Effect of NaCl Salinity on Growth, Nodulation and Total Nitrogen Content in *Sesbania sesban*

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Summary

A pot experiment was conducted to determine the effect of salinity on growth nodulation and nitrogen content of *Sesbania sesban*. Fifteen days old *S. sesban* seedlings grown in sandy loam soil were irrigated with NaCl solution of 0.034 mol/L, 0.069 mol/L, 0.103 mol/L, 0.137 mol/L and 0.172 mol/L. The plants were harvested after 80 days. Fresh and dry weight of shoots and roots and root- shoot ratio decreased progressively with the increasing salinity levels. Root showed more inhibition than shoot. Nodules were observed on the roots of plants growing in all five salinity levels but they showed morphological alterations in size and shape. The number and size of the nodules per plant and their fresh and dry weight decreased with increasing salinity levels. The percentage of tissue nitrogen also decreased progressively with the increasing salinity levels.

Key words

nodulation, nitrogen-fixation, salinity, *Sesbania sesban*, Papilionoideae, *Rhizobium*

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Introduction

Soil salinity affects adversely plant growth and development. An excess of salts in the soil leads to both osmotic and ionic stress (Munns, 2002; Benlloch-Gonzalez et al., 2005). The detrimental effect of salt is generally observed at the whole plant level. Suppression of growth occurs in all plants, but their tolerance levels at high salt concentrations vary widely among different plants (Rabie and Alamdini, 2005). Studies conducted on NaCl tolerance of leguminous plants that fix atmospheric nitrogen indicate that mainly herbaceous crop species have been examined in the past. Very few long-term studies have been conducted with multipurpose nitrogen fixing tree species growing in the tropics. Tropical deep rooted trees that can fix atmospheric nitrogen have the additional advantage of adding natural fertilizer to the deep layers of soil. *Sesbania sesban* is a Papilionoid tree legume that forms mutualistic symbiosis with root nodule bacteria and fix atmospheric nitrogen (Allen and Allen, 1981). *S. sesban* is grown as a perennial green manure crop. The foliage is used as a mulch. The leaves when used as green manure for about three years improve the productivity and physical condition of saline soil significantly and are reported to increase the humus content of soil and decrease the salt content. *S. sesban* can grow under different conditions such as waterlogged and acid soils (Allen and Allen, 1981).

Plants are generally most sensitive to salinity during germination and early seedling growth (Qureshi and Barrett-Lennard, 1998). In older plants tolerance to NaCl increases (Anthraper and Du Bois, 2003). The purpose of this study was to evaluate the effect of various concentrations of NaCl on nodule number and mass, fresh and dry tissue mass, percentage of tissue nitrogen and plant height measurements in eleven week old *S. sesban* plants.

Materials and methods

Seeds of *S. sesban* were collected from trees growing in the garden of the Department of Botany, University of Karachi. Healthy seeds were surface sterilized in 3.0% (w/v) mercuric chloride for 2 min., rinsed with sterilized water and germinated in plastic pouches filled with sandy-loam soil. Seeds germinated after one week in pouches. Twenty four pots lined on the inside with polythene bags were prepared. Each pot had a hole at the bottom allowing adequate drainage. The hole was plugged with cotton wool. Each pot was filled with 3 kg of air dried soil. The pots were arranged in a complete randomized design. Twenty four pots were used in the experiment (four replicate for each salinity treatment and the control). The pots were kept in a screen house under natural light and temperature conditions.

Table 1. Electrical conductivity values in the experiment

Molarity of NaCl (Mol/L)	Electrical conductivity (dSm^{-1})
0.034	5.18
0.069	8.62
0.103	10.20
0.137	14.24
0.172	16.22

The pots were irrigated with NaCl solutions of 0.034 mol/L, 0.069 mol/L, 0.103 mol/L, 0.137 mol/L and 0.172 mol/L NaCl prepared from sea salt. After attaining required salinity levels in the pots, the electrical conductivity of pot soil was measured (Table 1). Three seedlings were transferred to each pot after establishing the electrical conductivity of pot soil. The desired salinity levels were maintained throughout the growing period of plants by fortification with saline solutions at regular weekly intervals if necessary. The control sets were irrigated with tap water only.

Inoculum was prepared by isolating rhizobia from root nodules of *S. sesban* plants naturally growing in the garden of Botany Department following Somasegaran and Hoben (1994) Each pot was inoculated with100 mL of rhizobial culture near the root zone of the plants to ensure the root infection by rhizobia. The plants were irrigated with nitrogen free Hoagland solution once a week of transplantation of seedlings in pots. The soil was saturated at field capacity to avoid leaching.

Plants were harvested after 80 days of transplantation in the pots. They were uprooted taking great care not to break the secondary and tertiary roots and nodules. Growth parameters such as root length, shoot length, size of leaves, fresh and dry weight of roots and shoots and number, size, fresh and dry weight of nodules were recorded. The dry weight of roots, shoots and nodules was determined. Root/shoot ratio was calculated by dividing root length by shoot length. Total shoot nitrogen was estimated by micro Kjeldahl method following Somasegaran and Hoben (1994). Statistical analysis was carried out following Zar (1996).

Results and discussion

Effect of NaCl on plant growth

Plant growth in terms of height, root length, shoot length and leaf size was maximum in the control plants and declined progressively with increased salinity levels as compared to control (Table 2). Average plant height decreased from 53 cm to 30.62 cm. Root length decreased from 16.87 to 10.87 cm, shoot length decreased from 36.12 to 19.75 cm , leaf size decreased from 5.52 to 3.4 cm and root-shoot ratio decreased from 0.171 to 0.134 , when the root

Table 2. Effect of NaCl treatment on plant height, leaf size, root, shoot length and root-shoot ratio of *Sesbania sesban*

NaCl Concentration	Ece (ds. m^{-1})	Root length (cm)	Shoot length (cm)	Height of plant (cm)	Leaf size of plant (cm)	Root –Shoot ratio
Control	0.9	16.87 ± 0.314 (a)	36.12 ± 2.024 (a)	53.25 ± 2.174 (a)	5.525 ± 0.228 (a)	0.171 ± 0.027 (a)
0.034 mol/L	5.18	15. 75 ± 2.015 (ab)	28.75 ± 5.878 (ab)	44.5 ± 3.685 (b)	5.05 ± 0.132 (a)	0.176 ± 0.017 (a)
0.069 mol/L	8.62	14.00 ± 2.121 (abc)	23.75 ± 4.210 (b)	37.75 ± 2.594 (c)	4.82 ± 0.311 (a)	0.154 ± 0.027 (a)
0.103 mol/L	10.2	11.37 ± 0.544 (c)	24.05 ± 1.789 (b)	35.47 ± 0.475 (cd)	4.7 ± 0.575 (a)	0.161 ± 0.042 (a)
0.131 mol/L	14.24	12.37 ± 0.898 (bc)	21.25 ± 1.250 (b)	33.62 ± 1.068 (cd)	4.55 ± 0.320 (a)	0.122 ± 0.013 (a)
0.172 mol/L	16.22	10.87 ± 0.657 (c)	19.75 ± 3.502 (b)	30.62 ± 0554 (d)	3.4 ± 0.07 (b)	0.134 ± 0.027 (a)
		LSD 0.05 = 3.873	LSD 0.05 =10.393	LSD 0.05 = 6.269	LSD 0.05 = 0.944	LSD 0.05 = 0.081

Means in the vertical column followed by different letters are significant at P=0.05, as given by Duncan multiple range test. Means are followed by ± S.E.

Table 3. Effect of NaCl treatment on fresh weight, dry weight of roots and shoots of *Sesbania sesban*

NaCl Concentration	Ece (ds. m^{-1})	Fresh weight of root (g $plant^{-1}$)	Dry weight of root (g $plant^{-1}$)	Fresh weight of shoot (g $plant^{-1}$)	Dry weight of shoot (g $plant^{-1}$)
Control	0.9	7.42 ± 1.578 (a)	1.4 ± 0.204 (a)	18.0 ± 0.365 (a)	8.25 ± 0.478 (a)
0.034 mol/L	5.18	5.05 ± 1.132 (a)	1.3 ± 0.122 (a)	17.65 ± 1.741 (a)	7.5 ± 0.735 (a)
0.069 mol/L	8.62	4.77 ± 0.919 (ab)	1.1 ± 0.310 (ab)	14.95 ± 1.500 (a)	6.82 ± 0.77 (a)
0.103 mol/L	10.2	4.17 ± 0.720 (ab)	1.0 ± 0.21 (ab)	13.95 ± 1.629 (a)	6.425 ± 0.404 (a)
0.131 mol/L	14.24	3.72 ± 0.873 (b)	0.775 ± 0.313 (ab)	12.32 ± 1.719 (a)	6.25 ± 0.796 (a)
0.172 mol/L	16.22	2.90 ± 0. 533 (b)	0.55 ± 0.104 (b)	11.92 ± 3.3040 (a)	6.2 ± 1.386 (a)
		LSD 0.05 = 3.016	LSD 0.05 =0.581	LSD 0.05 = 5.461	LSD 0.05 = 2.455

Means in the vertical column followed by different letters are significant at P=0.05, as given by Duncan multiple range test. Means are followed by ± S.E.

zone salinity increased from 0.034 mol/L to 0.172mol/L. The plant height, root length, shoot length and leaf size decreased respectively to 57.7 %, 64.4%, 54.6% and 61.5 % and 78.3% of the control (Table 2).

Decrease in the height of plants grown in saline medium has been reported by Cordovilla et al. (1996), Mirza and Mahmood (1986) and Singla and Garg (2005). Reduction in growth of plants under high salinity is apparent since salinity increases the energy needs of the plant necessary to combat the osmotic and ionic stress for normal cellular maintenance and there is relatively less energy available for growth processes (O'Leary, 1986). The NaCl treatment did not show any adverse effects on plant growth during the first seven weeks of treatment in *Leucaena leucocephala* plants (Anthraper and Du Bio, 2003). At 14 weeks of treatment distinct differences began to occur between the various treatments with plants treated with NaCl. NaCl concentration of ≥ 0.025 mol/L showed the greatest decrease. A study by Singleton and Bohlool (1984) on soybeans corroborated this trend showing that NaCl concentration of 0.026 mol/L had a depressive effect on growth. A depressive effect at NaCl concentration ≥ 0.05 mol/L was also observed in chickpea (El-Sheikh and Wood, 1990) and in peas and faba beans (Delgado et al., 1994). Similar observations were made by Singla and Garg (2005) in *Cicer arietinum*. Keck et al. (1984) have reported that irrigation water salinity had greater effect on shoot growth than root growth in alfalfa. Similar observations were made by Mahmood and Mahmood (1989) in *Prosopis juliflora*. In the present investigation there was a stronger detrimental effect of salinity on root than shoot growth (Table 2). Our results corroborate with Keck et al. (1984), Mahmood and Mahmood (1989) and Singla and Garg (2005).

Effect of salinity on plant biomass

Fresh and dry weight of root and shoot showed a gradual decrease with the increasing salinity levels (Table 3). At 0.034 mol/L NaCl the total fresh weight of root decreased to 68 % and dry weight decreased to 92 % of the control, whereas at 0.172 mol/L total fresh weight and dry weight each decreased to 39 % of the control. In case of shoot at 0.034 mol/L salinity the total fresh and dry weight of shoot decreased to 98 % and dry weight to 90 % of the control, whereas at 0.172 mol/L total fresh and dry weight of shoot decreased to 66 % and 75 % of the control respectively. Singla and Garg (2005) observed that salinity significantly reduced dry matter accumulation in both roots and shoots in chickpea, root growth was more adversely affected than shoot growth.

Effect of salinity on nodulation

Nodules were found in all NaCl treatments used in present studies, which implies that growth of rhizobia in the rhizosphere, process of root hair infection and nodule development were not inhibited by salt concen-

Table 4. Effect of NaCl treatment on number, size, fresh weight, dry weight of nodules and tissue nitrogen of *Sesbania sesban*

NaCl Concentration	Ece (ds. m^{-1})	Nodule number per treatment	Nodule size (mm plant $^{-1}$)	Nodule fresh weight (g plant $^{-1}$)	Nodule dry weight (g plant $^{-1}$)	Nitrogen content (%)
Control	0.9	83.50 ± 4.941 (a)	14.3 ± 0.018 (a)	4.50 ± 0.446 (a)	0.85 ± 0.256 (a)	0.48 ± 0.032 (a)
0.034 mol/L	5.18	62.25 ± 11.448 (ab)	14.2 ± 0.062 (a)	4.21 ± 1.32 (a)	0.67 ± 0.138 (a)	0.38 ± 0.201 (ab)
0.069 mol/L	8.62	55.75 ± 11.338 (b)	13.9 ± 0.055 (a)	2.15 ± 0.123 (b)	0.62 ± 0.153 (a)	0.35 ± 0.116 (ab)
0.103 mol/L	10.2	52.75 ± 11.279 (b)	12.8 ± 0.033 (a)	2.04 ± 0.260 (b)	0.60 ± 0.150 (a)	0.34 ± 0.096 (ab)
0.131 mol/L	14.24	43.50 ± 5.041 (b)	15.1 ± 0.102 (a)	1.69 ± 0.457 (b)	0.54 ± 0.213 (a)	0.33 ± 0.094 (ab)
0.172 mol/L	16.22	39.25 ± 5.793 (b)	8.31 ± 0.252 (b)	1.02 ± 0525 (b)	0.26 ± 0.213 (a)	0.04 ± 0.005 (b)
		LSD 0.05 = 26.303	LSD 0.05 =0.349	LSD 0.05 = 1.931	LSD 0.05 = 0.599	LSD 0.05 = 0.363

Means in the vertical column followed by different letters are significant at P=0.05, as given by Duncan multiple range test. Means are followed by ± S.E.

trations used in the present studies. Similar results have been obtained by Roomi et al. (2002) with *Acacia ampliceps* growing under salt stress. Nodule number per plant decreased with increasing salinity levels. At 0.03 mol/L nodule number per plant decreased to 74% of the control whereas at 0.172 mol/L the nodule number decreased to 47% of the control (Table-4).

The depressive effect of NaCl at concentration ≥ 0.05 mol/L on nodulation and nitrogen fixation was also observed in *Vicia faba*, *Glycine max*, *Medicago sativa* and *Phaseolus vulgaris* (Abdel-Ghaffar et al., 1982; Cordovilla et al., 1996). El-Sheikh and Wood (1990), Rao and Tak (2002) and Soussi et al. (1999) observed similar effects on nodules in chickpea and Roomi et al. (1986) in *Acacia ampliceps.*

Nodule diameter decreased gradually with increasing salinity levels (0.03 mol/L to 0.172 mol/L). The average nodule diameter varied from 14.2 mm (0.034 mol/L) to 8.3 mm (0.172 mol/L). Fresh and dry weight of nodules decreased progressively with increasing salinity levels. At 0.034 mol/L, the fresh and dry weights of nodules decreased to 93% and dry weight decreased to 79 % of the control, whereas at 0.172 mol/L NaCl fresh and dry weight of nodules was 22% and 30% respectively of the control (Table 4). Decrease in fresh and dry weight of nodules at all salinity levels indicated a decrease in size and number of nodules (Mirza and Tariq, 1992). Decrease in number of nodules was accompanied by compensatory increase in nodule size (Buttery et al., 1990; Sprent and Zahran, 1988). In the present study decrease in fresh and dry weight of nodules, indicated a decrease in size of nodules.

Effect of salinity on total nitrogen

The total tissue nitrogen estimated in the control sapling was 0.048%. The total tissue nitrogen decreased gradually with increasing salinity levels. At 0.03 mol/L NaCl the nitrogen content decreased to 79% of the control whereas at 0.172 mol/L salinity the nitrogen content decreased to 8% of the control (Table 4).

The nitrogen contents of plants growing under salt stress show great diversity. Hopmans et al. (1983) showed gradual decrease in the nitrogen contents of *Acacia dealbata* with increase in salinity level. They showed that the salinity level of 11.1 mm hos /cm reduced acetylene reduction activity up to 98%. The growth of *Acacia dealbata* seedling was adversely affected by the high levels of NaCl. The decline in nitrogen content of plants subjected to salinity was significant in our experiments. Our results corroborate with Anthraper and DuBois (2003) and Hopmans et al. (1983).

It may be concluded that salinity levels used in present investigation affected all parameters of growth such as root growth, shoot growth, root-shoot ratio, nodule number and size and percentage of nitrogen in *S. sesban* plants that were 80 days old. These results were obtained with plants subjected to NaCl treatment at seedling stage before nodule formation. It will be interesting to repeat the experiments in which *S. sesban* plants are subjected to NaCl treatment after the nodules have been formed, and duration of treatment is extended. Work on these lines is in progress in our laboratory.

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