

## EFFECT OF SEA WATER SALINITY ON NODULATION AND NITROGEN FIXATION IN *PROSOPIS JULIFLORA* (Swartz) DC.

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### Abstract

Fifty six day old *Prosopis juliflora* seedlings were subjected to 40, 50, 60, 70 and 80% sea water treatment for one month. After 86 days the morphology of nodules, root-shoot ratio and total nitrogen content in root and shoot were analysed. Nodules showed morphological alterations in size and shape. Root-shoot ratio was lowest in the control and showed a gradual increase with increasing sea water concentrations. The amount of nitrogen in the stem and leaves showed a gradual decrease due to disturbance in the translocation of divalent cations to the aerial parts, while roots registered a gradual increase with increasing sea water concentrations due to accumulation of assimilated nitrogen in the roots.

### Introduction

*Prosopis* spp., the leguminous tree mesquite, exists on millions of hectares of semi-arid regions of the world (Felker & Clark, 1980). The potential of this mimosoid legume to supply biomass – derived energy, fuel and forage in arid and semiarid regions has been well recognized (Felker, 1979; Allen & Allen, 1981). An important feature of mesquite is that it can grow in saline areas and can tolerate salinity equivalent to sea water (Felker *et al.*, 1981). Mesquite species growing under natural conditions bear root nodules and can fix atmospheric nitrogen (Felker & Clark, 1980; Mahmood, 1985). In the present investigation the effect of different concentrations of sea water on growth, nodulation and nitrogen fixation in *Prosopis juliflora* is reported.

### Materials and Methods

*Preparation of seedlings:* Seeds collected from pods of *Prosopis juliflora* var. *juliflora* in mid April were treated with concentrated Sulphuric acid for 15 min. to break the dormancy, washed several times with water and sown in pots containing moist loamy soil collected from the vicinity of well-established plants of *P. juliflora*. Soil subjected to analysis (Anon., 1954) showed pH 8.4, CaCO<sub>3</sub> 10.20%, Total soluble salts 0.382%, Water holding capacity 23.87%, Organic matter 2.59%, Coarse sand 37.09%, Fine sand 31.69%, Silt 8.27%, and Clay 22.93%.

Twenty day old saplings were transferred into 25 cm. diam pots, one seedling per pot. Three replicates were taken for each treatment. The seedlings were irrigated daily with tap water. Nodulation in plants was facilitated by mixing the pot soil with 5 ml of

nodular extracts taken from nodules of *P. juliflora* plants growing under natural conditions.

Saplings 56 days old which had developed nodules were subjected to sea-water treatment on alternate days for 30 days period with 40, 50, 60, 70 and 80% sea water diluted in tap water @ 500 ml per pot. Tap water was used as control. After one month of sea water treatment the saplings were uprooted to determine root/shoot ratio, the frequency, shape and size of nodules.

*Estimation of Nitrogen:* Nitrogen content of leaf, stem and root portions of each plant was estimated with the help of micro-Kjeldahl apparatus (Bergersen, 1980). Crude protein contents were determined by multiplying the estimated nitrogen contents with the constant factor of 6.25 (Eggum & Juliano, 1975).

## Results and Discussion

*Root-shoot Ratio:* Root growth and its branching increased gradually with increasing salinity level. Maximum growth and branching of roots was observed in 80% sea water.

Mesquite develops a very deep root system under field conditions and opts for phreatophytic habit (Simpson, 1977). The root shoot ratio in two months old seedling of *Prosopis velutina* was 6.40 (Glendening & Paulsen, 1955). In the present study root-shoot ratio in 86 days old control plants was 1.36 and showed gradual increase in saplings grown in increasing concentrations of sea water (Table 1). Decrease in the growth of different plant species with increasing salinity has been reviewed by Alam *et al* (1986). Similar results on shoot growth of *Zea mays* have been reported by Kayani & Rehman (1988).

### *Nodulation:*

a) *Nodule number:* Mahmood (1985) reported on an average 15 nodules per *Prosopis juliflora* plant growing under field condition in various parts of Sind. In the present study, average number of nodules per plant was 12.33 (Table 1). This low average frequency could be attributed to excessively high pH of irrigation water, (9.05) low CaCo<sub>3</sub> content (10.20%) and poor aeration of soil under pot conditions (Table 1).

b) *Nodule diameter:* Nodule diameter decreased gradually with increasing salinity level (Table 2). There are reports that weight and number of nodules are reduced under salt stress (Wilson, 1970; Bernstein & Ogata 1966; Subba Rao *et al* , 1972; Hafeez *et al*, 1988). The average diameter of *P. juliflora* nodules growing under natural conditions was 1.5 mm (Mahmood, 1985) while in the present study the average nodule diameter varied from 1.4 mm in 40% sea water to 1.1 mm in 80% sea water treatment.

**Table 1. Effect of sea water irrigation on root shoot ratio, frequency and morphology of nodules of *Prosopis juliflora*.**

% Sea water	pH	EC dsm <sup>-1</sup>	Rootshoot Ratio	+nodule number	+ nodule diameter (mm)	÷ nodule shape
0 Tap water, control	9.05	0.35	1.36	12.00	1.3	globular
40	9.45	22.60	1.93	11.66	1.4	globular
50	9.45	28.50	2.04	12.33	1.2	globular cum elongated
60	9.45	33.60	3.25	10.00	1.2	elongated
70	9.45	36.00	3.92	11.00	1.1	deformed and reduced
80	9.45	44.00	3.99	11.66	1.1	deformed and reduced

+ Mean of 10 replicates; + Mean of 3 replicates.

\*The values of pH and EC of pure sea water were 9.50 and 54.0 dsm<sup>-1</sup> respectively.

c) *Nodule shape*: Mahmood (1985) reported globose nodules on the secondary roots of *P. juliflora* while Baird *et al* (1985) have reported spherical and elongate nodules, distributed evenly on both younger and older roots of mesquite. Shape of nodules showed variation in different sea water concentrations. In control and 40% sea water, globular nodules were seen, in 50% sea water the nodules were globular cum elongated and in 70% and 80% concentrations nodules showed rough and uneven margins (Table 1). The morphological changes in the shape of nodules could be due to severe water stress causing irreversible damage to symplastic connections between nodule cells (Sprent, 1971).

**Table 2. Amount of nitrogen and crude protein contents in *Prosopis juliflora* plants irrigated with different concentrations of sea water.**

Sea Water Conc. (%)	Nitrogen Conc. (%)				Protein Conc. (%)			
	Leaf	Stem	Root	Total	Leaf	Stem	Root	Total
0, Control	0.70	0.96	0.42	2.08	4.38	6.00	2.62	13.00
40	0.56	0.89	0.50	1.96	3.49	5.60	3.16	12.25
50	0.44	0.70	0.53	1.68	2.75	4.40	3.55	10.50
60	0.42	0.71	0.55	1.68	2.61	4.47	3.42	10.50
70	0.19	0.69	0.55	1.44	1.18	4.35	3.47	9.00
80	0.10	0.63	0.56	1.29	0.66	3.96	3.48	8.10

### *Nitrogen Estimation:*

The total nitrogen concentration in the control saplings was 2.08% with 13.00% crude protein. The average protein content reported in plants is usually 15% (Felker & Clark, 1980). Varying levels of nitrogen and protein contents have been reported for mesquite plants. For example, Felker & Clark (1980) reported mean total nitrogen percentage per dry weight in 13 varieties of *Prosopis* as 2.12% (13.25% of crude protein), while Glendening & Paulsen (1955) recorded 2.66% and 6.92% nitrogen (16.62% and 43.25% of crude protein) in young and mature tissues of *P. chilensis* and 4.0% to 7.7% nitrogen (25% to 48.12% of crude protein) in young and mature tissue of *P. velutina*. In *Acacia dealbata*, the nitrogen content was 1.79% (11.18% of crude protein) in control (Douglas & Chalk, 1983). In the present study the nitrogen contents present in leaf, stem and root of control plants was 0.64, 0.96 and 0.42%, respectively (Table 2). According to Merrill & Cowling (1966) 71% of the variation in nitrogen content among various angiospermous species could be accounted for by differences in volume of parenchyma cells.

In *P. juliflora*, the total nitrogen content gradually declined with increase in salinity of irrigation water (Table 2). Maximum nitrogen content was 2.08% in control, while the minimum value of 1.29% was obtained in 80% sea water. Our results corroborate with Douglas & Chalk (1983) who found gradual decrease in the nitrogen content of *Acacia dealbata* with increase in salinity. The results also indicate a gradual decrease in the nitrogen contents of leaves and stem while a gradual increase in the nitrogen contents of roots with increasing salinity levels of irrigation water (Table 2). The foliage was most affected. Similar results have been reported by Bernstein & Ogata (1966). Under high levels of salinity nitrogen fixation in mesquite is greatly impaired or totally inhibited (Jarrell & Virginia, 1984). Similar effects of short term salt stress on nodule activity and nitrogen content of *Glycine wightii* have been reported by Wilson (1970).

Salinity is known to disturb the balance of cations such as Na, Ca, K, Mg, Fe and Mn in root, stem and leaf. Salinity caused accumulation of Na in leaf and stem whereas Ca, K, Mg, Fe and Mn significantly decreased with increasing salinity levels in cucumber, snake melon and peanut plants (Alam *et al*, 1986). On the other hand Khan *et al* (1987) have shown that at increasing salt concentration in the irrigation medium concentration of Ca, K, Mg decreased while concentration of Na ions increased in roots of *P. juliflora* seedlings grown in various dilutions of amended sea water.

Divalent cations such as Mg, Mn and Fe have been recognised as one of the essential ingredients in the overall process of nitrogen fixation (Greulach, 1973). Hence any disturbance in their optimum level may result in decrease in the nitrogen content of stem and leaf as observed in *P. juliflora* (Table 2).

The observed increased levels of nitrogen contents in the root tissues of *P. juliflora* with increasing salinity levels would suggest that the plant is not a true halophyte, but usually considered to be a glycophyte (plant not completely adapted to salt stress), its physiology therefore is not as modified to behave normally under salt stress (Khan *et al*, 1987). The increased levels of nitrogen in the roots may thus be attributed to the disturbance in the translocation of assimilated nitrogen from the roots to the aerial parts. This hypothesis is substantiated by the work of Mass & Hoffman (1977) who found that in crop plants salinity normally restricts salt movement from the roots to the leaves.

#### References

- Alam, S.M., S. Ahmed and S.S.M. Naqvi. 1986. Effect of salinity on growth and mineral composition of cucumber, snake melon and peanut. *Pak.J.Bot.*, 18: 37-43
- Allen, O.N., and E.k. Allen. 1981. *The Leguminosae. A source book of characteristics, uses and nodulation*. Uni. of Wisconsin Press, Wisconsin.
- Anonymous. 1954. *Diagnosis and improvement of saline and alkali sols*. USDA Agric. Handbook. 60. U.S. Government Printing Office, Washington, DC.
- Baird, L.M., R.A. Virginia and B.D. Webster. 1985. Development of root nodules in a woody legume, *Prosopis glandulosa*. *Torr. Bot. Gaz.*, 146: 39-43.
- Bergersen, F.J. 1980. *Methods for evaluating biological nitrogen fixation*. John Wiley & Sons, New York.
- Bernstein, L. and G. Ogata. 1966. Effects of salinity on nodulation, nitrogen fixation and growth of soybeans and alfalfa. *Agron. J.*, 58: 201-203
- Douglas, L.A. and P.M. Chalk. 1983. Nitrogen fixation associated with *Acacia dealbata* Link., seedlings as estimated by the acetylene reduction assay. *Aust. J. Bot.*, 31: 331-339.
- Eggum, B.O. and B.O. Juliano 1975. Higher protein content from nitrogen fertilizer application and nutritive value of milled-rice protein. *J. Sci. Food Agri.*, 26: 425-427.
- Felker, P. 1979. Mesquite. An all-purpose leguminous arid land tree. In: *New Agricultural crops*. Westview Press, Boulder Colorado, pp. 90-132.
- Felker, P. and P.R. Clark. 1980. Nitrogen fixation (acetylene reduction) and cross inoculation in 12 *Prosopis* (Mesquite) species. *Plant & Soil*, 57: 177-186.
- Felker, P., P.R. Clark, A.E. Laag and P.F. Pratt. 1981. Salinity tolerance of the tree legumes: Mesquite (*Prosopis glandulosa* var. *torreyana*, *P. velutina* and *P. tamarugo*) grown in sand culture on nitrogen-free media. *Plant & Soil*, 61: 311-317.
- Glendening, C.E. and H.A. Paulsen. 1955. Reproduction and establishment of velvet mesquite as related to invasion of semi-desert grasslands. U.S.D.A. Tech. Bull 1127, pp. 50.
- Greulach, V.A. 1973. *Plant function and structure*. Macmillan Publishing Co., Inc. New York.

- 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.
- Jarrell, W.M. and R.A. Virginia. 1984. Salt-tolerance of Mesquite. *Calif. Agric.* 138: 28.
- Kayani, S.A and Mujeeb-ur-Rehman. 1988. Effects of NaCl salinity on shoot growth, stomatal size and its distribution in *Zea mays* L. *Pak. J. Bot.*, 20: 75-81.
- Khan, D., R. Ahmad and S. Ismail. 1987. Germination, growth and ion regulation in *Prosopis juliflora* (Swartz) DC., under saline conditions. *Pak. J. Bot.*, 19: 131-138.
- Mahmood, A. 1985. *A qualitative survey of nodulating ability of legumes of Pakistan*. Final Research Report (1982-1985). Deptt. of Botany, University of Karachi.
- Maas, E.V. and G.L. Hoffman. 1977. Crop salt tolerance-current assessment. *J. Irrig. Drain Div. Proc. Am. Soc. Civ. Engg.*, 103 (IR2): 115-134.
- Merrill, W. and E.B. Cowling. 1966. Role of nitrogen in wood deterioration, amounts and distribution of nitrogen in tree-stems. *Canad. J. Bot.*, 44: 1555-1580.
- Simpson, B.B. 1977. *Mesquite-Its biology in two desert scrub ecosystems*(U.S/IBP Synthesis Series 14). Dowden, Hutchinson and Ross, Inc. Stroudsburg pp. 250.
- Sprent, J.I. 1971. Effect of water stress on nitrogen fixation in root nodules. *Plant & Soil*, Special vol. 225-228.
- Subba Rao, N.S., M. Kalshmi - Kumari, C.S. Singh and S.P. Magu. 1972. Nodulation of lucerne (*Medicago sativa* L.) under the influence of sodium chloride. *Indian J. Agric. Sci.*, 42: 384-386.
- 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* (formerly *javanica*). *Aust. J. Agric. Res.*, 21: 571-582.

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