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ISOLATION AND EVALUATION OF SALT-TOLERANT MICROORGANISMS AND THEIR IMPACT IN ADAPTATION OF FABA BEAN TO SALINITY STRESS

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ABSTRACT

The target of the present study is the selecting salt tolerant *Rhizobium leguminosarum* biovar *viceae* through isolated them from salt-affected soils of North Delta of Egypt, and evaluated for their efficiency and salt tolerance, thereafter, the best efficient and salt-tolerant selected isolates along with A. mycorrhizae were used to inoculate faba bean plants grown in pots to elucidate their effects on ameliorating salt effects on the plant.

Rhizobial isolates varied in their tolerance to salinity. The isolates bringing from salt soils had more tolerance over those isolated from normal soil. The isolates of R2 and R6 were the superior. They confer the plants the highest nodules dry weight, plant dry weight and N-content. The plant exhibited the best growth and elements uptake (N, P and K-contents) when inoculated with the mixture of the tolerant rhizobia and mycorrhizae, especially R2+M treatment. Thus, we urged farmers to applying salt-tolerant inoculums in agricultural practices in order to increasing productivity of the crops under salinity condition. In addition, rather studies should be extended to explore the great benefits of microorganisms to alleviate different stresses on the crop.

Keywords: Salt affected soil, salt-tolerant microorganisms, faba bean, salinity.

INTRODUCTION

Increase of soil salinity is a big danger for man and the environment, whereas, desertification continuously enlarged and the fertility of lands decreased, therefore, the national income being in continuous deterioration. The poor peoples and deprives number rapidly increased, the main reason for this is the rises of salinization and desertification of soils. We must be fully aware that soil conservation and maintenance is the bases of continuation of the industrial, scientific and cultural renaissance, because the hungry person did not able to think, create or produce.

Salinization is considered one of the most threatening factors to the natural environment and to limiting agriculture crop production (Chinnusamy *et al.*, 2005; Zadeh and Naeini 2007). About forty percentage of the world's land surface are categorized as having potential salinity problems, most of these areas are confined to the tropics and Mediterranean regions (Cordovilla *et al.*, 1995). It has been estimated that 23% of agricultural soils are affected by problems related to high salinity. Most crops are sensitive to relatively low levels of salinity. In the case of legumes, not only the plant but also the symbiotic bacteria are sensitive to salinity, both at the free living stage and during the symbiotic process (Lloret , 1995). In Egypt the majority of salt-affected soils are located in the Northern-Center part of the Nile Delta. Nine hundred thousand hectares suffer from salinity problems (Abu-Zeid, 1988).

Legumes are classified as salt sensitive crop species (Zahran, 1999) and salinity may act as a water stress, which affects the photosynthetic rate, or may affect nodule metabolism directly. The depressive effect of salt stress on N_2-fixation by legumes is directly related to the salt-induced decline in dry weight and N-content in the shoot (Cordovilla *et al.*, 1995). However, legumes are generally more sensitive to salinity than their rhizobial counterparts and consequently, the symbiosis is more sensitive to salt stress than free-living rhizobia (Zahran, 1999). It is known that rhizobia increases the nitrogen nutrition of legumes and that one of the beneficial effects of the endo-mycorrhizal fungi is a better phosphorus uptake (Hatimi *et al.*, 1997). It is also known that the hyphae of these fungi play a role in the water movement from soil to roots (Graham and Syvertsen, 1984). There is an increasing evidence concerning the possibility of a better tolerance to drought and salinity in mycorrhizal plants, although it is difficult to distinguish the direct effects of fungi from those produced by a better nitrogen uptake induced by rhizobia (Cordovilla *et al.*, 1995).

Despite the importance and necessity of legume inoculation with rhizobia in increasing plant yield (Anjum *et al.*, 2006), the total annual terrestrial input of nitrogen from BNF (biological nitrogen fixation) ranged from 139 to 175 million tons of nitrogen (Burns and Hardy, 1975 and Paul, 1988), it is recently found that rhizobial inoculation has an additional importance in offering plant more tolerance against unsuitable environmental conditions such as high temperature, acidity, alkalinity, drought or salinity (Phillip, *et al.*, 2010). At the same context, Nour El-Din (2003 and 2010) found that inoculation of pea and faba bean plants with heavy metals tolerant rhizobial strains increased plants tolerance against these metals and increased the plants productivities. The matter which makes this process necessary to cope with the global ecological recession as well as the disastrous environmental pollution with residues of nitrates and nitrites (Kim *et al*, 1998) which cause dangerous diseases like cancer that initiated by allocation of nitrites in the human body which reacted with free amino acids resulting the carcinogenic nitrosamine compound .

Rhizobia like all biota negatively affect by the unfavorable environmental conditions especially salinity. Fortunately, some rhizobial strains can combat salinity stress. Alikhani and Leila (2010) found that rhizobial lentil symbiont strains differ in tolerance against salinity. Plasmid profile of salinity tolerant rhizobia were studied by Shamseldin (2008) and he concluded that these strains contain a common plasmid with a size of 250 kb which may be responsible for salinity tolerance. He reported that the apparent tolerance among the studied rhizobial strains may be genetically rather than adaptation.

The current investigation, therefore, aimed to isolation of different *R. leguminosarum* biovar *viceae* isolates from Egyptian salt-affected soils (North Delta region) for studying the probable differences in their N_2-fixing capacity and salinity tolerance, in addition to, investigating the effect of inoculation of faba bean plants with the most tolerant and efficient isolates along with inoculation with A. mycorrhizae on the plant growth under salinity circumstances.

MATERIALS AND METHODS

Study area:

The study area is located in North Delta region, Kafr El-Sheikh (31◦ 08´ North and 30◦ 56´East), Mutubas (31◦ 27´N and 31◦ 32´E), Elhamoul (31◦ 18´ N and 31◦ 09´E), and Baltim (31.33 N and 31.05 E). Climatic conditions of the study area are typically arid Mediterranean climate, characterized by aridity with long rainless summer, mild winter with low amounts of rainfall: other seasons are characterized by unstable climatic. The Nile River is the main source of irrigation water.

Seeds: Faba bean (*Vicia faba*) seeds (Nobaria 1) were kindly supplied from Department of Legumes, Sakha Agricultural Research Station. Kafr El-Sheikh, Egypt.

Medium used, Somasegaran and Hoben (1985): Yeast mannitol broth for rhizobial isolation and purification (Vincent, 1970). That contains the following by g/l: K_2HPO_4, 0.5; $MgSO_4$, 0.2; NaCl, 0.1; Mannitol, 10; Yeast extracts, 1; Distilled water, 1L. pH was adjusted to 6.8-7 and autoclaved at 121 °C for 15 minute. 15g agar was added when needed solid.

Nutrient solution (g/l): composed of K_2SO_4, 0 .486; K_2HPO_4 , 0.200 ; $MgSO_4.7H_2O$, 0.200; $FeCl_2$, 0.010 ; $CaCl_2$, 0.376; $H3PO_4$, 0.018; $ZnSO_4.7H_2O$, 0.0028 (Shrdeta *et al.*, 1984). All those contents were dissolved in 1 liter water and the pH of solution was adjusted to pH 6.9 using KOH.

Rhizobial isolates:*Rhizobium leguminosarum* bv. *viceae* were isolated from different locations in Kafr El-Sheikh Governorate soils using YMA medium (Vincent, 1970). The locations of different isolates are Kafr El-Sheikh, Mutubas, Elhamoul and Baltim.

A-mycorrhizae

A-mycorrhizal isolates as spores of three species (i.e. *Glomus mosseae*, *Gigaspora margarita*, *Acaulospora calaspora*) were supplied from Department of Microbiology, Soil, Water and Environment Research Institute, A.R.C., Giza, Cairo.

Soil and Plant sampling

The soil samples were air dried, crushed and sieved through 2 mm sieve, and subjected to chemical analysis. The characteristics of the soil are presented in Table 1. Using the spade, describe a circle with a radius of approximately 15 cm around the plant. This section was cut to a depth 20 cm at least, still using the spade slowly to left out the clump. Then the soil was removed carefully from the root avoiding detaching secondary roots from the plant where nodules may be found. The whole plant was placed into a plastic bag and then the roots were carefully washed under tap water.

Soil analysis: Chemical characteristics of soil were estimated according to Richards (1954).

Isolation and purification of *R. leguminosarum* bv. *viceae* from active nodules:

Samples were collected from saline habitats (Motobase, Balteim and El-Hamoul) and non-saline habitats (Kafr El-Sheikh). Active and healthy

nodules of faba bean plants were collected and rhizobia cells were isolated according to Vincent (1970).

In vitro evaluation of salt-tolerant rhizobial isolates:

A fixed number of the pure isolated *Rhizobium* ($5x10^8$) was dropped according to Somasegaran and Hoben (1985) onto plates containing YMA medium supplemented with different NaCl concentrations (0, 2, 4 and 8 dS/m). Plates were incubated at 30°C for 3 days till rhizobial colonies appeared. Rhizobial colonies were then counted.

In vivo evaluation of salt-tolerance and N_2-fixation capabilities of the isolates.

Rhizobial culture used

The pure isolates were grown in 500 ml flask containing 250-ml YMB medium on rotary shaker incubator at 28°C for 8 h daily. After 3 days of inoculations. The number of cells /ml of each culture were estimated using dropping plat method according to Somasegaran and Hoben (1985).

Leonard's jars were used for an *in vivo* evaluation of rhizobial isolates to salinity. Sand soil was washed several times with 0.1 HCl solution followed by washing with distilled water in order to remove nitrogen as well as other minerals. Leonard's jars were autoclaved twice at 1.5 par, 121°C for 4h (El Nady and Belal, 2005). Each jar was filled with 3 kg of sand soil.

Sterilized jars were arranged as complete randomize design with 5 replicates. Faba bean seeds were surface sterilized in order to eliminate possible contamination by native rhizobia by rinsing in ethanol (95%) for 3-5 minutes and soaking for 4 minutes in hydrogen peroxide (3% v/v) followed by washing in sterile distilled water several times. Four seeds per jar were sown. Seedlings were thinned to two per jar then inoculated with liquid cultures of different rhizobial isolates (5 ml x 10^8 cfu/ml / plant) prepared in addition to *Rhizobium* free liquid medium as control. Irrigation was carried out twice weekly by free of nitrogen nutrient solution according to prevailing climatic conditions (Shrdeta *et al.*, 1984). After 50 days of sowing plants were collected and subjected to the following determinations; fresh and dry weight of plant, dry weight of nodule, number of nodules per plant, nitrogen % and nitrogen content. The best tolerant and efficient isolates of rhizobia were selected and used in a pot experiment to study the effect of inoculation with salt-tolerant rhizobial isolates and/or A-mycorrhizae on plant growth and tolerance to different saline concentrations.

The pot experiment:

A green house split plot design pot experiment was carried out at Sakha Agricultural Research Station, Kafr El-Sheikh during season 2008 to investigate the effect of inoculation with the selected salt-tolerant as well as A-mycorrhizae on ameliorating salinity influence on the faba bean plants. The used pots were about 30 cm in diameters and 35 cm in height with capacity of 8 Kg clay soil. The used soil was collected from the experimental field of Sakha Agricultural Research Station, Kafr El-Skeikh. Composite surface soil sample (0-20) was taken just before conducting the experiment. The composite sample was air dried, crushed and sieved through 2 mm sieve, and subjected to some chemical analysis in the extraction of 1: 5 (1 part soil:5 part water) soil suspension (Table, 1).

The different treatments were arranged as follows:

1-Control 1 without inoculation, fertilized with 25% N, 100% P and 100%K.
2-Control 2 without inoculation, fertilized with 100%N, 100%P and 100 % K.
3-Inoculation with A-mycorrhizae, fertilized with 100% N, 50% P and 100% K.
4- Inoculation with rhizobial isolate R2, fertilized with 25%N, 100% P and 100%K.
5-Inoculation with rhizobial isolate R6, fertilized with 25%N, 100% P and 100%K.
6-Inoculation with A-mycorrhizae+R2,fertilized with 25%N, 50% P and 100%K.
7-Inoculation with A-mycorrhizae + R6, fertilized with 25%N, 50% P and 100%K.
8-Inoculation with A-mycorrhizae + R2 + R6, fertilized with 25%N, 50%P and 100%K.

Table 1: Some chemical properties of the experimental soil.

EC dS/m	Soluble anions (meq/L)				O.M.%
	CO_3^-	HCO_3^-	Cl^-	SO_4^-	
2.1	0.0	4.3	9.8	8.8	1.6
	Soluble cations (meq/L)				pH
SAR	Mg^{++}	K^+	Na^+	Ca^{++}	
8.39	4.0	0.2	15.7	3.0	8.0

SAR: Sodium adsorption ratio O.M: organic matter EC: Electric conductivity

The salinity levels expressed as electrical conductivity (EC). Artificial salinization with different levels (4, 8 and 12 dS/m) of EC was prepared using NaCl and $CaCl_2$ salts according to Manual of salinity research methods (1992).

Peat based cultures of rhizobia were prepared using the method described by (Vincent, 1970). Cell suspensions containing 10^8 cfu/ml of the best salt-tolerant and N_2-fixing isolates were used to impregnate sterilized peat at the rate of 52 ml liquid culture /100gm peat. Inoculated peat was well mixed and allowed to mature at room temperature for 48 hr.

Seeds wetted with 10% Arabic gum water solution as an adhesive material (Hamdi, 1982b) were inoculated with rhizobial peat-based preparation. Seeds were allowed to air dry in the shad for 30 minutes and sown immediately. Four seeds per jar were sown. Seedlings were thinned to two per jar.

Mycorrhizal inoculation

The mycorrhizal inoculum (*Glomus mosseae*, *Gigaspora margarita* and *Acaulospora calaspora*) was added to the soil at the sowing time in the pots. Each gm of mycorrhizal inoculum contained 20 spores.

Nitrogen fertilizer:

Nitrogen was added to control 1, treatments contained rhizobia; R2, R6, mycorrhizia+R2, A-mycorrhizae+R6 and A-mycorrhizae+R2+R6 pots received 25% N (0.25g urea/pot) with the rate of 31kg urea/fed. Control 2 and mycorrhizal inoculation received 100% N (1g urea/pot) with the rate of

125 kg urea/feddan (feddan = 4200 m^2). Nitrogen fertilizer dose was divided and applied at sowing time and after 15, 30, 45 days of sowing.

Phosphorus fertilization:

The soil was fertilized with super phosphate at the rate of 200 Kg /fed. (1.6 g/pot) except the treatments contained mycorrhizae which were fertilized with 100 Kg/ fed (0.8 g/pot), added to the soil before sowing.

Potassium fertilization:

Each pot was applied with 0.4g of potassium sulphate (with the rate of 50 kg/fed). This was added to inoculated and un-inoculated treatments at the time of flowering. Each pot was irrigated twice weekly with tap water to 60% of the water holding capacity.

Chemical analyses

Plant samples or seeds were dried and 0.2 g were grind, then digested in 5 ml H_2SO_4 and 1 ml perchloric acid in a conical flask as described by Chapman and Pratt (1963). The digested materials were completed to 50 ml and then distilled by micro-Kjeldahl methods and the nitrogen % of distillate was determined by titration against 0.02 normal H_2SO_4). The phosphorus was determined colorimetrically according to the methods described by Snell and Snell (1967). Sodium and potassium were determined in the digested solution by flame photometer (No, 712700 REG. DES No, 866150). N, P, K and Na contents were determined according to methods recorded by Chapman and Pratt (1963). Proline in dry leaves was determined at 50 days after sowing. Proline was determined following the method described by Batrs *et al.* (1973), and for rapid quantitative determination of IAA, the colorimetric method according to Pilet and Chollet (1970) was followed.

Statistical analysis

Data obtained were subjected to the analysis of variance and treatment means were compared using the L.S.D methods according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Data presented in Table I-1 showed the viable counts of rhizobia under increase of salinity. Increase of salinity severely injured rhizobial growth. Rhizobial numbers sharply decreased with the increase of salinity even at the lowest concentration (2dS/m), which exhibited significant decreases than un-amended controls. Rhizobial isolates response to salinity varied, whereas, salinity effect was more vigorous on the isolates brought about from non-saline locations (R7 and R8) which sharply affected at 4 dS/m level and there cell growth completely suppressed at 8dS/m. While, the isolates from salt-affected soils exhibited more tolerance even at 8dS/m level (isolates from R1 to R6). The growth of all isolates noted to be similar under control (without salinity), whereas there were no significant variations between them. In contrast, their counts varied under the impact of salinity. The isolates R2 and R6 were more tolerant at 8dS/m salinity level, but the isolates R7 and R8 did not show any growth at this level.

Table I-1: Effect of salinity levels on the viable counts (log number) of the local rhizobial isolates.

Isolates	Log viable counts				
	Salinity levels				
	Control	2 dS/m	4 dS/m	8 dS/m	Mean
R1	7.84a	6.71a	5.51ab	3.5ab	5.89
R2	7.82a	6.66ab	5.44b	3.38b	5.71
R3	7.80a	6.59ab	5.49ab	3.41b	5.83
R4	7.77a	6.74a	5.53ab	3.36b	5.87
R5	7.83a	6.63ab	5.57ab	3.44ab	5.85
R6	7.75a	6.61ab	5.49ab	3.6a	5.88
R7	7.75a	6.51b	5.14c	0c	4.87
R8	7.78a	6.49ab	5.07c	0c	4.85
Mean		6.61	5.40	2.58	5.50
Comparison		LSD 5%		LSD 1%	
2-S means at each l		0.1555		0.2066	
2-S means		0.054		0.073	

Rhizobial cells grown in liquid culture severely affected by salinity even at the lowest level (2 dS/m). This effect may be due to the direct contact of the cells with the saline culture. This result coincided with those cited by Peter *et al.* (2008) and Abolhasani *et al.* (2010), who reported that rhizobial cells sharply decreased with increase of culture salinity level. Therein study also found that the growth of all isolates was similar under un-saline culture, otherwise, under saline condition, growth of isolates collected from saline soils was best. This means that physiological processes of all isolates still of normal activities and the isolates brought about from saline soils were more tolerant to salinity stress. Dardanelli *et al.* (2009) showed that strains of the same species of *Bradyrhizobium* vary in their salt-tolerance to 100 mM NaCl. Osmotolerant rhizobia strains can support large modifications in the osmolarity without a decrease in the number of viable cells (Singleton *et al.*, 1982). This variation of rhizobia to salinity may be due to formation of intracellular accumulation of low-molecular-weight organic solutes called osmolytes as indicated by Csonka and Hanson (1991) such as an osmolyte, N-acetylglutaminyl-glutamine amide which accumulates in cells of *R. meliloti* as indicated by Smith *et al.* (1994). Dardanelli *et al.* (2009) found that at osmotic and saline stress, peanut rhizobia ATCCI0317, TALIOOO, TALI371 and SEMIA6144 showed a different response of potassium and trehalose content. Trehalose has been shown to protect cell membranes and proteins from inactivation or denaturation caused by a variety of stress conditions (McLntyre *et al.*, 2008).Glycine betaine concentration increased more in the salt-tolerant strains of *R. meliloti* than in sensitive strains (Smith *et al.*, 1988). The disaccharide trehalose plays a role in osmoregulation when rhizobia are growing under salt or osmotic stress were showed by El-Sheikh and Wood (1990). The content of polyamines (homospermidine) influenced in salt-tolerant cells and acid-tolerant strains of *R. fredii* is another salt-stress responses showed by Fujihara and Yoneyama (1994). This polyamine may function to maintain the intracellular pH and repair the ionic imbalance caused by osmotic stress and the formation of osmotic shock proteins was found in cells of rhizobia as indicated by Zahran *et al.* (1994). Exogenous

proline petaine (N, N-dimethylproline or stachydrine) highly stimulated the growth rate of *Rhizobium meloliti* in media of inhibitory concentrations of NaCl whereas poroline was ineffective. High levels of proline petaine uptake occurs in cells grown in media of elevated osmotic strength was noted by Gloux and Rudulier (1989). Proline protects membranes and proteins against the adverse effects of high concentration of inorganic ions and temperature extremes. It also functions as a protein-compatible hydrotope, and as a hydroxyl radical scavenger (Smirnoff and Cumbes, 1989). Accumulation of proline buffers cellular redox potential under environmental stresses (Wahid and Close, 2007). Mohammad *et al.* (1991), noted a differential response of *R. meliloti* to salinity and osmotic stress, indicating genetic variability in tolerance to these environmental constraints.

It is noted from records presented in Table I-2 that the increase of salinity levels significantly lowered dry weight of nodules. The inoculation of faba bean plants with all rhizobial isolates gave root nodules, but the un-inoculated plant did not nodulate. There were no significant variations between all isolates in their ability to produce nodules at the normal condition. Contrarily, significant variations appeared between the rhizobial isolates for nodule formation under the impact of salinity level. At 4dS/m level, significant variations were ,also, found in nodules dry weight for the plants inoculated with the different studied isolates, and the inoculation with R2 gave higher dry weight of nodules (0.085g/plant), followed by the inoculation with R6 which attained 0.067 (g/plant). While, the inoculation with R7and R8 isolates bringing from un-saline soils exhibited the lowest record (0.057 g/plant), and showed sharp decrease with the increase of salinity levels. There were no significant variations appeared, in the dry weight of nodules, between the plants inoculated with the different isolates under the highest salinity level (12dS/m).

The inoculation with all studied isolates nodulated the faba bean plants confirming efficiency of these isolates compared to un-inoculated plants that did not show any nodules, this result coincided with findings of Erman *et al.* (2009). The effect of inoculation with all addressed isolates were similar, but under salinity stress, the isolates of R2 and R6 attained the highest records, whereas, the isolates of R7and R8 which isolated from normal soil gave the lowest dry weight of nodules. This results confirmed that isolates brought about from saline soils not only nodulate plants grown under saline stress, but also well nodulate plants grown under normal conditions. In this context, Dardanelli *et al.* (2009) found that Peanuts subjected to osmotic stress presented nodulation parameters similar to those of control plants. Abolhasani *et al.* (2010) reported that *Sinorhizobium* strains isolated from saline soil acquired more ability to tolerate salinity. In this regard, Payakapong *et al.* (2006) and Shamseldin (2008) claimed that specific genes for salinity tolerance located on the plasmid of tolerant rhizobial strains, Payakapong *et al.* (2006), also, found that salt-tolerant *Sinorhizobium* strains had a remarkable amounts of glycine betaine, and the genes clusters encoded this compound were isolated. In the present study, there was no significant variations between different isolates under the highest salinity level (12dS/m), this may be due to the drastic harmful effect of this dose of salinity

on symbioses process ,whereas, El-Shiekh and Wood (1990) indicated that nodulation and nitrogen fixation activities are apparently more sensitive to salt than plant growth. Soybean root hairs show little curling or deformation when inoculated with *Bradyrhizobium japonicum* in the presence of 170 mM NaCl, and nodulation is completely suppressed by 210 mM NaCl (Tu, 1981). In addition, bacterial colonization and root hair curling of *Vicia faba* are reduced in the presence of 50 to 100 mM NaCl or 100 to 200 mm polyethylene glycol as osmotic, with infection threads reduced to 30 and 52% in the presence of these abiotic stresses respectively (Zahran and Sprent, 1986). Unsuccessful symbiosis under salt stress may be due to a failure in the establishment of rhizobia in the rhizosphere, or a failure of the infection process due to the effect of salinity (Singleton and Bohlool, 1984). Salt stress reduces the nodulation of legumes by inhibiting the very early symbiotic events (Zahran, 1999), whereas osmotic stress induces significant changes in water relations, growth and symbiotic N_2-fixation in stressed plants (Sassi Aydi *et al.*, 2008).

Table I-2: Effect of salinity levels on the dry weight of nodules (g/plant) of faba bean due to inoculation with different rhizobial isolates.

Treatments	Dry weight of nodules (g/plant)				
	Salinity levels				
	Control	4 dS/m	8 dS/m	12 dS/m	Mean
Un-inoculated	0.000b	0.0c	0.0b	0.0a	0.0d
R1	0.051ab	0.0390bc	0.028ab	0.009a	0.031c
R2	0.110a	0.0850a	0.066a	0.061a	0.0805a
R3	0.075a	0.0560bc	0.050ab	0.049a	0.058bc
R4	0.087a	0.066b	0.063ab	0.031a	0.0617bc
R5	0.063a	0.0610bc	0.044ab	0.037a	0.0513bc
R6	0.090a	0.0670b	0.071a	0.049a	0.0692b
R7	0.073a	0.057bc	0.054ab	0.020a	0.051bc
R8	0.071a	0.0570bc	0.054ab	0.024a	0.0515bc
Mean	0.0689	0.0653	0.0478	0.0313	0.0533
Comparison		LSD 5%		LSD 1%	
2-S means at each I		0.0559		0.0739	
2-S means		0.0186		0.0246	

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.
I : Inoculation S : Salinity R: Rhizobial isolate

The increase of salinity led to significant decrease in plant nitrogen content (Table I-3). The decrease was sharp in case of un-inoculation, whereas, salinity level of 8 dS/m led to a decrease evaluated 52.44%, while in case of inoculation with isolates R1 and R6, the percentages of decrease lowered to 25.82% and 29.94% respectively. In the same time, the inoculation with R2 and R6 isolates attained the highest level of nitrogen under the highest concentrations of salinity.

Nitrogen content of the plant decreased with the increase of salinity concentration. This harmful effect magnified in absence of inoculation. At inoculation with salt-tolerant isolates (R2 and R6), the deleterious effect remarkably decreased. Craig *et al.* (1991) found that salinity heavily lowered N-content of the plant. The results of Hatimi (1999), also, agreed with those

of the current study, whereas, they reported that rhizobial inoculation of *Phaseolus vulgaris* plants improved N-content of the plant under saline condition.

The dry weight of plant (g/plant) severely decreased due to the increase of salinity (Table I-4). The decreases were significant. In case of un-inoculated plants, the decrease was much higher than those of inoculated especially at moderate salinity level (8 dS/m). But, at the highest salinity level, the decrease in plant dry weight was as similar as those of inoculated plant. The salinity level of 8 dS/m decreased un-inoculated plant dry weight by 43.93%, while, at the inoculation with the isolates of R2 and R6 which isolated from salt-affected soils, the decreases were 25.34% and 15.82%, respectively. At the same time, the inoculation with these isolates resulted in the highest plant dry weight at both normal and salinity circumstances. The differences, mostly, were significant.

Table I-3: Effect of salinity levels on the N-content (mg/plant) of faba bean under inoculation with different rhizobial isolates.

Treatments	N-content (mg/plant)				
	Salinity levels				
	Control	4 dS/m	8 dS/m	12 dS/m	Mean
Un-inoculated	26.20d	17.35d	12.46d	9.37c	16.34d
R1	27.00d	22.20cd	20.03c	10.99bc	20.06c
R2	46.70a	34.75ab	27.71ab	19.67a	32.21a
R3	36.12c	27.85bc	20.48bc	11.50bc	23.99b
R4	36.89c	22.73cd	22.36bc	14.02abc	24.00b
R5	38.10c	32.67ab	19.74cd	14.84abc	26.34b
R6	46.10ab	35.64a	32.30a	18.53ab	33.14a
R7	37.19bc	31.46ab	19.80cd	14.63bc	24.84b
R8	36.80bc	29.40 ab	19.10cd	14.24bc	25.10b
Mean	36.78	28.24	21.55	13.41	25.17
Comparison		LSD 5%		LSD 1%	
2-S means at each I		6.9880		9.3060	
2-S means		2.3292		3.1020	

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

I : Inoculation S : Salinity R: Rhizobial isolate

Naeem *et al.* (2008) concluded that dry weight of pea plants decreased with increase of salinity. This finding coincided with the results of the present study. Therein results also showed that inoculation of the plant with the tolerant compatible rhizobia increased plant growth and potentially increased its tolerance to moderate concentrations of salinity. While in the presence of high salinity concentrations, there were no significant differences not only between the inoculated and un-inoculated plants but also between the plants inoculated with the different rhizobial isolates. This may be due to the drastic effect of high salinity level on the symbiotic relationship between the plant and rhizobia. Elshiekh and Wood (1990) reported that nodulation and nitrogen fixation activities are apparently more sensitive to salt than plant growth. Another cause of unsuccessful nodulation could be related to the inhibition by salt of one or more steps of the early events of the interaction symbiotic process. In a study of Naeem *et al.* (2008), they noted that 15 dS/m

soil salinity completely impaired nodules formation on inoculated pea plants with respective rhizobia. This confirm the beneficial and relief role of inoculation with salt-tolerant isolates on plant growth under salinity stress. The relief effect may resulted from supplementing the macrosymbiont with their needs of nitrogen in the suitable dose and time (Anjum *et al.*, 2006), release of growth promoting plant phyto-hormones (Vessey, 2003), in addition to release of bacteriocides offering the plant more resistance against pathogens (Gross and Vidaver, 1978). As shown in the present study, inoculation of faba bean plants with the tolerant rhizobia increased proline concentration in the plant (Table II-7). The increase of proline concentration in plant tissue potentially increased plant tolerance to salinity (Zahran, 1999).

The results obtained from Tables II-1 to II-3 indicated that inoculation of faba bean plants, generally, increased the plant tolerance against salinity. These results, also, indicated that rhizobial isolates bringing from saline soils especially those of R2 and R6, were more efficient in this respect. Thus, these two isolates were chosen for inoculation of faba bean plants in pots full with clay soil salinized with different concentrations of NaCl in presence or absence of inoculation with A-mycorrhizae, in order to address the role of these inoculants in ameliorating the drastic effect of salinity on the plant.

Data of nodules dry weight (g/plant) recorded in Table II-1 indicated that the un-inoculated N-fertilized treatment (100% N) gave the lowest nodules dry weight followed by that fertilized with 25% N under both normal and saline conditions, while the inoculation with mycrorrhizae improved the plant nodule dry weight ((0.48 for mycorrhizae treatment against 0.12 for 100% N treatment). A similar trend was found under saline condition, for example, at 12 dS/m mycorrhizal inoculation treatment attained 0.2 compared to 0.03 (g/plant) due to 100%N treatment. The best treatments improved nodules dry weight were inoculation with R2 under normal conditions and R2+M under saline condition. R2 achieved 0.63 compared to 0.12 and 0.24 for 100% N and 25% N treatments respectively. R2+M treatment attained the highest dry weight of nodules under 4 and 8 dS/m soil salinity levels (0.51 and 0.46, respectively). While, under the highest salinity (12 dS/m) concentration, R2 attained 0.33 followed by R2+M with 0.30. The salinity of soil caused sever deterioration in nodules dry weight reached to complete impaire of their formation for N-fertilized treatments under the highest salinity concentration (12 dS/m). Comparably, deterioration degree lowered due to inoculation treatments, whereas, rhizobia were able to form nodules until 12 dS/m salinity concentration. In this context, at 8 dS/m salinity, nodules dry weight for 100% N treatment decreased by 75%, while, the decrease reached about 20.7% only due to inoculation with R2+M treatment.

Table I-4: Effect of salinity levels on the faba bean dry weight (g/plant) of faba bean under inoculation with different rhizobial isolates.

Treatments	Dry weight (g/plant)				
	Salinity levels				
	Control	4 dS/m	8 dS/m	12 dS/m	Mean
Un-inoculated	1.35d	1.118c	0.757d	0.691a	0.980f
R1	1.46cd	1.206bc	1.090bc	0.687a	1.112e
R2	1.83a	1.441a	1.220b	0.899a	1.349ab
R3	1.71ab	1.430a	1.096bc	0.799a	1.261bc
R4	1.67abc	1.126c	1.052bc	0.718a	1.142de
R5	1.56bc	1.387ab	0.945cd	0.793a	1.172cde
R6	1.77ab	1.511a	1.490a	0.914a	1.422a
R7	1.61bc	1.476a	1.100bc	0.762a	1.237cd
R8	1.60bc	1.377ab	1.061bc	0.762a	1.2022cde
Mean	1.62	1.341	1.090	0.7806	1.2091
Comparison		LSD 5%		LSD 1%	
2-S means at each I		0.2025		0.2678	
2-S means		0.0675		0.0893	

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

I : Inoculation S : salinity R: rhizobial isolate

Dry weight of nodules decreased due to the treatment 100%N than the treatment of 25% N and other treatments. The reason may be due to the presence of nitrogen element in soil with high amounts which affect nodule formation (Burity *et al.*, 1999 & Tham and Tham, 2007). Likewise, mycorrhizal inoculation had stimulated effect on nodules dry weight, this finding agreed with those of Li-ShuMin, *et al.* (2004) who indicated that dual inoculation of faba bean plants with AM fungi and *Rhizobium leguminosorum* significantly increased the number and weight of nodules. the reason may attributed to *Rhizobium* symbiosis is dependant on high concentrations of P, where AM induced P nutrition and consequently increased nodulation and N_2-fixation (Va´zquez *et al.* (2002). The inoculation with tolerant rhizobia and /or mycorrhizae attained positive effect under salt-stress much more than their effect under normal conditions. The increase in nodules dry weight under 8 dS/m was about 13 fold due to inoculation with R2 and 15 fold for R2+M treatments over un-inoculated N-fertilized treatment. The reasons for these beneficial effect may be attributed to increasing number of tolerant, efficient, infective and competitive rhizobia into the rhizosphere of the plant which optimized symbiotic relationship and nodule formation. In addition, the mixed inoculation with mycorrhizae and rhizobia had an important role in supplement the host plant with their requirements from elements leading to good performance for symbiotic process (Ibibijen *et al.*, 1996). While, under salinity stress, the selected tolerant isolates used in the plant inoculation ameliorate the drastic effect of salinity on the plant (Estévez *et al.*,2009), in addition to release of plant phytohormones (Vessey, 2003) which promote plant growth and increased surface area of root giving the plant more ability to explore the soil and acquisition the nutrients , thus may inducing plant tolerance against salinity.

Table II-1: Influence of salinity levels on the dry weight of nodules (g/plant) of faba bean under inoculation with salt-tolerant *Rhizobium* isolates and/or A-mycorrhizae.

Treatments	Dry weight of nodules (g/plant)				
	Salinity levels				
	Control	4 dS/m	8 dS/m	12 dS/m	Mean
25% N	0.24e	0.11b	0.04f	0.00c	0.098c
100% N	0.12f	0.08d	0.03f	0.00c	0.062c
M	0.48d	0.31c	0.20e	0.00c	0.248c
R2	0.63a	0.44b	0.40cd	0.33a	0.45a
R6	0.55c	0.43b	0.37d	0.27b	0.40b
R2+M	0.58b	0.51a	0.46a	0.30ab	0.46a
R6+M	0.55bc	0.45b	0.41bc	0.29ab	0.42ab
R2+R6+M	0.55bc	0.44b	0.44ab	0.30ab	0.43ab
Mean	0.46	0.34	0.29	0.18	0.32
Comparison		LSD 5%		LSD 1%	
2-S means at each I		0.30		0.041	
2-S means		0.106		0.0144	

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

I : Inoculation S : Salinity R: *Rhizobium* isolate M : Mycorrhizae

Figures recorded in Table II-2 indicated that inoculation of faba bean plants with salinity tolerant rhizobial strains and/or mycorrhizae significantly increased seeds yield of the plant, and these increases were significant under normal conditions and salinity levels 4 and 8 dS/m, while, the increases did not reach to significance at 12 dS/m. The treatment of R2+R6+M gave the highest yield under 4, 8 dS/m and control, the readings reaches 9.64, 11.52 and 15.62 compared to 8.07, 10.7 and14.3 (g/plant) for 100% N treatment respectively. Salinity concentration of 8 dS/m lowered seed yield for plants of 100% N treatment by a percentage reached 43.7, while their yield lowered by 38.28% due to application of R2+R6+M treatment.

The effective role of inoculation of the faba bean plant with salt-tolerant rhizobia and/or mycorrhizae was much evident under normal conditions or moderate salinity degrees (6 and 8 dS/m), but the promoting effect disappeared under the highest salinity level (12 dS/m). The reason may be attributed to the influence of the introduced inoculants with the high levels of salinity as similar as the macrosymbiont (Zahran, 1999). Whereas, the level of 12 dS/m completely suppressed the endogenous rhizobia reflecting absence of nodules on the un-inoculated N-fertilized plants. But, with the inoculation with the tolerant rhizobial isolates, there were few nodules appeared, their efficiency may also be heavily reduced. Thereby, their positive effect on the host plant did not clearly appeared under this sever condition. These results were in harmony with those of Naeem *et al.* (2008) study, who concluded that high salinity concentrations (15 dS/m) drastically affected nodulation and yield of pea plant. Thus, we report that, under high salinity levels (more than 12 dS/m), faba bean plants should fertilized with their recommended dose of nitrogen rather than inoculation with un-tolerant rhizobia. Grover *et al.* (2010) reported that microorganisms can impart some degree of tolerance to plants towards abiotic stresses like salinity, They added that bacteria belonging to different genera including *Rhizobium*,

Bacillus, Pseudomonas, Pantoea, Paenibacillus, Burkholderia, Achromobacter, Azospirillum, Microbacterium, Methylobacterium, Variovorax, Enterobacter etc. have been reported to provide tolerance to host plants under different abiotic stress. Microorganisms can elicited stress tolerance in plant by a variety of mechanisms: Production of indole acetic acid, gibberellins and some unknown determinants by PGPR, result in increased root length, root surface area and number of root tips, leading to enhanced uptake of nutrients thereby improving plant health under stress conditions (Egamberdieva and Kucharova 2009). Some PGPR strains produce cytokinin and antioxidants, which result in abscisic acid (ABA) accumultion and degradation of reactive oxygen species. High activities of antioxidant enzymes are linked with oxidative stress tolerance (Stajner *et al.* 1997). Production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, conferred stress tolerance against drought and salt in pepper and tomato (Mayak *et al.* 2004a). Inoculation with ACC deaminase containing bacteria induce longer roots which might be helpful in the uptake of relatively more water from deep soil under drought stress conditions, thus increasing water use efficiency of the plants under drought conditions (Grover *et al.*, 2010).

Table II-2: Influence of salinity levels on the seeds yield (g/plant) of faba bean under inoculation with salt-tolerant *Rhizobium* isolates and/or A-mycorrhizae.

Treatments	Seeds yield (g/plant)				
	Salinity levels				
	Control	4 dS/m	8 dS/m	12 dS/m	Mean
25% N	11.86c	8.90c	6.467c	4.133b	7.842d
100% N	14.13b	10.70b	8.067b	4.993ab	9.473c
M	14.16b	10.63b	8.110b	4.953ab	9.466c
R2	15.26a	11.76a	9.233a	5.047ab	10.327b
R6	15.30a	12.10a	8.660ab	5.700a	10.440ab
R2+M	15.09a	12.20a	9.230a	5.700a	10.557ab
R6+M	15.70a	11.74a	9.550a	5.833a	10.708a
R2+R6+M	15.62a	11.52ab	9.640a	5.767a	10.640ab
Mean	14.64	11.195	8.62	5.266	9.932
Comparison		LSD 5%		LSD 1%	
2-S means at each I		0.989		1.319	
2-S means		0.350		0.466	

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

I : Inoculation S : Salinity R: *Rhizobium* isolate M : Mycorrhizae

The plant nitrogen content severely affected by salinity (Table II-3), whereas, the N-content due to 100% N and 25% N treatments under 8 dS/m decreased by 59.2% and 61.05%, respectively compared to 53.8 for R2 treatment and 54.06 for R2+R6+M one. On the other hand, the inoculation with the different treatments notably increased seeds N-content under normal conditions and in presence of 4 and 8 dS/m salinity levels over un- inoculated N-fertilized treatments. The variations were mostly significant. But, at the highest concentration (12 dS/m), the variations were not significant.

P-content of faba bean seeds raised due to the different inoculation treatments (Table II-4). The positive effects were obvious at normal

conditions (control), as all inoculation treatments attained significant variations than the un-inoculated treatments. Under, salinity levels of 4 and 8 dS/m, they, also, gave increases in seeds P-content, but, the variations were not consistent. While, under concentration of 12 dS/m there were no any significant variations between all treatments. In general, salinity caused sever harmful effect on P-content of seeds regardless presence or absence of inoculation.

Inoculation of faba bean plants with rhizobia and/or mycorrhizae had obvious effect in increase of seeds K-content (Table II-5), the increases were significant under normal condition. The promoting effect of inoculation lowered with the increase of salinity. However, the treatments R6+M and R2+R6+M showed significant increases over un-inoculated treatments (100%N an 25%N) under all studied levels of salinity. On the other hand, salinity largely decreased seeds K-content, the decreases values were significant.

Table II-3: Influence of salinity levels on the seeds N-content (mg/plant) of faba bean under inoculation with salt-tolerant *Rhizobium* isolates and/or A-mycorrhizae.

Treatments	N-content (mg/plant)				
	Salinity levels				
	Control	4 dS/m	8 dS/m	12 dS/m	Mean
25% N	336.56 d	207.56d	131.10d	72.03a	186.81
100% N	427.23 b	289.16bc	174.3bcd	98.77a	247.38
M	382.2 c	259.66c	166.10cd	87.50a	223.86
R2	491.53 a	340.43a	227.10a	106.13a	291.30
R6	482.0 a	337.10a	208.8abc	108.63a	284.15
R2+M	487.43 a	336.10a	212.20ab	114.30a	287.50
R6+M	475.4 a	322.00ab	232.26a	114.06a	286.05
R2+R6+M	483.3 a	312.90ab	222.03a	108.60a	281.70
Mean	445.77	300.61	196.75	101.25	261.09
Comparison		LSD 5%		LSD 1%	
2-S means at each I		42.229		56.335	
2-S means		1.493		19.920	

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

I : Inoculation S : Salinity R: *Rhizobium* isolate M : Mycorrhizae

The inoculation with rhizobia and/or mycorrhizae potentially increased NPK-contents of the plant seeds under normal conditions and moderate saline concentrations (4 and 8 dS/m). These results were in agreement with Vance (2001) who reported that inoculation with rhizobia and mycorrhizae greatly increased plant uptake of N, P and K, these inoculants promote plant growth. These microorganisms increased plant uptake of elements directly through N_2-fixation and solubility of un-available phosphates, and indirectly by improving plant growth (Kremer and Peterson, 1983) and increased plant protection against different pathogens (Vance, 2001). Therefore, increased root distribution and root efficiency in elements uptake.

Table II-4: Influence of salinity levels on the P-content (mg/plant) of seeds under inoculation with salt-tolerant *Rhizobium* isolates and/or A-mycorrhizae.

Treatments	P-content (mg/plant)				
	Salinity levels				
	Control	4 dS/m	8 dS/m	12 dS/m	Mean
25% N	20.17b	13.3b	7.53c	3.97a	11.25c
100% N	19.53b	13.9b	9.70bc	4.97a	12.0c
M	26.8a	15.9ab	10.8abc	5.60a	14.7b
R2	26.9a	16.1ab	12.00ab	5.47a	15.1ab
R6	28.0a	17.7a	11.00ab	5.63a	15.5ab
R2+M	27.7a	19.2a	11.70ab	6.77a	16.3ab
R6+M	28.9a	18.8a	13.00ab	6.97a	16.9ab
R2+R6+M	26.7a	18.0a	13.80a	6.37a	16.2ab
Mean	25.6	16.6	11.20	5.72	14.79
Comparison		LSD 5%		LSD 1%	
2-S means at each I		3.03		4.04	
2-S means		1.07		1.43	

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

I : Inoculation S : Salinity R: *Rhizobium* Isolate M : Mycorrhizae

Records of Table II-6 indicated that inoculation with rhizobia and/or mycorrhizae did not significantly affect Na-content of faba bean seeds under normal conditions, while, when salinity increased to 4 and 8 dS/m, the inoculation treatments of R2+M, R6+M and R2+R6+M attained significant increases in Na-content. But, at 12 dS/m level, there were no significant differences due to these treatments. On the other hand, a notable decrease in seeds Na-content occurred with the increase of salinity concentrations. These decreases were lowered in case of inoculation than those of un-inoculation treatments.

The absence of significant increases in Na under normal condition elucidate that Na was present in soil with low amounts, thus did not absorb by notable degree even in the presence of inoculants which aid in improving plant growth and absorption of elements. While, with increase of soil salinity, the inoculation caused significant increases in Na absorption especially at 4 dS/m. The reason may be due to the presence of Na in soil with remarkable amounts, and plants and rhizospheric microorganisms still un-injured. Thus, improving plant root efficiency for element absorption due to microbial inoculation, thereby, increased Na uptake too. This postulate assured because of absence of significant increases in the seeds at salinity level of 12 dS/m, whereas, the high concentration of salinity negatively affected microbial inoculants efficiency and potentiality of plant elements absorption.

Table II-5: Influence of salinity levels on the K-content (mg/plant) of faba bean seeds under inoculation with salt-tolerant *Rhizobium* isolates and/or A-mycorrhizae.

Treatments	K-content (mg/plant)				
	Salinity levels				
	Control	4 dS/m	8 dS/m	12 dS/m	Mean
25% N	152.0c	106.0d	73.0c	43.0c	93.9
100% N	186.0b	128.0c	92.0b	56.0abc	115.8c
M	187.0b	129.0bc	95.0b	51.0bc	115.0c
R2	206.0a	150.0a	111.0a	55.0abc	130.0b
R6	207.0a	150.0a	106.0ab	61.0ab	131.0b
R2+M	204.0a	152.0a	111.0a	65.0ab	133.0ab
R6+M	213.0a	141.0ab	114.0a	67.0a	134.0ab
R2+R6+M	213.0a	146.0a	118.0a	68.0a	136.0a
Mean	196.5	138.0	102.0	58.5	124.0
Comparison		LSD 5%		LSD 1%	
2-S means at each I		14.0		18.70	
2-S means		4.95		6.61	

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

I : Inoculation S : Salinity R: *Rhizobium* isolate M : Mycorrhizae

Inoculation with rhizobia and/or mycorrhizae significantly raises prolein concentration in the plant seeds (Table II-7). The promoting effect of proline increased with the increase of salinity. Likewise, R2+R6+M treatment achieved the highest proline level in the plant seeds under salinity condition. On the other hand, proline content increased with the increase of salinity levels. The increases at 12 dS/m were 74.3 due to the treatment of 100% N compared to 83.3% resulted from the treatment of R2+R6+M

Proline level in the plant seeds significantly increased with increase of salinity levels. Similar results were shown by Jin *et al.* (2010) who found that proline concentration in *Lathyrus sativus* leaves was significantly higher in salt-stressed conditions than unstressed condition. Ozturk and Demir (2002) concluded that proline is known to occur widely in the higher plants and normally accumulates in larg quantities in response to environmental stress. Sheteawi and Tawfik (2007), also, indicated that proline content generally increased in plants due to stress and the accumulation of proline may improve the cytoplasmic osmoregulation and thus, increase plant tolerance. On the other hand, inoculation with rhizobia and/or A-mycorrhizae significantly increased proline concentration compared to control. These results agreed with the findings of Sheteawi and Tawfik (2007) who showed that biofertilized plants revealed higher values of these metabolic products than non fertilized plants as their response for ameliorating and stimulating effect. Proline concentration regulation may be one of the mechanisms by which AM symbiosis can enhance host plant (Kaya *et al.*, 2009).

Table II-6: Influence of salinity levels on Na% and Na-content (mg/plant) of faba bean seeds under inoculation with salt-tolerant *Rhizobium* isolates and/or A-mycorrhizae.

Treatments	Na-content (mg/plant)				
	Salinity levels				
	Control	4 dS/m	8 dS/m	12 dS/m	Mean
25% N	9.10a	8.92c	8.32b	6.13a	8.12e
100% N	10.72a	9.99bc	9.38ab	6.30a	9.10cd
M	10.79a	9.13c	9.15ab	5.73a	8.70de
R2	9.66a	11.71ab	10.12ab	5.69a	9.29bcd
R6	9.67a	12.38a	9.76ab	7.27a	9.77abc
R2+M	10.56a	12.75a	10.61a	6.91a	10.21ab
R6+M	9.80a	12.83a	10.88a	6.90a	10.13ab
R2+R6+M	10.90a	12.43a	10.79a	7.37a	10.37a
Mean	10.16	11.27	9.88	6.53	9.46
Comparison		LSD 5%		LSD 1%	
2-S means at each I		2.040		2.722	
2-S means		0.721		0.952	

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

I : Inoculation S : Salinity R: *Rhizobium* isolate M : Mycorrhizae

Table II-7: Influence of salinity levels on the proline (mg/g dry wt.) of faba bean under inoculation with the salt-tolerant *Rhizobium* isolates and/or A-mycorrhizae.

Treatments	Proline (mg/g dry wt.)				
	Salinity levels				
	Control	4 dS/m	8 dS/m	12 dS/m	Mean
25% N	0.33e	0.40g	0.58f	0.61e	0.480
100% N	0.35d	0.42f	0.60e	0.61e	0.495
M	0.36c	0.43e	0.62c	0.63d	0.510
R2	0.355cd	0.44d	0.62c	0.64c	0.511
R6	0.36c	0.42f	0.61d	0.64c	0.522
R2 +M	0.367c	0.50c	0.64a	0.67b	0.554
R6+M	0.38a	0.51b	0.63b	0.68a	0.567
R2+R6+M	0.37b	0.52a	0.65a	0.68a	0.555
Comparison		LSD 5%		LSD 1%	
2-S means at each I		0.0091		0.0121	
2-S means		0.003		0.0042	

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

I : Inoculation S : Salinity R: *Rhizobium* isolate M : Mycorrhizae

CONCLUSION

It can be concluded that rhizobial isolates varied in their tolerance to salinity. The isolates bringing from salt soils had more tolerance over those isolated from normal soil. The plant exhibited the best growth and elements uptake (N, P and K-contents) when inoculated with the mixture of the tolerant rhizobia and mycorrhizae, especially R2+M treatment. Thus, we urged farmers to applying salt-tolerant inoculums in agricultural practices in order to increasing productivity of the crop under salinity condition.

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عزل و تقييم الميكروبات المتحملة للملوحة وتأثيرها على تحمل نبات الفول البلدي للملوحة

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تهدف الدراسة الحالية الى انتخاب ميكروبات ريزوبيا الفول البلدي المتحملة للملوحة عن طريق عزلها من تربة متحملة بالملوحة لمنطقة شمال دلتا مصر، و قيمت لتحديد كفاءتها و تحملها للملوحة، و بعد ذلك تم اختيار أفضل العزلات كفاءة و تحملا للملوحة و استخدمت مع الميكورهيزا الداخلية لتلقيح نباتات الفول البلدي في تجربة أصص لمعرفة تأثيرها المخفف للملوحة على النبات.

اختلفت عزلات الريزوبيا في درجة تحملها للملوحة و أظهرت العزلات المجلوبة من اراضي ملحية تحملا أكبر عن تلك المعزولة من تربة نظيفة. كانت العزلات R2 و R6 الاكثر تفوقا حيث منحت النبات أعلى قيم لوزن العقد الجاف ووزن النبات الجاف والمحتوى من النيتروجين للنبات. أعطت النباتات أعلى نمو و امتصاص للعناصر (محتوى النبات من N، P، K) عند تلقيحها بمخلوط الريزوبيا المتحملة و الميكورهيزا الداخلية خصوصا معاملة R2+M. لذلك فاننا نحث المزارعين لتلقيح الفول البلدي بالريزوبيا المتحملة لزيادة انتاجية المحصول المنزرع باراضي متأثرة بالملوحة. و يجب اجراء المزيد من الدراسات لاستكشاف الفوائد العظيمة للميكروبات لتخفيف الضغوط المختلفة على النبات.

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