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Full Length Research Paper

Role of bioinoculants in development of salt-tolerance of *Vicia faba* plants under salinity stress

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Through biological inoculation technology, the bacterial-mycorrhizal-legume tripartite symbiosis in saline conditions was documented and the effects of dual inoculation with *Azospirillum brasilense* (NFB) and Arbuscular mycorrhizal (Am) fungus *Glomus clarum* on the host plants (*Vicia faba*) in pot cultures were investigated at five NaCl levels (0.0 – 6.0 dSm^{-1}) in irrigating water. Am faba plants showed decreases in salinity tolerance, % of mycorrhizal infection and higher accumulation of proline with increasing levels of salinity. Am infection significantly increased tolerance of salinity, mycorrhizal dependency, phosphorus level, phosphatases enzymes, nodule number, nitrogen level, protein content and nitrogenase enzymes of all salinized faba plants in comparison with control and non-Am plants either in the absence and presence of NFB. In shoot system of non-Am plants, Na^+ concentration was increased while the concentrations of K^+, Mg^+ and Ca^+ were decreased with raising salinity stress. In Am plants, K^+/Na^+ , Mg^+/Na^+ and Ca^+/Na^+ ratios were higher than that of non-Am plants at all salinity levels. The Na^+ level in shoots of Am plants showed slight increase with raising salinity meanwhile, K^+ and Ca^+ concentrations showed noticeable increases especially at higher salinity levels. The results clearly showed that the inoculation of NFB to Am plants had potentiality to increase the effects of Am fungi under salinity stress. This study provides evidence for benefits of NFB to Am fungus in the protection of host plants against the detrimental effects of salt. If so, bacterial-Am-legume tripartite symbioses could be a new approach to increase the salinity tolerance of legumes plants under salinity conditions.

Key words: legumes mineral nutrition, mycorrhiza, nitrogen fixing bacteria, symbioses.

INTRODUCTION

Salt stress has become an ever-increasing threat to food production, irrigation being a major problem of agricultural fields due to gradual salinization. Salt stress has three fold effects: it reduces water potential, causes ion imbalance or disturbance in ion homeostasis and toxicity. This altered water status leads to initial growth reduction and limitation of plant productivity. Since salt stress involves both osmotic and ionic stress (Hayashi and Murata, 1998; Munns, 2002; Benlloch-Gonzales et al., 2005), growth suppression is directly related to total concentration of soluble salts or osmotic potential of soil water (Flowers, 2004; Pascale et al., 2005; Kaerji et al., 2005). The detrimental effect, as death of plants or decrease in productivity, is observed at the whole-plant level. Suppression of growth occurs in all plants, but their tolerance levels and rates of growth reduction at lethal concentrations of salt vary widely among different plant

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species. Salt stress affects all the major processes such as growth, photosynthesis, protein synthesis, and energy and lipid metabolism (Ramoliya et al., 2004; Parida and Das, 2005). Some researches showed that proline accumulation in plants may be a symptom of stress in less salinity-tolerance species and suggested that it plays multiple role in plant stress tolerance. Proline may act as a mediator of osmotic adjustment (Yoshiba et al., 1997), protects macromolecules during dehydration (Sanchez et al., 1998) and serve as a hydroxyl radical scavenger (Alia et al., 1995).

Many studies have demonstrated that inoculation with Am fungi improves growth of plants under a variety of salinity stress conditions (Diallo et al., 2001; Burke et al., 2003; Tain et al., 2004). Recently, Rabie, 2005 suggested that Am fungi protected the host plants against the detrimental effects of salt. When mungbean plants were irrigated with different dilution of seawater, Am plants showed higher growth than non-Am plants at all levels of irrigation. Mycorrhizae were involved in protection against salt stress, via better access to nutritional status (Zandavalli et al., 2004) and modification of plant physiology i.e. osmotic modifications (Roa and Tak, 2002) and photosynthesis (Meroguihae et al., 2002). To some extent, these Am fungi have been considered as bio-ameliorators of saline soils (Yano-Melo et al., 2003; Tain et al., 2004).

The application of bioinoculants like Am fungi and one of the plant growth-promoting rhizobacteria such as *Azospirillum, Agrobacteria, Pseudomonas,* several Gram positive *Bacillus* is an environment-friendly, energy efficient and economically viable approach for reclaiming wastelands and increasing biomass production. The beneficial effects of these bacteria in combination with Am fungi have been reported by a number of workers (Lesueur et al., 2001; Tain et al., 2002; Patreze and Cordeiro, 2004; Domenech et al ., 2004). It has been reported that these bacteria may affect Am fungi and their plant host through a variety of mechanisms that include (1) effects on the receptivity of the root; (2) effects on the root-fungus recognition; (3) effects on the fungal growth; (4) modification of the chemistry of the rhizospheric soil; and (5) effects on the germination of the fungal propagules. On the other hands, other reports stated that the presence of Am fungi is known to enhance nodulation and N fixation by legumes (Amora-Lazecano et al., 1998; Johansson et al., 2004). Moreover, Am fungi and NFB often act synergistically on infection rate, mineral nutrition and plant growth. Although the interaction between Am fungi and NFB was previously reported, less attention was paid to bacteria-Am-legume tripartite symbioses under salinity stress. Therefore, the present work was conducted to evaluate the efficacy of the Am fungus, *Glomus clarum*, and the nitrogen fixer, *Azospirillum brasilense*, in the development of salinity tolerance of one of the most popular legume of the world, *Vicia faba* (faba bean) plant under salinity stress.

MATERIALS AND METHODS

Soil

Agricultural topsoil obtained from a surface layer (0 – 20 cm) was used. The soil (pH 7.8), which was dried then ground and sieved (2.0 mm), contained 71.9% sand, 12.9% silt and 15.2% clay. Its composition includes 11.0 g kg^{-1} total organic carbon, 120 mg kg^{-1} total nitrogen, 14 mg kg^{-1} available nitrogen, 21 mg kg^{-1} available phosphorus, 17.5 mg kg^{-1} available potassium and 1.7 dSm^{-1} EC.

Seed

Seeds of *V. faba* cv. Geza 3 Rebaia were obtained from Agronomy Dept. Agric. Res. Center, Giza, Egypt.

Microorganisms

G. clarum, previously isolated from saline soil, was kindly provided by Professor Abd El-Fattah, G. M. (Mycology Lab., Mansoura University). *Azospirillum brasilense* was provided from Al-Humiany A., Assistant professor of bacteriology, Taief Teacher's College, Saudi Arabia.

Azospirillum treatments

Cultures of *A. brasilense* were grown on modified nitrogen-deficient semi soiled malate medium (Dobereiner, 1978) at 30°C for 48 h. The suspension obtained (1 X 10^8 cells/ml) was used for seeds inoculation at the rate of 10 ml pot^{-1}. The same dose was added to each pot after 10 days of sowing.

Mycorrhizal treatments

The mycorrhizal inoculum's consisted of spores, mycelium and root segments of *G. clarum* isolates propagated with onion (mycorrhizal infection = 70%) roots for four months. Each pot was inoculated with 20 g inoculum for the mycorrhizal treatments, or 20 g sterilized inoculum with 20 ml filtrates free from mycorrhizal propagules from the inoculum for the non-mycorrhizal treatments. The inoculum was placed 3 cm below the surface of the soil before sowing to produce mycorrhizal plants.

Growth conditions

The experiment was a 5×4×5 complete factorial which was comprised of 5 salinity levels and four inoculation treatments with 5 replicates for each treatment. The pots were arranged in a randomized block. It was carried out with the following treatments: non-inoculation control and inoculation with *G. clarum* and *A. brasilense* as a single and paired inoculum, each at five salt levels (five NaCl concentrations) of 0.0 (control), 1.5, 3.0, 4.5 and 6.0 dSm^{-1}. Each NaCl concentration, dissolved in nutrient solution containing nitrogen (N) at 100 mg L^{-1}, phosphorus (P) at 50 mg L^{-1} and potassium (K) at 50 mg L^{-1}, was added to the soil at a rate of 100 ml every week. 5 seeds were sown into each pot and were thinned to two seedlings per pot after emergence. Fertilization rates of all pots were 60 kg fed^{-1} (NH_4NO_3 33.5 %) and 48 kg K_2O fed^{-1} (K_2SO_4 48%). The pots were arranged in growth chamber at

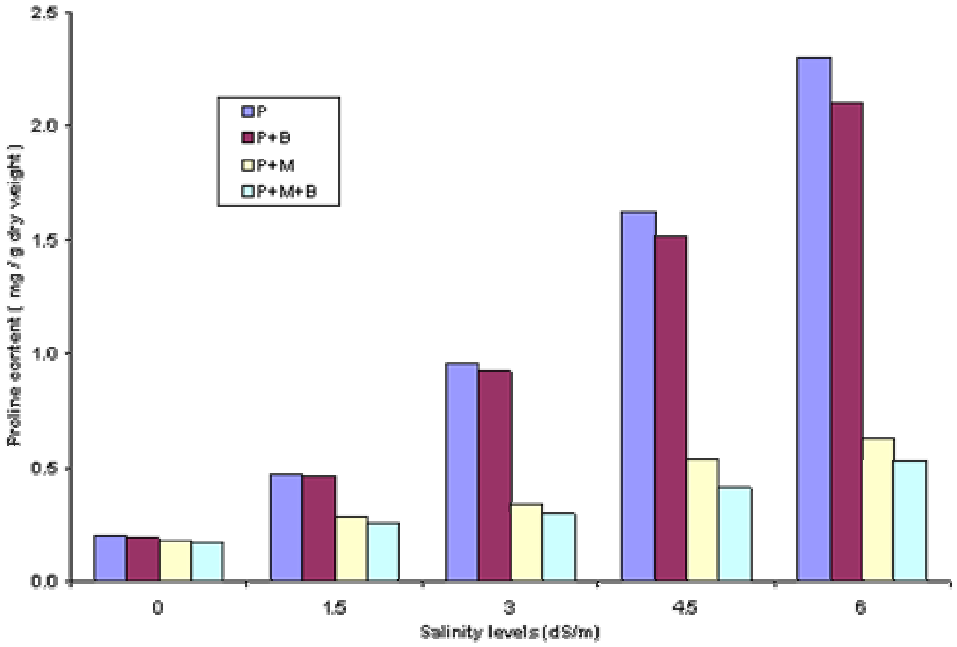

Figure 1. Effect of salinity levels on proline content of Am and non-Am plants with and without NFB.

25/20°C day/night, 11 h day, 60-70% relative humidity and watered to 75% of WHC 2-4 times week^{-1}. The plants were harvested 50 days after sowing date.

Analytical methods

At the end of the experiment, dry matter was measured. N was extracted from plants with sulfuric acid using the semi-micro Kjeldahl method (Jackson et al., 1973). P was extracted by nitric–perchloric acid digestion and measured using the vanadono-molybdophosphoric colorimetric method (Jackson, 1967). K and sodium (Na) of shoot system were assayed using a flame spectrophotometer, while calcium (Ca) and magnesium (Mg) of plant shoots were determined by atomic absorption (Allen et al., 1984). Protein contents of plant tissues were estimated according to Bradford (1976). Free proline of plant tissues was determined according to Bates et al., 1973.

Immediately after harvest, part of the root system of non-Am and Am plants was washed carefully in 4°C water to remove the adhering soil particles, and quantitatively assayed for soluble acid and alkaline phosphatase activities (Gianinazzi-Pearson and Gianinazzi, 1976). Values of phosphatase activities were recorded as units ml^{-1} min^{-1}. Nitrogenase activity was measured in root samples using the acetylene reduction assay (Hardy et al., 1973), and recorded as n moles C_2H_4 g dry root^{-1}h^{-1}.

The roots were cleared and stained by using the methods by Philips and Hayman (1970) and the percentage of mycorrhizal colonization was estimated by the methods of Trouvelot and Gianinazzi (1986). At each salinity level, the mycorrhizal dependency (M.D.) of the plants was calculated according to Gerdemann (1975) as:

$$M.D = \frac{\text{d.w Am plant at a particular level of salinity X100}}{\text{d.w.non-Am plant at the same level of salinity}}$$

Tolerance indices (Ti) of Am and non-Am faba plants to seawater were determined according to Shetty et. al. (1995) as:

$$Ti = \frac{\text{d.w. Plant at salinity level} \times 100}{\text{d.w. plant at 0.0 level of salinity}}$$

Statistical analysis

The results were subjected to ANOVA (Significance was set at $p<0.05$ and $p<0, 01$).

RESULTS

The results given in Figure 1 indicated that increasing salinity level resulted in an increase of the proline concentrations in Am and non-Am faba plants either in the absence or the presence of NFB. A close examination of the results showed that non-Am plants had a higher proline concentration than that of Am plants especially at high salinity level. In the absence of NFB, the proline contents of non-Am plants were raised from 0.2 to 1.287 mg g^{-1}dry weight at salinity level 0.0 (control) and 6.0 dSm^{-1} respectively, while it increased from 0.178 to 0.625 mg g^{-1}dry weight at salinity level 0.0 and 6.0 dSm^{-1} respectively in Am plants. On the other hands, in the presence of NFB, the proline content of non-Am plants recorded as 0.19 mg g^{-1}dry weight at 0.0 dSm^{-1} and 1.253 mg g^{-1} dry weight at 6.0 dSm^{-1}, while it was estimated as 0.172 mg g^{-1}dry weight at 0.0 dSm^{-1} and 0.529 mg g^{-1}dry weight at 6.0 dSm^{-1} in Am plants.

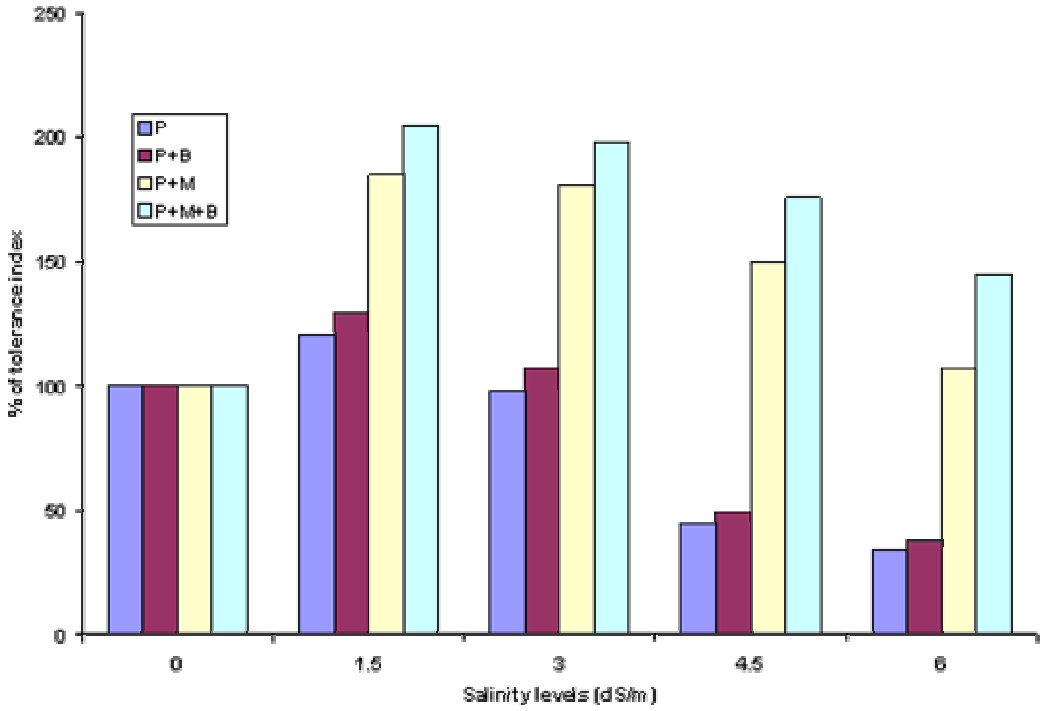

Figure 2. Effect of Salinity levels on salinity tolerance of Am and non-Am plants with and without NFB.

The data recorded in Figure 2 reveal that the tolerance indexes of faba plants were significantly reduced by increasing the salinity levels. The maximum reduction was obtained in control plants, while it was decreased from 121% at 1.5 dSm^{-1} salinity to 48% at 6.0 dSm^{-1} salinity. Plants inoculated with NFB did not exhibit a significant effect on the tolerance indexes of faba plants where the tolerance indexes was decreased from 130% – 52% at 1.5 and 6.0 dSm^{-1} salinity levels, respectively. On the other hand, the results in Figure 2 also clearly show that although the tolerance indexes of faba plants were significantly reduced by increasing the salinity levels, Am plants still had been a high salinity tolerance than that of non-Am plants at all salinity levels. It was also quite clear that the presence of NFB co-inoculated with Am fungi resulted in increasing the salinity tolerance of faba plants at all salinity levels. It reached 121% at salinity level of 6.0 dSm^{-1}.

The results given in Table 1 clearly showed that the frequency of mycorrhizal infection was significantly influenced by salinity. The mycorrhizal infection was reduced from 69% at 1.5 dSm^{-1} to 48% at 6.0 dSm^{-1} in the absence of NFB and from 74% to 52% at the same levels in the presence of NFB. It was observed that the highest infection was recorded at low and moderate levels of salinity either in the absence or the presence of NFB. Evidence from the data in Table 1 indicates that mycorrhizal dependencies for plant dry mass increased by raising salinity. The increase varied from 185% at lower salinity levels to 258% at higher salinity level in absence of NFB. While in the presence of NFB the increase varied from 195% to 273% at low and high salinity levels, respectively.

Phosphorus concentrations in shoots of non-Am faba plants were significantly decreased by increasing salinity during the experiment (Table 1). It was reduced by about 55% of the control in the absence of NFB and 41% of the control in the presence of NFB at high salinity level (6.0 dSm^{1}). The results in Table 1 also revealed that phosphorus concentrations in shoots of Am plants were significantly increased by raising levels of salinity and the highest concentrations were estimated in Am plants inoculated with NFB.

The activities of phosphates enzymes (acid and alkaline) were significantly reduced by raising salinity in non-Am plants either in the absence or the presence of NFB (Table 1). It is also quite obvious that the inoculation of faba plants with the Am fungus, *G. clarum*, resulted in stimulation of phosphatases enzymes, although the activities were slightly decreased in saline conditions. Moreover, the results also revealed that co-inoculation of *G. clarum* and *A. brasilense* to faba plants caused increases in acid and alkaline phosphatases compared with other treatments at all salinity levels.

The results of Table 2 showed that the number of nodules was significantly reduced in faba plants by

Table 1a. Effect of salinity levels on % mycorrhizal infection , mycorrhizal dependency (%), phosphorus content and phosphatases activities of mycorrhizal, and non- mycorrhizal faba plants with and without nitrogen-fixing bacteria.

Parameters	Salinity levels dS/m	Treatments			
		Plant (P)	P+NFB	P+VAM	P+NFB+ VAM
VAM infection (%)	0.0 control	0.0	0.0	63	65
	1.5 dS/m	0.0	0.0	69	74
	3.0 dS/m	0.0	0.0	69	73
	4.5 dS/m	0.0	0.0	59	65
	6.0 dS/m	0.0	0.0	48	52
Mycorrhizal dependency (M.D.) (%)	0.0 control	-	-	185	195
	1.5 dS/m	-	-	153	154
	3.0 dS/m	-	-	185	192
	4.5 dS/m	-	-	252	264
	6.0 dS/m	-	-	258	273
Phosphorus (P) Mg g^{-1} dry weight	0.0 control	0,64	0.69	1.35	1.39
	1.5 dS/m	0.60	0.64	1.50	1.52
	3.0 dS/m	0.57	0.60	1.53	1.56
	4.5 dS/m	0.45	0.53	1.57	1.61
	6.0 dS/m	0.29	0.41	1.63	1.65
Acid phosphatase activities (u /ml/min)	0.0 control	876	883	1450	1462
	1.5 dS/m	842	847	1512	1531
	3.0 dS/m	611	793	1486	1495
	4.5 dS/m	435	515	1113	1235
	6.0 dS/m	237	343	928	1180
Alkaline phosphatase activities (u /ml/min)	0.0 control	280	285	547	573
	1.5 dS/m	271	275	629	635
	3.0 dS/m	212	267	615	615
	4.5 dS/m	184	218	588	492
	6.0 dS/m	125	196	411	418

NFB = Nitrogen-fixing bacteria VAM = Vesicular arbuscular mycorrhiza

Table 1b. ANOVA for % of vesicular arbuscular infection, phosphorus and phosphatases of faba plants inoculated with single and dual NFB and vesicular arbuscular fungi under salinity stress.

treatments	% AM	M.D.	P	Phosphatase
Salinity	0.045*	0.0194*	0.001**	0.021*
Salinity x NFB	-	-	0.815	0.094
Salinity x VAM	0.006**	0.0231*	0.003**	0.026**
Salinity x NFB x VAM	0.007**	0.042*	0.005**	0.016*

* = significant differences (P<0.05) ** = significant differences (P<0.01)

increasing the level of salinity. At 6.0 dSm^{-1} salinity, the number of nodule decreased by about 92% of that formed by faba plants without any inoculants at 0.0 level of salinity. However, the presence of *G. clarum* caused amelioration nodules numbers by faba plants especially at higher salinity levels (4.5 and 6.0 dSm^{-1}). In addition, faba plants inoculated with *A. brasilense* caused a significant increase in nodule counts at low (1.5 dSm^{-1}) and moderate (3.0 dSm^{-1}) salinity levels, while at high salinity levels nodule counts were decreased. However,

Table 2a. Effect of salinity levels on number of nodules, nitrogen content protein content and nitrogenase activity of mycorrhizal, and non-mycorrhizal plants with and without nitrogen-fixing bacteria.

Parameters	Salinity levels dS/m	Treatments			
		Plant(P)	P+NFB	P+VAM	P+NFB+ VAM
Nodule number (no. plant^{-1})	0.0 control	13.0	21.0	13.0	29.0
	1.5 dS/m	10.0	34.0	13.0	37.0
	3.0 dS/m	9.0	28.0	11.0	33.0
	4.5 dS/m	2.0	10.0	8.0	25.0
	6.0 dS/m	1.0	3	4.0	15.0
Nitrogen (mg g^{-1} dry weight)	0.0 control	10.9	15.8	11.3	16.5
	1.5 dS/m	10.7	15.6	11.1	17.9
	3.0 dS/m	9.4	14.7	10.0	17.1
	4.5 dS/m	6.7	9.4	8.7	15.4
	6.0 dS/m	3.9	6.3	5.4	10.2
Protein content (mg^{-1} plant)	0.0 control	7.3	10.8	7.9	11.9
	1.5 dS/m	6.5	8.7	7.3	11.7
	3.0 dS/m	3.9	7.1	6,5	10.1
	4.5 dS/m	1.5	3.4	5.2	8.5
	6.0 dS/m	1.12	1.7	4.3	7.6
Nitrogenase activity (n-moles C_2H_4 g^{-1} dry root h^{-1})	0.0 control	57.4	76.1	65.4	77.3
	1.5 dS/m	52.0	63.3	69.1	86.6
	3.0 dS/m	42.9	59.0	64.9	81.2
	4.5 dS/m	31.0	37.6	58.0	77.9
	6.0 dS/m	17.8	22.1	51.5	75.4

NFB = Nitrogen-fixing bacteria VAM = Vesicular arbuscular mycorrhiza.

Table 2b. One way ANOVA for nodule, nitrogen, protein and nitrogenase of faba plants inoculated with single and dual NFB and Am fungi under salinity stress.

Treatments	nodule	Nitrogen	protein	Nitrogenase
Salinity	0·026*	0·038*	0.002**	0.049*
Salinity x NFB	0. 0216*	.0467**	0.039*	0.027*
Salinity x VAM	0.727	0.0481*	0.076	0.0591
Salinity x NFB x VAM	0.007**	0.009**	0.038*	0.031*

* = significant differences (P< 0.05) ** = significant differences (P< 0.01)

plants inoculated with NFB still have high counts of nodules especially in mycorrhizal plants at all salinity levels during this experiment.

The nitrogen content of uninoculated faba plants was significantly influenced by increasing salinity levels and it was reduced by about 64% at high salinity level (Table 2). Inoculation of faba plants with Am fungi caused slight amelioration of nitrogen content, especially at high salinity level, compared with uninoculated plants. On the other hands, inoculation of faba plants with NFB either singly or paired with Am fungi significantly increased nitrogen content compared to control plant at all salinity levels. Close examination of the results reveal that nitrogen content of paired inoculated faba plants at higher level of salinity was closely similar to that of uninoculated at 0.0 level of salinity.

Data in Table 2 clearly showed that protein content of faba plants was significantly reduced under salinity

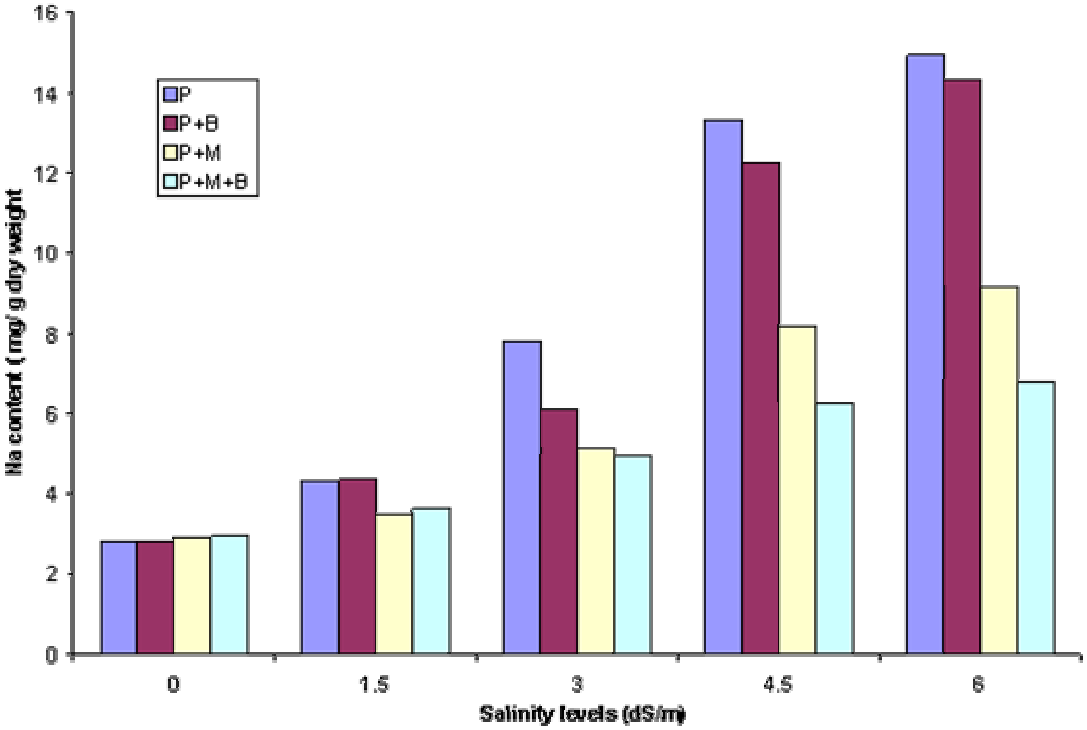

Figure 3. Effect of salinity levels on Na content of Am and non-Am plants with and without NFB.

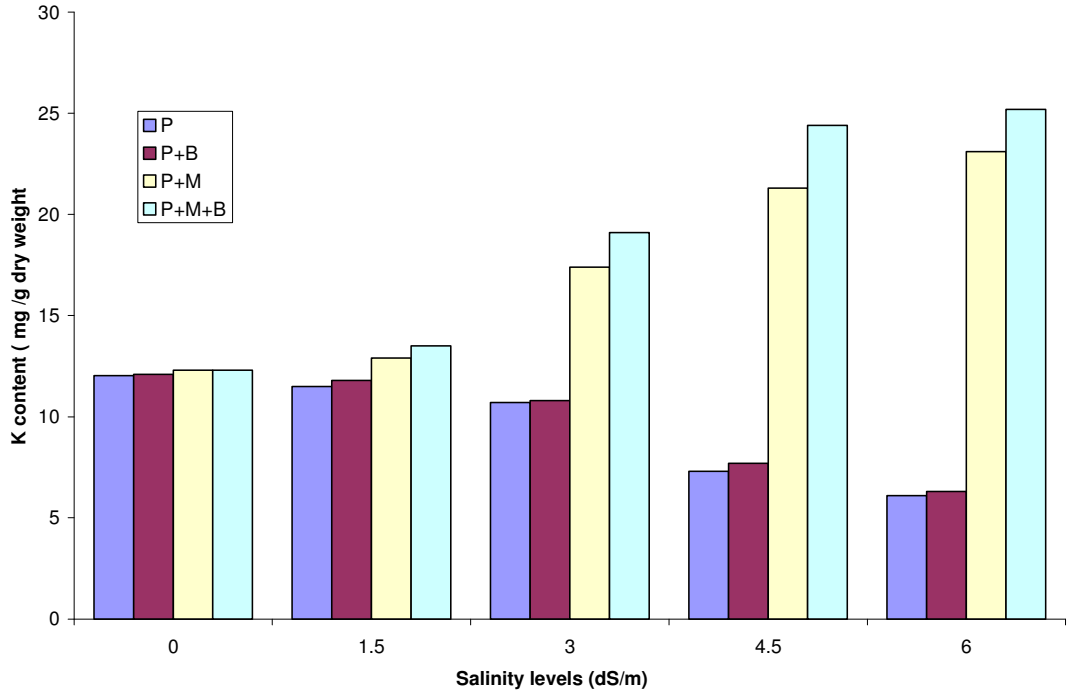

Figure 4. Effect of salinity on levels on K content in Am and non-Am plants with and without NFB.

Table 3. K/Na, Mg/Na, Ca/Na ratios of element contents in mycorrhizal, and non-mycorrhizal plants with and without nitrogen-fixing bacteria under different salinity levels.

Parameter	Treatments				
	Salinity levels (dS/m)	Plant(P)	P+NFB	P+VAM	P+NFB+ VAM
K/Na	0.0 control	4.3	4.3	4.22	4.19
	1.5 dS/m	2.7	2.72	3.72	3.74
	3.0 dS/m	1.4	1.87	3.39	3.87
	4.5 dS/m	0.55	0.62	2.61	3.91
	6.0 dS/m	0.41	0.44	2.52	3.73
Mg/Na	0.0 control	2.31	2.33	2.62	2.64
	1.5 dS/m	1.59	1.59	1.98	2.0
	3.0 dS/m	0.76	0.99	1.28	1.37
	4.5 dS/m	0.38	0.43	0.8	1.09
	6.0 dS/m	0.29	0.31	0.7	1.0
Ca/Na	0.0 control	1.17	1.31	0.7	1.12
	1.5 dS/m	0.79	0.9	1.07	1.14
	3.0 dS/m	0.4	0.56	1.12	1.0
	4.5 dS/m	0.21	0.24	0.84	0.92
	6.0 dS/m	0.14	0.19	0.64	0.88

conditions in all treatments in various degrees in this experiment. At high level of salinity, the protein content of uninoculated plants, non-Am plants with NFB, Am plants and Am plants with NFB was inhibited by about 85, 84, 46 and 36%, respectively. It can been seen that protein level in Am plants was higher than that in non-Am plant either in the presence or the absence of NFB at all salinity levels.

Nitrogenase activities were significantly inhibited with increasing salinity levels in non-Am plants either in the presence or the absence of NFB (Table 1). The results also revealed that nitrogenase activity in Am faba plants with or without NFB were higher than that corresponding of non-Am plants under salinity stress especially at higher levels of salinity. It was found that nitrogenase activities of Am plants in the absence and presence of NFB at 6.0 dSm^{-1} were closely similar to that corresponding to 0.0 (control) level of salinity. Thus, it could be concluded that nitrogenase activities was not affected by salinity in the faba plants co-inoculated with Am fungi and NFB.

The concentrations of minerals in faba plants were significantly influenced by microbial inoculants under salinity conditions. The results in Figures 3 and 4 reveal that Na^+ content in non-Am plants either with or without NFB inoculation was increased significantly by raising salinity. Na^+ content was about 7 folds at high salinity level compared to that in non-saline conditions. On the contrary, K^+, Mg^{2+}, and Ca^{2+} concentrations in these plants were significantly decreased with the rising levels of salinity. The data in Figures 5 and 6 indicate that significant increases of Na^+ concentration in Am plants with increasing salinity level but still had been much lower than that of non-Am plants especially at high levels. It can been seen that Na^+ concentration in Am faba plants inoculated with NFB at higher salinity levels are closely similar to that at lower levels of salinity. K^+ contents of Am plants were significantly increased with rising salinity and the highest contents were observed in the presence of NFB. The results of the Figures 5 and 6 also revealed that Mg^{2+} content of Am plants insignificantly and slightly decreases with raising salinity. On the other hand, in Am faba plants Ca^{2+} content in plant tissue was significantly increased by raising salinity levels and the highest content were observed in the presence of NFB inoculants.

The data recorded in Table 3 revealed that non-Am faba plants showed lower K^+/Na^+, Mg^{2+}/Na^+ and Ca^{2+}/Na^+ ratios than that of Am plants similarly in the absence and presence of NFB under salinity conditions especially at higher levels of salinity. Although these ratios were decrease by raising salinity level in all treatments, the percentage of this decreasing was lowered in Am plants especially those inoculated with NFB. It can been seen that under saline conditions the K^+/Na^+ ratio was observed as the highest ratios in Am plants at all levels of salinity .

DISCUSSION

Salinity is one of the major limitations on crop productivity and quality in the world. Katerji et al. (1998) and Hoorn et al. (2002) has shown that the negative effects of salinity are reducing the growth rate, biomass reduction, shorter stature, smaller leaves, osmotic effects, nutritional deficiency as well as mineral disorders. In the present study, increasing salt stress resulted in growth reduction in all treatments. At salinity levels of about 4.5 dSm^{-1}, the development of uninoculated faba plants was delayed, and at 6.0 dSm^{-1} some of the plants were killed. In general, Am faba plants were less affected by salinity than non-Am plants.

Plants develop a plethora of biochemical and molecular mechanisms to cope with salt stress. A number of nitrogen-containing compounds accumulate in plants exposed to saline stress. The most frequently accumulating nitrogen-containing compounds include amino acids, amide, proteins, quaternary ammonium compounds, and polyamines. The specific nitrogen-containing compounds that accumulate in saline environments vary with plant species. Many plants accumulate proline as a nontoxic and protective osmolyte under saline conditions (Jain et al., 2001; Pujol et al., 2001; Parida et al., 2002). In this study, the concentrations of proline in non-Am and Am plants either in the absence or the presence of NFB increased significantly by raising salinity. Non-Am faba plants accumulated much more proline than Am plants amongst the salinity levels used . The proline level in non-Am plants with and without of NFB were 4.0 and 3.7 fold higher than that of Am plants respectively at 6.0 dSm^{-1} level of salinity (Figure 1). Wang et al. (2004) reported that proline accumulation in plants may be a symptom of stress in less salinity-tolerance species and its contribution to osmotic adjustment was apparently negligible as compared with K^+. Based on these data, the authors concluded that non-Am faba plants are less salinity-tolerant than that of Am plants. If so, it is the experimental evidence that implicates that Am fungus *G. clarum* functions in salt adaptation of faba plants.

Mycorrhizal symbiosis is a key component in helping plants to cope with adverse environmental conditions. The benefit effects of mycorrhiza on the growth under saline conditions have been studied in various plant species and families (Muhsin and Zwiazek , 2002; Bohrer et al., 2003; Sanchez-Blanco, 2004; Rabie, 2005). In the present investigation the tolerance index of Am faba plants was higher than that of non-Am plants at all levels of salinity used in experiment (Figure 2). The tolerance index of salinity of Am plants was increased in the presence of NFB. Tolerance index of salinity in Am plants with and without of NFB were 3.8 and 3.1 fold higher than that of non-Am plants, respectively, at 6.0 dSm^{-1} level of salinity. The average values of tolerance index for Am plants were 155 and 181% in the absence and the presence of NFB, respectively, while for non-Am plants the values were 81 and 35% in the absence and the presence of NFB respectively. This result indicated that benefits of NFB were more evident for Am than non-Am plants which suggests that the dual inoculation of Am fungi and NFB may play an important role in increasing salinity tolerance of faba plants under salinity stress.

Previous research has shown that salinity may reduce mycorrhizal colonization by inhibiting the germination of spores (Hirrel, 1981), inhibiting growth of hyphae in soil and hyphal spreading after initial infection had occurred (McMillen et al., 1998), and reducing the number of arbuscules (Tian et al., 2004). In this study, although mycorrhizal colonization was reduced with increasing salinity levels, the dependency of faba plants on Am fungi was increased (Table 1). It is indicated that symbiosis association between Am fungus *G. clarum* and faba plants was strengthened in saline environment once the association was established. This may be a sign showing the ecological importance of Am association for plant survival and growth of plants under salinity stress. Moreover, mycorrhizal dependency was also increased with the inoculation of nitrogen fixer *A. brasilense* to Am faba plants. Thus, it could be concluded that the benefits of NFB to symbiotic association between Am fungi and faba plants increased under salinity conditions. NFB was assumed as mycorrhization helper bacteria and clearly had the potential to influence Am fungi. In this connection, NFB promotes mycorrhizal development (Duppi et al., 1994) improved spore germination (Moss, 1959) and colonization enhancement (Meyer and Linderman, 1986).

In the present investigation it was found that phosphorus levels in the plants and phosphatases enzymes were reduced with increasing salinity but the Am plants showed higher values of phosphorus and phosphatases activities at all salinity levels (Table 1). Thus it could be concluded that Am fungi increased phosphorus uptake, and saline stress in plants was thereby alleviated. This result indicated that the effect of Am fungi on phosphorus uptake constitutes one of the main mechanisms for increasing plant tolerance of salinity. This is consistent with previous findings that the main mechanism for enhanced salinity tolerance in Am plant was the improvement of phosphorus nutrition (Al-karaki et al., 2001; Yano-Melo et al., 2003; Tain et al., 2004). The results also showed that the phosphorus concentration and activities of phosphatases (acid and alkaline) enzymes in Am plants inoculated with NFB was higher than that in uninoculated Am plants at all salinity levels. These results are in agreement with the previous work of Rabie et al. (2005), who found that the presence of NFB increases phosphorus level and the activities of acid and alkaline phosphatases in Am cowpea plants under salinity stress. In general, the higher phosphorus level and also acid and alkaline phosphatases activities in Am plants irrespective with the absence and presence

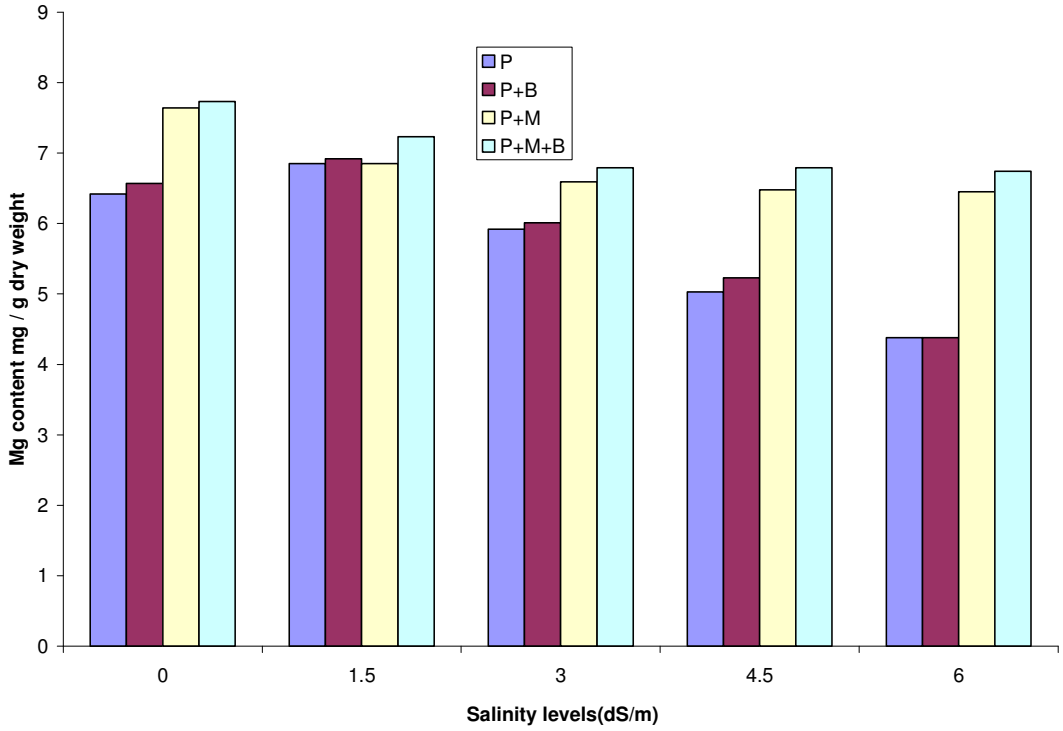

Figure 5. Effect of salinity levels on Mg content of Am and non-Am plants with and without NFB.

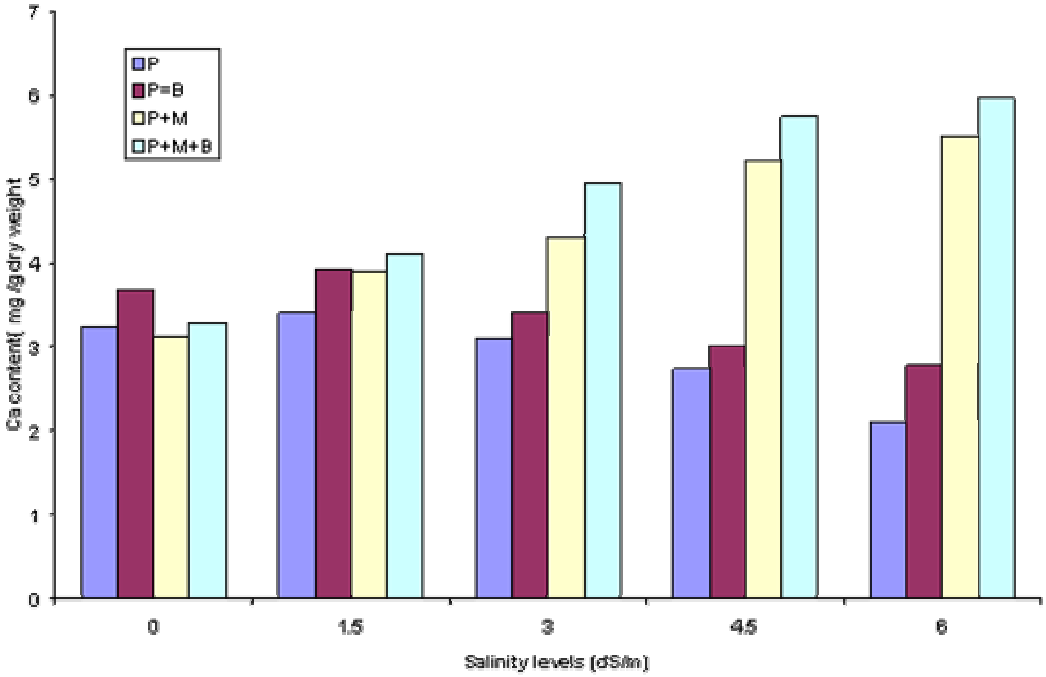

Figure 6. Effect of salinity levels on Ca content of Am and non-Am plants with and without NFB.

of NFB indicates that Am fungus *G. clarum* may still functionally be activated at all levels of salinity used in the present study. These results are also in harmony with those of previous workers (Jindal et al., 1993; Copeman et al., 1996; Yano-Melo et al., 2003).

Extrapolation of the results in Table 2 revealed that salinity inhibits nitrogen fixation by reducing nodulation, mineral nitrogen level, protein content and nitrogenase activity in faba bean plants. Considerable inhibition of nitrogen fixation had been reported in different legume plants by other workers (Soussi et al., 1999; Serraz et al., 2001; Parida and Das, 2005). It is interesting that the inhibitory effects of salinity on nitrogen fixation of faba plants was significantly reduced by preinoculation of these plants by Am fungus *G. clarum* .In this connection, evidence from the previous study (Amora-Lazcano et al., 1998; Johanson et al., 2004; Rabie et al., 2005) indicates that the presence of Am fungi is known to enhance nodulation and nitrogen fixation by legumes. The increased phosphorus uptake conferred by the Am symbiosis is beneficial for the functioning of the nitrogenase enzyme of the bacterial symbionts, leading to increased nitrogen fixation and consequently promotion of root and mycorrhizal development.

The other main results in Table 2 were that the potentiality of Am fungi to minimize the inhibitory effects of salinity on nitrogen fixation by faba plants was promoted by the presence of nitrogen fixer *A. brasilense*. In the presence of NFB, nodule formation, nitrogen level, protein content and nitrogenase activity of Am faba plants were measured as 4.0, 1.4, 3.8 and 2.9 fold higher than that in non-Am plants respectively at a level of salinity 6.0 dSm^{-1}. In addition, these parameters, in the presence of NFB were 5.0, 1.6, 4.5 and 3.4 fold higher in Am than non-Am plants at the highest level of salinity in the present study. This result is consistent with the suggestion that mycorrhizal and nodule symbioses often act synergistically on infection rate, mineral nutrition and plant growth (Belimov et al., 1999; Bero et al., 2000; Patreze and Cordeiro, 2004) which support both needs of nitrogen and phosphorus and increase the tolerance of plants to salinity stress (Rabie et al., 2005).

Experimental evidences from this study indicated that increased treatment of NaCl induced significant increase in Na^+ and decrease in K^+, Mg^{2+} and Ca^{2+} levels in shoot system of non-Am faba plants (Figures 3 to 6). These results are in agreement with the previous observations (Khan, 2001; Ferreira et al., 2001; Parida et al., 2004). These authors reported that high salt (NaCl) uptake competes with the uptake of other nutrient ions, especially K^+, leading to K^+ and other ions deficiency. The ions deficiency displayed by salinity stress, particularly by NaCl uptake indicated a nutritional imbalance. It has been generally accepted that Am fungi would enhance nutrient uptake by infected plants under salinity conditions (Roa and Tak, 2002; Yano-Melo et al., 2003; Zandavalli e t al., 2004). It appears from the present study that Am faba plants contained significant higher levels of K^+, Mg^{2+} and Ca^{2+} ions, particularly in the presence of NFB, than non-Am plants at all salinity levels. Based on these results and on existing literatures, the greater salt tolerance of Am plants may be the result of the plant nutrition improvement under salinity stress. It is also noteworthy that Na^+ concentration in shoot system of Am faba plants, especially in the presence of NFB, at high salinity level was comparable to that of non-Am plants at moderate salinity level. These results are consistent with the previous work (Zandavalli et al., 2004; Rabie et al., 2005), and suggest that Am fungi may protect shoot system, mainly leaves, from Na^+ toxicity either by regulating Na^+ uptake from the soil or by accumulating it in root thereby delaying its translocation onto shoot system of infected plants.

The most important results in Table 3 show that K^+/Na^+ and Ca^{2+}/Na^+ ratios were significantly increased in Am faba plants, especially in the presence of NFB, than that of non-Am plants. This may reflect the potential role of Am fungi for increasing plant K^+ and Ca^{2+} uptake more than Na^+ under salinity stress. The role of K^+ and Ca^{2+} in salt adaptation of plants has been previously discussed by several authors. Parida and Das (2005) reported that when under salt stress, plants maintain high concentrations of K^+ and low concentrations of Na^+ in the cytosol. They do this by regulating the expression and activity of K^+ and Na^+ transporters and of H^+ pumps that generate the driving force for transport. In addition, externally supplied Ca^{2+} reduces the toxic effects of NaCl, presumably by facilitating higher K^+/Na^+ selectivity. High salinity also results in increased cytosolic Ca^{2+} that is transported from the apoplast and intracellular compartments. The resultant transient Ca^{2+} increase potentialities stress signal transduction and leads to salt adaptation. Based on these data and existing literatures it is conceivable to conclude that Am symbioses may regulate the expression and activate K^+ and Na^+ transporters and H^+ pumps that generate the driving force for transport. Besides, it may increase the transient Ca^{2+} from apoplast and intracellular compartments. This inference needs further investigation to support it.

Extrapolations of the results in the present study and other unpublished data showed that Am faba plants grew in high saline environments without any detectable signs of toxic effects of NaCl, reflecting adaptation to high salinity. These extrapolations emphasized the prime role of Am fungi in increasing salinity tolerance of faba plants up to 6.0 dSm^{-l}. These data suggest that selective accumulation or exclusion of ions, control of ion uptake by roots and transport into leaves and compartmentalization of ions at the cellular and whole-plant levels are the most effective strategies of Am fungi for adaptation of faba plants to salinity stress. These strategies have been stimulated effectively by inoculating Am plants with NFB. Benefits of NFB to Am fungi under salinity stress were previously proved by Johanson et al.

(2004) and Rabie et al. (2005). These authors reported that in bacterial-Am-legume tripartite symbiosis relationships nodulation of NFB and establishment of Am often occur simultaneously and synergically. Besides, NFB provide fixed nitrogen not only to the plant, but also to the fungus. Moreover NFB can also assist in mobilizing nutrients from the soil and improving the growth of infected plants. If so the authors suggested that bacterial-Am-legume tripartite symbiosis could be a new approach to increasing the salinity tolerance of legumes plants under salinity conditions. However, many practical problems remain, such as the selection of better strains, optimization of strain dosages, salinity tolerance of symbionts, choice of good symbionts to plant and appropriate time for inoculation that needs further studies.

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