Effects of Seawater Salinity on Seedling Growth Nodulation and Tissue Nitrogen in *Acacia nilotica* (L.) Delile

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Summary

A pot experiment was conducted to determine the effect of sea water salinity on growth, nodulation and nitrogen content of *Acacia nilotica* (L.) Delile seedlings. Eight weeks old seedlings were irrigated with 40, 50, 60, 70 and 80% sea water for one month. After 12 weeks the plants were uprooted and the nodules were observed for their frequency, shapes and sizes. Nodules showed morphological alterations in size and shape in different salinity levels. The root-shoot ratio, nitrogen contents in the leaf, stem and root were analyzed. In general salt stress resulted in a decrease of plant growth, nodulation and percent tissue nitrogen in *A. nilotica* plants. Root-shoot ratio showed gradual increase with increasing sea water concentrations. Nitrogen contents decreased in leaf and stem, whereas it increased in roots. Nodules showed morphological alterations in size and shape with increasing salinity. *A. nilotica* accumulated NaCl in the xylem of the roots that may be considered as a preliminary salt tolerant mechanism adopted by the plant.

Key words

Acacia nilotica, legume tree, seedling growth, nodulation, nitrogen content, sea water salinity

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Introduction

Acacia nilotica (L.) Delile is a Mimosoid tree legume that exists on millions of hectares of semi-arid regions of tropical Africa, as well as India and Pakistan (Brewbaker et al., 1984). The plant is widely distributed in Sindh and Punjab provinces of Pakistan (Ali, 1973). *A. nilotica* is important in the rural economy of many of the world's arid and semiarid areas. The wood of *A. nilotica* is a very popular fuel in India and Pakistan and large quantities are consumed as firewood and charcoal. It has also been used extensively to fuel locomotives and river streamers, and it powers the boilers of some small industries as well. The hard, tough wood is resistant to termites, impervious to water and is popular for railroad sleepers, tool handles and carts. It is also used for boatbuilding in some countries. The leaves and pods of *A. nilotica* are widely used as fodder and, in arid regions of Pakistan constitute the main diet for goats and sheep. Pods contain as much as 15% crude protein. The bark and pods are widely used in the leather industry; their tannin content varies from 12-20%. *A. nilotica* is the commercial source of gum Arabic that is used in the manufacture of matches, ink, paints and confectionery. (Brewbaker et al., 1984). An important feature of *A. nilotica* is that it can grow in arid lands under stress conditions and can fix atmospheric nitrogen symbiotically in their root nodules in the presence of rhizobia (Mahmood, 1999). Besides *A. nilotica* is considered as a moderate salt tolerant legume tree and can form mycorrhizal association with the fungus *Glomus fasciculatum* showing increased absorption of P, Mg and Cu over non-mycorrizal plants under saline conditions (Giri et al., 2007). *A. nilotica* is planted for soil stabilization and control of erosion in the arid regions of the world (Zahran, 1999). In the present investigation short term effects of different dilutions of sea water on growth, nodulation and percent tissue nitrogen in leaf, stem and root of *A. nilotica* are presented.

Table 1. Edaphic characteristics of the garden soil

Soil texture	
Coarse sand	37.09%
Fine sand	31.69%
Silt	8.27%
Clay	22.93%
Edaphic characteristics	
pH	7.6
$CaCo_3$	10.20%
Total soluble salts	0.38%
Maximum water holding capacity	23.87%
Organic matter	2.59%

Table 2. Electrical conductivity of various sea water concentarions

Sea water concentration (%)	ECe (dSm^{-1})
Tap water (Control)	0.35
40	22.60
50	28.50
60	33.60
70	36.00
80	44.00
100	54.00

Table 3. EC_e , Na^+ , K^+ , Ca^{++} , Mg^{++} and SAR values of sea water

ECe	Na^+	K^+	Ca^{++}	Mg^{++}	SAR
54.00	324.78 (=7470 mg/L)	9.71 (=37.96 mg/L)	9.47 (=189.7 mg/L)	210.48 (=2450 mg/mL)	31.62 (mg/L)

Materials and methods

Seeds of *Acacia nilotica* were collected from pods of *A. nilotica* trees growing at the Karachi University campus. Healthy seeds were surface sterilized in 30% (w/v) mercuric chloride for two minutes, washed with sterilized water and germinated in plastic pouches filled with moist loamy soil. The edaphic characteristics are presented in Table 1.

Twenty days old seedlings were transferred into pots. Pots were lined on the inside with polythene bags. 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 garden soil. The pots were arranged in a complete randomized design. Twenty four pots were used (three replicates for each salinity treatment and a control). Six pots were kept for observation of nodules on roots after eight weeks of growth of seedlings. The seedlings were irrigated on alternate days with tap water initially up till 56 days of growth. Nodulation in seedlings was facilitated by mixing the pot soil with 5 ml of nodular extract collected from nodules of *A. nilotica* growing under natural conditions at the Karachi University campus.

Eight weeks old seedlings had already developed nodules. These seedlings were subjected to sea water treatment on alternate days for 30 days with 40, 50, 60, 70 and 80% sea water diluted with tap water @500 ml per pot. Tap water was used as control. Sea water was collected from Hawkes Bay beach near Karachi. Results of electrical conductivity of measurements are presented in Table 2. Concentration of Na^+, K^+, Ca^{++}, and Mg^{++} were measured using Petacourt PFPI Flame Photometer.

The seedlings were exposed to seawater treatment for four weeks. The seedlings were uprooted after 12 weeks, and roots were washed in a tray of water to clean adhering soil particles from the roots. Roots were floated in trays of water to facilitate nodule counting. Nodule frequency, shape and size were recorded. Roots and shoots were oven dried at 60°C for 72 hours and weighed to determine root-shoot ratio. Data were analyzed statistically to determine standard errors of means.

Estimation of nitrogen

Nitrogen content of leaf, stem and root portion of each plant was estimated with the help of micro-Kjeldahl apparatus (Hach et al., 1985).

Anatomy of root

Free hand sections of root of *A. nilotica* plants growing in sea water dilutions were obtained with a sharp razor blade. Sections were dehydrated by passing through ascending alcohol grades followed by three changes in absolute alcohol. Sections were stained with a weak safranin solution and mounted in Canada balsam.

Results and discussion

Root, shoot length and root-shoot ratio

Root length and shoot length showed different behavior under salinity stress. Root length increased while shoot length decreased progressively with increasing salinity level (Table 4). Effect of irrigation water salinity may be different on shoot growth and root growth in different plants. For example Keck et al. (1984) reported greater effect of salinity on shoot growth than on root growth in alfalfa and *Vicia faba*. Soussi et al. (1998) and Cordovilla et al. (1994) in chickpea, Tejera et al. (2005) in *Phaseolus vulgaris*, Jamil et al. (2007) in beet and cabbage and Sharif and Khan (2009) in four thorn forest tree species. On the other hand Singla and Garg (2005), Chachar et al. (2008) and Abdulmajid (2009) observed greater effect of salinity on root than on shoot growth. Our results corroborate with Keck et al. (1984), Tejara et al. (2005) and Sharif and Khan, (2009). The shoot growth of plants growing under saline conditions is suppressed because the ability of shoots to take up water is reduced under such environment (Chachar et al. 2008).

Root-shoot ratio

Abdulmajid (2009) observed a decrease in root-shoot ratio of chickpea at all levels of salinity used. In the present investigation root-shoot ratio increased gradually with increasing salinity levels, which indicates that root growth was less affected than shoot growth (Table 4). The higher sensitivity of shoots than roots to salinity results in an increase in root–shoot ratio (Tejera et al. 2005). Increase in root–shoot ratio in *Prosopis juliflora* with increasing salinity was also observed by Mahmood and Mahmood (1989).

Number of nodules

Mahmood and Iqbal (1994) reported an average of 6-15 nodules per *A. nilotica* plant growing under natural ecosystems from various parts of Sindh. In the present study nodule number averaged to 10 per plant (Table 5). There was no significant difference between nodule number in control plants and plants treated with different salinity levels (Table 5). Similar observations were made by Mahmood and Mahmood (1989) in the case of *Prosopis juliflora*. As pointed out by Bernstein and Ogata (1966) nodulation was only slightly affected by salinity when plants were established prior to initiation of salt treatment. In present study salt treatment started when the plants were 56 days old and had already developed nodules. Nodule number decreases with increasing salinity levels (Hafeez et al., 1988; Mahmood et al., 2008; Roomi et al., 2002; Soussi et al., 1999; Tejera et al., 2005).

Nodule size

Size of the nodule estimated from *A. nilotica* trees growing in the field was around 2 mm (Mahmood and Iqbal, 1994). Nodule size decreased gradually with increasing salinity. There was greater decrease between 50% and 60% salinity levels. It has been sported that size of nodules is reduced under salinity stress (Hafeez et al., 1988; Mahmood et al., 2008; Roomi et al., 2002). The size of *A. nilotica* nodules decreased from 1.8 mm (control) to 1.0 mm in plants subjected to 80% salinity. The reduction in size of nodules was 44% of the control (Table 5).

Nodule shape

Mahmood and Iqbal (1994) reported elongated nodules on the roots of *A. nilotica* growing in the field. Shape of nodules showed variation in different sea water concentrations. In control and 50% sea water elongated nodules were observed, in 60% and 70% sea water nodules were globular but in 70% sea water nodules were reduced in size, whereas in 80% sea water nodules were deformed (Table 5).

The morphological changes in shapes of nodules with increasing salinity levels could be due to severe water stress causing irreversible damage to symplastic connections between nodule cells (Sprent, 1971).

Table 4. Effect of sea water salinity on root length, shoot length and root-shoot ratio of *Acaca nilotica*

Sea water concentration (%)	ECe (dSm^{-1})	Shoot length (cm)*	Root length (cm)*	Root-shoot ratio*
Tap water (Control)	0.35	17.0 ± .18	2.5 ± 0.02	1.32
40	22.60	15.1 ± 0.11	2.8 ± 0.041	1.67
50	28.50	13.4 ± 0.32	3.4 ± 0.032	2.16
60	33.60	11.3 ± 0.27	3.7 ± 0.03	2.7
70	36.00	10.0 ± 0.41	4.1 ± 0.045	3.4
80	44.00	7.4 ± 0.22	4.5 ± 0.05	4.7

* Mean of three replicates

Table 5. Effect of sea water salinity on frequency and morphology of nodules of *Acacia nilotica*

Sea water concentration (%)	ECe (dSm^{-1})	Average frequency of nodules*	Average diameter of nodules*	Average shape of nodules*	Colour of nodules
Tap water (Control)	0.35	10.00 ± 0.12	1.8 mm ± .02	Oval or elongated.	Blackish brown
50	28.50	8.00 ± 0.02	1.7 mm ± .01	Elongated	Light brown
60	33.60	10.00 ± 0.23	1.3 mm ± .002	Globular	Dark pinkish
70	36.00	11.00 ± 0.17	1.0 mm ± .03	Globular	Pinkish brown
80	44.00	9.00 ± 0.11	1.0 mm ± .05	Deformed	Dark pinkish

*Mean of three replicates

Table 6. Effect of sea water salinity on nitrogen content of leaf, stem and roots of *Acacia nilotica*

Sea water concentration (%)	ECe (dSm^{-1})	Nitrogen content (%)			
		Leaf*	Stem*	Root*	Total
Tap water (Control)	0.35	0.58 ± 0.013	0.85 ± 0.002	0.29± 0.002	1.72
40	22.60	0.46 ± 0.005	0.77 ± 0.002	0.47± 0.003	1.70
50	28.50	0.40 ± 0.006	0.64 ± 0.015	0.49± 0.012	1.53
60	33.60	0.38 ± 0.011	0.61 ± 0.011	0.52± 0.012	1.51
70	36.00	0.21± 0.004	0.60 ± 0.001	0.57± 0.007	1.38
80	44.00	0.12± 0.011	0.57 ± 0.003	0.57± 0.006	1.26

*Mean of three replicates

Percent Tissue Nitrogen

Total plant nitrogen is a suitable indicator to estimate nitrogen fixation (Bliss, 1993). The tissue nitrogen percent in leaf, stem and root of control saplings of *A. nilotica* were 0.58, 0.85 and 0.29, respectively. Nitrogen concentration showed a progressive decrease with increasing salinity levels in leaf and stem but in roots the nitrogen concentration increased gradually with increasing salinity levels (Table 4). The nitrogen concentration percent in leaf and stem decreased to 32% and 97% of the control when salinity was raised to 80% seawater. On the other hand nitrogen content of roots increased to 49% of the control at 80% salinity. Similar observations have been made by Bernstein and Ogata (1966) for soybean and alfalfa, Wilson (1970) for *Glycine wightii*, Douglas and Chalk (1983) for *Acacia dealbata*, Mahmood and Mahmood (1989) for *Prosopis juliflora*, Jamil et al. (2005) for *Brassica* species and Jamil et al. (2007) for beet and cabbage plants. Tissue nitrogen estimated from foliage of *A. nilotica* growing under natural conditions in Karachi was found to be 1.9% (Mahmood, 1999). In the present investigation tissue nitrogen in the control saplings was 1.72% (Table 6). Douglas and Clark (1983) estimated 1.79% nitrogen in control saplings of *Acacia dealbata*. Mahmood and Mahmood (1989) have reported 2.08% tissue nitrogen in control saplings of *Prosopis juliflora*. The nitrogen contents of plants show a great diversity under salt stress. Bernstein (1962) reported an increase in the nitrogen content of plants at higher levels of NaCl while Shimose (1973) reported reduction in nitrogen content in rice under high levels of salinity. Nitrogen fixation in *Prosopis juliflora* is greatly impaired or totally inhibited under high levels of salinity (Jarell and Virginia, 1984). Nodulation was drastically affected at high salt concentrations in *Vigna radiata* (Hafeez et al., 1988). The combined values of nitrogen content of leaf, stem and root showed a decrease with increasing salinity (Table 6).

In general salt stress resulted in a decrease of plant growth, nodulation and percent tissue nitrogen in *A. nilotica* plants. Salt stress affects all the major processes such as growth, photosynthesis, protein synthesis and energy and lipid metabolism. Plants adopt different strategies to avoid salt injury (Ramoliya et al., 2004). However, the adaptation capacity may vary considerably between species as a result of sensitivity and/or tolerance of host plant, which may vary (Lopez et al., 2008). One of the strategies to avoid salt injury is to accumulate Na^+ and Cl^- ions mainly in the roots to ensure a limited Na^+ and Cl^- toxicity to shoots as observed in some Tunisian genotypes of chickpea (Abdelmajid, 2009). Accumulation of salt was also observed in the tracheids of roots of *A. nilotica* in the present investigation (Figs. A and B). The transport of water and mineral salts takes place through the tracheary elements of roots to shoots. Tracheary elements are comprised of single cells and are narrow often in the range of 10 to 25 μm in diameter. Vessels on the other hand are long cells comprising of subunits called vessel elements or vessel members, arranged end to end. Vessel members are usually wider than tracheids often in the range of (40 to 80 μm) and can be even much wider (Salisbury and Ross, 1986). Vessels are more efficient conduits of water than tracheids because water and dissolved mineral salts can flow relatively unimpeded from one vessel member to the next vessel member through large perfo-

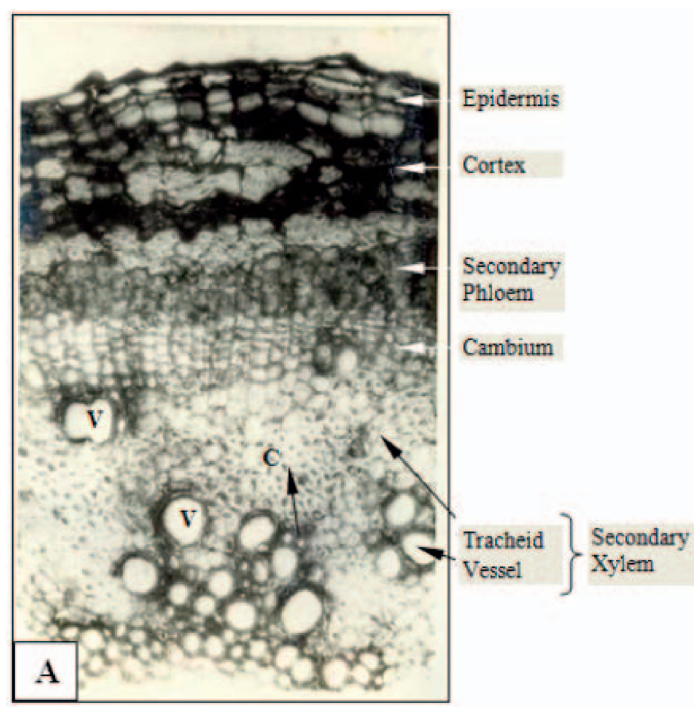

Figure A. Transverse section of a portion of root of *Acacia nilotica* grown in 60% NaCl (X 100), showing epidermis, cortex, secondary phloem, vascular cambium and vascular region comprising of tracheids (T) and vessels (V). The root has undergone secondary growth. Note accumulation of salt (c) in the tracheids

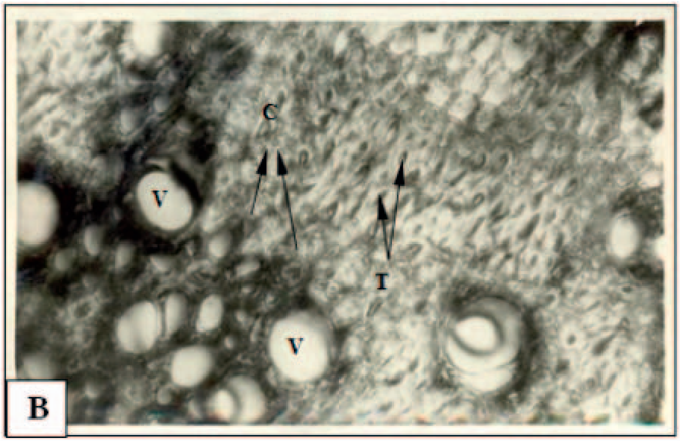

Figure B. Transverse section of a root of *Acacia nilotica* grown in 80% NaCl (X200), showing only the vascular regions. Note that the accumulation of salt (c) in tracheids (T) is more prominent than in Fig. A

rations at their end walls called perforation plates. By contrast water flowing from tracheid to tracheid must pass through pit membrane of the pit pairs in their developing cell walls that have small apertures (Evert, 2006). Salt accumulation in the tracheids of *A. nilotica* roots may be considered as primary salt tolerance mechanism adopted by the plant.

Salinity significantly reduces nodulation and nitrogen fixation (Ben Salah et al., 2009; El Sheikh, 1998; Soussi et al., 1998). The depressive effect of salt on plant growth and percent tissue nitrogen of *A. nilotica* may be explained on the basis of high contents of Na^+ ion (7470 mg/L) in comparison with K^+ ion (36.6 mg/L), Ca^{++} ion (189.7 mg/L) and Mg^{++} (2470 mg/L). Elevation of Na^+ in soil solution inhibits uptake of other nutrients (P, K, Fe, Cu and Zn) directly by interfering with various transporters in the root plasma membrane (Giri et al., 2007). Low $CaCO_3$ content (10.20%) and poor aeration of soil under pot conditions also add to the depressive effect of NaCl on plant growth, nodulation and nitrogen fixation in *A. nilotica* plants.

References

Abdelmajid K. (2009). Differences in response of some Tunisian chickpea genotypes (*Cicer arietinum* L.) to salinity. Pakistan J Bot 41, 3081-3091.

Ali S.I. (1973). Mimosaceae. Flora of Pakistan, 36, 1-41. Department of Botany, University of Karachi.

Ben Salah, A. Albacete, A. Andujar, C.M. Haouala, R. Labidi, N. Zribi, F. Martinez, V. Alfosea, C. Abdelly (2009). Response of nitrogen fixation in relation to nodule carbohydrate metabolism in *Medicago ciliaris* lines subjected to salt stress. J Plant Physiol 166, 477-488.

Bernstein L. (1962). Salt-affected soils and plants. In: Problems of the Arid Zones. Proc. 18th UNESCO Symposium, Paris, France, pp. 139-174.

Bernstein L., G. Ogata (1966). Effects of salinity on nodulation, nitrogen fixation and growth of soybeans and alfalfa. Agron J 58, 201-203.

Bliss F.A. (1993). Breeding common bean for improved biological nitrogen fixation. Plant and Soil 152: 71-79.

Brewbaker J.L., R.W.F. Hardy, J. Lymen (1984). Economically important nitrogen fixing trees. Nit Fix Tree Res Rep 1, 35-40.

Chachar Q., I.A. Solangi, A. Verhoef (2008). Influence of sodium chloride on seed germination and seedling root growth of cotton. Pakistan J Bot 40, 183-197

Cordavilla M. P., F. Ligero, C. Lluch (1994). The effect of salinity on N fixation and assimulation in *Vicia faba*. J Exp Bot 45, 203-210.

Douglas, L.A., P.M. Chalk (1983). Nitrogen fixation associated with *Acacia dealbata* Link. seedlings as estimated by the acetylene reduction assay. Australian J Bot 31, 331-339.

El Sheikh E.A.E. (1998). Response of *Legume-Rhizobium* symbiosis to salinity in Sudan: A review. UKJ Agric Sci 6, 142-155.

Evert R.F. (2006). Esau's Plant Anatomy. Willey-Interscience, John Wiley and Sons Inc. Hoboken, New Jersey. 600 pp.

Giri B., R. Kapoor, K.G. Mukerji (2007). Improved tolerance of *Acacia nilotica* to salt stress by arbuscular mycorrhiza, *Glomus fasciculatum* may be partly related to K/Na ratios in root and shoot tissues. Microbial Ecol 54, 753-760.

Hach C.C., S.C. Brayton, A.B. Kopelove (1985). A powerful Kjeldahl nitrogen method using peroxymonosulfuric acid. Jric Food Chem 33, 1117-1123.

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

Jamil M., C.C. Lee, S. Rehman, D.B. Lee, M. Ashraf, E.S. Rha (2005). Salinity (NaCl) tolerance of *Brassica* species at germination and early seedling growth. Electronic J Erviron Agric Food Chem 4, 970-976.

Jamil M., S. Rehman, E.S. Rha (2007). Salinity effects on plant growth, photosynthesis, photo chemistry and chlorophyll content in sugar beet (*Beta vulgaris* L.) and cabbage (*Brassica oleracea* L. var.*Capitata*). Pakistan J Bot 39, 753-760.

Jarell M.W, R.A. Virginia (1984). Salt tolerance of mesquite. Calif Agric 138, 28.

Keck J.J., R.J. Wagenet, W.F. Campbell, R.F. Knight (1984). Effects of water and salt stress on growth and acetylene reduction in alfafa. Soil Sci Amer J 48, 1310-1316.

Lopez M., J.A. Herrera-Cervera, A. Iribarec., N.A. Tejera, Lluch C. (2008). Growth and nitrogen fixation in *Lotus japonicus* and *Medicago truncata* under NaCl stress: nodule carbon metabolism. J Plant Physiol. 165, 641-650.

Mahmood A. (1999). A comparison of nitrogen concentration between wild and cultivated legumes of Sindh. Pakistan J Bot 31, 183-192.

Mahmood A., M. Athar, R. Qadri, N. Mahmood (2008). Effect of NaCl salinity on growth, nodulation and total nitrogen in *Sesbania sesban*. Agric Consp Sci 73, 137-141.

Mahmood A., P. Iqbal (1994). Nodulation status of leguminous plants in Sindh. Pakistan J Bot 26, 7-20.

Mahmood S.T., A. Mahmood (1989). Effect of sea water salinity on nodulation and nitrogen fixation in *Prosopis juliflora* (Swartz) DC. Pakistan J Bot 21: 68-73.

Ramoliya P., H. Patel, A.N. Pandey (2004). Effect of salinization of soil on growth and macro- and micro-nutrient accumulation in seedlings of *Salvadora persica* (Salvadoraceae). Forest Ecol Manage 22, 181-193.

Roomi D., R. Ahmed, S. Ismail, A. Ghaffar (2002). Effect of salt stress on *Rhizobium* and growth of *Acacia ampliceps* In: Prospects for saline agriculture (R. Ahmed and K.A. Malik, Eds.), Kluwer Academic Publishers, The Netherlands, pp.297-308.

Salisbury F.B., C.W. Ross (1986). Plant Physiology, 3rd. ed. CBS publishers and Distributors, New Delhi, pp. 540.

Sharif F. and A.U. Khan (2009). Alleviation of salinity tolerance by fertilization in four thorn forest species for the reclamation of salt affected sites. Pakistan J Bot 41, 2901-2915.

Shimose N. (1973). Salt tolerance of crops. Jarq Japan Agric Res 7, 178-184.

Soussi M., A. Ocana, C. Lluch (1998). Effect of stress on growth, photosynthesis and nitrogen fixation in chickpea (*Cicer arietinum* L.). J Exp Bot 49, 1329-1337.

Soussi M., C. Lluch and A. Ocana (1999). A comparative study of Nitrogen fixation and carbon metabolism in two chickpea (*Cicer arietinum* L.) cultivars under salt stress. J Exp Bot 50, 1701-1708.

Singla R., N. Garg (2005). Influence of salinity on growth and yield attributes in chickpea cultivars. Turkish J Agric For 29, 231-235.

Sprent J.I. (1971). The effects of water stress on nitrogen-fixing in root nodules. I. Effect on the physiology of detached nodules. New Phytol 70, 9-17.

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

Wilson J. R. (1970). Response to salinity in *Glycine*. VI. Some effects of a range of short-term salt stresses on the growth, nodulation and nitrogen fixation of *Glycine wightii*. Australian J Agric Res 21, 571-582.

Zahran H.H. (1999). *Rhizobium*- legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol Molec Biol Rev 63, 968-989.

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