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STUDIES ON SALT TOLERANCE IN *AVICENNIA MARINA* (FORSTK.) VIERH.: EFFECT OF NaCl SALINITY ON GROWTH, ION ACCUMULATION AND ENZYME ACTIVITY

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SUMMARY

The possible effects of salinity (0.0, 0.17, 0.34, 0.51, 0.85 M NaCl) on growth, ion accumulation and enzyme activity in *Avicennia marina* (Forstk.) Vierh. were investigated. Increase in salinity stimulated production of fresh mass. However, dry mass did not show significant enhancement. Optimum seedling growth (fresh and dry mass) was observed at 0.34 M NaCl. Water contributed to a large proportion of increase in fresh mass. Accumulation of Na^+ and Cl^- contributed substantially to the osmotic adjustment. Salinity decreased the concentration of K^+ in shoot. Na^+/K^+ ratio increased steadily with salinity. The peroxidase and acid phosphatase activities were stimulated by salinity, while superoxide dismutase and ATPase showed salt sensitivity.

Key words: **ATPase, *Avicennia marina*, ion accumulation, peroxidase, phosphatase salinity, SOD.**

INTRODUCTION

Mangroves are taxonomically diverse association of woody trees and shrubs that forms the dominant vegetation in tidal saline wet lands along the tropical and subtropical coasts and exhibit a broad spectrum of growth responses to salinity (Thomlinson, 1986). *Avicennia marina,* the grey mangrove has been exploited as a forage supplement in the coastal region of Gujarat and shows growth stimulation with moderate levels of added salts. However, differences in growth optima were reported by various investigators.

It has been shown that salinity does not modulate the properties of a number of enzymes (Krishnamurthy and Bhagwat, 1994-95) and there is no specific differences between glycophytes and halophytes (Greenway and Osmond, 1972). The high cytoplasmic sodium and chloride concentration and lower osmotic potential may affect the structure and function of enzyme proteins (Flowers *et al.*, 1977). When plants are subjected to environmental stress, the balance between the production of reactive oxygen species and the quenching activity of antioxidants is upset often resulting in oxidative damage. Plants with high levels of antioxidants have been reported a greater resistance to oxidative damage (Hernandez *et al.*, 1994). Relatively, little attention has been paid to salt tolerance of cytoplasmic enzymes in *A. marina* (Kylin and Gee, 1970). In the present experiment an attempt was made to study the growth responses, ion accumulation and changes in activity of enzymes in response to added NaCl in *A. marina.*

MATERIALS AND METHODS

Propagules of the grey mangrove *A. marina* (Forstk.) Vierh. were collected from the natural shrubs growing along inland saline marshes of Gulf of Cambay (21° C 45'

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N 72° 14' E). Uniform size propagules were raised on sand beds irrigated with 10% seawater of electrical conductivity (EC) approx. 5.5 dS/m and allowed to grow under natural photoperiod (day/night air temperatures 30/20°C) and the relative humidity ranged from 45 to 85%. Six week old seedlings of similar size were transferred to individual containers (polythene bags 10x30 cm) filled with acid washed river bed sand. After acclimation for two weeks, NaCl was added to the nutrient solution to give final concentrations of 0.0, 0.17. 0.34. 0.51 and 0.85 M NaCl of electrical conductivities (EC) 0.0, 16.0, 32.8, 46.7 and 62.7 dS/m. Salinity was imposed by stepwise increments of 0.1 M per day. Plants were irrigated alternatively with nutrient solution (Ball *et al.*, 1987) containing NaCl and without NaCl so as to keep soil salinity nearly constant. Control plants were irrigated with nutrient solution without added NaCl. Plants were gown under neutral photoperiod as described above. Plants were harvested after a period of 6 weeks and rinsed with distilled water to remove any salts and bottled dry. Plant parts were separated into leaves, stem and root and analyzed for growth (fresh and dry mass), various enzyme activities and ion accumulation. Three replicates were kept for each treatment.

Growth was determined as fresh and dry mass. Dry weight was determined after drying the plant parts at 70°C for 48 hours to a constant weight. Water content was determined as g of water per g dry weight of the tissue . Ions were extracted from dry material by the method of Wignarajah *et al.*, (1975b). Na^+, K^+ and Ca^{2+} contents were measured flame photometrically (Chemito 1020, India). Chloride was determined by titration against silver nitrate using potassium chromate as indicator (Clesari *et al.*, 1989).

Known weight (0.5g) of plant material was thoroughly ground in a cold mortar with a pestle in an ice bath (0-4°C), until no fibrous residue could be seen. The grinding medium (4-6 ml/g fresh weight) consisted of 100 mM phosphate buffer (pH 7.8) and 0.1 mM EDTA, 1%(w/v) polyvinylpyrrolidone and homogenizing glass beads. The homogenate was centrifuged, twice at 13,000g for 10 minutes at 0-4°C in a refrigerated centrifuge (Beckman-Avanti U.S.A). The supernatants were pooled and made to a final volume (10 ml) and referred as enzyme extract for activity assays. An aliquot of the extract was used for protein determination (Lowry *et al.*, 1951).

Peroxidase activity was measured according to the method of Shannon *et al.*, (1966). The amount of enzyme required to change the absorbance by 0.001 min^{-1} mg^{-1} protein was taken as unit enzyme activity. The activity of superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) using the method of Beauchamp and Fridovich (1971). Specific activity was expressed as units/mg protein. For the assay of acid phosphatase the procedure of Yamaya and Matsumato (1981) was adopted. The method of Kim and Weber (1980) was used for ATPase assay. All the data were subjected to one way analysis of variance and significance were determined at 5% level.

RESULTS AND DISCUSSION

In general *A. marina* gave better growth in the presence of NaCl salinity. Considerable enhancement in fresh mass was noticed with increase in salinity, however, dry mass accumulation did not show significant variation except at 0.34 M NaCl when compared to control. At 0.34 M NaCl growth was maximal, total fresh and dry mass being greater than the control values by 135% and 110% respectively. Salinity effect on growth was not uniform in all plant parts. In leaf and shoot both fresh and dry mass increased with increase in salinity. Whereas in root maximum fresh and dry weight was observed in control. Trends in plant water content (g water/g dry weight) paralleled those of fresh mass accumulation being lowest in control and increased with salinity (Table I). Growth stimulation at low to moderate external salinity has been reported for many halophytes (Flowers *et al.*, 1977, Reddy *et al.*, 1992, Al-Zahrani and Hajar, 1998). The observed growth response of *A. marina* was more or less consistent with the natural distribution of the species, where salinity exceeds 0.34 M NaCl. The increase in

TABLE I. Effect of NaCl on growth of *A. marina* plants were harvested after 6 weeks of growth in final salinity. Values mean ± S.D. of three independent determination of 5 plants.

Molarity	Fresh weight (g)				Dry weight (g)				Water Content
NaCl	Leaf	Shoot	Root	Whole plant	Leaf	Shoot	Root	Whole plant	($g.H_2O/g.DW$)
0.00	7.26 ± 0.58	5.95 ± 0.71	6.74 ± 0.10	19.88 ± 1.08	1.72 ± 0.27	1.52 ± 0.16	2.36 ± 0.25	5.63 ± 0.51	2.53
0.17	9.23 ± 0.79	6.53 ± 0.33	5.02 ± 0.02	20.75 ± 0.50	2.02 ± 0.27	1.57 ± 0.14	1.13 ± 0.02	4.75 ± 0.35	3.36
0.34	13.42 ± 0.67	7.84 ± 0.39	5.66 ± 0.18	26.94*±0.45	2.85 ± 0.37	1.86 ± 0.19	1.47 ± 0.13	6.20*±0.26	3.34
0.51	11.26 ± 0.45	7.94 ± 0.85	4.66 ± 0.21	23.60*±1.45	2.41 ± 0.12	1.82 ± 0.19	1.26 ± 0.05	5.51*±0.30	3.28
0.85	9.52 ± 1.19	7.35 ± 0.42	4.32 ± 0.28	21.22*±1.13	2.03±0.22	1.57±0.26	0.92 ± 0.15	4.53*±0.43	3.68

* Values significant at P = 0.05 level

fresh weight especially in leaf and shoot was mainly due to an increase in tissue water content as reflected by the change in fresh weight to dry weight ratio.

The result presented (Table II) shows that increase in NaCl concentration steadily increased Na^+ and Cl^- in all plants parts and the accumulation was significantly higher in leaf than in shoot or root. At 0.34 M NaCl, the concentration of Na^+ in leaf, shoot and root was 22.60, 14.28 and 26.5 g/kg dry weight, respectively as compared to 13.95, 9.41 and 13.79 g/Kg dry weight in the non saline treatment. Similarly Cl^- concentration increased significantly with external salinity in all plant parts (Table II). Massive ion accumulation is adaptive significance in most halophytes (Naidoo and Rughunanan, 1990). The salinity induced decrease in K^+ concentration

TABLE II. Effect of NaCl on ion content (g/Kg DW) in different plant parts of *A. marina*. Where, L. Leaf, S. Shoot and R. Root.

Molarity NaCl	Plant part	Na^+	K^+	Ca^{2+}	Cl^-	Na/K
0.00	L	13.95 ± 0.81	4.19 ± 0.18	14.16 ± 0.19	12.92 ± 1.07	3.32
	S	9.41 ± 0.41	3.79 ± 0.19	12.29 ± 0.38	5.75 ± 1.42	2.48
	R	13.79 ± 0.38	4.15 ± 0.19	20.70 ± 1.25	10.33 ± 1.43	3.32
0.17	L	14.41 ± 0.19	152 ± 0.03	19.54 ± 0.14	17.10 ± 1.55	9.48
	S	10.50 ± 0.37	1.78 ± 0.14	16.75 ± 0.57	13.95 ± 0.92	5.89
	R	13.12 ± 0.37	3.49 ± 0.12	13.79 ± 0.62	16.07 ± 1.35	5.89
0.34	L	22.60 ± 0.64	2.87 ± 0.12	11.41 ± 0.68	23.45 ± 0.84	7.87
	S	14.29 ± 0.38	2.41 ± 0.14	10.70 ± 0.19	14.99 ± 0.57	5.92
	R	26.5 ± 3.05	4.45 ± 0.26	28.25 ± 2.68	17.52 ± 0.56	5.95
0.51	L	21.54 ± 0.47	4.04 ± 0.31	18.50 ± 0.33	19.83 ± 0.57	5.33
	S	19.25 ± 0.33	3.50 ± 0.25	15.08 ± 0.64	12.68 ± 0.42	5.50
	R	20.29 ± 0.31	4.04 ± 0.31	16.41 ± 0.50	17.99 ± 0.35	5.02
0.85	L	23.99 ± 0.37	4.12 ± 0.33	14.37 ± 0.12	21.33 ± 0.58	5.82
	S	17.87 ± 0.89	3.04 ± 0.26	15.54 ± 0.38	14.65 ± 0.51	5.87
	R	20.16 ± 0.43	3.75 ± 0.25	11.83 ± 0.68	18.70 ± 0.42	5.37

observed in *A. marina* is in agreement with the results of Naidoo and Rughunanan (1990) and Al-Zahrani and Hajar (1998). In parallel with the Na^+ accumulation and decline in K^+ content, the Na^+/K^+ ratio increased at all levels of external salinity (Table II). The increase in Ca_2^+ ion concentration noticed in leaf and shoot might help in ameliorating to some extent, the inhibitory effect of NaCl concentration on nutrient transport and in turn may enable the plant to cope with high external salinity. Calcium is reported to reduce the toxic effect of NaCl salinity (Epstein, 1998).

The peroxidase activity increased under salt stress in *A. marina* root, whereas, in shoot the activity increased at 0.34 and 0.51 M NaCl, while decreased at 0.17 and 0.85 M NaCl as compared to control. In leaf no significant differences were noted with salinity (Fig. 1). NaCl was found inhibitory for superoxide dismutase activity in shoot and root. But in leaf the activity increased except at 0.17 M over control. In shoot and root the total activity decreased with increase in salinity. The increase in enzyme activity with external salinity may be due to increased synthesis of enzyme. The increased peroxidase and superoxide dismutase activity observed in the present study might be useful for adaptation under conditions requiring prevention of peroxidation of membrane lipids (Kalir and Poljakoff-Mayber, 1981).

Salinity had an inhibitory effect on ATPase activity in *A. marina*. The activity declined with increase in salinity. However, in leaf and shoot, the activity showed a slight increase at 0.51 and 0.85 M NaCl (Fig. 1). Kylin and Gee (1970) reported inhibition of ATPase activity in *Avicennia nitida* at higher levels of salinity. Salinity caused marked increase in acid phosphatse activity in *A. marina*. The stimulation of acid phosphatase activity observed in *A. marina* of the present study may play a vital role in osmoregulation. Our study reveals that the massive accumulation of inorganic ions and differential activity of enzymes may probably provide sufficient solutes for osmoregulation and could act as possible indicators for salt tolerance of *A. marina*.

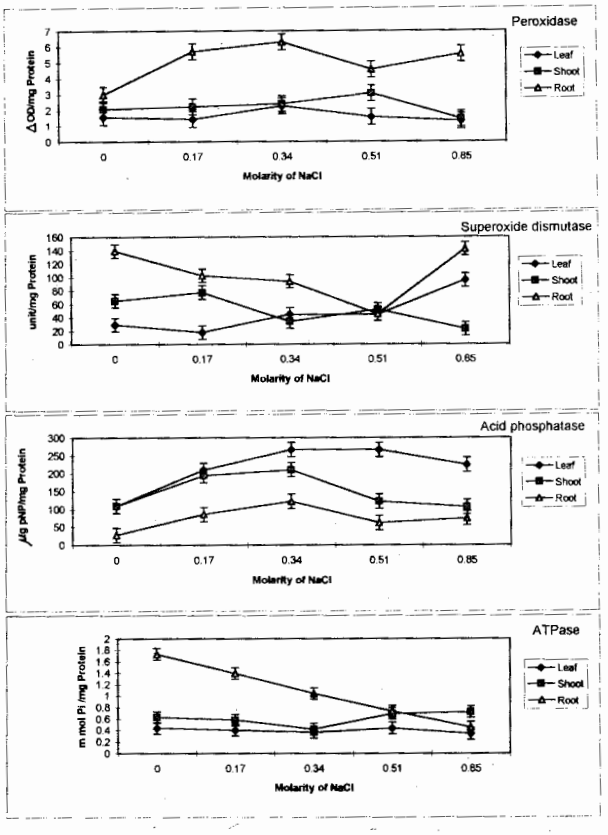

Fig. 1. Effect of NaCl on different enzyme activities of *Avicennia marina*.

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