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ARBUSCULAR MYCORRHIZA

The effect of inoculation with arbuscular mycorrhizal fungi on root traits and salt tolerance of *Tagetes erecta*

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Abstract. Soil salinization is a global environmental problem. Arbuscular mycorrhizal (AM) fungi are capable of enhancing plant resistance to stress, for instance, improving plant tolerance to salinity stress. A pot experiment was carried out to determine the effects of AM fungi (*Funneliformis mosseae*, *Rhizophagus intraradices*, or *F. mosseae* and *R. intraradices* combined) and five different NaCl levels on the growth and physiology of *Tagetes erecta* under greenhouse conditions. The results showed that dual inoculation with *F. mosseae* and *R. intraradices* significantly increased total root length, root surface area, root volume, and root tip number of *T. erecta* plants under salt stress. Inoculation with AM fungi inhibited Na^+ accumulation in leaves and promoted the absorption of N, P, K, and Ca in leaves, and thus increased ratios of K^+/Na^+, Ca^{2+}/Na^+, N/Na^+, and P/Na^+ in leaves under salt stress. Correlation analysis showed that the coefficient (0.964) between root volume and K^+ in leaves was the highest, while the correlation between root surface area and Na^+ content in leaves was remarkably negative (–0.95). AM fungi improved the osmotic regulation ability of plant cells, increased the activity of antioxidant enzymes, and reduced the damage of cell membranes. It is concluded that AM fungi improved salt tolerance of *Tagetes erecta* by changing root morphological traits, regulating uptakes of Na^+ and other nutrient elements, and enhancing antioxidant enzyme activities and osmotic adjustment.

Keywords: arbuscular mycorrhizal fungi, mineral element equilibrium, root traits, salt tolerance, *Tagetes erecta*.

1. INTRODUCTION

The ornamental flower industry has developed rapidly. However, with the increase of primary and secondary soil salinization since the beginning of the 21st century, soil salinization has become one of the major problems of flowers planted for urban greening (Li et al. 2014; Zhang et al. 2018; Redman et al. 2019), affecting the growth of many ornamental plants (Yang et al. 2018; Al-Farsi et al. 2021). Salt stress negatively impacts the ornamental value of plants (Acosta-Motos et al. 2014; Karagöz and Dursun 2021), leads to nutrient imbalance in plants, influences photosynthesis and produces reactive oxygen species (ROS), then reduces the biomass of the plant and the number and quality of its flowers, and affects the flowering period of plants (García-Caparrós and Lao 2018). Therefore, it is crucial to explore ways of improving salt tolerance in ornamental plants in order to promote the development of the flower industry. Recently, increasing attention is being paid to the role of plant symbiotic microbes in improving plant salt tolerance, particularly arbuscular mycorrhizal (AM) fungi (Chandrasekaran et al. 2019; Motaleb et al. 2020; Abbaspour et al. 2021; Fakhech et al. 2021).

AM fungi play important roles in promoting the growth and productivity of host plants by regulating their morphological, biochemical and physiological characteristics (Hussain et al. 2021; Thangavel et al. 2022). Plants colonized with AM fungi are able to obtain additional water and nutrients, thus alleviating the reduction of growth and

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leaf area caused by salt stress, and increase biomass, especially root biomass (Latef and He 2011; Xu et al. 2018; Gonçalves et al. 2021; Janah et al. 2021). It is well documented that AM fungi increase plant salt tolerance (Hashem et al. 2018; Murugesan et al. 2019; Wang H. et al. 2020). Under salt stress, AM fungi colonize roots, change the root morphology, increase the number and length of capillary and fibrous roots, further enhance root activity, and increase water absorption to improve plant's tolerance to salt stress (Murugesan et al. 2019; He et al. 2020; Parvin et al. 2020; Andrzejak and Janowska 2021; Liu et al. 2021). AM fungi also increase the absorption of minerals, maintain the balance of mineral elements in plants under salt stress, and thus enhance tolerance to salt stress (Mardukhi et al. 2015; Yarahmadi et al. 2018; Romero-Munar et al. 2019; Wang J. et al. 2020). Hashem et al. (2018) found that inoculation with AM fungi significantly promoted the absorption of K, Ca, Mg, Zn, Fe, Mn, and Cu in cucumber (*Cucumis sativus* L.), while significantly reducing the absorption of harmful ions, such as Na^+. Zai et al. (2021) reported that combined inoculation with AM fungus *Funneliformis mosseae* and phosphate-solubilizing fungus *Apophysomyces spartima* on *Prunus maritima* under 170 mM NaCl increased N, P, and K uptake, root growth and net photosynthetic efficiency, thus alleviating salt stress. AM fungi significantly promote the uptake of P, K, Ca, and Mg by roots and leaves and regulate K^+/Na^+ ratio in roots, thus alleviating the nutrient deficiency caused by osmotic stress (Wang, J. et al. 2020; Merieme et al. 2022; Thangavel et al. 2022). AM fungi regulate K, Ca, and P absorption and distribution as well as mineral element ion balance in plants, which is the key to improving salt tolerance of plants (Hidri et al. 2019; Yang et al. 2022). The absorption of mineral elements depends on root traits such as root growth and development; AM fungi have been found to promote both (Liu and Wang 2018; Sahur 2020; Rahimi et al. 2021).

In addition, the mechanisms of AM fungal colonisation on salt-affected plants also include the improved production of antioxidant enzymes and osmotic substances (Kaur et al. 2021; Kumar 2021). Increasing the activities of certain antioxidant enzymes and antioxidant molecules to limit oxidative damage is an important strategy of mycorrhizal plants to enhance salt tolerance (Laouane et al. 2019). Plant cells are protected by a complex antioxidant system that contains both non-enzyme and enzymatic antioxidants that reduce the harmful effects of ROS (Foyer and Noctor 2010; Evelin et al. 2019). AM fungi can protect plants from salt by reducing salt-induced oxidative stress (Dashtebani et al. 2014). Some studies reported higher activity of antioxidant defense enzymes in AM plants, which is associated with improved growth under salinity (Laouane et al. 2019; Latef et al. 2021). Inoculation with AM fungi promotes plant growth by reducing the damage caused by malondialdehyde (MDA, a product of lipid peroxidation) and H_2O_2 accumulation under salt stress (Yang et al. 2016; Boutasknit et al. 2020). Proline and soluble protein are important osmotic regulatory substances, which can reduce osmotic potential of cells, enhance water holding capacity, and maintain normal physiological metabolism of plants under stress (Gong et al. 2020). Higher proline concentrations indicate a better state of osmotic regulation, which may benefit the absorption of water and nutrients and higher concentrations of proline in AM plants than in non-AM plants have been reported at different salinity levels (Liu et al. 2016; Pollastri et al. 2018; Mosaddeghi et al. 2021). The increases in soluble protein content can also serve as an energy resource for plants under abiotic stress (Sami et al. 2016).

At present, studies have shown that AM fungi can improve the ability of horticultural crops and trees to resist salt stress (Diao et al. 2021; Sofy et al. 2021), but there are few studies on the application of AM fungi on ornamental plants in high salinity conditions. *Tagetes erecta* is an important plant with high ornamental, economic and medical value in the garden flower industry. However, its growth and production are adversely affected by soil salinity in the planting regions in northern China. Studies show that AM fungi promote the early flowering of *T. erecta* and improve its ornamental quality (Vaingankar and Rodrigues 2014); increase the yield of secondary metabolites and the content of economically important medicinal components (Engel et al. 2016; Johny et al. 2021); improve the tolerance of *T. erecta* to heavy metal stress (Castillo et al. 2011; Gong et al. 2011; Elhindi et al. 2018) and drought (Asrar and Elhindi 2011). However, whether AM fungi promote the growth and flowering of *T. erecta* under salt stress remains unknown. The objective of this study was to reveal the functional relationship between AM fungus-mediated root traits and mineral nutrient absorption, relative balance of nutrients and Na, and plant tolerance to salt. The outcomes of this study have a practical significance in improving salt tolerance of *T. erecta* and other garden plants.

2. MATERIALS AND METHODS

2.1. Experimental materials

Seeds of *Tagetes erecta* were purchased from Muyangdou Seed Research Industry Co., LTD, China. AM fungal inocula of *Funneliformis mosseae* and *Rhizophagus intraradices* composed uniform mixture of root segments, fungal mycelium, spores and sand, provided by Mycorrhizal Biotechnology Institute of Qingdao Agricultural University. Plastic pots (20 cm diameter × 25 cm depth) sterilized with 5% sodium hypochlorite were filled with autoclaved

(121 °C for 1.5 h) mixed soil (loam soil/vermiculite in 1:3 ratio) to grow *T. erecta* plants. The loam soil had 17.7% organic matter, 0.02% soil salt content, 156.1 mg kg^{-1} available N, 26.6 mg kg^{-1} available P, and 45.3 mg kg^{-1} available K, at a pH of 6.96.

2.2. Experimental design

A two-factor randomized block design consisting of four AM fungal inoculation variants and five levels of salt strength was used in this study with a total of 20 treatments. Three AM fungal inoculations were inoculation with *F. mosseae*, *R. intraradices*, and both *F. mosseae* and *R. intraradices* combined, and the fourth control group received no inoculation. Salt treatments were given with 0.2%, 0.4%, 0.6%, or 0.8% NaCl solution, the control group received no salt treatment. There were nine plants (pots) in each treatment, and seven of them were randomly selected for measuring growth and physiology parameters.

2.3. Sowing, inoculation, and management

The experiments were conducted in a sunlit greenhouse at Qingdao Agricultural University from March to October 2019 (23/15 °C day/night, 12/12 h light/dark, relative humidity 60–70%). For inoculation treatments, 12 000 units of inoculum potential (IP) of *F. mosseae*, *R. intraradices*, or *F. mosseae* combined with *R. intraradices* inocula were distributed to pots according to the experimental design by mixing in with the soil. The units of IP were measured with the methods described by Liu and Luo (2010) and calculated as IP = N × W × K + S, where IP is the inoculation potential, N is the number of vesicles in the root segment per unit length, W is the root weight (g), K is the root length per unit mass (cm), and S is the number of spores in the inocula per unit mass or volume. The uninoculated controls received 50 g of autoclaved inocula and 30 mL of filtrate (< 20 μm) of the same inocula in each pot.

Healthy and uniform sized seeds of *T. erecta* were soaked in a 10% H_2O_2 solution for two minutes, rinsed, and sown in seedling trays. Seedlings with 3–4 leaves were transplanted into pots. Seven days after transplanting, 30 mL of the salt solution was added every week, for a total of three times with the final NaCl concentration reaching the designed level, respectively. Plants were watered twice a week. Plants in each pot were given 200 mL of Hoagland's nutrient solution (half strength) every two weeks. Hoagland's nutrient solution (half strength) contains 607 mg/L K_2SO_4, 57.5 mg/L $(NH_4)_2PO_4$, 493 mg/L $MgSO_4$, 20 mg/L EDTA ferric-sodium salt, 15 mg/L $FeSO_4$, 2.86 mg/L H_3BO_3, 4.5 mg/L $Na_2B_4O_7 \cdot 10H_2O$, 2.13 mg/L $MnSO_4$, 0.05 mg/L $CuSO_4$, 0.22 mg/L $ZnSO_4$ and 0.02 mg/L $(NH_4)_2SO_4$.

2.4. Parameter measurements and methods

For the assessment of AM fungal colonisation and hyphal density six *T. erecta* plants were harvested from each treatment 30 days after adding NaCl. Fine roots were cut into 1 cm segments, cleared in 10% KOH, bleached in 2% HCl for 20 minutes, and stained with 0.05% trypan blue. Thirty fragments were examined for AM colonisation under a digital computerized microscope at eyepiece 10×, objective 10× and 40× (BX50 Olympus Microscope, Tokyo, Japan). All AM fungal structures including hyphae, arbuscules, and vesicles found in the roots were recorded. The total mycorrhizal colonisation percentage was determined with the method described by Biermann and Linderman (1981). Hyphal density was measured using the hyphal extraction and filtration method. After trypan blue staining, the grid crossing method was used to count the intersection points and calculate the hyphal density (Abbott et al. 1984).

Root morphological traits and root activity were examined as follows: root length, root tip number, total root surface area, and total root volume were analysed on scanned root images in the root scanner (WinRHIZO Version 2016a, Regent Instruments Inc., Canada). Root activity was determined by triphenyltetrazolium chloride (TTC) colorimetry (Li 2000). Five mL of 0%, 0.005%, 0.01%, 0.02%, 0.03% and 0.04% TTC solution was prepared and placed into the calibration test tube, mixed thoroughly with 5 mL of ethyl acetate and a small amount of $Na_2S_2O_4$, and then the red methyl hydrazine was produced at once. Next 5 mL of ethyl acetate was added and stirred well. Ethyl acetate solution was used as the control, and the optical density (OD) value of the solution at 485 nm wavelength was measured by spectrophotometer (UH5300, HITACHI Co., Ltd., Japan) and the stan- dard curve was drawn. Root samples (0.5 g) of each treatment were soaked in a 10 mL beaker with a mixture of 0.4% TTC and 66 mmol/L phosphate buffer (pH = 7.0), stored at 37 °C for 3 hours, and then 2 mL of 1 mol/L sulfuric acid was added to terminate the reaction. The roots were taken out and grinded with 2 mL ethyl acetate to extract methyl hydrazine, and OD value at 485 nm was recorded using a spectrophotometer (UH5300, HITACHI Co. LTD, Japan). The root activity was calculated according to the standard curve.

Plant growth parameters were assessed in full-bloom stage; plant height, diameter and the number of flowers were recorded. Plant dry weights (105 °C for 30 minutes and dried for 48 h at 80 °C) were determined in full-bloom stage. Leaf areas were obtained using Yaxin-1241 portable leaf area analyser (Beijing Yaxin Technology Co., Ltd., Beijing, China).

The content of mineral elements in leaves and roots was measured as follows: dried leaf or root tissue was

ground, and 0.5 g of the milled powder was mineralized with H_2SO_4 to determine N using the regular Kjeldahl method (Kirk 1950). After grinding the dried tissue and passing it through a 1 mm sieve, 0.5 g of tissue was added into a digestive tube containing 10 mL HNO_3 and 2 mL $HCOl_4$, and then the samples were left undisturbed overnight. The next day, the solution was dissolved in the temperature control digestion instrument, and was gradually heated to 170 °C to keep it boiling. One hour later, the lid was removed while heating was continued until 1 mL of solution was left. After the solution was slightly cooled down, 10 mL of deionized (DI) water was added in and heated to remove the acid. After boiling for five minutes, all the solution was transferred into a 25 mL volumetric flask, and the volume was topped up with DI water. Na, P, K, and Ca contents were then determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (Optima 8000, PerkinElmer Instrument Co., Waltham, MA, USA) (Tabatabai 1997).

For antioxidant enzyme extraction and assay fresh leaves and root samples were rinsed for five minutes in liquid nitrogen, and the frozen tissue samples were stored at –80 °C for further analyses. Enzymes were extracted from 1.0 g of tissue using a mortar and pestle with 5 mL of extraction buffer containing 50 mM potassium phosphate buffer (pH 7.6) and 0.1 mM Na-EDTA. The homogenate was centrifuged at 15 000 rpm for 15 minutes. The supernatant was used for the enzyme assays. All procedures to prepare the enzyme extracts were performed at 4 °C. The prepared enzyme extracts were used for the determination of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), and malondialdehyde (MDA) content as well as the soluble protein content.

SOD activity was assayed using the nitro blue tetrazolium reduction method (Giannopolitis and Ries 1977). The enzyme solution (20 μL) mixed with 3 μL reaction solution (phosphoric acid buffer: methionine: tetrazolium blue: EDTA-Na_2: riboflavin: water = 15: 3: 3: 3: 2.5) was kept under 4000 Lux light for 30 minutes, followed by shading, and the OD value at 560 nm was measured.

The reaction mixture (3 μL) contained 50 mL 0.1mol/L phosphoric acid buffer (pH 6.0), 28 μL guaiacol to dissolve completely, 19 μL of 30% H_2O_2 and 20 μL enzyme extract. POD activity was measured by recording the change of absorption values at 470 nm once every minute for three times (Chance and Maehly 1955).

The activity was assayed for one minute in a reaction solution composed of 2.5 mL reaction solution (0.1 mol/L H_2O_2: 0.1 mol/L pH 7.0 phosphoric acid buffer = 1: 4) and 0.1 mL enzyme extract. The change value of the absorbance of CAT enzyme activity was determined as a decrease in absorbance at 240 nm in one minute (Chance and Maehly 1955).

The 1 mL reaction solution consisted of 1 mmol/L ascorbic acid, 2.5 mmol/L H_2O_2, 50 mmol/L NaH_2PO_4 buffer (pH 7.0), and 0.1 mL enzyme extract and the change of absorption values was measured every 15 seconds within three minutes at 290 nm. The amount of ascorbic acid converted to protein per milligram per minute was calculated to indicate the activity of the APX enzyme (Eppley and Solorzano 1969; Nakano and Asada 1981). Osmotic regulatory substance and cell membrane permeability were assessed as follows: leaf soluble protein content was determined by the Coomassie Bright Blue G-250 method (Bradford 1976). The mixture of 20 μL enzyme solution and 3 mL of G-250 (0.1 g Coomassie brilliant blue G-250 dissolved in 50 mL of 90% ethanol, added 100 mL 85% phosphoric acid, constant volume to 1000 mL, filtered) was left undisturbed for two minutes. Absorbance values at 595 nm were measured to calculate the soluble protein content.

Leaf MDA content was determined by the thiobarbituric acid reaction (Li 2000). One mL of enzyme solution and 2 mL of reaction solution (0.6 g thiobarbituric acid was dissolved in a small amount of 1 mol/L NaOH, and the volume was fixed to 100mL with 10% trichloroacetic acid) were placed in a sealed tube and kept in a boiling water bath for 15 minutes. The tube was cooled down quickly after centrifugation. The supernatant was taken for measuring colorimetric values at 600 nm, 532 nm and 450 nm and the MDA contents were calculated.

Electrolyte leakage was assessed as described by Lutts et al. (1996). Leaf samples were collected and washed three times with deionized water to eliminate any surface-adhered electrolytes. The samples were placed in closed tubes containing 10 mL of deionized water and incubated at room temperature on a rotary shaker (100 rpm) for 24 hours. Then the samples were autoclaved at 120 °C for 20 minutes and the last electrical conductivity was obtained after equilibration at 25 °C.

Leaf proline content was determined by the method described by Bates et al. (1973). Approximately 1 g of leaves was weighed and homogenized in 2 mL of 3% sulfosalicylic acid solution. The homogenate was centrifuged at 13 000 rpm for 10 minutes and 1 mL of supernatant was placed in a test tube. Glacial acetic acid (1 mL) and acid ninhydrin (1 mL) were added to each tube, which were then closed and heated in a 100 °C water bath for one hour. After cooling the samples in an ice bath for 15 minutes, 2 mL of toluene was added to each sample and mixed on a vortex for 20 seconds under a fume hood. The test tubes were left undisturbed for at least 10 minutes to allow for the separation of the toluene and aqueous phases. The toluene phase was carefully pipetted out into a glass test tube and the absorbance was measured at 520 nm, using pure toluene as a blank. The standard curve was prepared using proline in a 3% sulfosalicylic acid solution.

2.5. Data analysis

Microsoft Excel (Microsoft Inc., Redmond, WA, USA), DPS 7.5, OriginPro 2021 and SPSS 11.5 software (SPSS Inc., Chicago, IL, USA) were used for data processing, graphing, and statistical analysis. All data were analysed by two-way ANOVA (NaCl stress, inoculation with AM fungi, and their interactions). A p-value < 0.05 was considered significant. Data are presented as mean ± standard error.

3. RESULTS

3.1. AM fungal colonisation status

Two-factor ANOVA showed that AM fungi, salt and their interaction had significant effects on the mycorrhizal colonisation, spore numbers per 50 g pot media and hyphal density ($p < 0.01$, Table 1). No mycorrhizal colonisation was observed in the treatment group that did not receive AM fungi inoculation. At zero salt concentration, mycorrhizal colonisation percentage of the plants inoculated with *F. mosseae*, *R. intraradices* and *F. mosseae* combined with *R. intraradices* were as 35%, 34% and 51%, respectively. As the salt concentration increased, mycorrhizal colonisation percentage, arbuscular colonisation, and hyphal density of the inoculated treatments decreased. Mycorrhizal colonisation of *F. mosseae* and *R. intraradices* double inoculation was superior to the other inoculation treatments under most NaCl levels (Table 1).

3.2. Effects of AM fungi and salt stress on root traits and root activity

Two-factor ANOVA showed that AM fungi and salt significantly influenced root tip numbers, root length, root surface area, root volume and root activity ($p < 0.01$), while their interactions were not significant ($p > 0.05$, Fig. 1). Salt stress significantly decreased the root tip numbers, root volume, root length, root surface area, and root activity, while inoculation with AM fungi reduced the adverse effects caused by salt stress, and the combined *F. mosseae* and *R. intraradices* treatment showed more profound effects on changing the root traits and root activities (Fig. 1). Under the same salt concentration conditions, inoculation with both *F. mosseae* and *R. intraradices* significantly increased the number of tips, root length, root surface area and root volume compared with non-inoculation treatment (Fig. 1a–d). At the salt concentration of 0.6%, inoculation with *F. mosseae* and *R. intraradices* showed the greatest promotion in root activity, it increased by 89.5% compared with non-inoculation treatment (Fig. 1e).

3.3. Effects of AM fungi and salt stress on mineral element contents

Two-factor ANOVA showed that AM fungi and salt significantly affected the content of N, P, K, Ca, Na, and the ratio of N/Na, P/Na, K/Na, Ca/Na in the leaves and roots ($p < 0.01$, Figs 2–5). The interaction between salt and AM fungi was significant on the above element contents and element ratios except the N content in the leaves, and N and P content in the roots (Figs 2–5). Leaf and root N, P, K, and Ca contents, and the ratios of these elements to Na decreased, while Na increased with the increase of salt concentration. Inoculation treatments increased N, P, K, and Ca contents and the element ratios to a certain degree, but decreased Na compared to the non-inoculation treatment regardless of salt concentrations. The combined *F. mosseae* and *R. intraradices* treatment produced the most beneficial effects on the leaves and roots (Figs 2–5). At 0.4% salt concentration, leaf Na content decreased significantly, and leaf N, P, K, and Ca contents increased significantly after inoculating with AM fungi (Fig. 2), and at zero salt concentration, the ratios of P/Na, K^+/Na^+ and Ca^{2+}/Na^+ increased significantly after inoculating with AM fungi. When the salt concentration was lower than 0.4%, the ratio of each element to Na in leaves decreased significantly with the increase of the salt concentration (Fig. 3). With the increase of salt concentration, the root Na content increased significantly in the same inoculation treatment (Fig. 4). Under 0–0.6% salt concentration, N content increased significantly, while under 0–0.2 %, Ca increased significantly and at 0.6%, K increased significantly in roots after inoculating with AM fungi (Fig. 4). When the salt concentration was lower than 0.4%, the ratio of each element to Na in roots decreased significantly with the increase of the salt concentration (Fig. 5). This result indicates that inoculation with AM fungi changed the absorption of nutrients and the nutrient contents under salt stress.

3.4. Correlation analysis of root traits and leaf element contents

Significant correlations were detected between root morphological traits (root length, root tip numbers, root surface area, root volume) and root activity and nutrient elements in leaves (N, P, K, and Ca), and the ratio of each element to Na (N/Na, P/Na, K^+/Na^+, Ca^{2+}/Na^+), with the highest correlation coefficient (0.964) between root volume and K. A significant negative correlation was observed between root traits (root length, root tip numbers, root surface area, root volume, and root activity) and leaf Na content, with the highest negative correlation coefficient (the absolute value of –0.95) between root surface area and Na (Table 2).

Table 1. Effect of AM fungi and salt on mycorrhizal colonisation, hyphal density and spore density

Treatments		Mycorrhizal colonisation (%)	Spore numbers per 50 g pot media	Hyphal density ($m \cdot g^{-1}$ pot media)
AM fungi	Salt concentration (%)			
–	0	0	0	0
	0.2	0	0	0
	0.4	0	0	0
	0.6	0	0	0
	0.8	0	0	0
Funneliformis mosseae	0	35 ± 0.5b	23.7 ± 0.7b	1.30 ± 0.03ab
	0.2	31 ± 0.6c	21.0 ± 0.6cde	1.11 ± 0.06c
	0.4	28 ± 0.6d	20.3 ± 0.3cde	0.98 ± 0.02d
	0.6	26 ± 0.4e	19.3 ± 0.7def	0.83 ± 0.04e
	0.8	23 ± 0.3fg	17.0 ± 1.0fghi	0.62 ± 0.04fg
Rhizophagus intraradices	0	34 ± 0.4b	22.7 ± 0.9bc	1.21 ± 0.06bc
	0.2	34 ± 0.7b	17.3 ± 1.2fgh	0.86 ± 0.05de
	0.4	28 ± 0.9d	20.7 ± 1.2cde	0.71 ± 0.06f
	0.6	23 ± 0.5fg	15.3 ± 0.3hi	0.69 ± 0.03f
	0.8	22 ± 0.8g	14.7 ± 0.9i	0.56 ± 0.04g
F. mosseae + *R. intraradices*	0	51 ± 0.4a	29.3 ± 1.8a	1.40 ± 0.06a
	0.2	35 ± 0.5b	28.3 ± 1.7a	1.28 ± 0.04b
	0.4	31 ± 0.7c	21.7 ± 0.9bcd	1.29 ± 0.02ab
	0.6	30 ± 0.9c	18.7 ± 0.9efg	1.21 ± 0.06bc
	0.8	24 ± 0.4ef	16.3 ± 0.9ghi	0.85 ± 0.04e
Significance Testing (F-Measure):				
AM fungi		157.321**	714.792**	884.588**
Salt concentration		380.600**	39.493**	76.917**
AM fungi*Salt concentration		38.456**	9.400**	11.901**

According to Tukey's post hoc test, different letters indicate significant differences at the 0.05 level and same letters within each column indicate no significant difference ($p \leq 0.05$) between treatments. "–" means no inoculation; * and ** indicate significant differences at $0.01 \leq p < 0.05$ and $p < 0.01$, respectively; and NS indicates no significant difference, $p > 0.05$. Values presented are the mean ± standard error of three replicates.

3.5. Effects of AM fungi and salt stress on the leaf and root antioxidant enzyme activities

Two-factor ANOVA showed that AM fungi, salt and their interactions significantly affected the activities of SOD, CAT, POD, and APX in both leaves and roots ($p < 0.01$, Tables 3 and 4). The activities of SOD, CAT, POD, and APX increased significantly in *T. erecta* leaves and roots as the salt concentration increased. Inoculation with AM fungi significantly increased the abovementioned enzyme activities in leaves under the same salt level compared to the no inoculation, except SOD in the treatment inoculated with *R. intraradices* without added salt (Table 3). When the salt concentration was higher than 0.6%, the activities of SOD, POD and APX in roots were significantly increased by the inoculation (Table 4). The combined *F. mosseae* and *R. intraradices* treatment produced the most effects (Tables 3 and 4).

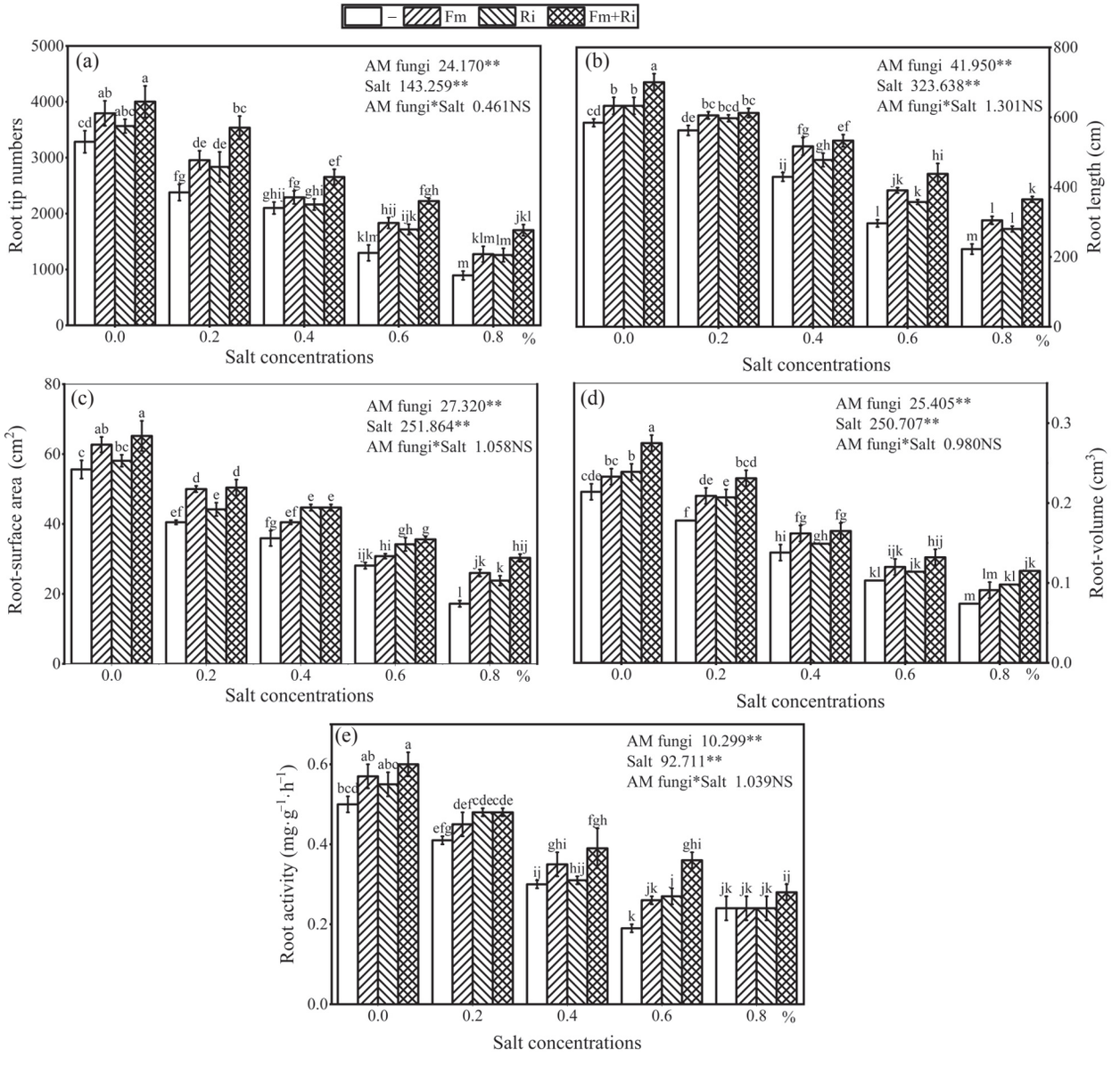

Fig. 1. Root traits [root tip numbers (a), root length (b), root surface area (c), root volume (d)] and root activity (e) of *Tagetes erecta* under different levels of salinity (0, 0.2%, 0.4%, 0.6% and 0.8%) in response to different inoculation treatments. "–" means no inoculation, Fm, Ri, and Fm + Ri, respectively signify *Funneliformis mosseae*, *Rhizophagus intraradices*, and *Funneliformis mosseae* and *Rhizophagus intraradices* combined. Letters indicate significant differences ($p < 0.05$) according to Tukey's post hoc test and the error bars represent the standard error (SE). * and ** indicate significant differences at $0.01 < p < 0.05$ and $p < 0.01$, respectively, and NS indicates no significant difference, $p > 0.05$.

3.6. Effects of AM fungi and salt stress on leaf osmotic regulatory substances and cell membrane permeability

Two-factor ANOVA showed that AM fungi and salt had significant effects on soluble protein, proline, relative conductivity and MDA contents in leaves ($p < 0.01$, Table 5). The interaction between AM fungi and salt had a significant effect on proline ($p < 0.01$) and relative conductivity ($0.01 \leq p < 0.05$) while there was no significant effect on soluble protein and MDA contents ($p > 0.05$) in leaves (Table 5). Soluble protein content decreased with the rising salt level, while proline, MDA contents, and leaf relative electrical conductivity increased. Inoculation with AM fungi raised the soluble protein and proline contents but lowered relative electrical conductivity and MDA contents to different degrees at the same salt level compared to non-inoculation treatment. The com-

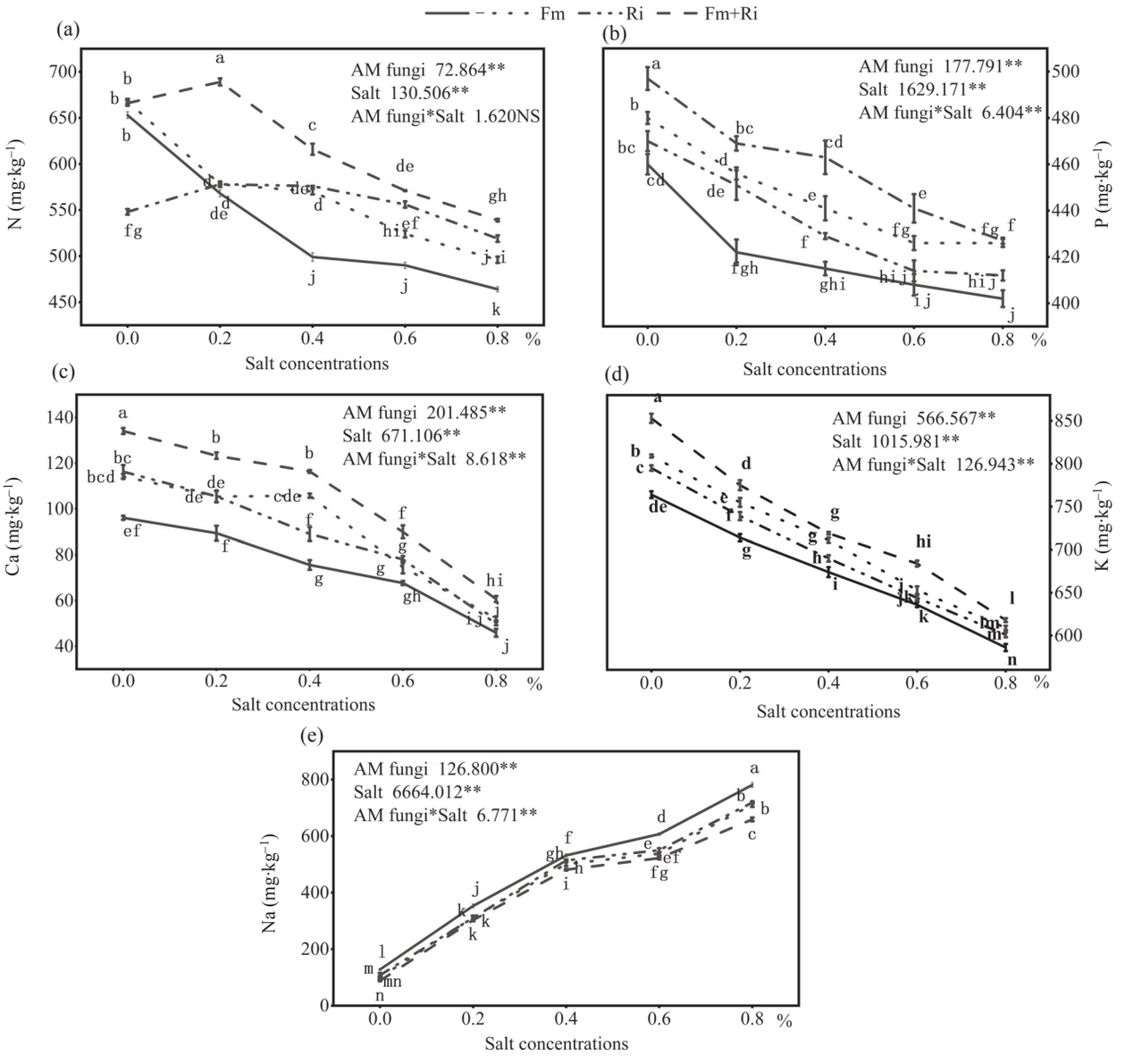

Fig. 2. Element contents [N (a), P (b), K (c), Ca (d), Na (e)] in the leaves of *Tagetes erecta* under different levels of salinity (0, 0.2%, 0.4%, 0.6% and 0.8%) in response to different inoculation treatments. "–" means no inoculation, Fm, Ri, and Fm + Ri, respectively signify *Funneliformis mosseae*, *Rhizophagus intraradices*, and *Funneliformis mosseae* and *Rhizophagus intraradices* combined. Letters indicate significant differences ($p < 0.05$) according to Tukey's post hoc test and the error bars represent the standard error (SE). * and ** indicate significant differences at $0.01 < p < 0.05$ and $p < 0.01$, respectively, and NS indicates no significant difference, $p > 0.05$.

bined *F. mosseae* and *R. intraradices* treatment produced the greatest beneficial effects (Table 5).

3.7. Effects of AM fungi and salt stress on plant growth and flowering

AM fungal inoculation treatments resulted in different effects on plant growth and flower diameter both with and without adding salt. Two-factor ANOVA showed that AM fungi and salt significantly affected shoot dry weight, root dry weight, and single leaf area ($p < 0.01$, Table 6), while their interactions significantly affected only single leaf area ($0.01 \leq p < 0.05$) (Table 6). *F. mosseae* and *R. intraradices* combined treatment produced more profound effects in plant growth and flower diameter among all inoculation treatments (Table 6). As the salt concentration increased (0–0.8%), the growth of *T. erecta* showed a decreasing trend for every inoculation treatment. Compared

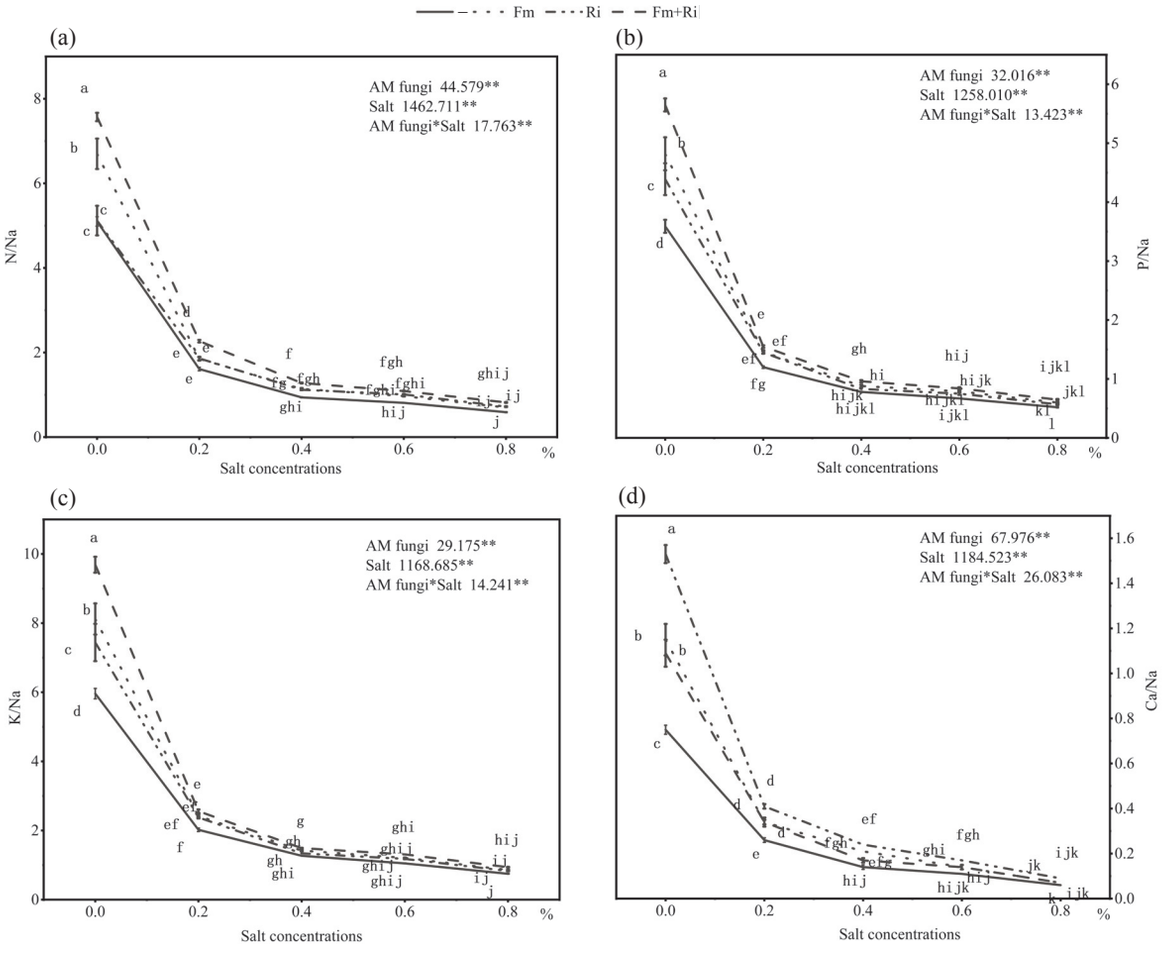

Fig. 3. Elemental ratio [N/Na (a), P/Na (b), K/Na (c), Ca/Na (d)] in the leaves of *Tagetes erecta* under different levels of salinity (0, 0.2%, 0.4%, 0.6% and 0.8%) in response to different inoculation treatments. "–" means no inoculation, Fm, Ri, and Fm + Ri, respectively signify *Funneliformis mosseae*, *Rhizophagus intraradices*, and *Funneliformis mosseae* and *Rhizophagus intraradices* combined. Letters indicate significant differences ($p < 0.05$) according to Tukey's post hoc test and the error bars represent the standard error (SE). * and ** indicate significant differences at $0.01 < p < 0.05$ and $p < 0.01$, respectively, and NS indicates no significant difference, $p > 0.05$.

Table 2. Correlation of root architecture (root surface area, root volume, root length, root tip numbers) and root activity with leaf element contents and element ratios

Root architecture	Na	N	P	K	Ca	N/Na	P/Na	K/Na	Ca/Na
Root surface area	–0.950**	0.823**	0.877**	0.963**	0.893**	0.852**	0.824**	0.825**	0.823**
Root volume	–0.943**	0.807**	0.870**	0.964**	0.898**	0.804**	0.773**	0.778**	0.787**
Root length	–0.917**	0.816**	0.858**	0.952**	0.923**	0.717**	0.681**	0.685**	0.696**
Tip numbers	–0.932**	0.837**	0.879**	0.953**	0.898**	0.814**	0.772**	0.772**	0.775**
Root activity	–0.905**	0.771**	0.834**	0.913**	0.828**	0.800**	0.778**	0.780**	0.780**

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

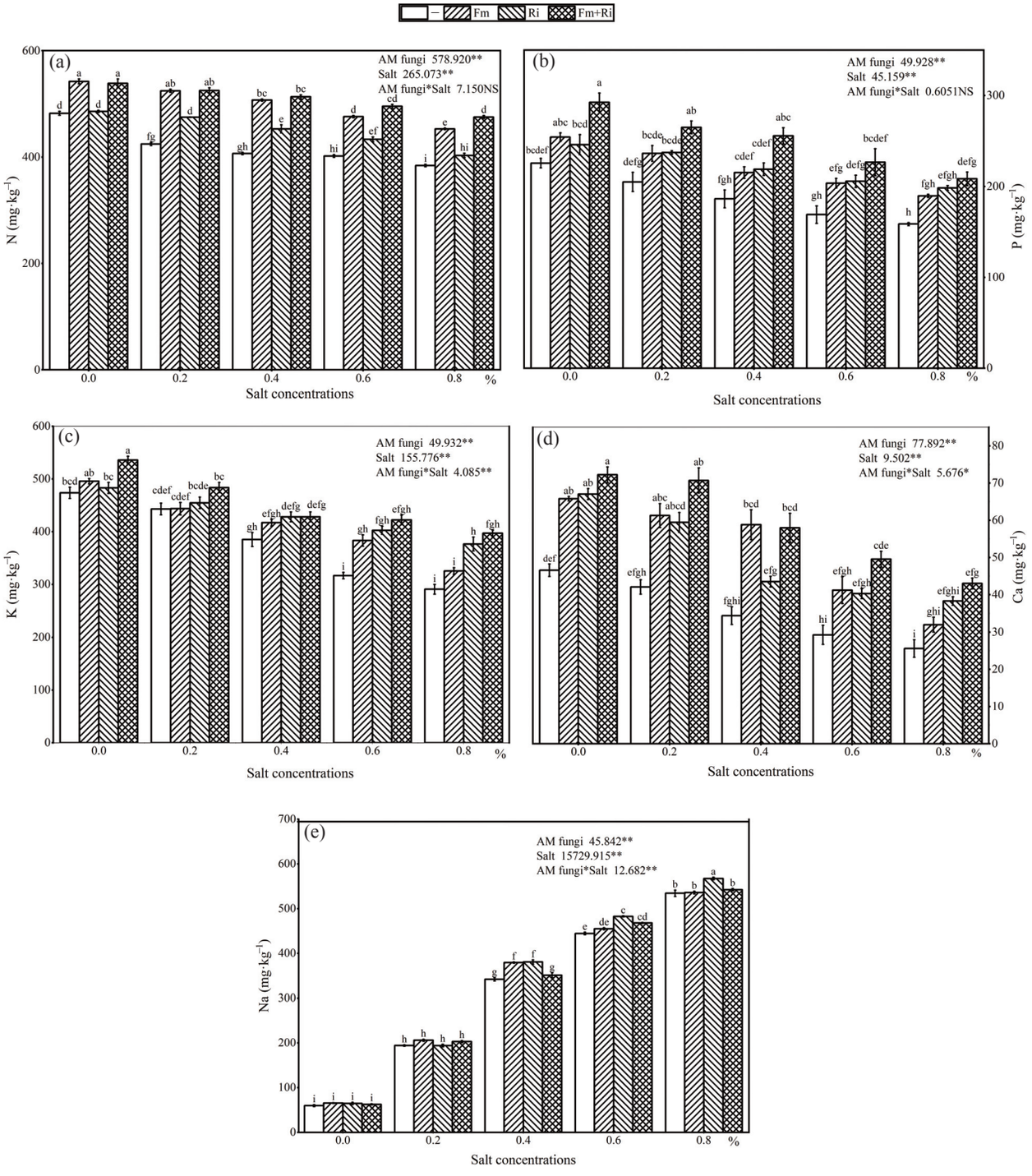

Fig. 4. Element contents [N (a), P (b), K (c), Ca (d), Na (e)] in the roots of *Tagetes erecta* under different levels of salinity (0, 0.2%, 0.4%, 0.6% and 0.8%) in response to different inoculation treatments. "–" means no inoculation, Fm, Ri, and Fm + Ri, respectively signify *Funneliformis mosseae*, *Rhizophagus intraradices*, and *Funneliformis mosseae* and *Rhizophagus intraradices* combined. Letters indicate significant differences ($p < 0.05$) according to Tukey's post hoc test and the error bars represent the standard error (SE). * and ** indicate significant differences at $0.01 < p < 0.05$ and $p < 0.01$, respectively, and NS indicates no significant difference, $p > 0.05$.

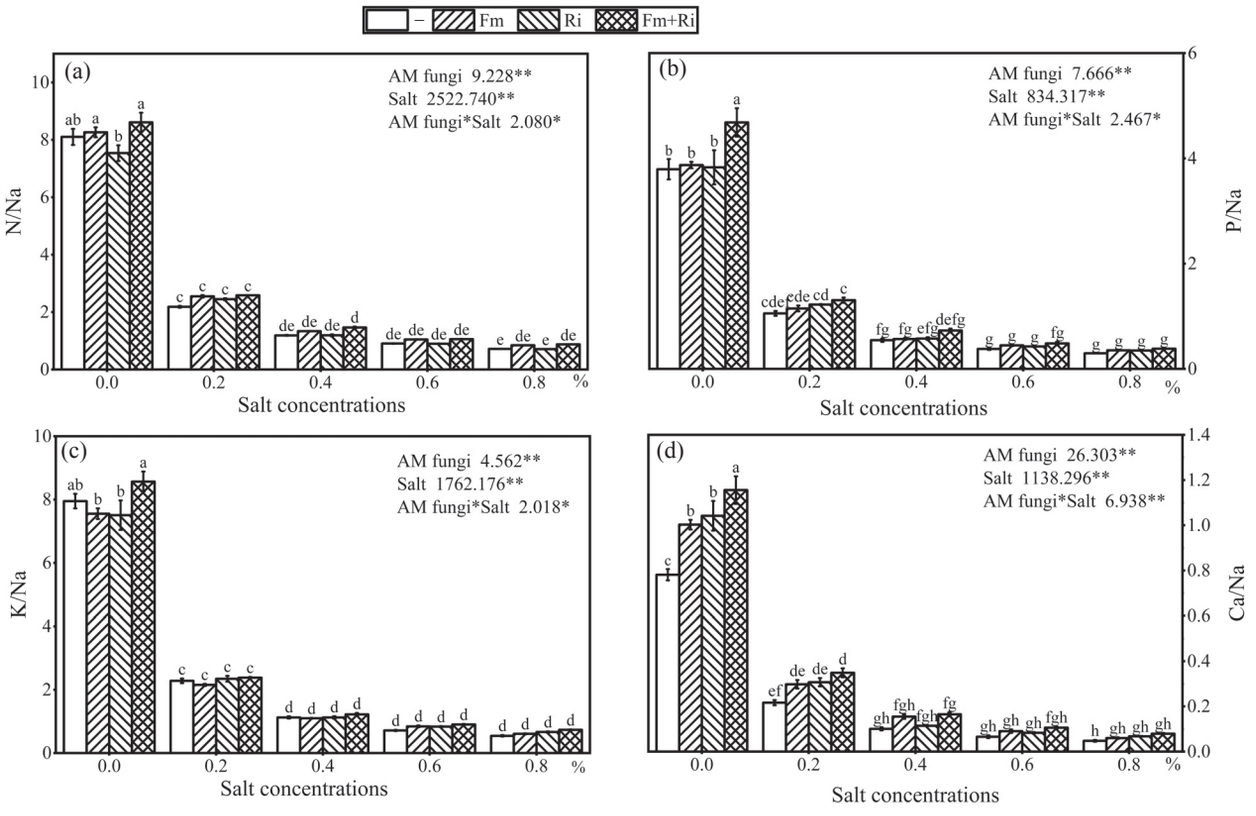

Fig. 5. Elemental ratio [N/Na (a), P/Na (b), K/Na (c), Ca/Na (d)] in the roots of *Tagetes erecta* under different levels of salinity (0, 0.2%, 0.4%, 0.6% and 0.8%) in response to different inoculation treatments. "–" means no inoculation, Fm, Ri, and Fm + Ri, respectively signify *Funneliformis mosseae*, *Rhizophagus intraradices*, and *Funneliformis mosseae* and *Rhizophagus intraradices* combined. Letters indicate significant differences ($p < 0.05$) according to Tukey's post hoc test and the error bars represent the standard error (SE). * and ** indicate significant differences at $0.01 < p < 0.05$ and $p < 0.01$, respectively, and NS indicates no significant difference, $p > 0.05$.

with the non-inoculated plants, shoot dry biomass and single leaf area of inoculated plants was significantly higher under all salt treatments; and at the 0.6% salt concentration, so was plant height and root dry biomass. Under no salt addition conditions, plant height, shoot dry weight, root dry biomass and single leaf area of *T. erecta* inoculated with both *F. mosseae* and *R. intraradices* were higher than that with the single AM fungus inoculation. Under 0.4% salt concentration, plant height, root dry biomass and single leaf area increased significantly with *F. mosseae* and *R. intraradices* combined inoculation compared to only *R. intraradices* inoculation treatment (Table 6).

4. DISCUSSION

This study showed that salt stress inhibited AM fungal colonisation; however, the influence of salt stress on AM fungus colonisation under dual inoculation was lesser than that under single inoculation. It has been demonstrated previously that AM fungi improve stress tolerance and growth, regulate morphological structure and improve the absorption of water and mineral nutrients in plants (Begum et al. 2019; Adeyemi et al. 2021).

The effectiveness of AM symbiosis in promoting plant growth and salt tolerance depends on host plants, AM fungal species and soil environmental conditions (Estrada et al. 2013; Pellegrino and Bedini 2014; Garg and Pandey 2015). On the whole, our experiment demonstrated more beneficial effects for *F. mosseae* than *R. intraradices*, and the dual inoculation with both *F. mosseae* and *R. intraradices* even further improved salt stress resistance of host plant *T. erecta*. At zero salt concentration, this dual inoculation performed better in promoting plant growth than the single species, while only at 0.4% salt concentration, *F. mosseae* and *R. intraradices* combined treatment showed a greater effect of enhancing plant growth and salt resistance compared with *R. intraradices*. So it is evident that *F. mosseae* should play a key role in the inoculation. In addition, the type of mixed species also plays a role in plant reaction. The ultimate effect of AM symbiosis on reducing salt

Table 3. Effect of AM fungi and salt on superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) activity in leaves

Treatments		SOD	CAT	POD	APX
AM fungi	Salt (%)	($U \cdot min^{-1} \cdot g^{-1}$)	($U \cdot g^{-1} \cdot min^{-1}$)	($U \cdot g^{-1} \cdot min^{-1}$)	($U \cdot g^{-1} \cdot min^{-1}$)
–	0	30.1 ± 0.9m	15.3 ± 0.7m	33.0 ± 0.6o	78.5 ± 4.1m
	0.2	33.1 ± 0.3kl	21.3 ± 0.5l	42.2 ± 0.6l	95.2 ± 0.8k
	0.4	35.8 ± 0.7ij	27.0 ± 1.2ij	52.5 ± 0.3i	113.8 ± 3.1i
	0.6	39.5 ± 0.3h	30.7 ± 0.5gh	56.6 ± 1.0h	129.7 ± 1.9g
	0.8	43.0 ± 0.3efg	36.0 ± 0.3d	69.6 ± 0.6de	153.0 ± 1.7e
Funneliformis mosseae	0	32.8 ± 0.7l	23.7 ± 0.7k	38.4 ± 0.6m	91.7 ± 1.2kl
	0.2	37.7 ± 1.3hi	27.4 ± 0.4ij	46.9 ± 0.6j	115.3 ± 3.8i
	0.4	42.0 ± 0.8g	32.3 ± 0.5fg	57.3 ± 0.7h	155.3 ± 0.3e
	0.6	45.3 ± 0.4d	34.3 ± 0.6def	68.2 ± 0.6e	189.0 ± 1.2b
	0.8	50.1 ± 0.7bc	39.4 ± 0.6c	80.3 ± 0.5b	183.3 ± 1.8c
Rhizophagus intraradices	0	29.5 ± 1.3m	21.3 ± 0.5l	36.3 ± 0.8n	87.9 ± 1.1l
	0.2	35.7 ± 0.2ij	25.4 ± 0.4jk	44.9 ± 0.7k	123.0 ± 1.5h
	0.4	42.7 ± 0.5efg	30.9 ± 0.2gh	61.5 ± 0.8g	134.3 ± 0.3fg
	0.6	44.6 ± 0.6de	33.7 ± 0.4ef	64.5 ± 0.6f	156.7 ± 1.8de
	0.8	49.2 ± 0.6c	43.8 ± 2.7b	74.9 ± 1.0c	181.3 ± 1.8c
F. mosseae + *R. intraradices*	0	35.1 ± 0.8jk	24.3 ± 0.6k	41.8 ± 0.7l	106.0 ± 2.2j
	0.2	42.4 ± 0.7fg	28.7 ± 0.2hi	52.4 ± 0.8i	137.3 ± 1.5f
	0.4	44.3 ± 0.6def	32.8 ± 0.2fg	62.4 ± 1.2g	161.3 ± 2.7d
	0.6	52.0 ± 1.1ab	35.3 ± 0.8de	70.3 ± 0.5d	194.3 ± 0.9a
	0.8	53.8 ± 1.3a	47.6 ± 0.5a	85.2 ± 0.3a	196.7 ± 1.8a
Significance Testing (F-Measure)					
AM fungi		120.741**	77.566**	238.225**	444.384**
Salt		302.205**	366.651**	1997.800**	1247.066**
AM fungi*salt		4.272**	4.289**	9.348**	19.749**

According to Tukey's post hoc test, different letters indicate significant differences at the 0.05 level and same letters within each column indicate no significant difference ($p \leq 0.05$) between treatments. "–" means no inoculation; * and ** indicate significant differences at $0.01 \leq p < 0.05$ and $p < 0.01$, respectively; and NS indicates no significant difference, $p > 0.05$. Values presented are the mean ± standard error of three replicates.

stress depends on the combination of inocula and plant species (Al-Karaki and Williams 2021). Thus, more combinations with various species need to be tested to find the most effective combinations.

Plants usually modify their root structure under salt stress to regulate the absorption of Na^+ by roots and enhance salt tolerance (Lata et al. 2019). AM fungi form arbuscule and mycelia in plant roots and rhizosphere, and the mycelium network of plant roots significantly enhances the ability of roots to enter the soil and increases the contact between roots and surrounding soil, thus promoting the absorption of water and mineral nutrients and therefore also plant growth (Bowles et al. 2016; Huang et al. 2020). Many experiments have shown that colonisation by AM fungi increases root length, root surface area, root tip numbers and root volume, improves absorption of mineral nutrients, and increases the utilization efficiency of nutrient elements (Abbaspour et al. 2021; Nacoon et al. 2021). Wang, J. et al. (2020) reported that salt tolerance in maize was positively correlated with taproot depth, but negatively correlated with Na^+ content in shoots. The present study showed that salt stress in-

Table 4. Effect of AM fungi and salt on superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) activity in roots

Treatments		SOD	CAT	POD	APX
AM fungi	Salt (%)	(U·min^{-1}·g^{-1})	(U·g^{-1}·min^{-1})	(U·g^{-1}·min^{-1})	(U·g^{-1}·min^{-1})
–	0	40.4 ± 0.6n	22 ± 0.7l	41.4 ± 0.5k	93.3 ± 0.8m
	0.2	41.3 ± 0.2mn	27.9 ± 0.5jk	49.8 ± 0.8j	107.8 ± 0.8kl
	0.4	43.5 ± 0.7klm	33.2 ± 0.9hi	55.5 ± 0.5gh	123.4 ± 1.2j
	0.6	46.7 ± 0.2hij	36.7 ± 0.7efgh	59.8 ± 0.8f	133.8 ± 1.8i
	0.8	48.4 ± 0.7hi	41.7 ± 0.7cd	70.1 ± 0.4de	159.8 ± 0.8g
Funneliformis mosseae	0	42.5 ± 0.6lmn	25.4 ± 0.5jkl	49.2 ± 0.7j	112.9 ± 0.5k
	0.2	45 ± 0.4jkl	29.6 ± 0.5ij	50.3 ± 0.5j	137.4 ± 0.6i
	0.4	49.6 ± 0.4fgh	34.3 ± 1.2hi	58.2 ± 0.5fg	166.5 ± 0.4f
	0.6	51.4 ± 0.4efg	39.6 ± 1.2defg	72.7 ± 0.5cd	193.1 ± 1.7c
	0.8	55.6 ± 0.4cd	45.8 ± 0.6bc	88.4 ± 0.6a	197.6 ± 1.2c
Rhizophagus intraradices	0	41.5 ± 0.6mn	23.7 ± 0.7kl	48.4 ± 0.6j	106.3 ± 0.8l
	0.2	42.6 ± 0.6lmn	27.5 ± 0.5jk	50.5 ± 0.5ij	144.4 ± 1.3h
	0.4	51.5 ± 1.0ef	35.3 ± 0.5gh	67.6 ± 0.5e	168.3 ± 0.8ef
	0.6	53.8 ± 0.2de	40.5 ± 0.2def	70.9 ± 0.9d	173.5 ± 1.8e
	0.8	59.5 ± 0.5b	46.6 ± 0.2b	83.1 ± 0.8b	192.5 ± 1.8c
F. mosseae + *R. intraradices*	0	46 ± 0.6ijk	27.4 ± 0.9jk	53.6 ± 0.6hi	124.5 ± 0.5j
	0.2	48.5 ± 0.7ghi	36.5 ± 0.9fgh	59.6 ± 1.0f	157.6 ± 1.2g
	0.4	55.4 ± 0.6cd	41.3 ± 1.8cde	67.7 ± 0.5e	183.7 ± 1.1d
	0.6	57.6 ± 0.2bc	42.5 ± 1.2bcd	75.3 ± 0.6c	208.6 ± 1.2b
	0.8	62.6 ± 0.6a	52.6 ± 1.4a	90.4 ± 0.6a	224.5 ± 1.2a
Significance Testing (F-Measure)					
AM fungi		283.713**	68.225**	397.624**	2000.213**
Salt		447.061**	369.550**	1840.334**	3243.047**
AM fungi*salt		13.151**	2.785**	29.660**	53.689**

According to Tukey's post hoc test, different letters indicate significant differences at the 0.05 level and same letters within each column indicate no significant difference ($p \leq 0.05$) between treatments. "–" means no inoculation; * and ** indicate significant differences at $0.01 \leq p < 0.05$ and $p < 0.01$, respectively; and NS indicates no significant difference, $p > 0.05$. Values presented are the mean ± standard error of three replicates.

hibited the development and elongation of *T. erecta* roots, hindered the development of the root system, and deteriorated the root morphological traits, which was not beneficial for enhancing salt tolerance and absoption of mineral nutrients. The AM fungi inhibited Na^+ absorption and in- creased K^+ absorption by increasing total root length and root tip number, root surface area, and root activities, so K^+/Na^+ tended to be balanced, which increases the active root absorption area to respond to the salt stress (Guo et al. 2017).

The accumulation of harmful salt ions in soil affects the uptake of mineral nutrients by plants (Loudari et al. 2020). Excess Na^+ in soil inhibits the acquisition of other nutrients by disrupting various transporters in the root plasma membrane (e.g., K-selective ion channels), and competes with K^+ for binding sites necessary for various cellular functions (Hajiboland 2013). Inoculation with AM fungi reduced the absorption and accumulation of Na^+ in *T. erecta* roots under salt stress and promoted the transport of Na^+ to shoots; thus, reducing osmotic stress on the roots

Table 5. Effects of AM fungi and salt on leaf soluble protein, proline, relative conductivity, and malondialdehyde (MDA)

Treatments		Soluble protein ($mg \cdot g^{-1}$ FW)	Proline ($mg \cdot g^{-1}$ FW)	Relative conductivity (%)	MDA ($mg \cdot g^{-1}$ FW)
AM fungi	Salt (%)				
–	0	13.3 ± 0.7bc	11.6 ± 1.0k	15 ± 0.8cdef	10.5 ± 1.2ghi
	0.2	10.1 ± 1.4d	15.0 ± 0.4j	16 ± 1.6cd	14.5 ± 1.1ef
	0.4	9.8 ± 0.8de	23.7 ± 0.3g	17 ± 0.7c	18.2 ± 0.5bc
	0.6	5.5 ± 0.8g	26.9 ± 0.4e	23 ± 1.0b	20.6 ± 1.0b
	0.8	5.3 ± 0.8g	30.0 ± 0.5d	27 ± 0.7a	25.3 ± 1.2a
Funneliformis mosseae	0	15.3 ± 1.2ab	20.3 ± 0.6h	9 ± 0.9h	9.6 ± 0.8hij
	0.2	13.7 ± 0.6bc	20.8 ± 0.3h	13 ± 0.9fg	10.6 ± 0.6ghi
	0.4	10.4 ± 0.6d	26.4 ± 0.5e	15 ± 0.5def	15.3 ± 1.2def
	0.6	9.3 ± 0.7de	32.3 ± 0.6c	18 ± 1.9c	17.7 ± 0.7cd
	0.8	7.6 ± 0.4efg	36.7 ± 0.4ab	21 ± 0.5b	20.5 ± 1.2b
Rhizophagus intraradices	0	14.5 ± 0.6ab	17.2 ± 0.3i	11 ± 1.0gh	11.5 ± 1.0gh
	0.2	11.5 ± 1.0cd	20.4 ± 0.6h	11 ± 1.2gh	13.1 ± 0.9fg
	0.4	9.7 ± 0.8de	25.0 ± 0.4fg	16 ± 0.8cde	16.3 ± 0.6cde
	0.6	9.5 ± 0.8de	30.3 ± 0.6d	22 ± 0.7b	18.6 ± 0.9bc
	0.8	6.8 ± 0.4fg	35.6 ± 0.3b	24 ± 0.4ab	24.4 ± 1.1a
F. mosseae + *R. intraradices*	0	16.4 ± 1.1a	21.3 ± 0.6h	9 ± 0.6h	7.7 ± 0.8j
	0.2	14.4 ± 1.2ab	25.5 ± 0.4ef	10 ± 0.8h	8.0 ± 0.6ij
	0.4	13.3 ± 0.7bc	28.9 ± 0.6d	14 ± 0.5ef	10.6 ± 1.0ghi
	0.6	10.8 ± 0.6d	32.6 ± 0.4c	15 ± 1.0def	12.8 ± 0.6fg
	0.8	9.2 ± 1.2def	37.2 ± 0.3a	16 ± 0.6cde	15.3 ± 1.2def
Significance Testing (F-Measure)					
AM fungi		19.066**	217.812**	53.550**	52.454**
Salt		49.048**	811.612**	100.732**	94.190**
AM fungi*salt		0.756NS	6.664**	3.220*	1.698NS

According to Tukey's post hoc test, different letters indicate significant differences at the 0.05 level and same letters within each column indicate no significant difference ($p \leq 0.05$) between treatments. "–" means no inoculation; * and ** indicate significant differences at $0.01 \leq p < 0.05$ and $p < 0.01$, respectively; and NS indicates no significant difference, $p > 0.05$. Values presented are the mean ± standard error of three replicates.

(Parihar et al. 2020). The absolute content and distribution pattern of key mineral elements plays an important role in plant salt tolerance. Additionally, the relative ratios of key mineral nutrients to Na^+, such as the K^+/Na^+ and Ca^{2+}/Na^+ ratios, better reflect the nutrient balance, physiological metabolism, and health status of plants. In general, under salt stress, the accumulation of harmful salt ions (Na^+) leads to element absorption deficiency, and low nutrient uptake and utilization are the major factors causing stunted growth. A low K^+/Na^+ ratio causes dehydration, membrane dysfunction, and ion toxicity in cells. Therefore, plants must maintain a high cytoplasmic K^+/Na^+ ratio under salt stress, which is essential for maintaining normal cell function, growth, and development (Zhao et al. 2020). The external hyphae of AM fungi are important for increasing the absorption of soil mineral cations (K^+, Ca^{2+}, Mg^{2+} and Fe^{3+}) (Alves et al. 2021). The difference in AM fungal mycelium length is the main driving force for nutrient absorption, and the external mycelia of AM fungi can obtain nutrients over

Table 6. Effect of AM fungi and salt on plant height, shoot dry weight, root dry weight, single leaf area and flower diameter

Treatments		Plant height (cm)	Shoot dry weight (g)	Root dry weight (g)	Single leaf area (mm^2)	Flower diameter (cm)
AM fungi	Salt (%)					
–	0	39.5 ± 2.2bc	1.24 ± 0.07efg	0.6 ± 0.02c	686.6 ± 4.2c	5.8 ± 0.09abc
	0.2	36.5 ± 1.1defg	1.12 ± 0.02hi	0.5 ± 0.02defg	607.0 ± 2.9h	5.7 ± 0.03abc
	0.4	32.6 ± 0.2ij	0.94 ± 0.01jk	0.4 ± 0.01hijk	606.6 ± 3.5h	5.5 ± 0.03bcde
	0.6	30.2 ± 1.2j	0.87 ± 0.03k	0.3 ± 0.01m	508.5 ± 4.1l	4.3 ± 0.09gh
	0.8	30.0 ± 1.2j	0.75 ± 0.03l	0.2 ± 0.01n	428.8 ± 1.5o	3.4 ± 0.07h
Funneliformis mosseae	0	40.3 ± 1.1bc	1.40 ± 0.09cd	0.7 ± 0.03b	717.5 ± 3.5b	5.8 ± 0.07abc
	0.2	38.2 ± 1.1cd	1.35 ± 0.05de	0.5 ± 0.02d	668.6 ± 2.3d	5.8 ± 0.12abc
	0.4	35.2 ± 0.6fghi	1.25 ± 0.03efg	0.5 ± 0.01efgh	648.8 ± 3.9fg	5.2 ± 0.12cdef
	0.6	35.4 ± 0.6defgh	1.23 ± 0.07fgh	0.4 ± 0.01ijk	538.7 ± 4.2j	4.5 ± 0.39fg
	0.8	33.5 ± 0.8hi	1.06 ± 0.04ij	0.4 ± 0.01lm	459.9 ± 2.5n	4.1 ± 0.09gh
Rhizophagus intraradices	0	41.0 ± 0.6b	1.47 ± 0.02bc	0.6 ± 0.06c	711.7 ± 2.1b	6.1 ± 0.03abc
	0.2	38.0 ± 1.1cde	1.32 ± 0.03def	0.5 ± 0.01def	657.5 ± 3.1ef	5.9 ± 0.03abc
	0.4	33.4 ± 0.6hi	1.21 ± 0.03fgh	0.5 ± 0.02ghij	641.5 ± 3.8g	5.3 ± 0.17bcdef
	0.6	34.2 ± 0.6ghi	1.17 ± 0.02gh	0.4 ± 0.01jkl	527.7 ± 3.6k	4.6 ± 0.19efg
	0.8	32.5 ± 1.4ij	0.95 ± 0.03jk	0.3 ± 0.01m	488.7 ± 1.8m	4.2 ± 0.09gh
F. mosseae + *R. intraradices*	0	45.3 ± 0.5a	1.71 ± 0.04a	0.8 ± 0.02a	785.6 ± 3.0a	6.4 ± 0.07a
	0.2	40.1 ± 0.6bc	1.52 ± 0.04b	0.6 ± 0.02c	663.5 ± 3.7de	6.2 ± 0.09ab
	0.4	37.7 ± 0.5cdef	1.30 ± 0.02def	0.5 ± 0.01de	657.5 ± 3.1ef	5.6 ± 0.12abcd
	0.6	35.4 ± 0.6efgh	1.25 ± 0.03efg	0.5 ± 0.01fghi	594.8 ± 2.9i	4.8 ± 0.42defg
	0.8	34.5 ± 0.8ghi	1.16 ± 0.03ghi	0.4 ± 0.01klm	515.3 ± 2.8l	4.7 ± 0.36efg
Significance Testing (F-Measure)						
AM fungi		21.558**	94.332**	38.131**	457.999**	10.463**
Salt		56.886**	94.831**	180.224**	3713.933**	91.601**
AM fungi*salt		1.060NS	0.929NS	1.023NS	25.522**	1.178NS

According to Tukey's post hoc test, different letters indicate significant differences at the 0.05 level and same letters within each column indicate no significant difference ($p \leq 0.05$) between treatments. "–" means no inoculation;* and ** indicate significant differences at $0.01 \leq p < 0.05$ and $p < 0.01$, respectively; and NS indicates no significant difference, $p > 0.05$. Values presented are the mean ± standard error of three replicates.

long distance and in narrow soil pores (Ortaş et al. 2019). In addition, AM fungal colonisation can enhance soil aggregation through external hyphae to filter soil water and nutrients, and some AM fungi can also secrete a variety of compounds to improve root colonisation and spore number of AM fungi, therefore further promoting the acquisition of soil nutrients by roots (Jiang et al. 2020; Moitinho et al. 2020). AM fungi promoted the absorption of N, P, K, and Ca in leaves, decreased the accumulation of Na^+, and increased the ratios of K^+/Na^+, Ca^{2+}/Na^+, P/Na, and N/Na under salt stress, and thus alleviated salt stress in *T. erecta*. This may be related to the increase of K^+ uptake due to up-regulation of the K^+ transporter in roots by AM fungi, while there is a competitive relationship between K^+ and Na^+ uptake (Fall et al. 2017). AM fungi limit the absorption and accumulation of Na^+ by regulating the expression levels of the AKT2, SOS1, and SKOR genes which regulate K^+ or Na^+ absorption and transport in the roots of host plants to maintain K^+ and Na^+ homeostasis (Estrada et al. 2013). Furthermore, Ca^{2+}

is an important cellular messenger of growth signals (Zhu et al. 2013). The accumulation of Ca^{2+} facilitates colonisation of AM fungi, resulting in better plant growth (Abbaspour et al. 2021). The present results showed that salt stress reduced the uptake and utilization of N, P, K and Ca in *T. erecta*, while inoculation with AM fungi improved the uptake and utilization of Ca and P. The possible reason for this could be that AM fungi regulate the expression of K^+/Na^+ transporter, and H^+ pump, creating conditions for transporting P and Ca and reducing Na^+ and Cl^- content, which are harmful to plant growth (Theerawitaya et al. 2020).

Plants under salt stress are often affected by Na^+ toxicity, osmotic stress, nutrient deficiency, and other factors resulting in poor growth (Yasmeen et al. 2019). Excessive Na^+ disrupts the internal balance of cells, resulting in degradation of proteins and damage to cell membranes, which affects the metabolic processes in cells (Hasanuzzaman et al. 2013). K^+ in plant cells is used not only to stabilize the pH level in the cytoplasm, but also to increase osmotic potential in vacuoles, which prevents cell membrane damage (Marschner 2012). In this study, inoculation with AM fungi inhibited the accumulation of Na^+, promoted the uptake of K^+, maintained a higher K^+/Na^+ ratio, prevented the destruction of the metabolic process and inhibited MDA synthesis. Therefore, selective ion absorption seems to be the main mechanism regulating plant cell permeability in AM plants (Hajiboland 2013). The cell membrane system is further damaged from the excessive accumulation of reactive oxygen species (O^{2-}) and superoxide radicals (OH^-) under salt stress (Nahar et al. 2016; Tiwari et al. 2016). However, the accumulation of hydrogen peroxide under salt stress was significantly reduced in plants inoculated with AM fungi, and the lipid oxidative damage in branches and roots was also low (Hajiboland et al. 2010; Abbaspour et al. 2021). AM fungal colonisation hinders the accumulation of ROS, and thus reduces lipid oxidative damage (Estrada et al. 2013; Bompadre et al. 2014). In the present study, we found that inoculation with AM fungi significantly promoted the activity of antioxidant enzymes under salt stress compared to non-inoculation treatment. Plants mitigate salt stress by producing nitric oxide (NO) and other compounds that counter the formation of ROS (Gupta et al. 2021). NO directly or indirectly triggers the expression of several redox-regulated genes, and helps activate many antioxidant enzymes, including CAT, APX, glutathione reductase, and SOD (Gupta et al. 2021) to maintain normal physiological and metabolic levels in plants (Liu et al. 2019; Santander et al. 2020; Qian et al. 2021). The increased accumulation of osmotic regulatory substances such as proline and soluble protein by AM fungi can maintain the normal metabolism of plants. Studies have shown that salt stress can lead to inhibition of plant protein synthesis or protein degradation, but inoculation with AM fungi can increase soluble protein content, maintain cell osmotic balance, and alleviate the damage of salt stress on plants (Medeiros et al. 2015; Islam et al. 2021). Soluble protein can be reused as nitrogen after stress and can be converted into free amino acids to help maintain physiological functions of plants under severe stress (Monreal et al. 2007; Abid et al. 2018; Islam et al. 2021).

The present experiment showed that the AM fungal inoculation, particularly with the combination of *F. mosseae* and *R. intraradices*, strongly enhanced the osmotic regulatory mechanisms, improved the activity of antioxidant enzymes, reduced membrane lipid peroxidation, and thus reduced salt stress damage and improved plant growth and salt tolerance. Further studies are required to investigate their regulating gene expression underpinning the molecular mechanism of increasing salt tolerance by AM symbiosis.

5. CONCLUSIONS

This study revealed the important role of AM fungi in alleviating salt stress in *T. erecta* plants through altering root morphological traits, accumulation of mineral nutrients, and the balance of nutrients and Na. AM fungi regulated antioxidant enzymes and the osmotic metabolism system, and thus enhanced the plant's tolerance to salt stress. The effect of dual inoculation with two AM fungal species was more profound than that of a single species. Research on the pathways of enhanced plant salt tolerance has practical significance and broad application prospects.

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Arbuskulaar-mükoriissete seentega inokuleerimise mõju *Tagetes erecta* juuretunnustele ja soolsustaluvusele

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Mulla sooldumine on ülemaailmne keskkonnaprobleem. Arbuskulaar-mükoriissetel (AM) seentel on võime suurendada taimede vastupidavust stressile, näiteks parandada taimede soolsustaluvust. Töös uuriti AM seente (*Funneliformis mosseae*, *Rhizophagus intraradices* või *F. mosseae* ja *R. intraradices* koos) ja viie erineva NaCl taseme mõju *Tagetes erecta* kasvule ja füsioloogiale kasvuhoonetingimustes.

Tulemused näitasid, et inokulatsioon ühtaegu kahe seeneliigiga (*F. mosseae* ja *R. intraradices*) suurendab oluliselt soolastressi all olevate *T. erecta* taimede juurte kogupikkust, juurte pindala ja ruumala ning juuretippude arvu. AM seentega inokuleerimine pärssis Na^+ kogunemist lehtedesse ja soodustas N, P, K ja Ca imendumist lehtedes ning suurendas seega K^+/Na^+, Ca^{2+}/Na^+, N/Na^+ ja P/Na^+ suhtelisi sisaldusi soolastressi all kannatavates lehtedes. Kõrgeim korrelatsioonikoefitsient (0,964) oli juurte ruumala ja lehtede K^+ sisalduse vahel, samas kui korrelatsioon juurte pindala ja lehtede Na^+ sisalduse vahel oli kõige madalam (–0,95). AM seened parandasid taimerakkude osmoosiregulatsiooni võimet, suurendasid antioksüdantsete ensüümide aktiivsust ning vähendasid rakumembraanide kahjustusi.

Järeldame, et AM seened parandavad *T. erecta* soolsustaluvust, muutes juurte morfoloogilisi tunnuseid, reguleerides Na^+ ja teiste toitainete omastamist ja osmoosi ning suurendades antioksüdantsete ensüümide aktiivsust.