



**SALT TOLERANCE IN SEEDLINGS OF THE MANGROVE *CYNOMETRA IRIPA*
KOSTEL**

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ABSTRACT: Present paper describes the growth and ion contents from seedlings of the mangrove *Cynometra iripa* Kostel. over a range of salinity, using NaCl and Na₂SO₄ as the major ion in a soil culture. The seedlings of *C. iripa* grew well at salinity levels up to 0.1M NaCl and 0.1M Na₂SO₄. Growth was inhibited at salinity levels above 0.2M NaCl and 0.2M Na₂SO₄. Chloride decreased in Na₂SO₄ treated seedlings. Proline accumulation was found to be increased with salinity in both treatments. Higher activities of catalase, peroxidase and polyphenol oxidase were noticed in seedlings. Effect of Cl salinity is more prominent than that of SO₄ salinity. Chlorophylls, carotenoids, TAN, polyphenol and free amino acids contents are decreased after critical concentration 0.1M NaCl and Na₂SO₄.

Key words: *Cynometra iripa*, mangrove, salinity, NaCl, Na₂SO₄.

INTRODUCTION

The mangrove *Cynometra iripa* kostel., a member of Fabaceae, is critically endangered species along west coast of Maharashtra. Only taxonomic account, ecological distribution & germination aspects have been studied by Gokhale (2004). Bhosale *et al.* (2002) have reported the species both in Sindhudurg & Ratnagiri districts of Maharashtra. Antibacterial activity & phytochemical screening from different parts of *Cynometra iripa* are carried out by Desai & Chavan (2010). This plant grows on estuary, where the salinity of the water at high tide is similar to that of sea water. The growth of this plant & the regulation of salt accumulation in its tissues responds to increasing salinity in sediment is not well understood. *C. iripa* is a facultative mangrove and can also grow in soils irrigated with fresh water. Present investigation deals with responses of growth & ion content of *C. iripa* seedlings exposed to a range of NaCl & Na₂SO₄ salinities.

A saline environment is required for stable mangrove ecosystems, as many species are less competitive under non-saline conditions (Lugo, 1980). Mangroves are considered to be facultative halophytes, i.e. they can often survive through not necessarily thrive in non-saline habitats (Cintron, G. and Schaeffer-Novelli, Y., 1983a; Walsh, G. B., 1974). It has often been reported that the growth of many halophytes is depressed without sodium chloride in the external environment (Flowers *et al.*, 1977; Greenway & Munns, 1980). The effect due to salinity & the resulting strongly negative osmotic pressure of soil water is a progressive stunting of the mangrove canopy inland from the water's edge. This can be almost universally recognized and takes place regardless of species composition.

Plants are equipped with diverse anti-oxidant substrates & enzymes that protect them against oxidative damage (Elstner, 1987) & their role in preventing salt induced oxidative damage in halophytes has been reported (Parida *et al.*, 2004; M'rah *et al.*, 2007). Many of the physiological adaptations of plants to saline conditions are similar to the adaptations shown by plants to desiccation stress & it has been suggested that plants showing drought resistance would also exhibit salinity tolerance (Munns, 2002).

MATERIAL AND METHODS

Plant material : mature fruits of *Cynometra iripa* were collected from Achara estuary, west coast of Maharashtra.

Method of plant culture: These fruits were washed under running tap water, sown in polythene bags, containing garden soil & farm yard manure, & irrigated with fresh water. After well establishment of these plants, seedlings of equal size are transferred into the pots, containing equal amount of garden soil & each with one seedling. After stabilization and establishment of seedlings for one month, replicates were treated with different concentrations of NaCl & Na₂SO₄ ranging from 0.025M to 0.3M on every alternate day. Control plants were maintained by giving tap water. After 25 treatments the further experiments were carried out.

Methods of analysis : Mature leaves of the same age from the identical position of the control plant and plants subjected to treatments were selected for the measurement of physical parameters like thickness, mass & for the calculation of density. Same precautions were taken while sampling the material for the study of ion uptake like Na, K, Ca & Cl & effect of salts on chlorophyll, carotenoid, polyphenols, TAN, proline, free amino acid content, osmotic potential & enzymes.

In order to keep uniformity of sampling all the samples were taken at 10 a.m. For the estimation of Na, K, Ca & Cl acid digestion method was followed Toth *et al.*, (1948). Oven dried leaves were used. Chlorids were estimated by the method given by Chapman & Pratt (1961). The method described by Arnon (1949) was followed for chlorophyll estimation while Titratable acid number (TAN) was determined by the method of Thomas & Beevers (1949). Free amino acid contents were estimated by the method of Moore & Stein (1948). Carotenoids was estimated by Kirk & Allen, (1965). The method of Folin & Denis (1915) was employed for determination of the total polyphenol contents. Proline was estimated following the method by Bates *et al.*, (1973). Osmotic potential was determined by the method described by Janardhan & Krishnamoorthy (1975). Enzyme catalase was assayed by following method of Luck (1974) as described by Sadasivam and Manikam (1992). Activity of enzyme polyphenol oxidase was studied according to the method of Mahadevan and Sridhar (1982). To study the enzyme peroxidase activity the method of Machly (1954) was followed. Samples from each treatment were analysed for all the above mentioned parameters.

RESULTS AND DISCUSSION

Effect of different concentration of NaCl & Na₂SO₄ treatments on physical properties of leaf are recorded in Table 1. It shows effect of varying concentration of NaCl & Na₂SO₄ on *C. iripa*. It is seen from the results that the effect of NaCl & Na₂SO₄ on physical structure of leaves is more pronounced. In *C. iripa* both the salinities induce succulence but effect of Cl salinity is more prominent than that of SO₄ salinity. It is evident from the studies that NaCl salinity has considerable effect on degree of succulence. The observations also suggest that water content of the leaf thickness, mass & volume of leaf increases along with the increasing concentration of NaCl. According to Tullin (1954) succulence is due to expansion of the cell wall, thus leading to increasing size of cells. NaCl was found to be most effective salt in promoting succulence. Van Eijk (1939) observed the effect of NaCl, CaCl₂, MgCl₂, NaNO₃, Na₂SO₄ & varies salt mixtures on succulence. Growth was significantly inhibited at a salinity of 0.2M NaCl & 0.2M Na₂SO₄ and above. Plants grown at a salinity lower than 0.2M NaCl & 0.2M Na₂SO₄ were insignificantly different in leaflet thickness, leaf area, leaf dry weight, but the mean values of these parameters remained higher in 0.1M NaCl & 0.1M Na₂SO₄ treatments than in the control and 0.2M NaCl & Na₂SO₄ treatments. It means sodium salt at 0.1M concentration can improve the growth of *C. iripa*. On the other hand, plants grown at 0.2M NaCl & 0.2M Na₂SO₄ and above have lower values for the above ground tissues. The results indicate that these salinities may possibly be stressful for *Cynometra iripa*.

Table 1. Effect of varying concentration of NaCl and Na₂SO₄ on physical properties of the leaves of *Cynometra iripa* Kostel.

Salt concentration (M)	Moisture (%)	Dry matter (%)	Mass of leaflet 'M' (g)	Area of leaflet 'A' (sq.cm.)	Leaflet thickness 'D'(mm)	Leaflet volume V=AD (cm ³)	Leaflet density M/V (g/cm ³)
Control	63.15	36.85	0.077	4.475	0.165	0.738	0.104
NaCl							
0.025	53.04	46.96	0.075	4.91	0.166	0.815	0.092
0.050	47.01	52.99	0.088	6.26	0.175	1.096	0.080
0.1	77.23	22.77	0.101	6.26	0.190	1.189	0.084
0.2	83.33	16.67	0.077	4.33	0.171	0.740	0.104
0.3	57.14	42.86	0.055	4.21	0.163	0.686	0.080
Na₂SO₄							
0.025	66.36	33.64	0.082	5.07	0.161	0.816	0.100
0.050	76.36	23.64	0.091	5.42	0.174	0.943	0.096
0.1	78.43	21.57	0.097	5.47	0.205	1.121	0.086
0.2	82.00	18.00	0.091	5.02	0.160	0.803	0.113
0.3	86.00	14.00	0.080	4.89	0.152	0.743	0.107

The distribution of Na, K, Ca & Cl in NaCl & Na₂SO₄ treated plants is given in Fig. 1. & Fig. 2. It was found that NaCl treatment caused accumulation of sodium & chloride more in *C. iripa*. Potassium content in the leaves is not affected by low concentrations of salt but its uptake is hampered by high concentrations of salts. From the major elements calcium uptake is lowered under salt stress condition.

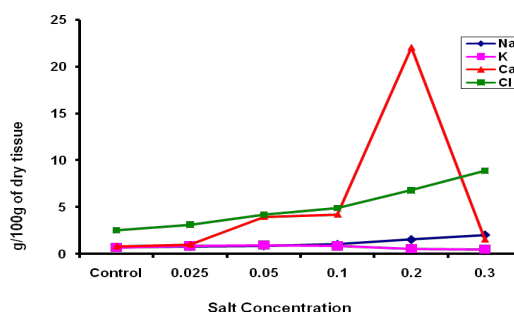


Fig.1 Effect of varying concentration of NaCl on Na, K, Ca, Cl.

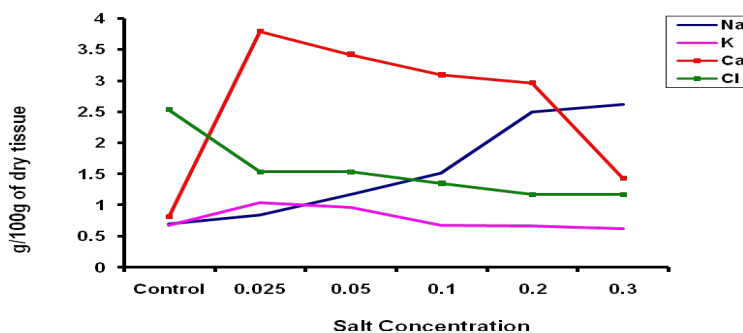


Fig.2 Effect of varying concentration of Na₂SO₄ on Na, K, Ca, Cl

It is seen from Fig.3 & Fig.4 that chlorophyll & carotenoid contents is decreased at high concentration of the NaCl in *C. iripa* plants. Na₂SO₄ stimulates the synthesis of chlorophyll even at higher concentrations. Kotmire & Bhosale (1980) have shown that NaCl decreases chloropylls in *Avicennia marina* & *A. officinalis*. The observations suggest that NaCl & Na₂SO₄ salinities influence chlorophyll metabolism differently. TAN & polyphenol of *C. iripa* is increased slightly with increasing NaCl & Na₂SO₄ concentrations. From Fig. 5, Fig. 6 and fig 7 it is seen that concentrations of cations in the plant greater than 0.1M may inhibit biochemical process such as enzyme activities (Flowers, 1972; Greenway & Osmand, 1972) and protein synthesis (Gibson et al., 1984). It has been demonstrated that most cations in the cells of halophytes are compartmentalized in the vacuole (Storey et al., 1983a; 1983b) & that organic solutes, e.g.- proline (Stewart & Lee, 1974). Proline accumulation is increased as salinity increases in both treatments. Higher activities of catalase, peroxidase & polyphenol oxidase are observed with increasing NaCl & Na₂SO₄ upto 0.1M concentrations. Free amino acids & osmotic potential is decreased at high concentrations of the NaCl & Na₂SO₄.

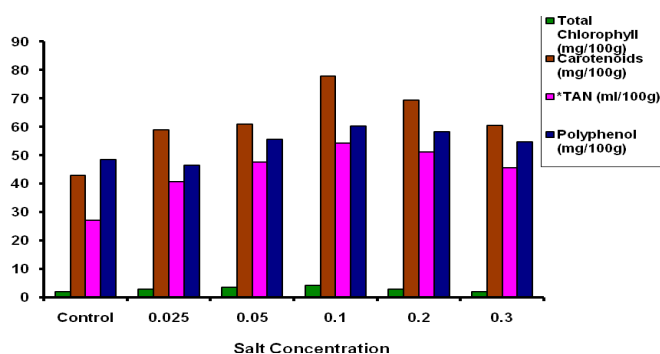


Fig.3 Effect of varying concentration of NaCl on Chlorophyll, Carotenoids, TAN and Polyphenols.

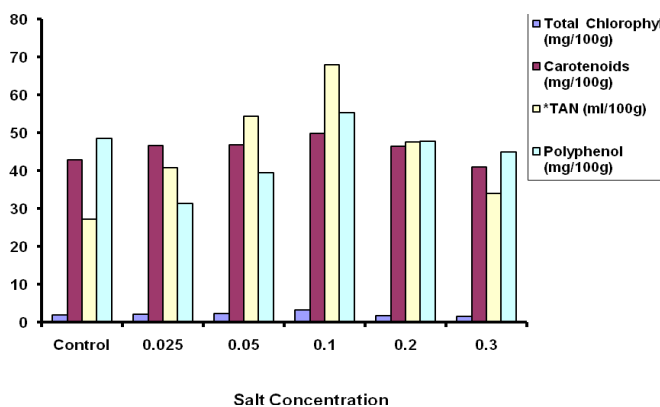


Fig.4 Effect of varying concentration of Na₂SO₄ on chlorophyll, carotenoids, TAN and Polyphenols

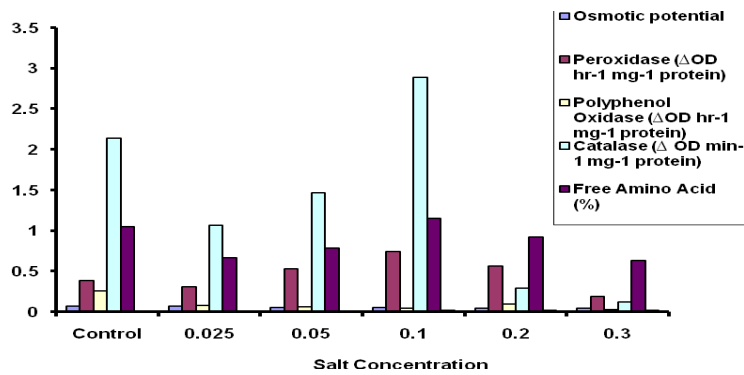


Fig. 5 Effect of varying concentration of NaCl on Osmotic potential, Enzymes, Free amino acids.

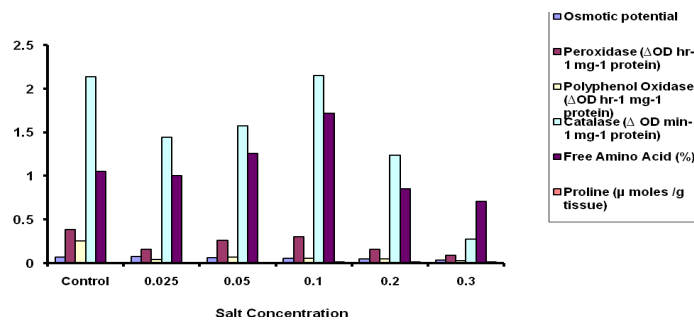


Fig.6 Effect of varying concentration of Na₂SO₄ on Osmotic potential, Enzymes, Free amino acids.

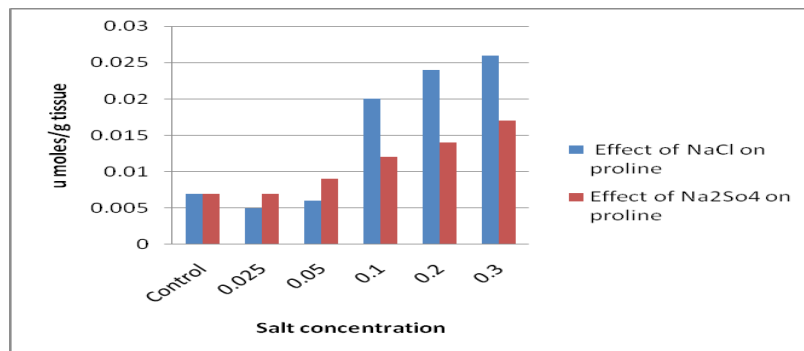


Fig.7 Effect of varying concentration of NaCl and Na₂SO₄ on proline.

Conclusion

It was observed that overall plant growth was not affected by lower doses of salts. However concentrations above 0.050M to 0.1M of NaCl and Na₂SO₄ salinities have adverse effect on plant growth. The effect of Cl salinity is more prominent than that of SO₄ salinity. Hence salt tolerance could be considered as an important adaptive strategy used by mangroves.

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