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Nodulation, Nodule Growth and Proton Release by Soybean Plants Cultivated in Hydroaeroponic System as Affected by Salinity and *Bradyrhizobium japonicum* Strains

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ABSTRACT

The present study aims to investigate the nodulation and nodule growth and proton release by soybean plants, Essor, inoculated with two strains of *Bradyrhizobium japonicum*, G49 and USDA122, tested for their salinity tolerance. The plants were cultivated in aerated saline solutions from the germination or 21 day after transplanting. By increasing salt concentrations, the strains were slowly grown. This effect was more obvious for strain USDA122 than G49 indicating that strain USDA122 was more sensitive to salts than the other one. Salt decreases the different parameters of plant growth. However, its effect was more pronounced in plants with early exposure to saline solutions. The nodule dry weight was the most affected. Nodule number was systematically less affected by salt than nodule mass. Plants inoculated with strain, G49 seem to be more tolerant than those inoculated with USDA122, especially for plants continuously growing in the high saline solution treatment. Salinity application significantly decreased the total proton release, whatever the strain used for inoculation. However, plants inoculated with strain G49 released more protons than those inoculated with the other bacterial strain. Increasing both of salinity concentration and time of application caused an increase in the values of specific proton release.

Key words: *Bradyrhizobium japonicum*, hydroaeroponic system, nodulation, salinity, soybean plants, proton.

INTRODUCTION

The vegetative production was highly limited by dinitrogen in saline zones. In the condition of limited available dinitrogen, the leguminous plants can grow better than the other plants since they can fix the atmospheric dinitrogen. Increasing salt concentrations limit and affect the dinitrogen symbiotic fixation (Lauter *et al*., 1981; Tu, 1981; Singleton and Bohlool, 1983, Abdel-Wahab *et al*., 2002).

The initiation of nodules was particularly sensitive to salinity. Survival and multiplication of rhizobia in the rhizosphere do not seem to limit the nodulation in saline medium. In fact, the majority of studied nodulating bacteria seem to be tolerant to salts rather than hosts plants. However, dinitrogen fixation by nodulated legumes which resulting from a molecular dialog between the micro and macrosymbiont and lead to nodules formation appears to be sensitive to salinity stress (Tu, 1981; Ikada, 1994).

The inhibition of infection may be related to a decrease of potential sites of infection which resulting from an inhibition either of root development (Ikada, 1994; Souissi, 2000) or elongation and curling of these organs (Lakashmi-Kumari *et al*., 1974; Tu, 1981; Zahran and Sprent, 1986; Ikada, 1994). Saline culture media limit the plant nutrition by calcium which leads to an inhibition in root emergence and growth (Zahran and Sprent, 1986; Zahran, 1999).

The inhibition of infection by salts seems to be due to the sensitivity of host plant rather than the direct effect on the microsymbiont (Singleton *et al*., 1982; Hafeez *et al*., 1988). However, other studies showed that salt effect occurs in late stages of the recognition plant host-bacteria. In white clover, the penetration of bacteria to root hair was inhibited by salts (Ikada, 1994). The ramification of infection filament and its migration through the root cortex is affected by salt stress (Bauer, 1981). Independent of its mode of action, the effect of salinity on nodulation was translated by a reduction in nodule number observed in several legumes, *Glycine max* (James *et al*., 1993; Abd-Alla *et al*., 1998); *Cicer arietinum* (Lauter *et al*., 1981; Ram *et al*., 1989; Elsheikh and Wood, 1990; Soussi *et al*., 1999); *Vigna radiata* (Hafeez *et al*., 1988)*; Phaseolus vulgaris* (Saadallah *et al*., 2001). However, stimulation in nodulation was observed in some legumes grown under salt stress: faba bean (Yousef and Sprent, 1983) and some varieties of chick-pea (Soussi *et al*., 1999).

Increased acidification of the rhizosphere by roots is a widespread response to the nitrogen (N) nutrition of the plant, as related to the balance of cations over anions taken up and, also, to the source of N taken up. Indeed, N can be positively charged (ammonium) and favor large proton release, negatively charged (nitrate) and favor hydroxyl release, or uncharged in the case of legumes depending on N_2 fixation (Raven and Smith, 1976; Hinsinger *et al*., 2003). In the latter case, proton release and, hence rhizosphere acidification is expected to occur because of legumes reliant on N_2 fixation take up more cations than anions (Raven *et al*., 1990; Tang *et al*., 2001a, b). The effect of N sources and rhizobial strains inoculation on proton release in legumes were reported before (Fan *et al*., 2002; Hinsinger *et al*., 2003). However the intrinsic rhizobial strains effect on the proton release by soybean plants under salinity stress need more studies.

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The majority of studies conducted on the interaction symbiotic dinitrogen fixation/salinity showed a decrease in nodule dry weight under salinity conditions. However, this behavior translates the simultaneous effects of salinity on either the nodules initiation or the growth of these organs. This study aims to determine: (1) the salinity tolerance of two *Bradyrhizobium japonicum* strains, (2) the respective part of the two previous mentioned components (nodule initiation and growth) in the reduction of nodule dry matter in the soybean plants cultivated at different levels of salinity and (3) to which extent the salinity and the two rhizobial strains enhance proton release.

MATERIALS AND METHODS

Tolerance of *Bradyrhizobium japonicum* strains, G49 and USDA122 to salinity stress

The two *B. japonicu*m, strains G49 and USDA122 were tested for their salinity tolerance by analyzing the growth curve parameters. Strains were pre-cultured in 96-well microplates in YEM medium without agar. The growth of strains was monitored using a Microplate Reader (Thermomax, Molecular Device, USA). Each well was filled with 150 µl of liquid medium, containing 0 (S^0), 1500 (S^{1500}), 3000 (S^{3000}) and 5000 (S^{5000}) mg NaCl l^{-1} and inoculated with 10 µl of the strain pre-cultures (Mohamed, 2004). Each treatment was replicated for 4 times. Plates were incubated at 20 °C for 192h. Optical density (A_{590nm}) was measured at 0, 24, 48, 72, 96, 120, 144, 168, 192 hours. Analyzed data were: (1) maximum growth (growth yield) estimated by the maximum optical density, (2) maximum growth rate estimated by the maximum increase in optical density per unit of time, and (3) apparent lag phase estimated by the time required to observe an increase in optical density (Mohamed *et al*., 2005). Variance analysis for maximal growth yield and maximal growth rate of each strain were performed using StatView Version 5 (SAS Institute Inc., USA) with the Fisher's PLSD test at the probability level of 5%.

Hydroaeroponic culture

Plants were grown in nutrient solution. Soybean seeds (*Glycine max* L. Merril) Essor, were sterilized in Ca hypochlorite solution (70% active chlorine) for 20 min, rinsed several times with sterile water. Seeds were germinated for 5 d in the dark on fly screens covered with paper towels above an aerated nutrient solution containing 800 µM $CaCl_2$ and 4 µM H_3BO_3. One seedling was transplanted (day 0) to 1 l bottle containing nutrient solution of the following composition (µM): KH_2PO_4, 250; K_2SO_4, 700; $MgSO_4$, 500; $CaCl_2$, 800; H_3BO_3, 4; Na_2MoO_4, 0.1; $ZnSO_4$, 1; $MnCl_2$, 2; $CoCl_2$, 0.2; $CuCl_2$, 1; and FeNaEDTA (ferric monosodium salt of ethylenediamine tetraacetic acid), 10 (Vadez and Drevon, 2001). Solution pH was adjusted every two days with KOH (0.01 M) to 7 during 0-42 day after transplanting (DAT) (Hinsinger *et al*., 2003). The bottles were wrapped with aluminum foil to keep the rooting environment in dark and to prevent the growth of algae. The roots were gently passed through the hole of a rubber stopper on the bottle neck, and cotton wool was fitted at the hypocotyl level. A dense water suspension of *B. japonicum*, strains G49 or USDA122 was added to the solution at a rate of approx 10^8 cells l^{-1}. This solution was unchanged for 7 d and then renewed with added rhizobia. Seven days later, plants were placed in solutions without added rhizobia; solutions were then changed every week. Urea (0.2 mM N) was added for the first 14 days after transplantation (DAT). Thereafter, the plants did not receive any external source of N. This low N supply did not prevent the formation of nodules (Saadallah *et al*., 2001).

The seedlings were distributed in 5 treatments from the first day of transplanting. In the 1st treatment, seedlings were grown in control nutrient solution described above. In the 2nd and 3rd treatments, NaCl was added to distilled water in order to raise the salinity levels to 1500 (S^{1500}), 3000 (S^{3000}) ppm, respectively. The salts of nutrient solution were added to these saline solution at the same concentrations described above. The addition of NaCl to the seedlings in the 4th (BS^{1500}) and 5th (BS^{3000}) treatments were at the same salinity levels of treatments 2 and 3, respectively, but it's application was delayed 21 DAT. Each treatment was repeated for four times.

The number of nodules was counted on intact plants 42 DAT and plants in the bottles were harvested. Plants were separated into shoots, roots and nodules. All plant tissues were oven-dried at 70 °C for 48 h.

The dry nodules were split up into three classes by sieving. First correspond to nodules which have diameter superior than 1mm, second contains nodules of diameter ranged between 0.5 and 1 mm and the third contains the nodules less than 0.5 mm in diameter.

Total amounts of protons released by plants were determined by summing up the amounts of KOH used for pH adjustment during plant growth and for titrating the used solution after plant growth to the initial pH. Specific H^+ release was expressed as the amount of H^+ released per unit root biomass.

Variance analysis for total and specific proton release were performed using StatView Version 5 (SAS Institute Inc., USA) with the Fisher's PSLD test at the probability level of 5%.

RESULTS

Tolerance of *B. japonicum* G49 and USDA122 to salinity stress

Growth curves at different salinity levels are shown in Figure (1A & 1B). The two strains exhibit the same rate of growth in the control media. By increasing salt concentrations, the strains were slowly grown with an indication of longer lag times. This effect was more obvious for strain USDA122 than G49. During the period ranged between 72-144h, all strains at the different salinity concentrations were in the exponential phase of growth; afterwards the cultures entered the stationary phase. For each strain, maximum growth rate was not significantly different between the salinity treatments indicating that the increased salt concentration had no effect on this parameter. However, the maximum growth was significantly affected by salinity for each strain with a probability less than 0.0001. The values of the maximum OD$_{590nm}$ ranged between 0.670-0.853 and 0.759-0.857 for USDA122 and G49 strain, respectively, indicating again that the strain USDA122 was more sensitive to the high salinity concentrations than the other one.

Plant growth

In the control nutrient solution, plants inoculated with the two rhizobial strains noticeably produced, in average, the same quantity of dry matter, a little more than 3.3 g plant^{-1} (Fig. 2). By increasing the salinity levels, the growth of plants decreased. In order to estimate the difference between plants inoculated with each strain, the index of saline sensitivity (SI) was used. This parameter corresponds to the difference between the dry matter production in saline solutions and those produced in control solution, expressed in a percentage of the latest. The values of this parameter were less than -35 % for plants inoculated with the two strains in the S^{1500} treatment. Plants express approx. the same sensitivity at this level of salinity. However, plants inoculated with *B. japonicum.* G49 seem to be more tolerant than those inoculated with USDA122 at the S^{3000} since the SI was -42 % for the first and -49 % for the second.

The delayed salinity treatments also reduced the growth of plants. However, its effect was less pronounced than when salinity treatments were applied at the germination. The values of SI for plants growing at the belated S^{1500} and S^{3000} treatments ranged between 17-25 %.

Values of the ratio root/shoot dry matters (R/S) were presented in Table (1). For plants inoculated with the two strains, this ratio tends to decrease in S^{1500} treatment as compared with control. This result seems to be related to the higher sensitivity of root than shoot at this level of salinity. However, R/S ratio in the S^{3000} treatment slightly increased as compared with S^{1500} treatment indicating that the shoot parts were more affected at this level of salinity than the roots. For the plants growing at the postponed salinities, the values of R/S ratio were more close to those of plants in control solutions than those growing in continual saline solutions. The plants presented the same variations of R/S ratio. However, the roots of plants inoculated with the strain *B. japonicum* USDA122 were slightly more affected by saline solutions than those inoculated with *B. japonicum* G49, especially for plants growing in continual saline solutions.

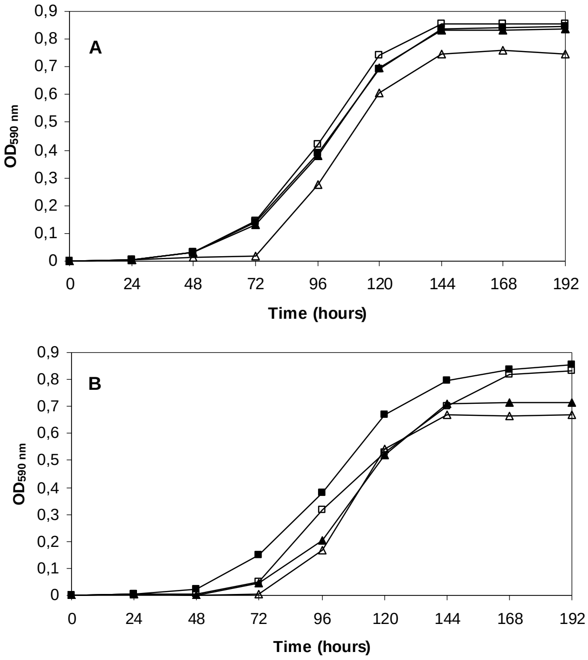

Figure (1): A and B: Growth curves of *Bradyrhizobium japonicum* strains: (a) G49 and (b) USDA122 at different salinity levels, the control (■), 1500 (□), 3000 (▲) and 5000 mg NaCl l^{-1} (Δ). Average of four replicates.

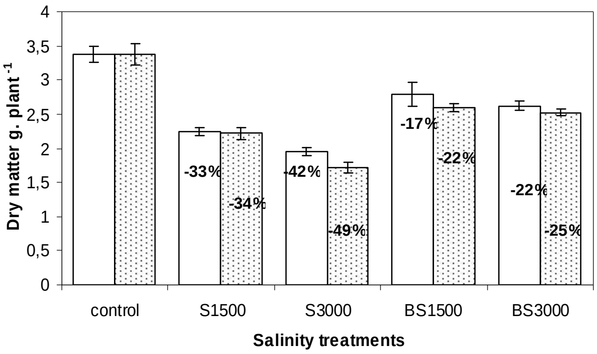

Figure (2): Variations of plant growth as a function of salinity levels and its application time. Plants inoculated with *Bradyrhizobium japonicum* strains: G49 (□) and USDA122 (▨). Bars represent s.e. of four replicates. The numbers within the histograms correspond to the index of saline sensitivity (SI).

Control, seedlings grown in nutrient solution (NS) without adding NaCL; S1500, seedling grown in (NS) plus 1.5g Nacl; S3000, seedling grown in (NS) plus 3g NaCL; BS1500, seedling grown in (NS) plus 1.5g NaCL applied 21 DAT; BS3000, seedling grown in (NS) plus 3g NaCL applied 21 DAT

Nodule initiation and developments

In the control solution, plants inoculated with the strain *B. japonicum* G49 slightly produce more nodule dry matter than those inoculated with the strain

USDA122 (Fig. 3A). The application of saline solution from the germination highly reduced nodule dry weight in plants inoculated with the two strains (plants inoculated with the strain G49 were less affected than those inoculated with the other one, especially at S^{1500} treatment). The delayed salt treatment caused also a decrease in nodule dry matter. However, its effect was less marked. Values of the ratio nodule/shoot dry matters (N/S) were presented in Table (1). For plants growing in control solution, the nodule dry matter represents approx 5-6 % of the shoot dry matter. This result showed a good nodule growth for plants inoculated with the two strains in the absence of salts. Increasing salinity from germination caused a high reduction of this ratio suggesting a particular sensitivity of nodules comparing with shoot parts. However, when the application of saline solution belated, the dry matter of the two organs reduced by the same manner.

The reduction of nodule dry weight results from either a decrease in nodule number, a reduction in the volume of these organs or as a result of these two parameters. The data shown in Figure (3B) demonstrate that the depressive action of salinity on nodule number was only obvious in plants growing continually in S^{3000} treatment. The delayed application of salinity does not seem to negatively affect the nodulation.

The reduction in nodule dry matter and number for plants growing in saline solutions are presented in Table (2). The decrease in nodule dry weight varied between -18 to -86.5 % according to salinity or strain treatments. Nodule number was less affected. Moreover, a slight stimulation in nodule number was observed for plants in belated salinity treatments. It seems that the reduction in nodule mass in these treatments is suggested to be due to the decrease in nodule growth more than the nodule initiation inhibition.

For the difference between plants inoculated with the two strains, it's noted that at S^{1500} treatment, nodule dry weight and nodule number were more affected for plants inoculated with strain USDA122 than strain G49. This leads us to suggest that the nodulation and nodule growth were more sensitive to salinity for plants inoculated with the first than the second strain. At S^{3000} treatment, plants inoculated with G49 do not seem to maintain its superiority, especially at the level of nodulation.

The distribution of dried nodules according to their sizes showed that, for plants in control solution, the nodules of big and medium sizes were predominant and represented more than 90 %, in average, of whole nodules (Fig. 4). For plants growing in saline solutions, the nodules of big sizes became minorities. This effect was less marked when saline solutions treatments were belated. This schema of distribution was identical for plants inoculated with the two strains.

Table (1): Variation of dry matter ratios R/S, N/S and N/R as a function of salinity concentrations and its application time for soybean plants inoculated with *Bradyrhizobium japonicum* strains, G49 and USDA122.

			Treatments		
	Control	**S^{1500}**	**S^{3000}**	**BS^{1500}**	**BS^{3000}**
Plants inoculated with *B. japonicum* strain G49					
R/S	0.26	0.22	0.23	0.24	0.24
N/S	0.06	0.03	0.01	0.05	0.05
N/R	0.25	0.16	0.06	0.21	0.2
Plants inoculated with *B. japonicum* strain USDA122					
R/S	0.29	0.22	0.25	0.31	0.30
N/S	0.05	0.01	0.01	0.06	0.06
N/R	0.18	0.06	0.05	0.19	0.19

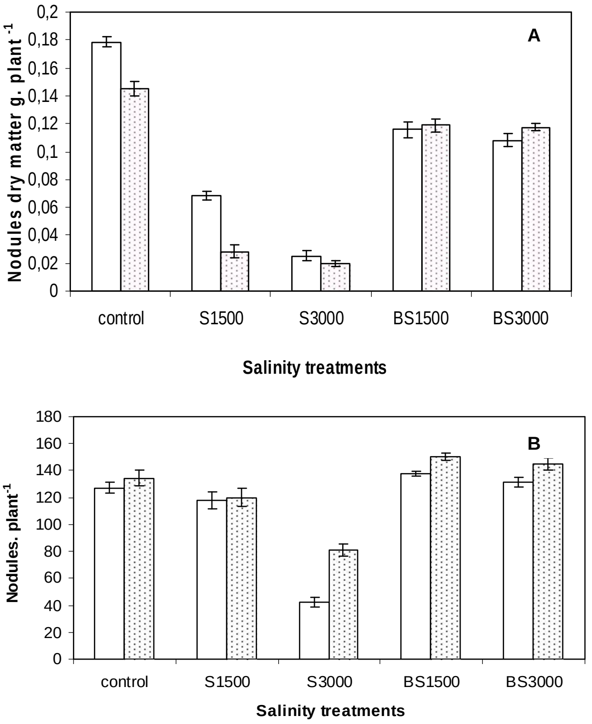

Figure (3): A and B: Effect of salinity (levels and time of application) on: (a) Nodule dry weight and (b) nodule number. Plants inoculated with *Bradyrhizobium japonicum* strains: G49 (☐) and USDA122 (▨). Bars represent s.e. of four replicates.

Control, seedlings grown in nutrient solution (NS) without adding NaCL; S1500, seedling grown in (NS) plus 1.5g Nacl; S3000, seedling grown in (NS) plus 3g NaCL; BS1500, seedling grown in (NS) plus 1.5g NaCL applied 21 DAT; BS3000, seedling grown in (NS) plus 3g NaCL applied 21 DAT

Proton release

There was a net proton release by plants relying on N_2-fixation. The amounts of total proton released by N_2 fixing plants (15-42 DAT) as measured by pH-meter are shown in Table (3). Growing plants released protons and decreased the solution pH. Salinity application significantly decreased the total proton release, whatever the strain used for inoculation. However, plants inoculated with the strain G49 released more proton than those inoculated with the other strain.

The amounts of specific proton release (*i.e.*, protons released per unit root biomass) at different salinity treatments are shown in Table (3). The lowest values of specific proton release were found in plants growing at control nutrient solution. Increasing both the salinity concentration and its time of application caused an increase in the values of this parameter. This effect was more obvious for plants inoculated with the strain G49 than those inoculated with the other one. Regardless of the salinity treatments, plants inoculated with the strain G49 release significantly more protons than the strain USDA122 since they released 1.06 and 1.00 nmol plant $^{-1}$.s^{-1}, respectively with a probability value of less than 0.0001. However, no significant difference was observed between the values of specific proton release for plants inoculated with strains G49 and USDA122 since the values of this parameter were 2.184 and 1.993 nmol g root DM^{-1}.s^{-1}, respectively, with a probability value of 0.2706.

DISCUSSION

The present study aimed to investigate the nodulation and nodule growth of soybean plants inoculated with two strains of *Bradyrhizobium japonicum* tested for their salinity resistant. The plants were cultivated at aerated saline solutions from the germination or at a belated manner after the establishment of the symbiotic organs. In soil system, the effect of salinity on the growth and survival of rhizobia and its distribution in the rhizospheres of plants was reported before (Jenkins *et al.*, 1989; Tate, 1995). However, in the hydroaeroponic system, as rhizobia were only added to the nutrient solution in the first 14 d, the rhizobial population was expected to be very low in the nutrient solution after day 14 (Tang *et al.*, 2001b). It is not

Table (2): Reduction in nodules dry matter and nodules number as affected by salinity concentrations and its application time for soybean plants inoculated with *Bradyrhizobium japonicum* strains, G49 and USDA122**.**

	Treatments			
	S^{1500}	**S^{3000}**	**BS^{1500}**	**BS^{3000}**
Reduction in nodules dry matter*:				
Plants inoculated with *B. japonicum* strain G49				
	-61.7	-85.8	-35.1	-39.5
Plants inoculated with *B. japonicum* strain USDA122				
	-80.5	-86.5	-18	-19
Reduction in nodules number*:				
Plants inoculated with *B. japonicum* strain G49				
	-7.3	-66.6	8.5	3.4
Plants inoculated with *B. japonicum* strain USDA122				
	-10.8	-39.7	11.9	8.2

*The reductions were calculated as a percentage of control treatment.

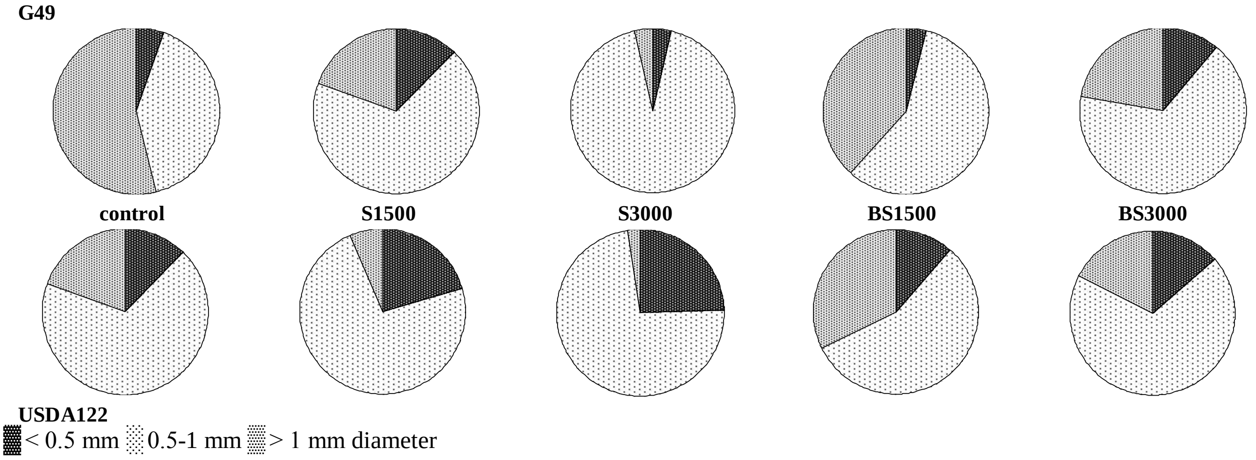

Figure (4): Distribution of nodules according to their size classes as affected by salinity levels and time of application. G49, plants inoculated with *Bradyrhizobium japonicum* strain, USDA122, and *Bradyrhizobium japonicum* strain, USDA122.

Control, seedlings grown in nutrient solution (NS) without adding NaCL; S1500, seedling grown in (NS) plus 1.5g Nacl; S3000, seedling grown in (NS) plus 3g NaCL; BS1500, seedling grown in (NS) plus 1.5g NaCL applied 21 DAT; BS3000, seedling grown in (NS) plus 3g NaCL applied 21 DAT

Table (3): Proton release by the roots of soybean plants as affected by salinity concentrations and its application time. Plants inoculated with *Bradyrhizobium japonicum* strains, G49 and USDA122.

Salinity treatments	**Total proton release (15-42) nmol plant^{-1}.s^{-1} (G49)[1]**	**Total proton release (15-42) nmol plant^{-1}.s^{-1} (USDA122)[2]**	**Specific proton release (15-38) nmol g root DM^{-1}.s^{-1} (G49)[1]**	**Specific proton release (15-38) nmol g root DM^{-1}.s^{-1} (USDA122)[2]**
Control	1.112	1.017	1.591	1.326
S^{1500}	1.020	0.983	2.507	2.385
S^{3000}	1.029	0.995	2.822	2.919
BS^{1500}	1.066	1.000	1.949	1.622
BS^{3000}	1.071	1.006	2.051	1.715
Values of probability[a]	0.0083*	0.0129*	<0.0001*	<0.0001*

(G49)[1] Plants inoculated with *B. japonicum* strain G49, (USDA122)[2] Plants inoculated with *B. japonicum* strain USDA122, [a] indicates a significant level at $P < 0.05$.

known whether the high salinity levels affect the nodule formation when the rhizobial population is low. In general, increasing salt concentrations may have a detrimental effect on microbial populations as a result of direct toxicity as well as through osmotic stress (Tate, 1995).

Successful *Rhizobium*-legume symbioses under salt stress require the selection of salt-tolerant rhizobial strain (Zahran, 1991a) since some legume hosts are less tolerant to salt than are their rhizobia. 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, 1983), whiles some rhizobia, e.g., *Rhizobium meliloti*, was tolerant to 300 to 700 mM NaCl (Mohammad, 1991; Embalomatis, 1994). The two tested rhizobial strains differed in their salinity tolerance since the strain G49 was more tolerant than the USDA122 strain. Variability in salt tolerance among crop legumes has been reported (Zahran, 1991a; Zahran, 1991b). Some legumes, e.g., *Vicia faba*, *Phaseolus vulgaris,* and *Glycine max*, are more salt tolerant than others, e.g., *Pisum sativum* (Zahran, 1999).

The legume-*Rhizobium* symbioses and nodule formation on legumes are more sensitive to salt or osmotic stress than are the rhizobia (El-Shinnawi, 1989). Salt stress inhibits the initial steps of *Rhizobium*-legume symbioses. Soybean root hairs showed little curling or deformation when inoculated with *Bradyrhizobium japonicum* in the presence of 170 mM NaCl, and nodulation was completely suppressed by 210 mM NaCl (Tu, 1981). In our experimental conditions, the different parameters of plant growth were affected even at 1500 ppm. This result confirms the particular sensitivity of soybean at this stressed condition. The modification of the dry matter distribution induced by salts between the shoot and root part was estimated by the variations between the ratio of roots /shoots dry matters. The results suggested that a specific inhibition of assimilates transport from shoot to root parts for plants growing at saline concentrations which translated by a decrease in the ratio of roots/shoots dry matters. This particular sensitivity of roots were found at saline conditions with other legumes; *Medicago sativa* (Brun, 1980; Bauer, 1981) *Cicer arietinum*), (Sléimi, 1996, Souissi, 2000).

The reduction in root growth was associated with an inhibition of nodulation, estimated by nodule number. This decrease was related to the diminution of potential sites of infection resulted essentially from an inhibition of root hair emergence and its development (Ikada, 1994; Souissi, 2000). Salt presence in culture medium limits the plant nutrition by calcium which leads to an inhibition in root emergence and growth (Zahran and Sprent, 1986).

The diminutions in nodule dry weight were more distinct than those of roots or shoots parts. The values of the ratios N/S or N/R were systematically reduced by salinity for plants inoculated with the two strains. According to Velagaleti and Marsh (1989), the diminution in nodule weights result from a reduction in quantity of photosynthetats which consecutively lead to a decrease in shoots parts development and a partial inhibition of photosynthetats transport to nodules. The results shown in the present study demonstrate that the reduction in nodule mass result from an inhibition of nodule initiation (estimated by nodule number) and/or the growth of these organs (estimated by nodule sizes). This second parameter seems to be more sensitive to salinity than the first one (Saadallah *et al*., 2001).

An interesting finding in this study is the intrinsic rhizobial strains difference in the relationship between root symbiosis and proton release. It was observed that plants inoculated with *B. japonicum*, strain G49 released more protons than those inoculated with the other strain (Table 3). However, no significant difference was observed between plants inoculated either with strain G49 or USDA122 when released protons expressed per unit root biomass. This indicates that the rhizobia strain had no specific effect on proton release, but an indirect effect via increased N_2 fixation and plant growth. The increased salinity caused a reduction in proton release. This may be explained by the observed reduction in root dry matter as an effect of salinity and by its negative effect on the potential sites of proton release which resulting from an inhibition of root development. Salinity stress in the growing root medium limits the plant nutrition by calcium which leads to an inhibition in root emergence and growth (Zahran and Sprent, 1986).

Several studies showed that the salinity increase the resistance to oxygen diffusion in the nodules (Vance and Heichel, 1991; Drevon *et al*., 1994; Vadez *et al*., 1996) which lead to an inhibition of the nitrogenase activity. They showed that the increase in oxygen pressure in the media of nodulated roots permit to eliminate the inhibitor effect of salinity on the acetylene reduction activity.

Generally, the salinity inhibits the N_2 symbiotic fixation, at least by limiting the nodule functioning through a decrease in its conductance of oxygen diffusion. In these conditions, the establishment of high number of small nodules ensures that a bigger contact surface with the exterior media which favorite an excellent entrance of oxygen to nodules. This may partially compensate the limitations in oxygen diffusion caused by salinity stress (Sheehy and Thornley, 1988).

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مدى تأثر التعقيد ونمو العقد والبروتونات المنطلقة من نباتات فول الصويا المنزرعة فى نظام الزراعة الهوائية المائية بالملوحة والتلقيح بالبرادي ريزوبيا

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الملخص العربــى

تم فى هذا البحث دراسه أثر التركيزات المتزايده من كلوريد الصوديوم فى مـدى من 1500 إلى 5000 جزء فى المليون على نمو سلالتين من برادى ريزوبيم جابونيكم هى USDA122 ، G49. إستخدمت هاتين السلالتين فى تلقيح نباتات فول الصويا (صنف ايسور) المنزرعة بنظام الزراعة الهوائية المائية فى محاليل ملحية طبقت من بداية الزراعة أو بعد استقرار تكون العقد (21 يوم من بداية الزراعة).

وقد أظهرت النتائج أن نمو النبات، وتكوين العقد الجذريه وأحجامها قد تأثرت بزيادة تركيزات الملوحة ومع ذلك فان هذا التأثير كان أكثر وضوحا على النباتات المنزرعة فى المحاليل الملحية من بداية الزراعة. وقد وُجد أن الأوزان الجافة للعقد الجذرية كانت أكثر تأثرا من أعداد العقد. كما لوحظ أيضاً تأخر نمو السلالاتين بزيادة تركيزات الملوحة وكان تأثير الملوحة أكبر على السلالة USDA122 مما يؤكد أن هذه السلالة أكثر حساسية للملوحة من السلالة الأخرى. وقد انعكست هذه الملاحظه على مدى تأثير هاتين السلالاتين على نمو نباتات فول الصويا فى المحاليل الملحيه ، فقد أعطت السلالة G49 زيادة ملحوظه للقياسات سابقه الذكر عنها للسلالة USDA122 خاصة للنباتات المنزرعة فى المحاليل عالية الملوحة. ان زيادة تركيزات الملوحة ادى الى انخفاض معنوى فى كمية البروتونات الكلية المنطلقة من جذور النباتات وذلك عند استخدام اى من السلالتين. وعلى الرغم من ذلك فان فقد أعطت النباتات الملقحة بالسلالة G49 زيادة ملحوظه فى كمية البروتونات الكلية المنطلقة من جذور النباتات عنها للنباتات الملقحة بالسلالة USDA122.