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Arbuscular mycorrhizal fungi inoculation impacts expression of aquaporins and salt overly sensitive genes and enhances tolerance of salt stress in tomato

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Abstract

Arbuscular mycorrhizal fungi (AMF) that establish reciprocal symbiosis with plant roots can enhance resistance to various stresses, including salt stress, but relevant mechanisms, especially at the molecular level, are scarce. The objective of this study was to analyze the effect of an arbuscular mycorrhizal fungus *Paraglomus occultum* on plant growth, leaf gas exchange, and expression of *plasma membrane intrinsic proteins* (*PIPs*), *tonoplast intrinsic proteins* (*TIPs*) and *salt overly sensitive* (*SOS*) genes in tomato under salt (150 mmol/L NaCl) and non-salt stress. Salt stress for 4 weeks inhibited root colonization rate of *P. occultum* and soil hyphal length by 0.21- and 0.57-fold, respectively. Salt stress also inhibited plant growth performance and leaf gas exchange, while inoculation with *P. occultum* significantly enhanced them under salt and non-salt stress conditions. AMF showed diverse regulation of root *SlPIPs* and *SlTIPs* expression, among which under salt stress, *SlPIP1;2*, *SlPIP1;5*, *SlPIP2;1*, *SlPIP2;6*, *SlPIP2;9*, *SlPIP2;10*, *SlTIP2;2*, *SlTIP3;2*, and *SlTIP5;1* were up-regulated by AMF colonization, and *SlPIP1;7*, *SlPIP2;5*, *SlPIP2;8*, *SlPIP2;11*, *SlPIP2;12*, *SlTIP2;3*, and *SlTIP3;1* were down-regulated, accompanied by no change in *SlPIP1;1*, *SlPIP1;3*, *SlPIP2;4*, *SlTIP1;1*, *SlTIP1;2*, *SlTIP1;3*, *SlTIP2;1*, and *SlTIP2;5*. Interestingly, salt stress inhibited the expression of *SlSOS1* and *SlSOS2* in non-mycorrhizal plants, while it increased the expression of *SlSOS1* and *SlSOS2* in mycorrhizal plants. AMF colonization down-regulated expression of *SlSOS1* and *SlSOS2* under non-salt stress while up-regulated expression of *SlSOS1* and *SlSOS2* under salt stress. It was concluded that AMF inoculation impacted the expression of stress-responsive genes, especially *SOS1* and *SOS2*, and enhanced salt resistance of tomato.

Keywords Arbuscular mycorrhizal fungi, PIPs, Salt stress, SOS, TIPs

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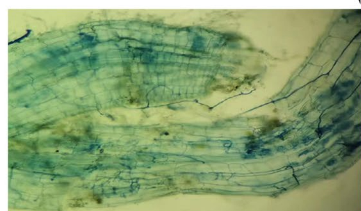
Graphical Abstract



Roots with arbuscular mycorrhizae

AMF plants exposed to 150 mM NaCl

Improved plant growth and leaf gas exchange

Diversified expression of *SlPIPs* and *SlTIPs*

Up-regulation: possibly accelerate the acquisition of water and other molecules

Down-regulation: possibly preserve cells from water loss

Activated *SlSOS1* and *SlSOS2* expressions for potential exchange of Na^+ and H^+

Introduction

Tomato (*Solanum lycopersicum*) is widely cultivated in the world and requires a large amount of water for its growth and development as well as for fruit set [1]. In recent years, the problem of secondary salinization has become increasingly serious due to irrational fertilization and irrigation in greenhouse cultivation, leading to successive crop replanting obstacles and salt damage in tomato [2]. The salt overly sensitive (SOS) signal transduction pathway can respond to the action of the Na^+/H^+ antitransporter SOS1, and then maintains the K^+/Na^+ ratio in cells of plants, thereby improving the salt tolerance of plants [3]. This regulation of SOS1 under salt stress also needs to be mediated by other members of the SOS pathway, namely SOS2 (serine/threonine protein kinase) and SOS3 (calmodulin) [4]. In addition, under salt stress, plant aquaporins (AQPs) are small and highly hydrophobic transmembrane proteins that promote bidirectional transmembrane movement of water, thereby regulating the flow of inter- or intracellular water molecules, as well as cell elongation and differentiation and stomatal movement [5]. AQPs can be divided into seven types, among which plasma membrane intrinsic proteins (PIPs) are highly conserved and are typical of highly water-selective channel proteins, and tonoplast intrinsic proteins (TIPs), which are localized on vacuolar membranes or vacuole formers, are key proteins for intracellular water transport, transporting not only water but also hydrogen peroxide (H_2O_2), urea and glycerol [6]. Therefore, it is very important to understand the salt tolerance of tomato by revealing the response of *SOSs* and *AQPs* in salt stress.

Arbuscular mycorrhizal fungi (AMF), an obligate trophic microorganism, can establish mutualistic symbionts with plant roots [7]. AMF occurs naturally in saline soils, and AMF strains isolated from saline habitats enhanced salt tolerance of plants [8]. The underlying mechanisms regarding AMF-enhanced salt tolerance of

plants have been proposed in the improvement of root architecture and nutrients, ion homeostasis, osmoregulation, antioxidant defense systems, and endogenous hormone regulation [9]. In trifoliate orange (*Poncirus trifoliata*) seedlings, AMF colonization up-regulated the expression of all the four *PtTIPs* and six *PtPIPs* under salt stress [10]. Nevertheless, in *Lactuca sativa* plants, AMF colonization did not affect *LsPIP2* expression, but it up-regulated *LsPIP1* expression under 100 mmol/L NaCl conditions [11]. This suggests that the regulation of AMF on *AQP* expression of host plants is varied, depending on the species of host plants and AMF as well as the *AQP* gene type. In addition to *AQPs*, AMF can also regulate the expression pattern of host *SOS*s in response to salt stress. In maize, mycorrhizal plants recorded similar expression of *ZmSOS1*, compared with non-mycorrhizal plants grown in 66 mmol/L NaCl; however, under 100 mmol/L NaCl, in four inoculated treatments, native *Claroideoglomus etunicatum* strain dramatically increased *ZmSOS1* expression in roots, coupled with lower Na^{+} levels, as compared with non-inoculation [12]. In pistachio plants, *Rhizophagus irregularis* inoculation did not impact *SOS1* expression under non-salt stress conditions, while it up-regulated *SOS1* expression under 250 μmol/L NaCl conditions [13]. Thus, host *SOS1* expression can be regulated by AMF under salt stress, but the effect seems to be influenced by salt levels as well as other factors such as mycorrhizal fungal species. These results indicate that *SOSs* and *AQPs* can play an important role in enhancing salt tolerance in mycorrhizal plants, but the relevant mechanisms and more experiments collectively need to be studied.

AMF has been demonstrated to increase salt tolerance in tomato plants [14, 15], while the underlying mechanism remains unclear. Since *SOSs* or *AQPs* are potentially involved in AMF-enhanced salt stress of plants, we hypothesized that the enhancement of salt tolerance in tomatoes by AMF is related to its regulation of *SOSs* and/or *AQPs*. The purpose of this study was to analyze the effects of AMF inoculation on plant growth, leaf gas exchange, and the expression of *PIPs*, *TIPs*, and *SOSs* in roots of salt-stressed tomato.

Materials and methods

Plant material culture

Seeds of tomato variety 'Huapiqiu' were provided by Hezhiyuan Seed Industry Co., Ltd. (Weifang, Shandong, China). On March 26, 2022, seeds were soaked in 75% ethanol for 5 min for surface disinfection, rinsed several times with distilled water, and then sown into a 32-hole disc. The disc was preloaded with the autoclaved (0.11 Mpa, 121 °C, 2 h) substrate (peat:vermiculite:perlite = 6 9:25:6, v/v/v). They were placed in an incubator with 28 °C/20 °C (day/night temperature, 16 h/8 h) and 80% of the relative humidity.

On April 15, 2022, two-leaf-old seedlings were transplanted into plastic pots (16 cm × 10 cm × 12.5 cm), in which 1.9 kg autoclaved growth substrates of soil and sand (3:1, v/v) were supplied. AMF inoculation was carried out at the time of transplanting. The arbuscular mycorrhizal fungus, *Paraglomus occultum*, was provided by the Institute of Root Biology, Yangtze University. The fungus was trapped using white clover under potted conditions for 10 weeks, and mycorrhizal fungal inoculum contained the fungus-colonized root segments, spores (15 spores/g), and hyphae. The inoculated treatment was supplied with 120 g of mycorrhizal inoculums. The uninoculated treatment also received 120 g of autoclaved mycorrhizal inoculums, plus 2 mL filtrates (25-μm) of the same weight of the inoculum.

One month after inoculation with *P. occultum*, salt treatments (0 and 150 mmol/L NaCl) were performed. To avoid salt shock, the given 150 mmol/L NaCl solution was gradually increased with a gradient of 50 mmol/L NaCl per day. After reaching 150 mmol/L NaCl intensity on the third day, the plants were watered every 3 days with 100 mL of 150 mmol/L NaCl per pot. Such NaCl treatments were continued for 4 weeks until plants were harvested, resulting in a total of 10 irrigations being applied during the experimental period. These seedlings were grown in a greenhouse (900 μmol/m^2/s of photosynthetic photon density, 28 °C/20 °C day/night temperatures (16 h/8 h), and 70% of the relative humidity). The plants were harvested on June 16, 2022, and then immediately stored in a −72 °C refrigerator.

Experimental design

The experiment consisted of four treatments: (1) the plants inoculated without *P. occultum* under 0 mmol/L NaCl (−AMF-NaCl); (2) the plants inoculated with *P. occultum* under 0 mmol/L NaCl (+AMF-NaCl); (3) the plants inoculated without *P. occultum* under 150 mmol/L NaCl (−AMF+NaCl); and (4) the plants inoculated with *P. occultum* under 150 mmol/L NaCl (+AMF-NaCl). Six replicates were set up for each treatment, and a total of 24 pots were randomly arranged.

Determinations of plant growth performance and leaf gas exchange

Gas exchange variables (photosynthesis rate, transpiration rate and stomatal conductance) were measured using a portable photosynthetic system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) on a sunny day prior to harvest, with the third leaf fully expanded at the top. Then, at harvest time, the plants

were divided into shoots and roots, whose weights were immediately recorded.

Determinations of root AMF colonization rate

Root mycorrhizal staining was performed using the method described by Phillips and Hayman [16]. After washing roots with distilled water, 1 cm root segments were collected and incubated with 10% of KOH at 95 °C for 1.5 h. After rinsing with distilled water, the roots were bleached with 10% of H_2O_2 for 10 min, and acidified with 0.2 mol/L of HCl for 15 min. After rinsing with distilled water, 0.05% of trypan blue in lactic acid solution was used to stain mycorrhizae in the roots for microscopic observation. Root AMF colonization rate was expressed as the percentage of the AMF-colonized root segment length versus total observed root segment length.

Determinations of soil hyphal length

Soil hyphal length was determined using the protocol described by Bethlenfalvay and Ames [17]. The 0.5 g of fresh soil sample was mixed with 6 mL of 0.1 mol/L phosphate buffer (pH 7.8). Subsequently, the 0.8 mL of the upper solution was well mixed with 0.4 mL of 0.05% of trypan blue in lactic acid solution in a water bath at 70 °C for 20 min and cooled to room temperature. Hyphae in the solution were microscopically observed, and hyphal length was recorded.

Determinations of *SlPIPs*, *SlTIPs*, and *SlSOSs* expression

Total RNA was extracted from roots and leaves (50 mg) using EASY spin Plus Plant RNA Rapid Extraction Kit (Aidlab, RN38, China) according to the manufacturer's protocol. The 2 µL of RNA was tested for RNA integrity by 1.0% agarose gel electrophoresis, and the RNA purity was calculated at 260 nm and 280 nm. The RNA was reverse transcribed to first-strand cDNA by using PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, RR047A, Beijing, China). At the tomato genome-wide level, 47 *AQP* genes were identified by Reuscher et al. [18], along with 14 *PIP* genes and 11 *TIP* genes. All *SlPIP* and *SlTIP* gene sequences were obtained from the Tomato Database (https://solgenomics.net/), and *SlSOS1* and *SlSOS2* gene sequences were obtained from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). Their primers were designed using the PrimerQuest™ tool (http://sg.idtdna.com/primerquest/Home/Index) and shown in Table 1. Real-time quantitative fluorescence PCR (qRT-PCR) analysis was performed using ChamQ Universal SYBR qPCR Master Mix (Vazyme, Q711) and fluorescent quantitative PCR detection system (FQD-96A, Hangzhou Bioer Technology Co., Ltd., Hangzhou, China). Expression levels of genes were

Table 1 Specific primer sequences of genes used for qRT-PCR

Gene names	Accession number	Primer sequences (5′→ 3′)
â-Actin	GQ339765	F: GTCCTCTTCCAGCCATCCA
		R: ACCACTGAGCACAATGTTACCG
SlPIP1;1	Solyc08g008050	F: ACCATCAAATAATCATCAGAGCA
		R: AGGATAAAATAAAAATTATTTTCAT
SlPIP1;2	Solyc01g094690	F: TAGAGACTCCCATGTCCCTATTC
		R: CTAGGCTTCTAGCAGGGTTAATG
SlPIP1;3	Solyc12g056220	F: GGTGTTGTGAAGGGTTTTATGGTT
		R: ACCCAGAAAATCCAGTGGTCATCC
SlPIP1;5	Solyc08g081190	F: CTATCATCTACAACGACGAGCA
		R: CATTGAAGGAGAAACTTGAACA
SlPIP1;7	Solyc03g096290	F: TTTCACTCACTAACTCCCATCAAT
		R: TAAAGAAAGAGGAAAGTAGCC ACA
SlPIP2;1	Solyc09g007770	F: CACATTAACCCTGCTGTTACATTC
		R: CAACCACAAATGGCTCCTAAAC
SlPIP2;4	Solyc06g011350	F: ACGTACCCGTGTTGGCACCTC TTCC
		R: ATGTTCGTCCCACGCCTTGTCACC
SlPIP2;5	Solyc10g084120	F: ATTGACCCTGAGGAACTTGGA AAA
		R: TCACCATCACTTTGGCTCTTGTAG
SlPIP2;6	Solyc11g069430	F: TACTCCGCAAAGGATTACACTGAT
		R: AGCCCAAGCAATACCAAGTAAACC
SlPIP2;8	Solyc01g111660	F: ATTCCCATATCCCTGTGTTGGCTCC
		R: AGCTGCAGCTCTCAAAATGTA TTGG
SlPIP2;9	Solyc10g055630	F: TCTTCTCTGCTACTGACCCTAA
		R: GTGGCCAAATGAACCATGAAA
SlPIP2;10	Solyc09g007760	F: CACATTAACCCTGCTGTTACATTC
		R: CAACCACAAATGGCTCCTAAAC
SlPIP2;11	Solyc02g083510	F: GTCCTCTTCCAGCCATCCA
		R: ACCACTGAGCACAATGTTACCG
SlPIP2;12	Solyc05g055990	F: ATACCCAACGTGTAGCATCACTCTC
		R: CCAGCAGTGGAATACACGAGA ACA
SlTIP1;1	Solyc06g074820	F: TCATCACTCCCCAACTTGTGCC
		R: AAAGCCATACCAGAACCCTGACCT
SlTIP1;2	Solyc06g075650	F: ATCCATAGCACATGCCTTTGCCCTT
		R: CCGATGTTTCCAGTCCACCAGTAG
SlTIP1;3	Solyc10g083880	F: CTATTCGTAGCGGTTTCGGTTG
		R: TTGTTCCCAAACTACCCTTCTT
SlTIP2;1	Solyc12g044330	F: TGACTGGAGGAATGGCGGTT
		R: ACCACAGCGGGTCCAAATGA
SlTIP2;2	Solyc03g120470	F: GATTCATTCAGCGTTGTCTCTCTT
		R: AAACGGCTACGAATAGAGCAAATC
SlTIP2;3	Solyc06g060760	F: AATGGTGAAGATTGCCTTTGGTAG
		R: TCAAATGTCCACCTGAGATGTTAG
SlTIP2;5	Solyc06g066560	F: TTTATCTCCACCTTGCTTTTCG
		R: CGGTAACAAACTTGAGGAGGCA
SlTIP3;1	Solyc06g072130	F: TTAGCGTCCTTCGTGCTATG
		R: AATCCCACTGGCCTCAATC

Table 1 (continued)

Gene names	Accession number	Primer sequences (5′→ 3′)
SlTIP3;2	Solyc03g019820	F: GCTGATTTATTGGTGTTATGGCTATG
		R: AGCAAGAACAGAGCCTTCACCG
SlTIP4;1	Solyc08g066840	F: TTATTCGTAAAATCAGTTTCATCA
		R: CAAGCAGCAACAGAAGCAAGTAAT
SlTIP5;1	Solyc03g093230	F: AGTGTACTGGATTGGACCTTTC
		R: GCAACAATTCCACCACCTATTC
SlSOS1	AJ717346	F: GTGCAGTACAGATGCTTTTACTTG
		R: AGGGCCACAACAGCCACA
SlSOS2	AJ717348	F: ATTTCCCGCCAACCTGCTAA
		R: TGCCGTTACCCCCTCAATTC

determined in three replicates. The $2^{-\Delta\Delta Ct}$ method [19] was used to evaluate relative expression of genes, along with the -AMF-NaCl treatment as the control.

Data analysis

Experimental data (means ± standard deviation; $n = 6$ for physiological variables; $n = 3$ for gene expression) were analyzed by the analysis of variance (ANOVA) according to SAS software, and the significance between treatments was compared according to the Duncan's range test at $P < 0.05$.

Results

Changes in root AMF colonization rate and soil hyphal length

No root mycorrhizae and soil hyphae were found in tomato without *P. occultum* inoculation. Root mycorrhizae (Fig. 1a) were found in roots inoculated with *P. occultum*, and root mycorrhizal colonization rate ranged from 38.6% to 49.1% (Fig. 1b). Mycorrhizal hyphae were observed in the soil of the inoculated plants, varied from 26.8 to 61.8 cm/g soil (Fig. 1c). Salt stress significantly inhibited root AMF colonization rate by 0.21-fold and soil hyphal length by 0.57-fold, compared with non-salt stress.

Changes in plant growth behavior

The growth behavior of tomato plants was strongly affected by salt stress and AMF inoculation (Fig. 2a). Salt treatment significantly decreased shoot biomass of non-AMF plants by 33.0%, respectively, compared with the 0 mmol/L NaCl treatment (Fig. 2b). Similarly, salt stress significantly inhibited shoot biomass and root biomass of AMF plants by 32.9%, and 32.0%, respectively, compared with non-salt stress (Fig. 2b–c). Under non-salt stress conditions, AMF inoculation dramatically increased shoot biomass and root biomass by 43.8% and 92.3%, respectively, compared with non-AMF inoculation; under salt stress conditions, AMF inoculation considerably increased shoot biomass and root biomass by 44.0% and 41.7%, respectively, compared with non-AMF treatment.

Changes in leaf gas exchange

Salt stress dramatically reduced leaf photosynthesis rate, transpiration rate, and stomatal conductance by 88.7%, 90.5%, and 72.2% in non-mycorrhizal plants and 81.6%, 82.5%, and 78.8% in mycorrhizal plants, respectively, compared with non-salt stress (Fig. 3a–c). However, AMF colonization distinctly elevated leaf photosynthesis rate, transpiration rate, and stomatal conductance by 39.4%, 36.7%, and 92.8% under non-salt stress and 5.0%, 61.2%, and 79.1% under salt stress, respectively, compared with non-AMF treatment.

Changes in root *SlPIPs* expression

In the *SlPIPs* from non-mycorrhizal plant roots, salt stress decreased the expression of *SlPIP1;2*, *SlPIP2;1*, *SlPIP2;9*, and *SlPIP2;10*, but increased the expression of *SlPIP1;1*, *SlPIP1;7*, *SlPIP2;4*, *SlPIP2;5*, *SlPIP2;8*, *SlPIP2;11*, and *SlPIP2;12* (Fig. 4). In the *SlPIPs* of mycorrhizal plants, salt stress decreased the expression of *SlPIP1;2*, *SlPIP1;3*, *SlPIP2;4*, and *SlPIP2;5*, but increased the *SlPIP1;1*, *SlPIP2;1*, *SlPIP2;6*, *SlPIP2;8*, *SlPIP2;9*, *SlPIP2;10*, and *SlPIP2;12* expression, compared with non-salt stress. In addition, under non-salt stress conditions, AMF up-regulated the expression of *SlPIP1;2*, *SlPIP1;3*, *SlPIP1;5*, *SlPIP2;4*, and *SlPIP2;5* by 0.19-, 1.04-, 0.45-, 3.43-, and 1.68-fold, but down-regulated the expression of *SlPIP2;1*, *SlPIP2;9*, and *SlPIP2;10* by 0.69-, 0.37-, and 0.72-fold, accompanied by no change in the expression of *SlPIP1;1*, *SlPIP1;7*, *SlPIP2;6*, *SlPIP2;8*, *SlPIP2;11*, and *SlPIP2;12*. Similarly, under salt stress conditions, AMF up-regulated the expression of *SlPIP1;2*, *SlPIP1;5*, *SlPIP2;1*, *SlPIP2;6*, *SlPIP2;9*, and *SlPIP2;10* by 5.53-, 0.71-, 1.51-, 0.65-, 3.54-, and 2.69-fold, but down-regulated the expression of *SlPIP1;7*, *SlPIP2;5*, *SlPIP2;8*, *SlPIP2;11*, and *SlPIP2;12*, each by 0.79-, 0.74-, 0.46-, 0.55-, and 0.42-fold, plus unchanged expression of *SlPIP1;1*, *SlPIP1;3*, and *SlPIP2;4*.

Changes in root *SlTIPs* expression

Salt stress treatment triggered up-regulated expression of *SlTIP1;2*, *SlTIP1;3*, *SlTIP2;3*, *SlTIP3;1*, *SlTIP3;2*, and *SlTIP5;1* in non-mycorrhizal plant roots, but also inhibited the expression of *SlTIP1;1*, compared with non-salt treatment (Fig. 5). Similarly, in mycorrhizal plant roots,

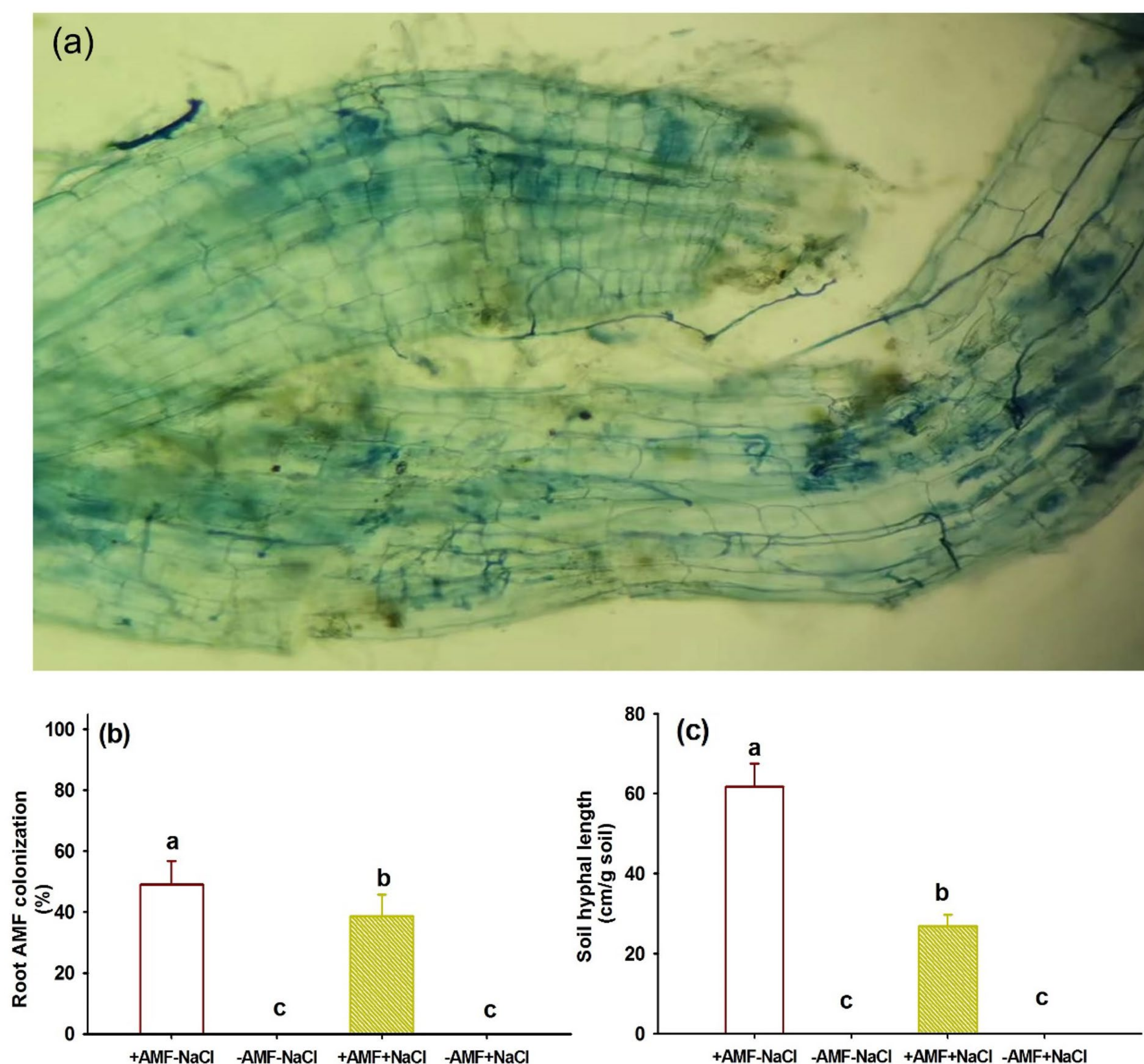


Fig. 1 Root mycorrhizae (**a**) of tomato by *Paraglomus occultum* and changes in root mycorrhizal colonization rate (**b**) and soil hyphal length (**c**) in response to salt stress (150 mmol/L NaCl). Data (means ± SD, *n* = 6) followed by different letters above the bars indicate significant ($P < 0.05$) differences between treatments. AMF-NaCl the plants inoculated without *P. occultum* under 0 mmol/L NaCl, +AMF-NaCl the plants inoculated with *P. occultum* under 0 mmol/L NaCl, −AMF+NaCl the plants inoculated without *P. occultum* under 150 mmol/L NaCl, +AMF-NaCl the plants inoculated with *P. occultum* under 150 mmol/L NaCl

additional NaCl treatment up-regulated the expression of *SlTIP2;5*, *SlTIP3;5*, and *SlTIP5;1* in roots, compared with non-salt treatment. On the other hand, compared with non-AMF inoculation, AMF inoculation under non-salt stress up-regulated the expression of *SlTIP1;1*, *SlTIP1;2*, *SlTIP1;3*, *SlTIP2;1*, *SlTIP2;2*, *SlTIP2;3*, and *SlTIP3;1* by 1.28-, 13.88-, 2.62-, 7.73-, 0.75-, 5.16-, and 20.23-fold, but down-regulated the expression of *SlTIP5;1* only by 0.71-fold; under salt stress, AMF inoculation significantly increased the expression of *SlTIP2;2*, *SlTIP3;2*, and *SlTIP5;1* by 0.89-, 0.62- and 0.46-fold, while it decreased the expression of *SlTIP2;3* and *SlTIP3;1* by 0.41- and 0.80-fold, respectively.

Changes in root *SlSOS1* and *SlSOS2* expression

The treatment with 150 mM NaCl significantly inhibited the expression of *SlSOS1* and *SlSOS2* in non-mycorrhizal roots by 0.68- and 0.38-fold, respectively, while the treatment with 150 mmol/L NaCl significantly increased the expression of *SlSOS1* and *SlSOS2* by 3.00- and 0.74-fold in mycorrhizal plants, compared with the treatment with 0 mmol/L NaCl (Fig. 6). On the other hand, under the

Fig. 2 Plant growth behavior (**a**) of tomato and changes in shoot biomass (**b**) and root biomass (**c**) in response to salt stress (150 mmol/L NaCl) and mycorrhizal colonization. Data (means ± SD, *n* = 6) followed by different letters above the bars indicate significant (*P* < 0.05) differences between treatments. See Fig. 1 for the abbreviations

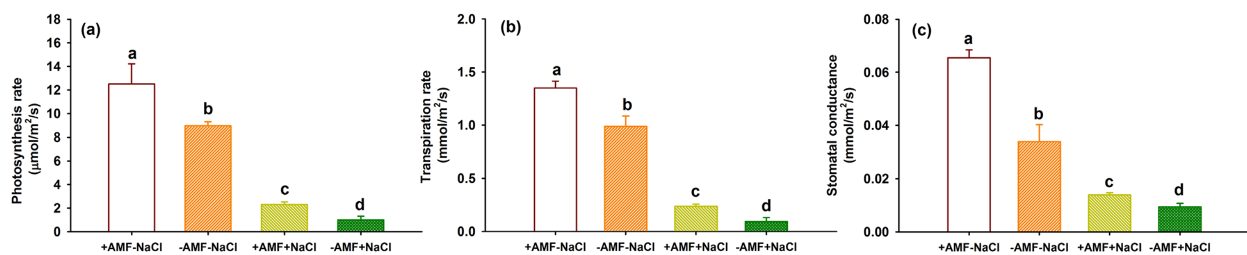


Fig. 3 Changes in leaf photosynthesis rate (**a**), transpiration rate (**b**), and stomatal conductance (**c**) of tomato in response to salt stress (150 mmol/L NaCl) and mycorrhizal colonization. Data (means ± SD, *n* = 6) followed by different letters above the bars indicate significant (*P* < 0.05) differences between treatments. See Fig. 1 for the abbreviations

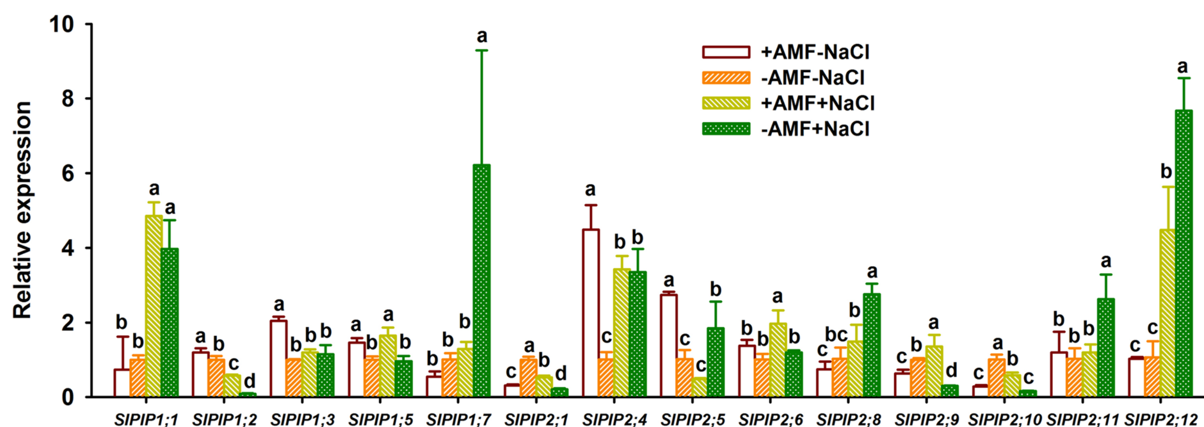


Fig. 4 Changes in *SlPIPs* expression in roots of tomato in response to salt stress (150 mmol/L NaCl) and mycorrhizal colonization. Data (means ± SD, *n* = 3) followed by different letters above the bars indicate significant ($P < 0.05$) differences between treatments. See Fig. 1 for the abbreviations

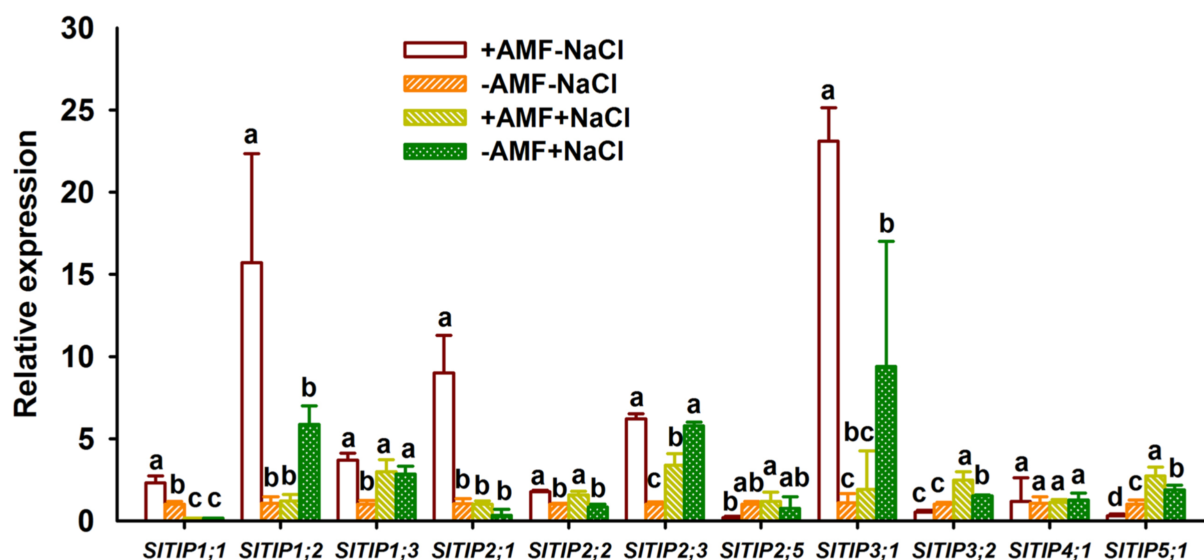


Fig. 5 Changes in *SlTIPs* expression in roots of tomato in response to salt stress (150 mmol/L NaCl) and mycorrhizal colonization. Data (means ± SD, *n* = 3) followed by different letters above the bars indicate significant ($P < 0.05$) differences between treatments. See Fig. 1 for the abbreviations

condition of 0 mmol/L NaCl, AMF colonization significantly reduced the expression of *SlSOS1* and *SlSOS2* in roots by 0.63-fold and 0.54-fold, compared with that non-AMF colonization. Under the condition of 150 mmol/L NaCl, AMF colonization significantly increased the expression of *SlSOS1* and *SlSOS2* in roots by 3.63- and 0.29-fold, respectively.

Discussion

Both root AMF colonization rate and soil hyphal length are important indicators of the affinity of symbiotic fungi for plants, which can reflect to a certain extent the ecological adaptability [20]. Salt stress (150 mmol/L NaCl) dramatically inhibited root AMF colonization rate and soil hyphal length in tomato, which agrees with earlier finding [21]. This may be due to the inhibited spore germination and reduced photosynthetic products and elongation of mycorrhizal extraradical hyphae by salt treatment [8, 9].

This study showed that salt treatment significantly inhibited the growth response of inoculated and uninoculated tomato plants, indicating that such NaCl concentration adversely affected tomato growth. However, AMF colonization substantially alleviated the inhibitory effect of salt treatment, and it was able to improve the accumulation of biomass in tomato, which is consistent with the finding of Ma et al. [22]. In general, mycorrhizal symbioses have well-developed extraradical mycelium

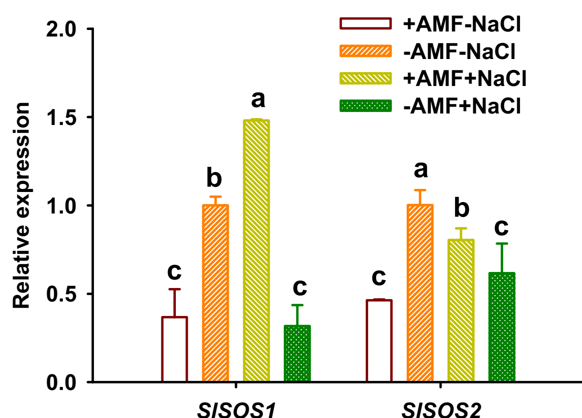


Fig. 6 Changes in *SlSOS1* and *SlSOS2* expression in roots of tomato in response to salt stress (150 mmol/L NaCl) and mycorrhizal colonization. Data (means ± SD, $n = 3$) followed by different letters above the bars indicate significant ($P < 0.05$) differences between treatments. See Fig. 1 for the abbreviations

on the root surface to help host plants absorb water and nutrients and thus promote plant growth [23]. Another possible explanation is that inoculation with AMF promotes leaf chlorophyll synthesis, root surface area, and root vigor of host plants [24, 25], thus accelerating plant growth behavior.

The water permeability of the plasma membrane and vacuolar membrane in plant cells is mainly through TIPs and PIPs in AQPs, where PIPs are mainly responsible for cellular water uptake or loss [26]. Salt stress and AMF inoculation diversely affected the expression of *SlPIP1* and *SlPIP2* genes in both mycorrhizal and non-mycorrhizal tomato plants. Chen et al. [27] also reported similar responses of *PIPs* and *TIPs* in black locust by *Rhizophagus irregularis* under salt stress. In trifoliate orange, AMF induced diverse responses of root *TIPs* to drought stress [28]. *PIP1* gene expression under salinity was down-regulated in *Lycopersicon esculentum* by AMF, while it was up-regulated in *Lactuca sativa* [11, 29]. In salinity, *SlPIP1;2*, *SlPIP1;5*, *SlPIP2;1*, *SlPIP2;6*, *SlPIP2;9*, and *SlPIP2;10* expression increased under mycorrhization. Li et al. [30] reported that *SlPIP2;1* was highly expressed in roots and characterized as water channels with high water permeability in *Xenopus oocytes*, along with transgenic tomato with high hydraulic conductivity. It suggests that the presence of arbuscular mycorrhizae may accelerate the root water uptake in salinity [31]. On the other hand, the down-regulation of expression of *SlPIP1;7*, *SlPIP2;5*, *SlPIP2;8*, *SlPIP2;11*, and *SlPIP2;12* in saline was under AMF colonization. Overexpression of *SlPIP1;7* in tomato accelerated root growth and root hydraulic conductivity and recorded less damage of cell membranes [32]. The overexpression of *SlPIP2;5* transgenic tomato exhibited greater water status and survival rate than wild plants under drought stress. Down-regulation of these *SlPIPs* under mycorrhization conditions may imply that the root cells of mycorrhizal plants reduce water permeability, which in turn preserves the cells from water loss [33]. Both mechanisms occurred in mycorrhizal tomato plants, showing the important function of mycorrhizae in saline conditions [10, 31]. However, further studies are required to determine whether these mycorrhizal-regulated *PIPs* are affected by different AMF species and how water uptake transfer occurs at the interface between plants and AMF.

In AQPs, TIPs also transport other molecules such as H_2O_2, urea, and glycerol, in addition to water [6]. In the present study, AMF inoculation still promoted more up-regulation of *SlTIPs* homologs than down-regulation, and the change was more prominent under non-salt stress than under salt stress. Ding et al. [34] also reported diverse expression patterns of *PtTIPs* homologs in roots of *Poncirus trifoliata* seedlings exposed to salt stress in response to AMF inoculation. They found that under salt stress, AMF only up-regulated *PtTIP4;1* expression in roots, along with no change in *PtTIP5;1* expression and down-regulated expression in *PtTIP1;1*, *PtTIP1;2*, *PtTIP1;3*, *PtTIP1;4*, *PtTIP2;1*, and *PtTIP2;2*. This suggests that the effects of AMF on *TIPs* and *PIPs* vary with host species, expressed tissue types, AMF, and salinity intensity [9, 35]. TIP5;1 is associated with the distribution of H_2O_2 in roots [36]. Mycorrhizal plants showed greater H_2O_2 effluxes in roots under drought stress [37]. In fact, under favorable environmental conditions, plants have low levels of H_2O_2, so *SlTIP5;1* expression was inhibited by AMF; under salt stress, *SlTIP5;1* was up-regulated by mycorrhizal fungi to transport H_2O_2 and its effluence to the rhizosphere, thus alleviating salt damage of mycorrhizal plants [38]. In addition, *TIP2;2* and *TIP5;1* are also involved in salinity tolerance in salt-sensitive and salt-tolerant plants by altering leaf gas exchange, especially transpiration rate [36]. Xin et al. [39] also observed that *SlTIP5;1*-overexpressed *Arabidopsis* plants represented higher salt tolerance than wild plants by regulating Na^+ and K^+ fluxes. It was also found that AMF-inoculated tomato plants had significantly higher photosynthesis rate, stomatal conductance, and transpiration rate under salt stress, accompanied by up-regulated expression of *SlTIP2;2* and *SlTIP5;1*. This is consistent with the results of Ding et al. [34] inoculated AMF on trifoliate orange under salt stress. More studies are to analyze how mycorrhizal fungi regulate these *SlTIPs* under salt stress and what the function of these *SlTIPs* is.

Our study also revealed that non-mycorrhizal tomato plants exhibited down-regulated expression of *SlSOS1*

and *SlSOS2* in roots in response to salt stress, while mycorrhizal plants represented up-regulated expression of *SlSOS1* and *SlSOS2* in roots in response to salt stress. This suggests that mycorrhizal plants are capable of activating the SOS pathway under saline conditions. Among them, *SOS1* is the Na^+/H^+ antitransporter, and its activation requires the participation of *SOS2* [3, 4]. *SlSOS1*-silenced tomatoes were more sensitive to salt stress, accompanied by a threefold higher rate of Na^+ uptake than wild-type plants, suggesting that the function of *SOS1* is to extrude Na^+ from roots [40]. *SlSOS2*-overexpressed tomatoes maintained the up-regulation of *SlSOS1* and endosomal–vacuolar Na^+/H^+ and K^+/H^+ antiports under salt stress [3]. Therefore, mycorrhizal tomato plants regulate intracellular Na^+ efflux through *SlSOS2-SlSOS1*, thus reducing the Na^+ toxicity. As a result, mycorrhizal tomato plants showed up-regulated expression of *SlSOS1* and *SlSOS2* in roots under salt stress compared with non-mycorrhizal plants. However, the expression of *SlSOS1* and *SlSOS2* in roots was inhibited by AMF colonization under non-salt stress conditions, because tomato plants were not subjected to salt stress and do not need to initiate the SOS pathway. Similar results were also reported by Abbaspour et al. [15] on *SOS1* of pistachio plants and Estrada et al. [12] on *SOS1* of maize in response to AMF colonization under salt stress conditions. However, *SlSOS1* and *SlSOS2* expression is tissue-specific [3, 40]. Therefore, future work needs to analyze the change of *SlSOS1* and *SlSOS2* expression in leaves, stems, and roots under salt stress and AMF colonization, in combination with the change in Na^+ and K^+ levels.

Conclusions

Although 150 mmol/L NaCl treatment significantly inhibited tomato growth and gas exchange, *P. occultum* inoculation significantly alleviated the inhibition, which was correlated with AMF activation of *SOS1* and *SOS2* expression and diversified regulation of *TIPs* and *PIPs* in roots. Such results clarify the role of mycorrhizae in salt tolerance of tomato, and also provide a new pathway for the future application of AMF in salt cultivation of tomato.

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Author contributions
MYL, QSL, WYD, LWD, MD, JHC and XT conducted the experiment. MYL wrote the original manuscript. MYL, QSW and QSL prepared figures. All authors reviewed and edited the manuscript.

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

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no potential conflict of interest regarding the publication of this work.

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