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BRIEF COMMUNICATION

Responses of *Ceriops roxburghiana* to NaCl stress

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Abstract

Responses of *Ceriops roxburghiana* Arn. leaves to the sodium chloride, applied at different concentrations (ranging from 100 to 600 mM), has been evaluated. Total amino acid content decreased with increasing NaCl concentration, while the protein content increased significantly up to 400 mM concentration and decreased thereafter. Total sugar content decreased at concentrations beyond 400 mM. Proline and glycine betaine were accumulated with increasing NaCl concentration. Protease and ATPase activities were increased whereas proline oxidase activity were decreased with increasing salinity. Peroxidase and malate dehydrogenase (NADH-MDH) activities did not significantly differ under various NaCl concentrations.

Additional key words: ATPase, glycine betaine, malate dehydrogenase, peroxidase, proline, proline oxidase, protease, salt stress.

Mangroves on the coastal region represent a fragile ecosystem. The salt tolerance due to osmotic adaptations of mangroves to the low and the variable water potential of their environment have long been a matter of interest. Osmotic adjustment involves both uptake and accumulation of ions and synthesis of low molecular mass organic solutes. The aim of present study, was to determine the organic constituents formed in the leaves and activities of some key enzymes in the seedlings of mangrove *Ceriops roxburghiana* Arn. under NaCl stress.

Seedlings of *Ceriops roxburghiana* Arn. of uniform size with a pair of leaves were collected from the Pichavaram mangrove forest on the northeast coast of India (11° 24' N and 79° 44' E). Seedlings were established in polythene sleeves in eight plots consisting of 30 seedlings per plot under sunlight [14-h photoperiod, maximum irradiance of 1 600 μmol (photon) m^{-2} s^{-1}], temperature 28 + 2 °C and treated with NaCl at varying concentrations ranging from 100 to 1000 mM besides an untreated control. The seedlings treated with NaCl above 600 mM did not survive.

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Leaf samples were collected randomly on 30th and 60th day after salt treatment, washed thoroughly with tap water followed by distilled water. The content of free amino acids was estimated according to Moore and Stein (1948), the content of protein according to Lowry *et al.* (1951), proline according to Bates *et al.* (1973), total phenols according to Bray and Thorpe (1954), and sugars according to Nelson (1944). Samples were oven-dried at 80 °C and then ground for the estimation of glycine betaine (Storey and Wyn Jones 1977). Enzymes activities were measured spectrophotometrically in a homogenate of the leaves, which was prepared by grinding approximately 500 mg of tissue in 10 cm^3 of a solution containing sucrose (400 mM) and Tris-HCl buffer (20 mM Tris, pH 7.0), centrifuged at 10 000 *g* for 1 h and then dialysed for 22 h against Tris-HCl (5 mM, pH 7.0). Peroxidase (EC 1.11.1.7) was assayed using *p*-phenyline diamine, the ATPase (EC 3.6.1.3) was assayed by determining the release of Pi from ATP (Flowers 1972). Protease (EC 3.4.2.2) was assayed by the measurement of releasing α-NH_2 due to hydrolysis of reserve protein at 570 nm (Prisco *et al.* 1975), while proline oxidase (EC 1.4.3) was assayed at 600 nm by following the reduction of DCPIP at room temperature (Huang and Cavalieri 1979). Malate dehydrogenase (NADH-MDH; EC 1.1.1.37) was assayed by following the oxidation of NADH at 340 nm at 27 °C (Flowers 1972).

A number of low molecular mass organic compounds (proline, glycine betaine and other quaternary ammonium compounds) called compatible solutes (Popp and Albert 1996). The type of compound stored by a certain species may have some consequence for its nitrogen and carbon economy as well as for its ecological distribution. The proline content progressively increased with increasing concentration of NaCl (Table 1). The high accumulation of proline with increasing salinity is associated with stimulation of proline synthesis from glutamate (Boggers *et al.* 1976) and inhibition of proline oxidation. In our study (Table 2), the increased proline content was well correlated with the decreased proline oxidase activity. Similar results were observed in *Salicornia brachiata* (Reddy *et al.* 1993), *Aegialitis annulata* and *Xylocarpus granatum* (Popp and Albert 1996).

In this present investigation, also glycine betaine accumulated significantly with increasing NaCl concentrations (Table 1). The content of amino acids progressively decreased with the increasing concentrations of NaCl (Table 1). The decrease in amino acids content up to 400 mM may be due to the active synthesis of proteins as a significant increase in total protein content was noticed up to 400 mM NaCl, beyond that concentration the protein content declined slightly (Table 1). The decreased protein content at higher concentrations of NaCl might be due to enhancement of the protease activity (Table 2). These results are comparable with the earlier studies in *Sesuvium portulacastrum* (Venkatesalu *et al.* 1994), *Salicornia brachiata* (Reddy *et al.* 1993) and *Phalaris arundinacea* (Maeda *et al.* 1995).

The accumulation of polyphenols increased with the increasing NaCl concentration upto 400 mM and beyond that concentration less phenol content was observed (Table 1). Polyphenol synthesis in mangroves is mainly controlled by the activity of polyphenol degrading enzymes and also partially by the age of the plants (Jamale and Joshi 1977). Similar results of increased polyphenols in natural saline

Table 1. Effect of NaCl stress on content of proteins, amino acids, total phenols, sugars, proline [mg g^{-1}(d.m.)], and glycine betaine [μmol g^{-1}(d.m.)] in the leaves of *Ceriops roxburghiana* on the 30th and 60th day after treatment (means ± SE, $n = 5$).

NaCl [mM]	Treatment [d]	Protein	Amino acids	Phenols	Sugars	Proline	Glycine betaine
Control	30	28.11 ± 0.10	16.54 ± 0.08	7.36 ± 0.17	16.17 ± 0.15	4.61 ± 0.18	53.28 ± 0.21
	60	28.52 ± 0.11	16.94 ± 0.12	7.55 ± 0.03	16.73 ± 0.06	4.85 ± 0.10	52.92 ± 0.23
100	30	28.52 ± 0.15	15.84 ± 0.20	8.00 ± 0.09	18.15 ± 0.05	5.57 ± 0.09	61.08 ± 0.31
	60	28.73 ± 0.23	16.25 ± 0.18	8.41 ± 0.13	18.46 ± 0.02	5.89 ± 0.20	63.58 ± 0.25
200	30	28.45 ± 0.09	14.42 ± 0.18	8.64 ± 0.27	19.21 ± 0.15	6.18 ± 0.28	68.54 ± 0.11
	60	27.49 ± 0.13	15.22 ± 0.22	8.88 ± 0.08	19.47 ± 0.13	6.44 ± 0.31	70.33 ± 0.03
300	30	27.43 ± 0.12	13.59 ± 0.19	8.88 ± 0.12	20.08 ± 0.13	6.66 ± 0.22	74.23 ± 0.15
	60	26.95 ± 0.20	14.55 ± 0.09	9.00 ± 0.04	20.63 ± 0.07	6.92 ± 0.18	76.36 ± 0.02
400	30	25.46 ± 0.08	12.94 ± 0.23	9.41 ± 0.25	22.60 ± 0.27	7.11 ± 0.08	78.50 ± 0.02
	60	26.34 ± 0.18	13.55 ± 0.14	9.58 ± 0.23	23.12 ± 0.06	7.14 ± 0.24	81.69 ± 0.09
500	30	24.53 ± 0.11	18.31 ± 0.23	8.42 ± 0.07	20.69 ± 0.19	7.82 ± 0.08	90.92 ± 0.09
	60	25.24 ± 0.18	19.00 ± 0.03	8.58 ± 0.11	20.91 ± 0.17	8.10 ± 0.11	93.41 ± 0.19
600	30	23.43 ± 0.21	18.96 ± 0.17	7.88 ± 0.03	19.77 ± 0.05	8.30 ± 0.27	104.43 ± 0.19
	60	24.63 ± 0.31	19.61 ± 0.16	8.03 ± 0.08	19.95 ± 0.18	8.70 ± 0.13	108.20 ± 0.14
CD ($P = 0.05$)		0.375	0.074	0.026	0.050	0.054	0.617

Table 2. Effect of NaCl stress on enzyme activities [μmol g^{-1}(protein) s^{-1}] in the leaves of *Ceriops roxburghiana* on the 30th and 60th day after treatment (means ± SE. $n = 5$).

NaCl [mM]	Treatment [d]	Protease	Proline oxidase	ATPase	Peroxidase	Malate dehydrogenase
Control	30	46.6 ± 0.01	0.93 ± 0.13	3.10 ± 0.05	34.3 ± 0.15	0.16 ± 0.06
	60	47.1 ± 0.08	1.00 ± 0.04	3.33 ± 0.02	35.0 ± 0.09	0.18 ± 0.02
100	30	44.6 ± 0.06	0.61 ± 0.11	3.30 ± 0.14	34.1 ± 0.07	0.20 ± 0.02
	60	45.3 ± 0.03	0.68 ± 0.05	3.00 ± 0.01	35.5 ± 0.03	0.20 ± 0.01
200	30	43.3 ± 0.11	0.56 ± 0.10	4.60 ± 0.07	34.5 ± 0.11	0.21 ± 0.04
	60	43.5 ± 0.08	0.65 ± 0.12	4.80 ± 0.04	35.3 ± 0.08	0.23 ± 0.01
300	30	42.8 ± 0.05	0.51 ± 0.03	5.30 ± 0.12	34.8 ± 0.07	0.28 ± 0.03
	60	43.3 ± 0.09	0.50 ± 0.07	5.30 ± 0.08	35.1 ± 0.01	0.28 ± 0.07
400	30	38.6 ± 0.02	0.50 ± 0.07	7.30 ± 0.06	34.5 ± 0.05	0.26 ± 0.01
	60	40.5 ± 0.05	0.48 ± 0.12	7.80 ± 0.03	35.8 ± 0.04	0.28 ± 0.03
500	30	50.6 ± 0.04	0.45 ± 0.02	6.60 ± 0.05	34.6 ± 0.12	0.28 ± 0.05
	60	51.3 ± 0.07	0.41 ± 0.07	6.80 ± 0.03	35.3 ± 0.09	0.26 ± 0.02
600	30	55.6 ± 0.13	0.40 ± 0.03	6.00 ± 0.05	35.0 ± 0.12	0.25 ± 0.01
	60	56.6 ± 0.08	0.33 ± 0.07	6.30 ± 0.03	34.8 ± 0.09	0.21 ± 0.03
CD ($P = 0.05$)		0.092	0.0014	0.011	0.0013	0.0010

fields were obtained in *Sonneratia alba*, *Rhizophora mucronata* and *Avicennia alba* (Jamale and Joshi 1977).

Carbohydrate storage is one of the three mechanisms proposed by Ackerson and Younger (1975) for salinity tolerance of plants. The results of the present study showed an increased sugar content at 400 mM NaCl, beyond that the sugar content declined (Table 1). Increased content of sugars was observed at low salinity in *Salicornia brachiata* (Reddy *et al.* 1993), *Suaeda fruticosa*, *Sesuvium sesuviodes* and *Cressa cretica* (Mohammed and Sen 1994).

High salinity induces a slight increase in ATPase activity (Table 2). The energy produced by the hydrolysis of ATP is very much required for water absorption, selective ion uptake and to maintain the metabolism in the NaCl-stressed plants. There are previous reports on salt stimulated ATPase activity in *Avicennia nitida* (Kylin and Gee 1970) and *Suaeda maritima* (Flowers 1972). Peroxidase activity did not differ much at different concentrations of NaCl (Table 2) which is important to protect the cells from accumulation of toxic oxygen. NaCl stress did not alter malate dehydrogenase activity significantly and moreover the activity was very less in *Ceriops roxburghiana* leaves. There are previous reports of inhibition of malate dehydrogenase activity at higher concentrations of NaCl (0.33 M) in *Suaeda maritima*, *Halimione portulacoides* and *Salicornia ramosissima* (Flowers 1972).

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