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RESEARCH ARTICLE

Alleviation of abiotic salt stress in *Ochradenus baccatus* (Del.) by *Trichoderma hamatum* (Bonord.) Bainier

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The current study was taken up to examine the role of bioagent (*Trichoderma hamatum*) in mitigating the deleterious effects of NaCl stress in *Ochradenus baccatus.* Varying concentrations of salt (0, 75, and 150 mM) were used to observe the effect on growth, pigments, some key metabolic attributes, antioxidant enzymes, and elemental accumulation in *O. baccatus*. The results indicated significant decrease in seed germination, plant growth, pigment content, membrane stability index, tissue water content, and total lipid content with salt stress. Lipid peroxidation increases with the increasing concentration of NaCl. Moreover, salinity stimulated the biosynthesis of phenols, diacylglycerol, sterol esters, nonesterified fatty acids, and enzymatic antioxidants like superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, glutathione reductase. The Na^+ content in shoot increases with elevated levels of NaCl concentration, accompanied with significant decreases in K^+, Mg^{2+}, and Ca^{2+}. Application of bioagent (*T. hamatum*) has been observed to alleviate the antagonistic effect of salt stress on plant growth and metabolic processes. In absence and presence of salt stress, the bioagent stimulated the plant growth and alter the plant metabolism through the modification of the above parameters.

Keywords: salinity; *Ochradenus baccatus*; *Trichoderma hamatum*; growth; lipids; antioxidants

Abbreviations: TL, total lipid; DG, diacylglycerol; TG, triacylglycerol; S, sterol; SE, sterol ester; FAA, nonesterified fatty acids; ROS, reactive oxygen species

Introduction

Ochradenus baccatus Del., belongs to family *Resedaceae*, is widely distributed in South-West and central regions of Saudi Arabia (SA; Al Qurainy et al. 2013). *O. baccatus* (Del.) is a semi-deciduous shrubby plant and grows in dry deserts as bushes (Bronstein et al. 2007). It is a very important medicinal plant as it contains high contents of antioxidants and anti-inflammatory agents (Alqasoumi et al. 2012).

Nowadays, rehabilitation of rangelands became national target in SA, so the main focus is to maintain range plants from extinction. Ecological factors affect the vegetation of *O. baccatus* so much that this plant is near to extinction (Al-Abbasi et al. 2010). Therefore, the demand of *O. baccatus* has increased tremendously during the last few decades (Al Qurainy et al. 2013). Range plants often experience abiotic stress and among these salt stress is most common that hampers the growth and biomass yield (Alqarawi, Hashem, Abd Allah, Alshahrani, et al. 2014). The primary effects of salinity stress are reduced germination percentage, fresh and dry weight of shoot and root, leaf water potential, chlorophyll (Chl) contents, photosynthesis, respiration, and protein synthesis (Neumann 2008; Ahmad & Prasad 2012a, 2012b; Rasool, Ahmad, et al. 2013; Rasool, Hameed, et al. 2013; Alqarawi, Hashem, Abd Allah, Alshahrani, et al. 2014). Salinity also cause nutritional disorders in plants which lead to deficiencies of several nutrients and drastically increasing Na^+ levels (Shahid et al. 2013). Moreover, the salt stress causes oxidative stress, through the production of variety of reactive oxygen species (ROS) like, singlet oxygen, superoxide ions, hydroxyl radical, H_2O_2, etc. These ROS are noxious molecules (Ahmad, Sarwat, et al. 2008; Ahmad, Umar, & Sharma 2010) and causes deleterious effects on mitochondria and chloroplast by disturbing cellular structures (Apel & Hirt 2004; Ahmad, Umar, & Sharma 2010, 2012; Azooz et al. 2011; Naz & Bano 2013). However plants are equipped with antioxidant machinery which includes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR; Apel & Hirt 2004; Ahmad, Jaleel, Salem, et al. 2010; Koyro et al. 2012; Rasool, Ahmad, et al. 2013). These antioxidants are present in different cellular compartments especially in chloroplast and mitochondria (Apel & Hirt 2004; Masood et al. 2006) and play a great role in resistance mechanism against salt stress.

Trichoderma spp. are commonly found in soil especially near the roots of plants. Some species form colonizations with the roots and some may live as parasites on other fungi. Interaction of *Trichoderma* spp. with plants accelerates their growth, crop production, and provides

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tolerance to different environmental stresses. They also enhance uptake of nutrients (Harman et al. 2004), production of many biochemical elicitors including various peroxidases (PODs), chitinases, β-1,3-glucanases, lipoxygenase-pathway hydroperoxide lyase, and compounds like phytoalexins and phenols to promote tolerance against both biotic and abiotic stresses (Gachomo & Kotchoni 2008; Shoresh et al. 2010). The beneficial effects of *Trichoderma* species on alleviation of adverse effects of salinity stress have been well documented (Hermosa et al. 2012; Rawat et al. 2013). The present study has been designed to observe the ability of *T. hamatum* to alleviate the negative effects of salinity stress.

Materials and methods

The experimental mold and its formulation

The bioagent (*Trichoderma hamatum* [Bonord.] Bainier) used in present study was isolated previously from tomato root grown in Sharkia governorate, Egypt (Abd-Allah & Ezzat 2005). *T. hamatum* was grown in potato dextrose broth (DIFCO) in flasks and was kept for seven days at 30°C and then shaked at 150 rpm. After the incubation period, the mycelium was lyophilized under vacuum and the lyophilized powder was mixed with talc powder and 1.0% carboxy methyl cellulose as an adhesive agent to give a final concentration of 3.2×10^6 cfu g^{-1} of carrier material. The formulated *T. hamatum* was added to the soil before sowing at a rate of 10 g/kg soil.

Experimental plant and seed germination test

Dry mature fruits of *O. baccatus* (Del.) were randomly collected from different rangelands in Riyadh region in 2013 (Figure 1). Healthy seeds were taken from the fruits and surface sterilized with sodium hypochlorite (5%, v/v) for two minutes at room temperature (+25°C), washed thoroughly with distilled water. After that the seeds were sown in plastic pots with different concentrations of NaCl (0, 75, and 150 mM). Another set was prepared with same concentration of NaCl but the soil in the pots was also supplemented with *T. hamatum*. The germination percentage is calculated on the basis of total seed percent:

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Pots experiment

Healthy plantlets from germinated seeds were selected and sown in separate plastic pots with 1.50 kg soil and sand (one plant/pot). Hoagland's solution (Hoagland & Arnon 1950) supplemented with sodium chloride to get concentration of 0, 75, and 150 mM was used for irrigation in pots with and without *T. hamatum*. The rate of irrigation was 100 ml for each treatment every three days for eight weeks at alternative temperature rate of (27°C day and 18°C night) with a photosynthetic photon flux density of 1500 µmol m^{-2} s^{-1} (18 h light and 6 h dark period). At the end of experiment period,

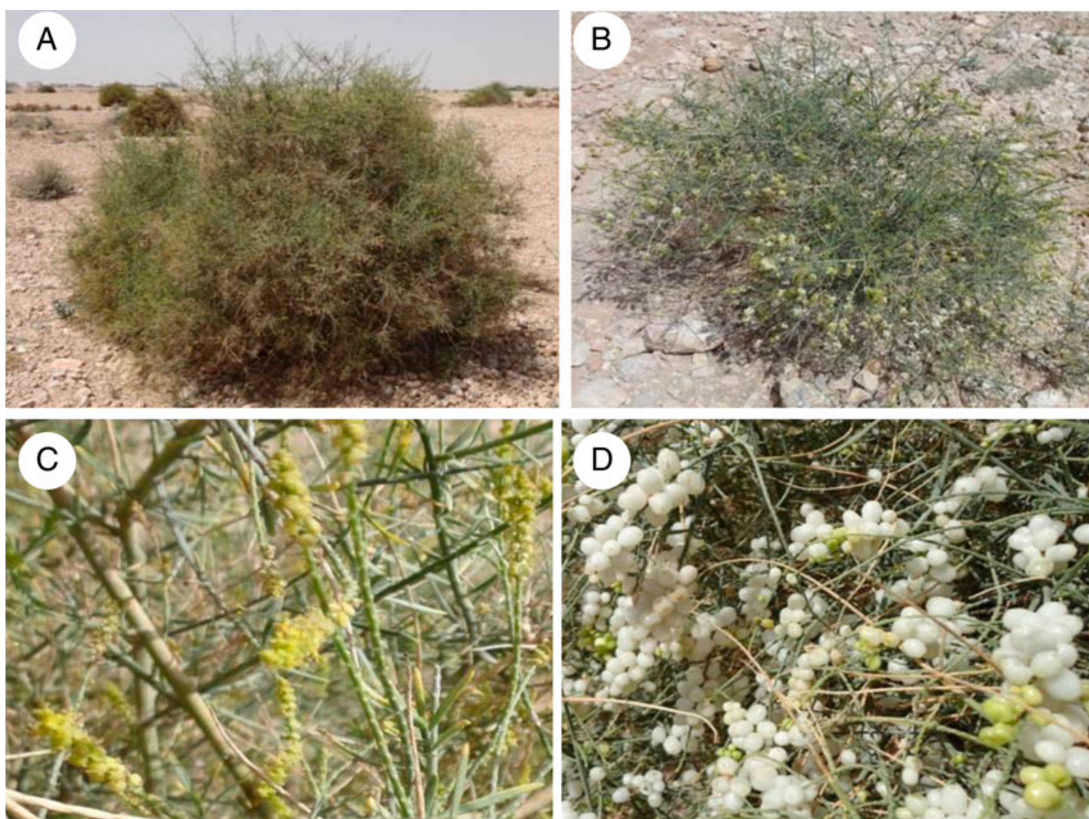

Figure 1. Incidence of *O. baccatus* (Del.) in the original habitat. (A) The vegetation stage of plant growth; (B) beginning of flowering and fruiting stage; (C) inflorescence stage; and (D) fruiting stage.

(eight weeks) the plants were harvested for growth and biochemical analyses.

The composition of the Hoagland solution is (mg l^{-1}): 270 N (KNO_3), 31 P (KH_2PO_4), 234 K (KNO_3), 200 Ca ($Ca(NO_3)_2.4H_2O$), 64 S ($MgSO_4.7H_2O$), 48 Mg ($MgSO_4.7H_2O$), 2.8 Fe (Fe-EDTA), 0.5 Mn ($MnCl_2.4H_2O$), 0.5 B (H_3BO_3), 0.02 Cu ($CuSO_4$), 0.05 Zn ($ZnSO_4.7H_2O$), and 0.01 Mo ($H_3MoO_4.H_2O$; Hoagland & Arnon 1950).

Photosynthetic pigments

The quantitative analysis of photosynthetic pigments was done by the method of Lichtenthaler and Wellburn (1983). The pigments were extracted from fresh leaves (100 mg) by acetone and the absorbance was read spectrophotometrically at 622, 664, and 440 nm wavelength.

Tissue water content (TWC)

The estimation of TWC was according to Smart and Bingham (1974) and described by Ahmad et al. (2012). TWC is calculated by:

$$\text{TWC} = \frac{\text{fresh weight} - \text{dry weight}}{\text{turgid weight} - \text{dry weight}}$$

Membrane stability index (MSI)

MSI was estimated according to the method of Sairam et al. (1997). The MSI was calculated by the following formula:

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

Lipid peroxidation

Lipid peroxidation in terms of concentration of malondialdehyde (MDA) was recorded by the method of Heath and Packer (1968). Absorbance at 532 and 600 nm were used for calculation of MDA equivalents. Blank sample was used as reference. MDA equivalent was calculated by the following equation:

$$\text{MDA equivalents } (\text{nmol} \cdot \text{cm}^{-1}) = 1000 \left[\frac{\text{Abs } 523 - \text{Abs } 600 \text{ nm}}{155}\right]$$

Determination of total phenolics

The total phenolics in the fresh leaves 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 Julkunen-Tiitto (1985). The optical density of the mixtures were read at 750 nm. Standard curve of pyrogallol was used as reference.

Estimation of ion accumulation

A known weight of oven dry leaf sample was 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. Standard curve of each mineral (10–100 μg ml^{-1}) used as reference.

Lipid analysis

Total lipids (TLs) were extracted by using chloroform: methanol (2:1, v/v), with 0.05% (w/v) of butylated hydroxytoluence (2.6 di-tert-butyl-p-cresol) and were estimated using the charring method of Marsh and Weinstein (1966), with stearic acid (Sigma) as the standard. The neutral lipids in the extracts were separated on thin layer chromatography (TLC) plates (chloroform:methanol:water, 65:35:3; v/v/v, used as mobile phase). The qualitative estimation was carried by reaction of TLC plates with acid dichromate for clarification. The quantitative estimation was carried out spectrophotometrically according to Amenta (1964).

Antioxidant enzyme assays

Fresh leaves of 500 mg were ground in sodium phosphate buffer (50 mM, pH 7.0) containing polyvinylpyrrolidone (1%, PVP). After 20 min of centrifugation (4°C) at 15,000 rpm the supernatant was collected and used to determine the activities of SOD, POD, CAT, and APX).

A method of Samantary (2002) was used to assay the activity of CAT (EC 1.11.1.6). Enzyme activity was expressed in M H_2O_2 destroyed mg^{-1} $protein^{-1}$ min^{-1}.

POD (EC 1.11.1.7) activity was assayed spectrophotometrically according to the method of Kar and Mishra (1976). 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 method of Nakano and Asada (1981) was used for the assay of APX (EC 1.11.1.11) activity. APX was assayed as a decrease in absorbance at 290 nm of ascorbate. One unit of enzyme was considered as the amount necessary to decompose 1 mol of substrate min^{-1} at 25°C.

The activity of GR (EC 1.6.4.2) was evaluated by the method of Carlberg and Mannervik (1985). The decrease in absorbance was read for 2 min at 340 nm. The activity of GR was calculated using the extinction coefficient of nicotinamide adenine dinucleotide phosphate (NADPH) of 6.2 mM^{-1} cm^{-1} and expressed as EU mg^{-1} protein.

SOD (EC 1.15.1.1) was determined by photoreduction of nitroblue tetrazolium (NBT) at 540 nm (Beyer & Fridovich 1987). One unit of SOD activity equaled to the amount required to inhibit photoreduction of NBT by 50%. The results of antioxidant enzyme assays were expressed as EU.

Statistical analysis

Data collected were analyzed by two-way analysis of variance followed by Duncan's Multiple range Test. The values obtained were the mean ± *SE* for five replicates in each group. *P* value at 0.05 was considered as significant.

Results

The seed germination decreases with increasing concentration of NaCl and the results are presented in Figure 2. The maximum decrease of 44.24% in seed germination was observed at 150 mM NaCl stress. Plants treated with NaCl in combination with *T. hamatum* showed minimum decrease in seed germination. The decrease of only 6.30% and 19.24% was observed at 75 mM and 150 mM NaCl supplemented with *T. hamatum*, respectively, as compared to control.

Salinity significantly decreased shoot and root length as well as shoot and root dry weight in the present study (Table 1). Maximum decline in length of shoot and root was 59.70% and 62.21%, respectively, at 150 mM NaCl. Shoot dry weight decreased to 75.00% and root dry weight to 76.56% at 150 mM NaCl. The application of *T. hamatum* mitigates the deleterious effect of NaCl stress and enhances the dry weight of root (48.4%) and shoot (125%) significantly as compared with control.

The results pertaining to the effect of salt stress and *T. hamatum* on Chl is depicted in Table 2. Significant decrease of 59.32%, 46.80%, 56.44%, was recorded in Chl a, Chl b, and total Chl, respectively, in *O. baccatus* at 150 mM NaCl. However, the soil amended with *T. hamatum* mitigated the antagonistic effect of NaCl stress on pigment content as compared to those treated with NaCl alone. Moreover, the application of *T. hamatum* alone (in absence of salt stress) caused significant increase in Chl a, Chl b, carotenoids, and total Chl as compared to control plants.

TWC of *O. baccatus* significantly decreased to 20.44% and 41.36% at 75mM and 150mM NaCl,

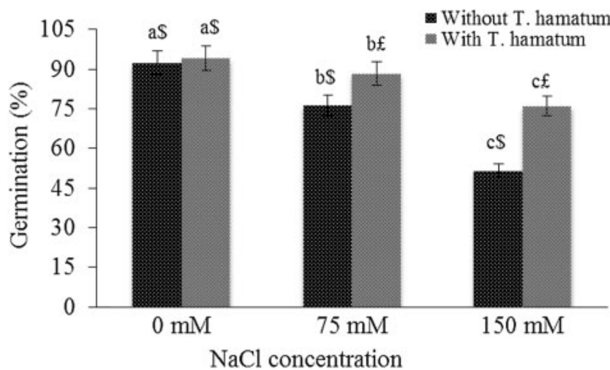

Figure 2. Effect of different salt concentrations on seed germination in *O. baccatus*. Data presented are the means ± *SE* (*n* = 5). Different letters indicate significant difference ($P < 0.05$) among the treatments. Symbols $ and £ denote significant change between with and without *T. hamatum* within the same treatment.

Table 1. Effect of NaCl in presence and absence of *T. hamatum* on growth and biomass yield of *O. baccatus* seedlings.

NaCl concentration	Growth and biomass							
	Without *Trichoderma*				With *Trichoderma*			
	Shoot		Root		Shoot		Root	
	Length (cm)	Dry wt (g)	Length (cm)	Dry wt (g)	Length (cm)	Dry wt (g)	Length (cm)	Dry wt (g)
0 mM	21.37 ± 1.97a$	0.36 ± 0.19a$	25.33 ± 2.12a$	0.64 ± 0.32a$	31.2 ± 2.33a£	0.81 ± 0.47a£	34.83 ± 2.61a£	0.95 ± 0.52a£
75 mM	12.60 ± 1.31b$	0.22 ± 0.06b$	15.13 ± 1.62b$	0.40 ± 0.25b$	18.9 ± 1.85b£	0.53 ± 0.29b£	23.40 ± 1.99b£	0.64 ± 0.32b£
150 mM	8.61 ± 0.90c$	0.06 ± 0.005c$	9.57 ± 1.02c$	0.15 ± 0.04c$	14.4 ± 1.21c£	0.31 ± 0.10c£	12.83 ± 1.37c£	0.37 ± 0.20c£

Note: Data presented are the means ± *SE* (*n* = 5). Different letters next to the numbers indicate significant difference ($P < 0.05$) among the treatments. Symbols $ and £ denote significant change between with and without *T. hamatum* within the same treatment.

Table 2. Effect of NaCl in presence and absence of *T. hamatum* on pigment content (mg g^{-1} fresh weight) of *O. baccatus* seedlings.

NaCl concentration	Pigment content (mg g^{-1} fresh weight)							
	Without *Trichoderma*				With *Trichoderma*			
	Chl. a	Chl. b	Total Chl	Car.	Chl. a	Chl. b	Total Chl	Car.
0 mM	4.72 ± 0.95a$^{\$}$	1.41 ± 0.13a$^{\$}$	6.13 ± 1.35a$^{\$}$	0.33 ± 0.05a$^{\$}$	5.35 ± 1.23a$^{£}$	1.85 ± 0.17a$^{£}$	7.20 ± 1.95a$^{£}$	0.36 ± 0.06a$^{£}$
75 mM	3.83 ± 0.81b$^{\$}$	1.22 ± 0.11b$^{\$}$	5.05 ± 1.15a$^{\$}$	0.21 ± 0.02b$^{\$}$	4.96 ± 1.12 b$^{£}$	1.48 ± 0.14b$^{£}$	6.44 ± 1.57a$^{£}$	0.30 ± 0.03 b$^{£}$
150 mM	1.92 ± 0.18c$^{\$}$	0.75 ± 0.09c$^{\$}$	2.67 ± 0.75a$^{\$}$	0.14 ± 0.007c$^{\$}$	2.74 ± 0.79 c$^{£}$	1.05 ± 0.10c$^{£}$	3.96 ± 0.84a$^{£}$	0.19 ± 0.009c$^{£}$

Note: Data presented are the means ± *SE* ($n = 5$). Different letters next to the numbers indicate significant difference ($P < 0.05$) among the treatments. Symbols $ and £ denote significant change between with and without *T. hamatum* within the same treatment.

respectively, as compared to control (Figure 3A). The pretreatment with *T. hamatum* could alleviate the negative effect of salt stress by decreasing TWC to only 7.92% at 75 mM and 30.25% at 150 mM NaCl stress as compared to the plants treated with NaCl alone.

MSI showed significant variation at all stress levels and the results are depicted in Figure 3B. Salt concentrations 75 and 150 mM caused significant decrease of 32.34% and 59.42%, respectively, in MSI as compared to control. The effect of *T. hamatum* was very effective in decreasing the deleterious effect of salinity stress. Salt stress 75 and 150 mM in combination with *T. hamatum* showed a decrease of only 12.91% and 26.43% in MSI, respectively, as compared to control.

The results pertaining to the effect of salt stress on MDA contents in presence and absence of *T. hamatum* is shown in Figure 3C. MDA content was significantly increased to 31.26% and 54.98% at 75 and 150 mM NaCl, respectively, as compared to control. Application of *T. hamatum* significantly decreased the MDA content to 22.75% at 75 mM and 43.15% at 150 mM NaCl. Hence the bioagent was much effective in decreasing MDA content as compared to salt-treated plants without bioagent.

The data presented in Figure 3D indicated that salinity stimulated the biosynthesis of phenols to 22.55% and 39.47% at 75 and 150 mM NaCl, respectively, as compared to control. Supplementation of *T. hamatum* further increased the total phenols to 30.09% at 75 mM and 46.51% at 150 mM NaCl. Moreover, in absence of salt stress, *T. hamatum* caused significant increase in total phenol content in control plants.

The results pertaining to the effect of salt stress and *T. hamatum* on TLs and neutral lipids are presented in Table 3. It was observed that 75mM NaCl caused significant decrease of 32.37%, 18.00%, and 10.20%, in TLs, triacylglycerol (TG), and sterol (S), respectively. However the higher concentration of NaCl (150mM) caused more decrease of 50.20%, in TL, 27.86% in TG, and 47.69% in S as compared to control. Application of *T. hamatum* in presence of NaCl (150 mM) decreased TL, TG, and S to 40.16%, 11.45%, and 23.43%, respectively, as compared to control. On the other hand salt stress caused remarkable rise in concentrations of diacylglycerol (DG), sterol ester (SE), and nonesterified fatty acids (FAA) and the increase is directly proportional to increasing salt concentrations. Supplementation of *T. hamatum* with 150 mM NaCl showed minimum increase of only 14.95% in DG as compared to control. The SE and FAA also showed less increase of 15.18% and 15.59%, respectively, at 150 mM NaCl supplemented with *T. hamatum*.

The results pertaining to the effect of salt stress on antioxidant enzymes are reported in Figure 4A–E. A significant increase in activity of enzymatic antioxidant has been reported in present study. The CAT, POD, APX, GR, and SOD increased to 32.92%, 41.50%, 30.69%, 30.18%, and 40.43%, respectively, at 75 mM NaCl as compared to control. Further increase in the antioxidant enzymes has been reported at 150 mM as

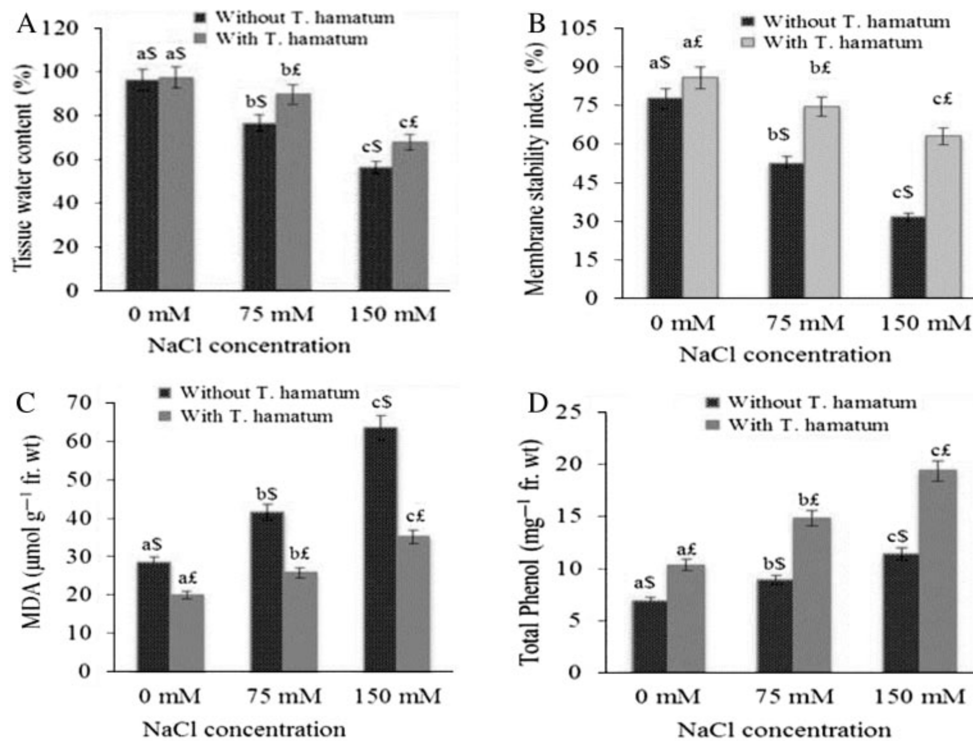

Figure 3. Effect of NaCl in presence and absence of *T. hamatum* on TWC (A), MSI (B), MDA (C), and total phenol contents (D) in *O. baccatus* seedlings. Data presented are the means ± *SE* ($n = 5$). Different letters indicate significant difference ($P < 0.05$) among the treatments. Symbols $ and £ denote significant change between with and without *T. hamatum* within the same treatment.

compared to 75 mM NaCl and control. Application of *T. hamatum* further increased the antioxidant enzyme activity at both concentrations of salt stress. This suggests that these enzymes are still supporting the *O. baccatus* to withstand the NaCl stress. Moreover, it is clear that the application of *T. hamatum* alone (in the absence of salt stress) caused a significant increase in activities of CAT, POD, APX, GR, and SOD.

The plants under salinity stress showed maximum accumulation of sodium ions and less uptake of potassium ions (Table 4). The present study also reported that Na/K ratio is directly proportional to elevated NaCl concentrations. Salt stress (150 mM) caused significant decrease of 58.51% and 52.38% in Mg^{2+} and Ca^{2+} as compared to control. The pretreatment of soil with *T. hamatum* was very effective in alleviating the deleterious effects of salinity stress by increasing K/Na ratio in plants. It also mitigates the deficiency of K^+, Mg^{2+}, and Ca^{2+}. Furthermore, the data indicated clearly that *T. hamatum* alone (in the absence of salt stress) could improve nutrient status of *O. baccatus*.

Discussion

Salt stress restricts the plant growth and therefore causes major threat to plant productivity. Seeds of *O. baccatus* subjected to different concentrations of NaCl stress showed decrease in germination at all stress levels. The results obtained in present study are in concurrence with those in wheat (Iqbal & Ashraf 2013) and tomato (Mastouri et al. 2010). Application of *T. hamatum* to the salt stressed seeds showed increased germination in present study. Delay in seed germination under saline conditions was attributed to decreased metabolism of seed reserves due to lower respiration rates (Azam et al. 2005), reduced water potential, and imbalance in plant nutrient uptake, which subsequently affected germination rate and plant growth. Specific ion toxicity also plays an important role in decreasing seed germination under salt stress (Iqbal & Ashraf 2013). *T. hamatum* increases the uptake of nutrients and also absorbs water from deep soil to increase the water potential (Martínez-Medina et al. 2014). This may also reduce the ion toxicity due to NaCl stress.

In the present study the length of shoot and root and dry weight decreased significantly under NaCl stress which may be attributed to increased osmotic stress, deficiency of nutrients, and disturbance of various physiological and biochemical mechanisms (Iqbal & Ashraf 2013; Rasool, Hameed, et al. 2013; Alqarawi, Hashem, & Abd Allah. 2014). Present study indicated that the application of *T. hamatum* efficiently increased growth parameters and could be an effective and easily adaptive strategy to alleviate the negative impact of salt stress. Our results corroborated with the findings of Mastouri et al. (2010) and Rawat et al. (2013) who reported that *Trichoderma* isolates alleviates the adverse effects of salt stress in different plants. *Trichoderma* associated with plants produces plant growth hormones like cytokinins-like molecules, e.g. zeatin and gibberellin GA_3 or GA_3 related (Zhang et al. 2013; Rawat et al. 2013) that have beneficial effects on plant growth under salt stress (Iqbal & Ashraf 2013). Moreover *Trichoderma*

Table 3. Effect of NaCl in presence and absence of *T. hamatum* on TLs and neutral lipids of *O. baccatus* seedlings.

NaCl concentration		Total lipids (mg g^{-1} dry weight)	Neutral lipids (μg g^{-1} fresh weight)				
		TL	DG	TG	S	SE	FAA
0 mM	Without *Trichoderma*	$36.29 \pm 2.97a^{\$}$	$665.41 \pm 9.21a^{\$}$	$867.18 \pm 10.41a^{\$}$	$754.75 \pm 9.75a^{\$}$	$799.47 \pm 9.91a^{\$}$	$219.82 \pm 3.69a^{\$}$
	With *Trichoderma*	$40.18 \pm 3.21a^{£}$	$477.26 \pm 5.57a^{£}$	$974.46 \pm 11.72a^{£}$	$805.83 \pm 10.05a^{£}$	$724.52 \pm 9.69a^{£}$	$194.73 \pm 3.74a^{£}$
75 mM	Without *Trichoderma*	$24.54 \pm 2.18b^{\$}$	$775.61 \pm 9.98b^{\$}$	$711.06 \pm 9.62b^{\$}$	$677.74 \pm 9.57b^{\$}$	$905.38 \pm 11.07b^{\$}$	$348.62 \pm 4.14b^{\$}$
	With *Trichoderma*	$30.49 \pm 2.55b^{£}$	$483.76 \pm 6.07b^{£}$	$822.56 \pm 10.18b^{£}$	$711.82 \pm 9.87b^{£}$	$834.19 \pm 10.62b^{£}$	$235.61 \pm 3.85b^{£}$
150 mM	Without *Trichoderma*	$18.07 \pm 1.69c^{\$}$	$827.19 \pm 10.22c^{\$}$	$625.50 \pm 9.01c^{\$}$	$394.74 \pm 4.77c^{\$}$	$1139.44 \pm 12.75c^{\$}$	$448.19 \pm 5.41c^{\$}$
	With *Trichoderma*	$24.04 \pm 2.01c^{£}$	$565.91 \pm 7.30c^{£}$	$767.85 \pm 9.82c^{£}$	$577.84 \pm 7.45c^{£}$	$942.55 \pm 11.14c^{£}$	$260.43 \pm 3.99c^{£}$

Note: Data presented are the means ± *SE* ($n = 5$). Different letters next to the numbers indicate significant difference ($P < 0.05$) among the treatments. Symbols \$ and £ denote significant change between with and without *T. hamatum* within the same treatment.

association increases root length thus helps the plant to absorb nutrients and water from the soil and thus enhancing the plant's efficiency to counteract salt stress (Arora et al. 1992). In many studies *Trichoderma* spp. could improve the growth of some medicinal plants by producing phytohormones such as gibberellins, auxin, and cytokinins (Sofo et al. 2011; Hanefat et al. 2012; Martínez-Medina et al. 2014; Resende et al. 2014).

Our results related to decrease in photosynthetic pigments of *O. baccatus* plants under NaCl are in agreement with Rasool, Ahmad, et al. (2013) for *Cicer arietinum*; and Alqarawi, Hashem, Abd Allah, Alshahrani, et al. (2014) for *Ephedra alata*. The reduction in the pigment content is attributed to the negative effect of salt stress on chloroplast (Zörb et al. 2009), increased activity of Chl-degrading enzymes such as chlorophyllase (Sultana et al. 1999) hence reduced synthesis of Chl and the instability of the pigment protein complex (Levitt 1980). Decrease in *β*-carotene and zeaxanthin formation is attributed to decrease in carotenoids due to NaCl stress thus increases photoinhibition (Sultana et al. 1999). The results also indicated the efficiency of *T. hamatum* to alleviate the negative effects of NaCl on pigment system and caused significant increase in Chl content in salt-treated plants as well as in control. The mitigation of negative effects of NaCl and improvement in the pigment system by *Trichoderma* spp. has also been reported by Rawat et al. (2011) and Zhang et al. (2013). *T. hamatum* associated plants showed improvement in photosynthetic pigments compared with control. The production of phytohormones such as gibberellins, auxin, and cytokinins plays an important role in stimulation of Chl content (Martínez-Medina et al. 2014; Resende et al. 2014).

Pretreatment with *T. hamatum* maintains the TWC that was decreased due to salt stress in the present study. Similar to our finding, the decrease in TWC caused by salinity stress is also reported by Josine et al. (2011) and Eisa et al. (2012) in *Rosa chinensis* and *Chenopodium quinoa*, respectively. Bae et al. (2009) also reported that *T. hamatum* increased TWC and improved water status, allowing seedlings to tolerate abiotic stress. One more reason may be the mycorrhizal fungi spreads their mycelium deep in to the soil to absorb water for the host plant.

Salinity stress is also having negative effect on MSI. Decrease in MSI content due to salinity stress in the present study is in accordance with Kafi et al. (2011), Rawat et al. (2013), and Rao et al. (2013) who reported that MSI decreases under salinity stress in sorghum, chickpea, and wheat, respectively. The cell injury by salt stress firstly appears in cell membranes (Ashraf & Ali 2008), hence MSI is considered one of the useful parameters to plant resistance against salinity stress (Farooq & Azam 2006). The positive impact of *Trichoderma* spp. on MSI is in accordance with Rawat et al. (2013). Lipid peroxidation expressed as MDA content significantly increased under salt stress. Such accumulation of MDA contents under salt stress is also reported by Hejazi Mehrizi et al. (2012) in *Rosmarinus officinalis*

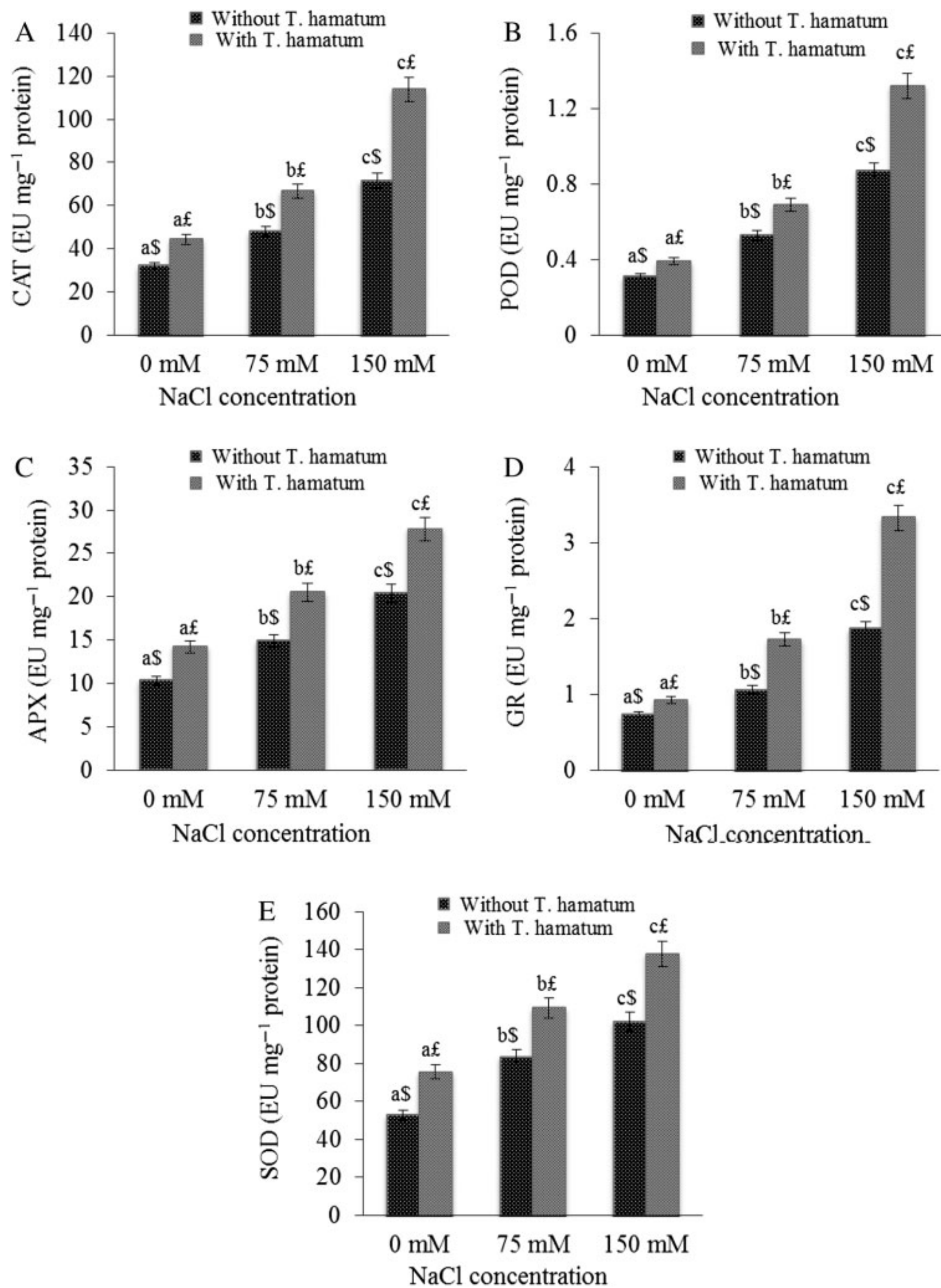

Figure 4. Effect of NaCl in presence and absence of *T. hamatum* on CAT (EU mg^{-1} protein) (A), POD (EU mg^{-1} protein) (B), APX (EU mg^{-1} protein) (C), GR (EU mg^{-1} protein) (D), and SOD (EU mg^{-1} protein) (E), in *O. baccatus* seedlings. Data presented are the means ± *SE* (n = 5). Different letters indicate significant difference ($P < 0.05$) among the treatments. Symbols $ and £ denote significant change between with and without *T. hamatum* within the same treatment.

and Rasool, Ahmad, et al. (2013) in *C. arietinum*. The pretreatment with *T. hamatum* caused significant decrease in MDA content in the present study. Salinity stress causes generation of ROS that attack the biomolecules including plasma membranes thus causing injuries and lipid peroxidation, hence the stability of the membranes is lost resulting in higher leakage of solutes. H_2O_2 generated through oxidative stress is responsible for higher leakage of solutes (Dionisio-Sese & Tobita 1998). The use of *T. hamatum* enhanced the antioxidant enzymes that quenches these ROS so the plasma membranes are affected least. *T. hamatum*-treated plants showed decrease in MDA content, which could be due to the expression of stress-related proteins like glutathione S-transferase, glutathione dependent formaldehyde dehydrogenase, and POD (Alqarawi, Hashem, & Abd Allah 2014).

These proteins scavenges ROS and protects the cell from damage. Reduced contents of MDA is an important indicator of stress tolerance as reported in mulberry (Ahmad, Jaleel, & Sharma 2010), chickpea (Rasool, Ahmad, et al. 2013), and mustard (Ahmad et al. 2012).

Phenolic compounds are important class of plant secondary metabolites, produced typically to enhance reproduction and give protection to plants against biotic and abiotic stresses (Pohjala & Tammela 2012). Plants

Table 4. Effect of NaCl in presence and absence of *T. hamatum* on elements accumulation of *O. baccatus* seedlings.

NaCl concentration	Elements accumulation (mg g^{-1} dry wt)									
	Without *Trichoderma*					With *Trichoderma*				
	Na^+	K^+	Na/K	Mg^{2+}	Ca^{2+}	Na^+	K^+	Na/K	Mg^{2+}	Ca^{2+}
0 mM	$7.97 \pm 0.85^{a\$}$	$70.13 \pm 4.14^{a\$}$	$0.11 \pm 0.02^{a\$}$	$4.17 \pm 0.37^{a\$}$	$4.83 \pm 0.41^{a\$}$	$5.70 \pm 0.52^{a£}$	$80.73 \pm 5.25^{a£}$	$0.07 \pm 0.003^{a£}$	$5.07 \pm 0.49^{a£}$	$5.43 \pm 0.51^{a£}$
75 mM	$16.37 \pm 1.21^{b\$}$	$56.57 \pm 3.07^{b\$}$	$0.29 \pm 0.05^{b\$}$	$2.73 \pm 0.29^{b\$}$	$3.53 \pm 0.32^{b\$}$	$8.90 \pm 0.92^{b£}$	$70.63 \pm 4.42^{b£}$	$0.12 \pm 0.009^{b£}$	$3.80 \pm 0.37^{b£}$	$4.33 \pm 0.42^{b£}$
150 mM	$25.77 \pm 2.25^{c\$}$	$42.19 \pm 2.55^{c\$}$	$0.61 \pm 0.12^{c\$}$	$1.73 \pm 0.19^{c\$}$	$2.30 \pm 0.23^{c\$}$	$11.47 \pm 1.01^{c£}$	$56.53 \pm 3.05^{c£}$	$0.20 \pm 0.08^{c£}$	$2.73 \pm 0.28^{c£}$	$3.77 \pm 0.34^{c£}$

Note: Data presented are the means ± *SE* (n = 5). Different letters next to the numbers indicate significant difference ($P < 0.05$) among the treatments. Symbols $ and £ denote significant change between with and without *T. hamatum* within the same treatment.

respond to abiotic stresses by synthesizing and accumulating phytoalexins, flavonoids, terpenoids, phenolic derivatives, aglycones, etc. The synthesis of phenolic compounds increased resistance against salt stress as reported by Wehner et al. (2010). Such increase in phenolic compounds reported due to NaCl in the present study are in agreement with the reports of Mehr et al. (2012) and Petridis et al. (2012) in *Anethum graveolens* and *Olea europaea*, respectively. The application of *T. hamatum* caused further increase in phenolic content as compared to control and salt-treated plants alone. Plants are stimulated by *Trichoderma* strains to manufacture some novel compounds, which are defensive in nature. The phenolic compounds possess antifungal, antibacterial, and antiviral as well as antioxidant properties that quenches the ROS generated through oxidative stress.

In the present study TL, TG, and S decreased significantly, however DG, SE, and FAA increased with increasing salt concentrations. Such inhibitory effect of salinity on lipid content (TL, TG, and S) might be attributed to destructive and negative effects of salt on chloroplasts and membrane permeability (Ahmad et al. 2012; Naz & Bano 2013). Sterols play an important role in maintenance of membrane permeability and fluidity (Hosono 1992), but are affected with abiotic stress including salinity (Kaya et al. 2001). Salinity induced oxidative stress resulting in enhanced membrane damage and electrolyte leakage (Ahmad, Sarwat, et al. 2008; Ahmad, Umar, & Sharma 2010; Rasool, Ahmad, et al. 2013). The scavenging nature of the antioxidants is one of the reasons to maintain the integrity of the membrane lipids under salt stress. Another reason might be the efficient absorption of nutrients through *T. hamatum* that maintains the growth and functioning of organelles related to photosynthesis. Through these mechanisms we may conclude that application of *T. hamatum* alleviates the deleterious effects of salinity on total and neutral lipids.

Salt stress increased the activity of antioxidant enzymes like CAT, POD, APX, GR, and SOD and the activities are directly proportional with increasing concentrations of salt. Similar results have also been observed in *Sesamum indicum* (Koca et al. 2007), *Pisum sativum* (Ahmad, John, et al. 2008), *Vicia faba* (Azooz et al. 2011), *Brassica juncea* (Ahmad et al. 2012), and *C. arietinum* (Rasool, Ahmad, et al. 2013). The application of *T. hamatum* further increased the antioxidant enzymes hence decreased the effects of salt stress in the present study. The potential of *T. hamatum* in alleviating the salt stress may be through an auxin-dependent mechanism (Zhang et al. 2013), which may depend on plant producing signals (Athar & Ashraf 2009). Iqbal and Ashraf (2013) also reported the pretreatment of wheat with auxin showed consistent promotive and beneficial effects on plant metabolism under salt stress. Increase in antioxidant enzymes denotes that more and more ROS must be scavenged and the effects of these ROS are minimized. SOD catalyzes the dismutation of $O_2^{\cdot-}$ to H_2O_2 and O_2. It protects the cells by removing $O_2^{\cdot-}$ that increases the risk of $OH^{\cdot}$ formation. CAT plays an important role in the removal of H_2O_2 from the

different organelles of the cell. One molecule of CAT can convert approximately six million molecules of H_2O_2 to H_2O and O_2 each minute. APX and POD is also involved in scavenging of H_2O_2 and plays an important role in stress tolerance. GR is a flavoprotein oxidoreductase and is a potential enzyme of the ascorbate-glutathione system.

Membrane proteins play a significant role in selective distribution of ions within the plant or cell (Ashraf & Harris 2004). In our study, salinity stress increased shoot Na^+ content however significant decrease in K^+, Mg^{2+}, and Ca^{2+} were observed. Our results are in accordance with the findings of Pandolfi et al. (2012) in pea, Kanwal et al. (2013) in wheat, and Iqbal and Ashraf (2013) in wheat. *T. hamatum* was very effective in increasing the accumulation of Ca^{2+} and K^+ instead of Na^+ content in the present study. The tolerance to salinity has been related with the accumulation of Ca^{2+} and K^+ in cereals (Iqbal & Ashraf 2013). The production of exogenous plant growth regulators by *T. hamatum* (Zhang et al. 2013) could explain the alleviation of salt stress via the biological impact of α-naphthaleneacetic acid, indole-3-acetic acid, and indole-3-butyric acid as reported by Iqbal and Ashraf (2013).

In conclusion, although the mechanisms explaining how *Trichoderma* spp. stimulates plant growth is not fully understood, the increase in total and neutral lipids and antioxidants by *T. hamatum* treatment decreases the negative effect of NaCl. Scavenging of ROS by antioxidants is the main defense mechanism involved in the sustainability of *O. baccatus* plants. The beneficial impact of *Trichoderma* spp. to plants provides new strategies to mitigate salt stress and also develop new ways to enhance the tolerance capacity against salinity.

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