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Physiological and Biochemical Responses of *Acacia Seyal* (Del.) Seedlings under Salt Stress Conditions

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ABSTRACT

In this study, the first experiment analyzed the effects of 0, 85, 171, 256, and 342 mM sodium chloride (NaCl) on the germination of *Acacia seyal* seeds. In the second in vitro experiment, the effects of inoculation with two rhizobial strains on growth, physiological, and biochemical responses of *A. seyal* seedlings was evaluated at four levels (0, 85, 171, and 256 mM) of NaCl. Results showed that at 342 mM NaCl, germination rate of *A. seyal* seeds declined by 56% from the control value, while any germination was recorded for *Acacia bivenosa* and *Acacia sclerosperma* seeds at the same salt level. Salt stress gradually decreased the growth, the soluble protein and the leaf-chlorophyll contents of *A. seyal* seedlings. However, rhizobial inoculation limits these adverse effects of salt on physiological and metabolic processes responses. *A. seyal* exhibited a moderate halophytic behavior; nodulation was enhanced by moderate salt stress.

Keywords: *Acacia seyal*, biochemistry, germination, physiology, salt stress

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INTRODUCTION

Salinity is an increasing environmental problem throughout the world and represents a major limiting factor in crop production and ecological environment building-up, especially in arid and semiarid regions (Zahran, 1999). Furthermore, climate change worsens the loss of forested land in these regions (Zeng, 2003). Thus, the supply of wood products from natural forests is inadequate to meet demand.

In Senegal, 6% of land, mainly in coastal areas, is salt affected (Barbiero et al., 2004). In saline environments, adaptation of plants to salinity during germination and early seedling stages is crucial for the establishment of species (Debez et al., 2004; Vicente et al., 2004). Indeed, seedlings are the most vulnerable stage in the life cycle of plants and germination determines when and where seedling growth begins (Kigel, 1995). Germination responses of halophytic species to environmental parameter determine their distribution in saline environments (Tobe et al., 2000). Thus, the germination response to sodium chloride (NaCl) concentrations is a useful and fast laboratory test for evaluation of salt tolerance.

The majority of salt stress studies have been carried out on annual plants and showed that salt stress adversely affected seed germination, plant growth and metabolism (Munns, 1993), nodulation (Cordovilla et al., 1999), rhizobia-legume symbiosis (Serraj, 2002; Zahran, 1999), and photosynthesis (Silveira et al., 2003). Several studies showed that excessive amounts of salt in soil lower the availability of water, inhibit the metabolic processes of plants and affect their nutrient composition, osmotic balance, and hydraulic conductivity resulting in stunted growth and low productivity (Al-Karaki, 2001; Apse et al., 1999). It has been reported that in sustainable agriculture, solutions to salinity-alkalinity problems include both plant breeding for salt tolerance and the application of biological factors such as reclamation of saline soils with multipurpose tree species such as *Acacia* spp, which are moderately salt-tolerant legume trees (Giri et al., 2003; Zahran, 1999). Though, studies conducted on *Acacia* species showed that, these species not only yield nutritious fodder for livestock and fuelwood; but have also the additional advantage of adding natural fertilizers to the soil through symbiotic association with compatible rhizobia and mycorrhizal fungi (Dommergues, 1995; Allen and Allen, 1981). These species have evolved adaptations to cope with nutritional deficiencies and other stresses (Doran and Turnbull, 1997). They are potentially useful for ecological applications, such as landscaping or rehabilitation of damaged ecosystems (Zahran, 2001; Midgley and Bond, 2001). The introduction of target indigenous species of plants, associated with a managed community of microbial symbionts, is a successful biotechnological tool to aid the recovery of damaged ecosystems (Requena et al., 2001). However, despite intense research efforts, few research works have recently reported results on the effects of microbial inoculation on sahelian acacias growth under salt stress conditions. Thus, research in biochemical and

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physiological responses of inoculated sahelian acacias under salinity stress is needed to improve the host plant performance in damaged environment.

Acacia seyal (Del.) is a multipurpose perennial legume, widespread in the semi-arid zone of tropical Africa from Senegal eastwards to Somalia and the Red Sea, and from the Nile valley south to Zambia (von Maydell, 1986; Hall, 1994). *A. seyal* is potentially salt tolerant. In Senegal, it is widely distributed in the salt-affected coastal steppes. However, little information is available on it level of salt tolerance.

This study was undertaken to investigate the salinity effects on seed germination, growth, nodulation, and nitrogen and carbon assimilation of *A. seyal* seedlings; with the intention of facilitating the on-going initiative of finding suitable species for plantation in the salt affected areas in Senegal. Plant growth, the glutamine synthetase (GS) and the phosphoenolpyruvate carboxylase (PEPC) activities, as well as the soluble protein and the chlorophyll contents were evaluated, as useful indicators of plants performance under salt stress (Zahran, 1999; Giri and Mukerji, 2004; Al-Karaki et al., 2001).

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MATERIALS AND METHODS

Seed Germination

Fresh seeds of *Acacia bivenosa*, *Acacia sclerosperma*, and *Acacia seyal* were provided by the Direction de Recherches sur les Productions Forestières (Institut Sénégalais de Recherches Agricoles). *A. bivenosa* and *A. sclerosperma* have been chosen as reference plants in this study due to their proven salt tolerance at different growth stages in various environments (Aswathappa et al., 1987).

Seeds of all species studied were scarified and surface sterilized by soaking them in 95% (v/v) sulfuric acid for 30 min (*A. bivenosa* and *A. seyal*) and 60 min (*A. sclerosperma*). Seeds (n = 25) were germinated as previously described (Diouf et al., 2007). Five concentrations of sodium chloride (NaCl) were tested: 0, 5, 10, 15, and 20 g L^{-1} (equivalent to 0, 86, 171, 257, and 342 mM NaCl). The experiment was conducted in three replications per salt treatment. Seeds were considered as germinated when emerging radical was visible. Germinated seeds were counted daily for 10 days. The germination rate was expressed in mean final percent germination, calculated from cumulative germinated seeds on the final day of assessment to that of total number of seeds in the sample at different salinity levels.

Growth and Nodulation of Inoculated *A. seyal* Seedlings

Seeds of *A. seyal* were germinated at room temperature as described above. The seedlings were transferred under sterile conditions (one seedling per tube) into 150 × 20 mm Gibson tube (Gibson, 1963) containing a sterile Jensen

nitrogen-free nutrient medium adjusted to pH 7 (Vincent, 1970). On the basis of the results of seeds germination, the four following NaCl concentrations were tested for this experiment: 0, 86, 171, and 257 mM. For each NaCl concentration, seedlings (N = 24) were maintained in a growth chamber with the following conditions (16 h photoperiod, day/night; air-temperature of 30 ± 1°C; relative humidity of 70 ± 5%; and photosynthetic photon flux density of 120 μmol m^{-2} s^{-1}).

After one week of culture, seedlings were inoculated with two *Mesorhizobium* sp. strains, ORS 3394 and ORS 3403, isolated from *Acacia seyal* roots nodules and phylogenetically close to *Mesorhizobium plurifarium*. GenBank accession numbers are FJ848985 and FJ848986, for the strains ORS 3394 and ORS 3403, respectively. Bacterial cultures in liquid yeast extract medium (Vincent, 1970) were obtained for inoculation. Seedlings were inoculated with 1 mL of the strain ORS 3394 or ORS 3403 suspensions, with approximately 10^9 *colony*-forming-unit (cfu) mL^{-1}, or 1 mL of the yeast-extract-mannitol (YEM) broth without bacteria for the control treatment.

Forty days after inoculation, plants were sampled and analyzed for the parameters outlined below. The growth was evaluated by measuring the height of the shoots. The effect of salt treatment on the rhizobial infection was evaluated by counting the nodules number per plant.

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Analytical Procedure

Leaves, roots, and nodules were weighed, frozen in liquid nitrogen and stored at −20°C until analysis. For assays of soluble proteins, and PEPC and GS activities; extraction was carried out at 4°C (Campa et al., 2000). Tissues samples were crushed at 4°C in an extraction buffer (4 mL per g) containing 25 mM Tris-hydrochloric acid (HCl; pH 7.6), 1 mM magnesium chloride ($MgCl_2$ $7H_2O$), 14 mM β-mercapto-ethanol, 500 mM ethylendiaminetetraacetic acid (EDTA), and 1% (w/v) water-soluble polyvinyl pyrrolidone (PVP 10). The mix was centrifuged at 15,000 *g* at 4°C for 20 min. Resulting supernatant was used for soluble proteins and enzyme assay. Extraction was performed in duplicate and measurements were made in triplicate. Centrifugations were done in a centrifuge (Model 3K15; SIGMA, Osterode am Harz, Germany) and the enzymes activities were assayed by UV/VIS spectrophotometry (Model Ultrospec; Pharmacia Biotech, Piscataway, NJ, USA). Glutamine synthetase activity (GS, EC 6.3.1.2) was assayed colorimetrically by the γ-glutamylhydroxamate biosynthetic reaction (O'Neal and Joy, 1973). The components of the modified reaction mixture were L-glutamate, 80 mM; hydroxylamine (NH_2OH), 6 mM; $MgCl_2$, 20 mM; EDTA, 4 mM; and adenosine triphosphate (ATP), 8 mM. The absorbance of supernatant was measured at a wavelength of 540 nm after at least 30 min incubation at 30°C. The GS activity was expressed as μmol γ-Glutamylhydroxamate (GGH) h^{-1} g^{-1} fresh weight.

The phosphoenolpyruvate carboxylase (EC 4.1.1.31) activity was assayed as a potential source of malate. *In vitro* PEPC activity was assayed by the nicotinamide adenine dinucleotide hydrogenase (NADH) oxidation (Nato and Mathieu, 1978). The activity of PEPC was assayed by spectrophotometer at a wavelength of 340 nm by following the oxidation of NADH at 30°C for 30 min. The assay mixture (pH 7.6) contained in a final volume of 1 ml; 15 mM Tris-HCl, 0.125 mM sodium bicarbonate ($NaHCO_3$), 0.025 mM NADH. The reaction was started by the addition of 0.312 mM Phospho-enol-Pyruvate (PEP). PEPC activity was expressed as mmol NADH h^{-1} g^{-1} fresh weight.

Soluble protein in the different extracts was quantified by the protein-dye binding with Coomassie brilliant blue (Bradford, 1976) by spectrophotometer at a wavelength of 595 nm after 15 to 20 min incubation, using bovine serum albumin (BSA) as standard (Merck, fraction V). Soluble protein content was expressed as mg g^{-1} fresh weight.

The procedure for chlorophyll determination was based on the absorption of light by aqueous acetone (80%) extracts of chlorophyll (Arnon, 1949). Leaves were crushed in a mixing acetone/water (80/20; v/v). After centrifugation 10,000 *g* at 4°C for 10 min, the resulting supernatant was used for the assay of chlorophyll content. The concentrations of total chlorophyll were determined by measuring the absorbance of chlorophyll b and a in acetone/water (80%, v/v) at the respective wavelengths 645 nm and 663 nm, in a spectrophotometer against acetone/water. Total chlorophyll concentration (chlorophyll a and b) was expressed as milligrams per grams fresh weight using the absorbance values (A_{663} and A_{645}) at the respective wavelengths and the specific absorption coefficients for chlorophyll a and b as calculated by the equation: $C = \{[20.2 (A_{645}) + 8.02 (A_{663})] \times V/M\}$. Where V and M are the extraction volume (L) and weight (mg) of crushed leaves.

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Statistical Analysis of Data

Data of plant height and nodules number were processed using SuperANOVA^{TM} software (Abacus Concepts Inc., Berkeley, CA, USA). Means were compared with the Student-Newman-Keuls range test ($P < 0.05$).

RESULTS

Seed Germination

The germination rate varied depending on salt concentration and the species (Table 1). In *A. seyal*, the germination rate decreased gradually with increasing salt stress. Increasing salinity from control to 85 mM NaCl decreased by 14% the germination rate in *A. seyal*. At 171 mM the highest germination

Table 1
NaCl effect on final percent germination (%) of *Acacia bivenosa*, *A. sclerosperma*, and *A. seyal*. The mean final percent germination was calculated from cumulative germinated seeds in gelosed-water (0.8%, w/v) after 10 days on the indicated NaCl concentrations (0, 85, 171, 256, and 342 mM) to that of total number of seeds in the sample

	Species		
NaCl (mM)	*Acacia bivenosa*	*A. sclerosperma*	*A. seyal*
0	100 ± 0.0	100 ± 0.0	100 ± 0.0
85	100 ± 0.0	100 ± 0.0	86 ± 6.0
171	100 ± 0.0	32 ± 7.8	64 ± 3.2
256	18 ± 4.3	04 ± 2.0	46 ± 3.0
342	00 ± 0.0	00 ± 0.0	44 ± 3.5

Values are means with standard error of the mean (n = 75).

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rate was observed in *A. bivenosa* (100%), followed by *A. seyal* (64%) and *A. sclerosperma* (32%). At high salt stress (256 and 342 mM NaCl), *A. seyal* showed excellent germination rate, better than *A. bivenosa* and *A. sclerosperma*. On the medium containing 342 mM NaCl, *A. seyal* seeds maintained a high germination rate (44%). In contrast, no germination was recorded in *A. bivenosa* and *A. sclerosperma* seeds.

Plant Growth and Nodulation

The roots nodulation by *Mesorhizobium* sp. strains enhanced the *A. seyal* growth, even for those growing in the medium with high level of NaCl (Table 2). At 256 mM NaCl, the percentages of the height increasing when comparing plants with and without rhizobial inoculation were 15% (ORS 3394), 18% (ORS 3403), and 19% (ORS 3394 + ORS 3403). Salinity significantly reduced the overall growth of plants of *A. seyal*, irrespective of the inoculation treatment. This was evident from the decline in the height of shoots with increasing salt stress. The plants growing in the medium with 256 mM NaCl appeared 30% shorter than those growing in the soil without NaCl. However, the root infection by rhizobial strains strengthened the capability of the plant to tolerate the salt stress in the medium comparatively to the uninoculated control.

For both NaC1 levels, inoculated plants were nodulated. Increasing the salinity level from the control to 85 mM NaCl had no significant effect on nodule initiation and development (Figure 1). While, similar to the plant height, high salinity level affected root nodulation. For plants growing in 171 mM and 256 mM NaCl, the salt stress reduced infection and nodule development. However, the adverse effects of salinity on the nodule initiation and development varied

Table 2
Effect of different levels of salinity (0, 85, 171, and 256 mM NaCl) on growth of *Acacia seyal* seedlings cultivated *in vitro* for 40 days of uninoculated and inoculated plants with two *Mesorhizobium* sp. strains ORS 3394 and ORS 3403

	Shoot height (cm)			
NaCl (mM)	Uninoculated	ORS 3394	ORS 3403	ORS 3394 + ORS 3403
0	11.24[a]	13.99[b]	13.53[b]	13.79[b]
85	10.1[a]	11.67[b]	10.88[ab]	12.2[b]
171	8.77[a]	9.53[ab]	10.29[b]	9.71[ab]
256	8.2[a]	9.45[ab]	9.79[b]	9.68[b]

For each NaCl treatment, values (means of 24 replicates) in the same line followed by the same letter are not significantly different according to Student-Newman-Keuls tests ($P < 0.05$).

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depending on the isolate used as bacterial inoculums. The nodule number of seedlings growing at 171 mM NaCl level and inoculated with ORS 3394 or ORS 3403 decreased by 24% and 28%, respectively, compared to the plants growing in the absence of NaCl. At 256 mM NaCl level, the nodule number of seedlings inoculated with ORS 3403 decreased to only 45% of the control.

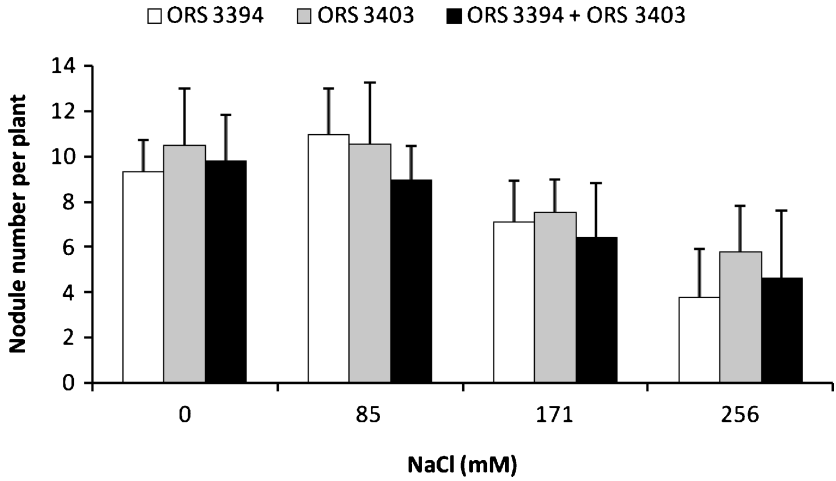

Figure 1. Effect of four levels of sodium chloride (0, 85, 171, and 256 mM) on nodule number per plant of *A. seyal* grown *in vitro* during 40 days and inoculated with two *Mesorhizobium* sp. strains (ORS 3394 and ORS 3403). Values are means of 24 plants and bars indicate standard error of the mean.

Table 3
Effect of different levels of salinity (0, 85, 171, and 256 mM NaCl) on soluble protein content (mg g^{-1} fresh weight) and glutamine synthetase (GS) activity (μmol γ-Glutamylhydroxamate h^{-1} g^{-1} fresh weight) of leaves, roots and nodules of *Acacia seyal* seedlings cultivated *in vitro* for 40 days and inoculated or not with strain of *Mesorhizobium* sp. ORS 3403

		Soluble protein		GS activity	
Organs	NaCl (mM)	Control	ORS 3403	Control	ORS 3403
Leaves	0	11.52 ± 0.7[a]	14.10 ± 0.3	113.71 ± 1.1	116.86 ± 2.1
	85	11.01 ± 0.5	12.60 ± 0.7	125.34 ± 0.2	129.92 ± 1.1
	171	9.16 ± 0.2	11.92 ± 0.5	129.77 ± 0.7	130.28 ± 2.8
	256	8.89 ± 0.3	10.77 ± 0.1	134.07 ± 1.41	134.07 ± 0.7
Roots	0	4.48 ± 0.7	4.40 ± 0.2	10.75 ± 1.4	11.11 ± 1.4
	85	3.57 ± 1.4	3.86 ± 0.7	10.22 ± 1.4	11.51 ± 0.5
	171	2.30 ± 0.7	2.32 ± 0.7	9.24 ± 0.3	9.22 ± 0.7
	256	2.03 ± 0.7	2.34 ± 0.7	4.33 ± 0.7	5.37 ± 1.4
Nodules	0	—	4.82 ± 1.4	—	77.24 ± 0.4
	85	—	4.02 ± 0.1	—	76.49 ± 1.4
	171	—	3.60 ± 1.4	—	74.77 ± 0.1
	256	—	2.32 ± 0.2	—	65.9 ± 2.3

[a]Values are means ± SE of three replicates.

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Whereas, the nodule number of the seedlings inoculated with ORS 3394 alone or dually inoculated with the two strains was decreased by 60% and 52%, respectively.

Soluble Proteins Content and GS Activity

The *A. seyal* plants inoculated with *Mesorhizobium* sp. strain ORS 3403 had higher leaf-protein content than the non-inoculated plants under the same NaCl levels (Table 3). In no NaCl conditions, the leaf-protein concentration of the nodulated plants was 29% more than that of the non-inoculated plants. In contrast, inoculation has no significant effect on root-protein content. Salt treatment significantly reduced the proteins concentration in all organs analyzed, proportionally to NaCl levels whatever the inoculation treatment. The increasing of leaf-protein content in inoculated plants was dropped at 5% when NaCl was at 256 mM. However, the reduction of protein concentration was organs dependent. The protein content in leaves was about 2-fold higher than in roots and nodules. At 256 mM NaCl, reduction was more important in nodules (50%) and roots (41%) than in leaves (32%) of inoculated plants when compared to that with zero NaCl, respectively.

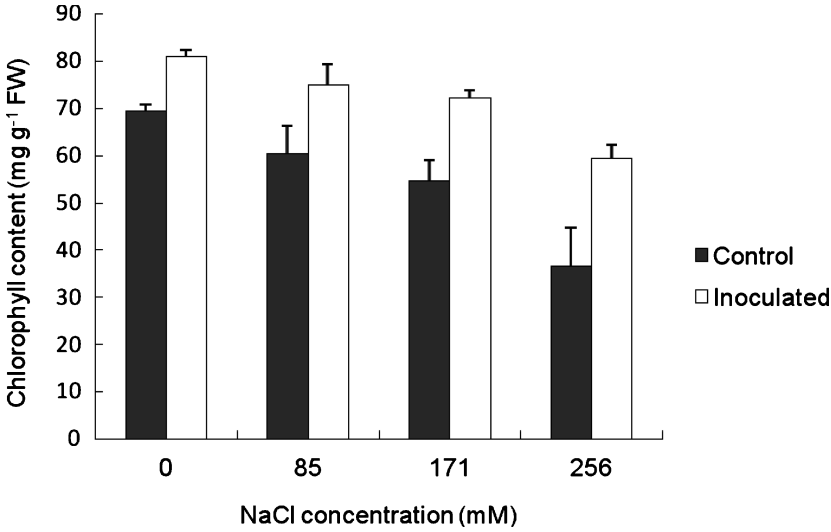

Figure 2. Effect of different levels of salinity (0, 85, 171, and 256 mM) on leaf-chlorophyll content (mg g^{-1} fresh weight) of *Acacia seyal* seedlings cultivated *in vitro* for 40 days, uninoculated (control) or inoculated with strain of *Mesorhizobium* sp. ORS 3403. Values are means ±SE of three replicates.

The leaf-GS activity was about 10-fold and 1.5-fold higher than in roots and nodules of inoculated plants, respectively. On the contrary to the leaf soluble proteins, inoculation did not increase leaf-GS activity. Our results showed also that, by contrast to root-GS and nodules-GS activities, the leaf-GS activity was slightly enhanced when increasing salt stress.

Leaf Chlorophyll Content and PEPC Activity

Leaf chlorophyll content was higher in inoculated plants. It was significantly reduced in both nodulated and non-nodulated plants as a result of increasing salinity. However, the decrease of leaf chlorophyll content was greater in uninoculated plants than in inoculated ones (Figure 2). At 256 mM NaCl, the decrease of leaf chlorophyll content reached 47% and 27% in uninoculated and inoculated plants, when compared to that with zero NaCl, respectively. Saline stress lowered the leaf number, led to the yellowing of leaves, which ultimately resulted in precocious leaf senescence compared with control plants (data not shown).

The leaf-PEPC activity was stimulated coincidently with the adding of more NaCl in the medium (Figure 3). There was a significant positive correlation between the medium salinity and the leaf-PEPC activity ($r = 0.916$ and $r = 0.943$, respectively, for uninoculated plants and inoculated ones). The

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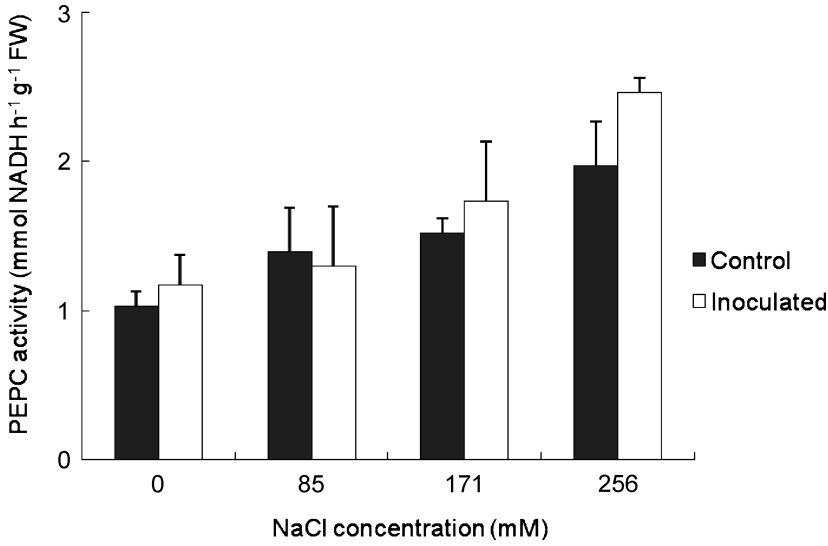

Figure 3. Effect of different levels of salinity (0, 5, 10, and 15 g l^{-1} NaCl) on phosphoenolpyruvate carboxylase (PEPC) activity (mmol NADH $h^{-1}g^{-1}$ fresh weight) in leaves of *Acacia seyal* seedlings cultivated *in vitro* for 40 days uninoculatd (control) or inoculated with strain of *Mesorhizobium plurifarium* ORS 3403. Values are means ± SE of three replicates.

increase of PEPC activity in the uninoculated plants was less important than inoculated ones. Comparing to non-salt control, 256 mM NaCl leaded to the increasing of the PEPC activity in uninoculated and inoculated plants by 67% and 92%, respectively.

DISCUSSION

For the successful establishment of plants in saline environments, seeds must remain viable at high salinity in an imposed secondary dormancy and germinate when salinity decreases (Vicente et al., 2004). In the present work, the effect of different concentrations of NaCl (0, 85, 171, 256, and 342 mM) on germination of *Acacia bivenosa*, *A. sclerosperma*, and *A. seyal* has been investigated. With increasing level of salinity, final percent germination of both of the species was markedly reduced. However, *A. seyal* showed the highest final percent germination (higher than 40%) at 342 mM NaCl. Seeds of *A. bivenosa* and *A. sclerosperma* were more sensitive to NaCl, as evidenced by the low final percent germination at 171 and 256 mM, which was inhibited at 342 mM NaCl. Our results showed that *A. seyal* would constitute viable seeds bank when salinity levels are high and that these seeds would germinate, after salt leaching from the soil surface by the rainy season (Tobe et al., 2000; Llanes et al., 2005).

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Salinity significantly decreases the growth of seedlings irrespective of the inoculation treatment. Recent studies reported depressive effects of NaCl on growth of agroforestry species (Mills, 2006; Villagra and Cavagnaro, 2005; Diouf et al., 2005). However, our results showed that inoculation improved seedlings growth comparatively to the uninoculated control, whatever the salt stress. Several reports emphasized that inoculation of legumes by salt tolerant strains of rhizobia may enhance their nodulation and nitrogen fixation under salt stress (Craig et al., 1991; Marcar et al., 1991; Lal and Khanna, 1994; Diouf et al., 2005). These reports pointed out that salt tolerant strains of rhizobia can nodulate salt-tolerant tree legumes and form effective nitrogen-fixing symbioses in saline soils with moderate salinity; this will improve fertility of saline soils in arid lands. Rhizobial inoculants are of prime importance in reclaimed damaged soil where wild legumes, especially trees, are cultivated (Zahran, 2001).

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We recorded root nodulation whatever the NaC1 levels. Thus, the growth of rhizobia in the rhizosphere, infection and nodule initiation were not affected by salinity (Singleton and Bohlool, 1984). Interestingly, moderate salt stress stimulated nodulation in *A. seyal* seedlings inoculated with the strain ORS 3394 (Figure 1). A similar stimulation in nodule number and nodule dry mass under salt stress has also been reported for other moderate halophytic species (Cordovilla et al., 1999; Soussi et al., 1999; Diouf et al., 2005; Garg and Singla, 2004).

Salinity seriously changes the soluble protein, the photosynthetic carbon metabolism, and the leaf-chlorophyll content. The reduction of soluble protein might result to an augmentation of the proteolytic activity for the synthesis of osmoprotectants (Silveira et al., 2003). However, the supply of carbon skeletons for amino acid biosynthesis tightly links nitrogen assimilation and carbon metabolism (Suarez et al., 2002). Therefore, enhanced growth of nodulated plants grown in saline environments has been contributed partly to an efficient mineral nutrition by the host plant, and subsequently lead to a higher production of photosynthates (Serraj, 2002; Zahran, 1999). In this study, we showed that the inhibitory effect of salt on chlorophyll content is alleviated by inoculation. Similar results were reported (Giri and Mukerji, 2004) on mycorrhizal plants. They suggested the explanation might be that mycorrhizal fungi are effective in the absorption of magnesium and suppression of sodium under salt stress conditions, reducing the antagonistic effect of sodium on magnesium absorption.

Salinity slightly increases the PEPC activity in the leaves of *A. seyal* seedlings. The induction of PEPC in leaves under saline treatment has also been reported by others workers (Soussi et al., 1998; Diallo and Queiroz-Claret, 1983). Higher PEPC activity could be used as a biochemical indicator of salt tolerance (Guerrier, 1988). Indeed, increasing capacity of organic acid synthesis might facility the production of organic solutes related to osmotic and pH adjustment under stress conditions (Venekamp, 1989).

As for *Faidherbia albida* (Campa et al., 2000), assimilation of ammonium into amino acids preferentially took place in shoots of *A. seyal*. In our work, inoculation had no effect on GS activity. We have observed that the leaf-GS activity was slightly enhanced when increasing salt stress as reported in cashew leaves (Silveira et al., 2003). The increase on GS activity might be related to release of ammonium during the degradation of proteins.

In summary, our results demonstrated that *A. seyal* is moderately salt tolerant, with an excellent germination rate (40%) at 342 mM NaCl, better than *A. bivenosa* and *A. sclerosperma* species. Interestingly, *A. seyal* would be able to constitute viable seeds bank in high salt level, and may facility the successful establishment of this species in saline environments. Salinity seriously affects growth and nodulation of *A. seyal* seedlings, as well as the soluble protein, the photosynthetic carbon metabolism, and leaf-chlorophyll content. However, inoculation improves plants growth and physiological and metabolic processes responses of *A. seyal* under salt conditions.

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