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ALLEVIATION OF ADVERSE IMPACT OF SALT IN *PHASEOLUS VULGARIS* L. BY ARBUSCULAR MYCORRHIZAL FUNGI

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Abstract

The present study was undertaken to evaluate the possible role of arbuscular mycorrhizal fungi (AMF) in enhancing the salt (0, 0.15; 0.25 M NaCl) tolerance in *Phaselous vulgaris*. The impact of AMF in presence and absence of salt stress was studied on growth, nodulation, and attributes of systemic acquired resistance in *P. vulgaris*. The results suggested that salinity caused significant decrease in growth performance, nodulation, pigment system, tissue water content, and membrane stability index. Also, salt stress caused significant decrease in phytohormones , polyamines, membrane stability index and tissue water content of *P. vulgaris*. On the other hand, lipid peroxidation (malondialdehyde), total phenol content and antioxidant enzymes (catalase, peroxidase, superoxide dismutase, ascorbate peroxidase, glutathione reductase) increases as salt concentration increases. The accumulations of sodium, chlorine were significantly increased by salt stress, however the concentration of potassium, phosphorous and calcium decreased. Overall, the results indicate that AMF alleviate the adverse impact of salinity on the plant growth, anabolic physiological attributes and nutrient uptake by reducing the oxidative damage of salt through strengthening and modulation the systemic acquired resistance.

Key words: Common bean; Salinity; Growth; Pigments; Phytohormones; Polyamines; Antioxidant defense system; Ion accumulation.

Introduction

The salinization of lands has become a major environmental issue and has been recognized as the most important economic, social and environmental problem in many regions of the world, including Saudi Arabia (Alqarawi *et al*., 2014a; Wu *et al*., 2014; Abd_Allah *et al*., 2015; Hashem *et al*., 2015). Salinity declines the vegetative growth equally as a result of water shortage and ionic toxicity (Wu *et al*., 2014) and induces sequential modifications in systemic acquired resistance system (Sai Kachout *et al*., 2013; Abd_Allah *et al*., 2015). The systemic acquired resistance system include many physiological and biochemical processes (Sai Kachout *et al*., 2013; Ahanger *et al*., 2014) and osmolytes and osmoprotectants (Hashem *et al*., 2014; Wu *et al*., 2014; Abd_Allah *et al*., 2015), which protects the biomolecules and other enzymatic reactions from osmotic and ionic damage such as photosynthesis, lipid peroxidation, phenol content and antioxidant enzymes (Ahanger *et al*., 2014; Hameed *et al*., 2014; Abd_Allah *et al*., 2015) which associated with perturbed carbon and nitrogen metabolism (Alqarawi *et al*., 2014a). Furthermore, exposure to high salinity triggers production of toxic reactive oxygen species (ROS) ultimately resulting in oxidative stress (Ahanger *et al*., 2014; Wu *et al*., 2014). Excess accumulation of ROS like superoxide, hydroxyl, and peroxide radicals is detrimental to normal metabolism and growth (Wu *et al*., 2014). ROS accumulate in leaves and leads to oxidation of various cellular molecules including lipids, proteins and chlorophylls. Hence peroxidation of membrane lipids, protein oxidation, and damage to nucleic acids are the immediate effects of the excess ROS (Alqarawi *et al*., 2014a; Hashem *et al*., 2014; Wu *et al*., 2014; Abd_Allah *et al*., 2015).

One of the important beneficial associations those forming a symbiotic relationship with the plant is the AMF association. AMF are ubiquitous and have proved to possess the potential to improve soil structure and plant growth under normal as well as stressful environments (Ahanger *et al*., 2014). Under salt stress conditions, AMF are believed to act as essential bio-ameliorators of stress and hence helping plants in alleviating stress induced damage (Ahanger *et al*., 2014; Hashem *et al*., 2014; Wu *et al*., 2014; Abd_Allah *et al*., 2014). Furthermore, AMF colonization was connected with antioxidant enzyme activities and antioxidant concentrations, thereby alleviating oxidative damage of plants grown under stress (Alqarawi *et al*., 2014c; Abd_Allah *et al*., 2015; Hashem *et al*., 2015). The AMF-mediated antioxidant defense systems may depend on the combination of the fungal species with the host plant, as well as the micronutrient availability (Ahanger *et al*., 2014; Wu *et al*., 2014).

Among major food legumes the common bean (*Phaseolus vulgaris* L.) is the third most important worldwide, it considered as an important protein and vegetable resource. The adverse impact of salt represents the major abiotic hazard that farmers confront in the salt marsh habitat. Abiotic stress dramatically declines the bean growth and productivity world-wide. Understanding the alleviation of abiotic stress response in plants by AMF application can help in devising strategies for improving stress tolerance in plants.

In the present investigation, special attention is given to bean metabolism under abiotic stress of salinity and role of AMF in mitigating the changes caused by salt stress in *P. vulgaris*.

Materials and Methods

The pot experiment: The present study was conducted under growth chamber conditions at Plant Production Department, College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia. The seed of common bean (*Phaselous vulgaris* L.) cultivar Giza 6 was obtained from Ministry of Agriculture, Egypt. The soil used was sandy loamy texture as described in our pervious experiment Alqarawi *et al*. (2014c). The seeds were surface sterilized with sodium hypochlorite (0.5%, v/v) for 3 min, washed thoroughly with distilled water before germination on blotter. Healthy germinating seeds were transferred to plastic pots with 2 kg autoclaved soil (1 plant/pot). The irrigation was applied to maintain the water level at 65% of the field capacity with Hoagland's solution (Hoagland & Arnon, 1950) supplemented with sodium chloride to get concentration of 0; 0.15 M/L and 0.25 M NaCl. The mycorrhizal fungi used in the present study were *Funneliformis mosseae* (syn. *Glomus mosseae*), *Rhizophagus intraradices* (syn. *Glomus intraradices*) and *Claroideoglomus etunicatum* (syn. *Glomus etunicatum*), as shown in Figure 1. AMF were isolated previously from salt march habitat in Alserw, Dakahlia, Egypt (Hashem *et al*., 2014).The seedlings were grown for eight weeks after sowing at 25 ± 1^{o}C with 12 h light (750 µmol m^{-2} S^{-1}) and 12 h dark photo-cycle, and RH of 70-75 %. The pot experiment was carried out by split-plot in randomized complete block design with five replications. At the end of pot experiment, the plants were harvested carefully, washed in distilled water, separated into shoots and roots. The samples of root and shoots were dried at 70^{o}C for 48 h and dry weight was recorded for both. The nodules collected, counted and used for extraction and estimation of leghemoglobin as will describe. Leaf samples were used for estimation of photosynthetic pigments, free polyamine, hormones and enzymes activity. Fresh root samples were used for collection rhizobial nodules.

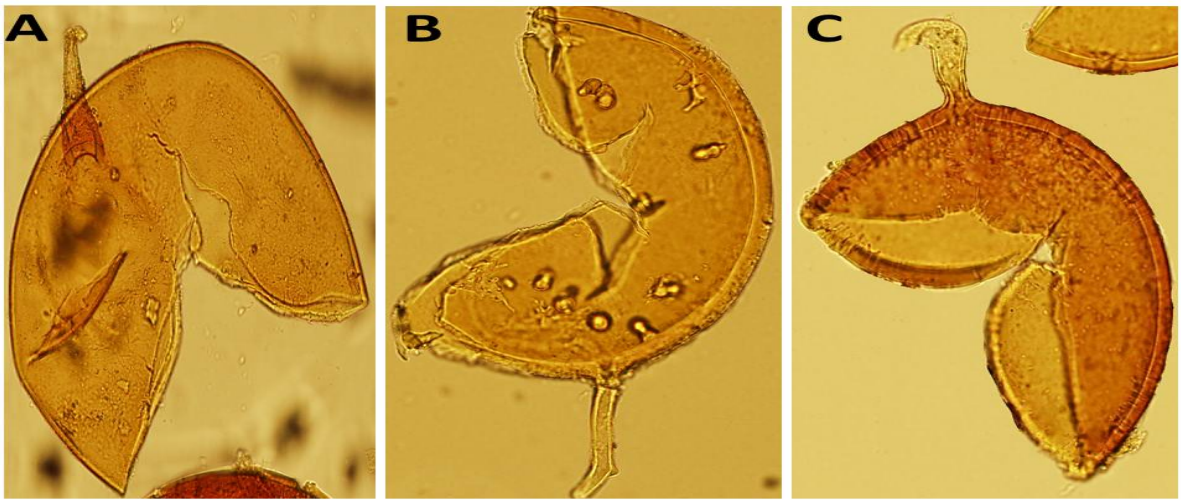

Fig. 1 A, B, C. Morphology of typical an intact crushed spores of arbuscular mycorrhizal fungi. **A**, *Funneliformis mosseae*; **B**, *Rhizophagus intraradices* and **B**, *Claroideoglomus etunicatum*.

Extraction and estimation of leghemoglobin: The leghemoglobin concentration of root nodules was estimated according to the method of Keilin & Wang, (1945). Frozen nodules (2.0 g) were ground to fine powder in liquid N_2 and transferred to 50 mM KPO_4 (pH 7.4) buffer that contained 1 mM EDTA. The mixture was stirred until it thawed into a homogenate at final temperature 2°C, transferred to centrifuge tubes. Following centrifugation at 4°C and 10,000xg for 10 min. The leghemoglobin-containing supernatant was collected, maintained to known volume with 50 mM KPO_4 (pH 7.4) buffer as above. The developed color intensity was recorded spectrophotometrically at 710 nm (T80 UV/VIS Spectrometer, PG Instruments Ltd, USA). The blank was 50 mM KPO_4 (pH 7.4) buffer that contained 1 mM EDTA. The leghemoglobin concentration was expressed as mg g^{-1} nodule fresh weight.

Determination of photosynthetic pigments: The photosynthetic pigments (chlorophyll a; chlorophyll b; carotenoids) were extracted and estimated by the method of Hiscox & Israelstam, (1979). Absorbance was determined spectrophotometrically at 480, 510, 645, 663 nm with DMSO as blank.

Determination of plant growth regulators: Plant growth regulators, indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and gibberellic acids (GA_1 & GA_4) were extracted in 80% aqueous acetone (4:1, v/v) supplemented with 10 mg^{-1} 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). IAA and IBA were expressed as mM g^{-1} fresh weight, however

GA_1 & GA_4 were expressed as ng g^{-1} fresh weight. Standard curves of IAA, IBA, GA_1 and GA_4 ranging from 10 to 200 ng ml^{-1} were used as references for quantification.

Extraction and determination of free polyamine: Leaf samples (300 mg) were homogenized in liquid nitrogen. The homogenate was suspended in 1.0 ml of perchloric acid 5% (v/v), incubated in ice for 30 min followed with centrifugation at 15,000×g for 15 min. Pellet was discarded and the supernatant was kept at 20°C. The free polyamine was determined by HPLC according to the method described by Jiménez-Bremont *et al*. (2007). Standard spermine, spermidine and putrescine polyamines were used as reference.

Estimation of lipid peroxidation: Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) produced by the thiobarbituric acid reaction as described by Heath & Packer, (1968). In this method, fresh leaves were ground in 1% (w/v) (10 ml g^{-1} fresh weight) trichloro acetic acid (TCA). The homogenate was centrifuged at 10,000xrpm for 5 minutes. The reaction mixture contains 1.0 ml of supernatant and 4.0 ml of 0.5% (w/v) thiobarbituric acid (TBA). The mixture was heated at 95°C for 30 min, cooled in an ice bath and centrifuged at 5000xrpm for 5 min for clarification. Absorbance at 532 and 600 nm were used for calculation of MDA equivalents. Blank sample was used as reference.

Tissue water content: Tissue water content (TWC) was estimated according to the method described by Smart & Bingham, (1974). Sample of leaf discs is taken from each treatment in replicates and the fresh weight is determined. The same leaf disc sample was kept on water for 4 hrs. The turgid weight was recorded. The leaf sample was dried in oven at 85°C for dry weight. Calculation of TWC was done by the following formula:

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

Membrane stability index: Membrane stability index (MSI) was determined according to the method of Sairam *et al*. (1997). 100 mg 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). Calculation of MSI was done by the following formula:

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

Determination of total phenolics: The total phenolics in the shoot system were extracted with 80% (v/v) acetone and estimated using (20%, w/v) sodium carbonate (Na_2CO_3) and Folin & Ciocalteau's phenol reagent following the method of Julkunen-Tiitto, (1985). The ODs of the mixtures were read at 750 nm. Standard curve of pyrogallol was used as reference.

Antioxidant enzymes assays: Fresh leaves (10 g) were homogenized in 50 mM sodium phosphate buffer (pH 7.0) containing 1% PVP-40 (Polyvinylpyrrolidone) as described by Malik & Singh, (1980). The homogenate was centrifuged at 15,000xrpm for 20 min at 4°C and the supernatant was used for the assays of enzymes activity. Protein in the enzyme extract was estimated according to Lowry *et al*. (1951). Superoxide dismutase [SOD] (EC 1.15.1.1) activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium adopting the method of Bayer & Fridovich, (1987). The reaction mixture consisted of 1 ml of enzyme extract, 40mM phosphate buffer (pH 7.8), 13mM methionine, 75mM NBT, 0.1mM EDTA, and 2μM riboflavin, which was added at the end. After mixing the contents, test tubes were shaken and placed 30 cm below light source consisting of two 20 W fluorescent lamps for 15 min. A tube with protein kept in the dark served as a blank, while the control tube was without the enzyme and kept in the light. The absorbance was measured at 540 nm. The activity of SOD is the measure of NBT reduction in light without protein minus NBT reduction in light with protein. One unit of activity is the amount of enzyme required to inhibit 50% initial reduction of NBT under light. The results were expressed as EU mg^{-1} protein. A method of Samantary, (2002) was employed for the assay of catalase [CAT] (EC 1.11.1.6). The enzyme extract (1 ml) was added to 1 ml of H_2O_2 and 3 ml of 0.1 sodium phosphate buffer (pH 7.0). The reaction was discontinued by adding 10 ml of 2% H_2SO_4 after 1 min of incubation at 20°C. The reaction mixture was then titrated against 0.01 M $KMnO_4$ to determine the quantity of H_2O_2 used by the enzyme. Enzyme activity was expressed as M H_2O_2 destroyed mg $protein^{-1}$ min^{-1}. Peroxidase (POD) (EC 1.11.1.7) activity was assayed spectrophotometrically according to the method of Kar & Mishra, (1976). The reaction mixture contained 0.5 ml of enzyme extract, 2.5 ml of 0.1M phosphate buffer (pH 7.0), 1.0 ml of 0.01M pyrogallol and 1.0 ml of 0.005M H_2O_2 were added. After incubation, the reaction was stopped by adding 1.0 ml of 2.5N H_2SO_4. The amount of purpurogallin formed was estimated by measuring the absorbance at 420 nm. The enzyme activity was expressed as EU mg^{-1} protein. The activity of ascorbate peroxidase (APX) (EC 1.11.1.11) was assayed according to the method of Nakano & Asada, (1981). The reaction mixture contained 1.0 ml of reaction buffer [potassium phosphate (pH 7.0) with 0.1 mM EDTA, 0.5 mM ascorbate, 0.1 mM H_2O_2 and 0.1 ml of enzyme extract]. APX was assayed as a decrease in absorbance at 290 nm of ascorbate. APX activity was expressed as unit mg^{-1} protein. For the calculation of APX enzyme activity, the extinction coefficient of 2.8 mM^{-1} cm^{-1} was used. One unit of enzyme was considered as the amount necessary to decompose 1 mol of substrate min^{-1} at 25°C. Activity of glutathione reductase (GR) (EC 1.6.4.2) was assayed by the method of Foyer & Halliwell, (1976). The reaction mixture contains, enzyme extract (0.1 ml), sodium phosphate buffer (pH 7.8) (0.025 mM), NADPH (0.12 mM) and GSSG (0.5 mM). 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.

Estimation of ion accumulation: A known weight of oven dry leaf sample were digested and Na^+, K^+ and Ca^{2+} were estimated according to the method of Wolf, (1982) using a flame photometer, (Bibby Scientific Ltd-Stone-Staffs-St15 0SA–UK). Standard curve of each mineral (10- 100 µg ml^{-1}) used as reference. The phosphorus (P) was extracted by nitric-perchloric acid digestion and measured using the vanado-molybdophosphoric colorimetric method (Jackson, 1962). Standard curve of each mineral (10–100 µg ml^{-1}) was used as reference. A chloride analyzer (Model 926, Sherwood Scientific Ltd., Cambridge, UK) was used to determine Cl^- concentration in the extracts.

Statistical analysis: Two-way analysis (ANOVA) was used for statistical analysis followed by Duncan's Multiple Range Test (DMRT). The values obtained were the mean ± SE for five replicates in each group. P value at 0.05 were considered as significant.

Results

Plant growth criteria: The impact of both NaCl stress and AMF on plant growth criteria is presented in Table 1. The salt stress (0.15 M) caused significant decrease in Shoot height, Shoot weight, Root weight and leaf area by percent of 13.06%, 29.71%, 35.81%, 39% and 58%, respectively as compared with control plants. The higher salt concentration (0.025 M) caused more significant decrease in plant growth criteria. The application of AMF improved all growth criteria of bean plant in both presence and absence of salt stress as compared with each control.

Nodulation and leghemoglobin content: The results in Table 2 show that salinity caused significant and drastic decline in number as well as fresh weight of nodules and leghemoglobin content. The decreases were in direct relation with increasing concentration of salt. At 0.15 M NaCl, number, fresh weight and leghemoglobin of nodules were decreased by percent of 61.56%, 35.47% and 53.64%, respectively as compared with control. The further salt concentration caused higher decrease in nodulation and leghemoglobin content. AMF inoculation has the potential to alleviate the adverse impact of salt on the nodulation and leghemoglobin content considerably compared with salt treated plants. In absence of salt stress, AMF caused visible and very significant enhancement in nodulation and leghemoglobin content as compared with control plants.

Pigment system: A significant decrease in leaf photosynthetic pigments was observed under saline regimes (0.15 M NaCl) by percent of 47.65%, 53.95%, 18.56%, 66.99% and 45.14% for chlorophyll a, chlorophyll b, chlorophyll a/chlorophyll b, carotenoids and total photosynthetic pigments, respectively (Table 3). The further concentration of salt (0.25 M NaCl) was most effective in reducing the accumulation of all photosynthetic pigments. Soil application of AMF considerably alleviated the adverse impact of salt on leaf contents of chlorophyll a, chlorophyll b, carotenoids and total photosynthetic pigments, as compared with salted plants (negative control). Under non saline condition, AMF were very effective and stimulated the leaves content of photosynthetic pigments significantly compared with non-treated control.

Table 1. Effect of different concentrations of NaCl on morphological criteria (shoot height [cm], stem weight [g], root weight [g] and leaf area [cm^2/plant] of *Phaseolus vulgaris* plants.

Salt concentrations (M/L)		Morphological criteria			
		Shoot height (cm)	Shoot weight (g)	Root weight (g)	leaf area (cm^2/plant)
Control	(Non)	25.53	2.49	1.29	111.9
	AMF	29.87	3.15	1.75	147.82
0.15 M/L	Control	17.6	1.757	0.828	67.6
	AMF	21.38	2.08	1.14	102.37
0.25 M/L	Control	10.0	0.76	0.49	36.96
	AMF	15.42	1.27	0.86	88.16
LSD at: 0.05		**2.75**	**0.29**	**0.13**	**8.47**

Table 2. Effect of different concentrations of NaCl on nodulation and nodules activities of *Phaseolus vulgaris* plants.

Salt concentrations (M/L)		Nodulation and nodules activities		
		Number/ Plant	Fresh weight (mg/Plant)	Hemoglobin (Unit)
Control	(Non)	21.67	51.67	30.33
	AMF	34.01	67.23	37.12
0.15 M/L	Control	8.33	33.34	14.06
	AMF	17.98	35.78	22.41
0.25 M/L	Control	1.33	17.67	7.43
	AMF	9.07	23.16	12.06
LSD at: 0.05		**5.02**	**9.11**	**4.32**

Table 3. Effect of different concentrations of NaCl on pigment system (mg/g fresh weight) of *Phaseolus vulgaris* plants.

Salt concentrations (M/L)		Pigment system (mg/g fresh weight)				Total pigments
		Chlorophyll a	Chlorophyll b	Chl.a/ Chl.b	Carotenoids	
Control	(Non)	1.49	0.89	1.67	0.203	2.583
	AMF	1.57	0.95	1.65	0.315	2.835
0.15 M/L	Control	0.78	0.57	1.36	0.067	1.417
	AMF	1.04	0.78	1.33	0.124	1.944
0.25 M/L	Control	0.34	0.18	1.89	0.037	0.5567
	AMF	0.72	0.34	2.11	0.087	1.147
LSD at: 0.05		**0.12**	**0.07**	**0.08**	**0.09**	**0.17**

Table 4. Effect of different concentrations of NaCl on on malondialdehyde (MDA) contents (µmol/g fr.wt.); membrane stability index [MSI] (%); tissue water content [TWC] (%) and Total phenols (mg/g fr. wt.) of *Phaseolus vulgaris* plants.

Salt concentrations (M/L)		Different physiological attributes			
		MDA	MSI	TWC	Total phenol
Control	(Non)	28.39	87.23	95.78	2.87
	AMF	25.01	91.47	97.11	3.25
	0.15 M/L	40.31	66.34	72.67	4.12
	AMF	31.55	78.15	78.42	5.78
	0.25 M/L	62.35	29.21	50.78	5.37
	AMF	43.16	41.03	62.35	7.14
LSD at: 0.05		**2.14**	**2.72**	**1.73**	**0.21**

Table 5. Effect of different concentrations of NaCl on polyamines content (nm/g fresh wt) of *Phaseolus vulgaris* plants.

Salt concentrations (M/L)		Polyamines content (nm/g fresh wt)		
		Spermine	Spermidine	Putrescine
Control	(Non)	490.73	899.93	618.27
	AMF	512.47	923.07	637.55
0.15 M/L	Control	250.74	459.03	321.03
	AMF	278.11	483.25	352.08
0.25 M/L	Control	326.83	531.63	432.13
	AMF	384.22	588.39	467.05
LSD at: 0.05		7.81	10.45	7.96

Malondialdehyde, membrane stability index, tissue water content and total phenol: In the present study (Table 4), under saline conditions (0.15; 0.25 M NaCl), significant increase was observed in lipid peroxidation measured as MDA (41.98%; 52.02%) and total phenols (43.54%; 87.11%), respectively as compared with control. However, membrane stability index (MSI) and tissue water content (TWC) were decreased in leaves of bean compared with control in directly proportional with salt concentration. AMF overcame the adverse impact of salt on MDA, MSI, TWC and total phenols, hence significant improvement was observed in such attributes compared with non mycorrhizal plants. Relative to control (non salted plants), AMF inoculated plants showed increase in MSI and TWC, however MDA content decrease significantly.

Polyamine content: The results in Table 5, indicated that 0.15 M of salt caused marked ($p \leq 0.01$) reduction in polyamines as spermine (48.90%), spermidine (48.99%), putrescine (48.07%) content of bean leaves as compared with control. Further decreases in polyamines (33.40%, 34.61%, 24.45%, respectively) were observed at the higher concentration (0.25M) of NaCl as compared to control plants. Under saline conditions, a significant impact of AMF was observed in improving polyamines content as compared with salt treated plants. Similarly in absence of salt stress, AMF caused significant improvement of polyamines contents as spermine (4.43%), spermidine (2.57%), putrescine (3.02%) content as compared with control.

Phytohormones: Salt adversely affected the phytohormones content in bean plants as shown by the present study (Table 6). The results suggested that phytohormones decreased with increasing NaCl concentration and played a positive role in mitigation of adverse effect of salt stress. Sodium chloride at 0.15 M and 0.25 M caused significant decrease in IAA (36.84%, 72.06%); IBA (34.02% and 65.14%); IAA/IBA (4.76% and 38.09%;); GA1 (17.97% and 44.66%;) and GA4 (27.35% and 61.63%,) contents, respectively as compared with control. However, GA1/GA4 increased by 13.05% and 44.36% with salinity at concentrations of at 0.15 M and 0.25 M, respectively as compared with control. As shown in Table 6, AMF application increased all phytohormones (IAA, IBA, GA1, GA4) content in bean plants significantly as compared with control. In presence of salt impact, AMF decreased the adverse impact of salinity stress on phytohormones content compared with mycorrhizal plants.

Table 6. Effect of different concentrations of NaCl on Hormonal balance of *Phaseolus vulgaris* plants.

Salt concentrations (M/L)		Hormonal balance					
		IAA	IBA	IAA/IBA	GA_1	GA_4	GA_1/GA_4
Control	(Non)	2.47	115.21	0.021	3.56	6.36	0.559
	AMF	2.83	121.34	0.023	3.88	6.87	0.564
0.15 M/L	Control	1.56	76.01	0.020	2.92	4.62	0.632
	AMF	2.03	87.93	0.023	3.16	5.12	0.617
0.25 M /L	Control	0.69	50.53	0.013	1.97	2.44	0.807
	AMF	1.17	68.91	0.017	2.34	2.83	0.826
LSD at: 0.05		**0.24**	**5.03**	**0.004**	**0.18**	**0.15**	**0.007**

Indole-3-acetic acid, IAA; Indole-3-butyric acid, IBA; Gibberellin A_1, GA_1; Gibberellin A_4, GA_4

Table 7. Effect of different concentrations of NaCl on antioxidant enzymes (EU/ mg protein) of *Phaseolus vulgaris* plants.

Salt concentrations (M/L)		Antioxidant enzymes (EU/ mg protein)				
		CAT	APX	POD	GR	SOD
Control	(Non)	3.11	9.24	4.24	6.93	1.11
	AMF	3.71	9.52	4.65	7.21	1.45
0.15 M/L	Control	4.35	10.41	5.81	8.18	1.85
	AMF	4.63	10.78	5.98	8.53	1.97
0.25 M/L	Control	5.82	11.62	6.53	9.73	2.37
	AMF	5.92	11.93	7.12	10.11	2.88
LSD at: 0.05		**0.09**	**0.21**	**0.11**	**0.09**	**0.13**

Catalase, CAT; Ascorbate peroxidases, APX; Peroxidases, POD; Glutathione reductase, GR; Superoxide dismutases, SOD

Table 8. Effect of different concentrations of NaCl on Elements accumilation (mg/g dry weight) of *Phaseolus vulgaris* plants.

Salt concentrations (M/L)		Elements accumilation (mg/g dry weight)					
		Na	K	Na/K	Ca	Cl	P
Control	(Non)	3.03	15.04	0.20	20.88	10.30	0.173
	AMF	2.74	17.98	0.15	26.11	9.78	0.372
0.15 M/L	Control	5.92	12.27	0.48	17.67	15.93	0.097
	AMF	4.68	14.23	0.32	20.34	12.23	0.251
0.25 M /L	Control	9.14	10.74	0.85	15.12	19.333	0.0467
	AMF	8.01	11.07	0.72	16.97	15.33	0.173
LSD at: 0.05		**0.21**	**0.43**	**0.03**	**0.84**	**0.38**	**0.07**

Antioxidant enzymes: The results pertaining to the effect of salinity (0.15M and 0.25M) in presence and absence of AMF on antioxidant enzymes (CAT, APX, POD, GR and SOD) activity in bean plants were given in Table 7. A significant increase of 39.87%; 12.66%; 37.02%; 18.03% and 66.67% in CAT; APX; POD; GR and SOD was observed at 0.15 M NaCl as compared to control. The further concentration of salinity (0.25 M) caused significant higher increment in activity of CAT; APX; POD; GR and SOD by percent of 87.13%; 25.75%; 54.01; 40.41% and 113.51%, respectively as compared with control. In the presence of AMF, the adverse impact of salinity on the antioxidant enzymes was counterbalanced and suppressed significantly. AMF caused slight increase in activity of all antioxidant enzymes in presence and absence of salt stress.

Elemental accumulation: Sodium chloride stress decreased the accumulation of essential elements in leaves of the bean plants (Table 8). Potassium; calcium and phosphorus contents of bean plants were decreased by 18.41%; 15.37% and 43.93%, respectively as compared with control at 0.15 M NaCl. The higher salt concentration (0.25 M NaCl) caused much decrease in the previous elements by percent of 28.59%; 27.58% and 73.01%, respectively as compared with control. On the other hand, the accumulation of sodium; chlorine and sodium/potassium ratio were increased significantly by percent of 95.37%; 54.66% and 140.00%, respectively at 0.15 M NaCl as compared with control. At 0.25 M NaCl, more significant increase in leaves contents of sodium; chlorine and sodium/potassium ratio by percent of 201.65; 87.67% and 325.00%, respectively as compared with control. Under absence of salt stress, the data clearly indicating the improving effect of AMF on the accumulation of elements, hence on the elemental balance. AMF caused significant increase in potassium; calcium and phosphorus contents of bean plants compared with control. Considerable ($p \leq 0.01$) improvement in elemental balance of bean leaves was observed in mycorrhizal under salt prevailing regimes (0.15 M and 0.25 M, NaCl). At the higher salt concentration (0.25 M)

the efficiency of AMF to alleviate the adverse impact of salt was lower than at the lower concentration (0.15 M), but it was also significant.

Discussion

Salinity is one of the common agricultural and biological abiotic stress faces every grower. Soil salinity results in the greater loss in agricultural productivity and disrupts the ecological balance of the area (Aggarwal *et al*., 2012). In our study, all plant growth criteria were decreased significantly due to salt impact, which corroborates with the findings of Hashem *et al*. (2014); Alqarawi *et al*. (2014b); Abd_Allah *et al*. (2015) who showed decrease in shoot length, root length, fresh weight of *Vicia faba*; *Ephedra aphylla* and *Solanum lycopersicum*, respectively with increasing concentration of NaCl. Plant growth and development mainly depends on photosynthesis, but this physiological process is greatly perturbed by stressful environments including salt stress (Hameed *et al*., 2014; Ahanger *et al*., 2014; Abd_Allah *et al*., 2015; Hashem *et al*., 2015). Application of AMF have been shown to alleviate the negative effect of NaCl on the growth are in accordance with Alqarawi *et al*. (2014c) and Abd_Allah *et al*. (2015); Hashem *et al*. (2015). AM fungi improve plant growth and yield as it provides adequate supply of mineral nutrients particularly phosphorous (Abd_Allah *et al*., 2015; Hashem *et al*., 2015).

In the present investigation, nodulation and nodules activity (leghemoglobin content) were reduced significantly in bean plants with increasing salinity. Our results are quite similar to previous observations in different legumes plants as *Vicia faba* (Hashem *et al*., 2014); *Cicer arietinum* (Egamberdieva *et al*., 2014) and *Sesbania sesban* (Abd_Allah *et al*., 2015). For the successful nodulation high number of rhizobia is necessary which are reduced under salinity stress (Severin *et al*., 2012; Abd_Allah *et al*., 2015). AMF induced improvement in nodulation and nodules activity in presence and absence of salt stress. In this context, in phosphorous deficient soils, Powell, (1976) observed that nitrogen fixation mainly depends on the availability of phosphorous provided by inhabiting AMF (Abd_Allah *et al*., 2015).

In view of several reports it is evident that salt stress can substantially suppress the concentration of photosynthetic pigments in many plants such as in *Morus alba* (Azooz *et al*., 2011); *Vicia faba* (Hashem *et al*., 2014); *Ephedra alata* (Alqarawi *et al*., 2014a). Decrease in the pigment content in present study is attributed to the negative effect of salt stress on chloroplast (Zörb *et al*., 2009) and increased activity of chlorophyllase enzyme (Sultana *et al*., 1999) and restricted supply of Mg^{2+}, Fe^{2+}, Zn^{2+} that are important for chlorophyll biosynthesis (Kupper *et al*., 1996). Stomatal limitation led to the reduction of CO_2 assimilation rate (Debez *et al*., 2008) consequently inhibits both synthesis and activity of photosynthetic pigments. The decrease in carotenoid content due to salinity leads to degradation of β-carotene and formation of zeaxanthins, which are apparently involved in protection against photoinhibition (Sultana *et al*., 1999). In the present investigation, AMF enhanced synthesis of photosynthetic pigments and considerably alleviated the adverse impact of salt on pigments system. The stimulatory mechanism of AMF is not completely understood yet (Hameed *et al*., 2014; Wu *et al*., 2014). Under normal as well as salinity stress conditions enhanced synthesis of photosynthetic pigments due to inoculation of AMF has earlier been reported by in Solanum lycopersicum (Hashem *et al*., 2015) and lettuce (Aroca *et al*., 2013). Aroca *et al*. (2013) observed enhanced uptake of essential mineral nutrients in AMF inoculated plants. Enhanced chlorophyll synthesis in AMF inoculated plants and subsequent amelioration of salinity stress induced deleterious effect may be due to the increased uptake of magnesium which forms an important part of chlorophyll pigment molecule (Aroca *et al*., 2013).

Lipid peroxidation is considered as one of the main criterions for systemic acquired resistance against salt stress (Abd_Allah *et al*., 2015). Increase in lipid peroxidation by NaCl in the present study corroborates with other findings in *Ochradenus baccatus* (Hashem *et al*., 2014); *Vicia faba* (Hashem *et al*., 2014) and *Sesbania sesban* (Abd_Allah *et al*., 2015). The enhancement of lipid peroxidation due to NaCl is attributed to generation of ROS, that attack the polyunsaturated fatty acids (PUFA) and disrupts the membrane structure and leads to cell leakage (Alqarawi *et al*., 2014a; Wu *et al*., 2014). Plants treated with NaCl in combination with AMF showed less increase in lipid peroxidation. One of the reasons might be antioxidant enzymes are able to scavenge the ROS before reacting with membrane lipids and minimizes peroxidation of lipids. Another reason might be up regulation of stress related proteins like glutathione stransferase (GST), glutathione dependent formaldehyde dehydrogenase (FALDH) and peroxidase.

Membrane stability index; tissue water content and total phenols of bean plants were decreased by salt stress and considered the useful parameters to cell injury and plant resistance against salinity stress. Our results were parallel to what has been previously reported in *Ephedra aphylla* (Alqarawi *et al*., 2014b); *Sesbania sesban* (Abd_Allah *et al*., 2015) and *Solanum lycopersicum* (Hashem *et al*., 2015). Soil application of AMF alleviated the adverse impact of salt on MSI, TWC and total phenols of bean plants. Mycorrhizal plants improve the stability as well as the integrity of membrane proteins by maintaining higher relative permeability of the cell (Kaya *et al*., 2009). This results in increased phosphorus uptake as well as antioxidant enzymes production (Feng *et al*., 2002). In the same context, our previous reports showed that AMF play an important and effective impact to increase the previous criteria and alleviation of adverse impact of salinity (Ahanger *et al*., 2014; Abd_Allah *et al*., 2015; Hashem *et al*., 2015).

Polyamines are small organic polycations present in all organisms and have a leading role in cell cycle, expression of genes, signaling, plant growth and

development and tolerance to a variety of abiotic stresses (Ahmad *et al*., 2012). Polyamines are key responses of salt tolerant plants, high accumulation of polyamines (putrescine, spermidine and spermine) in plants during abiotic stress has been well documented and is correlated with increased tolerance to abiotic stress (Alcazar *et al*., 2010). In the present study, salt stress induced significant increase in polyamines (spermine, spermidine and putricine) of bean plants. Soil application of AMF caused further increases these polyamines hence, suggests the defensive roles against NaCl stress. Some of the observations suggest that polyamines play a role in stabilizing membranes, scavenging free radicals, nucleic acids and protein synthesis, RNAse, protease and other enzyme activities, and interacting with hormones, phytochromes, and ethylene biosynthesis (Galston & Tiburcio, 1991). Because of these numerous biological interactions of polyamines in plant systems, it has been difficult to determine their precise role in plant growth and development (Alcazar *et al*., 2010; Hashem *et al*., 2014).

Plant hormones are signal molecules known to regulate many developmental processes in plants and are therefore suitable candidates to function in the systemic acquired resistance process (Iqbal & Ashraf, 2013a; Habib & Ashraf, 2014; Alqarawi *et al*., 2014b). In the present study, salinity caused significant alteration in the hormonal balance of bean plants shown as significant decrease in IAA, IBA, GA_1 and GA_4. Such alteration in hormonal balance reported due to NaCl in the present study are in agreement with the reports of Maggio *et al*. (2010) Iqbal & Ashraf, (2013b) and Alqarawi *et al*. (2014b). AM fungal colonization increases plants tolerance to salinity (Porcel *et al*., 2012; Abd_Allah *et al*., 2015) through alteration in hormonal profiles (Aroca *et al*., 2013). Endophytic fungi cause increase in endogenous levels of IAA and GA (Waqas *et al*., 2012). In the present study, AMF increase the IAA, IBA, GA_1 and GA_4 as suggested mechanism of alleviation of adverse impact of salt (under salt stress) and stimulation of plant growth (in absence of salinity). In the context, it have been reported that exogenously applied auxins and growth regulators alleviated the salt impact and stimulate the plant metabolism (Iqbal & Ashraf, 2013a; Habib & Ashraf, 2014).

Salt stress priming agents caused significant increase of antioxidant enzymes (CAT, APX, POD, GR and SOD) activity. Our results came in analogous to previous reports of Hashem *et al*. (2014); Alqarawi *et al*. (2014b); Abd_Allah *et al*. (2015) and Hashem *et al*. (2015) in different plants. AMF mitigated the adverse impact of salinity on the antioxidant enzymes of bean plants. Our earlier studies on *Ephedra aphylla* (Alqarawi *et al*., 2014c); *Capsicum annuum* (Kaya *et al*., 2009) and *Sesbania sesban* (Abd_Allah *et al*., 2015) demonstrated the positive impact and mitigation role of AMF on antioxidant enzymes which control the adverse impact of salt. Similarly, Hashem *et al*. (2015) reported that inoculation of AMF (*Funneliformis mosseae*; *Rhizophagus intraradices* and *Claroideoglomus etunicatum*) to *Solanum lycopersicum* plants reduced the adverse impact of salt with a significant increase in activities of antioxidant enzymes, leading to better growth through efficient scavenging of reactive oxygen species (ROS) generated.

The ionic imbalance was observed as main Impact of salt stress in bean plant during the present study. Our results were agreed with those reported in many other plants (Iqbal & Ashraf, 2013a; Hashem *et al*., 2014; Abd_Allah *et al*., 2015). The ionic balance has a key role in photosynthesis and other metabolic activities of the cell (Wu *et al*., 2014). The treatment of soil with AMF were very effective in alleviating the deleterious effects of salinity stress on ionic balance of bean and mitigates the deficiency of K^+ and increases K/Na ratio at all levels of salt stress. Similar results have been reported by other researchers (Alqarawi *et al*., 2014b; Hashem *et al*., 2014; Abd_Allah *et al*., 2015). Formally, Al-Karaki, (2000) also reported that AM fungal symbiosis plays a key role in higher K^+ accumulation and hence higher K/Na ratio in mycorrhizal plants. AMF spread the mycelium deep into the soil and absorb water and minerals for the host plant so increase the efficiency of the plants to withstand the NaCl stress High accumulation of Na^+ associated with a marked suppression of both K^+ and phosphorus contents due to NaCl was observed in present study.

In conclusion, NaCl caused delirious impact and oxidative stress on bean plants shown as decrease in growth criteria and photosynthesis, however both lipid peroxidation and antioxidative enzymes were enhanced. Soil applications of AMF have alleviated the adverse impact of NaCl on growth and all metabolic activities, consequently enhanced the anabolism. The obtained results suggested AMF as sustainable approach in alleviating the salt which can be used for rehabilitation of salt marsh degraded areas.

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