ revised the manuscript. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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Tables
### Table 1 Effects of *F. mosseae* on growth and root morphology parameters of *G. sinensis* seedling at different NaCl levels

| NaCl level (mM) | AMF status | Height growth (cm) | Diameter growth (mm) | Leaf area (cm²) | Root length (cm/plant) | Root surface area (cm²/plant) | Root tip number (/plant) |
|-----------------|------------|--------------------|----------------------|-----------------|------------------------|-----------------------------|-------------------------|
| 0               | NM         | 15.49±3.63Ba       | 0.77±0.21Ba          | 1.52±0.26Aa     | 510.4±12.7Ba           | 80.8±9.6Ba                  | 461±52Ba                |
|                 | AM         | 47.72±15.13Aa      | 2.00±0.95Aa          | 1.78±0.34Aa     | 4246.9±34.86Aa         | 887.6±152.5Aa              | 6145±1758Aa             |
| 50              | NM         | 12.01±2.18Bb       | 0.63±0.20Bb          | 1.14±0.32Aa     | 507.5±6.2Ba            | 80.4±9.7Ba                  | 439±85Ba                |
|                 | AM         | 47.58±16.35Aa      | 1.79±0.77Aa          | 1.53±0.26Aa     | 3949.6±208.4Aa         | 739.5±84.7Aa               | 6100±1201Aa             |
| 100             | NM         | 2.03±0.32Ba        | 0.34±0.17Bc          | 0.91±0.22Aa     | 400.4±45.7Bb           | 60.4±1.5Bb                  | 361±13Bab               |
|                 | AM         | 39.09±9.70Aa       | 1.10±0.55Bb          | 1.14±0.13AAb    | 3358.2±192.8Bb         | 583.4±121.1AAb             | 3875±225Aa              |
| 150             | NM         | 0.86±0.54Bc        | 0.21±0.07Bd          | 0.83±0.12Aa     | 324.8±62.7Bb           | 53.6±8.5Bb                  | 277±41Bb                |
|                 | AM         | 26.87±3.24Bc       | 0.63±0.20Aa          | 0.92±0.08Ac     | 2002.4±514.3Ac         | 454.5±172.2Ab              | 2955±656Bab             |

NM represents the groups without *F. mosseae* inoculation; AM represents the group with *F. mosseae* inoculation. Different capital letters indicate significant differences (*P < 0.05*) among inoculation treatments (NM and AM) within the same NaCl level. Different lowercase letters indicate significant differences (*P < 0.05*) among NaCl levels within the same inoculation treatment.

### Table 2 Effects of *F. mosseae* on antioxidant enzyme activities and osmoregulators of *G. sinensis* seedling at different NaCl levels

| Tissues | NaCl level (mM) | AMF status | POD (U/mg protein) | CAT (U/mg protein) | SOD (U/mg protein) | APX (U/mg protein) | Pro (µg/g) | SP (mg/g) |
|---------|-----------------|------------|--------------------|--------------------|--------------------|--------------------|------------|----------|
| Leaf    | 0               | NM         | 729.0±128.2Bc      | 1.98±0.34Bc        | 17.6±3.7Aa         | 11.9±5.2Ab        | 23.5±3.6Ac | 5.59±0.26Ac |
|         | AM              | 1112.3±54.0Ab | 3.99±1.20Aa      | 38.1±22.0Aa        | 14.6±5.0Ab        | 11.7±7.5Aa       | 5.38±0.39Aa |
|         | 50              | NM         | 1016.8±83.6Bc      | 3.29±0.61Bc        | 105.7±10.7Bc       | 23.5±5.0Aa        | 34.7±4.0Ab  | 7.00±0.30Ac |
|         | AM              | 1335.5±28.5Ab | 7.38±1.25Aa      | 121.0±22.9Ab       | 26.2±8.7Aa        | 18.3±4.0Ba       | 6.98±0.76Aa |
|         | 100             | NM         | 1433.9±222.2Aa     | 8.95±2.19Aa        | 132.7±13.5Ab       | 32.3±5.3Aa        | 52.4±8.8Ab  | 7.78±0.71Aa |
|         | AM              | 2452.9±633.6Aa | 16.69±5.89Ab      | 189.2±9.2Aa        | 34.8±1.0Aa        | 22.1±3.9Aa       | 6.99±0.63Aa |
|         | 150             | NM         | 1108.8±69.2Bab     | 5.25±0.55Bb        | 112.8±6.9Aab       | 47.1±18.8Aa       | 276.3±17.1Aa | 8.56±0.66Aa |
|         | AM              | 1737.8±143.4AAb | 9.89±0.73Aa       | 119.8±42.0Ab       | 34.1±7.9Aa        | 24.4±5.0Bb       | 6.65±0.93Bb |
| Root    | 0               | NM         | 763.5±179.2Ab      | 10.61±0.57Bb       | 45.8±6.1Bb         | 54.5±13.3Aa       | 27.2±2.2Ac  | 2.57±0.14Aa |
|         | AM              | 800.6±130.2Ac | 19.30±3.82Ab      | 112.3±9.0Aa        | 65.7±6.5Ac        | 20.3±3.5Bb       | 2.85±0.53Aa |
|         | 50              | NM         | 1245.3±207.6Ab     | 13.82±1.5Ba        | 56.0±2.2Bb         | 104.2±13.0Bc      | 45.6±4.3Ab  | 2.90±0.38Aa |
|         | AM              | 2076.6±237.1Aab | 23.09±2.67Aa      | 128.3±6.3Aa        | 312.3±18.9Bb      | 52.0±4.2Aa       | 2.91±0.28Aa |
|         | 100             | NM         | 649.6±131.9Bbc     | 23.45±6.49Bb       | 56.1±3.4Bb         | 235.4±12.7Ab      | 51.7±6.2Ab  | 2.55±0.36Aa |
|         | AM              | 2577.3±351.4AAb | 27.11±1.44Ab      | 96.6±8.2Ab         | 215.0±33.1Ab      | 62.2±10Aab       | 3.00±0.45Aa |
|         | 150             | NM         | 309.9±67.8Ab       | 10.77±2.15Ab       | 88.5±19.4Aa        | 343.5±18.3Aa      | 87.0±9.5Aa  | 2.18±0.29Bb |
|         | AM              | 1792.7±135.3Ab | 20.32±6.63Aa      | 93.0±10.5Aa        | 103.9±3.2Bc       | 92.9±31.8Aa      | 2.81±0.23Aa |

NM represents the groups without *F. mosseae* inoculation; AM represents the group with *F. mosseae* inoculation. Different capital letters indicate significant differences (*P < 0.05*) among inoculation treatments (NM and AM) within the same NaCl level. Different lowercase letters indicate significant differences (*P < 0.05*) among NaCl levels within the same inoculation treatment.

**Figures**
Fig. 1 Mycorrhizal colonization and mycorrhizal dependency of *G. sinensis* seedling at different NaCl levels. (a) Mycorrhizal colonization, (b) Mycorrhizal dependency. Different lowercase letters indicate significant differences ($P < 0.05$) among NaCl levels.

Figure 1

Mycorrhizal colonization and mycorrhizal dependency of *G. sinensis* seedling at different NaCl levels. (a) Mycorrhizal colonization, (b) Mycorrhizal dependency. Different lowercase letters indicate significant differences ($P < 0.05$) among NaCl levels.
Effects of F. mosseae on biomass of G. sinensis seedling at different NaCl levels. NM represents the groups without F. mosseae inoculation; AM represents the group with F. mosseae inoculation. In the same tissues, different capital letters indicate significant differences ($P < 0.05$) among inoculation treatments (NM and AM) within the same NaCl level, different lowercase letters indicate significant differences ($P < 0.05$) among NaCl levels within the same inoculation treatment.
Effects of F. mosseae on MSI, RWC and MDA contents of G. sinensis seedling at different NaCl levels. (a) Leaf relative water content, (b) Leaf membrane stability index, (c, d) Leaf and root MDA; NM represents the groups without F. mosseae inoculation; AM represents the group with F. mosseae inoculation. Different capital letters indicate significant differences (P < 0.05) among inoculation treatments (NM and AM) within the same NaCl level. Different lowercase letters indicate significant differences (P < 0.05) among NaCl levels within the same inoculation treatment.
Effects of F. mosseae on N, P concentrations, and N/P ratios of G. sinensis seedling at different NaCl levels. (a, b c) N concentration in leaf, stem, and root, (d, e, f) P concentration in leaf, stem, and root, (g, h, i) N/P ratio in leaf, stem, and root; NM represents the groups without F. mosseae inoculation; AM represents the group with F. mosseae inoculation. Different capital letters indicate significant differences (P < 0.05) among inoculation treatments (NM and AM) within the same NaCl level. Different lowercase letters indicate significant differences (P < 0.05) among NaCl levels within the same inoculation treatment.
Effects of F. mosseae on the ion concentrations of G. sinensis seedling at different NaCl levels. (a, b, c) Na+ concentration in leaf, stem, and root, (d, e, f) K+ concentration in leaf, stem, and root, (g, h, i) Ca2+ concentration in leaf, stem, and root, (j, k, l) Mg2+ concentration in leaf, stem, and root; NM represents the groups without F. mosseae inoculation; AM represents the group with F. mosseae inoculation. Different capital letters indicate significant differences (P < 0.05) among inoculation treatments (NM and AM) within the same NaCl level. Different lowercase letters indicate significant differences (P < 0.05) among NaCl levels within the same inoculation treatment.

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