Arbuscular mycorrhizal fungi enhances salinity tolerance of *Panicum turgidum* Forssk by altering photosynthetic and antioxidant pathways

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RESEARCH ARTICLE

Arbuscular mycorrhizal fungi enhances salinity tolerance of *Panicum turgidum* Forssk by altering photosynthetic and antioxidant pathways

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Present experiments were conducted to assess the response of *Panicum turgidum* to salinity and possible role of arbuscular mycorrhizal fungi (AMF) in enhancing the salt tolerance. The activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase (GR) and compatible solutes were increased by salt stress and were further enhanced by AMF inoculation. Hydrogen peroxide and malonaldehyde content increased in salt-stressed plants while a reduction was observed due to AMF inoculation. Salt-stressed plants showed higher activities of pyruvate orthophosphate dikinase (PPDK), phosphoenolpyruvate carboxylase (PEPC) and malate dehydrogenase as compared to control and AMF-inoculated plants. Salt stress caused significant decrease in phosphorous, potassium and calcium uptake but an increase in sodium uptake was observed. AMF alleviate salinity-induced negative impact on the plant growth and nutrient uptake by reducing the oxidative damage through strengthening of the antioxidant system.

Keywords: *Panicum turgidum*; AM fungi; salinity tolerance; antioxidants; enzymes

Introduction

Plants are continuously exposed to variety of environmental stresses resulting in perturbed growth and metabolism. Salinity is one of the important and adverse environmental constraint restricting growth and development of plant (Alqarawi et al. 2014; Fatma et al. 2014). At global level, salinity is an important problem particularly in arid and semiarid regions. Around 7% of the world's land is salt-affected (Ruiz-Lozano et al. 2001, 2012). Increasing industrialization and excessive use of saline water for crop irrigation further aggravates this problem. Salinity triggers osmotic effects resulting in reduced growth through alterations in physio-biochemical processes like photosynthesis, enzyme activity and ion homeostasis (Ahmad et al. 2012; Porcel et al. 2012; Fatma et al. 2014). Salt stress triggers excess production of toxic reactive oxygen species (ROS) and increased ROS levels induce oxidative stress in plants. Toxic ROS include superoxide, hydroxyl and peroxide radicals, and they pose detrimental effects to normal metabolism and growth (Mittler 2002; Ahmad et al. 2010). ROS produced accumulate in leaves and cause oxidation of various cellular molecules including lipids, proteins and chlorophylls. Peroxidation of membrane lipids, protein degradation and damage to nucleic acids are the immediate effects of the excess ROS (Mittler 2002; Ahmad et al. 2010). Moreover, photosynthetic apparatus and pigments are also often targeted by the toxic ROS (Ahmad et al. 2010; Fatma et al. 2014).

Nevertheless, plants are well equipped with different defense mechanisms to counteract the stress-induced oxidative damage. Upregulation of antioxidant enzymes is one of the prime defense mechanisms that help plants to combat stress-induced oxidative damage. Antioxidant defense system comprises superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and glutathione reductase (GR) (Ahmad et al. 2010; Liu et al. 2014). These intricate antioxidant enzymes mediate scavenging of toxic ROS so as to protect cells from the oxidative damage. In addition, increased synthesis and accumulation of compatible osmolytes is another tolerance mechanism employed by plants for ameliorating the damaging impacts of salinity (Ahmad & Sharma 2008; Ahanger et al. 2014b). Compatible solutes that contribute to osmoregulation through maintaining the cell water content include free proline, glycine betaine (GB), sugars and amino acids (Ahmad & Sharma 2008; Ahanger et al. 2014b). Accumulating compatible solutes or ions while bringing efficient sequestration and compartmentation of deleterious ions into the less sensitive parts like vacuole or apoplast is an important trait that determines salt tolerance in plants (Ahmad & Sharma 2008; Azooz et al. 2011). Most of the plants form symbiotic associations with the arbuscular mycorrhizal fungi

(AMF). AMF improves host plant growth by enhancing the rhizospheric soil characteristics (Asghari et al. 2005; Ahanger et al. 2014a; Hodge & Storer 2015) and enhances mobilization and uptake of several essential elements by modifying root architecture (Wu et al. 2010; Ahanger et al. 2014a; Hameed et al. 2014; Hodge & Storer 2015). AMF has been reported to alleviate the salt stress in plants through various mechanisms. In citrus plants, AMF colonization caused enhancement in leaf area, photosynthesis and phosphorous uptake by plants (Sherstha et al. 1995).

Panicum turgidum Forssk belongs to Poaceae family is a C_4 salt-tolerant perennial grass (Khan & Qaiser 2006; Koyro et al. 2013). It is commonly found in saline deserts of southern Pakistan but is also reported in other arid areas (Khan & Qaiser 2006). Halophytes are believed to be equipped well with several physiological and biochemical tolerance mechanisms and considered as suitable candidate plant for maintaining the sustainability of salt-affected soils (Khan et al. 2009). Present study was carried out to investigate the impact of salt stress on growth and key physio-biochemical attributes of *P. turgidum* and potential of AMF in ameliorating salt stress-induced deleterious changes.

Materials and methods

Soil, plant and mycorrhizal inoculums

The experiments were conducted under climate controlled growth chamber conditions at the Plant Production Department, College of Food & Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia. The soil used in the present investigation was sandy loam with the following properties (%): moisture content, 18.17; organic carbon, 0.74; total soluble salts, 0.26; total nitrogen, 0.058; electrical conductivity (EC), 6.8 dS/m and pH 7.8. Dried soil and fine sand (2:1; w/w) was autoclaved for 3 h at 121°C and then divided equally among plastic pots (10 cm in diameter, with 5 kg capacity). The seeds of *P. turgidum* were collected from Thomama region (N 25° 336,291 E 46° 533,651), Riyadh, Saudi Arabia. Seeds were surface sterilized with sodium hypochlorite (0.5%, v/v) for 3 min followed by thorough washing with distilled water. Healthy germinating seeds were transferred to pots (1 plant/pot). Hoagland's solution (Hoagland & Arnon 1950) supplemented with NaCl (250 mM) as described by Koyro et al. (2013) was used for irrigation every alternate day. The mycorrhizal inoculum [*Funneliformis mosseae* (syn. *Glomus mosseae*); *Rhizophagus intraradices* (syn. *Glomus intraradices*) and *Claroideoglomus etunicatum* (syn. *Glomus etunicatum*)] (Figure 1) was prepared according to Hashem et al. (2014). Fungal inoculums consisted of AM fungal spores, hyphae and colonized root fragments. The mycorrhizal inoculum was added to the experimental soil as 10 g of trap soil culture (approx. 100 spores/g trap soil, $M = 80\%$)/pot (1 Kg). Nonmycorrhizal soil served as control. The pots incubated for eight weeks in growth chamber

at 20/30°C as night/day alternative temperatures (12/12 h).

Determination of arbuscular mycorrhizal colonization

The mycorrhizal spores were extracted from the experimental soil of each treatment by wet sieving and decanting method as described by Daniels and Skipper (1982) and modified by Utobo et al. (2011). The intensity of fungal infection (mycelium, vesicles and arbuscules) and development within the infected regions of the roots were calculated as described in details (Hashem et al. 2014) according to the following formula:

$$\% \, \text{colonization} = \frac{\text{Total number of AM positive segments}}{\text{Total number of segments studied}} \times 100.$$

Photosynthetic pigments

The leaf samples (0.5 g) were extracted in aqueous acetone (80%) and extract was centrifuged at $10,000 \times g$ for 10 min. The optical densities of the supernatant were recorded at 480, 645 and 663 nm (T80 UV/VIS Spectrometer, PG Instruments Ltd, USA) using acetone as blank (Arnon 1949).

Extraction and estimation of carbohydrate fractions

The extraction of soluble carbohydrate fractions was carried out according to the method of Said et al. (1964). In this method, known weight of powdered leaf samples were homogenized in a mixture of 5.0 mL of 20% (w/v) phenol and 10.0 mL of 30% (w/v) trichloroacetic acid (TCA) and homogenate was left overnight in refrigerator at 4°C followed by centrifugation at 5000g for 15 min. The supernatant was used for estimation of water-soluble carbohydrates (monosaccharides and disaccharides), however the precipitate was oven dried (at 80°C for two successive constant weights) and used for estimation of polysaccharides. The content of soluble sugars (monosaccharides and disaccharides) and insoluble sugars (polysaccharides) was estimated according to the method of Nelson (1944) as modified by Naguib (1964).

Extraction and estimation of carboxylation enzymes

The fresh leaf samples were grinded in 50 mM Na acetate (pH 6.0) buffer as described by Omoto et al. (2012). NADP-MDH (NADP-dependent malate dehydrogenase, EC 1.1.1.82) and NAD-MDH (NAD-dependent malate dehydrogenase, EC 1.1.1.37) activities were determined using the methods described by Scheibe and Stitt (1988). NADP-ME (NADP-malic enzyme, EC 1.1.1.40) activity was assayed in accordance with Kanai and Edwards (1973) with minor modifications as described in details by Omoto et al. (2012). RuBPCase (ribulose-1,5-bisphosphate carboxylase, EC 4.1.1.39) activity was
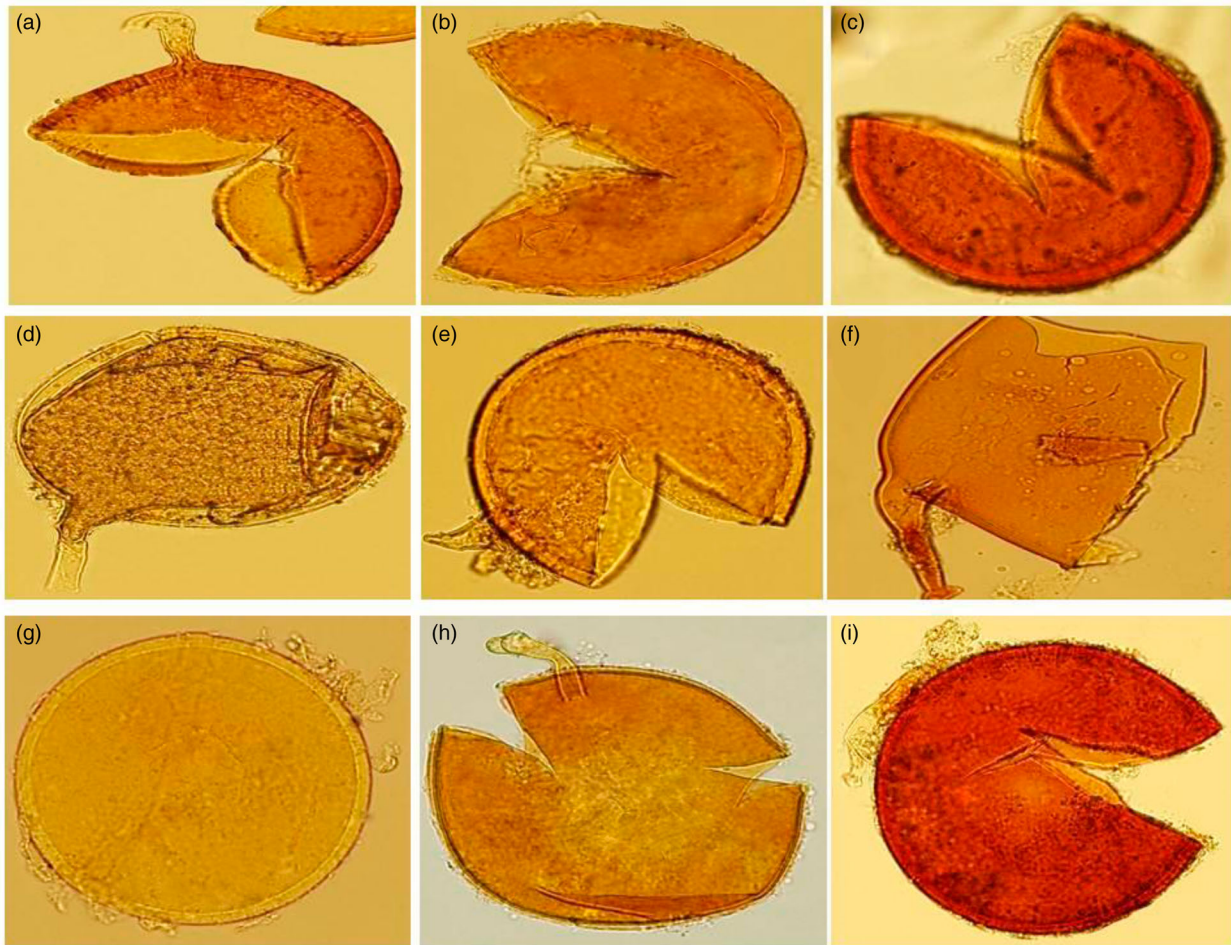
Figure 1. (a–i) Morphology of typical an intact crushed spores of AMF. (a, b, c) *C. etunicatum* (syn. *G. etunicatum*); (d, e, f) *F. mosseae* (syn. *G. mosseae*); (g, h, i), *R. intraradices* (syn. *G. intraradices*).

assayed according to Du et al. (1996). The method described by Fukayama et al. (2001) was used for assaying PPDK (Pyruvate orthophosphate dikinase, EC 2.7.9.1). PEPC (Phosphoenolpyruvate carboxylase, EC 4.1.1.31) activity was assayed following Du et al. (1996).

Extraction and estimation of antioxidant enzymes

The fresh leaf tissues (5.0 g) were macerated in 10 mL potassium phosphate buffer [pH 7.8; 50 mM] using an ice cooled sterilized pestle and mortar. The extract was centrifuged at $15,000 \times g$ for 15 min at 4°C and supernatant was used to determine the enzyme activity. Protein content in the extract was determined following Bradford (1976).

SOD (EC 1.15.1.1) was assayed by measuring inhibition in photoreduction of nitroblue tetrazolium (NBT) according to Giannopolitis and Ries (1977). The reaction mixture contained 500 μL phosphate buffer (pH 7.8), 0.5 mL distilled H_2O, 100 μL methionine (13 mM), 50 μL NBT (63 μM) and 50 μL enzyme extract and was exposed to light for 20 min. The OD of the irradiated aliquot was read at 560 nm. Activity that caused 50% inhibition in photoreduction of NBT was considered as one enzyme unit. The CAT (EC 1.11.1.6) activity was determined following method of Chance and Maehly

(1955). The reaction mixture contain 1.9 mL potassium phosphate buffer (50 mM; pH 7.0) and 1 mL H_2O_2 (5.9 mM) and 100 μL of the enzyme extract. Decrease in OD was observed at 240 nm for 2 min. The method of Chance and Maehly (1955) was used for determination of POD (EC 1.11.1.7) activity. One milliliter of reaction mixture contained 50 mM potassium phosphate buffer (pH 5.0), 100 μL guaiacol (20 mM), 100 μL H_2O_2 (40 mM) and 100 μL enzyme extract. The increase in OD at 470 nm was monitored for 2 min and activity was expressed as EU mg^{-1} protein. The activity of GR (EC 1.6.4.2) was monitored by observing decrease in absorbance at 340 nm for 2 min (Carlberg & Mannervik 1985). The activity of GR was calculated using the extinction coefficient of NADPH of 6.2 mM^{-1} cm^{-1} and expressed as EU mg^{-1} protein.

Glycine betaine content

The dry leaf sample (0.5 g) was shaken in 10 mL toluene (0.5%) and kept at 4°C overnight. After centrifugation, 1 mL of the filtrate was mixed with 1 mL of sulfuric acid (2N). 0.5 mL of solution was taken and then 200 μL of potassium tri-iodide (KI$_3$) solution (containing 7.5 g Iodine and 10 g Potassium iodide in 100 mL of 1 N HCl) was added to the tubes. The content was cooled in

a chiller. Afterwards 2.8 mL of ice cooled deionized H_2O and 5 mL of 1–2 di-chloroethane was added to the samples. The absorbance of the organic layer (lower layer) was recorded spectrophotometrically at 365 nm. The concentrations of GB were recorded against a standard curve developed with different concentrations of GB following Grieve and Grattan (1983).

Hydrogen peroxide

The fresh leaf tissue (0.5 g) was ground in a prechilled mortar with 5 mL of 0.1% (w/v) TCA. This homogenate was then centrifuged at $12,000 \times g$ for 15 min. To the supernatant (0.5 mL), 0.5 mL of potassium phosphate buffer (pH 7.0) and 1 mL of KI was added. The mixture was vortexed and the OD of suspension was read at 390 nm. H_2O_2 in the samples was determined following Velikova et al. (2000).

Malondialdehyde

The fresh leaf tissue (500 mg) was extracted in 5 mL 1.0% TCA. The homogenate was centrifuged at $20,000 \times g$ for 15 min. The supernatant (500 µL) was added with 2 mL 0.5% 2-thiobarbituric acid (prepared in 20% TCA). The samples were heated at 100°C for 1 h. Thereafter, the reaction was stopped by cooling the samples in ice, centrifuged at $10,000 \times g$ for 10 min and the supernatant was read at 532 and 600 nm (Carmak & Horst 1991). The level of thiobarbituric acid reactive substances was calculated using the absorption coefficient of 155 mmol cm^{-1}.

Total phenolics

Total phenolics in leaf tissues were estimated following method of Julkenen-Titto (1985). Fresh leaf (0.5 g) was extracted with 80% acetone (5 mL) and the extract was centrifuged at $10,000 \times g$ for 10 min. Aliquot (0.1 mL) was diluted to 2 mL using distilled H_2O.Thereafter, 1 mL of Folin and Ciocalteau's phenol reagent and 5 mL of 20% sodium carbonate was added and was read at 750 nm.

Free proline

Leaf samples (500 mg) were homogenized in 10 mL of 3% sulfosalicylic acid following the method of Bates et al. (1973). The crushed sample was filtered and 2.0 mL of filtrate were taken in a test tube, mixed with 2.0 mL acid ninhydrin solution and 2.0 mL glacial acetic acid. The reaction was started by incubating tubes at 100°C and terminated by keeping tubes on ice. After cooling, 4 mL toluene was added to the tubes and the upper phase containing toluene was read at 520 nm.

Estimation of ion accumulation

The estimation of Na^+, K^+, Mg^{2+} and Ca^{2+} concentrations was analyzed by flame photometrically described by Wolf

(1982). The phosphorus (P) was extracted by nitric-perchloric acid digestion and measured using the Vanadomolybdophosphoric colorimetric method (Jackson 1962). Standard curve of each mineral (10–100 µg/mL) was used as reference.

Statistical analysis

Duncan's Multiple Range Test was performed using One-Way ANOVA for a completely randomized design by SPSS-21 software and the differences in means (three replicates were used) were determined by the least significant differences (LSD) ($p = 0.05$) test.

Results

Mycorrhizal status

The typical morphological characters of an intact crushed spores of AMF used in the current study was shown in Figure 1(a)–(i), *C. etunicatum* (a, b, c,) [syn. *G. etunicatum*]; *F. mosseae* (d, e, f) [syn. *G. mosseae*]; and *R. intraradices* (g, h, i) [syn. *G. intraradices*]. The result of the root colonization of *P. turgidum* with AMF is presented in Figure 2(a)–(f).

A different AMF infection patterns in the roots of *P. turgidum* was observed, including colonization of spores (S) and vesicles (V), arbuscles (A), trank (T) and hyphae (H). As shown in Table 1, salinity caused significant decrease in mycelia, number of spores, vesicles and arbuscles by 77.1%, 60.2%, 38% and 58.6%, respectively, as compared to the control plants.

Photosynthetic pigments

Salinity reduced chlorophyll a, chlorophyll b and carotenoid contents by 26.1%, 48.5% and 51.2%, respectively (Table 2). However, AMF ameliorated the salinity-induced impact on these attributes (Table 2). The content of chlorophyll a, chlorophyll b and carotenoid in plants inoculated with AMF increased by 12.8%, 40.2%, and 21.9%, respectively, compared to the control plants.

Antioxidative enzymes

Considerable increase in activities of SOD, POD, CAT and GR was observed in AMF-inoculated plants. Percent increase in SOD, POD, CAT and GR due to AMF was 41.6%, 21.25%, 9.9% and 7.3%, respectively (Figure 3(a)–(d)). Salinity-induced increase in the level of SOD, POD, CAT and GR was 174.3%, 72.5%, 124.3% and 54.5%, respectively (Figure 3(a)–(d)).

Activities of carbon metabolizing enzymes

The activity of PPDK, PEPC, NADP-malate dehydrogenase (NADP-MDH) and NAD-malate dehydrogenase (NAD-MDH) was increased by 51.4%, 93.3%, 130.7% and 68.3% in salt-stressed *P. turgidum* plants as compared to the control (Figure 4(a)–(f)). However, AMF-inoculated plants maintained reduced activity of these
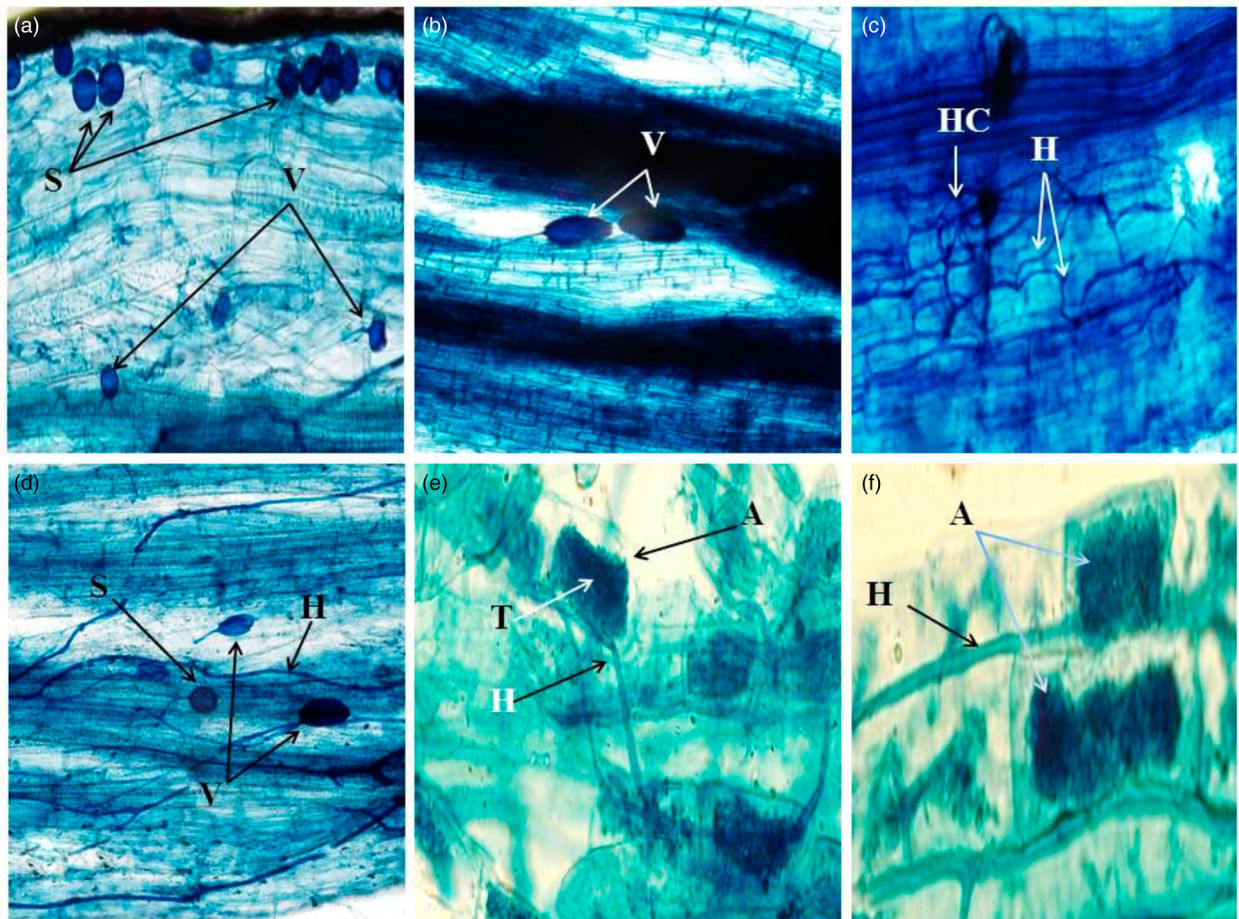
Figure 2. The colonization of AMF in the roots of *P. turgidum*. (a): vesicle (V) and spore (S) occurrence of the AMF-colonized root section, (b): Vesicles (V) formed in roots of *P. turgidum*, (c): Hyphae (H) and hyphal coils (HC) in roots of *P. turgidum*, (d): colonization of plant roots by AMF hyphae (H), vesicles (V) and spore density (S), (e): The root segments colonized by arbuscles (A), trank (T) and hyphae (H) of AMF, (f): Arbuscles (A), and hyphae (H) colonize the root.

Table 1. Effect of NaCl (250 mM) on total spores (spore/50 g soil) and structural colonization (%) of AMF in *P. turgidum*.

Treatments	Total spores (spore/50 g soil)	Structural colonization (%)		
		Mycelia	Vesicles	Arbuscules
AMF	68	90.33	50	46
NaCl + AMF	27	20.66	31	19
LSD at: 0.05[a]	9.8814	16.556	7.5186	6.4119

[a]Least significant difference. AMF – Arbuscular mycorrhizal fungi.

enzymes. Percent decrease was 21.4%, 15.5%, 14.3% and 6.6% in PPDK, PEPC, NADP-MDH and NAD-MDH, respectively (Figure 4(a)–(f)). An obvious reduction in activities of NADP-ME and Rubisco (up to 48.3% and 57%, respectively) was observed under salt stress conditions. However, AMF not only increased the activities

Table 2. Effect of NaCl (250 mM) in presence and absence of AMF on photosynthetic pigment contents (mg/g fresh weight) in *P. turgidum*.

Treatments	Chlorophyll a	Chlorophyll b	a/b	Carotenoids	Total
Control	1.210	0.383	3.174	0.082	1.675
AMF	1.388	0.537	2.588	0.100	2.025
NaCl	0.893	0.197	4.538	0.040	1.13
NaCl + AMF	1.03	0.295	3.491	0.064	1.389
LSD at: 0.05[a]	0.0652	0.0451	0.5002	0.011	0.0779

[a]Least significant difference. AMF – Arbuscular mycorrhizal fungi.
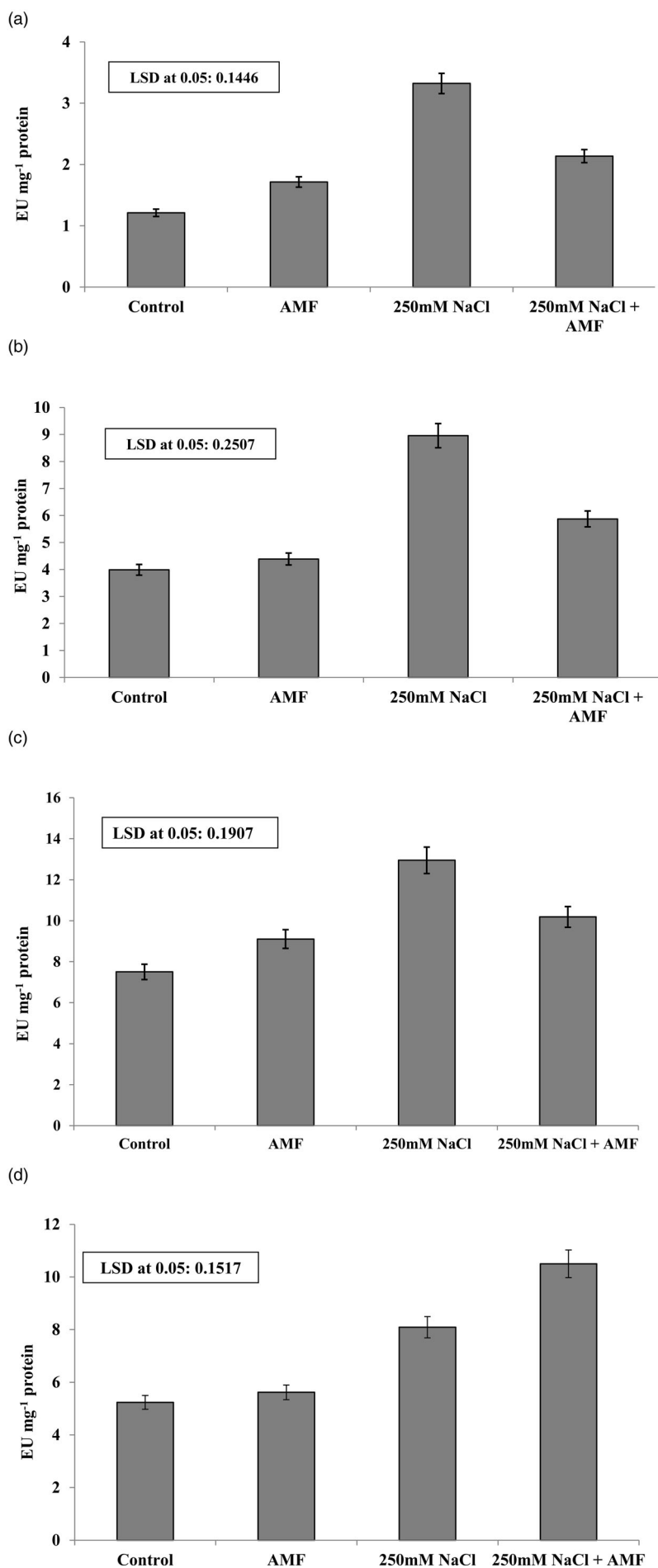
(a)

(b)

(c)

(d)

Figure 3. (a)–(d). The effect of NaCl (250 mM) in presence and absence of AMF on (a) superoxide dismutase (SOD), (b) catalase (CAT), (c) peroxidase (POD) and (d) glutathione reductase (GR) (EU mg^{-1} protein) activities in *P. turgidum*.
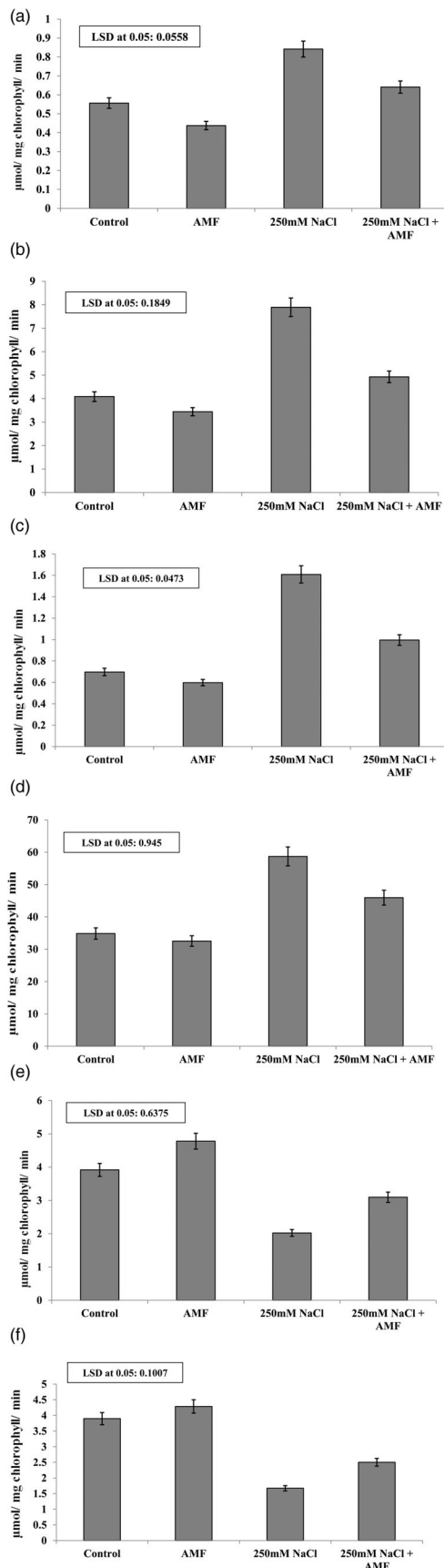
Figure 4. (a)–(f) Effect of NaCl (250 mM) in presence and absence of AMF on activities of carbon metabolizing enzymes (μmol/mg chlorophyll/min) in *P. turgidum*. (a) pyruvate orthophosphate dikinase; (b) phosphoenolpyruvate carboxylase; (c) NADP-malate dehydrogenase; and (d) NAD-malate dehydrogenase; (e) NADP-malic enzyme and (f) Rubisco.

of these enzymes but also ameliorated the salinity-induced changes to some extent (Figure 4(a)–(f)). Percent increase in NADP-ME and Rubisco in AMF-inoculated plants was 22% and 10%, respectively.

Hydrogen peroxide and malondialdehyde

In salt-stressed *P. turgidum* plants, H_2O_2 and malondialdehyde (MDA) content increased by 78.4% and 72.3%, while as in AMF-inoculated salt-stressed plants an increase observed by 5.2% and 24.1%, respectively (Table 3). In AMF-inoculated plants, H_2O_2 and MDA reduced by 2.9% and 35%, respectively, as compared to control plants (Table 3).

Proline, glycine betaine and total phenols

P. turgidum plants subjected to salt stress showed increased accumulation of proline, GB and total phenols. An increase in proline, GB and total phenols due to salinity was 142.6%, 39.8% and 85.3%, respectively (Table 3). The plants inoculated with AMF showed a slight decrease in proline (25.1%), GB (6.9%) and total phenols (43.6%) content (Table 3).

Sugars

The salt-stressed *P. turgidum* plants showed reduced total sugar content than control plants. In salt-stressed plants, total sugars and polysaccharides decreased by 16% and 34.9%, respectively, while as in salt-stressed AMF (250mM + AMF) inoculated plants a reduction of 11.4% and 17.4% was observed (Table 4). However, AMF-inoculated plants showed 20.7% and 26.8% increase in total sugar and polysaccharide content (Table 4). Salt-stressed plants showed 84.3% and 114.1% enhancement in soluble sugar and monosaccharide contents, respectively (Table 4). However, in AMF-inoculated plants, soluble sugar and monosaccharide contents reduced by 6.03% and 26.4%, respectively.

Elements accumulation

AMF inoculation resulted in increased uptake of essential elements like phosphorous, potassium and calcium, while as reduced the sodium ion. Percent increase in phosphorous, potassium and calcium content due to AMF was 228.3%, 20.09% and 7%, respectively (Table 5). Salinity stress increased sodium by 55.8%, while reduced phosphorous, potassium and calcium by 52.4%, 40.4% and 18.4%, respectively. In salinity-stressed AMF plants, percent decrease in phosphorous, potassium and calcium was 63.6%, 29% and 3.2%, respectively (Table 5).

Discussion

In our study, drastic decline in colonization and spore population in *P. turgidum* plants subjected to salt stress are in confirmation with the findings of Wu et al. (2010) for citrus seedlings, Miranda et al. (2011) for

Table 3. Effect of NaCl (250 mM) in presence and absence of AMF on systemic resistance attributes [H_2O_2 (μMol/g fresh weight), MDA (nMol/g fresh weight), TP (mg/g fresh weight), GB (μMol/g fresh weight), and proline (μMol/g fresh weight) in *P. turgidum*.

Treatments	H_2O_2	MDA	TP	GB	Proline
Control	217.70	42.992	2.860	32.835	0.711
AMF	211.32	27.911	4.103	35.106	0.890
NaCl	388.53	74.117	5.319	45.953	1.725
NaCl + AMF	229.03	53.391	6.737	38.007	1.250
LSD at: 0.05[a]	4.0754	0.5188	0.0872	0.8937	0.0485

[a]Least significant difference; AMF – Arbuscular mycorrhizal fungi; H_2O_2 –Hydrogen peroxide; MDA –malondialdehyde; GB – glycine betaine; TP – Total phenol.

Table 4. Effect of NaCl (250 mM) in presence and absence of AMF on sugars content (mg glucose/g dry weight) in *P. turgidum*.

Treatments	MonoS	DiS	TSS	PolyS	TS
Control	27.12	11.35	38.47	167.33	205.81
AMF	19.95	16.19	36.15	212.34	248.5
NaCl	58.07	5.94	64.01	108.81	172.83
NaCl + AMF	36.70	8.13	44.83	137.39	182.22
LSD at: 0.05[a]	2.6134	0.8921	3.1395	5.1197	5.0262

[a]Least significant difference; AMF – Arbuscular mycorrhizal fungi; MonoS –Monosaccharides; DiS –Disaccharides; TSS – Total soluble sugars: PolyS – Polysaccharides; TS – Total sugars.

ground cherry and Aroca et al. (2013) for lettuce. Salinity stress caused drastic decline in chlorophyll pigments. Our results of reduction in chlorophyll content due to salinity stress is in confirmation with the findings of Doganlar et al. (2010), Rasool et al. (2013) and Alqarawi et al. (2014) for tomato, *Cicer arietinum* and *Ephedra alata*, respectively. In *E. alata*, Alqarawi et al. (2014) demonstrated that chlorophyll contents reduced considerably with increasing salinity levels. Similarly, Azooz et al. (2011) also observed drastic decline in chlorophyll contents due to saline irrigation. Reduced chlorophyll contents under stress is attributed to increased activity of chlorophyllase causing degradation of pigments and hence resulting in reduced photosynthesis and hence growth. High salinity restricts *de novo* synthesis of proteins as well as functioning of pigment protein complex (Levitt 1980; Sultana et al. 1999; El-Tayeb 2005). Compared with stressed plants, AMF-inoculated plants maintained higher contents of chlorophyll pigments. Our results of enhanced chlorophyll contents in AMF-colonized plants are in support of the findings of Hajiboland et al. (2010) for *Solanum lycopersicum* L. and Aroca et al. (2013) for lettuce. In pepper, Kaya et al. (2009) demonstrated that inoculation of AMF increased chlorophyll content under normal as well as salt-stressed conditions. Enhancement in chlorophyll pigments due to

AMF is because of enhanced mineral uptake especially magnesium, an important component of chlorophyll molecule (Sheng et al. 2008). Higher contents of chlorophyll pigments in AMF-inoculated plants contribute to greater photosynthetic activity leading to maintained growth. Hence, it is clear from our results that AMF inoculation enhances chlorophyll contents and also mitigates the negative impact of salinity to some extent.

Activities of different antioxidant enzymes are upregulated on exposure to environmental stress. Antioxidant enzymes mediate scavenging of toxic ROS and hence allaying the oxidative stress-induced deleterious effects on several sensitive molecules like proteins, nucleic acids and lipids. In our results, increase in activities of SOD, CAT, GR and POD as a result of salt stress is in concurrence with the findings of Koca et al. (2007) for *Sesamum indicum* Mittal et al. (2012) for *Brassica juncea* and Rasool et al. (2013) for *C. arietinum* L. Enhancement in antioxidant enzyme activity under stress conditions helps in quick scavenging of ROS and maintaining their levels below the deleterious levels. SOD mediates scavenging of superoxide radicals into water and hydrogen peroxide and H_2O_2 produced is subsequently converted into water and oxygen by CAT (Mittler 2002). In our results, AMF inoculation increased the activity of the antioxidant enzymes studied thereby

Table 5. Effect of NaCl (250 mM) in presence and absence of AMF on ion accumulation (mg/g dry weight) in *P. turgidum*.

Treatments	Na^+	K^+	Na/K	Ca^{2+}	*p*
Control	3.10	16.67	0.186	23.46	0.187
AMF	2.68	20.02	0.134	25.11	0.614
NaCl	4.83	9.93	0.486	19.13	0.089
NaCl + AMF	3.38	11.83	0.286	22.70	0.306
LSD at: 0.05[a]	0.1335	0.5478	0.0153	0.4179	0.0218

[a]Least significant difference; AMF – Arbuscular mycorrhizal fungi.

mediating quick scavenging of ROS so that metabolic processes are least affected. Increased activities of antioxidant enzymes in AMF plants support the findings of Ghorbanli et al. (2004) and Abdel Latef and Chaoxing (2011) for tomato. In tomato, Qun et al. (2007) reported that salinity-stressed AMF-inoculated seedlings maintained higher activities of SOD, CAT and POD as compared to salt-stressed uninoculated seedlings. Similar results have been observed by Tang et al. (2009) in maize. AMF-induced increase in CAT activity has been reported in soybean (Ghorbanli et al. 2004). GR is among active and important components of ascorbate–glutathione pathway where the net electron flow is from NADPH to H_2O_2 resulting in conversion of H_2O_2 into water (Mittler 2002). Increased GR activity results in enhanced glutathione (reduced) production which acts as electro donor during the conversion of dehydroascorbate into ascorbic acid (Mittler 2002). Upregulation of GR maintains higher ratio of GSH/GSSG and $NADP^+$/NADPH. Higher ratio of $NADP^+$/NADPH reduces the formation of superoxide radicals through maintaining the photosynthetic electron transport (Noctor & Foyer 1998; Mittler 2002).

Salinity stress induced increase in H_2O_2 production corroborate with the findings of Ahmad (2010) and Ahmad et al. (2012), who in mustard demonstrated that H_2O_2 production enhanced considerably due to exposure to salinity with concomitant increase in membrane leakage. Tuna et al. (2008) confirmed that salinity enhances membrane leakage. Increased production of H_2O_2 causes disturbances in cellular homeostasis. AMF-treated plants showed lesser production of H_2O_2 which is possibly because of the increased activity of antioxidants in AMF-inoculated plants. In our results, increased peroxidation of membrane lipids in salt-stressed plants is in concurrence with the observations of Ahmad (2010) for mustard and Rasool et al. (2013) for chick pea. Exposure to salt stress enhances the production and accumulation of ROS. ROS cause peroxidation of unsaturated lipid component of membranes resulting in the loss of integrity, and hence leakage and desiccation. Present findings strongly support the ameliorative role of AMF in protecting the membrane lipids from peroxidation. Reduced MDA content in AMF-inoculated plants may be due to the substantial increase in antioxidant activities and phosphate metabolism (Qun et al. 2007; Tang et al. 2009).

Drastic decline in activities of photosynthetic carbon metabolizing enzymes Rubisco and NADP-dependent malic enzyme was obvious in salt-stressed *P. turgidum* seedlings. Similar reports of reduced activity of Rubisco due to salinity stress have earlier been reported by Soussi et al. (1998) in *C. arietinum* L. and Fang et al. (2013) in *Ulva linza*. Reduction in Rubisco activity is possibly due to its sensitivity to chloride ions (Seeman & Critchley 1985). In *P. turgidum*, reduced Rubisco activity due to salinity has been earlier demonstrated by Koyro et al. (2013). Inoculation of AMF induced the activity of NADP-ME resulting in enhanced stress tolerance. Earlier enhanced NADP-ME activity has been

reported to be correlated with enhanced stress tolerance. For example, in *Oryza sativa*, it has been demonstrated that exposure of stress enhances the transcript levels of NADP-ME (Cheng & Long 2007; Liu et al. 2007). NADP malic enzymes are believed to participate in several important metabolic pathways and plant defense through malate metabolism (Casati et al. 1999). Enhanced activity of NADP-ME in AMF-inoculated plants contributes to stress tolerance through enhancing carbon metabolism. PPDK, PEPC and malate dehydrogenase are among the key enzymes regulating carbon metabolism in C_4 plants. Our results of increased activities of PPDK, PEPC and MDH in salt-stressed plants corroborate with the findings of Fang et al. (2013) for *U. linza*. Winter (1974) also reported induction of PEPC activity in water-stressed *Mesembryanthemum crystallinum* L. Higher activities of C_4 enzymes is believed to be essential component for optimal growth under stress conditions (Doubnerova & Ryslava 2011).

Increase in accumulation of proline observed in our study corroborate with the findings of Ahmad (2010), Azooz et al. (2011) and Rasool et al. (2012). Increased synthesis of GB in salt-stressed *P. turgidum* seedlings reported in our study support the findings of Ahmad (2010) and Khan et al. (2014). In *Vigna radiata*, Khan et al. (2014) observed that enhanced GB accumulation results in improved salt tolerance and growth with subsequent increment in activity of photosynthetic attributes. GB is believed to stabilize the quaternary protein structures against the damaging effects of salt stress (Papageorgion & Murata 1995). In addition, GB protects several important components of photosynthetic apparatus including Rubisco and photosystem II from the deleterious impact of high salinity (Hossain & Fujita 2010). High salinity causes dissociation and inactivation of important photosynthetic components like Rubisco and PSII into subunits, and hence causing obstructions in normal photosynthetic pathway (Papageorgion & Murata 1995; Khan et al. 2014). GB has been reported to maintain higher activities of antioxidant enzymes, and hence lowering the levels of H_2O_2 (Hossain & Fujita 2010). Increased proline accumulation reported in our study may be due to the enhancement in the activity of proline synthesizing enzymes and reduction in catabolizing ones or its restricted incorporation during protein synthesis. Altered activities of proline synthesizing and proline degrading enzymes under salt-stress conditions has been observed by Ahmad et al. (2010) and Jaleel et al. (2007). Proline and GB help in maintaining the water balance of plants so that the stress-induced ravage is allayed (Ahmad 2010; Ahanger et al. 2014b). Proline influences protein turn over and directly regulates stress protective proteins (Thakur & Sharma 2005). AMF inoculation enhanced proline and GB accumulation. Further increment in proline and GB in AMF-inoculated salinity-stressed plants suggests the beneficial role of AMF in enhancing the stress tolerance by contributing to maintenance of cellular water content. Increased accumulation of compatible organic solutes is one of the important traits determining the tolerance potential

of plant. Our findings of increase in proline due to AMF are in support of the finding of Jindal et al. (1993) for *V. radiata* and Shekoofeh et al. (2012) for *Ocimum basilicum.* Colonization of AMF induces stress tolerance by inducing different degree of osmotic adjustment.

Phenolics encompass several groups of secondary metabolites which are implemented in several important functioning like defense. Phenols are included within the nonenzymatic antioxidants which carry the scavenging of toxic radicals and are involved in the interactions with several biotic and abiotic factors (Michalak 2006; Bartwal et al. 2013; Tomar & Agarwal 2013). Phenols mediate amelioration of stress through their role in protecting oxidative stress and increasing membrane stability (Michalak 2006; Khattab 2007). Enhanced synthesis of phenolics under stressful conditions considerably averts the stress-induced deleterious changes on important molecules like proteins. Similar to our results in *Anethum graveolens* L., Mehr et al. (2012) also demonstrated enhanced accumulation of phenols with increasing salt concentration. Greater accumulation of phenolics in plants subjected to stressful conditions is ascribed to the upregulation of the enzymes involved in their synthesis (Wada et al. 2014). In our study, increased phenols content in stressed plants corroborate with the findings of Mehr et al. (2012) for *A. graveolens* L., Tomar and Agarwal (2013) for wheat and Dawood et al. (2014) for *Vicia faba*. In confirmation with our results, Nell et al. (2009) also observed increased phenols content in AMF-inoculated *Salvia officinalis* L. plants as compared to uninoculated counterparts. Enhanced synthesis of phenols due to AMF inoculation under normal as well as stress conditions strongly supports the protective role of phenols in mitigating negative impact stressful environmental conditions.

Exposure of plants to salt stress results in impeded uptake of essential mineral elements. Our results of salinity-induced reduction in uptake of potassium and phosphorous in *P. turgidum* seedlings corroborate with the findings of Ghazi and Al-Karaki (2000) for tomato and Kohler et al. (2009) for lettuce. Increased sodium ion concentration within the root zone has direct influence on the uptake of several essential elements like potassium. Sodium shares antagonistic relationship with potassium (Kohler et al. 2009). AMF-inoculated seedlings showed lesser contents of sodium as compared to the control counterparts. Moreover, enhanced uptake of potassium and phosphorous as well as subsequent amelioration of salinity-induced restricted uptake of potassium and phosphorous observed in our study support the findings of Ghazi and Al-Karaki (2000), Kohler et al. (2009) and Wu et al. (2010). Wu et al. (2010) demonstrated that AMF inoculation to salt-stressed citrus plants considerably mitigated the deleterious impact on the uptake of essential elements like phosphorous, potassium and calcium. In lettuce plants subjected to severe salt stress, Kohler et al. (2009) also demonstrated that inoculation of AMF contributed significantly to growth maintenance by mediating enhanced uptake of essential mineral elements as compared to the uninoculated counterparts. Greater concentration of Na^+

in cellular tissue hampers several growth and metabolic processes in plants. Maintaining higher K/Na ratio is believed to be important strategy adopted by plants to mitigate stress-induced deleterious changes (Wu et al. 2010; Azooz et al. 2011; Tomar & Agarwal 2013). Optimal concentration of potassium is essential for several important metabolic processes (Tomar & Agarwal 2013; Ahmad et al. 2014). Efficient compartmentation or exclusion of deleterious ions like Na contributes to maintenance of tissue osmotic potential so that hyper-osmotic effects can be averted. In the present study, enhanced uptake of phosphorous, potassium and calcium due to AMF may be as result of selective absorption of these essential ions over deleterious sodium ion and hence resulting in maintaining lower Na/K ratio.

Conclusion

Salinity resulted in altered growth of *P. turgidum* due to its obvious effects on the physiological and biochemical parameters studied. Salinity increased production of hydrogen peroxide and lipid peroxidation causing loss of membrane integrity and at the same time also reduced the uptake of important mineral elements. In the present study, AMF ameliorated the negative impact of salinity on physio-biochemical parameters studied. AMF alleviated the salt stress-induced changes by its active involvement in antioxidant defense system, thereby protecting cells from oxidative damage. Further preventing the excess uptake of Na^+ and enhancing uptake of important ions confer the role of AMF in ensuring better growth under stress conditions. Besides this, AMF also increased proline and GB synthesis which have direct impact on the osmoregulation of the plants. Our study showed that salt stress-induced toxic effects on growth, antioxidant system and mineral nutrients in *P. turgidum* can be alleviated by AMF and the application of AMF technology is a practical option that could be used as tool for improving crop production under stress condition.

Disclosure statement

No potential conflict of interest was reported by the authors.

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