Recent Research in Science and Technology 2011, 3(1): 123-127
ISSN: 2076-5061
www.recent-science.com

BOTANY

EFFECT OF NaCl ON GROWTH, ION ACCUMULATION AND OXIDATIVE ENZYMES OF *SUAEDA NUDIFLORA* MOQ.

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Abstract

The effect of different concentration of NaCl (0-750 mM) salinity on growth, ion accumulation and enzyme activities were studied in a halophytic species of *Suaeda nudiflora*. The upper limit for the survival of this species at 750 mM NaCl and the optimum level for the better growth was 400 mM NaCl. Beyond, these concentrations the growth rate sharply declined Na^+ and Cl^- ions increased with increasing salinity upto the extreme level of NaCl, on the other hand K^+ content decreasing with increasing salinity. The Ca^{2+} content and Na^+/K^+ ratio increased steadily with salinity. The accumulation of cat ions was greater in leaves then in stem and root. NaCl treatment stimulated the activities of ATPase upto the extreme salinity level. However, catalase, peroxidase and polyphenol oxidase activities increased upto the optimal level and at higher concentrations showed decreasing trend. The changes of enzyme activities and their possible role in salt tolerance of *Suaeda nudiflora* have been studied.

Keywords: Halophyte, Ions, Oxidative enzymes, Salinity

Introduction

Salinity is a major environmental stress affecting plant growth and resulting in severe agricultural loss. The assiduous efforts made during the last few decades to increase the salt tolerance of conventional crops either through selective breeding or genetic manipulation have not yield any promising result. The idea of seawater agriculture and domestication of wild, salt tolerant plant (halophytes) for use as food, fodder and oil seed crops appears to be more feasible and economical them altering the basic physiology of traditional crops (Glenn *et al.,* 1998).

Suaeda nudiflora Moq. (Chenopodiaceae) is a succulent halophyte commonly occurring in saline marshes of the mangrove belt of Pitchavaram on the North East Coast by Tamil Nadu. India (Latitude 11º 24' N and Longitude 79º 44'E), various species of *Suaeda* has been suggested as an alternate for traditional oil seed crops and for sea water based agricultural system (Pasternak *et al*., 1985). In India, in certain areas, people use *Suaeda nudiflora* as a vegetable substituent. Though most of the halophytes of the family Chenopodiaceae show growth stimulation at moderate to higher levels of salinity (Glenn *et al.,* 1996). Relatively little alteration has yet been give to the salt tolerance mechanism of *S. nudiflora*, is sensitive to NaCl during germination and acquires salt tolerance at later stages of growth (Cherian, 1998). Therefore, to examine the effect of NaCl salinity on growth and metabolism during early stages of plant growth. The present investigation we report that the growth, ion accumulation and enzymes activities of *S. nudiflora* grown under different concentrations by NaCl salinity.

Materials and Methods

Seeds of *S. nudiflora* collected from the mangrove belt of Pitchavaram, were sown in winter (October-December, 2007) in polythene bags (9" × 7") were filled with homogenous mixture of garden soil it comprising of red earth, sand and farmyard manure in the ratio 1:2:1. The bags were placed in the garden and irrigated with distilled water until the first two pairs of leaves were developed (30 days). The seedlings were thinned at this stage and further grown for a period of 30 days with added nutrient solution (Hewitt, 1952). 60 days old plants were allocated at random to different salinity treatments. Salinity was imposed by stepwise increments by adding NaCl to the nutrient solution to final concentrations of 200, 400 and 750 mM. Control plants received only nutrient solution plants were irrigated alternatively with nutrient solution containing NaCl and without salt so as to keep soil salinity nearly constant and grown at natural photoperiod. Three replicates were kept for each treatment plants were harvested after a period of 30 days in final salinity and rinsed with deionized water and blotted dry. The plant parts were separated into leaf, stem and root were analyzed for growth, ion accumulation and enzyme activities by standard procedure.

Growth was determined as fresh and dry mass dry weight was determined after drying the plant parts at

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80^0 C for 48 h to a constant weight. Water content was determined as g of water per g of dry weight of the tissue (fresh weight – dry weight) dry weight, Ion contents were estimated in dry material by method of Wignarrajah *et al.* (1975). Na^+, K^+ and Ca^+ were measured by flame photometry chloride was determined by titration against silver nitrate using potassium chromate as indicator (Clesari *et al*., 1989). Catalase (Machly and Chance, 1967), Peroxidase, polyphooxidase (Kumar and Khan,1982) and ATPase – Jaffe and Galston(1966) were analysed. Growth data was subjected to one way analysis of variance and significance was determined at 5% level.

Results and Discussion

NaCl treatment enhanced the fresh and dry mass with increasing salinity upto 400 mM NaCl, beyond this, concentration the growth and dry mass declined, salinity effects on growth was not uniform in all plant parts. In leaf has more fresh and dry mass with increasing salinity upto the optimal level (Table 1) growth stimulation at low to moderate external salinity has been reported for any halophytes (Reddy *et al*., 1992; A1- Zaharani and Hajar, 1983 and Nagarajan *et al*., 2008). The large increase in fresh weight in the leaves was mainly due to an increase in tissue water content. The increase in water content can be a good reason for tissue succulence and salinity promotes succulence in a number of halophytes (Reddy *et al.,* 1992; Ayala and O.Leary, 1995; Gul *et al*., 2000 and Manikandan and Venkatesalu, 2004; Sivasankaramoorthy and Venkatesan, 2007) much of the dry weight increase in S.nudiflora was due to accumulation of Na^+ salts as reported in other species of *Suaeda* (Eshel, 1985). The inherent capacity to accumulate large quantities of ions from the external medium probably forms the basis for the extended growth stimulation at higher salinities in *S. nudiflora*.

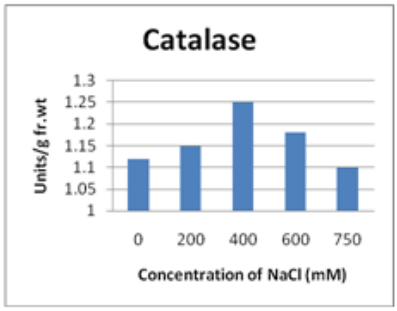
The results presented (Table II) shows that increase in NaCl concentration steadily increased Na^+ and Cl^- in all plant parts and the accumulation was significantly higher in leaf then in stem and root. At 750 mM NaCl, the concentration of Na^+ in leaf, stem and root was 25.40,15.12 and 23.5 g/kg dry weight, respectively as compared to 13.95, 9.41 and 12.16 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 Raghunathan, 1990). The salinity induced decrease in K^+ concentration at higher salinity observed in *Suaeda nudiflora* is an agreement with the results of Naidoo and Raghunathan (1990) and A1- Zaharani 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).

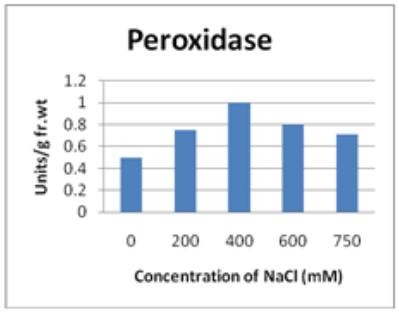
Suaeda nudiflora is able to maintain a high or favourable Na^+/K^+ ratio at higher salinity, high Na^+/K^+ ratio were also reported in Sarcocornia natalensis (Naidoo and Raghunathan, 1990) and other halophytes (Wu *et al*., 1998). Thus, it may be argued that the stimulated growth of *S. nudiflora* under saline conditions does not occur with ion exclusion, but there is a true tolerance as evidenced by high concentrations of inorganic ions with Na^+ being used as the major vacuolar *Osmoticum*.

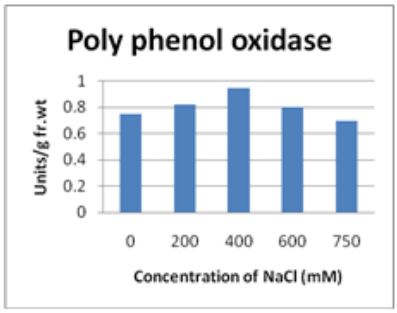
The activity of catalase, peroxidase and polyphenol oxidase increased upto optimum level of 400 mM NaCl and at higher concentrations showed decreasing trend many salt tolerant species are reported to reduce the membrane damage by increasing enzymatic defense against oxygen radical (Smirnoff, 1993). The activity of antioxidant enzmes associated with detoxification of active oxygen species can be increased via appropriate gene transfer and the possible effects of such transformations on plant resistance to environmental stresses have been investigated (Noctor and Foyer, 1998; Mukerje *et al.,* 1999). Decline in catalase activity with the progress of water stress has been reported in *Oryza sativa* (Lin and Hueikaw, 2000) and in certain halophyte, such as *Avicennia marina* (Ashihara *et al*., 1997) in *Bruguiera gymnorrhiza* (Takemura *et al.,* 2000).Significant increase in the peroxidase activity in the halophytes, such as *Suaeda nudiflora* (Sam Cherian and Reddy, 2000). *Bruguiera conjugata* and *Ceriops roxburghiana* (Sivasankaramoorthy and Chellappan, 2006).

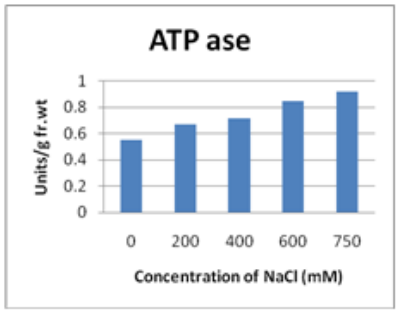
The significant increase in the ATPase activity upto extreme level of 750 mM NaCl salinity. There was a significant increase of plasma membrance H^+-ATPase activity on *Spartina patens* under callus grown NaCl condition (Wu and Seliskar, 1998). This, enzyme plays a major role in the osmoregulation under saline condition (Reuveni *et al*., 1987). In conclusion, reveals that the salt tolerance of *S. nudiflora* is characterized by massive accumulation of inorganic ions, succulent leaves, stimulated activities of enzymes are responses to the external salinity.

Fig I, Effect of NaCl on Catalase, Peroxidase, Polyphenol oxidase (Units min^{-1} g^{-1} fr.wt) and ATPase (mM No_3 reduced g^{-1} fr.wt h^{-1}) in the leaf of *S. nudiflora*









Concentration of NaCl (mM)	Fresh weight (g)				Dry weight (g)				Water Content (g/g dry Wt)
	leaf	Stem	Root	Whole Plant	Leaf	Stem	Root	Whole Plant	
(0) Control	8.40 ±0.105	7.15 ±0.089	6.17 ±0.077	21.72 ±0.271	1.75 ±0.021	1.55 ±0.019	1.15 ±0.014	4.45 ±0.055	12.82
200	8.56 ±0.114	7.25 ±0.097	6.25 ±0.083	22.06 ±0.295	1.80 ±0.024	1.69 ±0.022	1.30 ±0.017	4.79 ±0.064	12.48
400	10.15 ±0.145	8.15 ±0.116	7.50 ±0.107	25.80 ±0.369	1.90 ±0.027	2.75 ±0.025	1.40 ±0.020	5.05 ±0.072	15.70
650	9.12 ±0.138	8.10 ±0.123	7.40 ±0.112	24.62 ±0.374	1.70 ±0.025	1.60 ±0.024	1.35 ±0.020	4.65 ±0.070	15.32
750	8.35 ±0.134	7.50 ±0.120	7.17 ±0.115	23.02 ±0.370	1.64 ±0.026	1.50 ±0.024	1.18 ±0.018	4.32 ±0.069	14.02

Table I. Effect of NaCl on growth of *S. nudiflora*

*Values significant at P=0.05 level

Table - II. Effect of NaCl on ion Content (g/Kg^{-1} dry Wt.) of *S.nudiflora*

Concentration of NaCl (mM)	Plant part	Na^+	K^+	Ca^+	Cl^-	Na/K
(0)Control	L	10.15 ±0.118	4.40 ±0.051	5.27 ±0.061	9.17 ±0.106	2.37 ±0.027
	S	7.10 ±0.082	3.15 ±0.036	5.10 ±0.059	8.00 ±0.093	2.25 ±0.026
	R	7.05 ±0.081	3.00 ±0.034	4.17 ±0.048	7.12 ±0.082	2.35 ±0.027
200	L	15.25 ±0.190	3.17 ±0.039	6.13 ±0.076	14.10 ±0.176	4.81 ±0.060
	S	14.15 ±0.177	3.00 ±0.014	5.75 ±0.072	14.00 ±0.175	4.71 ±0.058
	R	13.25 ±0.165	2.75 ±0.034	5.50 ±0.068	12.20 ±0.152	4.81 ±0.060
400	L	17.15 ±0.230	3.00 ±0.040	7.19 ±0.096	16.25 ±0.218	5.71 ±0.076
	S	15.12 ±0.202	2.75 ±0.036	6.50 ±0.087	16.10 ±0.216	5.43 ±0.072
	R	14.10 ±0.189	2.50 ±0.033	5.75 ±0.077	13.27 ±0.178	5.64 ±0.075
650	L	19.25 ±0.275	2.75 ±0.039	7.15 ±0.102	18.25 ±0.261	7.00 ±0.100
	S	16.20 ±0.231	2.50 ±0.035	6.25 ±0.089	16.15 ±0.231	6.48 ±0.092
	R	15.12 ±0.216	2.41 ±0.034	5.50 ±0.078	14.19 ±0.203	6.27 ±0.089
750	L	20.50 ±0.293	2.29 ±0.034	7.00 ±0.106	20.10 ±0.305	8.35 ±0.126
	S	18.17 ±0.276	2.19 ±0.033	6.10 ±0.092	17.17 ±0.261	8.23 ±0.125
	R	16.10 ±0.244	2.00 ±0.030	5.25 ±0.079	13.12 ±0.199	8.05 ±0.122

L = Leaf S= Shoot R= Root

Table - III, Effect of NaCl on Catalase, Peroxidase, Polyphenol oxidase (Units min^{-1} g^{-1} fr.wt) and ATPase (mM No_3 reduced g^{-1} fr.wt h^{-1}) in the leaf of *S. nudiflora*

Concentration of NaCl (mM)	Catalase	Peroxidase	Polphenol oxidase	ATPase
(0)Control	1.12 ±0.013	0.50 ±0.005	0.75 ±0.008	0.55 ±0.006
200	1.15 ±0.014	0.75 ±0.009	0.82 ±0.010	0.67 ±0.008
400	1.25 ±0.016	1.00 ±0.013	0.95 ±0.012	0.72 ±0.009
650	1.18 ±0.016	0.80 ± 0.011	0.80 ±0.011	0.85 ±0.012
750	1.10 ±0.016	0.71 ±0.010	0.70 ±0.010	0.92 ±0.013

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