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EFFECT OF SALINITY ON NODULATION, GLUTAMINE SYNTHETASE AND GLUTAMATE SYNTHASE ACTIVITY IN NODULES OF ALFALFA (*MEDICAGO SATIVA* L.)

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ABSTRACT. Bami cultivar of alfalfa (*Medicago sativa*) was inoculated with salt-tolerant *Sinorhizobium meliloti* in solution culture with different salt concentrations (0, 50, 75 and 100 mmoles l^{-1}NaCl) added immediately at the time of inoculation. The results indicated that *S. meliloti* formed an infective and effective symbiosis with alfalfa under saline and nonsaline conditions. Salinity significantly decreased shoot and root dry weight, nodule weight and mean nodule weight. Roots were more sensitive than shoots, and N_2 fixation was more sensitive to salinity than was plant growth. Analyses of ammonium assimilating enzymes in the nodule showed that glutamine synthetase appeared to be more tolerant to salinity than glutamate synthase, and that it limits ammonium assimilation under saline stress.

Key words: Alfalfa; Glutamine synthetase; Glutamate synthase; N_2 fixation; Salinity; *Sinorhizobium meliloti*.

INTRODUCTION

Saline soils and saline irrigation constitute a serious production problem for vegetable crops as saline conditions are known to suppress plant growth, particularly in arid and semiarid areas (Tejera *et al.,* 2005). Chloride and sulfate salts are predominant in saline soils. Plant growth, nutrient uptake and metabolism, and protein synthesis are all thought to be adversely affected under salt stress conditions (Wenxue *et al.,* 2003). Retarded plant growth, resulting in reduced crop yield, has been reported by many investigators (Zhu, 2002; Ashraf, 2002) as a major cause of abnormal nutrient metabolism and impaired protein synthesis in plants under stress conditions.

Legumes have been suggested as appropriate crops for the enhancement

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of bioproductivity and the reclamation of marginal lands, because these plants not only yield nutritious fodder, protein-rich seeds and fruits, but they also enrich soil nitrogen in symbiotic association with *Rhizobium* (Dashti *et al.,* 1998). Nodulation and nitrogen fixation in legume-*Rhizobium* associations are adversely affected by salinity, which can preclude legume establishment and growth, or reduce crop yield (Mohammad *et al.,* 1991).

The specific sensitivity of the symbiotic nitrogen fixation (SNF)-dependent legumes to salinity, is well documented for initiation, development and function of nodules. Unsuccessful symbiosis under salt-stress may be due to failure in the infection process because of the effect of salinity on the establishment of rhizobia (Garg and Gupta, 2000). Legumes and the process of nodule initiation are both more sensitive to osmotic stress than are rhizobia (Georgiev and Atkins, 1993).

The effect of NaCl salinity on legume growth, nodulation, and nitrogen fixation has been the subject of several investigations (El-Hamdaoui *et al.,* 2003; Diouf *et al.,* 2005). However, there are few studies on relating effects of NaCl salinity on the root nodule structure (Serraj *et al.,* 1998; El-Hamdaoui *et al.,* 2003). Alfalfa (*Medicago sativa*) is a perennial flowering plant in the pea family *Fabaceae* cultivated as an important forage crop in many countries around the world. The alfalfa is native to a warmer temperate climate such as that of Iran (where it is thought to have originated).

During nodulation, both glutamine synthetase (GS) and NADH-dependent glutamate synthase (NADH- GOGAT) have been shown to increase in activity in parallel with nitrogenase and other enzymes in the nitrogen assimilatory pathway (Schubert, 1986; Cullimore and Bennett, 1988). *In vitro* studies indicate that GS and GOGAT are involved in the assimilation of NH_3 to produce glutamate and glutamine (Groat and Vance, 1981). Ohyama and Kumazawa (1978) using the ^{15}N tracer technique, reported that GS/ GOGAT played a predominant role in the assimilation of N in soybean. Treatments that reduce N_2 fixation also decrease NADH-GOGAT (Groat and Vance, 1981). In addition, nodule GOGAT activity in ineffectively nodulated plants is strikingly lower than in effectively nodulated plants (Schubert, 1986).

The aim of the present report was to assess the effect of continuous salinity on alfalfa growth, nodulation and N_2 fixation in solution cultures inoculated with salt-tolerant *Rhizobium*. We also examined the effect of salt on the activity of cytosolic glutamine synthetase (GS) and NADH-dependent glutamate synthase (NADH-GOGAT) extracted from nodules of salt-stressed alfalfa plants.

MATERIALS AND METHODS

Plant material and growth conditions

The cultivar Bami of alfalfa was used in this study. Pre-germinated seeds were planted in modified Leonard jar assemblies (two per jar) containing vermiculite and nutrient solution consisting of 200 mg KH_2PO_4, 200 mg MgSO47H2O, 200 mg KCl, 120 mg $CaSO_42H_2O$, 25 mg Na_2-FeEDTA, 4 mg $Na_2MoO_42H_2O$, 2 mg $MnSO_42H_2O$, 2 mg $CuSO_45H_2O$, 3 mg $ZnSO_47H_2O$, 18 mg H_3BO_3 and 120 mg $CoCl_24H_2O$ per liter of water (Rigaud and Puppo, 1975). Each seedling was inoculated with 1 ml of broth from a log-phase culture of *Rhizobium leguminosarum bv. viciae* containing 10^9 cells ml^{-1}. The *R. leguminosarum* strains used were 3841, previously described as salt tolerant (Cordovilla, 1993). The jars were arranged randomly in a growth chamber with a 16-8 h light-dark cycle, 23-16°C day-night temperature, relative humidity 55- 75% and photosynthetic photon flux density (400- 700 nm) of 450 mmol $m^{-2}s^{-1}$ supplied by fluorescent lamps and incandescent lamps (30% fluorescent wattage).

Experimental design

Four concentrations of salt (0, 50, 75 and 100 mmoles l^{-1}) in the form of NaCl were added to the growth medium immediately after transplanting. The osmotic strengths of the nutrient solutions ranged from 0.02 to 0.27 MPa at 23°C were maintained for 8 weeks. The plants were harvested every week for 3 weeks during the vegetative growth period. Harvesting started 6 weeks after transplanting; three plants were included per harvest. The plants were removed from the jars, the roots were horoughly rinsed with water, blotted dry on filter paper, and nodules picked and kept on ice. Shoot, root and nodule dry weights were recorded after drying for 24 h at 70°C. Three plants per treatment were used for nodule dry weight.

The experimental design was a randomized block factorial consisting of four salinity treatments maintained for six, seven and eight weeks. All values are means of four replicates per treatment. Results were subjected to two-way analysis of variance, including a least significant difference (LSD) test between means. Sources of variance (treatments or time) were compared by Dankan's multiple-range test.

Assays

Nodule samples (1 g fresh weight) were homogenized on ice with acid-washed quartz sand and 12 ml (ice-cold) of an extraction medium containing 100 mmoles l^{-1} maleic acid-KOH, pH 6.8, 100 mmoles l^{-1} sucrose, 2% (v/v) 2-mercaptoethanol and 15% (v/v) ethylene glycol, plus 0.5 g polyvinyl-polypyrrolidone. The homogenate was filtered through four layers of cheesecloth and the nodule debris removed; the filtrate was centrifuged at 3500 g at 2°C for 8 min. The resulting supernatant was centrifuged once more at 30000×g for 20 min, producing a clear solution of host cytoplasm and its organelles, and this solution was used for the enzyme assay.

Glutamine synthetase was determined by the hydroxamate synthetase assay (Farnden and Robertson, 1980; Kaiser and Lewis, 1984). Because glutamine synthetase activity differs from one legume to another, the assays were optimized using different amounts of nodule extract to maintain the enzymatic reaction for 30 min, without any of the substrates used by the enzyme limiting the reaction and, thus, avoiding misleading

results. Two blanks without enzyme and without L-glutamate were also analysed. The NADH-glutamate synthase activity was assayed spectrophotometrically at 30°C by monitoring the oxidation of NADH at 340 nm, essentially as indicated by Groat and Vance (1981) and Singh and Srivastava (1986), consistently within 2 h after extraction. Two controls (without a-ketoglutarate or glutamine) were used to correct for endogenous NADH oxidation. The decrease in absorbance (linear at least 10 min) was recorded for 8 min in a Beckman DU-70 spectrophotometer.

Nitrogenase activity was determined by acetylene reduction on the entire root systems of six plants, using a gas chromatograph (Perkin Elmer 8600) equipped with a column of Poropak R (Ligero *et al.,* 1986), as described by Cordovilla *et al.* (1995). Soluble protein content was determined in cell-free nodule extracts by protein-dye binding with Coomassie brilliant blue (Bradford, 1976), using bovine serum albumin (Merck, fraction V) as a protein standard.

The products of acid digestion from a modified Kjeldahl procedure were steam-distilled, after which the nitrogen content was determined by mass spectrometry as described by Bremner (1965), and Pessarakli and Tucker (1985).

RESULTS

Salinity significantly reduced the dry weights of both shoots and roots ($p<0.05$) (*Table 1*). The inhibitory effect of salt was apparent after 6 weeks of salt treatment in shoots, whereas root dry weight reflected inhibition after 6 weeks with 100 mmoles l^{-1} NaCl, and after 8 weeks with 50 mmoles l^{-1} salt. Shoots appeared to be more sensitive to salinity than did roots. In all harvests, root dry weight and shoot dry weight showed similar degrees of inhibition with 100 mmoles l^{-1} NaCl, whereas inhibition was less marked with 50 and 75 mmoles l^{-1} salt. In plants grown with NaCl, shortening of the internodes was more marked at higher salt concentrations; in addition, leaves were smaller and roots darker and less ramified, due to the accumulation of phenols (data not shown).

Salinity significantly reduced the total nodule number per plant ($p<0.05$) (*Table 1*). After 8 weeks a 52.17% and 69.56% reduction in nodule number was caused by NaCl concentrations of 75 and 100 mmoles l^{-1}, respectively. In general, total nodule number redused throughout the growth period in each treatments. Nodule differentiation was affected by salt. Nodules lost their pink color (leghemoglobin content), and by inference N_2 fixation activity, becoming white and inactive. Salinity significantly decreased total nodule weight per plant after 6 weeks (*Table 1*). Reductions of 41.23%, 72.64% and 88.8% in nodule weight were caused by salt concentrations of 50, 75 and 100 mmoles l^{-1}, respectively, as a consequence of the smaller size of the nodules formed at the higher salt concentrations.

Salinity significantly decreased total N in both shoots and roots of alfalfa ($p<0.05$, *Table 2*), although inhibition was more marked in shoots with all saline treatments. Nitrogenase (acetylene reduction) activity was higher in control plants than in all

saline treatments (*Table 1*). Both total and specific activity were severely depressed by salinity. Salt treatment reduced acetylene reduction activity (ARA) per plant in response to dose. After 8 weeks, NaCl at 50 mmoles l^{-1} led to a 26.66% reduction, and concentrations of 75 and 100 mmoles l^{-1} led to an 49% and 52% reduction, respectively .

Table 1 - Effects of saline treatments on dry-matter accumulation (g $organ^{-1}$) in shoots and roots, total nodule number ($plant^{-1}$), nodule weight (mg DW $plant^{-1}$), acetylene reduction activity (ARA) per plant (mmol C_2H_4 $plant^{-1}h^{-1}$) and ARA per weight of nodule (mmol C_2H_4 $g^{-1}h^{-1}$) in nodules of alfalfa plants infected by *Sinorhizobium meliloti*

Parameter	NaCl treatment (mmoles l^{-1})	Weeks after inoculation		
		Week 6	Week 7	Week 8
Shoot dry weight (g $organ^{-1}$)	0	1.36	1.83	2.65
	50	1.16	1.53	2.22
	75	0.98	1.29	1.85
	100	0.85	1.12	1.63
	LSD (0.05) 0. 38			
Root dry weight (g $organ^{-1}$)	0	0.74	0.83	1.12
	50	0.7	0.76	0.88
	75	0.68	0.72	0.8
	100	0.48	0.55	0.72
	LSD (0.05) 0.45			
Total nodule number ($plant^{-1}$)	0	14	18	23
	50	12	15	18
	75	8	10	11
	100	2	4	7
	LSD (0.05) 3			
Total nodule weight (mg DW $plant^{-1}$)	0	8.29	10.28	12.1
	50	5.95	6.33	7.11
	75	2.72	3.26	3.31
	100	0.48	0.85	1.35
	LSD (0.05) 1.63			
ARA per plant (mmol C_2H_4 $plant^{-1}h^{-1}$)	0	1.17	1.73	2.25
	50	1.12	1.4	1.65
	75	0.72	1.06	1.14
	100	0.57	0.83	1.08
	LSD (0.05) 0. 97			
ARA per unit weight of nodule (mmol C_2H_4 g^{-1}nodule h^{-1})	0	9.86	11.43	13.37
	50	8.23	9.35	10.87
	75	5.87	7.23	8.8
	100	4.11	5.67	6.23
	LSD (0.05) 1.82			

Table 2 - Effect of saline treatments on glutamine synthetase (GS) (mmol g-glutamyl-hydroxamate $g^{-1}FW\ h^{-1}$), glutamate synthase (GOGAT) (mmolNADHox $g^{-1}FW\ h^{-1}$) activities and soluble protein content (mg $g^{-1}FW$) in nodules, and reduced nitrogen content in shoot and root (mg $organ^{-1}$) of 8-week-old of alfalfa plants infected by *Sinorhizobium meliloti*

Parameter	NaCl treatment (mmoles l^{-1})			
	0	50	75	100
Glutamine synthetase (mmol g-glutamyl-hydroxamate $g^{-1}FW\ h^{-1}$)	168	147	126	108
	LSD (0.05) 8.42			
Glutamate synthase (mmol NADHox $g^{-1}FW\ h^{-1}$)	128.2	85.4	76.1	58.7
	LSD (0.05) 3.27			
Protein (mg $g^{-1}FW$)	12.4	12.1	9.4	8.6
	LSD (0.05) 1.64			
Nitrogen content in shoot (mg $organ^{-1}$)	74.3	53.8	41.3	27.5
	LSD (0.05) 5.21			
Nitrogen content in root (mg $organ^{-1}$)	47.2	33.8	28.1	21.6
	LSD (0.05) 3.23			

Ammonium assimilation activity was measured in nodule cytosol (*Table 2*). After 8 weeks, GS activity was, on average, 2-fold higher than NADH-GOGAT, and both activities were significantly decreased $p<0.05$) by salinity. A 12.5% reduction in GS activity was seen after treatment with 50 mmoles l^{-1} NaCl and a 35.71% reduction after 100 mmoles l^{-1}. NADH-GOGAT appeared to be more sensitive than GS to salinity. Concentrations of 50, 75 and 100 mmoles l^{-1} led to reductions of 33.38%, 40.63% and 54.21%, respectively, in NADH-GOGAT activity. Soluble protein content of the nodules (*Table 2*) was also significantly reduced ($p<0.05$) by salt.

DISCUSSION

A favourable rhizosphere environment is highly important to the interaction between root hairs and *Rhizobium*, as it not only encourages the growth and multiplication of rhizobia but also ensures the healthy development of root hairs. Any environmental stress that affects these processes is also likely to influence infection and nodulation (Alexander, 1984).

Salinity reportedly reduces shoot and root weights in several legumes, such as faba bean (Zahran and Sprent, 1986), soybean (*Glycine max*) (Grattan and Maas, 1988), and bean (*Phaseolus vulgaris*) (Wignarajah, 1990). With 90 mmoles l^{-1} NaCl, chick-pea plants (*Cicer arietinum*) survived for only 4 weeks (Elsheikh and Wood, 1990), and cowpea plants (*Vigna radiata*) died at maturity (65 d) (Hafeez *et al.,* 1988). In the present study, at maturity (after 8 weeks), growth was inhibited but the plants did not die, confirming that the alfalfa cultivar Bami is less sensitive to saline conditions than are many other

legumes. Salinity affected shoot growth more than root growth, as was also reported for beans (Wignarajah, 1990).

Reduction of nodulation (Zahran and Sprent, 1986) and inhibition of N_2-fixing activity in legumes (Cordovilla *et al.,* 1994) are typical effects of salinity. Total nodule number per plant was adversely affected only by high concentrations of salinity (75 and 100 mmoles l^{-1}), indicating tolerance of *Rhizobium leguminosarum* to low levels of salinity. This contrasts with the findings, in which even low levels of NaCl led to a 60% decrease in nodulation (Elsheikh and Wood, 1990). However, nodulation in alfalfa after 8 weeks was completely depressed in plants that received the highest saline treatment. Similar findings were reported for *Vigna radiata*: nodulation was reduced by about half after exposure to low levels of salinity (below 50 mmoles l^{-1}) (Hafeez *et al.,* 1988).

In our experiments, the increase in the total nodule weight per plant under salt stress and in the weight of nodules per gram of root suggest that the nodules grew to the detriment of root growth. The increase in average nodule weight as salinity declined corroborates earlier observations for faba bean (Yousef and Sprent, 1983), and more recent findings for chick pea (Elsheikh and Wood, 1990). Other authors observed no increase in total nodule weight (Vessey and Buss, 2002). Reduced nodule formation by alfalfa at low levels of salinity could have been due to adverse effects on the process of nodule initiation, an event in *Rhizobium*-legume symbiosis which is very sensitive to osmotic stress (Öğütçü *et al.*, 2008). Also, nodule differentiation was affected by salt as evidenced by the appearance of white nodules that must have been newly formed, incompletely differentiated or senescent; consequently, the reduction in plant growth under saline conditions can be explained by the reduction or failure in nodulation.

The notable decline in ARA with low level salt stress agrees with earlier observations for cowpea (Hafeez et al., 1988), chick pea (Elsheikh and Wood, 1990) and peanut (*Arachis hypogea*) (Leidi *et al.,* 1992). The effect of 100 mmoles l^{-1} of salt on specific nitrogenase activity was more severe than on the nodule dry weight; this may be attributable to a direct effect of salt on nitrogenase. Diouf *et al*. (2005) reported that NaCl directly affected nitrogenase purified from *Azotobacter*.

The reduction in nitrogen content was greater than the reduction in growth. Similarly, Vessey and Buss (2002) found that the nitrogen content in soybean was more strongly reduced than growth. By contrast, other authors (Weil and Khalil, 1986; Dixon, 1993) found no reduction in nitrogen content.

The GS/GOGAT cycle is thought to be responsible for the assimilation of most of the NH_4 derived from N_2 fixation in the nodules of legumes. Giller and

Flowers (2002) found that salt treatment substantially inhibited the glutamine-synthase-cycle enzymes in bean leaves. In contrast to the response in halophytes, the addition of salt to the nutrient medium creates unfavourable conditions for the growth of bean, and lowers the activity of this enzymatic pathway. In faba bean nodules the activities of GS and NADH-GOGAT, which are responsible for ammonium absorption (Cullimore and Bennett, 1988; Cordovilla, 1993) were also inhibited by salinity. Similar results were reported by Bourgeais-Chaillou *et al.* (1992) for soybean (*Glycine max*). Our findings show that NADH-GOGAT activity was more markedly inhibited than GS activity, suggesting that the latter enzyme is the limiting factor in ammonium assimilation by nodules in *Vicia faba* under salt stress.

The decrease in soluble protein content of the nodules may be due to a protein break-down , or to an alteration in the incorporation of amino acids into proteins (Lee *et al.,* 2008). In this connection, the selective accumulation of intracellular amino acids is a prominent physiological response of many organisms to osmotic stress (Yancey *et al.,* 1982).

REFERENCES

Alexander M., 1984 - Ecology of rhizobium. *In:* Alexander, M.(Ed.), Biological Nitrogen Fixation: Ecology, Technology and Physiology. Plenum Press, New York pp. 39-50.

Ashraf M., 2002 - Salt tolerance of cotton: some new advances. Crit. Rev. Plant. Sci., 21:1-30.

Bourgeais-Chaillou F., Perez-Alfocea F., Guerrier G., 1992 - Comparative effects of N-sources on growth and physiological responses of soyabean exposed to NaCl-stress. J. Exp. Bot., 43, 1225-1233.

Bradford M.M., 1976 - A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye-binding. Anal. Biochem., 72: 248- 254.

Bremner J.M., 1965 - Organic forms of nitrogen. *In:* Methods of soil analysis, Part II. Black, C.A. et al. (Eds.), Amer. Soc. Agron., Madison, Wisconsin, USA, pp.1238-1255.

Cordovilla M.P., 1993 - Estudios fisiologicos y bioquimicos del ciclo de la glutamato sintasa en nodulos de *Vicia faba*: efecto del estres salino. Ph.D. Thesis, University of Granada, Spain.

Cordovilla M.P., Ligero F., Lluch C., 1994 - The effect of salinity on N_2 fixation and assimilation in *Vicia faba.* J. Exp. Bot., 45: 1483-1488.

Cordovilla M.P., Ligero F., Lluch C., 1995 - Influence of host genotypes on growth, symbiotic performance and nitrogen assimilation in faba bean (*Vicia faba* L.) under salt stress. Plant Soil, 172: 289-297.

Cullimore J.V., Bennett M.J., 1988 - The molecular biology and biochemistry of plant glutamine synthetase from root nodules of *Phaseolus vulgaris* L. and other legumes. Plant Physiol., 132:387-393.

Dashti N., Zhang F., Hynes R., Smith D.L., 1998 - Plant growth promoting rhizobacteria accelerate nodulation and increase nitrogen fixation activity by field grown soybean [*Glycine max* (L.) Merr.] under short season conditions. Plant Soil, 200: 205-213.

Diouf D., Duponnois R., Tidiane B.A., Neyra M., Lesueur D., 2005 -

Symbiosis of *Acacia auriculiformis* and *Acacia mangium* with *Arbuscular mycorrhizal* fungi and *Bradyrhizobium* spp. improves salt tolerance in greenhouse conditions. Funct. Plant. Biol., 32:1143-1152.

Dixon R.K., Garg V.K., Rao M., 1993 - Inoculation of *Leucaena* and *Prosopis* seedlings with *Glomus* and *Rhizobium* species in saline soil: rhizosphere relations and seedlings growth. Arid Soil Res. Rehabil., 7:133–144.

El-Hamdaoui A., Redondo-Nieto M., Rivilla R., Bonilla I., Bolaños L., 2003 - Effects of boron and calcium nutrition on the establishment of the *Rhizobium leguminosarum*–pea (*Pisum sativum*) symbiosis and nodule development under salt stress. Plant Cell Environ., 26: 1003-1012.

Elsheikh E.A.E.., Wood M., 1990 - Effect of salinity on growth, nodulation and nitrogen yield of chickpea (*Cicer arietinum* L.). J. Exp. Bot., 41: 1263-1269.

Farnden K.J.F., Robertson J.G., 1980 - Methods for studying enzymes involved in metabolism related to nitrogenase. *In:* Bergersen, F.J. (Ed.), Methods for evaluating biological nitrogen fixation. Wiley, New York, pp. 265-314.

Garg B.K., Gupta I.C., 2000 - Nodulation and symbiotic nitrogen fixation under salt stress, Curr. Agric. 24: 23-35.

Georgiev G.I., Atkins C.A., 1993 - Effects of salinity on N_2 fixation,nitrogen metabolism and export and diffusive conductance of cowpea root nodules. Symbiosis, 15, 239-55.

Rao D.L.N., Giller K.E., Yeo A.R., Flowers T.J., 2002 - The effects of salinity and sodicity upon nodulation and nitrogen fixation in chickpea (*Cicer arietinum*). Ann. Bot., 89: 563-570.

Grattan S.R., Maas E.V. 1988 - Effect of salinity on leaf P accumulation and injury in soybean: I. Influence of varying $CaCl_2$/NaCl. Plant Soil.,105: 25-32.

Groat R.G., Vance C.P., 1981 - Root nodule enzymes of ammonia assimilation in alfalfa (*Medicago sativa* L.). Plant Physiol., 67: 1198-1203.

Hafeez F.Y., Aslam Z., Malik K.A., 1988 - Effect of salinity and inoculation on growth, nitrogen fixation and nutrient uptake of *Vigna radiata* L. Wilczek. Plant Soil, 106: 3-8.

Kaiser J.J., Lewis O.A.H. 1984 - Nitrate reductase and glutamine synthetase activity in leaves and roots of nitrate fed *Helianthus annuus* L. Plant Soil, 70: 127-130.

Lee G.J., Carrow R.N., Duncan R.R., Eiteman M.A., Rieger M.W., 2008 - Synthesis of organic osmolytes and salt tolerance mechanisms in *Paspalum vaginatum*. Environ. Exp. Bot., *63*: 19-27.

Leidi E.O., Silberbush M., Soares M.I.M., Lips S.H., 1992 - Salinity and nitrogen nutrition studies on peanut and cotton plant. J. Plant Nutr., 15: 591-604.

Ligero F., Lluch C., Olivares J., 1986 - Evolution of ethylene from roots of *Medicago sativa* plants inoculated with *Rhizobium meliloti*. Plant Physiol., 125: 361-365.

Mohammad R.M., Akhavan-Kharazian M., Campbell W.F.,Rumbaugh M.D., 1991 - Identification of salt and drought tolerant *Rhizobium meliloti* L. strains. Plant Soil, 134: 271-276.

Öğütçü H., Algur Ö.F., Elkoca E., Kantar F. 2008 - The determination of symbiotic effectiveness of rhizobium strains isolated from wild chickpeas collected from high altitudes in Erzurum. Turk. J. Agric. For., 32: 241- 248.

Ohyama T., Kumazawa K. 1978 - Incorporation of ^{15}N into various nitrogenous compounds in intact soybean nodules after exposure to $^{15}N_2$ gas. Soil Sci. Plant Nutr., 24: 525-533.

Pessarakli M., Tucker T.C., 1985 - Ammonium (^{15}N) metabolism in cotton under salt stress. J.Plant Nutr., 8: 1025-1045.

Rai R., Prasad. V., 2003 - Salinity tolerance of *Rhizobium* mutants: growth and relative efficiency of symbiotic nitrogen fixation. Soil Biol. Biochem., 15: 217-219

Rigaud J., Puppo A., 1975 - Indole-3-acetic acid catabolism by soybean bacteroids. J. Gen. Microbiol., 88: 223-228.

Schubert K.R., 1986 - Products of biological nitrogen fixation in higher plants: synthesis, transport, and metabolism. Ann. Rev. Plant Physiol., 36: 539-574.

Serraj R., Vasquez-Diaz H., Drevon J.J., 1998 - Effects of salt stress on nitrogen fixation, oxygen diffusion and ion distribution in soybean, common bean and alfalfa. J. Plant Nutr., 21: 475-488.

Singh R.P., Srivastava H.H., 1986 - Increase in glutamate synthase (NADH) activity in maize seedlings in response to nitrate and ammonium nitrogen. Physiol. Plant, 66: 413-416.

Tejera N.A., Campos R., Sanjuan J., Lluch C., 2005 - .Effect of sodium chloride on growth, nutrient accumulation, and nitrogen fixation of common bean plants in symbiosis with isogenic strains. J.Plant Nutr., 28: 1907-1921.

Vessey J.K., Buss T.J., 2002 - *Bacillus cereus* UW85 inoculation effects on growth, nodulation, and N accumulation in grain legumes: controlled-environment studies. Can. J. Plant Sci., 82: 282-290.

Weil R.R., Khalil N.A., 1986 - Salinity tolerance of winged beans as compared to that of soybean. Agron. J., 78: 67-70.

Wenxue W., Bilsborrow P.E., Hooley P., Fincham D.A., Lombi E., Forster B.P., 2003 - Salinity induced differences in growth, ion distribution and partitioning in barley between the cultivar Maythorpe and its derived mutant Golden Promise. Plant Soil, 250:183-191.

Wignarajah K., 1990 - Growth response of *Phaseolus vulgaris* to varying salinity regimes. Environ. Exp. Bot., 30: 141-147.

Yancey P.H., Clark M.E., Hand S.C., Bowlus R.D., Somero G., 1982 - Living with water stress: evolution of osmolyte systems. Science, 217: 1214-1222.

Yousef A.N., Sprent J.I., 1993 - Effect of Nacl on growth, nitrogen incorporation and chemical composition of inoculated and NH4NO3 fertilized *Vicia faba* L. plants. J. Exp. Bot., 143: 941-950.

Zahran H.H., Sprent J.I., 1986 - Effects of sodium chloride and polyethylene glycol on root-hair infection and nodulation of *Vicia faba* L. plants by *Rhizobium leguminosarum*. Planta, 167: 303-309.

Zhu J.K., 2002 - Salt and drought stress signal transduction in plants. Annu. Rev. Plant Biol., 53: 247-274.