

## EFFECT OF SOIL SALINITY ON SURVIVAL AND PERFORMANCE OF ALFALFA RHIZOBIA

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### ABSTRACT

The effect of salt stress on the growth and survival of four introduced *Sinorhizobium meliloti* strains in the rhizosphere of alfalfa *Medicago sativa* L. was studied. Response to inoculation with these strains and the competition among them and against native rhizobia were investigated in two salt affected soils with different salt concentrations of 10 and 36 dS m<sup>-1</sup>. Results showed that none of the introduced rhizobial strains was affected by the soil salinity up to 36 dS m<sup>-1</sup> as they were able to grow and survive in the rhizosphere of alfalfa plants. However, nodule formation by both native and introduced rhizobial strains was negatively affected by salt concentration. This indicates that the first step in nodule formation is extremely sensitive to salinity, likely due to the effect of salinity on the root infection sites but not on the survival of rhizobia. Acetylene reduction of nodulated roots and nitrogen accumulation were adversely affected by salt concentration in the soil. This may show the negative effect of salt stress on plant growth and subsequently nodule function. Patterns of rhizosphere colonization by the introduced rhizobial strains declined markedly with increased salt concentration of the soil. Data showed that the introduced rhizobial strains could grow, survive and fix nitrogen at salt concentrations inhibitory to the growth of alfalfa. Therefore, we recommend that the future studies on the effect of salinity on symbiotic N<sub>2</sub> fixation should focus more on aspects related to the symbiosis rather than the study of each separate partner.

**Keywords:** Alfalfa, N<sub>2</sub>-fixation, nodulation, saline soils, competition, survival of rhizobia.

### INTRODUCTION

Salinity is a serious threat to agriculture in arid and semiarid regions (Rao and Sharma, 1995). Nearly 40 % of the world's land surface can be categorized as having potential salinity problems (Cordovilla *et al.*, 1994). Most of these areas are confined to the tropics and Mediterranean regions. Increases in the salinity of soils or water supplies used for irrigation result in decrease the productivity of most crop plants and lead to marked changes in the growth pattern of plants (Cordovilla *et al.*, 1994). Increasing salt concentrations may have a detrimental effect on soil microbial populations as a result of direct toxicity as well as through osmotic stress (Tate, 1995). Soil infertility in arid zones is often due to the presence of large quantities of salt, and the introduction of plants capable of surviving under these conditions (salt-tolerant plants) is worth investigating (Delgado *et al.*, 1994). There is currently a need to develop highly salt-tolerant crops to recycle agricultural drainage waters, which are literally rivers of contaminated water that are generated in arid zone irrigation districts (Glenn *et al.*, 1999). Salt tolerance in plants is a complex phenomenon that involves morphological and developmental changes as well as physiological and biochemical processes.

Salinity decreases plant growth and yield, depending upon the plant species, salinity levels, and ionic composition of the salts (Delgado *et al.*, 1994).

The legume-*Rhizobium* symbiosis and nodule formation on legumes are more sensitive to salt or osmotic stress than are the rhizobia (Zahran, 1991). Salt stress inhibits the initial steps of *Rhizobium*-legume symbiosis. The effect of salt stress on nodulation and nitrogen fixation of legumes have been examined in several studies (Abdel-Wahab *et al.*, 1991; Delgado *et al.*, 1994; and Ikeda *et al.*, 1992). The reduction of N<sub>2</sub>-fixing activity by salt stress is usually attributed to a reduction in respiration of the nodules (Waish, 1995) and a reduction in cytosolic protein production, specifically leghemoglobin, by nodules (Delgado *et al.*, 1994). The depressive effect of salt stress on N<sub>2</sub> fixation by legumes is directly related to the salt-induced decline in dry weight and N content in the shoot (Correa and Barneix 1997). The salt-induced distortions in nodule structure could also be reasons for decline in the N<sub>2</sub> fixation rate by legumes subject to salt stress (Zahran and Abu-Gharbia, 1995). Reduction in photosynthetic activity might also affect N<sub>2</sub> fixation by legumes under salt stress (Georgiev and Atkias, 1993).

Although the root nodule-colonizing bacteria of the genera *Rhizobium* and *Bradyrhizobium* are more salt tolerant than their legume hosts, they show marked variation in salt tolerance. Growth of a number of rhizobia was inhibited by 100 mM NaCl (Yelton *et al.*, 1983), while some rhizobia, e.g., *Sinorhizobium meliloti* were tolerant to 300 – 700 mM NaCl (Embalomatis *et al.*, 1994). Salt-tolerant strains of *Rhizobium* can nodulate legumes and form effective N<sub>2</sub>-fixing symbiosis in soils with moderate salinity. Therefore, inoculation of various legumes with salt-tolerant strains will improve N<sub>2</sub> fixation in saline environments (Zou *et al.*, 1995). However, tolerance of the legume host to salt is the most important factor in determining the success of compatible *Rhizobium* strains to form successful symbiosis under conditions of high soil salinity (Criag *et al.*, 1991). Evidence presented in the literature suggests a need to select plant genotypes that are tolerant to salt stress and then match them with the salt tolerant and effective strain of rhizobia (Cordovilla *et al.*, 1995). In fact, the best results for symbiotic N<sub>2</sub> fixation under salt stress are obtained if both symbiotic partners and all the different steps in their interaction (nodule formation, activity, capacity of nitrogen fixation, etc.) resist such stress (Georgiev and Atkias, 1993; & Zahran *et al.*, 1994).

In Saudi Arabia, salt affected soils occupy vast areas especially in the eastern part. In addition, spots of deteriorated soils are now scattered along the north and middle area (Bashour *et al.*, 1983). Such saline conditions may limit the symbiosis through: (1) affecting survival and proliferation of rhizobia in the soil and rhizosphere, (2) inhibiting the infection process, (3) directly affecting root nodule function, (4) reducing plant growth, photosynthesis, and demand for nitrogen (Criage *et al.*, 1991).

The aim of the present study is to investigate the effect of salt stress on the growth and survival of four *Sinorhizobium meliloti* strains in the rhizosphere of alfalfa *Medicago sativa* L. The establishment of inoculant

strains in alfalfa nodules and their efficiency in relation to indigenous rhizobia was also evaluated.

## MATERIALS AND METHODS

The experiments were conducted in the greenhouse of Agriculture College, King Saud Univ., Saudi Arabia. Two salt affected soils from AL-Qassiem were included in the study. Soil samples were collected from two sites. Site I is 48 Km north Buraydah, 3 Km east Buraydah Hail road, while site II is 48 Km north Buraydah, 3 Km west Buraydah Hail road. The values of paste extract electrical conductivity (EC) in these sites were 10 and 36 dS m<sup>-1</sup>, respectively. The physical and chemical analyses of the soils are presented in Table 1. Each soil was put in plastic pots of 30 cm in diameter. Every pot was filled with 10 Kg of the previously mentioned soils. Four strains of *Sinorhizobium meliloti* were used either singly or in combination. Alfalfa seeds *Medicago sativa* L. were surface sterilized. Peat based inoculums was used to inoculate seeds before planting. Each pot was planted with 250 mg (60 kg hectare<sup>-1</sup>) of alfalfa seeds. Four replicates were used for each treatment. Water was added to be stable at 60 % of soil water holding capacity (17.5%).

### Rhizobial strains:

Four strains of *Sinorhizobium meliloti* were used in the present study. Two strains were local and isolated from host Lucerne grown on Saudi Arabian soils. These strains were isolated, purified, and stored according to the protocol of Allen (1971). The other two strains were foreign strains TAL 1372 and TAL 1373. These strains were kindly supplied from Nifal project, Hawaii, USA.

**Table 1: physical and chemical properties of the soils.**

Determinations	Site I	Site II
Sand %	65	63
Silt %	24	19
Clay %	11	18
Textural class	Sandy loam	Sandy loam
pH*	8.2	7.5
E.C.** (dS m <sup>-1</sup> )	10.00	36.00
CaCO <sub>3</sub> %	20.0	17.0
Organic matter (%)	0.39	0.26
Total nitrogen (ppm)	312.00	265.00

\* In soil paste.

\*\* In soil paste extract.

### The treatments were as follows:

1. Uninoculated control.
2. Inoculated with strain No. 1 (local isolate).
3. Inoculated with strain No. 2 (local isolate).
4. Inoculated with strain TAL 1372.
5. Inoculated with strain TAL 1373.
6. Inoculated with a mixture of four strains.

Shoot was harvested in three cuts after 75, 110 and 145 days from planting. Nitrogen content of shoot was determined according to Jackson (1973). At each cut, 10 alfalfa plants were taken out to determine nodule fresh weight and nitrogenase activity in nodules according to the method described by Hardy *et al.* (1973). Statistical analyses were carried out for the studied parameters according to Snedecor and Cochran (1978).

Rhizosphere samples were taken periodically 7, 15, 30 and 45 days after planting to determine the population densities of the four introduced rhizobial strains. The procedure of Kingsley and Bohlool (1981) was used to separate the rhizobial strains from the rhizosphere soils using gelatin-ammonium phosphate. Soil-buffer mixtures were treated with 5 drops of tween 80 and shaken for 30 min. after being shaken, inner rhizosphere soil suspensions were sub sampled for dry weight determination, and samples of all suspensions were sedimented by centrifugation at 5000 rpm for 15 min. Appropriate volumes (1-3 ml) of each supernatant were passed through polycarbonate membrane filters pretreated with irgalan black to reduce auto fluorescence. Material retained on the membrane filter was treated with gelatin-rohdamine conjugate (Bohlool and Schmidt, 1968), before the application of fluorescence antibodies (FAs) to avoid non-specific absorption and to improve the background contrast of the membrane filter. Procedure of FA staining and microscopic enumeration of fluorescing rhizobial cells were carried out as described by Schmidt (1974).

#### **Nodule serotyping:**

Strain specific fluorescent antibodies (FAs) were used for nodule serotyping. Gelatin-Rhoda mine conjugate (Bohlool and Schmidt, 1968) was used to control non-specific staining and auto fluorescence. Fifty nodules from each treatment were carefully washed and crushed in sterilized water. The slides were air dried and heat fixed. With a Pasteur pipette, a drop of gelatin-Rhoda mine conjugate was placed on the smears. Before the Rhoda mine gel was dried, one drop of FA stain was added. Stained nodule smears were examined with a Zeiss universal microscope equipped for epifluorescence and phase contrast. A strong positive reaction was indicated by brilliant yellow green fluorescence of the smear on a dark purple background. No cells would be visible if the specific strain was not present on the smear. The presence of more than one serogroup per nodule was detected by using the dual lighting system of reflected fluorescent and transmitted phase-contrast light. The switching from phase-contrast to fluorescent light, clearly shows the presence of one or more than one strain in the same nodule. The dominance of one strain over the other within the same nodule was based on the ratio between the stained and non stained cells with each FA. Nodule smears with 5% or more of non fluorescing cells in the presence of FA-positive cells were considered evidence of a mixed infection.

## RESULTS AND DISCUSSION

### Dry matter yield:

Yield responses of alfalfa to inoculation with four strains in two salt affected soils are presented in Fig. 1. Results clearly show that the alfalfa dry matter yield decreased with increasing soil salinity. These results are in harmony with those of Delgado *et al.*, (1994) and Cordovilla *et al.*, (1995) who stated that salts induced decline in dry weight of four grain legumes.

Data in Fig. 1 also show that, inoculation of alfalfa with *Sinorhizobium meliloti* strains induced a significant increase in dry matter yield as compared with uninoculated treatments. These results are in accordance with the finding of Young *et al.* (1986) and El-Mokadem *et al.*, (1991) and contradict with results of Gaur and Lowther (1982) and Sheath *et al.* (1984).

Significant differences in alfalfa dry matter production were observed with the different inoculated treatments (Fig. 1). In all inoculated treatments, local strain No. 2 was superior to other tested strains in the three cuts. Multistrain inoculant gave the lowest dry matter yield as compared with the single strain inoculants. However, multistrain still gives significantly higher yields than the uninoculated treatments. This may be due to the interactions between the four strains within the host roots and the competition between them for the energy required for their nodule maintenance. It is also clear from Fig. 1, that the dry matter yield increased with inoculation even at the highest salt concentration, however, the yield obtained at higher salt concentration ( $36 \text{ dS m}^{-1}$ ) was much lower than the less salt affected soil ( $10 \text{ dS m}^{-1}$ ). This indicates that alfalfa growth was more affected by the salinity than the inoculant rhizobia. These results are in agreement with those of Cordovilla *et al.*, (1995) who indicated that many strains of rhizobia can grow and survive at salt concentrations which are inhibitory to their legume hosts.

### Nodule fresh weight:

Results presented in Fig. 2, clearly show that the uninoculated plants recorded the lowest nodule fresh weight. However, inoculation with rhizobia exerted significant increase in nodule fresh weight. This was correlated with the data of dry matter yield, with local strain No. 2 being the highest nodule forming strain throughout the growth of alfalfa followed by strains TAL 1373, local strain No. 1, TAL 1372 and multistrain inocula in respective order.

Regarding the effect of salt stress on nodulation, a significant decrease in nodulation was observed with increased salt stress. This indicates that the nodule formation by both native and introduced rhizobial strains was affected by salt stress. This may be due to the effect of high salt content on the proliferation of rhizobia in the rhizosphere and/or to the inhibition of the infection process. These results are in line with those of Bajpai and Gupta (1979), and Singleton and Bohlool (1984) who found that nodule numbers and weight were reduced by increasing salt concentration. They attributed this to the salt sensitivity of root infection sites. In this respect, Zahran and Sprent (1986) stated that salinity stress showed little curling or deformation of root hairs. These results are also in accordance with

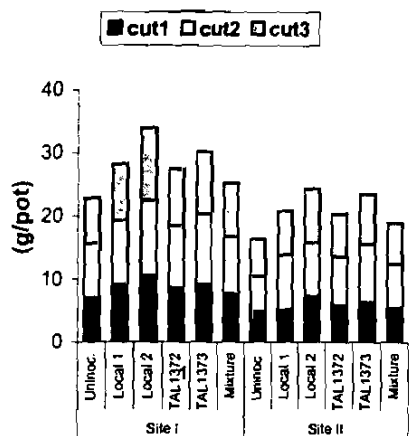


Fig.(1): Dry matter yield of inoculated alfalfa grown in two salt affected soils.

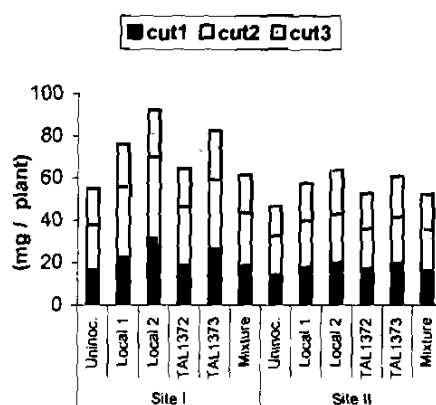


Fig.(2): Nodule fresh weight of inoculated alfalfa grown in two salt affected soils.

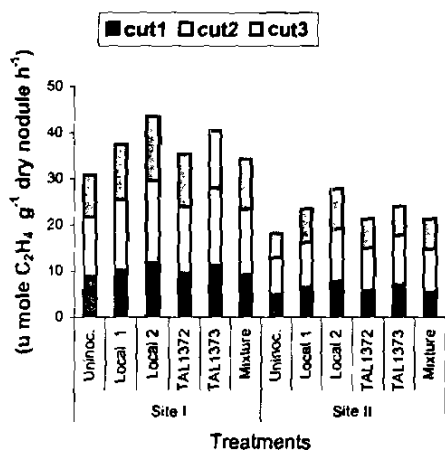


Fig.(3): Nitrogenase activity of nodule from inoculated alfalfa grown in two salt affected soils.

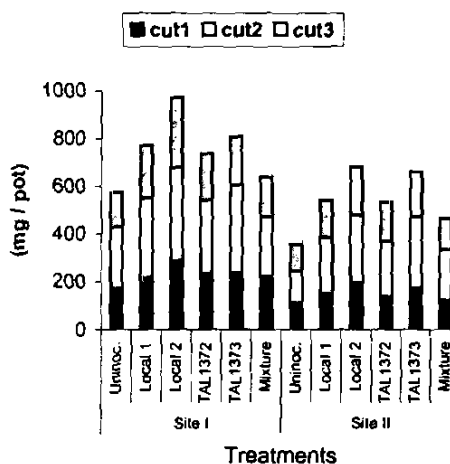


Fig.(4): Nitrogen content of inoculated alfalfa grown in two salt affected soils.

LSD at 0.05 level for:				
Factor	Dry weight	Nodule	Nitrogenase	N-Content
Strains (St)	1.98	1.862	0.79	58.13
**Salinity level(SI)	2.81	1.023	1.17	49.12
SIXSI	3.89	2.974	2.09	137.25

\*\* Salinity levels of site I and II were 10 and 36 dS/m. respectively.

Mashhady *et al.*, (1998) who found that growing Lucerne (alfalfa) in sandy soil of high salt concentration (250 mM NaCl) completely prevented the formation of nodules.

**Nitrogenase activity:**

Data presented in Fig. 3 indicate that, although a considerable number of nodules were formed on the roots on uninoculated plants, they exhibited lower values of nitrogenase activity as compared with the nodules of inoculated treatments in both tested soils. This may be due to the dominance of less effective strains among the native population of *Sinorhizobium meliloti* in Saudi Arabian salt affected soils. These results are in accordance with the finding of Materon (1989), who stated that, the native *Rhizobium* in many localities were not fully effective in nitrogen fixation.

Nitrogenase activity of nodules from inoculated plants decreased with increasing salt concentration. The reduction of N<sub>2</sub> fixing activity (nitrogenase activity) by salt stress is usually attributed to a reduction in respiration of the nodules (Walsh, 1995), and a reduction in cytosolic protein production, specifically leg hemoglobin, by nodules (Delgado *et al.*, 1994). The depressive effect of salt stress on N<sub>2</sub>-fixing capacity is also related to the negative effect of salt stress on the plant growth and subsequently on the transferred carbohydrates to root nodules. The nodules formed on the alfalfa plants inoculated with different strains, differed in their nitrogenase activity with local strain No. 2 being the highest in this respect. The highest nodule function was observed in the second cut followed by third cut at which nodules still functioning at high rate N<sub>2</sub> fixation as indicated by nitrogenase activity.

**N-content:**

Results in Fig. 4 show that inoculation of alfalfa with selected rhizobial strains enhanced nitrogen fixation since N-contents of inoculated plants were higher than those in uninoculated ones. The values of nitrogen content in the different inoculated treatments were found to be parallel to those values of the dry matter yield, nodules fresh weight and nitrogenase activity (Fig. 1, 2, & 3). Results in Fig. 4 indicate that the introduced rhizobial strains can grow, survive and fix nitrogen at high salt concentrations in soils. It is not surprising that the maximum nitrogen content was found in plant samples at the second and third cuts since; the maximum yield was also recorded at these cuts (Fig. 1).

In the light of the obtained results it could be concluded that strains of rhizobia not only can survive but may even grow at salt concentrations in excess of those tolerated by alfalfa host. This in part shows the environmental adaptation of the microsymbiont to its habitat. Whereas the host legume produces seed and enters dormancy at the onset of the dry season, its microsymbiont, *Sinorhizobium meliloti* in order to survive, must be able to encounter much higher levels of salts in the soil particularly as the soils dries.

**Survival and growth of rhizobia in alfalfa rhizosphere:**

The fluorescent antibody technique which allows for the identification and enumeration of specific strains of *Rhizobium* directly in the environment provided a unique tool for the study of population changes of *Rhizobium* in the rhizosphere. Moawad *et al.* (1984) using this technique was able to follow the numerical changes in the rhizosphere populations of soybean rhizobia and its relation to competitive ability in soils of US upper Midwest. They came to conclusion that the competitive ability of a certain strain is not correlated with its specific stimulation.

The population dynamics of four introduced *Sinorhizobium meliloti* strains were studied in the rhizosphere of alfalfa and the changes in densities of each strain are presented in Table 2. Local strains No.1 and 2 found in the rhizosphere of uninoculated treatments. This could be explained by the fact that, both strains are local isolates and likely to be dominant in many Saudi Arabian soils. The population densities of the local strains No. 1 and 2 in the uninoculated soils remained relatively constant not exceeding  $10^2$  cell  $g^{-1}$  dry soil. However, in the inoculated treatments either with single or multistrains inocula, the population densities of the four introduced rhizobial strains reached  $10^5$  cell  $g^{-1}$  dry soil. There were no clear differences in the population densities of the four introduced strains in the inoculated treatments.

As shown in Table 2, the patterns of rhizosphere colonization by the introduced strains were more or less similar. The population densities of the introduced strains increased during the period of active nodulation (0-15 days after seeding) due to root secretion, then decreased slightly till 45 days but still higher than the 7 days count.

The presence of inoculant rhizobial strains in the rhizosphere of alfalfa at higher salt concentration in the soil strengthen the suggestion that the salinity stress even at high levels up to 36 dS  $m^{-1}$  does not suppresses the growth and survival of the strains in the rhizosphere. Many species of bacteria adapt to saline conditions by the intracellular accumulation of low-molecular-weight organic solutes called osmolytes (Csonka and Hanson, 1991). The accumulation of osmolytes is thought to counteract the dehydration of low water activity in the medium but not to interfere with macromolecular structure or function (Smith *et al.*, 1994). Rhizobia utilize the mechanism of osmotic adaptation (Zahran, 1997). In the presence of high levels of salt, the levels of intracellular free glutamate and /or  $K^+$  were greatly increased (sometimes up to six fold in a few minutes) in cells of *Sinorhizobium meliloti* (Jian *et al.*, 1993). Potassium ions strictly control  $Mg^{2+}$  flux during osmotic shock. The accumulation of the osmolytes is dependent on the level of osmotic stress, the growth phase of the culture, the carbon source, and the presence of osmolytes in the growth medium. However, it seems that the first step in nodule formation is extremely sensitive to salinity and this is mainly due to the effect of salinity on the root infection sites (Zahran, 1991).



Table(2): Population densities of four introduced *Sinorhizobium meliloti* strains in the rhizosphere of alfalfa grown in two salt affected soils.

Days after Seeding	Uninoculated soils x 10 <sup>2</sup>				Inoculated with Single strains x 10 <sup>5</sup>				Inoculated with Multi strains x 10 <sup>5</sup>			
	1	2	1372	1373	1	2	1372	1373	1	2	1372	1373
Site I (E.C= 10 dS m <sup>-1</sup> )												
7	1.3	3.0	0.0	0.0	4.1	3.7	3.6	4.6	2.2	1.4	1.1	2.1
15	3.2	7.2	0.0	0.0	7.8	8.3	5.6	7.9	5.7	6.4	4.7	4.3
30	2.8	4.5	0.0	0.0	7.2	6.9	3.5	6.2	4.6	5.4	2.8	3.2
45	2.9	4.9	0.0	0.0	7.0	7.3	4.2	7.0	4.0	4.7	2.2	3.6
Site II (E.C= 36 dS m <sup>-1</sup> )												
7	1.2	3.2	0.0	0.0	2.5	1.5	5.2	3.2	1.2	1.8	2.1	2.4
15	2.0	7.9	0.0	0.0	5.4	3.4	9.5	7.3	3.4	3.3	6.8	4.2
30	1.8	5.4	0.0	0.0	3.6	2.8	7.2	6.1	1.6	1.6	5.6	3.8
45	1.6	5.8	0.0	0.0	3.4	1.9	5.5	6.4	1.8	1.7	4.2	3.8

**Competition between inoculants and native rhizobia:**

An important objective in legume inoculation research is to select highly effective strains of rhizobia for a particular host plant. These strains must also be able to establish themselves in the rhizosphere and compete successfully for nodule sites against the indigenous soil rhizobia which often include ineffective strains.

Results given in Table 3 show the serological typing of nodule from alfalfa inoculated with four inoculants strains. In the uninoculated soils, the percentage of nodules occupancy with local strains No. 1 and 2 were 4 & 8 and 2 & 3 for site I (E.C=10 dS m<sup>-1</sup>) and site II (E.C=36 dS m<sup>-1</sup>), respectively. Strains TAL 1372 and TAL 1373 were not found within nodules formed in the uninoculated soils.

Table(3): Serotyping of nodules from alfalfa inoculated with single or multistrains inoculants of *Sinorhizobium meliloti* in two salt affected soils.

Inocula strains	% Nodule occupancy									
	Local No. 1		Local No. 2		TAL 1372		TAL 1373		Native rhizobia	
	Site I	Site II	Site I	Site I	Site II	Site I	Site II	Site I	Site I	Site II
Uninoc.	4	2	8	3	0	0	0	0	88	95
Local No. 1	74	56	0	0	0	0	0	0	26	44
Local No. 2	0	0	82	68	0	0	0	0	18	32
TAL 1372	0	0	0	0	60	48	0	0	40	52
TAL 1373	0	0	0	0	0	0	79	62	21	38
Mixture	20	18	30	30	17	15	27	23	6	14

Data of the nodule serotyping (Table 3) show variable degrees of competitive ability in the two salt affected soils. The use of the strains as single inoculant shows that these strains were capable of competing with the native rhizobia. In site I (EC = 10 dS m<sup>-1</sup>) local strains No. 2 and TAL 1373 occupied 82% and 79% of the nodules. In site II (EC = 36 dS m<sup>-1</sup>), the same

strains occupied 68%, and 62% respectively. These results indicate that local strain No. 2 and TAL 1373 were strong competitors against native alfalfa rhizobia. These results are in line with those of Robinson (1969), who found that some introduced rhizobial strains were able to outcompete the native rhizobia. Gibson *et al.* (1976) also stated that the single strain inoculant of *R. trifolii* was present at higher frequency in the sampled nodules from the inoculated plots.

Results from Table 3 also show that nodule occupancy by native rhizobia markedly decreased in case of the multistrain inoculant as compared with the single ones. Similar results were also reported by Hamdi *et al.* (1984). In the present study, local strain No. 2 was superior in competition with the other introduced strains in the mixture of the four strains.

It should be stated that although, the population densities of the introduced *Sinorhizobium meliloti* strains were not affected by soil salinity (Table 2), the proportion of nodules formed by these strains declined markedly in the most saline soil as compared with the less saline one. This may be attributed to the ability of native rhizobia to compete with the introduced strains under these saline conditions.

The successful formation of one nodule by competing rhizobia is usually achieved by one strain but, on occasion two (or more) can be found in the same nodule. The occurrence of dual occupancy was once considered a rare event, but evidence has accumulated which demonstrates its more frequent occurrence. Fluorescent antibody technique is particularly useful in the detection of mixed infections in nodules. Data in the present study, (Table 4) show that, double strain occupancy in nodules in case of multistrain inoculant ranged from 8-14, and 10-16% of the total nodules at site I and II, respectively. These results are in line with those of Marques Pinto *et al.* (1974) and Labandera & Vincent (1975). Local strains No. 2 and TAL 1373 were found at high frequencies in doubly infected nodules. These results are in accordance with those of May and Bohlool (1983) who suggested a close relationship between the competitiveness of a strain and its involvement in the formation of doubly infected nodules.

From the aforementioned results it can be concluded that some strains of *Rhizobium* can nodulate legumes and form effective N<sub>2</sub>-fixing symbiosis in salt affected soils. This work proved that the relative success of the strains in producing nodules appeared to be independent of their rate of multiplication in the rhizosphere of the host plant. It is worthy to mention that tolerance of the legume host to salt is the most important factor in determining the success of compatible *Rhizobium* strains to form successful symbiosis under conditions of high soil salinity. So, inoculation of various legumes with salt-tolerant strains will improve N<sub>2</sub> fixation in saline environments. Therefore, this work recommends that the future studies on the effect of salinity on symbiotic N<sub>2</sub> fixation should focus more on aspects related to the symbiosis rather than the study of each partner apart.

**Table(4): Percent of nodule occupancy in alfalfa nodules by multistrain inoculant in two salts affected soils.**

Inocula strains	% Nodule occupancy									
	Local No. 1		Local No. 2		TAL 1372		TAL 1373		Total	
	Site I	Site II	Site I	Site I	Site II	Site I	Site II	Site I	Site I	Site II
Local No. 1	0	0	6	7	0	0	4	5	10	12
Local No. 2	6	7	0	0	8	9	0	0	14	16
TAL 1372	0	0	8	9	0	0	4	5	12	14
TAL 1373	8	5	0	0	4	5	0	0	8	10

## REFERENCES

- Abdel-Wahab, S. M.; M. T. El-Mokadem; F. A. Helemish and M. M. Abou El-Nour (1991). The symbiotic performance of *Bradyrhizobium japonicum* under stress of salinized irrigation water. *Ain Shams Sci. Bull.*, 28B: 469-488.
- Allen, O. N. (1971). *Experiment in Soil Bacteriology*. Burgess, Minneapolis, Minnesota.
- Bajpai, P.D. and B.R. Gupta (1979). Effect of salinization, phosphorus fertilization and the irinteraction on the symbiosis between *R. trifolii* and berseem *Trifolium alexandrinum*. *J. Indian Soc. Soil Sci.*, 27: 462-469.
- Bashour, I. I.; A. S. Al-Mashhady; J. Deviprasad; T. Miller and M. Mazora (1983). Morphology and composition of some soils under cultivation in Saudi Arabia. *Geoderma*, 29: 327-340.
- Bohlool, B.B. and E.L. Schmidt (1968). Non specific staining: Its control in immunofluorescence examination in soil. *Science*, 162:1012-1014.
- Cordovilla, M. P.; F. Ligeró and C. Lluch (1994). The effect of salinity on N<sub>2</sub> fixation and assimilation in *Vicia faba*. *J. Exp. Bot.*, 45: 1483-1488.
- Cordovilla, M. P.; A. Ocana; F. Ligeró and C. Lluch (1995). Salinity effects on growth analysis and nutrient composition in four grain legumes-*Rhizobium* symbiosis. *J. Plant Nutr.*, 18: 1595-1609.
- Correa, O. S. and A. J. Barneix (1997). Cellular mechanisms of pH tolerance in *Rhizobium loti*. *World J. Microbiol. Biotechnol.*, 13: 153-157.
- Criag, G. F.; C. A. Atkins and D. T. Bell (1991). Effect of salinity on growth of *Rhizobium* and their infectivity and effectiveness on two species of *Acacia*. *Plant Soil*, 133: 253-262.
- Csonka, L. N. and A. D. Hanson (1991). Prokaryotic osmoregulation: genetics and physiology. *Annu. Rev. Plant Physiol.*, 45:569-606.
- Delgado, M. J.; F. Ligeró and Lluch (1994). Effect of salt stress on growth and nitrogen fixation by faba-bean, common bean and soybean plants. *Soil Biol. Biochem.*, 26: 371-376.
- El-Mokadem, M. T.; A. Helemish; S. M. Abdel-Wahab and M. M. Abou-El-Nour (1991). Salt response of clover and alfalfa inoculated with salt tolerant strains of *Rhizobium*. *Ain Shams Sci. Bull.*, 28B:441-468.
- Embalomatis, A.; D. K. Papacosta and P. Katinakis (1994). Evaluation of *Rhizobium meliloti* strains isolated from indigenous populations northern Greece. *J. Agric. Crop Sci.*, 172: 73-80.

- Gaur, Y.D. and W.L. Lowther (1982). Competitiveness and persistence of introduced rhizobia on oversown clover *Trifolium repens* influence of strain inoculation rate and line pelleting. *Soil Biol. Biochem.*, 14: 99-102.
- Georgiev, G. I. and C. A. Atkias (1993). Effect of salinity on  $N_2$  fixation, nitrogen metabolism and export and diffusive conductance of cowpea root nodules. *Symbiosis*, 15: 239-255.
- Gibson, A.H.; R.A. Date; J.A. Ireland and J. Brokwell (1976). A comparison of competitiveness and persistence amongst five strains of *R. trifolii*. *Soil Biol. Biochem.*, 14: 395-401.
- Glenn, E. P.; J. J. Brown and E. Blumwald (1999). Salt tolerance and crop potential of halophytes. *Crit. Rev. Plant Sci.*, 18: 227- 255.
- Hamdi, Y.A.; M.E. El-Hadoad; Y.Z. Ishac and M.A. Abdel-Daiem (1984). Studies on Ina competition between different strains of *R. leguminosarum* and native *Rhizobium* in Egyptian soils. Second conf. of the Agric. Res. Centre, Giza, Egypt, 9-11 April.
- Hardy, R.W.F.; R.C. Burns and R.D. Holsten (1973). Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. *Soil Biol. Biochem.*, 5:47-81.
- Ikeda, J. L.; M. Kobayashi and E. Takahashi (1992). Salt stress increases the respiratory cost of nitrogen. *Soil Sci. Plant Nutr.*, 38: 51-56.
- Jackson, M.L.(1973). *Soil Chemical Analysis*. Constable and Co.London, pp. 113-115
- Jian, W.; Y. Susheng and L. Jilun (1993). Studies on the salt tolerance of *Rhizobium meliloti*. *Acta Microbiol. Sin.*, 33:260-267.
- Kingsley, M.T. and B.B. Bohlool (1981). Release of *Rhizobium* spp. from tropical soils and recovery for immunofluorescence enumeration. *Appl. Environ. Microbiol.*, 42: 241-248.
- Labandera, C.A. and J.M. Vincent (1975). Competition between an introduced strain and native Uruguyan strains of *R.trifolii*. *Plant and Soil*, 42: 327-347.
- Marques Pinto, C.M.; Y.Y. Plaik and J.M. Vincent (1974). Nodulating competitiveness amongst strains of *R. meliloti* and *R. trifolii*, *Aust. J. Agric. Res.*, 25: 317-329.
- Mashhady, A. S ; S. H. Salem; F. N. Barakh and A. M. Heggo (1998). Effect of salinity on survival and symbiotic performance between *Rhizobium meliloti* and *Medicago sativa* in Saudi Arabian soils. *Arid Soil Res. Rehabil.*, 12: 3-14.
- Materon, L.A. (1989). Constraints to nodulation of annual medics by indigenous populations of *Rhizobium meliloti* in West Asian soils. *Introducing Ley farming to the Mediterranean basin. Proceedings of an International Workshop 26-30 June 1989, Perugia, Italy.*
- May, N. and B.B. Bohlool (1983). Competition among *R. leguminosarum* strains for nodulation of lentils. *Appl. And Environ. Microbiol.*, 45 (3): 960-965.

- Moawad, H.; W.R. Ellis and E.L. Schmidt (1984). Rhizosphere response as a factor in competition among three serogroups of indigenous *R. japonicum* for nodulation of field-grown soybean. *Appl. Environ. Microbiol.*, 47: 607-612.
- Rao, D. L. N. and P. C. Sharma (1995). Effectiveness of rhizobial strains for chickpea under salinity stress and recovery of nodulation on desalinization. *Indian J. Exp. Biol.*, 33: 500-504.
- Robinson, A.C. (1969) Competitive ability and effectiveness of *Rhizobium* strains. *Aust. J. Agric. Res.*, 20: 1053.
- Schmidt, E.L. (1974). Quantitative autecological study of microorganisms in soil by immunofluorescence. *Soil Sci.*, 118: 141-143.
- Sheath, G.W.; M.J. Macfarlane and P.M. Bonish (1984). Evaluation of arrowleaf clover *Trifolium vesiculosum* in north island New-Zealand hill country nodulation and reseeded. *NZJ. Exp. Agric.*, 12: 209-218.
- Singleton, P.W. and B.B. Bohlool (1984). Effect of salinity on nodule formation by soybean cultivar Davis. *Plant Physiol.*, 74: 72-76.
- Smith, L. T.; G. M. Smith; M. R. Desouza; J. M. Pocard; D. Le Rudulier and M. A. Madkour (1994). Osmoregulation in *Rhizobium meliloti*: mechanism and control by other environmental signals. *J. Exp. Zool.*, 268:162-165.
- Snedecor, C. W. and J. Cochran (1978). *Statistical Method* 6 th . ed. Oxford and FBH Publishing Co. Calcetta , India .
- Tate, R. L. 1995. *Soil Microbiology (symbiotic nitrogen fixation)*, p. 307-333. John Wiley & Sons, Inc., New York, N. Y.
- Walsh, K. B. 1995. Physiology of the legume nodule and its response to stress. *Soil Biol. Biochem.*, 27: 637-655.
- Yelton, M. M.; S. S. Yang S.A. Edie and S. T. Lim (1983). Characterization of an effective salt-tolerant fast-growing strain of *Rhizobium japonicum*. *J. Gen. Microbiol.*, 129: 1537-1547.
- Young, N.R.; D.M Hughes and L.R. Mytton (1986). The response of white clover to different strains of *R. trifolii* in hill land reseeded a second trial. *Plant and Soil*, 94: 277-284.
- Zahran, H. H. (1991). Conditions for successful *Rhizobium*-legume symbiosis in saline environments. *Biol. Fertil. Soils*, 12: 73-80.
- Zahran, H. H. (1997). Diversity, adaptation and activity of the bacterial flora in saline environments. *Biol. Fertil. Soils*, 25:211-223.
- Zahran, H. H. and M. A. Abu-Gharbia (1995). Development and structure of bacterial root-nodules of two Egyptian cultivars of *Vicia faba* L. under salt and water stresses. *Bull. Fac. Sci. Assiut Univ.*, 24: 1-10.
- Zahran, H. H. and J. I. Sprent (1986). Effect of sodium chloride and polyethylene glycol on root hair infection and nodulation of *Vicia faba* L. plants by *Rhizobium leguminosarum*. *Planta*, 167:303-309.
- Zahran, H. H.; L. A. Rasanen; M. Karsisto and K. Lindstrom (1994). Alteration of lipopolysaccharide and protein profiles in SDS-PAGE of rhizobia by osmotic and heat stress. *World J. Microbiol. Biotechnol.*, 10: 100-105.

Zou, N.; P. J. Dort and N. E. Marcar (1995). Interaction of salinity and rhizobial strains on growth and  $N_2$ - fixation by *Acacia ampliceps*. Soil Biol. Biochem., 27: 409-413.

تأثير ملوحة التربة على حيوية وأداء ريزوبيا البرسيم الحجازي  
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تم دراسة أثر ملوحة التربة على نمو وحيوية أربعة سلالات مختلفة من ريزوبيا البرسيم الحجازي في الريزوسفير وكذلك دراسة التنافس بين هذه السلالات الأربعة بعضها البعض وبينها وبين السلالات المتوطنة في نوعين من الترب الملحية تحتوي على ١٠ و ٣٦ ديسي سيمنز / متر. وقد بينت النتائج عدم تأثر أي من السلالات الأربعة الملقح بها بالملوحة حتى في وجود التركيز العالي من الأملاح بالتربة (٣٦ ديسي سيمنز / متر) حيث استطاعت هذه السلالات النمو والبقاء بحيوية في ريزوسفير نباتات البرسيم الحجازي وعلى العكس من ذلك فقد انخفضت النسبة المئوية للعقد الجذرية المتكونة بواسطة السلالات الملقح بها أو تلك المتوطنة بالتربة مع ازدياد نسبة الملوحة بها وهذا يعني ان الخطوة الأولى في عملية تكوين العقد الجذرية تكون أكثر حساسية للملوحة ويرجع ذلك الى تأثير الملوحة على مواقع اللكتين الموجودة على جذور البرسيم الحجازي والتي تتكون منها العقد الجذرية أكثر من تأثير الملوحة على حيوية الرزوبيا ونموها في ريزوسفير النبات البقولي النامي في مثل هذه الترب الملحية. كما بينت النتائج التأثير السلبي للملوحة على نشاط انزيم النيتروجينيز ومحتوى نباتات البرسيم الحجازي من النيتروجين وربما يرجع ذلك الى تأثير الملوحة على نمو النباتات ومن ثم على كفاءة العقد الجذرية المتكونة. وقد أوضحت النتائج قدرة سلالات الريزوبيا على النمو والحيوية وتثبيت نيتروجين الهواء الجوي عند تركيزات من الملوحة تفوق التركيزات التي تستطيع النباتات البقولية النمو عندها مما يعني أن النباتات أكثر حساسية للملوحة من بكتريا العقد الجذرية الرزوبيا.