Agriculture (Poľnohospodárstvo), 60, 2014 (1): 10−21
DOI: 10.2478/agri-2014-0002

EFFECTS OF PLANT GROWTH-PROMOTING RHIZOBACTERIUM (PGPR) AND ARBUSCULAR MYCORRHIZAL FUNGUS (AMF) ON ANTIOXIDANT ENZYME ACTIVITIES IN SALT-STRESSED BEAN (*PHASEOLUS VULGARIS* L.)

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YOUNESI, O. – MORADI, A.: Effects of plant growth-promoting rhizobacterium (PGPR) and arbuscular mycorrhizal fungus (AMF) on antioxidant enzyme activities in salt-stressed bean (*Phaseolus vulgaris* L.). Agriculture (Poľnohospodárstvo), vol. *60*, 2014, no. 1, pp. 11–21.

Plant Growth-Promoting Rhizobacterium (PGPR) represents a wide variety of soil bacteria that, when grown in association with a host plant, result in stimulation of growth of their host. The aim of this study was to investigate the influence of inoculation with a PGPR, *Pseudomonas fluorescence*, alone or in combination with an arbuscular mycorrhizal fungus, *Glomus mosseae* (Nicol. & Gerd.), on antioxidant enzyme activities (catalase (CAT) and peroxidase (POX)), phosphatase activity, solutes accumulation, growth and minerals nutrient uptake in shoots of bean (*Phaseolus vulgaris* L.) affected by three levels of salt stress. Salinity decreased bean growth, regardless of the biological treatment and the salt stress level. The plants inoculated with *P. fluorescence* had significantly greater shoot biomass than the control plants at all salinity levels, whereas the mycorrhizal inoculation treatments were only effective in increasing shoot biomass at a low salinity level. The plants inoculated with P. fluorescence presented higher concentrations of shoots' K^+ and lower concentrations of shoots' Na^+ under high salt conditions. Salt stress increased shoots' proline concentration, particularly in plants inoculated with the PGPR. Increasing salinity stress raised significantly the antioxidant enzyme activities, including those of total POX and CAT, of bean shoots compared with their corresponding non-stressed plants. The PGPR strain induced a higher increase in these antioxidant enzymes in response to severe salinity. Inoculation with selected PGPR could serve as a useful tool for alleviating salinity stress in salt-sensitive plants.

Key words: arbuscular mycorrhizal fungi, bean, plant growth-promoting rhizobacterium, salinity

Saline soils and saline irrigation constitute a serious production problem for vegetable crops. Saline conditions are known to suppress plant growth, particularly in arid and semiarid areas (Parida & Das 2005). Common bean (*Phaseolus vulgaris* L.) in a symbiotic association with *Rhizobium leguminosarum* bv. *phaseoli* can fix atmospheric nitrogen and represents one of the world's most important sources of dietary protein (Hantngston *et al.* 1986). Common bean growth is sensitive to salinity (Hantngston *et al.* 1986). Salt induces osmotic stress by limiting absorption of water from soil and ionic stress resulting from high concentrations of potentially toxic salt ions within plant cells. Salt stress is also linked to an oxidative stress as a consequence of the generation of reactive oxygen species, such as superoxide ion, hydrogen peroxide and hydroxyl radicals, which are detrimental to plant survival under salt stress. Salt-stressed plants display a complex oxidative defence strategy, with catalase (CAT) and peroxidise (POX) enzymes being involved in scavenging of the hydrogen peroxide generated in

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response to oxidative stress. Saline stress is also known to affect many physiological activities related to the accumulation of ions and osmolytes such as proline (Lee *et al.* 2008). The accumulation of these compounds plays a major role in the process of osmotic adjustment, limiting water loss and ion toxicity. Biochemical alterations in plants due to salt stress may affect the nutritional balance and consequently growth and development.

The use of plant growth-promoting bacteria (PGPR) and symbiotic microorganisms, especially arbuscular mycorrhizal (AM) fungi, may prove useful in developing strategies to facilitate plant growth in saline soils. For example, Glick *et al*. (1998) showed that under different stresses using PGPR such as *Pseudomonas fluorescence* can alleviate the adverse effects of stress on plant growth. It has been indicated that some PGPR are able to produce polysaccharide products, binding Na^+ in the root zone and hence alleviating the effects of salt stress on plant and enhancing microbial growth and activities (Tank & Saraf 2010). Many studies have demonstrated that inoculation with AM fungi improves growth of plants under salt stress (Sharifi *et al*. 2007). The improved growth of AM plants has been attributed to enhanced nutrient uptake, particularly of N and P and subsequent increased growth (Jeffries *et al*. 2003). However, in some cases, plant salt tolerance was not related to P concentration (Ruiz-Lozano & Azcón 2000). Thus, it has been proposed that salt-tolerance mechanisms, such as enhanced osmotic adjustment and reduced oxidative damage or improved nutritional status, can explain the contribution of AM symbioses to the salinity resistance of host plants (Augé 2001).

In this study, we hypothesise that inoculation with *P. fluorescence* and *Glomus mosseae*, alone or in combination, can induce salinity tolerance to bean and such tolerance is correlated with changes in antioxidant enzyme activity (CAT and POX activities), accumulation of solutes (proline and soluble sugars) and mineral nutrient uptake such as P.

MATERIALS AND METHODS

The used soil for pots was collected from an uncultivated site located in Qom province, Iran. The basic soil properties were as follows: organic matter content 1.08%, total N 0.062%, total K 740.8 mg/kg, total P 10.90 mg/kg, available P ($NaHCO_3$-extractable) 2.78 mg/kg, water-soluble K 13.43 mg/kg and electrical conductivity 8.1 dS/m.

The plant used in the experiment was bean (*Phaseolus vulgaris* L.). Seeds of bean were grown for 10 days in peat substrate under nursery conditions, without any fertilisation treatment.

Microorganisms

The mycorrhizal fungi used were obtained from the Institute of Soil and Water Research, Karaj, Iran. *G. mosseae* species was multiplied in pots using a mixture of sterile sepiolite/vermiculite (1:1, v:v) as growing substrate and *Sorghum* sp. as host plant. Trap cultures were maintained under greenhouse controlled conditions for 4 months. AM fungal inoculum consisted of a mixture of rhizospheric soil from the trap cultures containing spores, hyphae and mycorrhizal root fragments and was stored in polyethylene bags at 5°C. The inoculum was subjected to a most probable number test (Sieverding 1991) to determine potential infectivity and equalise application doses. Sources of inoculum had a potential infectivity of about 35 infective propagules/g inoculum.

P. fluorescence was isolated from field soil located in Qom province, Iran, and was grown in liquid Tryptic Soy Broth medium at 30°C. The bacterial culture was centrifuged at 4000 rpm for 5 min at 2°C and the sediment was re-suspended in sterilised tap water. The bacterial suspension contained 10^9 colony forming units/ml.

Microbial inoculation and salt stress treatments

The experiment was arranged as a factorial in completely randomised design with two factors and 3-fold replications. The first factor had four levels: control soil, soil inoculated with the AM fungus (AMF) *G. mosseae*, soil inoculated with the bacteria *P. fluorescence*, and a combination of soil inoculated with the bacteria *P. fluorescence* and with *G. mosseae*. The second factor had three levels of salt stress: non-salt stress, moderate salt stress and severe salt stress.

An amount of 700 g of substrate consisting of soil and vermiculite at a ratio of 2:1 (v:v) sterilised by autoclaving at 105°C for 60 min in three consec-

utive days was placed in 1-l pots. Bean seedlings were transplanted to the pots (three per pot). The AM inoculum was mixed with the potting substrate at a rate of 5% (v/v). The same amount of the autoclaved inoculum was added to non-AM plants, supplemented with a filtrate (Whatman no. 1 paper) of the culture, to provide the microbial populations accompanying the mycorrhizal fungi. *P. fluorescence* was inoculated two times during the growth period. The dose of inoculum applied corresponded to 10^{10} colony forming units per plant. Two concentrations (2 and 4 g NaCl/kg soil) of saline solution were applied to the saline pots. To avoid osmotic shock, the NaCl concentration was gradually increased for four consecutive days until the desired concentration was attained. A plastic bag was put underneath each pot to collect excess water due to drainage. This water was reapplied to the respective pot. All seedlings were grown for 4 weeks without any fertiliser treatment. At the end of experiment, the electrical conductivity of the non-saline pots and the pots cultivated under medium and high salinity levels was about 0.340, 0.620 and 0.920 dS/m, respectively. The experiment was conducted in a greenhouse (average temperature ranged from 20 to 25°C and the relative humidity from 70 to 80%). Midday photosynthetically active radiation averaged 260 E/m^2/s.

Plant analyses

One month after planting, two plants per treatment were harvested. The roots were washed free from soil under a stream of cold tap water and fresh and dry (105°C, 5 h) weights of shoots and roots were recorded. Plant tissues were ground before chemical analysis. The foliar contents of phosphorus were determined, after digestion in nitric–perchloric acid (5:3) for 6 h, by colorimetry (Murphy & Riley 1962) and the plant K was estimated by flame photometry. The N concentration was determined by colorimetry after the Kjeldahl digestion.

Roots were sub-sampled in three 2-cm cross-sections of the upper, middle and lower root system. To assess colonisation, roots were cleared with 10% KOH and stained with 0.05% trypan blue (Phillips & Hayman 1970). The percentage of root length colonised by AM fungi was calculated by the gridline intersect method (Giovannetti & Mosse 1980). Positive counts for AM colonisation included the presence of vesicles, arbuscules or typical mycelium within the roots.

Acid phosphatase activity was determined using p-nitrophenyl phosphate disodium (0.115 M) as substrate. An amount of 2 ml of 0.5M sodium acetate buffer at pH 5.5 using acetic acid and 0.5 ml of substrate were added to 100 mg of fresh root tissue and incubated at 37°C for 90 min. The reaction was stopped by cooling at 2°C for 30 min. Then, 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.5M NaOH were added, and the mixture was centrifuged at 4,000 rpm for 5 min. The p-nitrophenol formed was determined by spectrophotometry at 398 nm (Tabatabai & Bremner 1969). Controls were made in the same way, although the substrate was added after incubation.

Proline and total soluble sugars

Free proline and total soluble sugars were extracted from 1 g of fresh shoots (Bligh & Dyer 1959). The methanolic phase was used for the quantification of both substances. Proline was estimated by spectrophotometric analysis at 515 nm of the ninhydrin reaction, according to Bates *et al*. (1973). Soluble sugars were analysed by 0.1 ml of the alcoholic extract reacting with 3 ml freshly prepared anthrone (200 mg anthrone + 100 ml 72% (w:w) H_2SO_4) and placed in a boiling water bath for 10 min according to Irigoyen *et al*. (1992). After cooling, the absorbance at 620 nm was determined in a spectrophotometer. The calibration curve was made using glucose in the range of 20–400 μg/ml.

Measurement of CAT and POX activity

All operations were performed at 4°C. Shoots (2 g) were homogenised with a mortar and pestle in 4 ml of ice-cold 50 mM Tris-acetate buffer pH 6.0, containing 0.1 mM ethylenediaminete-traacetic acid, 5 m Mcysteine, 2% (w/v) polyvinylpyrrolidone, 0.1 mM phenylmethylsulphonyl fluoride and 0.2% (v/v) Triton X-100. The homogenate was centrifuged at 14,000 × g for 20 min and the supernatant fraction was filtered through Sephadex G-25 columns (NAP, Pharmacia Biotech AB, Uppsala, Sweden), equilibrated with the same buffer used for the homogenisation.

CAT activity was determined at 25°C according to Aebi (1984). The reaction mixture contained 10 mM H_2O_2 in a 50 mM phosphate buffer pH 7.0, and 100 μl of leaf enzyme extract in a total volume

of 3 ml. CAT activity was estimated by decrease in absorbance of H_2O_2 at 240 nm. Total POX was determined in assays containing 50 mm Tris-acetate buffer (pH 5.0) and 0.5 mm H_2O_2, using 1.0 mm 4-methoxynaphtol as electron donor (E_{595} = 21,600 m/cm; Ros-Barceló 1998). The reaction was initiated by adding enzyme. Controls were carried out in the absence of H_2O_2.

Statistical analysis

Data were log transformed to achieve normality. Microbial inoculation, salinity level and their interaction effects on measured variables were tested by a two-way analysis of variance and comparisons among means were made using Dunkan's test calculated at $P < 0.05$. Statistical procedures were carried out with the software package SPSS 10.0 for Windows.

RESULTS

Growth, shoot water content, nutrient assimilation and mycorrhizal colonisation

The data revealed significant ($P \leq 0.01$) effect of salinity and microbial inoculation on shoot dry weight of bean (Table 1). Interactions among salinity × microbial treatments were also significant ($P \leq 0.05$). Salinity decreased the dry weight of the shoots and roots for all bean plants (Table 2). The plants inoculated with *P. fluorescence* had significantly greater shoot biomass than the control plants at both salinity levels, whereas the mycorrhizal inoculation treatments only were effective in increasing shoot biomass at the medium salinity level. Under non-saline conditions, shoot dry weight of bean was increased by the inoculation with *P. fluorescence*, while no significant effect was found with the mycorrhizal inoculation treatments (Table 2). The mycorrhizal and bacterial inoculation produced similar increases in plant growth. Neither of the microbial treatments had an effect on the root biomass (Table 1).

Statistical analysis of the data showed significant ($P \leq 0.01$) effect of salinity and microbial treatments on shoot water content of bean (Table 1). Leaf hydration was changed by the salt and microbial treatments. The bacterial inoculation increased significantly ($P \leq 0.01$) the shoot water content in the non-salinised plants. At the highest salinity level, the water content was higher in shoots of plants treated with *P. fluorescence* or *G. mosseae* (Table 2).

In non-salinised plants, mycorrhizal inoculation treatment produced active colonisation in root systems of bean seedlings. The level of colonisation in roots of mycorrhizal plants was not affected by bacterial inoculation but decreased significantly ($P \leq 0.05$) with increasing NaCl concentration (Table 1). The results indicate that different levels of salinity had inhibitory effects on mycorrhizal colonisation. High mycorrhizal colonisation was observed at the control condition (non-salt stress) (Table 2).

The assayed salinity and microbial inoculations significantly affected ($P \leq 0.01$) the foliar N and P concentrations of bean plants (Table 1). Interactions among salinity × microbial treatments were also significant ($P \leq 0.05$). A 22.38% and 47.55% reduction in foliar N concentration was caused by medium and severe salinity levels as compared with the control (non-salt stress), respectively (Table 3). The mycorrhizal inoculation treatment in combination with *P. fluorescence* was the most effective treatment for increasing the foliar N concentration. Also, the highest foliar concentrations of phosphorus were seen in the plants inoculated with *G. mosseae*, alone or in combination with *P. fluorescence*. At the medium salinity level, *G. mosseae*-colonised plants showed the highest concentration of foliar P.

Data regarding Na, K and Ca concentrations showed significant effect of salinity ($P \leq 0.01$) and microbial ($P \leq 0.05$) treatments on Na, K and Ca contents of beans (Table 1). Interactions among salinity × microbial treatments were also significant ($P \leq 0.05$). Salinity increased the foliar, Na concentration and decreased the foliar K and Ca concentrations in bean plants (Table 3). The increased Na due to salt stress was higher in the control plants than in the plants treated with *P. fluorescence* or AM fungi. *P. fluorescence*- and *G. mosseae*-colonised plants presented the highest concentrations of foliar K and lowest concentrations of foliar Na under high salt-stress conditions.

Table 1

Effect of salinity and microbial inoculation on shoot and root dry weight, shoot water content, colonisation, nutrient contents, acid phosphatase, proline, total soluble sugars, catalase (CAT) and peroxidase (POX) activity of salt-stressed bean plant

Source of variability	Shoot dry weight	Root dry weight	Shoot water content	Colonisation	Nutrient contents					Phosphatase	Proline	Total soluble sugars	CAT	POX
					Na	K	Ca	N	P					
Salinity	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Microbial inoculation	++	ns	++	ns	++	+	+	++	++	ns	+	++	+	+
Salinity × microbial inoculation	+	ns	+	ns	+	+	+	+	+	ns	+	+	+	+

+, ++ and ns indicate the level of significance at $P \leq 0.05$, 0.01 and non-significant effect, respectively

Phosphatase activity, proline and solute accumulation

Statistical analysis of the data showed significant ($P \leq 0.01$) effect of salinity on phosphatase activity of bean (Table 1). But neither bacterial nor mycorrhizal inoculation had any significant ($P \geq 0.05$) effect on the phosphatase activity of non-salinised plants (Table 1). Phosphatase activity in roots of the *P. fluorescence*-inoculated and control plants was increased significantly at the medium salinity level (Table 4).

Proline concentrations increased significantly ($P \leq 0.01$) as a consequence of salinity (Table 1). Statistical analyses of the data revealed that no significant ($P \geq 0.05$) differences among the different treatments were found for proline accumulation in bean shoots under non-saline conditions (Table 4). Proline accumulation was considerably less for mycorrhizal plants than non-mycorrhizal plants at the medium salinity level (Table 4). In the case of *P. fluorescence*-inoculated plants, the increase in proline concentration was higher than control plants at the highest salinity level. At medium and high salinity levels, plants co-inoculated with *Pseudomonas* and AMF showed 11% and 21% greater proline than the control plant, respectively.

Data revealed significant ($P \leq 0.01$) effect of salinity and microbial treatments on soluble sugar accumulation of bean (Table 1). Interactions between salinity × microbial treatments were also significant ($P \leq 0.05$). Salt stress decreased sugar accumulation in all plants (Table 4). Under high salt stress conditions, the control plants accumulated the lowest concentration of total soluble sugars. Under non-saline conditions, the inoculation with the microbial inoculation caused differences in the total soluble sugar accumulation when compared with the control plants. At the medium and high salinity levels, only plants co-inoculated with *Pseudomonas* and AMF had significant effect on soluble sugar accumulation and showed 63.67% and 95.57% greater proline as compared with the control plants.

Antioxidant enzyme activities

Statistical analysis of the data showed significant ($P \leq 0.01$) effect of salinity and microbial treatments on antioxidant enzyme activities of bean (Table 1). Interactions among salinity × microbial treatments

T a b l e 2

Effect of inoculation with *Pseudomonas fluorescence* and *Glomus mosseae* on shoot and root dry biomass, shoot water content and colonisation of bean seedlings grown at three levels of salinity

Treatment	Shoot dry biomass [g dw]	Root dry biomass [g dw]	Shoot water content [%]	Colonisation [%]
Without NaCl				
Control	1.23	0.65	64.00	0.00
P. fluorescence	1.60	0.65	82.50	0.00
G. mosseae	1.40	0.72	80.00	65.60
P. fluorescence + *G. mosseae*	1.67	0.68	84.40	66.20
2 g NaCl/kg soil				
Control	0.80	0.52	60.00	0.00
P. fluorescence	1.23	0.55	81.10	0.00
G. mosseae	1.15	0.60	78.40	48.20
P. fluorescence + *G. mosseae*	1.35	0.61	83.80	48.50
4 g NaCl/kg soil				
Control	0.63	0.38	58.70	0.00
P. fluorescence	1.17	0.45	78.30	0.00
G. mosseae	1.05	0.47	63.50	35.00
P. fluorescence + *G. mosseae*	1.30	0.45	80.20	36.30
$LSD_{0.05}$	0.14	0.09	5.26	9.34

T a b l e 3

Effect of inoculation with *Pseudomonas fluorescence* and *Glomus mosseae* on potassium, sodium, calcium and phosphorus content of bean seedlings grown at three levels of salinity

Treatment	N [mg/g]	P [mg/g]	K [mg/g]	Na [mg/g]	Ca [mg/kg]
Without NaCl					
Control	14.30	1.80	41.30	5.33	3.87
P. fluorescence	17.60	2.85	46.60	3.27	4.30
G. mosseae	16.50	2.90	51.34	5.64	5.30
P. fluorescence + *G. mosseae*	18.00	3.50	56.28	3.12	5.85
2 g NaCl/kg soil					
Control	11.10	1.10	36.23	7.35	2.63
P. fluorescence	16.90	2.55	41.50	4.87	3.80
G. mosseae	16.20	3.23	45.67	6.80	4.90
P. fluorescence + *G. mosseae*	17.00	2.80	48.81	4.38	5.13
4 g NaCl/kg soil					
Control	7.50	0.90	24.20	9.16	1.88
P. fluorescence	12.30	1.38	36.23	5.65	3.17
G. mosseae	10.20	2.10	34.47	7.55	3.78
P. fluorescence + *G. mosseae*	14.70	2.53	38.43	5.30	4.26
$LSD_{0.05}$	2.40	0.98	2.16	1.72	1.04

were also significant ($P \leq 0.05$). Salinity notably increased the specific total POX and CAT activities in the non-inoculated and inoculated plants (Table 5). The inoculation with *P. fluorescence* and AMF increased CAT and POX activities in shoots of non-salinised bean plants, as shown in Table 5. The total POX activity of plants treated with *P. fluorescence* in combination with the AM fungi was increased by all salt levels. At the high salinity level, POX and CAT activities were, on average, 3- and 1.65-fold higher than the control plant, respectively.

DISCUSSION

Salinity adversely affected the growth of bean, regardless of the biological treatment and the salt-stress level. However, when the plants were inoculated with an AMF or a PGPR, the extent of growth suppression was decreased and these treated plants had greater dry weights than untreated plants. Our results show that the AM fungi assayed stimulated significantly the growth of bean plants only under moderately saline conditions, while inoculation with *P. fluorescence* increased plant biomass even under severely saline conditions. On the other hand, the effect of dual inoculation with PGPR and AM fungi on shoot biomass of bean was similar to that of PGPR colonisation alone. These observations clearly suggest that the selected microorganisms can differentially alleviate some of the deleterious effects of salt stress.

Increased salt concentration decreases the osmotic potential of a growth medium, thus reducing the water availability. The use of water content is a good indicator of water stress (Mayak *et al*. 2004). In the control plants, leaf water content decreased as the external salt concentration increased. However, plants inoculated with *P. fluorescence* were more hydrated than the control plants under saline conditions. These results demonstrated that the bacterial treatment influenced the extent of water stress and that *P. fluorescence* efficiently protected the host plants against the detrimental effects of salt. Greater hydration induced by the PGPR strain might be attributable to increased water use efficiency and/or

Table 4

Effect of inoculation with *Pseudomonas fluorescence* and *Glomus mosseae* on acid phosphatase, proline and total soluble sugars of bean seedlings grown at three levels of salinity

Treatment	Acid phosphatase [µmol PNP* g/h]	Proline [µmol/g FW]	Total soluble sugars [mg/g FW]
Without NaCl			
Control	28	5.0	30.5
P. fluorescence	30	5.5	40.0
G. mosseae	30	5.1	43.3
P. fluorescence + *G. mosseae*	31	5.5	47.6
2 g NaCl/kg soil			
Control	34	9.6	22.3
P. fluorescence	46	12.8	28.2
G. mosseae	42	6.7	24.4
P. fluorescence + *G. mosseae*	39	10.7	36.5
4 g NaCl/kg soil			
Control	35	11.8	11.3
P. fluorescence	44	14.8	16.2
G. mosseae	43	12.6	13.0
P. fluorescence + *G. mosseae*	53	14.4	22.1
$LSD_{0.05}$	12	3.16	8.87

*p-nitrophenol

enzymatic lowering of plant ethylene concentrations thereby decreasing the ethylene inhibition of seedling root biomass (Saravanakumar & Samiyappan 2007). The root biomass of control plants was decreased as a consequence of salinity, while those of inoculated plants was not decreased at the medium salinity level and suffered a lesser reduction at the highest level.

The increased shoot biomass of seedlings inoculated with AM fungi could be partly related to the increase in water uptake that a mycorrhizal fungus provides under saline conditions (Ruiz-Lozano & Azcón 1996): the higher shoot water content in plants inoculated with *G. mosseae* supports this possibility. Nutritional mechanisms may have played a role in the differential modulation of host water status by the different AMF species. Improved nutritional status can lead to specific stimulation of photosynthetic capacity over stomatal conductance, which, in turn, influences positively the plant water status (Querejeta *et al*. 2003). In particular, the protection of mycorrhizal plants against salt stress was related to the effect that the added endophytes had on increasing P uptake. The concentration of foliar P in mycorrhizal plants was particularly increased under moderately saline conditions. Enhanced P nutrition is considered as one of the major mechanisms by which AMF can improve the water status of their host plants (Augé 2001). Non-nutritional mechanisms by which AMF can also improve the water status of their host plants include hormonal signalling, osmotic adjustment, changes in root hydraulic conductivity, direct contributions of extraradical hyphae to water uptake and changes in the moisture characteristics of rhizosphere soil (Augé 2001). It has been hypothesised that mycorrhizae can alter the morphology of the root system, yielding a more extensive absorbing area, which may be considered a mechanism of salinity tolerance (Ibrahim *et al*. 1990). Likewise, mycorrhizae are known to increase the xylem pressure potential by increasing root biomass and therefore improving water uptake (Augé 2001). It is worth noting that the root biomass of *G. mosseae*-colonised plants was about double that of the control plants under moderately saline conditions.

In many plants, salt stress has been shown to affect carbohydrate partitioning and metabolism,

T a b l e 5

Effect of inoculation with *Pseudomonas fluorescence* and *Glomus mosseae* on peroxidase (POX) and catalase (CAT) activities of bean seedlings grown at three levels of salinity

Treatment	POX [μmol/min/g FW]	CAT [μmol/min/g FW]
Without NaCl		
Control	136	31
P. fluorescence	387	35
G. mosseae	300	32
P. fluorescence + *G. mosseae*	410	36
2 g NaCl/kg soil		
Control	143	35
P. fluorescence	420	41
G. mosseae	385	38
P. fluorescence + *G. mosseae*	462	44
4 g NaCl/kg soil		
Control	152	38
P. fluorescence	438	51
G. mosseae	418	46
P. fluorescence + *G. mosseae*	480	63
$LSD_{0.05}$	64	18

leading to the synthesis of new compounds (Sharma *et al*. 1990). In particular, various solutes have been shown to accumulate during salinity. Their accumulation might be of importance for the adjustment of the cellular water potential under conditions of reduced water availability, and they can act as scavengers of reactive oxygen species. In plants exposed to salinity, the total non-structural carbohydrate content in the shoots was reduced significantly compared with plants not exposed to salinity. This could indicate that salinity induces a preferential partitioning of carbohydrates to the roots, as demonstrated by Schellenbaum *et al*. (1998). The decrease in total soluble carbohydrates due to salinity could be related also to limited carbohydrate availability, as a consequence of a decline in photosynthesis (Goicoechea *et al*. 2005). The control plants presented the highest reductions in shoot total soluble sugars throughout the salt-stress experiment, which could indicate that these plants were less salt tolerant. The total soluble carbohydrates of plants inoculated with *P. fluorescence* and/or the AM fungi were not affected under moderate salinity.

In contrast to soluble carbohydrates, moderate and severe salinity increased shoot proline accumulation in non-mycorrhizal plants. A high level of proline enables the plants to maintain an osmotic balance when growing under low water potentials. It is reported that proline protects higher plants against salt/osmotic stresses, not only by adjusting osmotic pressure, but also by stabilising many functional units such as complex II electron transport, membranes and proteins and enzymes such as RUBISCO (Mäkelä *et al*. 2000). However, no changes in proline concentrations were observed in plants inoculated with the selected AM fungi in response to moderate salinity. This could indicate that moderate salinity affected to a lesser extent the mycorrhizal plants, so they accumulated less proline. The concentrations of proline are always not high enough to adjust the osmotic potential in some plants under stress (Hoque *et al*. 2007). In fact, the inhibition of growth of bean was higher in non-inoculated plants than in inoculated plants in spite of the fact that the levels of proline were similar for all plants under severe salinity. Thus, our data suggest that the accumulation of proline itself cannot confer salt tolerance in bean plants.

Salinity dominated by Na^+ and Cl^- not only reduces Ca^{2+} and K^+ availability, but also reduces Ca^{2+} and K^+ mobility and transport to the growing parts of plants, affecting the quality of both vegetative and reproductive organs. Moreover, many studies have shown that high concentrations of NaCl in the soil solution may increase the ratios of Na^+/Ca^{2+} and Na^+/K^+ in plants, which would then be more susceptible to osmotic and specific ion injury as well as to nutritional disorders that result in reduced yield and quality (Sivritepe *et al*. 2003). Salt tolerance in plants has been related to their ability to decrease leaf osmotic potential, which has been linked to Na^+ and/or Cl^- ion exclusion mechanisms or to the retention of salt ions in roots, preventing the accumulation of Na^+ and/or Cl^- in the shoot. The results of this study show that salinity caused an increase in Na concentration and a decrease in K^+ and Ca^{2+} regardless of the microbiological treatment. However, both microbiological treatments reduced the Na^+ uptake of plants and/or increased the K^+ uptake, compared with the control plants under salt stress, thus increasing the K^+:Na^+ ratio. Previous studies indicate that an increase in concentration of K^+ in plants under salt stress could ameliorate the deleterious effects of salinity on growth and yield (Giri *et al*. 2007). Potassium plays a key role in plant water stress tolerance and has been found to be the cationic solute responsible for stomatal movements in response to changes in bulk leaf water status (Caravaca *et al*. 2004). This means that the *P. fluorescence* strain and AM fungi could alleviate the effects of salinity stress in bean. Reduced Na concentration in bean plants exposed to salinity, due to AM inoculation, may have helped the plants prevent accumulation of cellular Na to a toxic concentration. There are several reports of lower Na^+ concentrations in AM plants, compared with non-AM plants, under salinity (Giri & Mukerji 2004; Ashraf *et al*. 2004; Sharifi *et al*. 2007). In the case of *P. fluorescence*, this PGPR strain can produce exopolysaccharides that bind cations, including Na^+ (Kohler *et al*. 2006), thus decreasing the content of Na^+ available for plant uptake. We have shown the beneficial effect of this PGPR strain on soil aggregate stabilisation (Kohler *et al*. 2006). Previous studies have described a negative relationship between soil aggregation and percentage of Na^+ saturation in the ex-

change complex (Lax *et al*. 1994). In this context, the reduced Na^+ concentrations recorded in shoots of *P. fluorescence*-colonised plants could be due to the lower percentage of exchangeable Na^+ in the soil cultivated with such plants. It has been indicated that salt stress affects the physiology and biochemistry of plant cells under *in vitro* and *in vivo* conditions. In this context, increased acid phosphatase has been reported in plants grown under salt stress (Duff *et al*. 1994). We have observed that moderate salinity enhanced the phosphatase activity of non-mycorrhizal bean plants. Ehsanpour and Amini (2003) also demonstrated that osmotic and salt stresses increase acid phosphatase activity in *Medicago sativa*. Acid phosphatase is known to act under stress by maintaining a certain level of inorganic phosphate in plant cells (Olmos & Hellin 1997). The fact that mycorrhizal plants showed the highest levels of foliar P could explain why salinity did not affect the phosphatase activity of such plants. However, our results show that acid phosphatase did not play a role in responses of inoculated and non-inoculated lettuce to severe salinity.

Salt stress may induce a combination of negative effects on salt-sensitive plants including osmotic stress, ion toxicity and oxidative stress. The induction of antioxidant enzyme such as CAT and POX can be considered as one mechanism of salt tolerance in plants (Hernández *et al*. 2003). These antioxidant enzymes are involved in eliminating H_2O_2 from salt-stressed roots (Kim *et al*. 2005). CAT, which is localised in peroxisomes, decomposes hydrogen peroxide to water and molecular oxygen without consuming reductants and, thus, may provide plant cells with an energy-efficient mechanism to remove hydrogen peroxide (Scandalios *et al*. 1997). Hydrogen peroxide can be removed also by "non-specific" POX in the apoplast of lignifying tissues (Ros-Barceló *et al*. 2006), which use hydrogen peroxide as an electron donor to metabolise phenolic compounds. These latter enzymes are ubiquitous and are involved in various processes such as cell growth control and tolerance of environmental stress (Quiroga *et al*. 2000). Thus, decreased activity of POX can prevent or reduce lignification processes caused by salinity, thereby enabling continuity of water uptake through the cell walls (Yazici *et al*. 2007). Reduced CAT activity under stress conditions has been reported in other plants, such as sunflower (Mike *et al*. 1992); this reduction is parallel to a rise in the H_2O_2 content. Increasing salinity stress significantly increased the antioxidant enzyme activities of bean shoots, including those of POX and CAT, compared with their respective non-stressed controls. Remarkably, the PGPR strain induced a higher increase in these antioxidant enzymes in response to severe salinity. Based on these findings, a PGPR, *P. fluorescence*, was more effective than an AMF with respect to alleviating the negative effect of salinity on the growth of bean. Co-inoculation with PGPR and AMF had no additive effect on the plant growth. The contribution of the PGPR and AM fungi to plant salt tolerance was closely related to salinity avoidance mechanisms, including increased water uptake and restricted Na uptake. The protection of mycorrhizal plants against moderate salt stress was also related to the effect that the added endophytes had on increasing P uptake. Induction of antioxidant enzyme activities was involved in the ability of the PGPR strain to increase the tolerance of lettuce grown under severe salt stress. Therefore, inoculation with selected PGPR could serve as a useful tool for alleviating salinity stress in salt-sensitive plants.

CONCLUSIONS

The results of this study showed that both PGPR and AMF can modify salt stress in salt-stressed bean. It seems the positive effects of the microbial inoculation on tolerance of salt-stressed bean may be through improving reactive oxyradical scavenging system, and increasing osmotic adjustment compounds and mineral nutrient contents under salt stress conditions. Thus, inoculation with selected PGPR and AMF could serve as a useful tool for alleviating salinity stress in salt-sensitive plants.

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Received: September 4, 2013