World Applied Sciences Journal 13 (2): 256-265, 2011
ISSN 1818-4952

Evaluation of Growth, Nodulation and Nitrogen Fixation of Two *Acacia* Species under Salt Stress

[1]T.S. Al-Shaharani and [2]N.D. Shetta

[1]Plant Production, Food and Agriculture Sciences Collage, King Saud University, KSA
[2]Forestry Department, Faculty of Agriculture, Alexandria University, Egypt

Abstract: Seedlings of two *Acacia* species; *Acacia ehrenbergiana* (Hayne.) and *Acacia tortilis* (Forssk.), grown in Dirab Valley (N.24° 24′ 33″, E.46° 39′ 40″), South Riyadh, KSA, were inoculated with four *Rhizobium* strains; (AER), isolated from nodulated roots of *Acacia ehrenbergiana*, (ASR) strain isolated from *Acacia saligna*, (ATR) strain, isolated from *Acacia tortilis* and (LLR) strain isolated from *Leucaena leucocephala* to evaluate growth response and ability of such species to form root nodules under salt stress and to obtain an indication of nitrogen fixation potential during two successive seasons. Results demonstrated that, salt concentration affects growth parameters of seedling, root nodulation and nitrogen fixation of *Acacia ehrenbergiana* (Hayne.) and *Acacia tortilis* (Forssk.). Increasing of salt concentration led to decrease growth, nodulation and nitrogen fixation. The harmful concentration of salt was 3% NaCl, since *Rhizobium* strains were affected by salt stress in the soil, yet the most salinity-tolerant strain was AER followed by strain ASR and the least tolerant strain of salinity was LLR. *Acacia ehrenbergiana* displayed the highest tolerance of salt stress than *Acacia tortilis*. Our experiment suggested that the inoculated legume trees with salinity-tolerant *Rhizobium* strains in the nursery should establish an effective nitrogen fixing symbiosis, which may be more tolerant of salinity after outplanting. It has been emphasized that the selection of legume host from highly saline or non saline habitats appeared to be the most important factor governing compatible *Rhizobium* strains to nodulation and N_2 fixation under salinity hazards.

Key words: *Acacia* trees · *Rhizobium* inoculation · Salt stress · Nodulation · Nitrogen fixation · Saudi Arabia

INTRODUCTION

Several environmental conditions are considered as limiting factors which may inhibit growth and activity of N_2-fixing plants. Salinity is the major environmental factor limiting plant growth and its productivity; it may act as a water stress factor which may affect photosynthesis or directly inhibits nodule metabolism [1]. In the case of *Rhizobium*-legume symbioses, the process of N_2-fixation is strongly related to the physiological status of the host plant. Root nodules are the site of a beneficial symbiotic association between legume plants and soil bacteria commonly denominated as *Rhizobia*. Plant supplies bacteria with an energy source, malate or succinate. In turn, bacteria fix the atmospheric N_2 gas to NH_4^+, providing the later to plant for assimilation into amino acids, protein and other essential nitrogenous compounds. Typical environmental stresses faced by legumes and their symbiotic partner (*Rhizobium*) may include photosynthetic deprivation, water stress, salinity and temperature [2]. Salinity constrains seriously the production in irrigated agriculture throughout the world. Nearly one third of the world's irrigated agricultural land is saline and current estimates put forwards as a salt-affected. The relationship between soil moisture, growth of legumes and their potential for nodulation and N_2-fixation has long been recognized, increasing the salinity of soils results in decreased productivity of most economic crops. The physiological features of 'salt-stress' and responses of plants in adapting to and surviving under saline conditions have been extensively studied. Although a limited number of legume species have been assessed, most have been relatively sensitive to salt [3]. Some salt-tolerant lines of certain legumes have been reported by Abdel Wahab and Zahran [4] and Cordovilla *et al.* [5]. Other legumes are salt tolerant, but legume hosts are less tolerant of salt than their *Rhizobia* [6, 7]. Legumes and woody legumes have been ranked as appropriate crops for the enhancement of bioproductivity and the reclamation of marginal lands because these

Corresponding Author: Thobayet Al-Shaharani {thobayet@yahoo.com}, Nader Shetta {n.shetta@yahoo.com}, Plant Production, Food and Agriculture Sciences Collage, King Saud University, Saudi Arabia.

plants not only yield nutritive fodder, protein-rich seeds and fruits, but they enrich soil nitrogen in symbiotic association with *Rhizobium* as well. Nodulation and nitrogen fixation in legume-*Rhizobial* associations are adversely affected by salinity, which can preclude legume establishment and growth, or reduce crop yield [8]. 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* [9]. Nodulation and nitrogen fixation are more sensitive to osmotic stress than are *Rhizobia* itself. The effects of salt stress on nitrogen fixation have been examined by Lauter *et al.* [10], Rai and Prasad [11] and Velagaleti and Marsh [12], but leguminous woody tree species have responded differently to salinity. Some woody legumes, e.g. *Prosopus* spp., *Acacia* spp. and *Leucaena purpureus* produce indeterminate (meristematic) nodules which are more salt tolerant than determinate (non-meristematic) nodules formed by other legumes [13]. Only the indeterminate nodules have the potential to regenerate activity and structure distorted by stress treatments, while *Rhizobia* might be able to withstand and successfully multiply under severe salinity, their infectibility and nodulating ability may change [14]. The formation of effective N_2-fixing nodules under salt stress depends on the presence of effective strains of *Rhizobia* and leguminous plants, which have the ability to form early nodules. Abdel-Wahab *et al.* [15], Sprent [16], Vance and Heichel [17], Drevon *et al.* [18], Gonza´lez *et al.* [19] and Serraj *et al.* [20] concluded that exposure to salt or drought stresses decreased nodule permeability. This decrease is associated with a contraction of nodule inner-cortex cells and an increase in abscisic acid content of nodule [9, 21, 22, 23]. Salt induced inhibition of nitrogenase activity and nodule respiration is compensated by raising PO_2 in the nodulated-root rhizosphere [24]. The specific sensitivity of the symbiotic nitrogen fixation (SNF)-dependent legumes to salinity is well documented for initiation, development and function of nodules. Abiotic stress such as salinity or drought led to decreases nodule permeability [25, 26].

Saudi Arabia (Lat. 32°34′ N-16° 83′ N, Long. 34° 36′ E-56′ E) is a vast arid desert with an area of about 2250,000 sq kms covering the major part of the Arabian Peninsula [27], which subjected to dry wind. Drought, scarce of precipitation and extreme high temperature are the most common characters of this region within the country. Under these conditions soils are affected by salinization and drought. Nonetheless the importance of *Acacia* species in Saudi Arabia, no attention has been given to their root-nodule symbiosis especially under salinity, drought and high temperature. The objectives of this study were to evaluate growth response and ability of two local woody legume trees; *Acacia ehrenbergiana* (Hayne.) and *Acacia tortilis* (Forssk.) to form nodules when planted under salt stress in Riyadh region; and to obtain an indication of the nitrogen fixation potential of the species.

MATERIALS AND METHODS

Site and Woody Legume Trees Used in the Study: The experiment was conducted in Forest Physiology Laboratory and Greenhouse Facilities, University of King Saud at Dirab Valley, (N.24° 42′ 33″, E.46° 44′ 40″, Alt.600m), South Riyadh, KSA. The climate in this part has been classified as arid environment with cold winter and hot summer. Mean, maximum and minimum temperature and maximum relative humidity during two successive seasons (2009-2011) are shown in Table 1. The aims of the study were to evaluate growth response and ability of two woody legume trees namely; *Acacia ehrenbergiana* (Hayne.) and *Acacia tortilis* (Forssk.) to form nodules when planted under salt stress in Riyadh region and to obtain an indication of the nitrogen fixation potential of the species. Physical and chemical characteristics of soil are given in Table 2.

Bacteriological Studies: Bacteria were chosen and isolated from the roots of the two woody legume trees (mentioned below). Isolates were named by an abbreviation of the host tree followed by the name of *Rhizobium* strains. Nodules were sampled from roots of young trees and seedlings. Strains were abbreviated as *AER, ASR, ATR and LLR* for isolates from, *Acacia ehrenbergiana* (Hayne.), *Acacia tortilis* (Forssk.), *Acacia saligna* (Labill.) and *Leucaena leucocephala* (Lam.), respectively. Root nodules were immersed in 95% ethanol for 10 seconds and 0.1% mercuric chloride for 5 min to disinfect the surface, then rinsed several time with sterilized water. Each nodule was crushed on plates containing yeast extract mannitol agar (YEMA) with 0.0025% (w/v) Congo red [28]. After incubation for 3 days at 28 °C single colonies were selected and transferred several times on YEMA plates to ascertain purity of *Rhizobia* strains isolated and kept in YEMB (yeast extract-mannitol broth) liquid with 20% glycerol at-20°C for further processing.

Table 1: Mean, maximum and minimum temperature and maximum relative humidity during two seasons of study

Experimental season	Temperature (°C) Maximum	Minimum	Mean	Maximum Relative Humidity (%)
2009/2010	25.78	11.75	18.77	65.26
2010/ 2011	28.38	12.20	20.29	58.80

Table 2: Physical and chemical analyses of soil used

Particle size distribution (%)						Soluble cations (meq/l)				Soluble anions (meq/l)			
Sand	Silt	Clay	Soil texture	pH	EC (dS/m)	Na^+	K^+	Ca^{++}	Mg^{++}	HCO_3^-	CO_3^{-}	SO_4^{-}	Cl^-
62.92	22.32	14.76	Sandy loam	7.86	6.53	30.43	2.47	25.0	7.40	2.16	-	7.20	30.60

Seedlings Production and Incubation with *Rhizobium* Strains and Salt Treatments: To break mechanical dormancy owing to seed coat, seeds of *Acacia ehrenbergiana* and *Acacia tortilis* were surface-sterilized and treated with hot water then soaked for two days and germinated in sterilized mixture soil of sand and vermiculite 1:1(V/V) in the greenhouse maintained at day /night temperatures of approximately 25°C. Two weeks after germination, the seedlings were transplanted to pots of 16 cm diameter containing approximately 3 kg soil and watered manually with tap water. After six weeks, seedlings growing in pots were inoculated with 20 ml (approximately 10^8 bacterial cells ml/plant) of *Rhizobia* strains grown in YEM broth incubated for 3 days with shaking (200 rpm) at 28°C. Seedlings were allowed to establish for 15 days after which time the salt (NaCl) treatment was started. Afterwards, seedlings were separated into four groups (three plants per treatment) and irrigated with nutrient solution supplemented with 0 (control), 1, 2 and 3% of NaCl in the base of volume. Avoidance of salinity shock, the 3% concentration was increasingly exercised in 6 applications by 0.5% salt increase each. Seedlings were harvested at 60 days of the artificial inoculation with *Rhizobium* strains. Seedlings and *Rhizobial* strains were evaluated for their symbiotic potential (nodulation) under salinity stress. The numbers of nodules were counted, stem, root and nodule dry mass measured for each seedling after oven-drying at 80°C for 48 hours. The height of seedlings and total N content of the seedlings at the final harvest was measured using the Kjeldahl method as specified by Bergersen [29].

Nitrogen Fixation Assays: Nitrogen-fixation activity in root nodules was determined by C_2H_2 reduction assay (ARA) using a gas chromatograph equipped with a flame ionization detector (Carlo ERBA strumentazione) to measure the nitrogenase activity of root-nodules. Root nodules were placed in 100 ml rubber-capped vials containing C_2H_2 (10% v/v) in air. After an hour of incubation, the amount of ethylene was determined by taking 1 ml gas samples and the acetylene reduction rate was measured as described by Ligero *et al.* [30].

Statistical Analysis: Data were statistically analyzed in each season for growth parameters, nodules and nitrogen fixation using analysis of variance procedure for split-plot design in RCBD as described by Gomez and Gomez [31]. Means of treatments were compared with the Least Significant Difference Test (L.S.D) at 0.05 level of probability [32].

RESULTS

Effect of Salt Concentrations on Growth, Nodulation and Nitrogen Fixation: Data presented in Table 2 indicated that seedling parameters were varied with the salt concentrations within the two seasons. Highly significant differences were found between seedling height and total nitrogen content, while significant differences were found between number of nodules, nodules dry weight and nitrogen fixation. Moreover, the interaction between species and salt concentrations were significant in seedlings height and total nitrogen content at first season. On the other hand, in the second season, highly differences were found at seedlings height, total nitrogen content, number of nodules per plant and nitrogen fixation and significant differences was in stem dry weight. The interaction between species and salt concentrations were significant at total nitrogen content and stem dry weight. Seedling parameters of *Acacia* species in the presence of salt concentrations are summarized in Table 2. Without salt (control treatment), growth parameters, nodulation and nitrogen fixation were given the highest mean values compared with the salt treatments used in the study at

Table 3: Mean values of growth, nodulation and nitrogen fixation as affected by Salt concentrations during two seasons

	Salt concentration (%)									
	First Season					Second Season				
Seedling parameters	Control	1	2	3	L.S.D	Control	1	2	3	L.S.D
Height (cm)	38.54	29.83	28.71	27.13	3.40	38.04	31.61	27.67	25.92	4.17
Total nitrogen content /plant	18.99	16.88	15.14	13.61	0.99	18.31	16.94	14.98	12.92	0. 01
Number of nodules	45.67	33.46	23.79	19.29	15.84	33.92	21.35	11.92	5.17	8.51
Nodules dry weight (mg)	0.61	0.28	0.23	0.18	0.29	0.32	0.72	0.09	0.26	0.73
Root dry weight (gm)	2.72	2.51	2.88	3.09	0.80	3.04	3.25	2.54	2.48	0.91
Stem dry weight (gm)	6.37	5.23	6.22	5.61	1.47	6.05	5.66	4.11	4.10	1.20
Nitrogen fixation (μml/l)	1.05	1.03	0.82	0.57	0.33	1.31	1.25	0.84	0.36	0.32

Table 4: Effect of salt concentrations on *Acacia* species, growth parameters, nodulation and nitrogen fixation as affected by salt concentrations during two seasons

		Salt concentrations (%)									
		First season					Second season				
Seedling Parameters	Species	control	1	2	3	L.S.D interaction	control	1	2	3	L.S.Dinteraction
Height (cm)	*Acacia ehrenbergiana*	43.25	31.08	27.33	26.92	4.80	45.25	39.75	32.58	31.75	5.89
	Acacia tortilis	33.83	28.58	27.33	30.75		30.75	23.78	22.78	20.08	
Total nitrogen content (mg/plant)	*Acacia ehrenbergiana*	19.38	16.75	15.50	13.90	0.007	18.55	16.73	15.70	12.90	0.008
	Acacia tortilis	18.60	17.00	14.78	13.33		18.08	17.15	14.25	12.94	
Number of nodule plant^{-1}	*Acacia ehrenbergiana*	32.33	26.58	18.25	11.75	22.39	39.75	21.53	14.58	6.50	12.03
	Acacia tortilis	59.00	40.33	25.33	26.83		28.08	21.17	8.00	3.83	
Nodule dry weight (mg)	*Acacia ehrenbergiana*	0.77	0.20	0.16	0.10	0.42	0.37	0.26	0.13	0.02	1.04
	Acacia tortilis	0.44	0.37	0.19	0.36		0.27	1.18	0.05	0.03	
Root dry weight (gm)	*Acacia ehrenbergiana*	2.94	3.01	2.92	3.30	1.13	3.67	3.01	2.67	2.61	1.28
	Acacia tortilis	2.40	1..99	2.83	2.89		2.42	3.49	2.29	2.46	
Stem dry weight (gm)	*Acacia ehrenbergiana*	5.66	4.87	4.70	4.39	2.08	7.22	4.88	4.36	4.47	1.69
	Acacia tortilis	7.08	5.59	7.73	6.83		4.89	6.45	3.85	3.76	
Nitrogenase activity (nmol/min/g)	*Acacia ehrenbergiana*	1.12	1.01	0.85	0.67	0.46	1.19	1.03	0.91	0.41	0.46
	Acacia tortilis	0.93	1.08	0.79	0.47		1.43	1.48	0.77	0.31	

two seasons. Only root dry weight in the salt concentration 3% gave the highest mean average than control treatment in the first season, while in the second season salt concentration 1% gave the highest mean average at nodule dry weight than the control treatment (Table 3). *Acacia ehrenbergiana* was the most tolerant of salt concentrations than *Acacia tortilis* and the most effective salt concentration was varied from 2-3% between the species in the first season, while it was 3% in the second one (Table 4). Results demonstrated that, salt concentration affects growth, nodulation and nitrogen fixation of two *Acacia* species used in this study. With increasing salt concentrations, growth and nodulation and nitrogen fixation were decreased. The most harmful concentration of salt was 3% under condition of Riyadh region (Fig. 1).

Effect of *Rhizobium* Strains on Growth, Nodulation and Nitrogen Fixation: Data presented in Table 5 revealed that there are highly significant differences between *Rhizobium* strains in both seasons. Seedlings height, total nitrogen content, number of nodules, nodules dry weight and nitrogen fixation showed highly significant differences, while no significant was found of root and stem dry weights in two studied seasons. The interaction between species and *Rhizobium* strains were not significant in two seasons, except the total nitrogen content (Table 5). *Rhizobium* strain AER excelled in growth parameters, nodulation and nitrogen fixation than the strains ASR and LLR. Studied *Rhizobium* strains were able to cause significant responses for seedling parameters in the both seasons moreover, nodulation and nitrogen fixation (Table 5). The most species response to *Rhizobium* strain was *Acacia ehrenbergiana* followed by

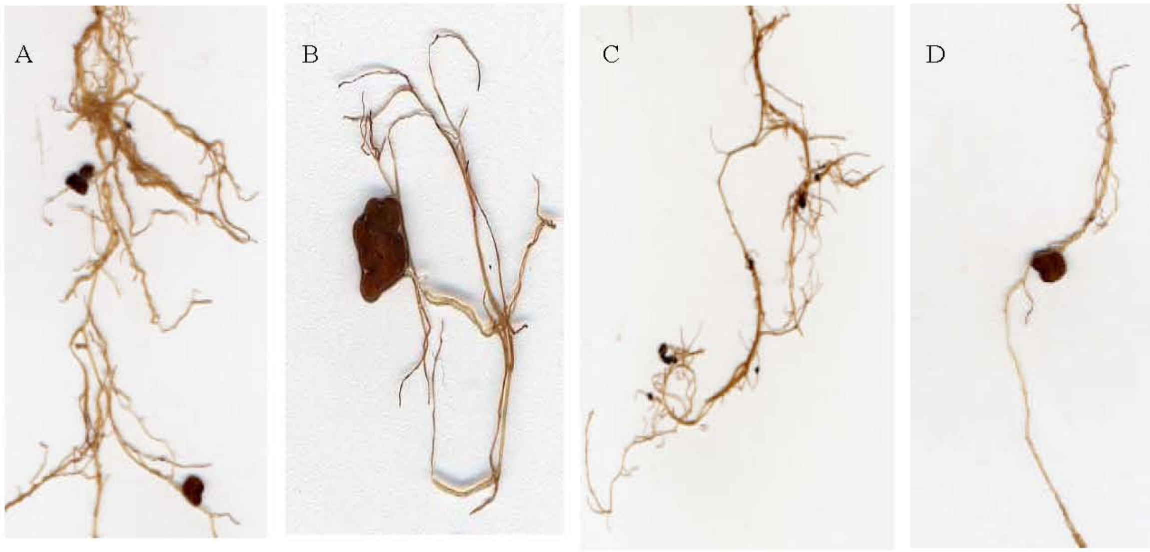

Fig. 1: Nodules isolated from seedlings of *Acacia ehrenbergiana* (A and B) and *Acacia tortilis* (C and D) under salt concentration 3% NaCl.

Table 5: Mean values of growth, nodulation and nitrogen fixation as affected by *Rhizobium* strains during two seasons

	Rhizobium strains									
	First Season					Second Season				
Seedling parameters	AER	ASR	ATR	LLR	L.S.D	AER	ASR	ATR	LLR	L.S.D
Height (cm)	34.67	32.04	30.50	27.00	3.40	35.96	33.61	28.25	25.42	4.17
Total Nitrogen content /plant	17.85	17.40	15.75	13.63	0.30	17.70	15.53	15.01	14.91	0.006
Number of nodules	57.04	41.80	12.46	10.92	15.84	33.83	20.18	12.33	5.83	8.51
Nodules dry weight (mg)	0.70	0.38	0.11	0.10	0.29	0.28	0.25	0.08	0.55	0.73
Root dry weight (gm)	3.07	2.92	2.91	2.30	0.3	2.93	2.93	2.41	3.03	0.91
Stem dry weight (gm)	6.52	5.40	5.22	6.27	1.47	4.84	4.40	4.88	5.27	1.92
Nitrogen fixation (nmol/min/g)	1.26	1.06	0.45	0.69	0.33	1.57	0.86	0.70	0.63	0.32

Table 6: Mean values of growth, nodulation and nitrogen fixation as affected by tress species during two seasons

	Tree species					
	First Season			Second season		
Seedling parameters	*Acacia ehrenbergiana*	*Acacia tortilis*	L.S.D	*Acacia ehrenbergiana*	*Acacia tortilis*	L.S.D.
Height (cm)	32.83	29.27	5.99	37.35	24.26	4.21
Total nitrogen content /plant	16.38	15.92	0.37	15.97	15.60	0.01
Number of nodules	22.30	38.88	20.87	20.59	15.27	16.87
Nodules dry weight (mg)	0.31	0.34	0.21	0.20	0.38	1.25
Root dry weight (gm)	3.05	2.55	0.99	2.99	2.66	2.20
Stem dry weight (gm)	4.90	6.81	3.82	5.23	4.74	1.23
Nitrogen fixation (nmol/min/g)	0.91	0.81	0.21	0.88	0.99	0.57

Acacia tortilis and the most effective strain was AER followed by ASR and ATR. The least strain was LLR reacted on *Acacia* species (Fig. 1).

Strain isolated from *Acacia ehrenbergiana* (AER), strain isolated from *Acacia saligna* (ASR), strain isolated from *Acacia tortilis* (ATR) and strain isolated from *Leucaena leucocephala*(LLR).

Effect of *Acacia* Species on Growth, Nodulation and Nitrogen Fixation under Salt Concentration: The statistical analysis of the results indicated that the differences between species were not significant as well as interaction among species, salt concentrations and *Rhizobium* strains for all seedling parameters (Table 6). Results demonstrated that, *Acacia ehrenbergiana* had

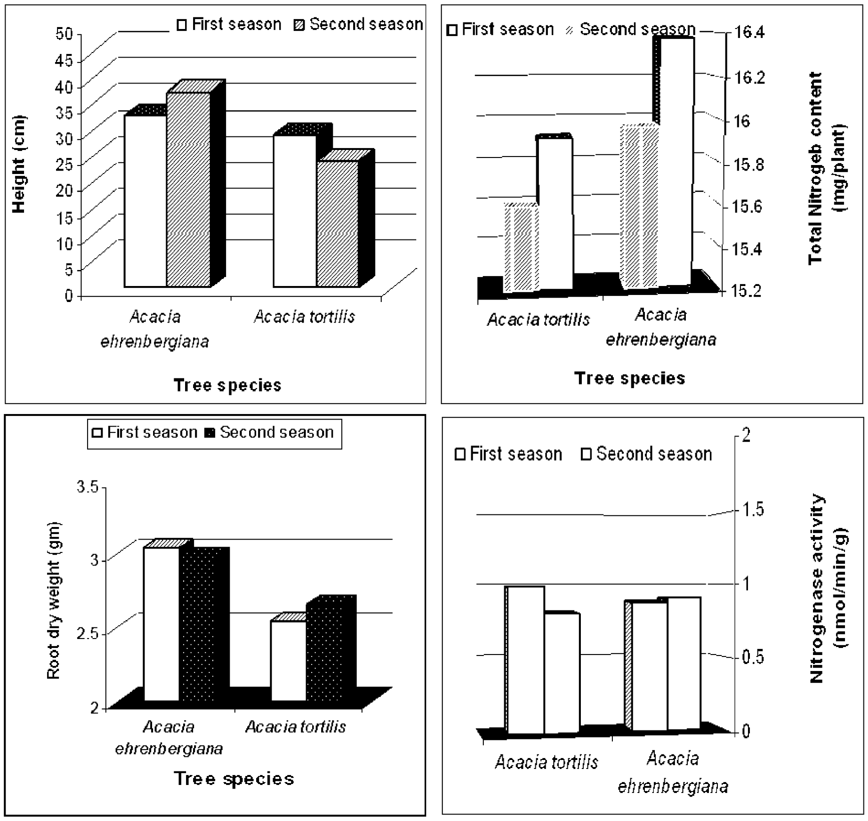

Fig. 2: Response of two *Acacia* species to growth, nodulation and nitrogen fixation under salt stress

the highest mean values of height, total nitrogen content, root dry weight and nitrogen fixation, while *Acacia tortilis* had the highest mean values of number of nodules, nodules dry weight and stem dry weight in the first season. In the second season, the same results were found except for nodules dry weight and nitrogen fixation, since *Acacia tortilis* was higher significantly than *Acacia ehrenbergiana* in such parameters (Table 6).

Generally, the results from the two seasons showed that, the *Acacia ehrenbergiana* exhibited growth, nodulation and nitrogen fixation better than those of *Acacia tortilis* under salt stress (Fig. 2).

DISCUSSION

In this study, two *Acacia* species; *Acacia ehrenbergiana* (Hayne.) and *Acacia tortilis* (Forssk.), grown in Dirab Valley, South of Riyadh, KSA, were inoculated with four *Rhizobium* strains; (AER), isolated from *Acacia ehrenbergiana*, (ASR) strain isolated from *Acacia saligna*, (ATR) strain, isolated from *Acacia tortilis* and (LLR) strain isolated from *Leucaena leucocephala* to evaluate growth response and ability of this species to form root nodules when planted under salt stress; and to obtain an indication of the nitrogen fixation potential of the species during two successive seasons (2009-2011).

Salt stress results in obvious stunting symptoms of plants. The immediate response of salt stress decreased rate of leaf surface expansion led to cease of shoot expansion with increasing salt concentration. Salt stress also decreased fresh and dry weights of leaves, stems and roots. Symptoms of salinity described above have been reported by Wang and Nil [33], Chartzoulakis and Klapaki [34] and Parida and Das [35]. For *Acacia* species, seedling height (growth) was more affected by salt stress compared with that obtained in case of root dry and stems weight of beans. Salinity was also reported that reduces shoot and root weights in several legumes [24, 36]. Results indicated that salt stress caused a significant depression in seedling growth parameters (height and dry weight of stem as well as root parts of both studied *Acacia* species and it seemed to reduce the availability of the nutrients required for the growth and then development of the plants comparing with the control. In the present study, salinity affected seedling parameters, nodulation and nitrogen fixation in both *Acacia ehrenbergiana* and *Acacia tortilis*. Growth limitation at high salinity may be due to depletion of energy that is needed for growth. These results confirm the previous reported of Cordovilla *et al.* [5], Tejera *et al.* [37], Bouhmouch *et al.* [38] and Imada *et al.* [39]. In fact, under salt stress conditions of soil, chlorine ions limit the absorption of NO_3^-. Antagonistic effect between Cl^-/ NO_3-is well known in glycophytes than halophytes plants; the latter are able to absorb efficiency NO_3-even under high salt conditions [38]. Horst and Taylor [40] studied the effect of salinity (a mixture of NaCl and $CaCl_2$) on 44 cultivars of Kentucky bluegrass and reported that growth reduced by 50% at a salt concentration of 7500 ppm (about 11 dSm^{-1}), which was higher than the value obtained in this study. Qian *et al.* [41] reported that at 2 dSm^{-1} differences in salinity caused 50%shoot growth reduction in two Kentucky bluegrass cultivars.

Total nitrogen content in both *Acacia* species was affected by salt concentrations. The control treatment (no salt) had the highest total nitrogen content, while total nitrogen content per plant was decreased with increasing salt concentrations. Similar results were reported by Cordovilla *et al.* [5]. By contrast, other studies found no reduction in nitrogen content under salt stress [9, 42].

A favorable 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 [5]. The effect of salinity was more on nodules than on vegetative parts and N_2-fixation was more sensitive to salinity than plant growth. Our results showed that, nitrogen fixation were more sensitive to salinity. The nodulation and nitrogen fixation were significantly affected by salt concentrations for both two studied *Acacia* species. Similar findings were reported for *Vigna radiata*: nodulation which was reduced by about half after exposure to low levels of salinity. In absence of NaCl, nitrogen fertilized plants had significantly higher dry weight accumulation in their shoots and roots than did N-fixing plants. Other authors observed no increase in total nodule weight [14]. Reduced nodule formation by faba bean at low levels of salinity could be attributed to adverse effects on the process of nodule initiation, an event in *Rhizobium*-legume symbiosis which is very sensitive to osmotic stress [43]. 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. These results are confirmed with those reported previously by Serraj and Drevon [20], Tejera *et al.* [37] and Ashraf and Bashir [44]. In contrast findings L'taief *et al.* [24] reported that salinity did not alter nodule conductance in chickpea variety. This tolerant variety showed no significant differences in nodule conductance under salinity.

The notable decline in ARA with low level salt stress agrees with earlier observations for cowpea [45]. The effect of 100 mM 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. Burns *et al.* [46] reported that NaCl affected directly nitrogenase purified from *Azotobacter*. Results demonstrated that nitrogenase activity of nodules under salt stress was decreased with increasing salt concentrations and the most affected salt concentration was 3%. Our results were in harmony with those obtained by Tejera *et al.* [37], Hafeez *et al.* [45] and Burns *et al.* [46].

In general, it was found that with increasing salt concentrations, growth, nodulation and nitrogen fixation were decreased. The most detrimental concentration of salt was 3% under condition of Riyadh region. The *Rhizobium* strain AER enhanced growth parameters, nodulation and nitrogen fixation than ASR and LLR one did. Studied *Rhizobium* strains were able to cause significant responses for seedling parameters in both seasons moreover, nodulation and nitrogen fixation. Results showed that local *Rhizobium* strain (AER) had the superiority effect to fix and nodulate the *Acacia ehrenbergiana* and *Acacia tortilis* than the (ASR, ATR and LLR) strains which was unable to compete with the AER strain under field condition that may be due to adaptation of the this strain to the environmental factors and salt stress. Although, Shetta [47] found that local *Rhizobia* strains had superior effect on nitrogen fixation and nodulation in *Acacia karroo* than the foreign one which was unable to compete with the native strains under field condition that may be due to adaptation of the native strains to the environmental habitats and site conditions. For salt tolerance, the results indicated that AER, AST, ATT and LLR strains are considered as tolerant of salt concentration until 3% [48]. By contrast, Shetta [49] reported that the nodulation and nitrogen fixation of *Rhizobium* isolated from *Acacia saligna* was more tolerant of salinity than that isolated from *Leucaena leucocephala* and NGR234 strain.

In the present study, *Acacia ehrenbergiana* had the highest mean values in height, total nitrogen content, root dry weight and nitrogen fixation than *Acacia tortilis.*

CONCLUSIONS

From the above mention results, it can be concluded that, salt concentrations affected seedling parameters, nodulation and nitrogen fixation of *Acacia ehrenbergiana* (Hayne.) and *Acacia tortilis* (Forssk.). Increasing salt concentrations, growth, nodulation and nitrogen fixation were decreased. The most affect concentration of salt was 3% under condition. Although, *Rhizobium* strains were affected by salt stress in the soil, the most salinity-tolerant strain was AER followed by strain ASR and the least strain tolerant to salt stress was LLR. *Acacia ehrenbergiana* had the better tolerant to salt stress, than *Acacia tortilis* under the condition of Riyadh region. Our experiment suggest that inoculating tree legumes with salinity-tolerant *Rhizobium* strains in the nursery should establish an effective nitrogen fixing symbiosis which may be more tolerant of salinity after outplanting. Also selection of legume host from highly saline or non saline habitats appeared to be the most important factor governing compatible *Rhizobium* strains to nodule and fix nitrogen in conditions of high soil salinity. These findings may be directly related to the maintenance of nitrogen fixation and therefore to the adaptation of *Acacia* nodules of salinity especially in arid region of Saudi Arabia.

REFERENCES

1. Allakhverdiev, S.I., Y. Nishiyama, S. Miyairi, H. Yamamoto, N. Inagaki, Y. Kanesaki and N. Murata, 2002. Salt stress inhibits the repair of photodamaged photosystem II by suppressing the transcription and translation of psbA genes in Synechocystis. Plant Physiol., 130: 1443-1453.
2. Walsh, K.B., 1995. Physiology of the legume nodule and its response to stress. Soil Biochemistry, 27: 662-669.
3. Zahran, H.H., 1991. Conditions for successful *Rhizobium*-legume symbiosis in saline environments. Biology and Fertility of Soils, 12: 73-80.
4. Abdel Wahab, A.M. and H.H. Zahran, 1983. The effects of water stress on N_2 (C_2H_2) fixation and growth of *Medicaga sativa* L. Acta Agronomica Academiae Scientiarum Hungaricae, 32: 114-118.
5. Cordovilla, M.P., A. Ocana, F. Ligero and C. Lluch, 1995. Salinity effects on growth analysis and nutrient composition in four grain legumes-*Rhizobium* symbiosis. Journal of Plant Nutrition, 18: 1595-1609.
6. Zahran, H.H., 1997. Diversity, adaptation and activity of the bacterial flora in saline environment. Biology and Fertility of Soils, 25: 211-223.
7. Aouani, M.E., R. Mhamdi, M. Mars and R. Ghrir, 1998. Nodulation and growth of common bean under NaCl-stress. Soil Biol. Biochem., 30: 1473-1475.
8. Mohammad, R.M., M. Akhavan-Kharazian, W.F. Campbell and M.D. Rumbaugh, 1991. Identification of salt-and drought tolerant *Rhizobium meliloti* L. strains. Plant Soil, 134: 271-276.
9. Singleton, P.W. and B.B. Bohlool, 1984. Effect of salinity on nodule formation by soybean. Plant Physiol., 74: 72-76.
10. Lauter, D.J., D.N. Munns and K.L. Clarkin, 1981. Salt response of chickpeas influenced by N supply. Agric. J., 73: 961-966.
11. Rai, R. and V. Prasad, 1983. Salinity tolerance of *Rhizobium* mutants: growth and relative efficiency of symbiotic nitrogen fixation. Soil Biol. Biochem., 15: 217-219.
12. Velagaleti, R.R. and S. Marsh, 1989. Influence of host cultivars and *Bradyrhizobium* strains on the growth and symbiotic performance of soybean under salt stress. Plant Soil, 119: 133-8.
13. Sprent, J.I., 1985. Nitrogen Fixation in Arid Environments. In: Plants for Arid Lands, pp: 215-229. Published by Kew, London: Royal Botanic Gardens, pp: 225.
14. Zahran, H.H. and J.I. Sprent, 1986. Effects of sodium chloride and polyethylene glycol on roothair infection and nodulation of *Vicia faba* L. plants by *Rhizobium leguminosarum*. Planta, 167: 303-309.
15. Abdel-Wahab, A.M., M.S.A. Shabebw and M.A.M. Younis, 2002. Studies on the effect of salinity, drought stress and soil type on nodule activities of *Lablab purpureus* (L.) sweet (Kashrangeeg). J. Arid Environ., 51: 587-602.
16. Sprent, J.I., 1972. The effects of water stress on nitrogen fixing root nodules. New Phytol., 71: 451-60.
17. Vance, C.P. and G.H. Heichel, 1991. Carbon in N_2 fixation: limitation or exquisite adaptation. Ann. Rev. Plant Physiol. Plant Mol. Biol., 42: 373-392.
18. Drevon, J.J., C. Deransart, H. Irekti, H. Payer, G. Roy and R. Serraj, 1944. La salinite´ (NaCl) abaisse la conductance des nodosites de legumineuse a` la diffusion de l'oxygene, Facteurs Limitant la Fixation Symbiotique de l'Azote dans le Bassin Mediterraneen, INRA, Editions, les colloques, 77: 73-84.

19. Gonzalez, E.M., L. Galvez and C. Arrese-Igor, 2001. Abscisic acid induces a decline in nitrogen fixation that involves leghaemoglobin, but is independent of sucrose synthase activity. J. Exp. Bot., 52: 285-93.
20. Serraj, R., H. Vasquez-Diaz, G. Hernandez and J.J. Drevon, 2001. Genotypic difference in the short-term response of nitrogenase activity (C_2H_2 reduction) to salinity and oxygen in the common bean. Agronomie, 21: 645-51.
21. Garg, B.K. and I.C. Gupta, 2000. Nodulation and symbiotic nitrogen fixation under salt stress. Curr. Agric., 4: 23-35.
22. Saadallah, K., J.J. Drevon and C. Abdelly, 2001. Nodulation et croissance nodulaire chez le Haricot (*Phaseolus vulgaris* L.) sous contrainte saline. Agronomie, 21: 627-34.
23. Irekti, H. and J.J. Drevon, 2003. Acide abcissique et conductance a` la diffusion de l'oxyge`ne dans les nodosite´s de haricot soumises a` un choc salin. In: J.J. Drevon, B. Sifi, editors, Fixation Symbiotique de l'Azote et De´veloppement Durable dans le Bassin Me´diterrane´en, INRA Editions, les colloques, 100: 371-80.
24. L'taiefa, B., B. Sifi, M. Zaman-Allah, J.J. Drevonc and M. Lachaala, 2007. Effect of salinity on root-nodule conductance to the oxygen diffusion in the *Cicer arietinum-Mesorhizobium ciceri* symbiosis. J. Plant Physiol., 164: 1028-1036.
25. Nogales, J., R. Campos, H. Ben Abdelkhalek, J. Olivares, C. Lluch and J. Sanjuan, 2002. *Rhizobium tropici* genes involved in free-living salt tolerance are required for the establishment of efficient nitrogen-fixing symbiosis with *Phaseolus vulgaris*. Mol. Plant-Microbe. Interact., 15: 225-232.
26. Noel, A., R.C. Tejera1, J. Sanjuan and C. Lluch1, 2004. Nitrogenase and antioxidant enzyme activities in Phaseolus vulgaris nodules formed by *Rhizobium tropici* isogenic strains with varying tolerance to salt stress. J. Plant Physiol., 161: 329-338.
27. El-Ghanim, W.M., L.M. Hassan, T.M. Galal and B. Abdel-fattah, 2010. Floristic composition and vegetation analysis in Hail region north of central Saudi Arabia. Saudi J. Biol. Sci., 17: 119-128.
28. Vincent, J.M., 1970. A Manual for the Practical Study of Root Nodule Bacteria. IBP Handbook No. 15. Blackwell, Oxford, pp: 164.
29. Bergersen, F.J., 1980. Measurement of Nitrogen Fixation by Direct Means. In: F.J. Bergersen, (Ed.), Methods for Evaluating Biological Nitrogen Fixation. John Wiley and Sons, New York, pp: 65-110.
30. Ligero, F., C. Lluch and J. Olivares, 1996. Evolution of ethylene from root of *Medicago sativa* plants inoculated with *Rhizobium meliloti*. J. Plant Physiol., 125: 361-365.
31. Gomez, K.A. and A. Gomez, 1984. Statistical Procedure for Agriculture Research. Handbook, John Wiley and Sons, New York.
32. Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. 7th Ed.-Iowa State Univ. Press, Ames, Iowa.
33. Wang, Y. and N. Nil, 2000. Changes in chlorophyll, ribulose biphosphate carboxylase-oxygenase, glycine betaine content, photosynthesis and transpiration in Amaranthus tricolor leaves during salt stress. J. Hort. Sci. Biotechnol., 75: 623-627.
34. Chartzoulakis, K. and G. Klapaki, 2000. Response of two green house pepper hybrids to NaCl salinity during different growth stages. Sci. Hort., 86: 247-260.
35. Parida, A.K. and A.B. Das, 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxicol. Environ. Safety, 60: 324-349.
36. Alshammarya, S.F., Y.L. Qian and S.J. Wallner, 2004. Growth response of four turfgrass species to salinity. Agricultural Water Management, 66: 97-111.
37. Tejera, N.A., R. Campos, J. Sanjuan and C. Lluch, 2004. Nitrogenase and antioxidant enzyme activities in *Phaseolus vulgaris* nodules formed by *Rhizobium tropici* isogenic strains with varying tolerance to salt stress. J. Plant Physiol., 161: 329-338.
38. Bouhmouch, I., B.S. Mouhsine and F. Brhada, 2005. Influence of host cultivars and *Rhizobium* species on the growth and symbiotic performance of *Phaseolus vulgaris* under salt stress. J. Plant Physiol., 162: 1103-1113.
39. Imada, S., N. Yamanaka and S. Tamai, 2009. Effects of salinity on the growth, Na partitioning and Na dynamics of a salt-tolerant tree, *Populus alba* L. J. Arid Environ., 73: 245-251.
40. Horst, G.L. and R.M. Taylor, 1983. Germination and initial growth of Kentucky bluegrass in soluble salts. Agron. J., 75: 679-681.
41. Qian, Y.L., S.J. Wilhelm and K.B. Marcum, 2001. Comparative responses of two Kentucky bluegrass cultivars to salinity stress. Crop Sci., 41: 1890-1895.
42. Weil, R.R. and N.A. Khalil, 1986. Salinity tolerance of winged beans as compared to that of soybean. Agron. J., 78: 67-70.
43. Velagaleti, R.R., S. Marsh and D. Krame, 1990. Genotypic differences in growth and nitrogen fixation among soybean (*Glycine max* (L.) Merr.) cultivars grown under salt stress. Trop. Agric., 67: 169-177.

44. Ashraf, M. and A. Bashir, 2003. Salt stress induced changes in some organic metabolites and ionic relations in nodules and other plant parts of two crop legumes differing in salt tolerance. Flora, 198: 486-498.
45. Hafeez, F.Y., Z. Aslam and K.A. Malik, 1988. Effect of salinity and inoculation on growth, nitrogen fixation and nutrient uptake of (*Vigna radiata* (L.) Wilczek). Plant Soil, 106: 3-8.
46. Burns, A., G.D. Watt and Z.C. Wang, 1985. Salt inhibition of nitrogenase catalysis and salt effects on the separate protein components. Biochem., 24: 3932-3936.
47. Shetta, N.D., 2010. Bio-role of *Acacia karroo* in nitrogen fixation at different locations of North-West Egypt region. American-Eurasian J. Agric. Environ. Sci., 7(4): 471-477.
48. Shetta, N.D., T.S. Al-Shaharani and M. Abdel-Aal, 2011. Identification and characterization of *Rhizobium* associated with woody legume trees grown under Saudi Arabia condition. American-Eurasian J. Agric. Environ. Sci., 10(3): 371-379.
49. Shetta, N.D., 2002. Biological and genetical studies on *Rhizobium* species isolated from woody trees grown in Egypt. PhD, Faculty of Agriculture, Alexandria University.