Pak J. Bot. 33 (special issue) 2001

SEAWATER TOLERANCE IN *RHIZOPHORA MUCRONATA*

*IRFAN AZIZ AND M. AJMAL KHAN

Department of Botany,
University of Karachi, Karachi – 75270, Pakistan.

Abstract

Rhizophora mucronata Lam. is found in the Miani Hor estuary, Balochistan and the Indus delta. Its propagules were collected from Miani Hor (Sonmiani beach) and grown for one year in sandy soil, salinized with 0, 25, 50, 75 and 100% seawater fortified with nitrogen. The optimum growth of plants was obtained at 50% seawater. Plant height and leaf area was promoted at 50% seawater and significantly decreased with increasing salinity of the growth medium. The plants progressively adjusted their water potential in response to change in external water potential i.e. responded as osmoconformer. Concentrations of Na+ and Cl- increased, while stomatal conductance decreased with increases in salinity.

Introduction

Rhizophora mucronata Lam. (Rhizophoraceae) is a moderate sized tree, up to 10 meter tall, supported by adventitious prop roots (Ghafoor, 1984). Its few small populations are located in the Miani Hor estuary, Balochistan and the Indus delta, Pakistan (Saifullah, 1982; Atkinson, 1987; Ansari, 1987). It helps in coastal stabilization and provides nursery areas for economically important fishes and crustaceans in the tropics and sub-tropics (Tomlinson, 1986). The forest stature of this species is regulated by residual effects of tidal and waves energy, soil salinity, nutrient availability and flooding frequency (Lugo *et al.*, 1989; Cintron *et al.*, 1985; Feller 1995).

Mangroves grow in salinity stress following shifts between flooding by ocean water and fresh water introduced by rain and run-off (Lugo *et al.*, 1989). Such spatial and temporal changes in salinity could affect growth and physiology of plants (Naidoo, 1985). Mangrove growth usually declines at high salinities, and optimal growth is obtained at moderate salinities (Clough *et al.*, 1984). This pattern of growth is primarily a reflection of low external water potentials on the water relations of the plants (Luttge, 1997). Leaf water potential of plants become more negative when there is salinity stress (Clough *et al.*, 1984). Other responses associated with salt stress in plants besides retarded growth and lowered water potentials include changes in sap osmotic pressure, salt exclusion at root level and active salt excretion through leaves (Hutchings and Saengar 1987, Stewart and Popp 1987). Salt tolerant plants also maintain low stomatal conductance (Clough, 1984; Ball and Farquhar, 1984; Naidoo, 1985) through the accumulation of ions like sodium and chloride in their tissues. Such ions could have serious effects on plant metabolism, however, in salt tolerant plants, ions

*Current Address: Govt. Degree Science & Commerce College, OrangiTown, Karachi.

are usually sequestered in the vacuole and their lower osmotic potential is effectively countered by synthesis of small organic solutes (Popp *et al.*, 1984). These organic solutes serve as compatible solutes.

Rhizophora mucronata is rapidly disappearing from Pakistani coasts and it may face extinction if not properly rehabilitated. The present study was designed to understand the morphological and physiological responses of *R. mucronata* when exposed to various concentrations of seawater. This study will help us in rehabilitation of the plants in suitable coastal areas. Following questions were addressed in our study: 1) How does *Rhizophora mucronata* cope with change in seawater concentrations? 2) What is the mechanism of osmoregulation in response to various seawater concentrations?

Materials and methods

Propagules of *R. mucronata* were collected from Miani Hor, Balochistan, during the summer season and the bottom 5-cm of each was embedded in a 36-cm diameter plastic bucket filled with acid washed beach sand. Plants were grown in a greenhouse under natural conditions and watered for two weeks through sub-irrigation. After two weeks, plants were progressively treated with seawater (0, 25, 50, 75 and 100%) fortified with nutrient solution (Popp and Polania, 1989). The levels of culture solutions were maintained in the pots through daily sub-irrigation with water and the solutions were replaced every seven-day to avoid salinity built up in pots. Growth parameters were measured after one year at sapling level. Leaves were rinsed with distilled water prior to the determination of water potential. Leaf discs, 5 mm in diameter, were punched from the middle of the lamina and placed in a C-52 sample chamber. After equilibration, water potential of five shoots of each treatment was determined with the help of Wescor HR 33 T dew point micro-voltmeter. Stomatal conductance was measured using AP-4 porometer (Delta-T devices, Cambridge, UK) on the adaxial surface of fully expanded leaves at first node. For ionic measurements, half gram of plant material was boiled in 10 ml of water for two h at 100 °C using a dry heat bath. This hot water extract was cooled and filtered using Whatman no. 42 filter paper. One ml of hot water extract was diluted with distilled water for ion analysis. Ion contents of plants were analyzed by using Ion analyzer (Radiometer, Ion-85). The results were analyzed using linear regression and the significance of individual treatment means measured using Bonferroni test (SPSS, 1999).

Results and discussion

Plant height of *R. mucronata* was promoted at 50% seawater and significantly ($P < 0.05$) decreased with increasing salinity of the growth medium (Fig. 1). Leaf area per plant was higher at 50% seawater and significant differences ($P < 0.05$) were observed among their means (Fig. 2). Reduced growth was obtained in 100% seawater and no mortality was recorded. *R. mucronata* form South Africa showed an optimal growth at 25% seawater (Naidoo, 1985) and similar responses were reported for other mangroves (Downton, 1982; Clough, 1984; Smith *et al.*, 1995; Ogrady *et al.*, 1996). However, in *A. marina* optimum growth was observed in 50% seawater (Karim and Karim, 1993). Growth optima for other mangrove species from Pakistan were 50% seawater (Aziz and Khan, 2000). These results showed a greater spatial variation in the range of tolerance indicating that mangroves from mesic sites of Australia and South Africa are relatively less tolerant to salinity than the species from the arid coasts of Pakistan.

A linear regression showed that water potential in both young and old leaves became more negative with the increase in media salinity ($P < 0.003$; Fig. 3). In other studies, water potential became more negative in mangroves when subjected to increasing salinity (Naidoo, 1985; Rada *et al.*, 1989; Werner and Von villart, 1995). A negative water potential in plants is caused by accumulation of inorganic solutes (Popp, 1983) as well as low molecular weight organic solutes such as proline (Stewart and Lee, 1974), betaine (Storey and Wyn Jones, 1975; Popp, 1983). In addition, sequestration of the main part of cellular NaCl within the vacuole is also caused in some mangroves (Werner and Stelzer, 1990). Increasing concentration of ions in *R. mucronata* caused progressively more negative values for water potential in leaves and xylem tension in stem. For this purpose, a sufficient amount of cyclitols was found in many *Rhizophora* species that serves as osmolyte in response to salt stress (Popp, 1983). Re-allocation of nitrogenous organic resources to osmoregulation could severely limit plant growth rate (Naidoo, 1985). In our results, retarded growth of *R. mucronata* at higher salinity might be due to the presence of such organic solutes.

Higher stomatal conductance was observed in 0% seawater and an increase in salinity of rooting medium caused a decrease in stomatal conductance of young and old leaves ($P < 0.0001$; Fig. 4). Similar results were reported for other mangrove species (Aziz and Khan, 2000; Werner & Von Villert, 1995; Naidoo, 1985). It is assumed that lowering in osmotic potential in external medium due to increased salt concentration, results in decreased stomatal conductance (Naidoo, 1985). It may reduce water absorption hence causing stomatal closures and increase water use efficiency of the plants (Werner and Stelzer, 1990; Gordon, 1993). The lowering in conductance decreases the rate of carbon dioxide accumulation and uptake (Apahalo and Jarvis, 1993) and an increase in xylem tension (Ball and Farquhar, 1984).

Sodium and chloride concentrations significantly ($P < 0.05$) increased with increases in media salinity ($P < 0.05$; Fig. 5). High concentration of ions in tissues of *R. mucronata* at high salinities (75 and 100%) may have caused a reduction in growth, because they may inhibit biochemical processes such as enzyme activities and protein synthesis (Gibson *et al.*, 1994). Mangroves grow best in moderately saline solutions (Werner and Stelzer, 1990) because they maintain turgor by accumulating salts in the tissues. The reduction in leaf area and plant height of *R. mucronata* in salinity higher than 50% seawater also corresponds to the increasing accumulation of sodium and chloride in the plants. Similar response in *R. stylosa* was reported above 25% seawater and it was suggested that osmotic effect could cause water stress at growing points of the plants (Clough, 1984). *Rhizophora* sp. does not have specialized salt glands on leaf and stem hence it can be classified as an excluder (Scholander *et al.*, 1962).

Our results suggest that *R. mucronata* is a highly salt tolerant species, which grows best when fresh water mixes with seawater, and it maintains its salt balance like a true halophyte by lowering its water potential and decreasing stomatal conductance, though its growth is retarded at very high salinity. *R. mucronata* is an excellent candidate for the rehabilitation of the Indus delta and other coastal areas of

Pakistan. This species is an osmoconformor requiring a slow change in soil salinity and therefore, it would be better suited for the sea front.

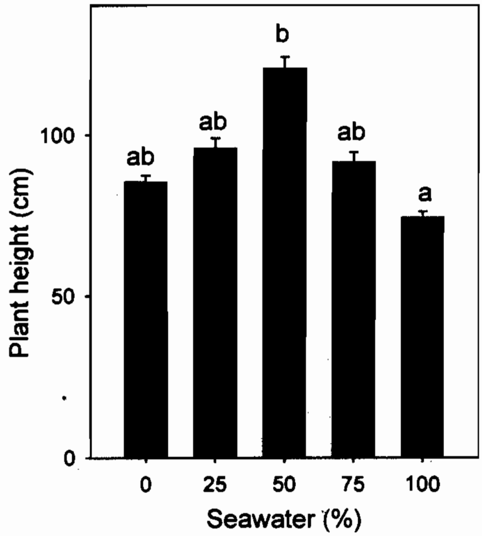

Fig. 1. Effect of NaCl (0, 25, 50, 75 and 100% seawater) on plant height (cm) in *Rhizophora mucronata* plants. Different letters on error bars represent significant difference at $P < 0.05$ (Bonferroni test).

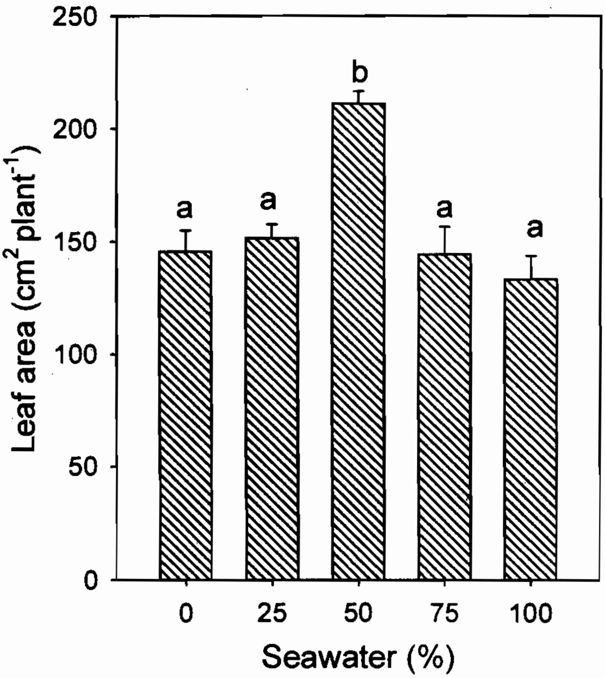

Fig. 2. Effect of NaCl (0, 25, 50, 75 and 100% seawater) on leaf area in *Rhizophora mucronata* plants. Different letters on error bars represent significant difference at $P < 0.05$ (Bonferroni test)

^{1} while the trees account for 0.08 t ha^{-1} in a year. The area multiplied with the salt removal can give the tentative estimate.

It is clear from the aforementioned discussion that the outgoing salts amount to 80.59 mt (61.14+16.5+2.95 removed by irrigation + rainfall, drainage and crops, respectively), while the addition of salts through canal water, groundwater extraction and shallow water table amounts to 150.19 mt. This gives a net addition of 69.60 mt of salts to the Punjab Irrigation Basin.

The disturbed salt balance is giving rise to Secondary Salinity, which is now gaining a dominant position over the genetic salinity. The salt-balance mechanism if not properly managed, it will turn our most of the irrigated lands unproductive within 80 years or so. Need therefore, be felt to look into the balance and adopt such means and ways that can effectively control the problem without serious quality concerns to the system.

Conclusions and recommendations

The following tentative and general conclusions and recommendation can be drawn from this study.

1. Punjab Irrigation Systems (PIS) large scale irrigation developments have brought great prosperity to the people but it also mobilized salts and yielded to salinity and waterlogging problems.
2. Salinity / sodicity has surfaced up as water short, water excess dilemma due to defective management at various levels and for economic reasons. At present about 12.38 % of the CCA is salt affected and 0.22% waterlogged.
3. Canal water is excellent in quality. It has low Na/Ca but high HCO_3/Ca ratios. Besides its excellent quality, canal water is adding salts to the basin because of restricted drainage.
4. About 150.19 mt salts are added while 80.59 mt are removed per year. Net addition of 69.60 mt per year thus may become a concern in future. Thus salt balance is disturbed and requires attention of the irrigation managers.
5. The irrigation water applied should take care of both crops and leaching requirements.
6. The measures should be taken to lower down the shallow water table.
7. Drainage effluent loaded with salts should not be directly unloaded to the rivers furnishing water to the canals for irrigation purpose. The re-use potential of drainage surplus should be assessed.
8. Rights of farmers over groundwater exploitation should be defined to prevent over pumpage and intrusion from saline zone to the fresh water zone.
9. Water storage or reservoirs need immediate construction to minimize dependence on groundwater of poor to marginal quality. Otherwise, forced by the canal water shortage, the farmers would continue the practice of exploiting poor water quality ground water.
10. Adequate knowledge on water quality is must. The water with low Na/Ca but high HCO_3/Ca ratio or waters of even negative RSC may sodicate the soil through geochemical precipitation process.
11. Unless the surface water resources are not harnessed, the use of spatio-temporal salinity and ground water database should be made effectively in developing targeted reclamation programmes in canal commands.

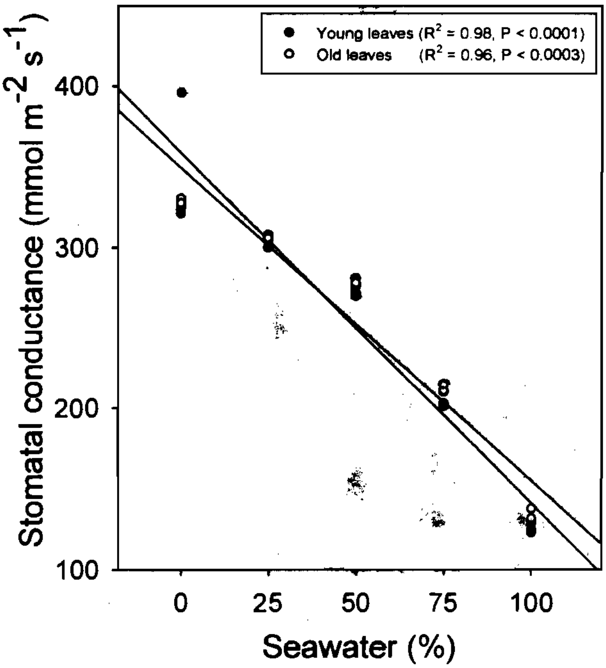

Fig. 4. Effect of NaCl (0, 25, 50, 75 and 100% seawater) on stomatal conductance in *Rhizophora mucronata* plants. A linear regression represent significant difference at P < 0.003

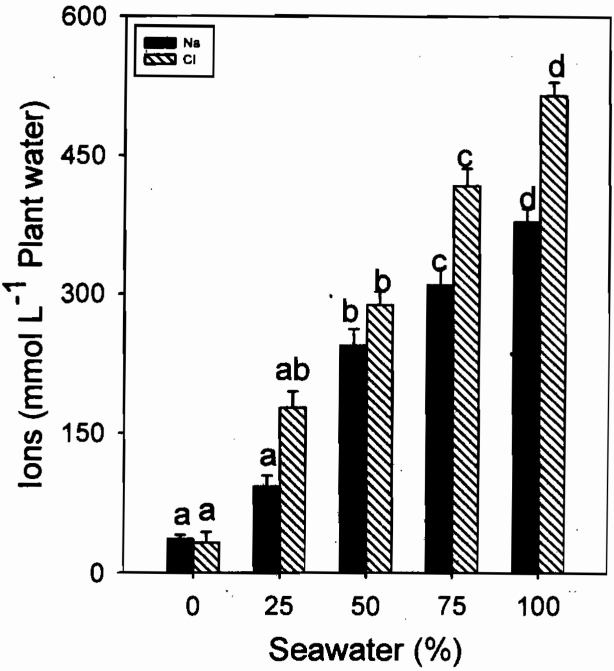

Fig. 5. Effect of NaCl (0, 25, 50, 75 and 100% seawater) on the ionic content in *Rhizophora mucronata* plants. Different letters on error bars represent significant difference at $P < 0.05$ (Bonferroni test)

References

Adam. P. 1990. *Salt marsh ecology*. Cambridge University press, London.

Ansari, T.A. 1987. *Mangroves of the Asia and the Pacific. In Mangrove Ecosystem in Asia and Pacific.* (Ed): T.A. Ansari. Tokyo, Japan. pp. 151-173.

Apahalo, P.J., and P.G. Jarvis. 1993. Separation of direct and indirect responses of stomata to light: Results from a leaf inversion experiment at constant intercellular CO_2 molar fraction. *J. Exp. Bot.*, 44: 791-800.

Atkinson, M.R., G.P. Findlay, A.B. Hope, M.G. Pitman, H.D.W. Saddler, and K.R. West. 1967. Salt regulation in the mangroves *Rhizophora mucronata* Lam. and *Aegialitis annulata* R.Br. *Aust. J. Biol.* Sci., 20: 589-699.

Aziz, I., and M.A. Khan. 2000. Physiological adaptations to seawater concentration in *Avicennia marina* from Indus delta, Pakistan. *Pak. J. Bot.*, 32: 151-169.

Ball, M.C., and G.D. Farquhar. 1984. Photosynthetic and stomatal responses of two mangrove species, *Aegiceras corniculatum* and *Avicennia marina* to long term salinity and humidity conditions. *Plant Physiol.*, 74: 1-6.

Cintron, G., A.E. Lugo and R. Martinez. 1985. Structural and functional properties of mangrove forests. In *The botany and natural history of Panama.* Monographs in Systematic Botany. (Ed): W.G.D. Arcy and M.D. Correa. Missouri Botanical Gardens, St. Louis, Missouri. pp. 53-66.

Clough, B.F. 1984. Growth and salt balance of the mangroves *Avicennia marina* (Forssk.) Vierh. and *Rhizophora stylosa* Griff. in relation to salinity. *J. Plant Physiol.*, 11: 419- 430.

Downton, W.J.S. 1982. Growth and osmotic relations of the mangrove *Avicennia marina,* as influenced by salinity. *Aust. J. Plant Physiol.*, 9: 519-528.

Feller, I.C. 1995. Effects of nutrient enrichment on growth and herbivory of dwarf red mangrove *Rhizophora mangle. Ecol. Monog.* 65: 477-505.

Ghafoor, A. 1984. Rhizophoraceae. In *Flora of Pakistan.* (Ed): E. Nasir and S.I. Ali. University of Karachi, Karachi, Pakistan. pp. 683.

Gibson, T.S., R. Spiersc and C.J. Brady. 1994. Salt tolerance in plants. II. In vitro translation of mRNAs from salt tolerant and salt sensitive plants on wheat germ ribosomes: Responses to ions and compatible organic solutes. *Plant Cell Environ.*, 7: 579-587.

Gordon, D.M. 1993. Diurnal water relations and salt content of two contrasting mangroves growing in hypersaline soils in tropical-arid Australia. In *Towards the rational use of high salinity tolerant plants.* (Ed) H. Lieth and A. Al Masoom. The Netherlands. pp. 193-216.

Hutchings, P.A., and P. Saenger. 1987. *Ecology of mangroves.* University of Queensland and Press, St. Lucia, Australia.

Karim, J., and A. Karim. 1993. Effect of salinity on the growth of some mangrove plants in Bangladesh. In *Towards the rational use of high salinity tolerant plants.* (Ed): *H. Lieth and A. Al Masoom.* The Netherlands. pp. 187-192.

Lugo, A.E., M.M. Brinson and S.Brown. 1989. Synthesis and search of paradigms in wetland ecology. In *Forest Wetlands*: Ecosystems of the world. (Ed): A.E. Lugo, M. Brinson, and S. Brown. Elsevier, Amsterdam, The Netherlands. pp. 447-460.

Luttge, U. 1997. *Physiological Ecology of Tropical Plants.* Springer-Verlag, Berlin Heidelberg, Germany.

Naidoo, G. 1985. Responses of the mangrove *Rhizophora mucronata* to high salinities and low osmotic potentials. *S. Afr. J. Bot.*, 52: 124-128.

Ogrady, A.P., K.A. Mcguiness and D. Eamus. 1996. The abundance and growth of *Avicennia marina* and *Rhizophora stylosa* in the low shore zone of Darwin Harbour, Northern Territory. *Aust. J. Ecol.*, 21: 272-279.

Popp, M., and J. Polania. 1989. Compatible solutes in different organs of mangrove trees. *Ann. Soc. For.*, 46: 842 - 844.

Popp, M. 1983. Chemical composition of Australian mangroves. I. Inorganic ions and organic acids. *Zeit. fur Pflanzenphysiol.*, 113: 395-409.

Popp, M., F. Larher and P. Weigel. 1984. Chemical composition of Australian mangroves. III. Free amino acids, total methylated onium compounds and total nitrogen. *Zeit. fur Pflanzenphysiol.*, 114: 15-25.

Rada, F., G. Goldstein, G. Orozcoc, M. Montilla, M. Zabala and A. Azocar. 1989. Osmotic and turgor relations of three mangrove ecosystem species. *Aust. J. Plant Physiol.*, 16: 477-486.

Saifullah, S.M. 1982. Mangrove ecosystem of Pakistan. *The third research report on mangroves in middle west, Japan Corporation Institute, Center for Middle East.* Tokyo, Japan.

Scholander, P.F., H.T. Hammel, E. Hemmingson, and W. Garey. 1962. Salt balance in mangroves. *Plant Physiol.*, 37: 722-729.

SPSS Inc. 1999. SPSS: SPSS 9.0 for Windows 98. SPSS Inc. USA.

Smith, S.M., Y.Y. Yang, , Y. Kamiya and S.C. Snedaker. 1996. Effect of environment and gibberellins on the early growth and development of the red mangrove, *Rhizophora mangle* L. *Plant Growth Regul.*, 20: 215-223.

Stewart, G.R., and M. Popp. 1987. The ecophysiology of mangroves. In *Plant life in aquatic and amphibious habitats.* (Ed): R.M, Crawford. Blackwell, Oxford. pp. 333-345.

Stewart, G.R., and J.A. Lee. 1974. The role of proline accumulation in halophytes. *Planta.*,120: 279-289.

Storey, R., and R.G. Wyn Jones. 1975. Betaine and choline levels in plants and their relationship to NaCl stress. *Plant Sci. Lett.*, 4: 161-168.

Tomlinson, P.B. *The Botany of Mangroves.* Cambridge University Press. London.

Werner, B.H., and D. J. Von Villert. 1995. Dynamic changes in bulk water relations during stomatal oscillations in mangrove species. Continuous analysis using a dew point hygrometer. *Physiol. Plant.*, 94: 479-485.

Werner, A., and R. Stelzer. 1990. Physiological responses of the mangrove *Rhizophora mangle* grown in the absence and presence of NaCl. *Plant Cell Environ.*, 13: 243-255.