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ARBUSCULAR MYCORRHIZAL FUNGI MITIGATES NaCl INDUCED ADVERSE EFFECTS ON *SOLANUM LYCOPERSICUM* L.

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Abstract

The present study aimed to investigate the effects of AMF on the growth and physio-biochemical attributes, antioxidant enzyme activities, plant growth regulators and inorganic nutrients in tomato grown under salt stress condition. Tomato plants were exposed to different concentrations of NaCl alone (0, 50 and 150 mM) and in combination with AMF (0mM+AMF, 50mM+AMF and 150mM+AMF). Spore population and colonization, growth and biomass yield, pigments, membrane stability index and malondialdehyde were negatively affected. Exposure of plants to combination of NaCl and AMF showed positive impact on the above parameters. Proline and antioxidant enzyme activity increased with increasing concentration of NaCl and further increase was observed in plants treated with NaCl in combination with AMF. Acid and alkaline phosphatase, hydrolytic enzymes and pectinase are also affected with increasing concentration of salt. However plants treated with NaCl in combination with AMF balances the above enzymatic activity. Salt stress decreases the auxin concentration in plants but application of AMF has been shown to restore the auxin content. ABA increases with salt concentration but less accumulation of ABA have been found in plants treated with AMF. Regarding the nutrient uptake, Na^+ and Na;K ratio increased and P, K, Mg and Ca decreases with increasing concentration of NaCl. Enhanced accumulation of P, K, Mg, Ca and K:N ratio and less uptake of Na^+ was observed in presence of AMF. The results confirm that NaCl imposes threat to the survival of tomato plants and application of AMF mitigates the negative effect to an appreciable level.

Key words: NaCl; AMF; Pigments; Proline; Antioxidants; Lipid peroxidation; Phosphatases; Hydrolytic enzymes; Growth regulators; Ion uptake.

Introduction

Plants being sessile often experience fluctuations in external environment and disturb the plant's normal growth and development (Ahmad *et al*., 2014a). Abiotic and biotic stress is engulfing the cultivated land at an alarming rate. Among the abiotic stresses salt stress is having a greater impact on farmlands worldwide. It is reported that about 7% of the total land on earth and 20% of the total arable area are affected with high salt content (Anon., 2010). On the other hand the human race is increasing and is believed to reach 8.3 billion by 2030 (Anon., 2010). It is difficult to feed this increasing population as the productive land is decreasing day by day. So to combat with these problems scientists are looking for an alternative to bring the uncultivated land under cultivation.

Salinity stress hampers the plant growth and development due to: (i) decrease in osmotic potential of the soil solution that leads to water stress, (ii) imbalance of essential nutrients, (iii) specific ion effect (salt stress), or (iv) combination of these factors (Rasool *et al*., 2013; Hashem *et al*., 2014a). During osmotic stress plants also synthesize low molecular weight compounds known as compatible solutes or osmolytes like proline, protein, glycinebetaine etc. (Ahmad *et al.,* 2014a,b). These compatible solutes don't interfere with normal biochemical reactions and makes plants to tolerate the stress. Salt stress is also responsible for oxidative stress through the generation of reactive oxygen species (ROS) (Ahmad *et al*., 2010a,b). The ROS are singlet oxygen ($_1O^2$), superoxide ions (O_2^-) and peroxides like hydrogen peroxide (H_2O_2) (Ahmad *et al*., 2014b). ROS are deleterious to biomolecules like proteins, DNA, RNA, membrane lipids etc. To combat with these ROSs plants are equipped with defense mechanisms like enzymatic (superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and non-enzymatic non-enzymatic (ascorbic acid (ASA), glutathione (GSH), glutathione S-transferase) antioxidants that neutralize the harmful effects of these ROSs (Apel & Hirt, 2004). Na/K ratio increases and the elements like K, P, Ca^{2+}, Mg^{2+} etc are decreasing with increasing concentration of NaCl. Na^+ replaces the other cations and hampers their uptake through root system.

Arbuscular mycorrhizal fungi (AMF) are ubiquitous and they are made up by a different variety of land plants, including at least 80% angiosperms and fungi belonging

to glomeromycota. AMF have been shown to promote plant growth (Hameed *et al*., 2014; Hashem *et al*., 2014b), enhance nutrient uptake such as nitrogen, phosphorus, magnesium, and micronutrients from the soil (Evelin *et al*., 2012), improve soil structure, and also able to enhance plant tolerance under different stresses such as drought and salinity (Wu *et al*., 2014), and protect host plants against pathogens (Sikes *et al*., 2009). Plants treated with AM fungi have been shown to enhance the growth and yield, and maintains the osmotic and ionic balance to a normal level so that plants will thrive well under these stress conditions (Hameed *et al*., 2014).

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop and are consumed fresh or cooked throughout the world. Tomatoes are also consumed as processed food products such as sauce, ketchup, tomato juice; puree etc. Tomato is full of phytochemicals like lycopene, β-carotene, flavonoids, vitamin C and essential nutrients. Most abundant is lycopene, which represents 80-90% of total pigments and is responsible for the deep red color of the ripe tomato fruits and other tomato products (Shi & Le Maguer, 2010). Tomato is moderately tolerant to salinity stress (Al-Karaki, 2000). NaCl stress leads to growth inhibition, decreased photosynthetic pigments and induction of oxidative stress in tomato plants (Giannakoula & Ilias, 2013).

Materials and Methods

The experimental plant and soil: Seeds of salt tolerant tomato (*Solanum lycopersicum* L. var. Castle rock) were surface sterilized with sodium hypochlorite (0.5%, v/v) for 3 min, washed thoroughly with distilled water before germination on blotter. The sandy was divided among plastic pots (1 kg capacity). Hoagland's solution supplemented with different concentrations of NaCl to get concentration of 0, 75 and 150 mM/ L was used for irrigation.

Mycorrhizal inoculums: The mycorrhizal fungi used in the present study were isolated previously from salt march habitat soil [(EC) = 9.2 dS m^{-1}] sown with wheat plants in Alserw, Dakahlia, Egypt at summer of 2012 (Alqarawi *et al*., 2014c). The extraction and quantification of AM fungi was carried by wet sieving, decanting and sucrose density gradient centrifugation techniques as described by Daniels & Skipper (1982) and modified by Utobo *et al*. (2011). The selected AM fungi [*Funneliformis mosseae* (syn. *Glomus mosseae*); *Rhizophagus intraradices* (syn. *Glomus intraradices*), and *Claroideoglomus etunicatum* (syn. *Glomus etunicatum*)] were inoculated singly with tomato plants using autoclaved soil (clay:sand, 1:1, w/w) as a host plant for three generations in pot cultures in a greenhouse for propagation of fungal spores. Fungal inoculums potential was determined by the most probable numbers method (Alexander, 1982) and each trap culture contained ~10.2 x 10^3 propagules per pot (1 Kg capacity)**.** 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 (1Kg). Non-mycorrhizal soil was used as reference.

Pot experiment: The pot experiment was carried out by split-plot in randomized complete block design with five replications. Healthy similar germinating seedlings were selected for the experiments. The seedlings were grown for eight weeks at 27 ± 2°C with 18 h light (750 µmol m^{-2} S^{-1}) and 6 h dark photo-cycle, and RH of 70-75% after transplantation. At the end of pot experiment, the plants were harvested carefully, washed in distilled water, separated into leaves, shoots and roots and were used for analyses of different parameters.

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 & 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 according to the following formula:

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

Determination of growth parameters: Fresh weight (FW) of root and shoot was taken instantly after harvesting, whereas dry weight (DW) was determined by drying the plant samples at 65°C for 72 h and then weighed.

Determination of photosynthetic pigments: The photosynthetic pigments were extracted from leaves of tomato plants in dimethyl sulfoxide (DMSO) as described by Hiscox & Israelstam (1979). Absorbance was determined spectrophotometrically at 480, 510, 645, 663 nm (T80 UV/VIS Spectrometer, PG Instruments Ltd, USA), DMSO was used as blank.

Determination of proline and Leaf water content: Proline was estimated following the method of Sadasivam & Manickam (1996). The mixture was boiled for 1 hour and then absorbance determined spectrophotometerically at 520 nm. Standard curve of pure proline was used as a reference.

Leaf water content (LWC) was estimated according to the method described by Smart & Bingham (1974). Calculation of LWC was done by the following formula:

$$\text{TWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}}$$

Determination of membrane stability index and malondialdehyde: Membrane stability index (MSI) was determined according to the method of Sairam *et al*. (1997). 0.1 g of fresh leaf samples was taken in test tubes in two sets and 10 ml of double distilled water was added to each. One set was kept in water bath for half an hour at 40°C and the electric conductivity was recorded (C_1). Water bath with boiling temperature (100°C) was used for 2nd set of test tubes and the EC was also recorded (C_2).

$$(\text{MSI}) = [1 - (C_1/C_2)] \times 100$$

The method of Heath & Packer (1968) was used to determine the concentration of malondialdehyde (MDA). Absorbance at 532 and 600 nm were used for calculation of MDA equivalents. Blank sample was used as reference. MDA equivalent was calculated according to the following equation:

$$\text{MDA equivalents (nmol.cm}^{-1}) = 1000 [(\text{Abs 523- Abs 600nm})/155].$$

Antioxidant enzymes assays: Fresh leaves (10 g) were homogenized in 50 mM sodium phosphate buffer (pH 7.0) containing 1% soluble Polyvinyl pyrrolidone (PVP). The homogenate was centrifuged at 15,000 rpm for 20 min at 4°C and the supernatant was completed to known volume with sodium phosphate buffer and used for the assays of enzymes activity. Protein in the enzyme extract was estimated according to Lowry *et al.* (1951).

CAT (EC 1.11.1.6) was assayed by the method of Samantary (2002). Titration of the reaction mixture against $KMnO_4$ (0.01M) was performed to observe the quantity of H_2O_2 used by the enzyme.

APX (EC 1.11.1.11) was assayed by the method of Nakano & Asada (1981). Decrease in absorbance at 290 nm of ascorbate was used for APX assay. APX activity was expressed at Units mg^{-1} protein.

The method of Kar & Mishra (1976) was employed for the peroxidase (POD) (EC 1.11.1.7) assay. Absorbance was measured at 420 nm. The enzyme activity was expressed as EU mg^{-1} protein.

The method of Bayer & Fridovich (1987) was used for the assay of SOD (EC 1.15.1.1) activity. The absorbance was measured at 540 nm. The activity of SOD was expressed as enzyme unit (EU) mg^{-1} protein. One unit of SOD was defined as the amount of protein causing 50% decrease of the SOD-inhibitable NBT- reduction.

Activity of GR (EC 1.6.4.2) was assayed by the method of Foyer and Halliwell (1976). Oxidation of NADPH was measured at 340 nm for 2 min. the GR activity was calculated using the extinction coefficient of NADPH of 6.2 mM^{-1} cm^{-1} and expressed as EU mg^{-1} protein.

Determination of Acid phosphatase and alkaline phosphatase activity: The method of Gianinazzi-Pearson & Gianinazzi (1976) was used for the assay of acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) activity. Absorbance was measured at 410 nm. The acid phosphatase activity was expressed as µmol-p-nitrophenol released min^{-1} mg $protein^{-1}$.

Hydrolytic enzyme assays: The method of Cuglielminetti *et al*. (1995) was used for the assay of α-amylase (EC 3.2.1.1). Standard curve of glucose used as reference. Enzyme unit expressed as mg reducing sugar liberated h^{-1} mg protein.

Carboxy methyl cellulase (E.C. 3.2.1.4) was assayed using the method described by Shewale & Sadana (1978). Standard curve of glucose (10-100µg/ml) was used as reference. Enzyme unit expressed as mg reducing sugar $liberated^{-1}$ $1h^{-1}$ mg $protein^{-1}$.

The method of Shewale & Sadana (1978) used for estimation of cellulase activity. Standard curve of glucose (10-100µg/ml) used as reference. Enzyme unit expressed as mg reducing sugar $liberated^{-1}$ $1h^{-1}$ mg $protein^{-1}$.

Invertase (E.C. 3.2.1.26) activity was assayed by the method of Pressey & Avants (1980). Standard curve of glucose (10-100 µg/ml) used as reference. Enzyme unit expressed as mg reducing sugar $liberated^{-1}$ $1h^{-1}$ mg $protein^{-1}$.

Proteinase (EC 3.4.24.4) activity was assayed according to the method of Anson (1938). Enzyme unit was expressed as the amount of enzyme required to produce an increase in liberate folin-positive amino acids and peptides by 1.0 µmol tyrosine $^{-1}$ min^{-1}. Standard curve of tyrosine (10-100 µg/ml) used as reference.

Polygalacturonase [PG] (EC3.2.1.15) activity was assayed by the method of Wang & Pinckard (1971). Standard curve of polygalacturonic acid (10-100 µg/ml) used as reference. Enzyme unit expressed as mg reducing sugar $liberated^{-1}$ $1h^{-1}$ mg $protein^{-1}$.

Pectin lyase [PL] (EC 4.2.2.10) activity was assayed using the thiobarbituric acid technique according to the method of Olutiola & Akintunde (1979). The absorbance was recorded at 540 nm. Enzyme unit was expressed as the increase in absorbance of 0.01 1h mg protein under assay conditions.

Pectin methylesterase [PME] (PME, EC 3.1.1.11) activity was assayed by the method of Bateman (1963). Enzyme unit was expressed as the amount of enzyme equivalent 0.01 N $NaOH^{-1}$ $1h^{-1}$ mg^{-1} protein.

Determination of plant growth regulators: Plant growth regulators (IAA, IBA and ABA) were extracted in 80% aqueous acetone (4:1, v/v) supplemented with 10 mg/L butylated hydroxytoluene and purified using EtOAc and $NaHCO_3$ as described by Kusaba *et al*. (1998). The purified extract residue was subjected to HPLC on a column of PEGASIL ODS (6 mm i.d. x 150 mm, Senshu Kagaku, Tokyo, Japan). Standard curves of IAA, IBA and ABA ranging from 10 to 200 ng/ml were used as references for quantification.

Estimation of ion accumulation: A known weights of oven dry leaf samples were digested and Na^+, K^+, Mg^{2+} and Ca^{2+} were estimated according to the method of Wolf (1982) using a flame photometer Jenway Flame Photometer, Bibby Scientific Ltd-Stone-Staffs-St15 0SA–UK. The Phosphorus (P) was extracted by nitric-perchloric acid digestion and measured using the Vanado-molybdophosphoric colorimetric method. 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 were determined by the least significant differences (LSD) (p=0.05) test.

Results

Spore population and total colonization: The total spore population showed decrease as NaCl concentration increases. At 75 mM NaCl a decrease of 31.9% and at 150 mM a decrease of 72.2% was observed in total spore population/100gm of soil (Table 1). Salt stress increased mycelium, vesicles and arbuscles by 12.8%, 6.9% and 11.5% respectively at 75 mM NaCl stress. However 150 mM NaCl decrease mycelium to 30.3%, vesicles to 21% and arbuscles to 25.6% as compared to the control (Table 1).

Growth and biomass yield: The results related to the effect of salt stress and AMF on biomass yield of *S. lycopersicum* is depicted in table 2. The shoot length decreases by 42.9% and 67.9% at 75 and 150 mM NaCl stress respectively as compared to the control. Salt treated plants in presence of AM fungi showed less decrease in shoot length by 28.9% at 75 mM and 56.5% at 150 mM as compared to plants treated alone with NaCl. A decrease of 32.7% and 28.8% in root length was observed in plants treated without AM fungi and with AM fungi respectively at 75 mM NaCl stress. The decrease was more pronounced in 150 mM NaCl stress. The shoot and root DW also decreased with increasing concentration of NaCl stress. Salt stress decreases the shoot dry weight to 76.6% in plants treated with 150 mM NaCl alone. The AMF treated showed a decrease of only 56.1% at the same concentration of salt. Root DW is also showing the same decrease but the decrease was more pronounced in plants not treated with AM fungi (Table 2).

Pigments: Table 3 deals with the effect of different salt concentrations and AMF on pigments contents. The results showed that all the pigments chl. 'a', 'b', carotenoids, total pigments decreased with increasing concentration of salt but plants treated with AMF showed less decrease. A decrease of 72.2%, 63.3%, 74.4% and 69.9% was observed in chl. 'a','b' carotenoids and total pigments, respectively at 150 mM NaCl stress. However a decrease of only 45.3% in chl. 'a', 34.1% in chl. 'b', 60.3% in carotenoids and 49.4% in total pigments were observed in plants treated with AMF.

Table 1. Effect of different concentrations of NaCl in presence and absence of AM fungi on total spore population and structural root colonization in *S. lycopersicum*.

Salt treatment (mM)	Total spore population/100g soil	Total colonization (%)		
		Mycelium	Vesicles	Arbuscules
Control	1486 ± 13.5a	76.2 ± 2.2a	34.6 ± 1.4a	26.1 ± 1.0a
75mM	1011 ± 11.3b	87.4 ± 2.9b	37.2 ± 1.6b	29.5 ± 1.3a
150 mM	412 ± 6.4c	53.1 ± 3.2c	27.3 ± 1.1c	19.4 ± 0.87b

Data presented are the means ± SE (n = 5)
Different letters next to the number indicate significant difference (p<0.05)

Table 2. Effect of different concentrations of NaCl in presence and absence of AM fungi on length (cm) and dry weight (g) of shoot and root of *S. lycopersicum* seedlings.

Salt treatment (mM)	Growth and Biomass yield							
	Without AM fungi				With AM fungi			
	Shoot		Root		Shoot		Root	
	Length (cm)	Dry wt. (g)	Length (cm)	Dry wt. (g)	Length (cm)	Dry wt. (g)	Length (cm)	Dry wt. (g)
Control	26.84 ± 1.37a	0.34 ± 0.05a	12.44 ± 1.13a	0.23 ± 0.03a	42.8 ± 2.4a	0.57 ± 0.09a	21.43 ± 1.29a	0.47 ± 0.07a
75 mM	15.31 ± 1.27b	0.19 ± 0.02b	8.36 ± 0.72b	0.14 ± 0.01b	30.4 ± 1.48b	0.36 ± 0.06b	15.24 ± 1.25b	0.32 ± 0.05b
150 mM	8.61 ±0.75c	0.08 ± 0.007c	5.52 ± 0.54c	0.08 ± 0.007c	18.6 ± 1.29c	0.25 ± 0.04c	10.73 ± 1.1c	0.18 ± 0.02c

Data presented are the means ± SE (n = 5)
Different letters next to the number indicate significant difference (p<0.05)

Table 3. Effect of different concentrations of NaCl in presence and absence of AM fungi on photosynthetic pigments of *S. lycopersicum* seedlings.

Salt treatment (mM)	Pigments content (mg g^{-1}fr. Wt.)							
	Without AM fungi				With AM fungi			
	Chl. 'a'	Chl. 'b'	Carotenoids	Total pigments	Chl. 'a'	Chl. 'b'	Carotenoids	Total pigments
Control	0.87 ± 0.09a	0.41 ± 0.05a	0.17 ± 0.009a	1.46 ± 0.19a	1.12 ± 0.12a	0.59 ± 0.06a	0.24 ± 0.01a	1.96 ± 0.59a
75 mM	0.47 ± 0.05b	0.27 ± 0.03b	0.09 ± 0.005b	0.83 ± 0.07b	0.92 ± 0.09b	0.49 ± 0.04b	0.18 ± 0.007b	1.44 ± 0.14b
150 mM	0.24 ± 0.02c	0.15 ± 0.007c	0.04 ± 0.001c	0.43 ± 0.06c	0.61 ± 0.05c	0.39 ± 0.02c	0.09 ± 0.005c	0.99 ± 0.10c

Data presented are the means ± SE (n = 5)
Different letters next to the number indicate significant difference (p<0.05)

Proline: Proline content showed an increase with increasing concentration of NaCl. An increase of 46.5% and 55% in proline content was observed at 75 and 150 mM NaCl stress respectively in non-AMF treated plants. Plants treated with NaCl in presence of AMF showed further increase of 51.6% at 75 mM and 62.9% at 150 mM NaCl (Fig. 1A).

Leaf water content (LWC): LWC also showed decreasing trend with increasing concentration of NaCl. An increase of 14.01% at 75 mM and 35.08% at 150 mM NaCl in LWC was observed in plants in present study. However AMF treated plants showed less decrease of 6% and 12.9% at 75 and 150 mM NaCl respectively (Fig. 1B).

Membrane stability index (MSI): Plants treated with AMF showed 12.9% and 29.9% decrease in MSI at 75 and 150 mM NaCl stress respectively while plants without AMF showed more decrease of 22.6% at 75 mM and 44.9% at 150 mM NaCl stress (Fig. 2A).

Malondialdehyde (MDA): The results related to the effect of NaCl in presence and absence of AMF on MDA content is depicted in figure 2B. The MDA content increased with increasing concentration of NaCl. An increase of 30.8% and 53.2% in MDA content was observed at 75 mM and 150 mM NaCl respectively in plants without AMF. However a less amount of MDA content 23.8% at 75 mM and 37.4% at 150 mM NaCl was observed in plants treated with AMF.

Antioxidants: Salt stress induces the activity of different antioxidant enzymes. All the enzymes showed increasing trend with increasing concentration of salt. CAT activity increased by 22.1% and 57.7%, APX by 14.1% and 25.3%, POD by 5.6% and 23.7%, GR by 28.9% and 37.1% and SOD by 23.9% and 36.8%, at 75 and 150 mM NaCl respectively in plants without AMF. Plants treated with AMF showed further increased activity of 59.6%, 53.8%, 43.7%, 68.2% and 48.7% in CAT, APX, POD, GR and SOD respectively at 150 mM NaCl stress (Fig. 3A-E).

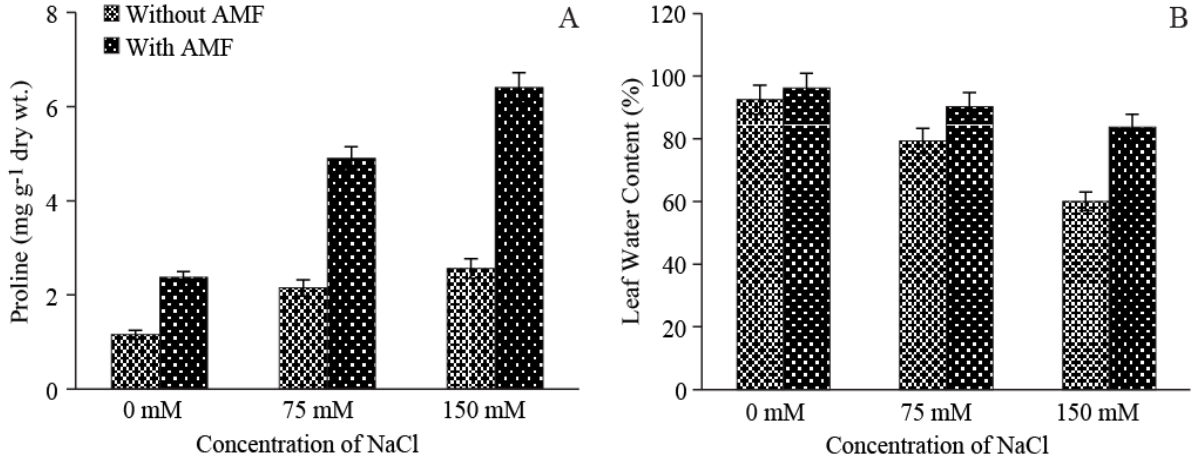


Fig. 1. Effect of different concentrations of NaCl on (A) proline content (mg g^{-1} DW) and (B) leaf water content (%) in presence and absence of AM fungi in *S. lycopersicum* seedlings. Data presented are the means ± SE (n = 5). Different letters indicate significant difference (p<0.05).

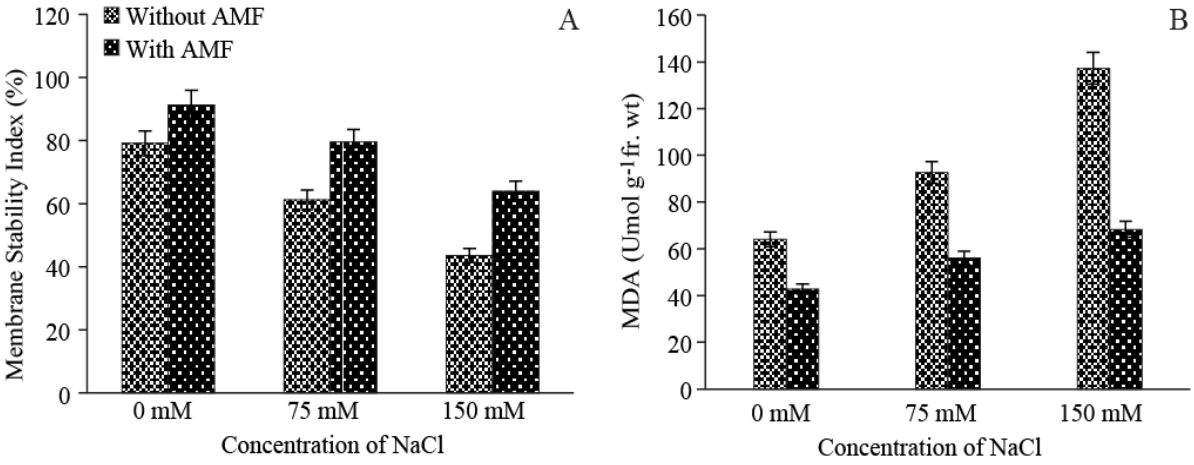


Fig. 2. Effect of different concentrations of NaCl on (A) membrane stability index [MSI] (%) and (B) MDA content (µmol g^{-1} FW) in presence and absence of AM fungi in *S. lycopersicum* seedlings. Data presented are the means ± SE (n = 5). Different letters indicate significant difference (p<0.05).

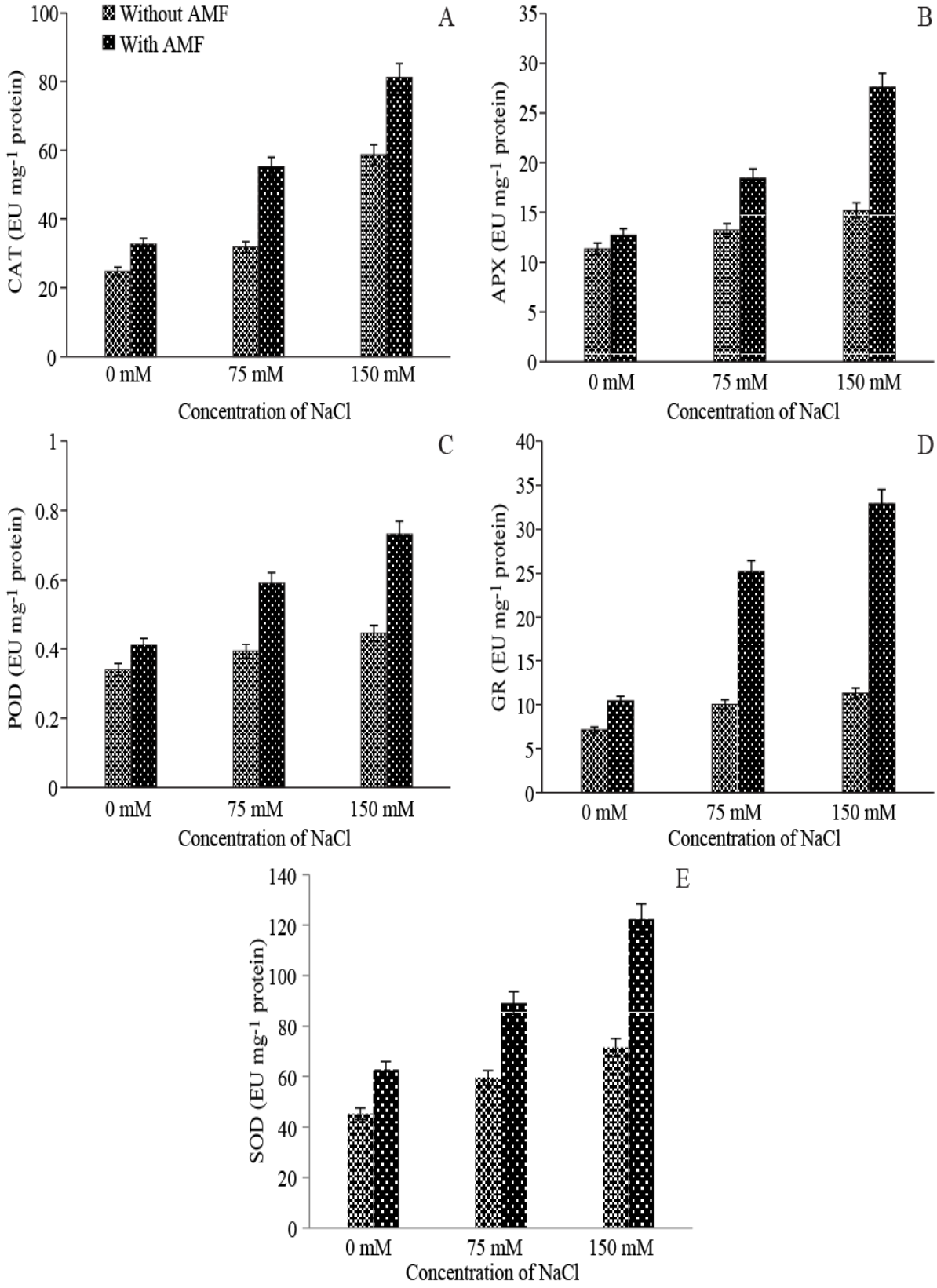


Fig. 3. Effect of different concentrations of NaCl on (A); CAT (EU mg^{-1} protein); (B) APX (EU mg^{-1} protein); (C) POD (EU mg^{-1} protein), (D) GR (EU mg^{-1} protein) and (E) SOD (EU mg^{-1} protein) in presence and absence of AMF in *S. lycopersicum* seedlings. Data presented are the means ± SE (n = 5). Different letters indicate significant difference (p<0.05).

Acid and alkaline phosphatase: The results pertaining to the effect of NaCl on phosphatase enzymes in presence and absence of AMF is depicted in figure 4A-B. Acid phosphatase showed an increase of 53.5% at 75 mM and 67.8% at 150 mM NaCl stress in plants without AMF. Plants treated with AMF showed only 26.9% and 46.4% increase in acid phosphatase at 75 mM and 150 mM NaCl respectively. Alkaline phosphatase also showed the similar response but the decrease was less in AMF treated plants as compared to non AMF treated.

Hydrolytic enzymes: The hydrolytic enzyme activity with respect to NaCl in presence and absence of AMF is shown in table 4. The amylase activity decreases by 29.6% and 79.7% in plants treated with 75 and 150 mM NaCl alone as compared to control. However, plants treated with NaCl in presence of AMF showed decrease of only 22.9% at 75 mM and 35.5% at 150 mM NaCl. Other hydrolytic enzymes showed increase with NaCl in both cases with and without AMF. An increase of 32.2%, 29.3%, 40.8% and 51% in CMase, cellulose, invertase, proteinase respectively was observed at 150 mM NaCl alone. Plants treated with NaCl in presence of AMF also showed an increase in hydrolytic enzyme activity but the increase is less as compared to plants not treated with AMF.

Pectinase enzymes: Figure 5A-C explains the results related to pectinase enzyme under salt stress in presence and absence of AMF. Salt stress increased the pectin lyase (PL) to 71.6% and 79%, pectine methyl esterase (PME) to 17.4% and 32.51% and polyglacturonase (PG) to 22.4 and 44.7% at 75 and 150 mM NaCl respectively in non AMF treated plants. AMF treated plants showed increase of only 55.8% and 67.1% in PL, 7.5% and 16.4% in PME and 5.8% and 16.3% in PG at 75 and 150 mM NaCl stress respectively. AMF treated plants showed less increase in pectinase enzymes as compared to plants treated with NaCl alone.

Plant growth regulators: The results pertaining to the effect of NaCl in presence and absence of AMF on plant growth regulators is presented in figure 6A-C. Salt stress alone decreases IAA and IBA to 58.5% and 40.2% respectively at 150 mM NaCl. However plants treated with NaCl in presence of AMF showed less decrease of 42.5% in IAA and 37.9% in IBA at 150 mM NaCl. ABA were increased to 86.4% and 93.5% at 75 mM and 150 mM NaCl alone. Plants treated with AMF showed 80.1% at 75 mM and 88% increase in ABA at 150 mM NaCl stress.

Ion accumulation: The results related to the effect of NaCl in presence and absence of AMF on elemental accumulation is depicted in table 5. Among the elemental accumulation only Na^+ and Na/K ratio showed increase with increasing concentration of NaCl in absence as well as in presence of AMF. Maximum accumulation of Na^+ and Na/K was 65.5% and 86.6% respectively at 150 mM NaCl alone. 150 mM NaCl along with AMF showed increase of 61.6% in Na^+ and 76.7% in Na/K ratio. Other elements like K^+, Mg^{2+}, P, Ca^{2+} decreases with increasing concentration of salt and AMF treated plants showed increment in uptake of above elements to an appreciable level.

Table 4. Effect of different concentrations of NaCl in presence and absence of AM fungi on hydrolytic enzyme activity of *S. lycopersicum* seedlings.

Salt treatment (mM)	Hydrolytic enzyme activity (EU mg^{-1} protein)									
	Without AMF					With AMF				
	Amylase	CMCase	Cellulase	Invertase	Proteinase	Amylase	CMCase	Cellulase	Invertase	Proteinase
Control	6.17 ± 0.51a	25.19 ± 0.51a	4.78 ± 0.22a	2.63 ± 0.11a	2.92 ± 0.11a	1.35 ± 0.17a	22.12 ± 1.20a	3.14 ± 0.21a	2.11 ± 0.10a	2.43 ± 0.14a
75 mM	4.34 ± 0.32b	31.22 ± 0.70b	5.18 ± 0.41b	3.97 ± 0.29b	4.11 ± 0.30b	1.04 ± 0.05b	24.35 ± 1.91b	4.23 ± 0.31b	2.74 ± 0.12b	3.01 ± 0.20b
150 mM	1.25 ± 0.11c	37.18 ± 0.80c	6.77 ± 0.61c	4.45 ± 0.33c	5.96 ± 0.49c	0.87 ± 0.09c	27.88 ± 2.11c	4.96 ± 0.39c	3.25 ± 1.24c	3.78 ± 0.25c

Data presented are the means ± SE (n = 5)

Different letters next to the number indicate significant difference (p<0.05)

Table 5. Effect of different concentrations of NaCl in presence and absence of AM fungi on elemental accumulation of *S. lycopersicum* seedlings.

Salt treatment (mM)	Elemental accumulation (mg g^{-1} dry wt.)											
	Without AMF						With AMF					
	Na^+	K^+	Na/K	Mg^{2+}	P	Ca^{2+}	Na^+	K^+	Na/K	Mg^{2+}	P	Ca^{2+}
Control	8.12± 1.42a	34.81± 5.51a	0.23± 0.07a	1.78± 0.92a	1.37± 0.73a	4.27± 0.82a	14.32± 2.95a	41.63± 6.52a	0.34± 0.14a	3.92± 0.84a	3.85± 0.81a	5.18± 1.51a
75 mM	17.79± 3.62b	22.75± 4.02b	0.78± 0.03c	1.04± 0.51b	1.24± 0.62b	3.16± 0.73b	25.41± 4.32b	36.78± 5.8b	0.69± 0.22b	2.14± 0.65b	3.61± 0.75b	4.53± 1.33b
150 mM	23.55± 4.11b	14.22± 2.91c	1.65± 0.40c	0.67± 0.31c	0.73± 0.28c	1.25± 0.11c	37.31± 5.93c	25.41± .35c	1.46± 0.52c	1.63± 0.49c	2.43± 0.21c	3.01± 0.69c

Data presented are the means ± SE (n = 5)

Different letters next to the number indicate significant difference (p<0.05)

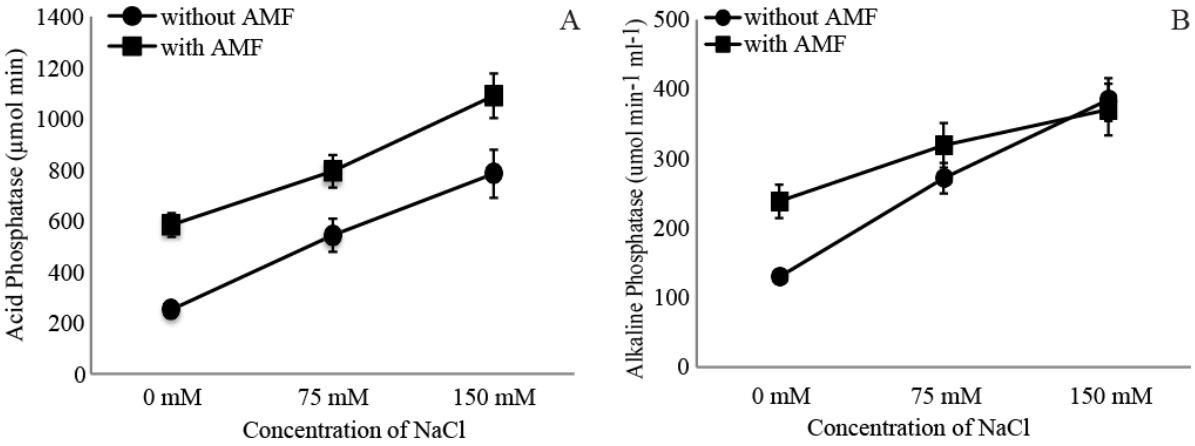


Fig. 4. Effect of different concentrations of NaCl on activities of (A) acid phosphatase and (B) alkaline phosphatase enzymes (µmol min^{-1} ml^{-1}) in presence and absence of AM fungi in *S. lycopersicum* seedlings. Data presented are the means ± SE (n = 5).

Discussion

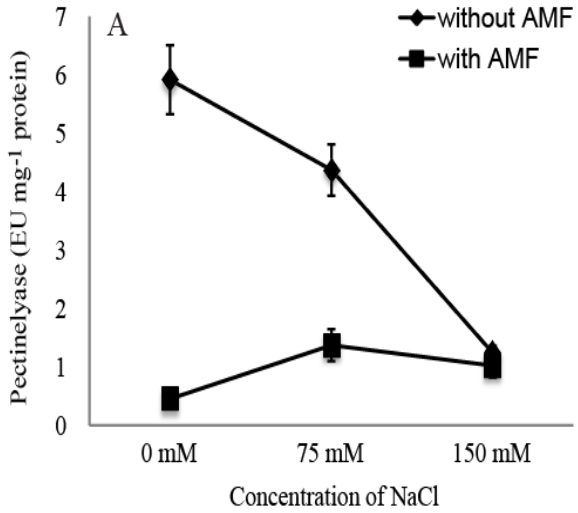
AMF is reported to alleviate toxic effects caused by salinity stress and improves growth rate, biomass, nutrient uptake of plants (Evelin *et al*., 2009; Hameed *et al*., 2014). Our results of decreasing root colonization corroborates with the findings of Juniper & Abbott (2006), they also reported that salt stress can affect AM fungi by slowing down root colonization, spore germination and hyphal growth. Our results showed increase in colonization at 75 mM NaCl and decrease at 150 mM NaCl stress. The reason behind the increase of colonization at low concentrations of salt and decrease at high concentrations of salt may be AM fungal species have varying tolerance to salinity. Johnson-Green *et al*. (2001) reported that arbuscular mycorrhizal fungi could resist 50 mg total salt ml^{-1} soil water. Root coloization by AMF is reduced by NaCl and is reported by Giri *et al*. (2007) in *Acacia nilotica*, Sheng *et al*. (2008) in *Zea mays*. It indicates that AMF is suppressed by NaCl stress (Sheng *et al*., 2008). From the reports it is concluded that the hyphal growth is sensitive to increasing concentration of NaCl.

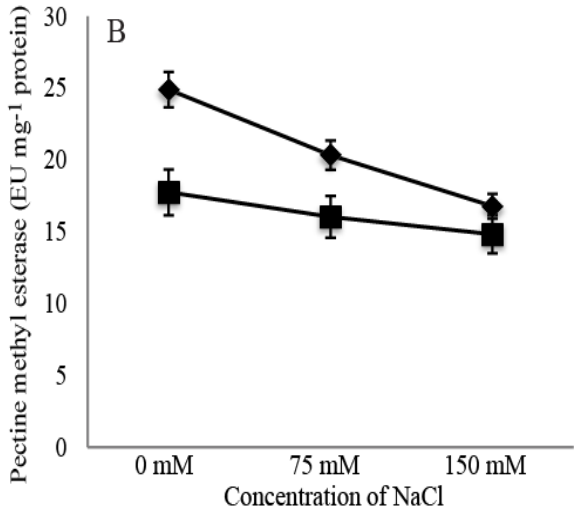
The length of shoot and root decreased with the increasing concentration of NaCl and the results are in accordance with Rohanipoor *et al*. (2013) who also reported decrease in shoot length under salt stress in maize. Ahmad *et al*. (2014b) also observed decrease in shoot length with increasing concentration of salt in mulberry. Our results of decreasing shoot and root dry weight corroborates with the findings of Ahmad *et al*. (2012) on different cultivars of *Brassica juncea*. Plants inoculated with AMF have been reported to improve plant growth and yield even under stress conditions (Wu *et al*., 2010; Alqarawi *et al*., 2014c). Ying-Ning *et al*. (2013) observed that citrus plants inoculated with AMF overcome the adverse effects of NaCl stress. The enhanced growth in AM fungi treated plants is due to adequate supply of mineral nutrients, particularly phosphorous, increase in surface area for absorption caused by profuse branching of hyphae and uptake of water from soil with low water potential.

Increasing concentration of salt decreases the pigment concentration in the present study. Salt stress decreases the photosynthetic pigment is also reported in chickpea genotypes (Rasool *et al*., 2013), broad bean (Azooz *et al*., 2011) and mustard (Ahmad *et al*., 2012). Our results also showed that plants inoculated with AMF have less impact on pigment system and is reported by Al-Qarawi *et al.* (2014 a,b) in lettuce. Mycorrhizal plants in response to salt stress have been observed to increase the chlorophyll content (Al-Qarawi *et al*., 2014 a,b) suggesting the less interference of salt with chlorophyll biosynthesis. Mycorrhization increases the absorption of Mg^{2+} that was negatively affected by salt stress hence the synthesis of chlorophyll increases in mycorrhizal plants. Zhu *et al*. (2010) reported that maize plants inoculated with *Glomus etunicatum* showed decreased Na^+ level. The increased photosynthetic pigments by mycorrhizal colonization in plants are due to the inhibition of Na^+ transport, which leads to better functioning of photosynthetic machinery.

Proline accumulation is one of the natural means to adapt to environmental stress conditions. Proline is a non-toxic osmolyte and maintains the osmoregulation under salt stress (Rasool *et al*., 2013; Ahmad *et al*., 2010b). Plants when colonized by AM fungi show high degree of protection by accumulating more and more solute (Evelin *et al*., 2009). Proline also acts as energy storage (i.e., C & N) during salt stress (Aggarwal *et al*., 2012). Enhanced proline accumulation can be linked with increased N-fixing ability of plants.

Water status is disrupted by salt stress, however, mycorrhizal colonization prevents the host plant from dehydration and thereby increases the root hydraulic conductivity at low water potential (Aroca *et al*., 2007). Kumar *et al*. (2010) also demonstrated normal levels of water in leaves of mycorrhizal *Jatropha curcas* under salt stress. This symbiosis results in efficient water conductance in roots and simultaneously increases stomatal conductance and hence transpiration (Jahromi *et al*., 2008). AM inoculation help the host plant to acquire nutrients through their hyphal network and thereby improves the photosynthetic rate as well as water osmotic homeostasis.





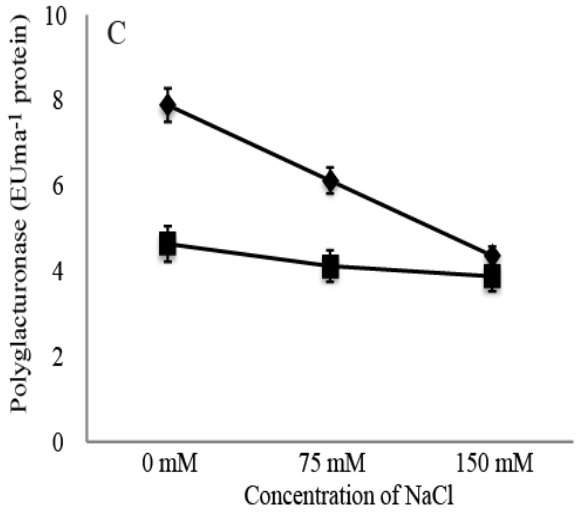
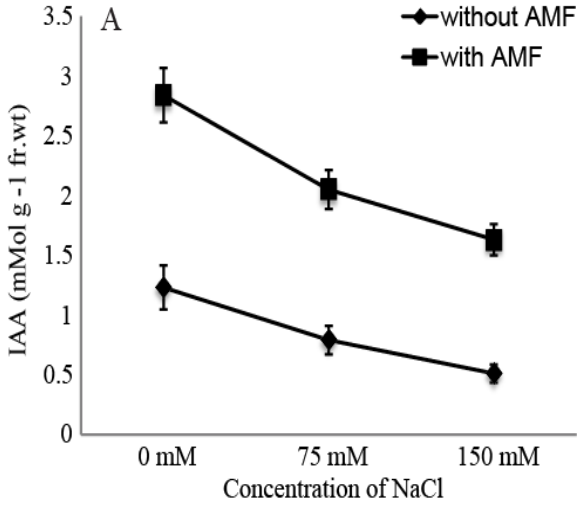
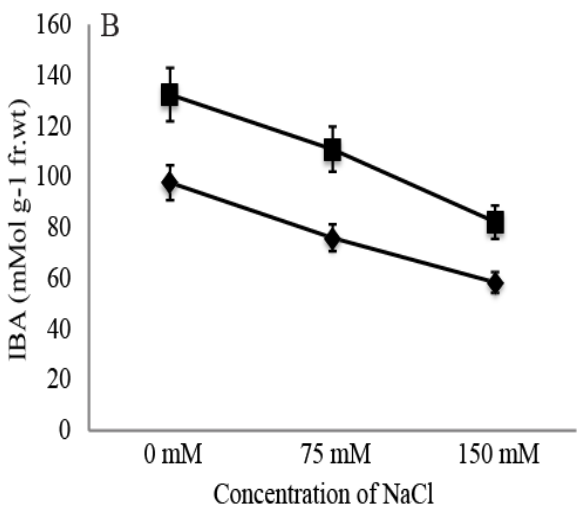


Fig. 5. Effect of different concentrations of NaCl on activities of (A) pectinelyase (EU mg^{-1} protein); (B) pectine methyl esterase (EU mg^{-1} protein) and (C) polyglacturonase (EU mg^{-1} protein) enzymes in presence and absence of AM fungi in *S. lycopersicum* seedlings. Data presented are the means ± SE (n = 5).





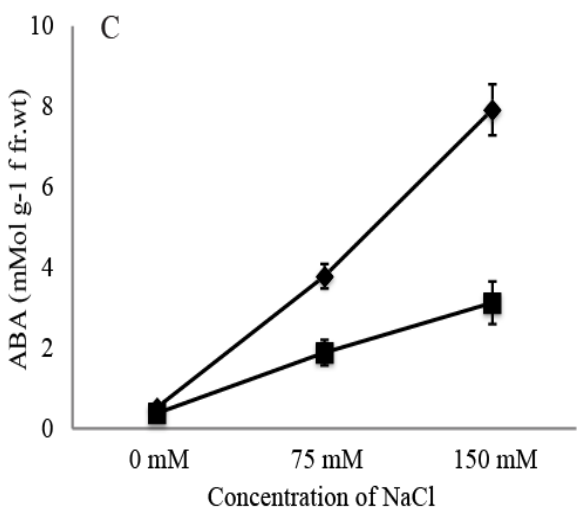


Fig. 6. Effect of different concentrations of NaCl on (A), auxins (mM g^{-1} FW); (B), IBA (mM g^{-1} FW) and (C), ABA (mM g^{-1} FW) in presence and absence of AM fungi in *S. lycopersicum* seedlings.

Cell membranes are the organelles that are susceptible to stress injury and are the important parameter to plant resistance against salt stress. Plants treated with NaCl in presence of AMF showed less decrease in MSI in the present study. Rawat *et al*. (2013) also demonstrated that *Trichoderma* spp. have positive impact on MSI in chickpea under salt stress. Mycorrhizal plants improve the stability as well as the integrity of membrane proteins by maintaining the relative permeability of the cell. NaCl induced ROS reacts with membrane proteins leads to fragmentation of peptide chain and proteolysis so the membrane stability is disrupted. Plants inoculated with AMF showed enhanced activities of antioxidants that counterbalance the negative effect of ROS on MSI.

Lipid peroxidation is often seen when cell is undergoing some stress. Increased lipid peroxidation with increasing concentration of NaCl was also reported by Rasool *et al*. (2013) in different genotypes of chickpea. Plants treated with NaCl in combination with AMF showed less increase in MDA content as compared to plants treated alone with NaCl (Wu *et al*., 2010). AMF accumulate less MDA content than non-AMF plants so helped in decreasing oxidative damage to lipids (Evelin & Kapoor, 2013). ROS reacts with polyunsaturated fatty acids (PUFAs) linoleic (18:2) and lenolenic (18:3) and forms complex mixture of lipid hydroperoxides. Increased activities of antioxidants like SOD, CAT, POD, GR in AMF treated plants showed less lipid peroxidation. The reason might be that antioxidants scavenges the radical production before reacting with the membrane lipids and minimize the lipid peroxidation.

Salt stress increases activity of antioxidant enzymes in chickpea genotypes (Rasool *et al*., 2013). Similar results have also been confirmed in different plants (Ahmad *et al*., 2010a). Increase in antioxidant activity under salt stress is an important criterion of salt tolerance (Ahmad *et al*., 2010a). Salt treated plants in combination with AMF further increases the antioxidant activity in present study and coincides with the results of Evelin & Kapoor (2013) who also showed an increase in antioxidant activity with *Glomus intraradices* colonization in *Trigonella foenum-graecum*. AMF inoculated plants were observed to have elevated activities in SOD, CAT, POD, APX, and GR. SOD is the first line of defence that catalyzes the disproportionation of $O_2^{\cdot-}$ to H_2O_2 and O_2. It removes $O_2^{\cdot-}$ and hence decreases the risk of $OH^{\cdot}$ formation from $O_2^{\cdot-}$ via the metal catalyzed Haber-Weiss type reaction. CAT is present in all aerobic eukaryotes and is responsible for the H_2O_2 removal from the different organelles of the cell. APX and POD is involved in scavenging of H_2O_2 and have been reported to have a leading role in ROS management under stress. GR is a flavo-protein oxidoreductase and is very important enzyme of ascorbate-glutathione system. GR catalyzes the reduction of glutathione, a molecule involved in many metabolic regulatory and oxidative processes in plants. GR catalyzes the NADPH dependent reaction of disulphide bond of GSSG and is thus important for maintaining the reduced pool of glutathione. The increase in SOD, CAT, APX and GR in the present study suggested that AMF inoculation increased enzymatic antioxidant production to protect the plants against oxidative damage, thus helps in enhancing salt tolerance.

Acid phosphatase is an important hydrolytic enzyme, which is widely distributed in plants. Increased activity of acid phosphatase with NaCl was also reported by Ehsanpour & Amini (2003) in alfalfa. Deficiency of phosphorous is responsible for the increase in acid phosphatase activity. The higher acid phosphatase activity might be due to the high resistance of the pre-existent acid phosphatase to stress induced degradation or due to stress-stimulated new acid phosphatase synthesis (Pan & Chen, 1988). The increased activity of alkaline phosphatase in present study corroborated with the findings of Rai & Sharma (2006) who also showed NaCl increases extracellular alkaline phosphatase and decreased intracellular phosphorous content and P uptake in *Anabaena doliolum*. Abiotic stress including NaCl hampers the uptake of P from the soil. This leads to activation of cellular phosphatases that helps in release in soluble phosphate from its insoluble form and helps in osmotic adjustment. Fujita *et al*. (2010) showed that when P of the soil decreases root phosphatase activity increases. AMF provides the P content to the plant and the activity of the cellular phosphatases decreases (Beltrano *et al*., 2013).

Amylase is an important enzyme in carbohydrate metabolism and has a role in degradation of starch that can be used for the growth of seedling. Salt stress decreases the α-amylase content in the present study. 50% reduction in amylase activity was observed in seeds of *Zea mays* treated with 100 mM NaCl (Sangeetha, 2013). The reduction in α-amylase is one of the main factor for reduced assimilate transportation and reduction in growth. The AMF inoculated plants have been shown to increase the α-amylase activity, may be due to the increased levels of phytohormones like IAA (Kim *et al*., 2006), absorption of nutrients through hyphal network that compensates the negative impact of NaCl stress in plant cell.

NaCl negatively affects the hydrolytic enzymes CMCase, cellulase, invertase and protease in the present study. Thakur & Sharma (2005) reported the increase in invertase activity in sorghum under NaCl stress. Our results of increased protease activity under NaCl stress corroborates with the findings of Kennedy & De Fillippis (1999) in *Grevillea* species. Proteases hydrolysed proteins to form amino acids, which are used in osmotic adjustment, storage form of nitrogen and maintenance of cellular pH. Application of AMF in combination with salt stress maintains the hydrolytic enzyme activity to normal. This may be due to AMF inoculated plants maintain relatively higher water content as compared to uninoculated plants. AMF helps the plant in uptake of soil nutrients, higher stomatal conductance thereby increasing the demand for transpiration (Sheng *et al*., 2008). Plants inoculated with AMF showed lower osmotic potential, which is maintained by fungal accumulating solutes, consequently resulting in improved plant osmotic adjustment (Evelin *et al*., 2009).

Pectin is one of the main components of the plant cell and functions as barrier against environmental stress (An *et al*., 2008) and is degraded by pectinases. Pectin lyases are the only pectinase, which has the capability of degrading pectin polymer directly. Transgenic arabidopsis expressing pepper methylesterase inhibitor protein (*caPMEII*) exhibit enhanced resistance to abiotic and biotic stress (An *et al*., 2008). In the present study NaCl leads to increase in PL, PME and PG which resulted in degradation of pectin. Application of AMF has resulted in less increase in these pectinases, thus giving stability to the plant cell wall. PME (pectin methyle esterase) activity controls the methylation of pectins, cell wall cation exchange capacity and thus the ion dynamics in this compartment (Gerendas, 2007).

Phytohormones have been observed to modulate the effects of different biotic and abiotic stresses. Our results of decreased IAA due to salt stress corroborates with the findings of Sakhabutdinova *et al*. (2003). IAA has been reported to alleviate the NaCl stress in different plants like wheat (Egamberdieva, 2009), maize (Kaya *et al*., 2010). Ghodrat *et al*. (2012) showed that priming with IBA improves germination and increase the resistance against salt stress. Jung & Park (2011) also reported the role of auxin in modulating seed germination under high salinity. AMF inoculated plants have showed enhanced auxin (IAA and IBA) content as compared to control and the results are in conformity with Luo *et al*. (2009). Plant hormones also act as signaling molecules and have a role in plant developmental processes and are having main function in colonization process (Ludwig-Mueller, 2000). IBA, an auxin increases the number of lateral roots thus facilitates the colonization of host (Kaldorf & Ludwig-Mueller, 2000). Our results of increased ABA under NaCl stress corroborates with the findings of Zörb *et al*. (2013) in maize leaves. Jia *et al*. (2002) reported that salt stress resulted in ABA accumulation in roots up to 10 fold. Plants inoculated with AMF showed lower ABA levels than non-inoculated ones under salt stress, suggesting that they are less stressed (Aroca *et al*., 2013). AM fungi have the capacity to alter the levels of ABA and thereby adapt to different environmental stresses including salinity.

Salt stress resulted in significant increase in Na^+ and reduced accumulation of K^+ and Ca^{2+} in leaves of cowpea cultivars (Patel *et al*., 2010). Talei *et al*. (2012) also reported that salinity increases Na^+ accumulation and decreases phosphorous, potassium, calcium and magnesium in *Andrographis paniculata*. According to Azcon-Aguilar *et al*. (1979) phosphorous becomes unavailable as phosphorous ions precipitate with Ca^{2+}, Mg^{2+} and Zn^{2+} ions in salt stressed soil. So for the plant growth and development, this unavailable phosphorous must be made available to plants. AMF inoculation has been reported to increase the P content in plants through the enhanced uptake by the hyphae (Ruiz-Lozano & Azcon 2000). This suggests that AMF increased P uptake may reduce the negative effect of NaCl in plants. Increased Na^+ always hampers the uptake of K^+ because Na^+ ions compete with K^+ for binding sites. AMF inoculated plants have been reported to enhance K^+ ions under NaCl stress (Kadian *et al*., 2013). AMF increases K^+:Na^+ ratio in roots and shoots of plants (Evelin *et al*., 2009). High K^+:Na^+ ratio helps the plants to maintain the normal K^-mediated enzymatic processes and protein synthesis. NaCl stress hampers the uptake of $Ca^{2+}$and Mg^{2+}ions from the soil. Lettuce inoculated with mycorrhizal fungi showed enhanced uptake of Ca^{2+} (Cantrell & Linderman, 2001). Higher Ca^{2+} concentration mitigates the negative effects of NaCl and maintains high K^+:Na^+ ratio which leads to salt adaptation (Rabie & Almadini, 2005). Mg^{2+} being the component of chlorophyll help to enhance the photosynthetic rate. Mycorrhizal plants have been shown to enhance more Mg^{2+} than non-mycorrhizal plants.

In conclusion, NaCl has detrimental effect on the growth and development of tomato plants. NaCl greatly affected biomass yield, biochemical attributes, enzymatic activities and also affected the plant growth regulators and ion homeostasis. Applications of AMF have reversed the negative effect of NaCl in the present study and are also reported by other workers. Utilization of AMF will be a sustainable approach in alleviating the salt stress in plants and at the same time salt affected land will be converted to cultivable land.

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References

Aggarwal, A., N. Kadian, K. Neetu, A. Tanwar and K.K. Gupta. 2012. Arbuscular mycorrhizal symbiosis and alleviation of salinity stress. *J. Appl. Nat. Sci*., 4(1): 144-155.

Ahmad, P., C.A. Jaleel and S. Sharma. 2010b. Antioxidative defense system, lipid peroxidation, proline metabolizing enzymes and biochemical activity in two genotypes of *Morus alba* L. subjected to NaCl stress. *Rus. J. Plant Phys*., 57(4): 509-517.

Ahmad, P., C.A. Jaleel, M.A. Salem, G. Nabi and S. Sharma. 2010a. Roles of enzymatic and non-enzymatic antioxidants in plants during abiotic stress. *Critical Rev. Biotech*., 30(3): 161-175.

Ahmad, P., K.R. Hakeem, A. Kumar, M. Ashraf and N.A. Akram. 2012. Salt-induced changes in photosynthetic activity and oxidative defense system of three cultivars of mustard (*Brassica juncea* L.). *Afr. J. Biotechnol*., 11(11): 2694-2703.

Ahmad, P., M. Ashraf, M.M. Azooz, S. Rasool and N.A. Akram. 2014a. Potassium starvation-induced oxidative stress and antioxidant defense responses in *Brassica juncea. J. Plant Inter.*, 9(1): 1-9.

Ahmad, P., M. Ozturk, S. Sharma and S. Gucel. 2014b. Effect of sodium carbonate-induced salinity-alkalinity on some key osmoprotectants, protein profile, antioxidant enzymes, and lipid peroxidation in two mulberry (*Morus alba* L.) cultivars. *J. Plant Inter*., 9(1): 460-467.

Alexander, M. 1982. "Most probable number method for microbial populations", in *Methods of Soil Analysis*, (Ed.): Black, C.A. (American Society of Agronomy, Madison, WI, USA). 815-820.

Al-Karaki, G.N. 2000. Growth and mineral acquisition by mycorrhizal tomato grown under salt stress. *Mycorrhiza*., 10: 51-54.

Alqarawi, A.A., A. Hashem, E.F. Abd_Allah, T.S., Alshahrani and A.A. Al-Huail. 2014a. Effect of salinity on moisture content, pigment system, and lipid composition in *Ephedra alata* Decne. *Acta Biol. Hungarica*, 65(1): 61-71.

Alqarawi, A.A., E.F. Abd_Allah and Abeer Hashem. 2014c. Alleviation of salt-induced adverse impact via mycorrhizal fungi in *Ephedra aphylla* Forssk. *J. Plant Interact*., 9(1): 802-810.

Alqarawi, A.A., E.F. Abd_Allah, Abeer Hashem, Asma A. Al Huqail and A.A. Al Sahli. 2014b. Impact of abiotic salt stress on some metabolic activities of *Ephedra alata* Decne. *J. Food Agric. Environ*., 12(2): 620-625.

An, S.H., K.H. Sohn, H.W. Choi, L.S. Hwang, S.K. Lee and B.K. Hwang. 2008. Pepper pectin methylesterase inhibitor protein CaPMEI1is required for antifungal activity, basal disease resistance and abiotic stress tolerance. *Planta*, 228: 61-78.

Anonymous. 2010. FAO Land and plant nutrition management service. http://www.fao.org, 2010.

Anson, M.I. 1938. The estimation of pepsin, tripsin, papain and cathepsin with hemoglobin. *J. Gen. Physiol*., 22: 79-89.

Apel, K. and H. Hirt. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol*., 55: 373-399.

Aroca, R., J.M. Ruiz-Lozano, A.M. Zamarren, J.A. Paz, J.M. García-Mina, M.J. Pozo and J.A. López-Ráez. 2013. Arbuscular mycorrhizal symbiosis influences strigolactone production under salinity and alleviates salt stress in lettuce plants. *J. Plant Physiol*., 170: 47-55.

Aroca, R., R. Porcel and J.M. Ruiz-Lozano. 2007. How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in *Phaseolus vulgaris* under drought, cold or salinity stresses?. *New Phytolo*., 173: 808-816.

Azcón-Aguilar, C., R. Azcón and J.M. Barea. 1979. Endomycorrhizal fungi and *Rhizobium* as biological fertilizers for *Medicago sativa* in normal cultivation. *Nature*, 279: 325-327.

Azooz, M.M., A.M. Youssef and P. Ahmad. 2011. Evaluation of salicylic acid (SA) application on growth, osmotic solutes and antioxidant enzyme activities on broad bean seedlings grown under diluted seawater. *Int. J. Plant Physiol. Biochem*., 3(14): 253-264.

Bateman, D.F. 1963. The "Macerating enzyme" of *Rhizoctonia solani*. *Phytopathol*., 53: 1178-1186.

Bayer, W.F. and J.L. Fridovich. 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Anal. Biochem*., 161: 559-566.

Beltrano, J., M. Ruscitti, M.C. Arango and M. Ronco. 2013. Effects of arbuscular mycorrhiza inoculation on plant growth, biological and physiological parameters and mineral nutrition in pepper grown under different salinity and P levels. *J. Soil Sci. Plant Nut*., 13(1): 123-141.

Borde, M., M. Dudhane and P.K. Jite. 2010. AM fungi influences the photosynthetic activity, growth and antioxidant enzymes in *Allium sativum* L. under salinity condition. *Not. Sci. Biol*., 2(4): 64-71.

Cantrell, I.C. and R.G. Linderman. 2001. Preinoculation of lettuce and onion with VA mycorrhizal fungi reduces deleterious effects of soil salinity. *Plant Soil*, 233: 269-281.

Cuglielminetti, L., J. Yamaguchi, P. Perata and A. Alpi. 1995. Amylolytic activities in cereal seeds under aerobic and anaerobic conditions. *Plant Physiol*., 109: 1069-1076.

Daniels, B.A. and H.D. Skipper. 1982. "Methods for the recovery and quantitative estimation of propagules from soil", *In*: *Methods and Principles of Mycorrhizal Research*, Ed. Schenck, N. C. (The American Phytopathological Society), 29-36.

Egamberdieva, D. 2009. Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. *Acta Physiol. Plant*., 31(4): 861-864.

Ehsanpour, A.A. and F. Amini. 2003. Effect of salt and drought stress on acid phosphatase activities in alfalfa (*Medicago sativa* L.) explants under *in vitro* culture. *Afr. J. Biotechnol*., 2 (5): 133-135.

Evelin, H. and R. Kapoor. 2013. Arbuscular mycorrhizal symbiosis modulates antioxidant response in salt-stressed *Trigonella foenum-graecum* plants. Mycorrhiza. Doi: 10.1007/s00572-013-0529-4.

Evelin, H., B. Giri and R. Kapoor. 2012. Contribution of *Glomus intraradices* inoculation to nutrient acquisition and mitigation of ionic imbalance in NaCl-stressed *Trigonella foenum-graecum*. *Mycorrhiza*, 22: 203-217.

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

Foyer, C.H. and B. Halliwell. 1976. The presence of glutathione and glutathione reductase in chloroplast: a proposed role in ascorbic acid metabolism. *Planta*, 133: 21-25.

Fujita, Y., B.J.M. Robroek, P.C. De Ruiter, G.W. Heil and M.J. Wassen. 2010. Increased N affects P uptake of eight grassland species: the role of root surface phosphatase activity. *OIKOS Synthesizing Ecol*., 119: 1665-1673.

Gerendas, J. 2007. "Significance of polyamines for pectin-methylesterase activity and the ion dynamics in the apoplast: The significance of the apoplast for the mineral nutrition of higher plants", In: (Eds.): Sattelmacher, B. Horst, W.J. *The apoplast of higher plants: compartment of storage, transport and reactions* (Springer Drodretch) 67-83.

Ghodrat, V., M.J. Rousta and M.S. Tadaion. 2012. Effect of priming with indole-butyric acid (IBA) on germination and growth of wheat under saline conditions. *Int. J. Agri. Crop Sci*., 4(6): 289-292.

Gianinazzi-Pearson, V. and S. Gianinazzi. 1976. Enzymatic studies on the metabolism on vesicular arbuscularmycorrhiza. 1. Effect of mycorrhiza formation and phosphorus nutrition on soluble phosphatase activities in onion roots. *Physiol. Veg*., 14: 833-841.

Giannakoula, A.E. and I.F. Ilias. 2013. The effect of water stress and salinity on growth and physiology of tomato (*Lycopersicon esculentum* Mill.). *Arch. Biol. Sci., Belgrade*., 65 (2): 611-620.

Giri, B., R. Kapoor and K.G. Mukerji. 2007. Improved tolerance of *Acacia nilotica* to salt stress by arbuscular mycorrhiza, *Glomus fasciculatum*, may be partly related to elevated K^+/Na^+ ratios in root and shoot tissues. *Microbial. Ecol*., 54: 753-760.

Hameed, A., D. Egamberdieva, E.F. Abd_Allah, A. Hashem, A. Kumar and P. Ahmad. 2014. "Salinity stress and arbuscular mycorrhizal symbiosis in plants". In: (Ed.): Miransari M. *Use of microbes for the alleviation of soil stresses* (Springer New York). 1: 139-159.

Hashem, Abeer, E.F. Abd-Allah, A.A. Alqarawi, Asma A. Al Huqail and D. Egamberdieva. 2014a. Alleviation of abiotic salt stress in *Ochradenus baccatus* (Del.) by *Trichoderma hamatum*. *J. Plant Interact.*, 9(1): 857-868.

Hashem Abeer, E.F. Abd-Allah, A.A. Alqarawi, G. El-Didamony, Mona S Alwhibi, Dilfuza Egamberdieva and P. Ahmad. 2014b. Alleviation of adverse impact of salinity on faba bean (*Vicia faba* L.) by arbuscular mycorrhizal fungi. *Pak. J. Bot*., 46(6): 2003-2013.

Heath, R.L. and L. Packer. 1968. Photoperoxidation in isolated chlorplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys*., 125: 189-198.

Hiscox, J.D. and G.F. Israelstam. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot*., 57: 1332-1334.

Jahromi, F., R. Aroca, R. Porcel and J.M. Ruiz-Lozano. 2008. Influence of salinity on the in vitro development of *Glomus intraradices* and on the *In vivo* physiological and molecular responses of mycorrhizal lettuce plants. *Microbial Ecol*., 55: 45-53.

Jia, W., Y. Wang, S. Zhang and J. Zhang. 2002. Salt-stress-induced ABA accumulation is more sensitively triggered in roots than in shoots. *J. Exp. Bot*., 53(378): 2201-2206.

Johnson-Green, P., N.C. Kenkel and T. Booth. 2001. Soil salinity and arbuscularmycorrhizal colonization of *Puccinellia nuttalliana*. *Mycological Res*., 105: 1094-1110.

Juniper, S. and L.K. Abbott. 2006. Soil salinity delays germination and limits growth of hyphae from propagules of arbuscular mycorrhizal fungi. *Mycorrhiza*, 16: 371-379.

Kadian, N., K. Yadav, N. Badda and A. Aggarwal. 2013. AM fungi ameliorates growth, yield and nutrient uptake in Cicer arietinum L. under salt stress. *Russian Agri. Sci*., 39: 321-329.

Kaldorf, M. and J. Ludwig-Müller. 2000. AM fungi might affect the root morphology of maize by increasing indole-3-butyric acid biosynthesis. *Physiol. Plant*., 109: 58-67.

Kar, M. and D. Mishra. 1976. Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. *Plant Physiol*., 57: 315-319.

Kaya, C., A.L. Tuna and A.M. Okant. 2010. Effect of foliar applied kinetin and indole acetic acid on maize plants grown under saline conditions. *Turk. J. Agric. For*., 34: 529-538.

Kennedy, B.F. and L.F. De Fillippis. 1999. Physiological and oxidative response to NaCl of the salt tolerant *Grevillea ilicifolia* and the salt sensitive *Grevillea arenaria. J. Plant Physiol*., 155: 746-754.

Kim, S.K., T.K. Son, S.Y. Park, I.J. Lee, B.H. Lee, H.Y. Kimand S.C. Lee. 2006. Influences of gibberellin and auxin on endogenous plant hormone and starch mobilization during rice seed germination under salt stress. *J. Environ. Biol*., 27(2): 181-186.

Kumar, A., S. Sharma and S. Mishra. 2010. Influence of arbuscular mycorrhizal (AM) fungi and salinity on seedling growth, solute accumulation and mycorrhizal dependency of *Jatropha curcas* L. *J. Plant Growth Regul*., 29: 297-306.

Kusaba, S., Y. Kano-Murakami, M. Matsuoka, M. Tamaoki, T. Sakamoto, I. Yamaguchi and M. Fukumoto. 1998. Alteration of hormone levels in transgenic tobacco plants overexpressing a rice homeobox gene OSH1. *Plant Physiol*., 116: 471-476.

Lowry, O.H., N.S. Rosebrough, A.L. Farrand and RJ. Randall. 1951. Protein measurement with folin phenol reagent. *J. BiolChem*., 193: 263-275.

Ludwig-Müller, J. 2000. "Hormonal balance in plants during colonization by mycorrhizal fungi", In: (Eds.): Douds, D.D., Kapulnik, Y. *Arbuscular Mycorrhizas: Physiology and function (*Kluwer Academic Publishers, The Netherlands) 263-285.

Luo, Z.B., D. Janz, X. Jiang, C. Gö bel, H. Wildhagen, Y. Tan, H. Rennenberg, I. Feussner and A. Polle. 2009. Upgrading root physiology for stress tolerance by ectomycorrhizas: insights from metabolite and transcriptional profiling into reprogramming for stress anticipation. *Plant Physiol*., 151: 1902-1917.

Nakano, Y. and K. Asada. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol*., 22: 867-880.

Olutiola, P.O. and O.A. Akintunde. 1979. Pectin lyase and pectin methyl esterase production by *Penicillium citrinum*. *Trans. Br. Mycol. Soc*., 72: 49-55.

Pan, S.M. and G.R. Chen. 1988. The effect of salt stress on acid phosphatase activity of *Zea mays* seedlings. *Bot. Bull. Acad. Sinica*., 29: 33-38.

Patel, P.R., S.S. Kajal, V.R. Patel, V.J. Patel and S.M. Khristi. 2010. Impact of salt stress on nutrient uptake and growth of cowpea. *Braz. J. Plant Physiol*., 22(1): 43-48.

Pressey, R. and J.K. Avants. 1980. Invertase in oat seedlings. Separation, properties, and changes in activities in seedling segments. *Plant Physiol*., 65: 136-140.

Rabie, G.H. and A.M. Almadini. 2005. Role of bioinoculants in development of salt-tolerance of *Vicia faba* plants under salinity stress. *Afr. J. Biotech*., 4: 210-222.

Rai, A.K. and N.K. Sharma. 2006. Phosphate metabolism in the cyano-bacterium *Anabaena doliolum* under salt stress. *Curr. Microbiol*., 52: 6-12.

Rasool, S., A. Ahmad, T.O. Siddiqi and P. Ahmad. 2013. Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. *Acta Phys. Plant*., 35(4): 1039-1050.

Rawat, L., Y. Singh, N. Shukla and J. Kumar. 2013. Salinity tolerant *Trichoderma harzianum* reinforces NaCl tolerance and reduces population dynamics of *Fusarium oxysporum* f.sp. *ciceroin* chickpea (*Cicer arietinum* L.) under salt stress conditions. *Arch. Phytopathol. Plant Prot*., 46(12): 1442-1467.

Rohanipoor, A., M. Norouzi, A. Moezzi and P. Hassibi. 2013. Effect of silicon on some physiological properties of maize (*Zea mays*) under salt stress. *J. Biol. Envron. Sci*., 7(20): 71-79.

Ruiz-Lozano, J.M. and R. Azcón. 2000. Symbiotic efficiency and infectivity of an autochthonous arbuscular mycorrhizal Glomu ssp from saline soils and *Glomus deserticola* under salinity. *Mycorrhiza*, 10: 137-143.

Sadasivam, S. and A. Manickam. 1996. Biochemical methods. 2nd Edn., New Age International Publishers (P) Ltd., New Delhi, India. pp. 107-109.

Sairam, R.K., P.S. Deshmukh and D.S. Shukla. 1997. Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. *J. Agron. Crop Sci*., 178: 171-178.

Sakhabutdinova, A.R., D.R. Fatkhutdinova, M.V. Bezrukova and F.M. Shakirova. 2003. Salicylic acid prevents the damaging action of stress factors on wheat plants. *Bulg. J. Plant Physiol*., Special Issue: 314-319.

Samantary, S. 2002. Biochemical responses of Cr-tolerant and Cr-sensitive mung bean cultivars grown on varying levels of chromium. *Chemosphere.*, 47: 1065-1072.

Sangeetha, R. 2013. Effect of salinity induced stress and its alleviation on the activity of amylase in the germinating seeds of *Zea mays*. *Int. J. Basic. Life Sci*., 1(1): 1-9.

Sheng, M., M. Tang, H. Chan, B. Yang, F. Zhang and Y. Huang. 2008. Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. *Mycorrhiza*. 18: 287-296.

Shewale, J.G. and J.C. Sadana. 1978. Cellulase and β-glucosidase production by a basidomycete species. *Can J. Microbiol*., 24: 1204-1216.

Shi, J. and M. Le Maguer. 2000. Lycopene in tomatoes: chemical and physical properties affected by food processing. *Crit. Rev. Biotech.*, 20(4): 293-334.

Sikes, B.A., K. Cottenie and J.N. Klironomos. 2009. Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. *J. Ecology*, 97: 1274-1280.

Smart, R.E. and G.E. Bihgham. 1974. Rapid estimates of relative water content. *Plant Physiol*., 53: 258-260.

Talei, D., M.A. Kadir, M.K. Yusop, A. Valdiani and M.P. Abdullah. 2012. Salinity effects on macro and micronutrients uptake in medicinal plant King of Bitters (*Andrographis paniculata* Nees.). *Plant Omic J*., 5(3): 271-278.

Thakur, M. and A.D. Sharma. 2005. Salt stress and phytohormone (ABA)-induced changes in germination, sugars and enzymes of carbohydrate metabolism in *Sorghum bicolor* (L.) moench seeds. *J. Agricul. Social. Sci*., 1: 89-93.

Utobo, E.B., E.N. Ogbodo and A.C. Nwogbaga. 2011. Techniques for extraction and quantification of arbuscular rmycorrhizal fungi*. Libyan Agric. Res. Center. Int*., 2: 68-78.

Wang, S.C. and J.A. Pinckard. 1971. Pectic enzymes produced by *Diplodia gossypina In vitro* and in infected cotton bolls. *Phytopathol*., 61: 1118-1124.

Wolf, B. 1982. A comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. *Comm. Soil Sci. Plant Anal*., 13: 1035-1059.

Wu, Q.S., Y.N. Zou and E.F. Abd_Allah. 2014. Mycorrhizal Association and ROS in Plants. In: (Ed.): P. Ahmad. Oxidative Damage to Plants. Elsevier Inc. pp. 453- 475. DOI: http://dx.doi.org/10.1016/B978-0-12-799963-0.00015-0

Wu, Q.S., Y.N. Zou, W. Liu, X.F. Ye, H.F. Zai and L.J. Zhao. 2010. Alleviation of salt stress in citrus seedlings inoculated with mycorrhiza: changes in leaf antioxidant defense systems. *Plant Soil Env.*, 56(10): 470-475.

Ying-Ning, Z., L. Yong-Chao and Q.S. Wu. 2013. Mycorrhizal and non-mycorrhizal responses to salt stress in trifoliate orange: plant growth, root architecture and soluble sugar accumulation. *Int. J. Agric. Biol*., 15: 565-569.

Zhu, X.C., F.B. Song and H.W. Xu. 2010. Arbuscular mycorrhizae improves low temp stress in maize via alterations in host water status and photosynthesis. *Plant Soil*, 331: 129-137.

Zörb, C., C.M. Geilfus, K.H. Mühling and J. Ludwig-Müller. 2013. The influence of salt stress on ABA and auxin concentrations in two maize cultivars differing in salt resistance. *J. Plant Physiol*., 170(2): 220-224.

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