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ORIGINAL PAPER

# Modulation of nutrient acquisition and polyamine pool in salt-stressed wheat (*Triticum aestivum* L.) plants inoculated with arbuscular mycorrhizal fungi

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**Abstract** Two wheat (*Triticum aestivum* L.) cultivars, Sids 1 and Giza 168, were grown under non-saline or saline conditions (4.7 and 9.4 dS $m^{-1}$) with and without arbuscular mycorrhizal fungi (AMF) inoculation. Salt stress considerably decreased root colonization, plant productivity and N, P, $K^+$, Fe, Zn and Cu concentrations, while it increased $Na^+$ level, particularly in Giza 168. Mycorrhizal colonization significantly enhanced plant productivity and N, P, $K^+$, Fe, Zn and Cu acquisition, while it diminished $Na^+$ uptake, especially in Sids 1. Salinity increased putrescine level in Giza 168, however, values of spermidine and spermine increased in Sids 1 and decreased in Giza 168. Mycorrhization changed the polyamine balance under saline conditions, an increase in putrescine level associated with low contents of spermidine and spermine in Giza 168 was observed, while Sids 1 showed a decrease in putrescine and high increase in spermidine and spermine. Moreover, mycorrhizal inoculation significantly reduced the activities of diamine oxidase and polyamine oxidase in salt-stressed wheat plants. Modulation of nutrient acquisition and polyamine pool can be one of the mechanisms used by AMF to improve wheat adaptation to saline soils. This is the first report dealing with mycorrhization effect on diamine oxidase and polyamine oxidase activities under salt stress.

**Keywords** Arbuscular mycorrhiza · Salinity · Wheat · Productivity · Nutrient acquisition · Polyamines pool

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## Introduction

Soil salinization is considered to be one of the most important abiotic stresses that adversely affects crop productivity. High salt depositions in the soil generate a low water potential zone in the soil, making it increasingly difficult for the plant to acquire both water as well as nutrients, moreover elevated $Na^+$ in soil solution inhibits and disrupts the uptake of other nutrients (P, K, Fe, Cu and Zn) by interfering with various transporters in the root plasma membrane (Marschner 1995). Salinity also changes polyamines (PAs) composition (El-Bassiouny and Bekheta 2005). Polyamines, namely diamine putrescine (Put), triamine spermidine (Spd) and tetraamine spermine (Spm), are low molecular weight, aliphatic polycations found in the cells of all living organisms (Pang et al. 2007; Kusano et al. 2008). Plant PAs are involved in a variety of divergent processes, such as gene expression, protein and DNA synthesis, cellular homeostasis, cell division and differentiation, growth and developmental processes such as embryogenesis, organogenesis, senescence, and also responses to abiotic and biotic stresses (Moschou et al. 2008). Diamine oxidase (DAO; EC 1.4.3.6) and polyamine oxidase (PAO; EC 1.4.3.4) are thought to play a major role in production of $H_2O_2$, which is toxic and lead to oxidative stress (Goyal and Asthir 2010).

Exploitation of soil microbes for utilizing salt-stressed lands is of great importance. Arbuscular mycorrhizal fungi

(AMF), obligate biotrophs of higher plants, constitute one of the most widespread groups of these soil microorganisms. They colonize the root cortex of the most plant species and develop an extraradical mycelium which spreads through the soil surrounding plant roots (Ruiz-Lozano and Azcon 2000). By increasing the interface between plants and the soil environment, they enhance plant uptake of low mobile ions, improve quality of soil structure, enhance plant community diversity, improve rooting and plant establishment, improve soil nutrient cycling and enhance plant tolerance to biotic and abiotic stress (Smith and Read 2008). Although salinity negatively affects mycorrhizal colonization, many reports show improved growth and productivity of mycorrhized plants under saline conditions (Wu et al. 2010b; Shekoofeh et al. 2012; Talaat and Shawky 2012). This positive effect was explained by improved host plant nutrition (Wu et al. 2010b; Hajiboland et al. 2010; Abdel-Fattah and Asrar 2012; Shekoofeh et al. 2012). Under saline conditions, AMF may discriminate against $Na^+$ as $Na^+$ was found at moderate levels in AMF, and in contrast, disproportionally accumulate mineral ions capable of osmoregulation, such as $K^+$ and $Ca^{2+}$, in their tissues (Hammer et al. 2011). The external hyphae of AMF can deliver up to 80 % of plant P, 25 % of plant N, 10 % of plant K, 25 % of plant Zn and 60 % of plant Cu (Marschner and Dell 1994). Change in polyamines balance is a frequent response of plant metabolism to the mycorrhizal colonization influencing many physiological aspects including stress resistance (Smith and Read 2008). However, the information regarding PAs in plant–fungal symbiotic interactions is very limited; they may take part in the molecular signalling events between the symbiotic partners (El Ghachtouli et al. 1995). Similar pathways for Put synthesis to those described in plants and bacteria have been found in an AM fungus (Sannazzaro et al. 2004). Role of PAs in the mycorrhizal symbiosis was first shown by El Ghachtouli et al. (1995), who detected that exogenously applied PAs increased colonization frequency in pea. According to Wu et al. (2010a), exogenous Spm, Spd, and Put significantly increased the number of arbuscules and vesicles, moreover, in the three PAs species, the stimulated effects were highest in Put-applied seedlings, which might be explained in two ways: that Put is a precursor in the Spd and Spm biosynthesis, or that Put is the most abundant PA in un-germinated spores of *Glomus mosseae*. In addition, mycorrhization may change the PA balance of plants. Sannazzaro et al. (2007) showed higher content of total free polyamines in mycorrhized plants compared to non-AM ones. They suggested that since PAs have been proposed as candidates for the regulation of root development under saline situations, it is possible that AM plants (which contained higher PA levels and showed improved root growth) were better shaped to cope with salt stress. However, the roles of PAs in plant-AM fungus interactions, especially wheat mycorrhizal interactions, are not well understood.

Although there are some evidences for a positive involvement of PAs in plant response to salt stress, there is poor information regarding the influence of mycorrhizal symbiosis on PAs pool in plants under saline conditions. Thus, our goal was to evaluate the contribution of mycorrhizae to PAs balance in two wheat cultivars exposed to saline conditions. Moreover, the ability of AMF to improve food nutritional value through enhanced uptake of macro- and micro-nutrients currently merits more attention. So, the other goal was to evaluate the efficacy of mycorrhizal colonization on the nutrient acquisition by mycorrhized plants grown in saline soils. The hypothesis tested is that AMF application may make an important contribution to ameliorate the deleterious effects generated by salinity on plant productivity, confirm the modulation of stress management by AMF via regulating the nutrient acquisition and the PA pool resulting in better performance of plants under stress conditions.

## Materials and methods

### Plant material and experimental design

Two pot experiments were performed in the greenhouse of the National Research Center at Giza, Egypt, during the two successive seasons of 2010/2011 and 2011/2012. Each experiment included 12 treatments (two cultivars × three salinity levels × two mycorrhizal treatments). Treatments in each experiment were replicated four times and arranged in a complete randomized design. Two wheat cultivars (*T. aestivum* L. cv. Sids 1 and cv. Giza 168) were used. For each cultivar, three levels of salinity [0.1 dS $m^{-1}$ (non-saline), 4.7 and 9.4 dS $m^{-1}$] were used; saline conditions were obtained by adding to the soil a mixture of NaCl, $CaCl_2$ and $MgSO_4$ at the molar ratio of 2:2:1, respectively. Plants from each salinity level were either grown with (AM+) or without mycorrhizal fungi (AM−).The spores of arbuscular mycorrhizae were collected from the rhizosphere soil of maize plants by the wet sieving and decanting technique (Gerdemann and Nicolson 1963). The mycorrhizal spores were identified as a mixture of *Glomus* spp. according to their morphological characteristics including color, size, surface, number of spore wall layers, hyphae, and hyphal attachments as described by Schenck and Perez (1990). In all mycorrhizal treatments, the inocula were standardized by previous estimation of total spores in the prepared spore suspension using a haemocytometer. The inoculated dosage was 3.5 ml of the inoculum per pot, corresponding to approximately 550 spores. Propagule infectivity was tested according to the method of Sharma et al. (1996). The

inoculum was placed 2–3 cm below the planting hole. Non-mycorrhizal treatments received 3.5 ml of filtered spore-free suspension to provide the same microflora without mycorrhizal fungi. Chemical analysis of the soil under investigation is presented in Table 1 and was determined according to Cottenie et al. (1982). The soil used was a clay loam (sand 37 %, silt 28 %, clay 35 %), collected from the National Research Center Experimental Station, sieved (pore size 2 mm), diluted with quartz sand (particle diameter <1 mm; 2:1, soil to sand, v/v), and sterilized by steaming the mixture at 100 °C for 1 h on three consecutive days. Fertilization was carried out by adding ammonium sulfate (20.5 % N), calcium superphosphate (15.5 % $P_2O_5$), and potassium sulfate (48 % $K_2O$) at the rate of 2.0, 2.2, and 1.1 g $pot^{-1}$, respectively, before planting, as well as 2.0 g $pot^{-1}$ ammonium sulfate 30 days after planting. For each pot (containing 10 kg of the soil mixture), ten grains thinned to six after germination were planted on November 18 in both seasons. Grains were obtained from the Wheat Research Department, Agriculture Research Center, Ministry of Agriculture, Giza. All pots were irrigated to soil saturation before planting. After planting, irrigation was applied at the appropriate times with tap water to maintain soil moisture near maximum water-holding capacity. The plants were sampled after 70 days of mycorrhizal inoculation to assess the following parameters.

### Estimation of mycorrhizal colonization

Representative samples of the fresh roots were taken immediately after harvest, washed, cut into 1 cm pieces, and fixed by formalin acetic-acid alcohol solution. Root samples were cleared with 10 % KOH solution and stained with 0.05 % trypan blue in lactophenol (Phillips and Hayman 1970), and microscopically examined for root colonization. The mycorrhizal colonization was evaluated by the method of Giovanetti and Mosse (1980). Data are given as percentage of root length colonized.

### Plant productivity analysis

At maturity, number of grains $plant^{-1}$, grain yield $plant^{-1}$ and weight $grain^{-1}$, were recorded.

### Determination of nutrient status

Dried ground shoots and grains (1 g) were digested in a mixture of boiling perchloric acid and hydrogen peroxide for 8 h. When the fumes were white and the solution was completely clear, it was cooled to room temperature and filled up to 10 ml with deionized water. Reagent blanks were prepared by carrying out the whole extraction procedure but in the absence of sample. Nitrogen was determined by the modified micro-Kjeldahl method following Pregl (1945), and P was determined by the ammonium molybdate blue method (Allen 1989). Potassium and Na were determined using a flame photometer (ELE UK). Iron, Zn and Cu were measured with an atomic-absorption spectrophotometer (Unicam 989-AA Spectrometer-UK).

### Extraction and estimation of polyamines

Polyamines were extracted, separated and detected after dansylation as described by Reggiani et al. (1990) using silica plates (60 $F_{254}$, Merck, Germany) with cyclohexane–ethylacetate (3:2 v/v) as the solvent. Spots, demarcated under UV light, were scraped from the plates and extracted with ethylacetate. Fluorescence was measured in an LS 30-Luminescence Spectrofluorimeter (Perkin-Elmer, UK) at an excitation wave length of 360 nm and emission wave length of 506 nm and the results were compared with dansylated standards. Polyamine content was expressed as nmol $g^{-1}$ FW.

### Extraction and assay of diamine oxidase and polyamine oxidase

Leaf samples were homogenized in 100 mM K-phosphate buffer (pH 6.5) containing 5 mM dithiothreitol and the extract was centrifuged at 16,000×*g* for 20 min at 4 °C. The supernatant was used as source of enzyme. Diamine oxidase (DAO; EC 1.4.3.6) and polyamine oxidase (PAO; EC 1.4.3.4) activities were assayed as per Asthir et al. (2002) using Put (for DAO) and Spd (for PAO) as substrates. The reaction mixture of 2.0 ml consisted of 0.1 ml of enzyme extract, 50 U of CAT, 0.1 % *o*-aminobenzaldehyde and the reaction started with one of the two different buffer and

**Table 1** Chemical analysis of the used soil under different salinity levels

| Salinity levels EC (dS $m^{-1}$) | pH | $HCO_3^- + CO_3^{2-}$ (mg $kg^{-1}$) | $Cl^-$ (mg $kg^{-1}$) | $SO_4^{2-}$ (mg $kg^{-1}$) | $Ca^{2+}$ (mg $kg^{-1}$) | $Mg^{2+}$ (mg $kg^{-1}$) | $Na^+$ (mg $kg^{-1}$) | $K^+$ (mg $kg^{-1}$) | N (mg $kg^{-1}$) | P (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.1 | 7.20 | 204.3 | 315.9 | 434.2 | 91.0 | 40.1 | 3.8 | 30.8 | 19.2 | 3.1 |
| 4.7 | 7.50 | 270.5 | 998.1 | 908.6 | 377.2 | 155.6 | 275.0 | 37.0 | 16.0 | 2.8 |
| 9.4 | 7.64 | 244.6 | 1,599.0 | 1,399.3 | 558.8 | 203.7 | 503.2 | 41.0 | 12.3 | 2.4 |

substrate combinations i.e., 10 mM Put in 50 mM K-phosphate buffer (pH 7.5) for DAO; 10 mM Spd in 50 mM K-phosphate buffer (pH 6.0) for PAO. The reaction was incubated at 30 °C for 3 h, and then stopped with 2.0 ml of 10 % (v/v) perchloric acid and the tubes were centrifuged at 6,500×*g* for 15 min. Formation of the Δ-pyrroline product was determined by reading the absorbance at 430 nm in spectrophotometer. Control reactions were carried out with inactivated enzyme prepared by heating for 20 min in a boiling water bath. Activities are expressed as nmol Δ-pyrroline $g^{-1}$ FW $h^{-1}$ for DAO and PAO.

### Statistical analysis

All data were statistically analyzed using a three-factorial completely randomized design (Snedecor and Cochran 1980). Combined analysis was made for the two growing seasons, since the results of the two seasons followed a similar trend. All values are means of four replicates. Significant differences were calculated using the least-significant-difference (LSD) test at $p < 5$ % level.

## Results

Uninoculated plants did not show any colonization. Structures characteristic of AMF were detected in the roots after inoculation. Sids 1 inoculated plants cultivated under different salinity levels showed 83 to 62 % of mycorrhizal root length while Giza 168 plants colonized by AMF showed a percentage of root colonization ranging from 71 to 42 % of root length (Fig. 1).

Salt stress markedly reduced number of grains $plant^{-1}$ and grain yield $plant^{-1}$ (Fig. 2). Interestingly, these attributes were significantly higher in mycorrhizal than non-mycorrhizal plants in presence as well as in absence of salt stress. Mycorrhization significantly ($p < 0.05$) increased grain yield $plant^{-1}$ by 75.0, 85.1 and 96.1 % in Sids 1 and by 47.6, 58.6 and 73.3 % in Giza 168 at 0.1, 4.7 and 9.4 dS $m^{-1}$ salinity level, respectively, when compared with uninoculated plants.

Nitrogen, P and $K^+$ concentrations were markedly decreased in shoots and grains of non-mycorrhizal plants in response to salt exposure, but the reduction was much less in Sids 1 compared to Giza 168 plants (Fig. 3). Mycorrhizal inoculation alleviated the adverse effect of salt stress and significantly ($p < 0.05$) enhanced N, P, and $K^+$ accumulation, particularly in Sids 1. Nitrogen level was increased in shoots of Sids 1 inoculated plants by 31.3, 46.5 and 53.9 %, that of P by 48.6, 56.7 and 62.5 % and that of K by 28.4, 41.6 and 46.2 % at 0.1, 4.7 and 9.4 dS $m^{-1}$ salinity level, respectively, when compared with non-mycorrhized plants. An increase in shoot and grain sodium concentration of non-mycorrhized plants was observed

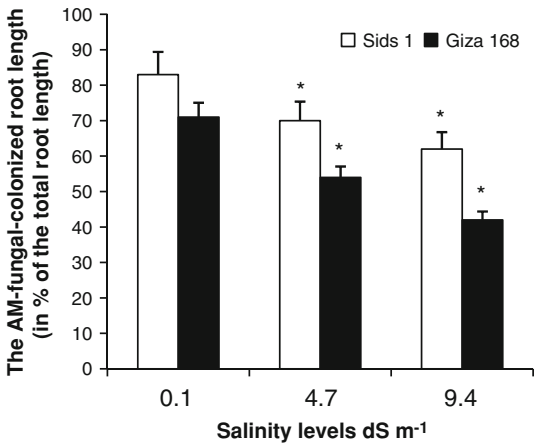


**Fig. 1** Percentage of total root length infected by arbuscular mycorrhizae under different salinity levels. The column value represents the mean (±SE) of four replicates. *Asterisks* indicate significant differences at the 0.05 level compared with the non-saline plant (0.1 dS $m^{-1}$ salinity level)

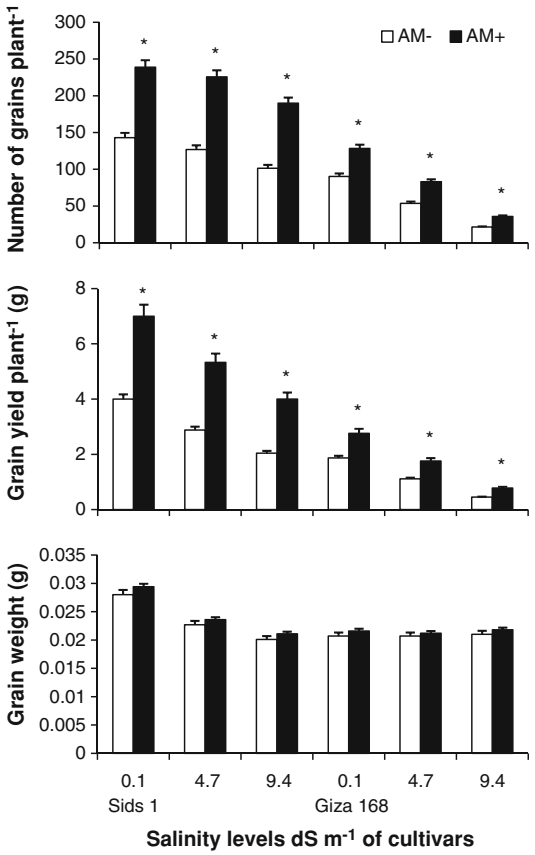


**Fig. 2** Changes in the grains number $plant^{-1}$, grain yield $plant^{-1}$ (g) and grain weight (g) of two wheat cultivars as affected by mycorrhizal inoculation under different salinity levels. *Every column in each graph* represents the mean (±SE) of four replicates. *Asterisks* indicate significant differences at the 0.05 level compared with the uninoculated plant

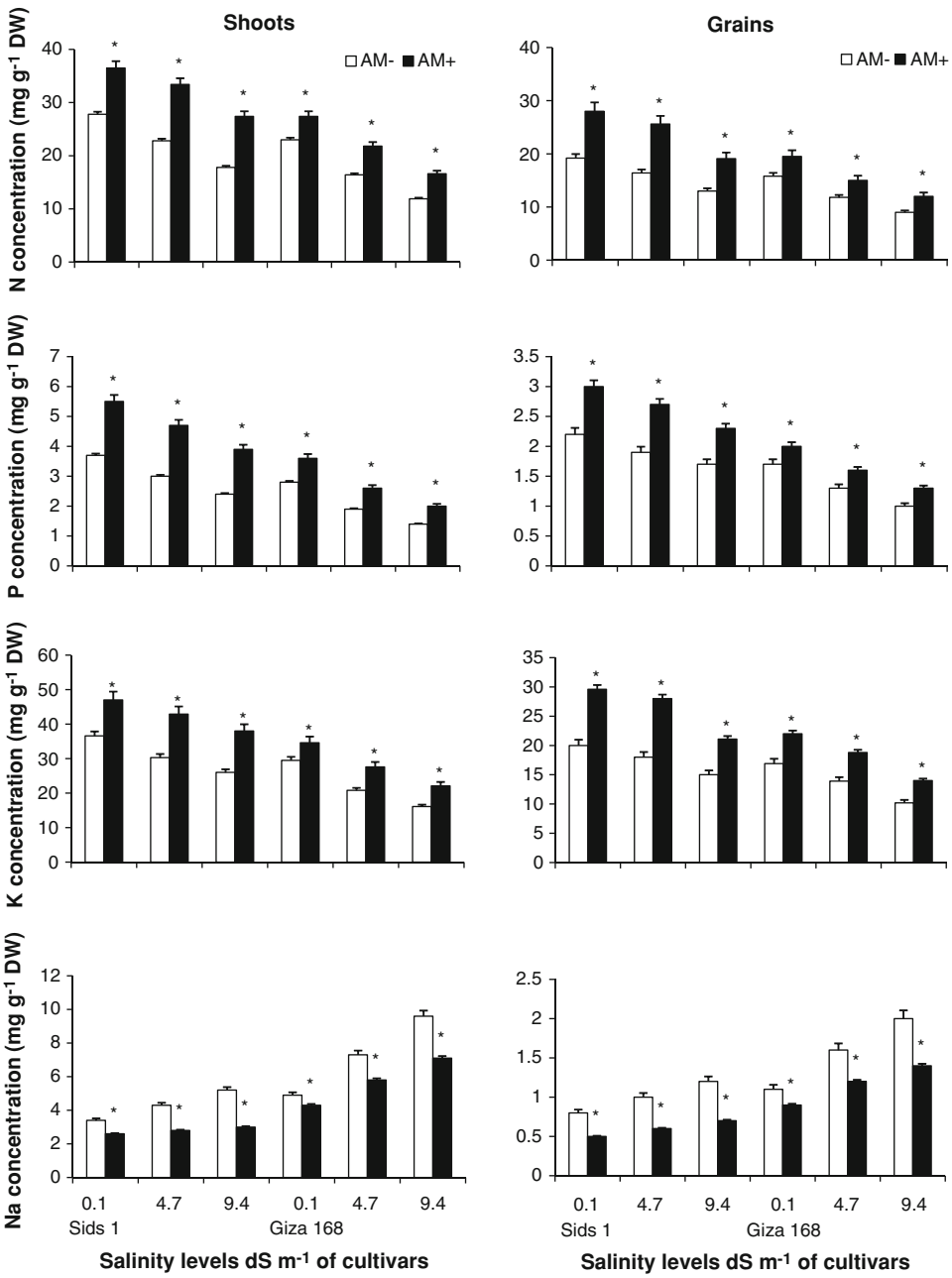


**Fig. 3** Changes in the concentrations of nitrogen, phosphorus, potassium and sodium (mg $g^{-1}$ DW) in shoots and grains of two wheat cultivars as affected by mycorrhizal inoculation under different salinity levels. *Every column in each graph* represents the mean (±SE) of four replicates. *Asterisks* indicate significant differences at the 0.05 level compared with the uninoculated plant

under salinity, particularly in Giza 168 (Fig. 3). Mycorrhization significantly ($p < 0.05$) decreased its concentration by 23.5, 34.9 and 42.3 % in Sids 1 shoots and by 12.2, 20.5 and 26.0 % in Giza 168 shoots compared to values of uninoculated plants at 0.1, 4.7 and 9.4 dS $m^{-1}$ salinity level, respectively, indicating lower absorption by the roots and lesser translocation to the shoots. Lower concentrations of Fe, Zn and Cu were detected in shoots and grains of stressed non-mycorrhized plants, especially in Giza 168 (Fig. 4). Mycorrhizal symbiosis protected wheat against the detrimental effect of salinity and significantly ($p < 0.05$) improved Fe, Zn and Cu acquisition, particularly in Sids 1. The level of Fe was increased in shoots of Sids 1 mycorrhized plants by 29.8, 39.0 and 54.1 %, that of Zn by 25.1, 33.8 and 57.2 % and that of Cu by 28.2, 35.8 and 50.9 % at 0.1, 4.7 and 9.4 dS $m^{-1}$ salinity level, respectively, when compared with non-mycorrhized plants.

Salt stress increased Put level in Giza 168 (Fig. 5). Values of Spd and Spm were increased in Sids 1 and decreased in Giza 168 by salinity (Fig. 5). Mycorrhization changed the PAs balance; it increased Spd and Spm contents in Sids 1 plants under non-saline condition. Furthermore, the accumulation pattern of PAs was not consistent in both cultivars due to mycorrhizal inoculation under saline conditions. Mycorrhizal salt-stressed Sids 1 plants showed higher levels of Spd and Spm as compared to non-mycorrhizal plants.

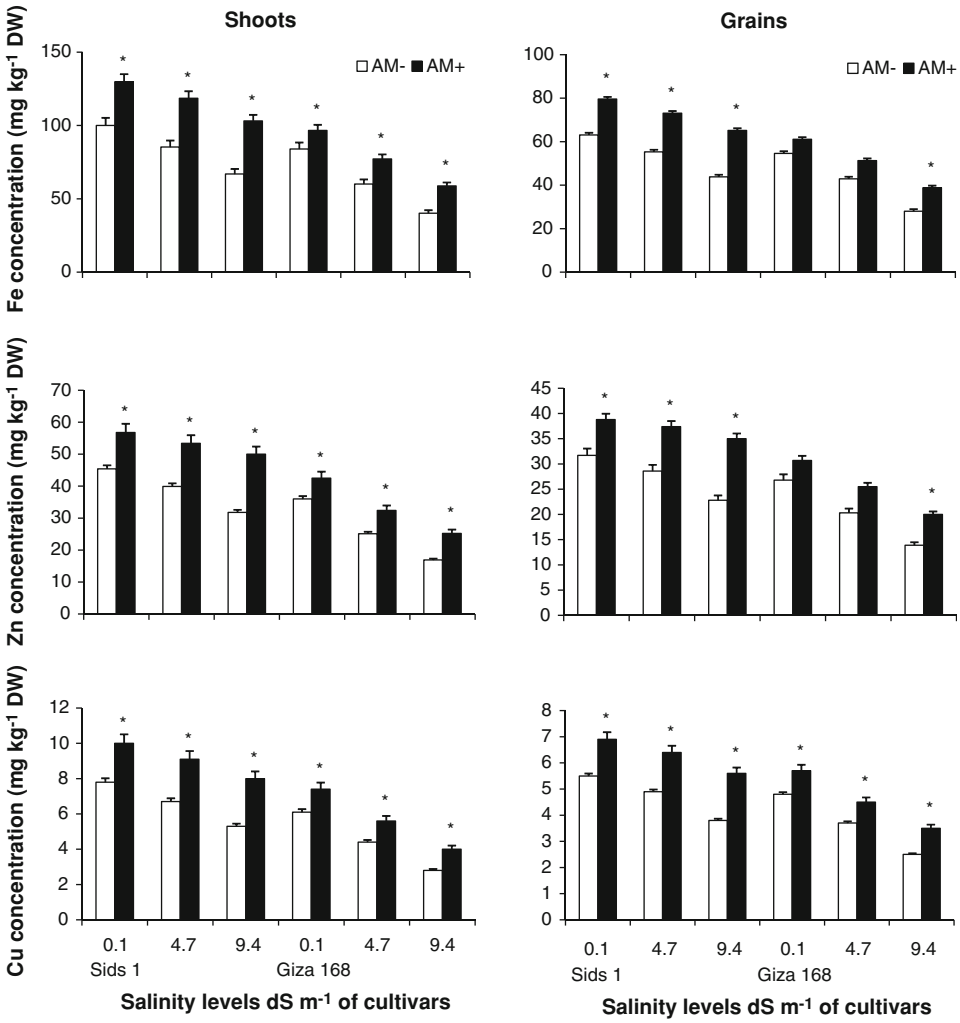


**Fig. 4** Changes in the concentrations of iron, zinc and copper (mg $kg^{-1}$ DW) in shoots and grains of two wheat cultivars as affected by mycorrhizal inoculation under different salinity levels. *Every column in each graph* represents the mean (±SE) of four replicates. *Asterisks* indicate significant differences at the 0.05 level compared with the uninoculated plant

Mycorrhizal salt-stressed Giza 168 plants showed higher Put and lower Spd and Spm levels than the corresponding non-mycorrhizal plants. The maximum increase was observed in Sids 1 mycorrhized plants exposed to 9.4 dS $m^{-1}$ salinity level that had 40.8 and 45.9 % higher levels of Spd and Spm, respectively, over uninoculated plants.

Salinization increased DAO and PAO activities, the effect was more pronounced in Sids 1 (for DAO) and in Giza 168 (for PAO) (Fig. 5). Mycorrhization significantly ($p < 0.05$) decreased their activities, particularly in Giza 168 (for DAO) and in Sids 1 (for PAO). Mycorrhizal colonization reduced the values of DAO in Giza 168 by 7.0, 17.5 and 19.0 % and that of PAO in Sids 1 by 10.3, 20.8 and 25.0 % at 0.1, 4.7 and 9.4 dS $m^{-1}$ salinity level than non-mycorrhized plants, respectively.

## Discussion

Mycorrhizal symbiosis is an essential component of most plants and the challenge for agriculture today lies in the possibility to take advantage of the numerous ecosystem services of soil stabilization, biofertilization, bioprotection, bioregulation offered by this natural resource.

As expected, the extent of AM-fungal root colonization was lower when roots grew in saline conditions; this effect could be explained by the direct effect of salt stress on the fungi. Salinity can hamper colonization capacity not only by inhibiting spores germination, hyphae growth in soil, or hyphal spreading after initial infection (Hajiboland et al. 2010; Wu et al. 2010b; Abdel-Fattah and Asrar 2012; Shekoofeh et al. 2012) but also by increasing $H_2O_2$ accumulation in the mycorrhized roots; $H_2O_2$ could diffuse across the thin hyphal wall of arbuscular branches and might be able to initiate the fungal programme for senescence, thus, the accumulation of ROS in the cytoplasm of arbuscule-containing cells might ultimately lead to arbuscular degradation (Fester and Hause 2005).

Soil salinity poses a severe threat to wheat productivity, which has been attributed to toxicity of excessive $Na^+$ and $Cl^-$, disturbance in the accumulation of nutrients, disturbance in water and osmotic potential, disruption in the

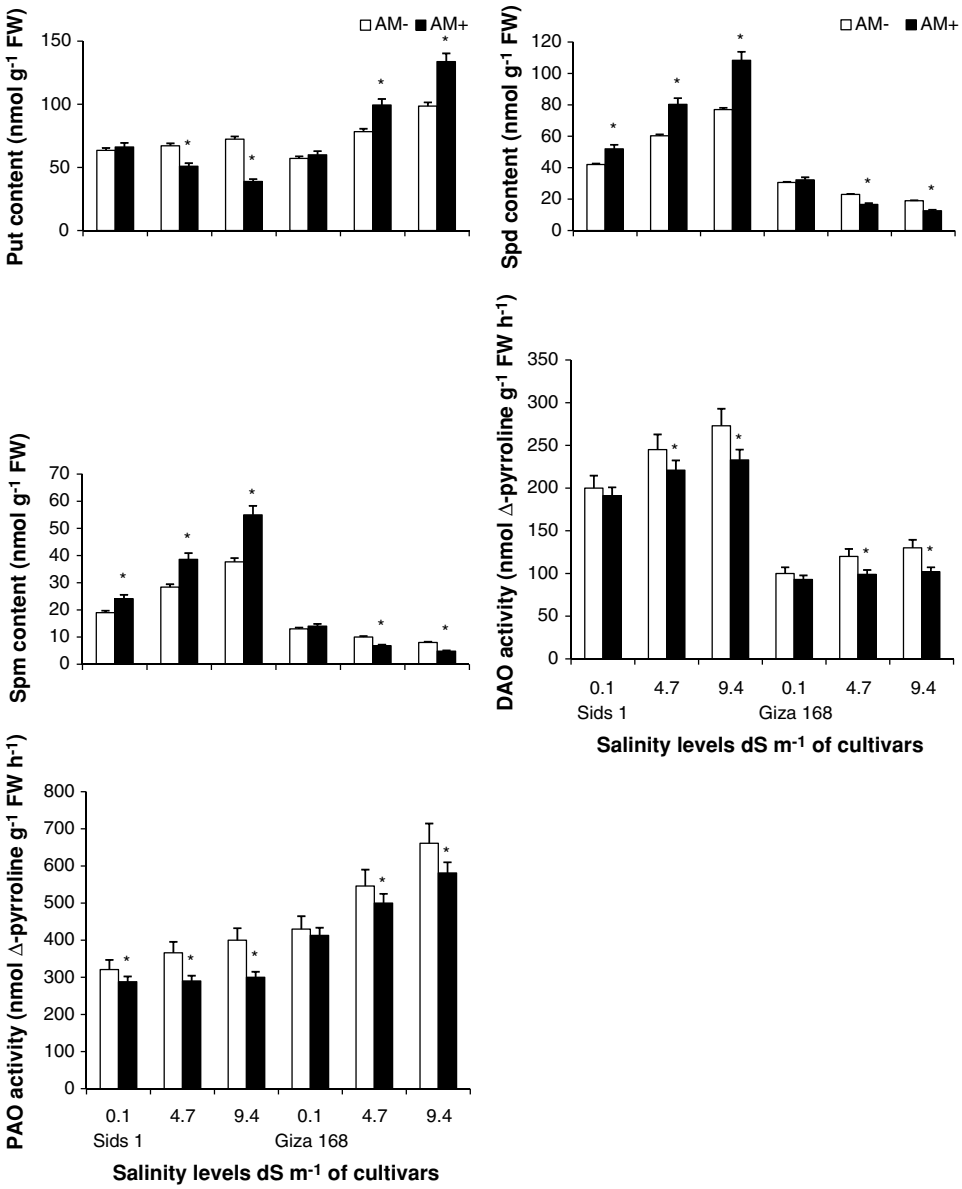


**Fig. 5** Changes in the endogenous polyamine contents (Put, Spd, Spm) (nmol $g^{-1}$ FW) and the diamine oxidase (DAO) and polyamine oxidase (PAO) activities (nmol Δ-pyrroline $g^{-1}$ FW $h^{-1}$) in leaves of two wheat cultivars as affected by mycorrhizal inoculation under different salinity levels. *Every column in each graph* represents the mean (±SE) of four replicates. *Asterisks* indicate significant differences at the 0.05 level compared with the uninoculated plant

structure of enzymes, damage in cell organelles and plasma membrane, disruption in photosynthesis, respiration and protein synthesis, reduction in assimilates translocation to the sink and increasing in the production of ROS in the chloroplast (Hajiboland et al. 2010; Wu et al. 2010b; Shekoofeh et al. 2012). Salinity-induced suppression of wheat grain yield has been attributed to a decline in grain weight in Sids 1, but in Giza 168 it was due to a decline in grain number. This result may support the finding that the decrease in grain number of salt-sensitive genotypes could be due to the lack of photo-assimilates accumulation before anthesis; however, in salt-tolerant genotypes, reduction in grain weight might be attributed to the lack of assimilate availability in grain filling period (Rahnama et al. 2011). Interestingly, we found that mycorrhization increased the fitness of wheat plant to salt stress and enhanced its productivity by improving host plant nutrient status (it increased N, P, $K^+$, Fe, Zn and Cu acquisition, while it diminished $Na^+$ uptake) and by altering polyamine balance (it changed Put, Spd and Spm content as well as reduced the activities of diamine oxidase and polyamine oxidase). This is consistent with other findings (Giri and Mukerji 2004; Sannazzaro et al. 2007; Daei et al. 2009; Hajiboland et al. 2010; Wu et al. 2010b; Abdel-Fattah and Asrar 2012).

Salt stress affected plant physiological traits through changes in ionic status in the plant cells; it interfered with nitrogen uptake and reduced its accumulation, which might be attributed to a direct competition of chloride with nitrate at the membrane level and/or an effect on the membrane proteins and change plasmalemma integrity (Köhler and Raschke 2000). Deviating from the response generated by

salt, AMF had a favorable impact on nitrogen acquisition. Our results are consistent with previous reports (Giri and Mukerji 2004; Abdel-Fattah and Asrar 2012). Indeed, the extraradical mycelia take up inorganic nitrogen from the soil in the form of nitrate and assimilate it via nitrate reductase, located in the arbuscule-containing cells (Kaldorf et al. 1998). Improved N nutrition by mycorrhiza may reduce the toxic effects of Na ions by reducing its uptake and this may indirectly help in maintaining the chlorophyll content (Giri and Mukerji 2004). Salinization reduced phosphorus absorption, because phosphate ions precipitate with $Ca^{2+}$, $Mg^{2+}$ and $Zn^{2+}$ ions and become unavailable to plants (Azcon-Aguilar et al. 1979). In this study, we found that mycorrhiza significantly enhanced P uptake facilitated by the extensive hyphae of the fungus; which extend beyond the depletion zone around roots and acquire nutrients that are several centimeters away from the root surface, as described by Ruiz-Lozano and Azcon (2000). The effective P acquisition by the external hyphae is related to the (a) formation of polyphosphates within the hyphae, resulting in maintenance of low internal phosphate (Pi) concentrations, (b) production of extracellular acid phosphatases, which catalyze the release of P from organic complexes in the soil and (c) encoding of a phosphate-transporters genes such as GvPT, GiPT and GmosPT in AMF (Marschner and Dell 1994; Selvaraj and Chellappan 2006). Enhanced P uptake by mycorrhiza may reduce the negative effects of $Na^+$ and $Cl^-$ ions by maintaining vacuolar membrane integrity, which facilitates compartmentalization within vacuoles and selective ion intake (Rinaldelli and Mancuso 1996).

Increasing $Na^+$ concentration in saline soils led to a substantial reduction of potassium accumulation in wheat tissues, which could be attributed to the competition between $Na^+$ and $K^+$ at the level of absorption sites (Epstein and Rains 1987). Mycorrhizal colonization significantly enhanced $K^+$ absorption, which is accomplished by regulating the expression and activity of $K^+$ and $Na^+$ transporters and of $H^+$ pumps that generate the driving force to transport ions (Parida and Das 2005). This positive effect of mycorrhization on $K^+$ acquisition was also reported by (Daei et al. 2009; Hajiboland et al. 2010; Wu et al. 2010b; Abdel-Fattah and Asrar 2012). Increased $K^+$ accumulation in stressed mycorrhized plants may maintain a high $K^+/Na^+$ ratio which (a) prevents the disruption of various K-mediated enzymatic processes and inhibition of protein synthesis (Evelin et al. 2009) and (b) is beneficial in the ionic balance of the cytoplasm or $Na^+$ efflux from plants (Giri and Mukerji 2004). Salinization enhanced $Na^+$ accumulation in wheat tissues; however, mycorrhizal colonization significantly reduced its uptake. Our results corroborate the findings of Giri and Mukerji (2004), Daei et al. (2009), Hajiboland et al. (2010), Wu et al. (2010b) and Abdel-Fattah and Asrar (2012). Indeed, mycorrhization reduced $Na^+$ acquisition via improving membrane integrity and, therefore, facilitate compartmentalization within vacuoles and selective ion uptake (Rinaldelli and Mancuso 1996), stimulating plant growth, which have dilution effect on $Na^+$ level (Al-Karaki 2006), discriminating against $Na^+$; as $Na^+$ was found at moderate levels in AMF, AM-fungal mycelium might have the possibility to pre-select nutrients for the plants (Hammer et al. 2011), preventing $Na^+$ allocation to the shoots by keeping it inside root cell vacuoles and intraradical fungal hyphae (Cantrell and Linderman 2001), inducing overexpression of $Na^+/H^+$ antiporters and lowering expression of *LsLea* gene; late embryogenesis abundant protein act as stress markers, which suggest that mycorrhized plants suffer less stress than non-mycorrhized plants and able to avoid $Na^+$ accumulation (Evelin et al. 2009). Prevention of $Na^+$ accumulation and enhancement of $K^+$ concentration could be a part of the general mechanism of salt stress alleviation in wheat by AMF.

Salt stress markedly reduced Zn, Cu and Fe acquisition; however, mycorrhizal symbiosis improved their mobilization, uptake and transfer to the shoot especially in the stressed plants. This positive effect might be attributed to the extensive root development and hyphae that reduce the distance for diffusion of nutrients (Subramanian et al. 2009), the binding of the metal to the fungal mycelium, or possibly due to fungal-induced changes in rhizosphere pH, which could alter the solubility and, therefore, availability of the metal (Li and Christie 2001), the increased shoot P content that could increase Cu and Zn sink size, which might induce uptake and translocation of Cu and Zn to plant shoots (Liu et al. 2000) and the enhanced expression of Zn transporter gene; the gene *MtZIP2* that encodes a plasma membrane-localized Zn transporter (Burleigh et al. 2003). Trace elements such as Cu, Fe and Zn are essential for normal growth and development of plants. Thus, modulation of nutrient acquisition can be one of the mechanisms used by AMF to improve wheat productivity under saline conditions.

Polyamines are considered as growth regulators implicated in a wide range of plant growth and developmental processes (Moschou et al. 2008). They can play a role in stress reactions and resistance by its ability to (a) associate with anionic components of the membrane such as phospholipids thereby stabilizing the bilayer surface and retarding membrane deterioration, (b) stabilize the conformation of nucleic acids, resulting in improved translation and protein synthesis, (c) prevent chlorophyll loss and the inhibition of photochemical reactions of photosynthesis, (d) maintain cellular pH and ion homeostasis, (e) improve water and nutrient uptake like phosphorus, nitrogen and micronutrients, (f) scavenge free radicals and (g) modify expression of some stress-related genes (Pang

et al. 2007; Smith and Read 2008) and thus improve crop productivity. Polyamines might also enhance mycorrhizal symbiosis by involvement in signalling events in the plant-fungus interaction (El Ghachtouli et al. 1995). Variations in PAs level were observed in wheat cultivars under soil salinization, Sids 1 accumulated more Spd and Spm than Giza 168; however, greater Put accumulation was detected in Giza 168 than in Sids 1. This observation indicates that the individual PAs may have different roles during the response of plants to salt stress. This corresponds with the findings of El-Bassiouny and Bekheta (2005), who reported that the excessive accumulation of Put in the saline-sensitive cultivars could be explained as a result of (a) the starvation for $K^+$ under saline conditions; Put was formed in leaves to replace $K^+$, the major inorganic cation, reflecting a homostatic mechanism for controlling cellular pH in higher plants and/or (b) the inhibition of Spd and Spm synthesis by the inhibition of the activity of the enzyme *S*-adenosylmethionine decarboxylase. Likewise, the high level of Spd and Spm induced by salinity in salt-tolerant cultivars suggested stimulation of Spd and Spm synthesis or inhibition of polyamine oxidase. Furthermore, variations in individual PAs in response to salinity and mycorrhization depending on the plant genotypes were detected. In mycorrhized salt-stressed Sids 1 plants, the increased Spm content might be due to increased ABA content (Sannazzaro et al. 2007), while the decreased Put level might result from Put conversion into different amino acids, based on the fact that amino acids and PAs are related in their metabolic pathways and affected by alteration in enzymatic levels caused by salinity (Flores and Filner 1985). In mycorrhized stressed Giza 168 plants, higher Put accumulation could play a protective role in the plant cell. Interestingly, our results showed that mycorrhizal inoculation significantly reduced the activities of diamine oxidase and polyamine oxidase in salt-stressed wheat plants. In mycorrhized salt-stressed Sids 1 plants, decreased PAO activity corresponded to increased Spm and Spd content. However, in mycorrhized stressed Giza 168 plants, decreased DAO activity corresponded to increased Put level. Differences in free PAs and PA catabolizing enzymes could play a vital role on antioxidative defense mechanism in inoculated plants under saline conditions.

## Conclusions

The outcome of the present study clearly illustrates the importance of mycorrhizal symbiosis in altering the plant physiology in salt-stressed wheat plants by reducing $Na^+$ uptake and by increasing the absorption of N, P, $K^+$, Fe, Zn and Cu as well as the content of PAs, which indicates that beneficial mechanisms are operating and suggests that the promotion of this symbiotic association could aid wheat plants to cope with saline conditions. It is interesting to note that this is the first study, which has shed light on the effect of mycorrhizal colonization on DAO and PAO activities in salt-stressed plants. Moreover, the present results show that mycorrhization might be a promising and an environmentally friendly approach to obtain higher wheat grain yield in saline croplands.

**Author contribution** N. Talaat designed the experiment, ran the experiment, determined nutrients concentration and polyamines content, analyzed data, and wrote the manuscript. B. Shawky designed the experiment, ran the experiment, and determined diamine oxidase and polyamine oxidase activities.

## References


Abdel-Fattah GM, Asrar AA (2012) Arbuscular mycorrhizal fungal application to improve growth and tolerance of wheat (*Triticum aestivum* L.) plants grown in saline soil. Acta Physiol Plant 34:267–277

Al-Karaki GN (2006) Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water. Sci Hortic 109:1–7

Allen SE (1989) Chemical analysis of ecological materials, 2nd edn. Blackwell, Oxford

Asthir B, Duffus CM, Smith RC, Spoor W (2002) Diamine oxidase is involved in $H_2O_2$ production in the chalazal cells during barley grain filling. J Exp Bot 53:677–682

Azcon-Aguilar C, Azcon R, Barea JM (1979) Endomycorrhizal fungi and rhizobium as biological fertilizers for *Medicago sativa* in normal cultivation. Nature 279:325–327

Burleigh SH, Kristensen BK, Bechmann IE (2003) A plasma membrane zinc transporter from *Medicago truncatula* is up-regulated in roots by Zn fertilization, yet down-regulated by arbuscular mycorrhizal colonization. Plant Mol Biol 52:1077–1088

Cantrell IC, Linderman RG (2001) Preinoculation of lettuce and onion with VA mycorrhizal fungi reduces deleterious effects of soil salinity. Plant Soil 233:269–281

Cottenie A, Verloo M, Kiekens L, Velghe G, Camerlynck R (1982) Chemical analysis of plants and soils. In: Laboratory of Analytical and Agrochemistry. State University, Ghent, Belgium, pp 14–24

Daei G, Ardekani MR, Rejali F, Teimuri S, Miransari M (2009) Alleviation of salinity stress on wheat yield, yield components, and nutrient uptake using arbuscular mycorrhizal fungi under field conditions. J Plant Physiol 166:617–625

El Ghachtouli N, Paynot M, Morandi D, Martin-Tanguy J, Gianinazzi S (1995) The effect of polyamines on endomycorrhizal infection of wild-type *Pisum sativum*, cv. Frisson (nod + myc+) and two mutants (nod−myc+ and nod − myc−). Mycorrhiza 5:189–192

El-Bassiouny HMS, Bekheta MA (2005) Effect of salt stress on relative water content, lipid peroxidation, polyamines, amino acids and ethylene of two wheat cultivars. Int J Agric Biol 7:363–368

Epstein E, Rains D (1987) Advances in salt tolerance. In: Gabelman HW, Loughman BC (eds) Genetic aspects of plant mineral nutrition. Martinus Nijhoff Publ., Boston, pp 113–125

Evelin H, Kapoor R, Giri B (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann Bot 104:1263–1280

Fester T, Hause G (2005) Accumulation of reactive oxygen species in arbuscular mycorrhizal roots. Mycorrhiza 15:373–379

Flores HE, Filner P (1985) Polyamine catabolism in higher plants. Characterization of pyrroline dehydrogenase. Plant Growth Regul 3:277–291

Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. Trans Br Mycol Soc 46:235–238

Giovanetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol 84:489–500

Giri B, Mukerji KG (2004) Mycorrhizal inoculant alleviates salt stress in *Sesbania aegyptiaca* and *Sesbania grandiflora* under field conditions: evidence for reduced sodium and improved magnesium uptake. Mycorrhiza 14:307–312

Goyal M, Asthir B (2010) Polyamine catabolism influences antioxidative defense mechanism in shoots and roots of five wheat genotypes under high temperature stress. Plant Growth Regul 60:13–25

Hajiboland R, Aliasgharzadeh N, Laiegh SF, Poschenrieder C (2010) Colonization with arbuscular mycorrhizal fungi improves salinity tolerance of tomato (*Solanum lycopersicum* L.) plants. Plant Soil 331:313–327

Hammer EC, Nasr H, Pallon J, Olsson PA, Wallander H (2011) Elemental composition of arbuscular mycorrhizal fungi at high salinity. Mycorrhiza 21:117–129

Kaldorf M, Schemelzer E, Bothe H (1998) Expression of maize and fungal nitrate reductase in arbuscular mycorrhiza. Mol Plant Microbe Interact 11:439–448

Köhler B, Raschke K (2000) The delivery of salts to the xylem. Three types of anion conductance in the plasmalemma of the xylem parenchyma of roots of barley. Plant Physiol 122:243–254

Kusano T, Berberich T, Tateda C, Takahashi Y (2008) Polyamines: essential factors for growth and survival. Planta 228:367–381

Li XL, Christie P (2001) Changes in soil solution Zn and pH and uptake of Zn by arbuscular mycorrhizal red clover in Zn-contaminated soil. Chemosphere 42:201–207

Liu A, Hamel C, Hamilton RI, Ma BL, Smith DL (2000) Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. Mycorrhiza 9:331–336

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic Press, New York

Marschner H, Dell B (1994) Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159:89–102

Moschou PN, Paschalidis KA, Roubelakis-Angelakis KA (2008) Plant polyamine catabolism. Plant Signal Behav 3:1061–1066

Pang X, Zhang Z, Wen X, Ban Y, Moriguchi T (2007) Polyamines, all-purpose players in response to environment stresses in plants. Plant Stress 1:173–188

Parida SK, Das AB (2005) Salt tolerance and salinity effects on plants. Ecotoxicol Environ Saf 60:324–349

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–160

Pregl F (1945) Quantitative organic micro analysis, 4th edn. A. Churchill Ltd., London

Rahnama A, Poustini K, Tavakkol-Afshari R, Ahmadi A, Alizadeh H (2011) Growth properties and ion distribution in different tissues of bread wheat genotypes (*Triticum aestivum* L.) differing in salt tolerance. J Agron Crop Sci 197:21–30

Reggiani R, Giussani P, Bertani A (1990) Relationship between the accumulation of putrescine and the tolerance to oxygen-deficit stress in Gramineae seedlings. Plant Cell Physiol 31:489–494

Rinaldelli E, Mancuso S (1996) Response of young mycorrhizal and non mycorrhizal plants of olive tree (*Olea europaea* L.) to saline conditions. 1. Short term electro physiological and long term vegetative salt effects. Adv Hortic Sci 10:126–134

Ruiz-Lozano JM, Azcon R (2000) Symbiotic efficiency and infectivity of an autochthonous arbuscular mycorrhizal *Glomus* sp from saline soils and *Glomus deserticola* under salinity. Mycorrhiza 10:137–143

Sannazzaro AI, Alvarez C, Menendez A, Pieckenstain F, Alberto E, Ruiz O (2004) Ornithine and arginine decarboxylase activities and effect of some polyamine biosynthesis inhibitors on *Gigaspora rosea* germinating spores. FEMS Microbiol Lett 230:115–121

Sannazzaro AI, Echeverria M, Alberto EO, Ruiz OA, Menendez AB (2007) Modulation of polyamine balance in *Lotus glaber* by salinity and arbuscular mycorrhiza. Plant Physiol Biochem 45:39–46

Schenck NC, Perez Y (1990) Manual for the identification of VA mycorrhizal fungi. Synergistic Publications, Gainesville, p 286

Selvaraj T, Chellappan P (2006) Arbuscular mycorrhizae: a diverse personality. J Cent Eur Agric 7:349–358

Sharma MP, Gaur A, Bhatia NP, Adholeya A (1996) Growth responses and dependence of *Acacia nilotica* var. cupriciformis on the indigenous arbuscular mycorrhizal consortium of a marginal wasteland soil. Mycorrhiza 6:441-446

Shekoofeh E, Sepideh H, Roya R (2012) Role of mycorrhizal fungi and salicylic acid in salinity tolerance of *Ocimum basilicum* resistance to salinity. Afr J Biotechnol 11:2223–2235

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic Press, London

Snedecor GW, Cochran WG (1980) Statistical methods, 7th edn. Iowa State University Press, Ames

Subramanian KS, Tenshia V, Jayalakshmi K, Ramachandran V (2009) Role of arbuscular mycorrhizal fungus (*Glomus intraradices*)—(fungus aided) in zinc nutrition of maize. J Agric Biotechnol Sustain Dev 1:29–38

Talaat NB, Shawky BT (2012) Influence of arbuscular mycorrhizae on root colonization, growth and productivity of two wheat cultivars under salt stress. Arch Agron Soil Sci 58:85–100

Wu QS, Peng YH, Zou YN, Liu CY (2010a) Exogenous polyamines affect mycorrhizal development of *Glomus mosseae*-colonized citrus (*Citrus tangerine*) seedlings. ScienceAsia 36:254–258

Wu QS, Zou YN, He XH (2010b) Contributions of arbuscular mycorrhizal fungi to growth, photosynthesis, root morphology and ionic balance of citrus seedlings under salt stress. Acta Physiol Plant 32:297–304