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SOME PARAMETERS OF GROWTH OF RIVER COOBA SEEDLINGS UNDER SALT STRESS

Zulfiqar Ali Sahito, D. Khan and Naeem Ahmed

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

ABSTRACT

The experiment was conducted to observe the influence of Sea salt salinity (0, 0.15, 0.3, 0.6, 0.9 and 1.2% corresponding to ECiw of 0.6, 3.51, 5.24, 9.23, 12.81 and 16.67 $dS.m^{-1}$, respectively) on seedling growth and the physiological, biochemical and mineral parameters of growth in *Acacia stenophylla* A. Cunn. Ex. Benth. On average basis, 50% reduction in seedling growth performance corresponded to 12.51 ± 1.51 $dS.m^{-1}$. Phyllode concentrations of protein, sugars, proline and phenols increased significantly with the salt stress and the pigments (chlorophylls and carotenoids) concentrations declined. There was substantial increase in Na and Cl contents of phyllode (303.9 and 145.9 % over control, respectively) in extreme salinity. There was very low variation in K contents – only 4.74% decline over control under extreme salinity. *A. stenophylla* appeared to be potassiophillic as K / Na ratio was always much higher than one in magnitude although declined from 10.20 in control to 2.26 under extreme salt stress of ECiw: 16.67 $dS.m^{-1}$. The results are discussed in physiological context.

Key Words: *Acacia stenophylla* A. Cunn. Ex. Benth., Salt stress, Diluted Sea Water Irrigation, Physiological and Biochemical Parameters of Growth, Salt Tolerance.

INTRODUCTION

The drought and salinity of water and soil are the main impediments to the agriculture in arid regions. A great deal of research on the subject with a view of selection of promising plants for fuel, fodder and food and their cultivation in the halo-xeric environments has been undertaken in many countries of the World. The Acacias are the most successful survivors in arid and semi-arid areas as they possess features required to withstand severe desert conditions (El-Amin, 1976) many of which are tolerant to saline conditions and promisingly suitable for cultivation in arid areas to provide fodder or browse for livestock, fuel wood, edible seeds, gum, tannins, shade, shelter, live fences, soil stabilization and ornamentals (Wickens, 1995). Desert shrubs may acquire water held with high metric forces and utilize and retain water efficiently and conservatively (Moore *et. al*., 1972). Although various desert plants may vary with respect to the toxicity of different salts, owing to their capability to withstand high osmotic effects, salinity tolerance of desert plants / xerophytes may be quite higher than that of many agronomic species (McKell, 1979). The cultivation of salt-tolerant, under-exploited plants by utilizing saline water for irrigation can provide an economic use of abandoned semi-arid and arid lands (Dagar *et al.*, 2006).

The mechanisms of salt tolerance are of two main types: those minimizing the entry of salt into the plant and other minimizing the cytoplasmic concentration of salt (Munns, 2002). The later mechanism may include exclusion, succulence, transport, compartmentalization and excretion (Popp, 1995). These processes bring osmotic adjustments which is crucial for plant survival in the saline environment (Flowers and Colmer, 2008). The ability of plants to tolerate salts involves multiple biochemical pathways that facilitates retention or acquisition of water, protect chloroplast functions and maintain ion homeostasis and scavenging of oxygen radicals (Parvaiz and Satyawati, 2008; Zielinska, 2012). The influence of abiotic stress signals on secondary metabolism in plants have been reviewed by a number of researchers recently (Ramakrishna and Ravishankar, 2011; Mane *et al.*, 2011; Aslam *et al.*, 2011; Rahdari and Hoseini, 2011).

Some 48 species of *Acacia* have been screened although only few have been tested both in field and glasshouse (Niknam and McComb, 2000). Craig *et al.* (1990) have tested ten taxa of *Acacia*. *A. cyclops, A. brumalis, A. redolens* and *A.* aff. *lineolata* had survival rate of 100% after 12 weeks irrigation with saline solution of 9.5 $dS.m^{-1}$. *A. saligna, A. stenophylla* and *A. salicena* have also been shown to be salt tolerant in field (Aswathappa *et al.*, 1987; Hussain and Gul, 1991; Gill and Abrol, 1991; Hafeez, 1993; Singh *et al.*, 1994; Shirazi *et al.*, 2006). Six species of *Acacia* have been grown in Riyadh under irrigation for four years including *A. stenophylla* and compared for their growth and biomass production (Aref *et al.*, 2003). *A. stenophylla* has high coppicing ability (not exactly known under salinity), moderate rate of growth, 4-12m in height, long-lived, grows in Australia on creek banks, on heavy soils, often alkaline clays under mean annual temperature 11 – 23 ^{o}C (coldest 4 – 9 ^{o}C and hottest 25 – 35 ^{o}C (Muslin and Mc Donald, 2004). *Acacia stenophylla* is reported to tolerate salinity of the order of >16$dS.m^{-1}$ (ECe) and occasionally waterlogging. It lives for more than 50 years (Thomson, 1987). Survival and growth of 24 native

species of Australia was tested near Wellington in Central-West-New South Wales on a saline discharge site by Marcar *et al.* (2003). *Acacia stenophylla* showed no growth decline up to ECe of 10 $dS.m^{-1}$.

Experimental investigations related to growth of River Cooba seedlings (*Acacia stenophylla* A. Cunn. Ex. Benth.) in pots while irrigated with seawater dilutions are undertaken here to assess its salt tolerance and its possible scope in afforestation under saline irrigation in sandy soils. Besides some important biochemical parameters of growth, phyllode contents of Na^+, K^+ and Cl^- ions are also investigated.

MATERIALS AND METHODS

The seeds of *A. stenophylla* collected from its tree in Botany Department, University of Karachi, during March, 2012 were sterilized with sodium Hypochlorite (2%) for two minutes and stored in brown aseptic bottles for around two months.

Germination of seeds

The after-ripened and sterilized seeds were slightly clipped at one end manually to break impervious testa were germinated in distilled water in petri plates over Whatman filter paper. The seeds germinated c 77%. They were allowed to grow for a week prior to their transplantation in pots (initially four seedlings per pot).

Sand Culture Experiment

The present work was conducted during July- September 2012 in the green house of the Biosaline Research Laboratory, Department of Botany, University of Karachi. The sand was collected from sand dunes of Sandspit, Karachi. The sand was passed through a 2 mm sieve to remove gravels and other materials. The sand was washed with acid solution and then 5-6 times with running tap water in order to make it free from all nutrients and minerals. Approximately, 3 Kg of this washed sand was filled in pots measuring 20 cm in diameter and 24 cm in height. The bottom of pots was provided with a hole for drainage of surplus water. A filter paper was placed at the bottom of pots. Four replicate pots for each treatment were placed on bench in the green house in random fashion. The seedling before the commencement of treatment were irrigated with modified (Epstein, 1972) half strength Hoagland solution for two times at an interval of three days and subsequently with tap water for 10 days.

Preparation of irrigation medium

Out of the crop of seedlings, three seedlings of more or less similar vigour were selected and transplanted into pots equidistantly. Twenty four pots were so prepared for six treatments. A series of solutions of sea salt concentrations (0, 0.15, 0.3, 0.6, 0.9 and 1.2% corresponding to (ECiw of 0.6, 3.51, 5.24, 9.23, 12.81 and 16.67 $dS.m^{-1}$, respectively) was prepared by dissolving appropriate amount of sea salt in tap water. The irrigation medium of 0.6 $dS.m^{-1}$ was considered as control. In order to avoid the shock effects of saline irrigation, the plants were pre-conditioned by gradual increment of salinity to desired levels. The control and treatments consisted of four replicates. Before commencement of treatment thinning of seedlings was practiced to leave one healthiest seedling per pot. After six weeks (20 irrigations of un-amended Seawater dilutions) the seedlings were harvested for growth measurement and other analyses.

Growth analysis

Growth analysis besides morphometric measurements also included number of leaves and phyllode per plant, fresh and dry weight of root, stem and leaves and phyllodes. For dry weights, plant material was dried at 60 ^oC for 48 h in oven.

Photosynthetic pigments

The phyllode samples were excised from the plants and immediately frozen in liquid nitrogen and stored at -20 ˚C until used for photosynthetic pigments. The phyllode samples (0.1 g) were grounded in liquid nitrogen and then homogenized in 5 ml 80% cold acetone, centrifuged at 3000 g for 5 minutes. The supernatant was separated and the residue was again dissolved in 3 ml of 80% cold acetone and centrifuged. The process was repeated until all the photosynthetic pigments were extracted. All supernatant fractions were pooled and final volume was adjusted. The absorbance of the extracts was recorded at 649 and 665 nm for chlorophylls determination while 480 and 510 nm for carotenoids determinations, respectively. The absorbance was recorded on spectrophotometer. The chlorophyll and carotenoids contents were determined according to the equations described by Strain *et al*., (1971) and Duxbury and Yentsch (1956), respectively.

Chlorophyll a (µg/ml) = 11.63 (A_{665}) – 2.39 (A_{649})

Chlorophyll b (µg/ml) = 20.11 (A_{649}) – 5.18 (A_{665})

Total Chlorophylls (µg/ml) = 6.45 (A_{665}) + 17.72 (A_{649})

Carotenoids (µg/ml) = 7.6 (A_{480}) – 2.63 (A_{510})

The chlorophyll and carotenoids contents were expressed as $mg.g^{-1}$ fresh weight of leaves.

Biochemical analysis

Proteins

The fully expanded phyllode immediately after harvest was frozen in liquid nitrogen and stored at -20 °C until use. The sample (0.5 g) was grounded in liquid nitrogen and homogenized in 5 ml of ice chilled potassium phosphate buffer (pH = 7, 0.1 M) containing 1mM EDTA and 1% PVP (w/v). The homogenate was filtered through a muslin cloth and then centrifuged at 21,000 x g at 4 °C for 20 min in refrigerated centrifuge. The supernatant was separated and stored at -20 °C. The protein contents were determined by using Bradford Assay reagent method (Bradford, 1976). The proteins were determination against Bovine Serum Albumin as standard and the value of proteins was calculated from a following best-fitted standard curve equation.

$$\text{Proteins } (\mu g.ml^{-1}) = -3.29196 + 114.2755\ OD \pm 5.3436$$
$$(t = 16.76,\ F = 280.93,\ p < 0.0001,\ R^2 = 0.9723)$$

The concentration of protein contents were mentioned in $mg.g^{-1}$ fresh weight of leaves.

Total sugars

Fresh phyllode samples were boiled in 80% ethanol at boiling water bath to kill the tissues. Then, samples were homogenized in 80% ethanol and centrifuged at 4000 g for 10 minutes. The supernatant was separated and the residue was again extracted with 80% ethanol. Both supernatants were combined and then the volume was made up to desired level by distilled water. The extract was used for the determination of total sugars by the method of Fales (1951). The total sugars were determined were determined against glucose as standard and the total sugars were calculated from a following best-fit standard curve equation.

$$\text{Total sugars } (\mu g.ml^{-1}) = 228.462.\ OD^{0.97275} \pm 0.04455$$
$$(t = 49.28,\ F = 2428.32,\ p < 0.0001,\ R^2 = 0.9967)$$

The concentration of total sugars was expressed as $mg.g^{-1}$ fresh weight of leaves.

Phenols

Soluble phenols were determined by the method of Singleton and Rossi (1965). The fresh phyllode material was homogenized in 80% methanol and centrifuged. To 1 ml of diluted extract 5 ml of Folin-Ciocalteu reagent (1:9 ratio in distilled water) and 4 ml of 7.5% Na_2CO_3 were added. The absorbance was recorded at 765 nm after incubation of 30 minutes at 25 °C. The soluble phenols concentration in leaf tissues was determined against Gallic acid and calculated from a following best-fit standard curve equation.

$$\text{Phenols } (\mu g.ml^{-1}) = 1.62724 + 94.5284\ OD - 17.19352\ (OD)^2 \pm 0.3425$$
$$(t = 35.57) \quad (t = -4.17),$$
$$(p < 0.0001) \quad (p < 0.0051)\ \ F = 8786.10,\ p < 0.0001\ \&\ 0.0051,\ R^2 = 0.9996)$$

The concentration of total phenols was mentioned in $mg.g^{-1}$ fresh weight of leaves.

Proline

The proline contents were determined by the method of Bates *et al*. (1973). The dried phyllode powder sample (0.1 g) was homogenized with 5 ml of 3% (w/v) sulphosalicylic acid and centrifuged at 5000 g for 20 minutes. Two ml of extract was transferred in caped test tube, and then 2 ml glacial acetic acid and 2 ml ninhydrin reagent (prepared by dissolving 2.5 g ninhydrin in 60 ml of glacial acetic acid and 40 ml 6 M phosphoric acid) were added while vortex. The mixture was boiled for 1 hour at 100 °C, cooled and then 4 ml of toluene was added to each tube. Two layers were appeared, the chromophore layer of toluene was removed and their absorbance was recorded against reference blank of pure toluene. The proline concentration was determined from a predictive equation of the standard curve prepared from extra pure proline from Sigma.

$$\text{Proline (microgram / 2 ml)} = -0.740092 + 16.60767\ (OD_{520}) \pm 0.54031$$
$$t = 35.07$$
$$p < 0.00001;\ F = 1230.16\ (p < 0.00001)$$

Mineral analysis:

The mineral ions in phyllode samples were determined according to the method of Chapman and Pratt (1961). The phyllodes were dried at 60 ˚C for 48 h. The dried phyllodes (100 mg) were powdered and transferred into porcelain crucibles. The crucibles were placed in a muffle furnace at 550 ˚C for 6 h. The ash was dissolved in 5 ml

of 2 N HCl. After 20 min the solution was diluted with deionized water. This solution was filtered through a Whatman No. 1 filter paper and the concentrations of Na^+ and K^+ ions were determined with flame photometer. The best-fit standard curve equations are as follows:

$$Na\ (ppm) = 0.016135.X^{1.879824} \pm 0.04433$$
$$(t = 49.528, F = 2453.01, p < 0.0001, R^2 = 0.9968)$$

$$K\ (ppm) = 0.244346.X^{1.314603} \pm 0.04433$$
$$(t = 29.47, F = 868.54, p < 0.0001, R^2 = 0.9909)$$

Where X = Reading on the flame photometer. The concentration of Na and K ions were expressed as $meq.g^{-1}$ dry weight of leaves.

Chlorides

One hundred milli gram dried phyllode powder was dissolved in 20 mL deionized water. The solution was boiled for one hour. For Cl determination , 100 µL of hot water extract, 4 mL of acid reagent (900 mL deionized water, 6.4 mL conc. Nitric acid and 100 mL of glacial acetic acid) were taken in vial. Four drops of gelatin reagent (0.62 % boiling water) were also added in it. The concentration of Cl ion in the solution was determined by silver nitrate precipitation with chloridometer (HBI, model No. 4425150).

RESULTS AND OBSERVATION

The sixty day-old seedlings of *A. stenophylla*, which were irrigated with various dilutions of un-amended Seawater (ECiw: 0.60 to 16.67 $dS.m^{-1}$) at sandy soil in pots for around six weeks of their life, exhibited gradual loss of growth with the increase of Sea salt stress. All growth parameters including shoot and root lengths, their dry weights and number of leaves / phyllodes and their weight per seedling declined progressively with the rise of salinity of the irrigation medium (Table 1). The relationship of various growth parameters with salinity in terms of significant best fit regression equations are given in Table 2 and Fig. 1. Maximum reduction was observed in case of root length and consequently its weight and the number of leaves / phyllodes per seedling and their dry weight. Such a behaviour of growth parameters resulted in loss of seedling weight with increase of the salinity of the irrigation medium (Fig.1). The EC of the irrigation medium corresponding to 50% reduction in various growth parameters, as calculated on the basis regression equations presented in Table 2 and Fig. 1, varied substantially from 8.35 to 18.52 $dS.m^{-1}$. The root growth was more susceptible to salinity than shoot growth. On average basis 50% reduction in seedling growth performance corresponded to 12.51 ± 1.51 $dS.m^{-1}$ (Table 3).

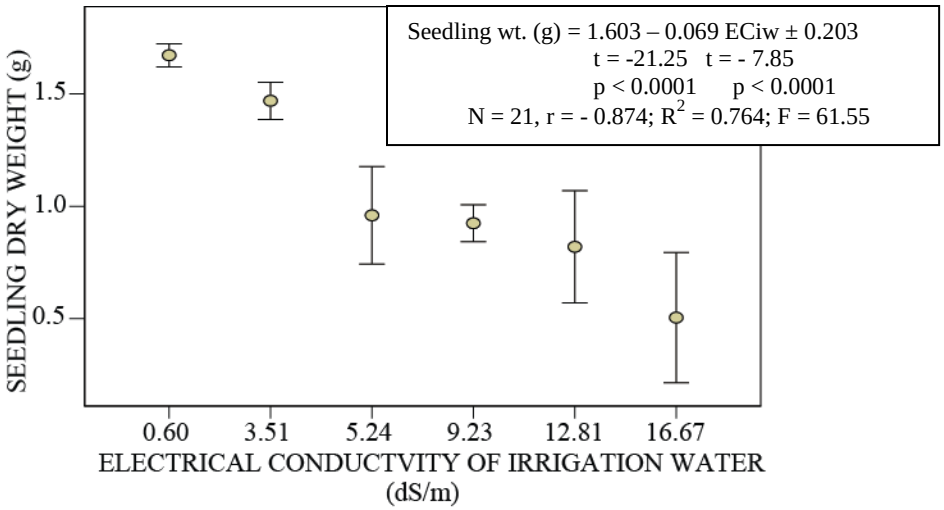

Fig. 1. Relationship of seedling size with salinity of the irrigation medium.

The photosynthetic pigments with some irregularity tended to decline in concentration with increase of salinity. Chlorophyll-a concentration reduced by 9.6%, chlorophyll-b by 28.55% and total chlorophyll and carotenoids contents declined over control by 14.27 and 12.29%, respectively under irrigation with seawater dilution corresponding to EC_{iw}: 16.67 $dS.m^{-1}$ (Table 4).

Table 1. Effects of Seawater dilutions irrigation on seedling growth of *A. stenophylla*.

ECiw (dS /m) of Irrigation Medium	Statistics	Shoot Length (cm)	Root Length (cm)	Shoot Dry Wt. (g)	Root Dry Wt. (g)	phyllodes Dry Wt (g).	Seedling Dry Wt. (g)	Number of leaves / phyllodes
0.60	Mean	35.000	77.500	0.6575	0.4175	0.5975	1.6725	32.7500
	SE	1.0801	7.1356	0.05662	0.01436	0.0520	0.02562	2.39357
3.51	Mean	34.125	16.500	0.5725	0.2100	0.6875	1.4700	29.00
	SE	1.9830	0.9574	0.04589	0.02582	.03301	.04143	1.58114
	P / R (%)	-2.50	- 78.71	- 12.93	- 49.71	15.06	- 12.11	- 11.31
5.24	Mean	22.500	22.000	0.4575	0.1900	0.3125	0.9600	22.75
	SE	3.0687	5.5827	.05282	.02483	.05329	0.10870	2.86865
	P / R (%)	- 37.14	- 71.61	- 30.42	- 54.49	- 47.70	- 42.60	- 30.53
9.23	Mean	21.500	13.750	0.4925	0.0800	0.3525	0.9250	18.00
	SE	3.4278	4.7500	.02097	0.01780	.02175	0.04113	1.47196
	P / R (%)	- 38.57	- 82.26	- 25.09	- 80.84	- 41.00	- 44.69	- 45.04
12.81	Mean	26.000	30.250	0.3775	0.1625	0.1800	0.7200	13.00
	SE	2.7386	7.4764	.07889	0.03301	.07292	0.13342	4.79583
	P / R (%)	- 25.71	- 60.97	- 42.59	- 61.08	- 69.87	- 56.95	- 60.31
16.67	Mean	23.000	11.500	0.3400	0.0600	0.0725	0.4725	5.25
	SE	1.7795	2.0616	.04509	.00577	0.04956	.06263	3.35099
	P / R (%)	- 34.29	- 85.16	- 48.29	- 85.63	- 87.87	- 71.75	- 83.97

P / R (%), Promotion or reduction over control (%).

Under irrigation with seawater dilutions (EC_{iw}: 0.60 to 16.67 $dS.m^{-1}$) phyllode protein contents increased gradually progressively up to 674.8% over control in high salinity. Similar trends of increase in phyllode sugar, phenols and proline concentrations were exhibited with promotion over control as high as 53.12, 156.02 and 87.83%, respectively (Table 5).

Sodium concentration in phyllode increased with salinity in substantial amounts – over 380 % over control under salinity treatment of EC_{iw}: 12.81 and 304 % over control under EC_{iw}: 16.67 $dS.m^{-1}$. K concentration, on the other hand, varied but little under saline treatments and control (fluctuating from average value of 4.53 meq/L in control and 4.32 meq/L in high salinity treatment (EC_{iw}: 16.67 $dS.m^{-1}$). The maximum concentration of K was 5.12 meq/L in low salinity regime of ECiw: 3.51 $dS.m^{-1}$ (Table 6). The Chloride ion concentration increased regularly with salinity exhibiting promotion in concentration around 150% over control in salinity regime of EC_{iw}: 16.67 $dS.m^{-1}$ (Table 6). K / Na ratio although declined with salinity from 10.2 in the control to 2.26 in the high salinity regime, such a decline was due to increased absorption of sodium with salinity (Table 7).

The biochemical and mineral parameters (Yi) studied here related directly with the magnitude of salinity (Xi) in simple linear model (Table 8). K /Na ratio, however, best related with salinity in accordance with a negative power model (Fig. 2).

Table 2. Equations of significant linear regression between salinity (Xi) and various growth parameters (Yi).

Stem Length (cm) =	33.385 – 0.901ECiw ± 6.2217 t = 14.42 t = -3.34 p < 0.0001 p < 0.0.003	r = - 0.609; R^2 = 0.0.370; F = 11.17 N = 21	EQ. # 1
Root Length (cm) =	49.264 -2.680 ECiw ± 22.75 t = 5.82 t = - 2.73 p < 0.001 p < 0.014	r = - 0. 529; R^2 = 0.280; F = 7.04 N = 21	EQ. # 2
Stem wt. (g) =	0.633 - 0.018 ECiw ± 0.09679 t = 17.58 t = -4.30 p < 0.001 p < 0.001	r = - 0.702; R^2 = 0.493; F = 18.46 N = 21	EQ. # 3
Root dry wt. (g) =	0.334 – 0.020 ECiw ± 0.077653 t = 11.56 t = -5.91 p < 0.001 p < 0. 001	r = - 0.804; R^2 = 0.647 F = 34.85 N = 21	EQ. # 4
Phyllode Dry Wt. (g) =	0.636 – 0.031Ciw ± 0.1306; t = 13.09 t = - 5.50 p < 0.62 p < 0. 001	r = - 0.784; R^2 = 0.614, F = 61.55 N = 21	EQ. # 5
Seedling Dry Weight (g) =	1.603 – 0.069 ECiw ± 0.203; t = -21.25 t = - 7.85 p < 0.001 p < 0.0001	r = - 0.874; R^2 = 0.764; F = 61.55 N = 21	EQ. # 6
Phyllodes Per seedling =	32.353 -1.344 ECiw ± 4.403; t = 19.75 t = - 7.05 p < 0.0001 P < 0.0001	r = - 0.851; R^2 = 0.723; F = 50 N = 21	EQ. # 7

Table 3. Salinity of irrigation medium corresponding to 50% reduction in growth of *A. stenophylla* seedlings on the basis of regression equations given in Table 2.

Parameters of growth	ECiw ($dS.m^{-1}$) corresponding to 50% reduction in growth	Mean ± SE
Shoot Length	18.52	
Root Length	9.19	
Stem dry weight	17.58	
Root dry weight	8.35	12.51 ±1.51 $dS.m^{-1}$
Weight of Phyllodes	10.26	
Number of Phyllodes per seedling	12.04	
Seedling dry weight	11.62	

In our experiment, one seedling died in ECiw: 12.81 $dS.m^{-1}$ and 2 seedlings died in ECiw: 16.67 $dS.m^{-1}$. Soil salinity (ECe) associated with these seedlings was 16.50 in earlier treatment and 17.2-and 18.5 $dS.m^{-1}$, respectively in the later treatment.

DISCUSSION

The experiment was conducted to observe the influence of Sea salt stress on the seedling growth and the physiological, biochemical and mineral parameters of growth in *Acacia stenophylla*. Seawater irrigation inhibited all the growth parameters significantly as a direct function of Sea salt stress. A decrease in plant growth under salinity is a commonly observed phenomenon (Ahmad *et al.,* 1985; 1987; Khan *et al.,* 1987, 1989a and b). Growth reduction under salinity has been reported even in halophytes such as *Nitraria retusa* and *Atriplex halimus* in NaCl concentration of 400-800 mM (Boughalleb and Denden, 2011). The growth suppression in plants under salinity is thought to be related with the increased energy expenditure by plants to combat osmotic and ionic stresses due to salt (s) and consequently lesser energy available for normal physiological processes of plants (O' Leary, 1986). Fifty percent reduction in growth corresponded to 12.51 ± 1.51 $dS.m^{-1}$ which is somewhat comparable to that reported in

Australian literature on salt tolerance of this species. Its growth is generally reduced at ECe: 10-15 and survival is reduced at 15-20 $dS.m^{-1}$ (Marcar *et al*., 1995). Its growth showed no decline up to ECe: 10.0 $dS.m^{-1}$ (Marcar et al., 2003). The level of growth inhibition in salinity may depend on several factors – plant type, magnitude of salinity, duration of salinity exposure, the ionic composition of soil solution and / or irrigation medium, frequency of irrigation, edaphic and climatic conditions, etc. (Ahmad *et al.*, 1985, 1987; Heimann, 1958; Gupta, 1990; Khan, 1987; Khan *et al.*, 1989a and b). It was the contention of Heimann (1958) reported that within certain limits it is not the absolute quantity of the ions in water which is determinant to growth and life limits but it is the relative quantity of the components in composition of the solution which is the most decisive one. Some mortality of seedlings, in our experiment, in soils of ECe above 16.50 is, however, in agreement with Marcar *et al.* (1995).

There was decline of photosynthetic pigments, particularly chlorophylls and carotenoids under salinity. A decrease in plant growth and chlorophyll contents have been reported even in halophytes such as *Nitraria retusa* and *Atriplex halimus* in NaCl concentration of 400-800 mM NaCl (Boughalleb and Denden, 2011). The decrease in chlorophyll-b is often reported (Ahmad *et al.*, 1985; Ali *et al*., 2013) which is suggested to be due to inhibition of iron-containing enzymes which activates the biosynthesis of chlorophyll (Rubin and Artiskhovaskaya, 1964). Anthocyanins are reported to increase under salinity (Parida and Das, 2005). In salt sensitive species anthocyanins, in contrast, are reduced (Daneshmand *et al*., 2010). Relatively better salt tolerance of *N. retusa* has, however, been suggested to be related to higher carotenoids accumulation in *N. retusa* (Boughalleb and Denden, 2011).

Table 4. Concentration of photosynthetic pigments (mg per g fresh weight of phyllode) in *A. stenophylla* under seawater dilutions irrigation.

Irrigation Medium: ECiw (dS/ m)	Statistics	Chlorophyll - a	Chlorophyll - b	Total chlorophyll	Carotenoids
0.60 (Control)	Mean	0.5292	0.2877	0.8169	0.2254
	SE	0.01795	0.02283	0.03911	0.01117
3.51	Mean	0.4967	0.2610	0.7577	0.2114
	SE	0.01778	0.01489	0.02098	0.00763
	P / R %)*	- 6.14	- 9.28	- 7.25	- 6.21
5.24	Mean	0.3203	0.1453	0.4656	0.1438
	SE	0.02201	0.01573	0.01724	0.00489
	P / R (%)	- 39.47	- 49.50	- 43.00	- 36.20
9.23	Mean	0.3159	0.2555	0.5715	0.1598
	SE	0.05106	0.03522	0.04070	0.01451
	P / R (%)	- 40.31	- 11.92	- 30.04	- 29.10
12.81	Mean	0.5017	0.3528	0.8546	0.2375
	SE	0.14612	0.05514	0.20107	0.05558
	P / R (%)	- 5.20	22.62	4.61	5.38
16.67	Mean	0.4890	0.2113	0.7003	0.1977
	SE	0.03080	0.06457	0.03377	0.01606
	P / R (%)	- 9.59	- 26.55	- 14.27	- 12.29

*, P / R (%), Promotion or Reduction (%) over control (0.60 dS/m).

There was increase in proteins, total soluble sugars, proline and phenol contents under salt stress in phyllodes of *A. stenophylla*. There are several reports where increase in sugar concentration is observed, particularly under salinity treatments (Rozema, 1978; Ahmad *et al.*1987; Khan and Ahmad, 1998, 2002). Total sugars content has been reported to increase in *Medicago arborea* (Boughalleb *et al.*, 2011). Munns and Termaat (1986) have reported that the concentrations of sugars always rise in growing as well as expanded tissues after plants are exposed to salinity.

The utilization of sugars in growing tissues is blocked which subsequently results in accumulation of sugars in the plant body. The decrease in sugar content has, however, also been reported in *Melia azedarach* under saline conditions by Ahmad *et al*. (1985). Rozema (1978) reported larger increase in sugar concentration under salinity stress in relatively salt sensitive species, *Juncus alpinoarticularis* ssp. *articappilus*. Shannon and Qualset (1984) reported that accumulation of sugar in leaf is generally larger in salt excluding plants. Khan and Ahmad (2002) also reported significant promotion in sugar accumulation in salt excretive *Sporobolus arabicus*. Relatively salt tolerant legume, *Indigofera oblongifolia* (reducing growth by 50 % at ECiw: 12.05 ± 0.92 $dS.m^{-1}$) also showed increase of sugar level in leaves, which became fleshy with age under saline environment (Khan and Ahmad, 1998). A moderately salt tolerant grass *Panicum turgidum* with tendency of excluding Na from shoot, on the other hand, showed substantial decrease in foliar sugar level under salinity (Khan and Ahmad, 2007). However, it is certain that sugars not only serve as resource food materials but also as cellular osmoticum (Shannon, 1984; Jeffereies *et. al*., 1979), besides proline, glycinebetaine and other organic solutes.

Table 5. Effect of Seawater dilutions irrigation on some biochemical parameters of *A. stenophylla*.

Irrigation Medium (ECiw)	Statistics	Protein ($mg.g^{-1}FW$)	Sugar ($mg.g^{-1}FW$)	Phenols ($mg.g^{-1}FW$)	Proline ($mg.g^{-1}DW$)
0.60	Mean	0.23635	5.4005	3.7259	1.0169
	SE	0.018215	0.25951	0.36490	0.07664
3.51	Mean	0.49776	6.9015	4.6911	1.2923
	SE	0.045610	0.29314	0.29100	0.06092
	P/R (%)*	110.60	22.24	25.91	12.61
5.24	Mean	0.69060	7.2594	5.8148	1.3756
	SE	0.080148	0.17581	0.15232	0.07803
	P/R (%)*	192.19	34.42	56.06	35.27
9.23	Mean	0.97600	7.8384	6.4178	1.5296
	SE	0.056410	0.18477	0.19727	0.06425
	P/R (%)*	270.40	45.14	72.25	50.42
12.81	Mean	1.46910	8.3695	8.1677	1.8733
	SE	0.099761	0.16044	0.20041	0.13317
	P/R (%)*	521.58	54.98	119.21	84.22
16.67	Mean	1.83135	8.2695	9.5401	1.9100
	SE	0.027426	0.77932	1.49044	0.26870
	P/R (%)*	674.85	53.12	156.05	87.83

*P / R (%), Promotion or Reduction (%) over control (0.60 dS/m).

Salinity may influence the protein system, free amino acid pool and accumulation of intermediate products. The effects, however, appear to be dependent on the nature of plant as both decrease (Eder *et al.,* 1977; Poljakoff-Mayber, 1982) and increase (Ahmad *et al.,* 1984; Singh and Vijaykumar, 1974; Helal *et al.,* 1975) in protein level have been reported under salinity. The increase in protein level has been suggested due to increase in respiration rate (Nieman and Paulsen, 1967). Besides, tremendous promotion of protein concentration, proline also increased substantially (87.8 % over control) under salt stress. The accumulation of proline has been reported under different stressful conditions and its accumulation in saline environment (Strogonov, 1964; Rozema, 1978 ; Rains *et al.,* 1982; Joshi *et al.,* 2005) is considered beneficial for plant growth (Rozema, 1978 ; Rains *et al.,* 1982; Nawaz *et al.,* 2010). Total proline contents were reported to increase in *Medicago arborea* by Boughalleb *et al.,* 2011). Aziz *et al.* (1998) reported correlation between proline accumulation and salt tolerance in *Lycopersicon esculentum* and *Aegiceras corniculatum*. Petrusa and Winicov (1997) had demonstrated that salt tolerant alfalfa plants rapidly doubled their proline contents in roots whereas such increase in salt-sensitive plants was slow. Proline accumulation may take place either due to protein degradation or inhibition of proline conversion under salinity (Singh *et al.,* 1973). It is assumed that proline increases the protein solubility (Schobert and Tschesche,

1978), it is compatible in permeability to cytoplasm and prevents the dehydration of enzymes and other essential structures (Gorham *et al.*,,1981), it controls the ion-fluxes (Stewart and Lee, 1974) and regulates the intracellular Na distribution and storage of nitrogen (Jeffereies, 1980; Ahmad *et al.*, in Jeschke, 1984).

Table 6. Concentration of Na, K, and Cl ions (meq /l) in the phyllode of *A. stenophylla* irrigated with seawater dilutions.

Irrigation Medium (ECiw)	Statistics	Na	K	Cl
0.60	Mean	0.4625	4.5363	3.375
	SE	0.04383	0.29554	0.784
3.51	Mean	0.6928	5.1171	3.900
	SE	0.16999	0.31369	0.432
	P / R (%)	49.79	12.80	15.56
5.24	Mean	0.8532	4.1767	7.125
	SE	0.22579	0.69863	0.312
	P / R (%)	84.48	- 7.93	111.11
9.23	Mean	1.4610	4.7001	7.575
	SE	.19199	0.78745	0.317
	P / R (%)	215.89	3.61	123.70
12.81	Mean	2.2219	5.0115	8.475
	SE	.22974	0.61465	0.540
	P / R (%)	380.41	10.48	151.11
16.67	Mean	1.8678	4.3212	8.300
	SE	0.08998	2.12335	0.540
	P / R (%)	303.85	- 4.74	145.93

P / R (%), Promotion or Reduction (%) over control (0.60 dS/m).

Table 7. K / Na ratio in phyllodes of *A. stenophylla* irrigated with seawater dilutions.

EC_{iw} (dS/m)	Mean	SE	P / R (%)
0.60	10.2048	1.59355	-
3.51	9.2924	2.84160	- 8.90
5.24	5.4684	0.84818	- 46.41
9.23	3.2107	0.44109	- 68.54
12.81	2.3320	0.42493	- 77.15
16.67	2.2640	1.0277	- 77.81

The availability, uptake and transportation of ions in plants in saline environment are affected by a multitude of factors. The inter-ionic interactions are complex in root zone and governed by such factors as temperature, areration and the presence of other ions and several other abiotic and biotic factors (Gratten and Grieve, 1999). Na, K, and Chloride contents increased greatly under salinity in phyllodes of *A. stenophylla*. There was, however, decrease in K concentration with rising concentration of Na. Leaves are more vulnerable than roots to Na because Na and Cl more accumulate in shoots than in roots (Tester and Davenport, 2003) - even in halophytes (Boughalleb and Denden, 2011). Higher levels of Na may disrupt various enzymatic processes in cytoplasm. Several studies suggest that the plasma membrane may be the primary site of salt injury (Mansour, 1997). Non-electrolytes and water permeability get altered markedly altered upon salt exposure. He and Cramer (1992) have also recorded reduction in K concentration under salinity. High Na / K ratio exerts metabolic toxicity by competition between Na and K for the binding sites of many enzymes (Tester and Davenport, 2003). At a high concentration, Na can replace Ca from the plasma membrane, resulting in change of the plasma membrane structure and permeability. Salinity coupled with waterlogging is known to decrease the ability of sodium exclusion and selection of K over sodium (Kriedemann and Sand, 1984). Under such conditions significant increase of mortality of *Acacia* plants has been

reported (Niknam and Mc Comb, 2000). The maintenance of adequate concentrations of K is necessary for plant survival in saline soils. Sufficient amounts of K in leaves are considered to indicate better tolerance of a species for saline environment. In spite of high Na content in the irrigation media prepared from Arabian Sea salt, *A. stenophylla* in our experiments absorbed K in high concentrations i.e. 10.21 ± 1.59 times higher than that of Na in control and 2.264 ± 1.03 times to Na in 1.2% Sea salt (ECiw: 16.67 $dS.m^{-1}$). It follows from the results that *A. stenophylla* is a potassiophillic plant in which K absorption although declines with the increase of salinity (increasing Na preponderance) but remains quite higher than that of Na even in very high salinity. Shirazi *et al.* (2006) grew *A. stenophylla* and some other species in saline sodic silty clay to clay loam type of soils with salinity (ECe) 15.5 to 60.0 $dS.m^{-1}$ in upper soil layer (0-30 cm) and 9.10 to 51.2 $dS.m^{-1}$ in lower layer (30-60 cm) under pH of 7.5 to 8.2 with SAR 14.81 – 37.6 and 40-243.0 in upper and lower layer, respectively. Na in these soil layers ranged from 165 – 2890 and 152.2 – 1695 meq/L^{-1}, respectively and K from 1.41 – 5.0 and 1.03 to 1.92 $meq.L^{-1}$, respectively. The maximum concentration of K recorded in phyllodes of *A. stenophylla* by these workers was 0.96% against Na 0.32% that is K / Na ratio of 3.0. This concentration of K was maximum in this species compared to other species tested viz. *Acacia ampliceps, A. nilotica, Eucalyptus camaldulensis* and *Conocarpus lancifolius.* Higher K/Na ratios have been considered (and found in) tolerant varieties of higher plant species (Hedge and Joshi, 1974; Giriraj *et al.,* 1976; Chauhan *et al*., 1980; Lopez and Setti, 1997) although high salinity tolerances in plants with high Na and Cl contents have also been found (Figdore *et al*., 1989). Higher K/Na ratio is known to improve leaf water potential (Devitt *et al.,* 1981). Giri *et al.* (2007) have reported an arbuscular mycorrhiza, *Glomus fasciculatum*, to alleviate deleterious effects of salinity in *Acacia nilotica* by improving nutrition due to improved K / Na ratio in root and shoot under the salinity of 4.5 to 9.5 $dS.m^{-1}$. Such an association of *Glomus* sp. with *A. stenophylla* could be suspected to help plant in protecting disruption of K-mediated enzymatic processes under the salt stress.

Table 8. Equations of significant linear regression between salinity (Xi) and biochemical parameters (Yi).

Protein($mg.g^{-1}.FW$) =	= 0.155 + 0.099 ECiw ± 0.1148 t = 3.62 t = 19.85 p < 0.0001 p < 0.0001	r = 0.977; R^2 = 0.954; F = 394.10 N = 21	EQ. # 1
Total Sugars($mg.g^{-1}.FW$) =	5.942 + 0.181 ECiw ± 0.6422 t = 3.62 t = 19.85 p < 0.0001 p < 0.0001	r = 0.831; R^2 = 0.691; F = 42.43 N = 21	EQ. # 2
Phenols($mg.g^{-1}.FW$)=	3.558 + 0.3521 ECiw ± 0.7050 t = 13.57 t = 11.54 p < 0.0001 p < 0.0001	r = 0.934; R^2 = 0.875; F = 133.94 N = 21	EQ. # 3
Proline($mg.g^{-1}.FW$) =	= 1.041 + 0.058 ECiw ± 0.14935 t = 18.13 t = 8.93 p < 0.0001 p < 0.0001	r = 0.899; R^2 = 0.808; F = 79.74 N = 21	EQ. # 4
Na($meq.L^{-1}$) =	0.355 + 0.115 ECiw ± 0.1.282 t = 7.62 t = 7.16 p < 0.019 p < 0.0001	r = 0.854; R^2 = 0.730; F = 51.25 N = 21	EQ. # 5
Cl ($meq.L^{-1}$) =	3.634 + 0.372 ECiw ± 0.3716 t = 2.57 t = 6.71 p < 0.0001 p < 0.0001	r = 0.854; R^2 = 0.730; F = 51.25 N = 21	EQ. # 6

To effectively counteract the toxic and osmotic effects of increased Na level, the role of vacuole as hypothesized by Jennings (1968) could be of utmost physiological significance in *A. stenophylla*. Under saline conditions sequestration of Na in vacuole i.e., intracellular compartmentalization of cations and Na -K exchange at cellular membrane are known processes in many halophytes and glycophytes as well (Jeschke, 1984). Furthermore, under such conditions the increased concentration of proline could not only have prevented dehydration and degradation of enzymes and proteins within cytoplasm counteracting the osmotic effects of the increased vacuolar sap, but also could have been important in regulating the intracellular Na distribution

(cf. Ahmad *et al*., in Jeschke, 1984). At higher salinity the effects of Na accumulation in the treated plants, should have resulted in mortality of plants as observed here in case of few plants under irrigation with water of ECiw: 12.81 and 16.67 $dS.m^{-1}$. Ionic effects bring accumulation or reduction of specific secondary metabolites (Mahajan and Tuteja, 2005) such as phenols which are known to increase under stressful conditions and help plants to bring osmotic balance. In present studies, there was an increase of phenolic contents up 156.05 % over control under extreme salt stress. Boughalleb and Denden (2011) have reported the role of higher polyphenol content in better salt tolerance of *Nitraria retusa*. The benefits of increased phenol contents may be thought to be available to *A. stenophylla*.

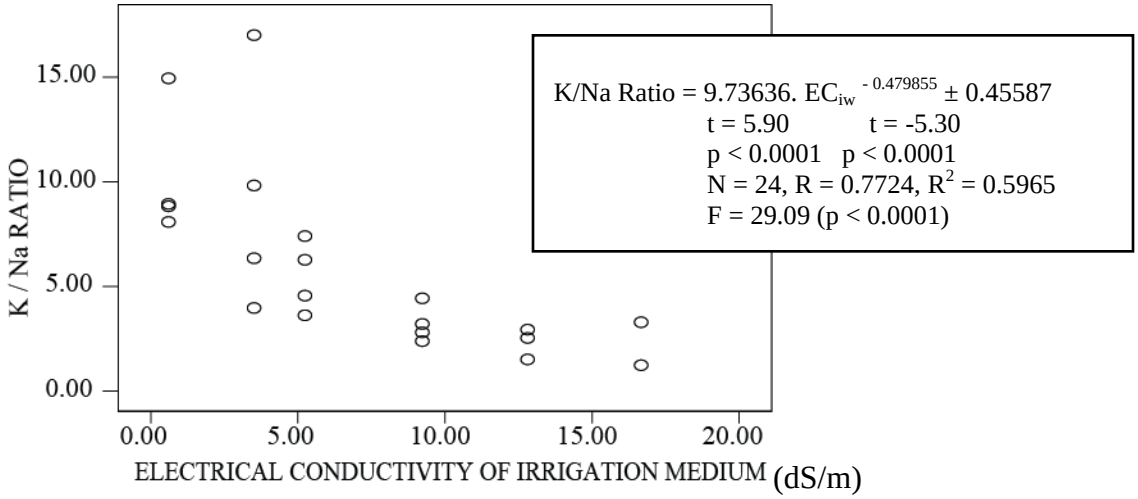

Fig. 2. Relationship of foliar K / Na ratio in *A. stenophylla* phyllodes under irrigation with Seawater dilutions.

Taken together the results, the physiological phenomena such as increase of concentration of protein, sugars, proline and secondary metabolites like phenols and larger K / Na ratio in the phyllodes under saline conditions may play significant role in the salt tolerance of *Acacia stenophylla*.

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