GROWTH OF WIRY WATTLE SEEDLINGS UNDER SALT STRESS

ZULFIQAR ALI SAHITO AND D. KHAN

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 coriacea subsp. pendens.* On average basis, 50% reduction in seedling growth performance in coastal sandy soil corresponded to ECiw: 14.94 ± 2.18 $dS.m^{-1}$. Phyllode concentrations of protein, total soluble sugars, proline and phenols increased significantly with the salt stress and the pigments (chlorophylls and carotenoids) concentrations posed a fluctuating behaviour. There was substantial increase in Na and Cl contents of phyllode (306.59 and 213.67 % over control, respectively) in extreme salinity of ECiw: 16.67 $dS.m^{-1}$. K contents declined under saline environment. K/Na ratio although declined in salinity treatments as compared to the control, it didn't vary practically amongst the salinity treatments of ECiw: 3.51 to 16.67 $dS.m^{-1}$. The results are discussed in physiological context.

Introduction

Some 48 species of *Acacia* have been screened in relation to salinity (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 (Aswathappa *et al.,* 1987; Hussain and Gul, 1991; Gill and Abrol, 1991; Hafeez, 1993; Singh *et al.,* 1994; Marcar *et al.,* 1995; Shirazi *et al.,* 2006). Sahito *et al*. (2013) have reported 50% decline in growth of *A. stenophylla* to correspond with Seawater dilution of ECiw: 12.51 ± 0.51$dS.m^{-1}$. Six species of *Acacia* have been grown in Riyadh under irrigation for four years and compared for their growth and biomass production by Aref *et al. (*2003). Survival and growth of 24 native species of Australia including some Acacias have been tested near Wellington in Central-West-New South Wales on a saline discharge site by Marcar *et al.* (2003).

Acacia coriacea (wire wood, desert oak, dogwood or wiry wattle) is one of the desert species of Australia - reported from the Pilbara coast as well (www.kimseed.com.au) - has been studied for its salt tolerance at germination stage. Rahman *et al*. (1997) found the effects of NaCl on seed germination of *Acacia coriacea* to be adverse due to internal osmotic and toxicity rather than from a restriction of imbibition. Harris *et al.* 1998) tested effects of hardening by soaking seeds of nine species of *Acacia* in water. The treatment showed varying effects of hardening on seed germination in distilled water and NaCl – high rate of germination in *A. tortilis*; low reduced rate of germination in *A. elata* and no significant effect on germination in other species including *A. coriacea* were recorded. LD_{50} concentration of NaCl in case of *A. coriacea* seed germination was reported to be 125mM (Rahman *et al.,* 1998a). It was rated as salt sensitive species at germination stage (Rahman *et al.,* 1998 b).

Since many species including some halophytes are reported to be differentially tolerant to salinity at germination and subsequent growth phase (Ayers and Hayward, 1948; Azizov, 1974; Ungar, 1974; Mahmood and Malik, 1986; Khan and Ahmad, 1998; Khan *et al.,*1987; Prado *et al*., 2000; Ali *et al.,* 2013a) and there is involvement of multiple biochemical pathways in the salt tolerance of plants facilitating retention or acquisition of water, protection of chloroplast functions, maintenance of ion homeostasis, scavenging of oxygen radicals and the peculiarities of secondary metabolism under salt stress (Parvaiz and Satyawati, 2008; Ramakrishna and Ravishankar, 2011; Mane *et al.,* 2011; Aslam *et al.,* 2011; Rahdari and Hoseini, 2011; Zielinska, 2012), experimental investigations related to the growth of wiry wattle (*Acacia coriacea* DC. *subsp. pendens* Cowan and Maslin) at seedling stage while irrigated with Seawater dilutions in pots have been undertaken to assess its salt tolerance and its possible scope in afforestation on Pakistan coast under multiple salts salinity of Seawater dilutions in coastal sandy soil. 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. coriacea* subsp. *pendens* collected from its tree growing in the Botanical Garden, 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 and were germinated in distilled water in petri plates over Whatman filter paper. The seeds germination was c 77%. The seedlings were allowed to grow for a week prior to their transplantation in pots (initially three 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 each pot 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 to be 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 measurements.

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

Biochemical analysis

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 estimation. The leaf samples (0.1 g) were ground 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 in phyllodes 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.

Proteins: The fully expanded phyllodes immediately after harvest were frozen in liquid nitrogen and stored at -20 °C until use. The leaf 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.
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Proteins ($\mu g.ml^{-1}$) = -3.29196 + 114.2755 OD ± 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 leaf 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 against glucose as standard and the total sugars were calculated from a following best-fit standard curve equation.

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: The 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.

Phenols ($\mu g.ml^{-1}$) = $1.62724 + 94.5284\ OD - 17.19352\ (OD)^2 \pm 0.3425$

($t = 35.57$) ($t = -4.17$),

($p < 0.0001$) ($p < 0.0051$); $F = 8786.10$, $p < 0.0001$ & 0.0051, $R^2 = 0.9996$)

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

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. The mixture was boiled for 1 hour at 100 °C, cooled and then 4 ml of toluene was added to each tube while vortex. 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.

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 leaf samples were determined according to the method of Chapman and Pratt (1961). The leaves of the plants were dried at 60 ˚C for 48 h. The dried leaves (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 were 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 mg dried leaf 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. coriacea* 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. The growth parameters generally 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 length and the dry wt. of stem declined with salinity but the length of roots exhibited promotion particularly at lower salinities. The magnitude of promotion of root growth, however, lessened in higher salinities. Similarly, dry mass of roots showed promotion in low salinities (up to 5.24 dS.m^{-1}) but it declined thereafter gradually to c 65% over control in high salinity of 16.67 dS/m^{-1}. The number of phyllodes declined progressively. There was 71.9% decline in number of phyllodes over control in extreme salinity. The dry mass of phyllodes also exhibited a pattern of decline with salinity (-87.87% over control under extreme salt stress). The relationship of various growth parameters with salinity in terms of significant best fit linear regression equations are given in Table 2 and Fig. 1. The root length, however, related with salinity curvilinearly – initial promotion under low salinity regime but decline thereafter. There was 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 11.40 to 24.38 dS.m^{-1}. On average basis 50% reduction in seedling growth performance corresponded to 14.94 ± 2.18 dS.m^{-1} (Table 3).

Chlorophyll-a concentration substantially promoted in high salinity (Table 4). The concentration of chlorophyll –b was somewhat erratic, it however increased under high salt stress. The behaviour of total chlorophyll contents was similar to chlorophyll – b. Carotenoids contents declined more in lower salinities and then lessened in higher salinities. Chlorophyll-a concentration increased by 30.43%, chlorophyll – b by 32.19% and total chlorophyll by 31.08% under extreme salt stress (Table 4).

Under irrigation with Seawater dilutions (EC_{iw}: 0.60 to 16.67 dS.m^{-1}) phyllode protein contents increased gradually progressively up to 436.88% over control in high salinity. Similar trends of increase in phyllode total soluble sugars, phenols and proline concentrations were exhibited with promotion over control as high as 63.11, 135.43 and 89.52%, respectively under extreme salt stress (Table 5).

Sodium concentration in phyllode increased with salinity in substantial amounts – around 230 % over control under salinity treatment of EC_{iw}: 12.81 and 307% over control under EC_{iw}: 16.67 dS.m^{-1}. K concentration, with some irregularities followed a declining trend with increase of salt concentration. There was, however, some promotion in K concentration (15.4% over control) in extreme salinity. There was regular increase in chloride concentration along the salinity gradient of 0.6 to 16.67 dS.m^{-1}. On the other hand, it 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 213.67% over control in salinity regime of EC_{iw}: 16.67 dS.m^{-1} (Table 6). K/Na ratio although declined in salinity treatments as compared to the control, didn't vary practically amongst the salinity treatments of ECiw: 3.51 to 16.67 dS.m^{-1} (Table 7). K absorption reduced along the increasing concomitant Na absorption in saline environment.

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, related with salinity in accordance with a negative power model (Fig. 2).

Table 1. Effects of irrigation with seawater dilutions on the growth of *A. coriacea* subsp. *pendens*.

Irrigation Medium ECiw	Statistics	Stem Length (cm)	Root Length (cm)	Stem dry wt. (g)	Root dry wt (g)	Leaves / Phyllodes dry wt. (g)	Number of leaves / Phyllodes
0.60	Mean	31.8750	27.7500	0.5225	0.2900	0.6825	44.5
	SE	1.55958	1.10868	.04110	.04183	.11672	2.72336
3.51	Mean	27.00	33.5000	0.3675	0.4125	0.4875	40.0
	SE	1.77951	5.00833	.06210	0.15766	0.12236	5.47723
	P/R (%)	-15.29	20.72	- 29.67	42.24	- 28.57	- 10.11
5.24	Mean	28.25	48.0000	0.4000	0.4050	0.4575	33.0
	SE	2.71953	7.07107	0.05730	.14027	.09481	4.79583
	P/R (%)	- 11.37	74.55	- 23.44	39.66	- 32.96	- 25.84
9.23	Mean	27.25	41.5000	0.3725	0.2775	0.4575	37.5000
	SE	1.56125	2.62996	0.02869	0.05977	0.10547	1.89297
	P/R (%)	- 14.56	49.55	-28.71	- 4.31	- 32.96	- 15.73
12.81	Mean	25.60	43.3750	0.3150	0.2000	0.4100	22.0000
	SE	1.74851	2.70320	0.02661	.03536	.03894	3.36650
	P/R (%)	- 19.62	56.31	- 39.71	- 31.03	- 50.07	- 50.56
16.67	Mean	18.60	32.2500	0.1700	0.1000	0.0828	12.5000
	SE	2.58501	7.87798	0.04378	0.03189	0.02764	1.04083
	P/R (%)	- 41.60	16.22	- 67.46	- 65.52	- 87.87	- 71.91

*, % promotion or reduction over control.

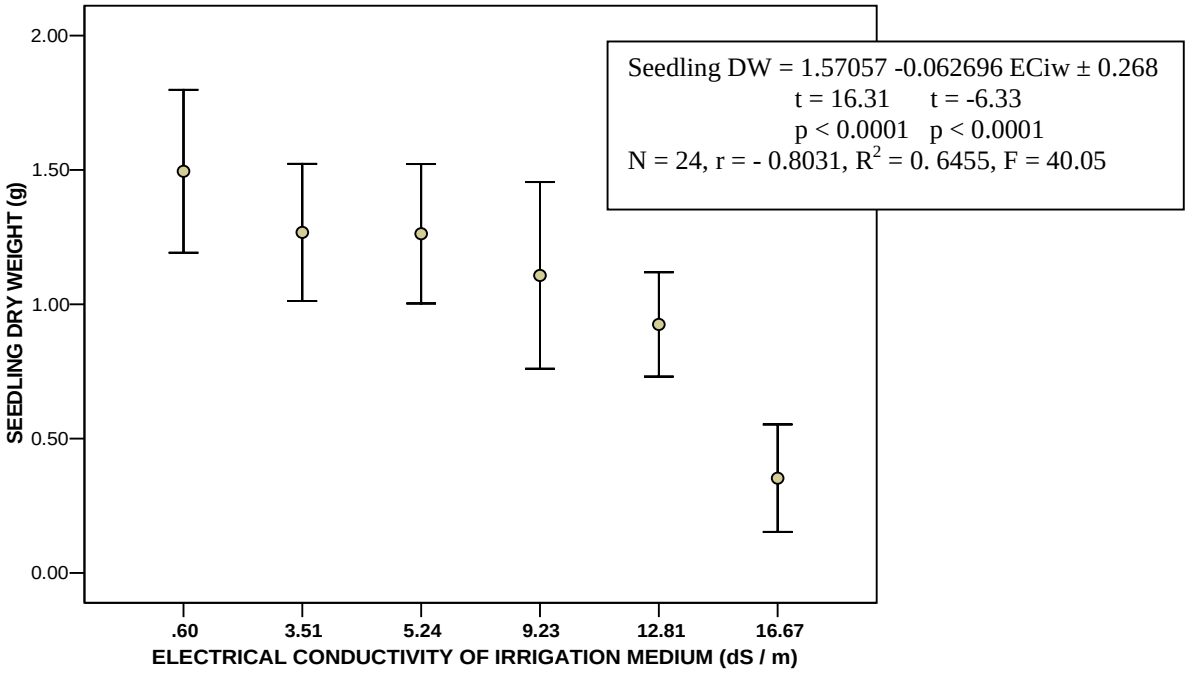

Fig. 1. Relationship of seedling size with the salinity of the irrigation medium.

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

Stem Length = 31.639 – 0.649 ECiw ± 4.1375 (cm) t = 21.24 t = -4.24 p < 0.0001 p < 0.0001	r =- 0.670; R^2 = 0. 450; F = 17.97 N = 23	EQ. # 1
Root Length : r – 0.131 (NS)*		-
Stem wt. (g) = 4980- 0.018 ECiw ± 0.09261 t = 14.94 t = - 5.11 p < 0.0001 p < 0.0001	r = - 0.737; R^2 = 0.543; F = 26.10 N = 23	EQ. # 2
Root wt. (g) = 0.41096 - 0.016246 ECiw ± 0.18107 t = 6.30 t = -2.42 p < 0.0001 p < 0. 024	r = - 0.804; R^2 = 0.647 F = 34.85 N = 23	EQ. # 3
Leaf dry wt. (g) = 0.6610 – 0.029 ECiw ± 0.18582 t = 9.89 t = - 4.21 p < 0.0001 p < 0. 0001	r = - 0.668; R^2 = 0.446, F = 17.68 N = 23	EQ. # 4
Seedling wt. (g) = 1.570567 – 0.062696 ECiw ± 0.26755 t = 16.31 t = - 6.33 P < 0.001 p < 0.0001	r = - 0.803; R^2 = 0.764; F = 40.05 N = 23	EQ. # 5
leaves / seedling = 46.586 -1.873 ECiw ± 7.630 t = 16.96 t = - 8.63 p < 0.0001 P < 0.0001	r =- 0.851; R^2 = 0.666; F = 43.95 N = 23	EQ. # 6

*, Root length = 25.059 + 4.37923 (ECiw) – 0.23698 $(ECiw)^2$ ± 10.0644
t = 4.88 t = 2.98 t = - 2.89
P < 0.001 p < 0.0071 p < 0.0087; R = 0.545, R^2 = 0.2974, F = 4.45 (p < 0.0246).

Table 3. Salinity of irrigation medium corresponding to 50% reduction in growth of *A. coriacea* subsp. *pendens* seedlings.

Parameters of growth	ECiw ($dS.m^{-1}$) corresponding to 50% reduction in growth	Mean ± SE
Shoot Length	24.38	14.94 ±2.18 $dS.m^{-1}$
Root Length	-	
Stem dry weight	13.83	
Root dry weight	12.65	
Weight of Leaves	11.40	
Number of leaves per seedling	12.44	
Seedling dry weight	12.53	

Discussion

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).

An experiment was here conducted to observe the influence of Sea salt salinity on the seedling growth and the physiological, biochemical and mineral parameters of growth in *Acacia coriacea subsp. pendens*. Seawater irrigation inhibited the growth parameters significantly as a direct function of the Sea salt stress which is a

Table 4. Effects of Irrigation with Seawater irrigation on photosynthetic pigments (mg.g^{-1}.FW) in *Acacia coriacea* subsp. *pendens.*

Irrigation Medium (ECiw: dS.m^{-1})	Statistics	Chlorophyll – a	Chlorophyll - b	Total Chlorophyll	Carotenoids
0.60	Mean	0.45792	0.26599	0.72391	0.20848
	SE	0.04927	0.08111	0.05139	0.01054
3.51	Mean	0.45827	0.22412	0.68240	0.16108
	SE	0.01950	0.03263	0.05202	0.02152
	P / R (%) *	0.0764	-15.74	- 5.73	- 22.74
5.24	Mean	0.35498	0.24528	0.60025	0.14884
	SE	0.01695	0.05888	0.06548	0.01530
	P / R (%) *	- 22.70	- 7.79	- 17.08	- 28.61
9.23	Mean	0.53419	0.15668	0.69088	0.18121
	SE	0.04292	0.11544	0.12478	0.01178
	P / R (%) *	16.66	- 41.09	- 4.56	- 13.08
12.81	Mean	0.57735	0.36906	0.94641	.19471
	SE	0.02165	0.05163	0.05304	.00896
	P / R (%) *	26.08	38.74	38.74	- 6.60
16.67	Mean	0.59726	0.35162	0.94889	0.19933
	SE	0.01205	0.02455	0.02061	0.00374
	P / R (%) *	30.43	32.19	31.08	- 4.39

*, % promotion or reduction over control.

Table 5. Effects of irrigation of seawater dilutions on some biochemical parameters of *A. coriacea* ssp. *pendens*.

Irrigation Medium (ECiw)	Statistics	Protein (mg.g^{-1}.FW)	Sugar (mg.g^{-1}.FW)	Phenols (mg.g^{-1}.FW)	Proline (mg.g^{-1}.FW)
0.60	Mean	0.29721	34.68353	0.99349	0.98406
	SE	0.037879	1.074580	0.262314	0.068143
3.51	Mean	0.48919	39.29503	1.25175	1.53605
	SE	0.08119	1.54549	0.16874	0.07901
	P / R (%) *	64.59	13.30	25.99	56.09
5.24	Mean	0.76259	42.4593	1.77579	0.97243
	SE	0.06207	.849353	0.13719	0.036211
	P / R (%)	156.98	22.42	78.74	- 1.18
9.23	Mean	1.07971	46.2880	1.75989	1.10675
	SE	0.04848	1.441244	0.071815	0.08935
	P / R (%)	263.28	33.460	77.42	11.25
12.81	Mean	1.26826	48.4052	2.30746	1.47004
	SE	0.03010	2.76595	0.091615	0.056895
	P / R (%)	326.72	39.56	131.97	49.39
16.67	Mean	1.59566	56.5720	2.33787	1.86505
	SE	0.02605	1.39708	0.116435	0.077625
	P / R (%)	436.88	63.11	135.32	89.52

*, % promotion or reduction over control.

common phenomenon in plants under salinity (Ahmad *et al.,* 1985; Khan *et al.,* 1987, 1989 a and b) even in halophytes (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) (O' Leary, 1986). The fifty percent reduction in growth of *A. coriacea* seedlings corresponded to Seawater dilution

of ECiw: 14.94 ± 2.18 dS.m-1 which is somewhat comparable to *Acacia stenophylla* as reported to be 12.51 ± 1.51 $dS.m^{-1}$ (Sahito *et al.,* 2013).

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.,* 2013b) 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 (Boughalleb and Denden, 2011).

There was increase in proteins, total soluble sugars, proline and phenol contents under salt stress in phyllodes of *A. coriacea*. This metabolic response of *A. coriacea* resembles to *A. stenophylla* (Sahito *et al*., 2013). 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 have been reported to increase in *Medicago arborea* (Boughalleb *et al.,* 2011) under salinity. 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.

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) have 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).

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 Cl contents increased greatly under salinity in phyllodes of *A. coriacea*. 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 upon salt exposure. He and Cramer (1992) have also recorded reduction in K concentration under salinity. High Na / K ratio exerts metabolic toxic effect due to competition between Na and K for the binding

Table 6. Effects of irrigation with seawater dilutions on ionic Concentration in leaf.

Irrigation Medium ECiw	Statistics	Na (meq /L)	K (meq/L)	Cl (meq/L)
0.60	Mean	0.95332	2.30969	3.475
	SE	0.222926	.504437	.383786
	Mean	1.94387	1.25025	6.50
3.51	SE	0.15954	.292039	0.30277
	P / R (%)*	103.91	- 45.87	87.05
	Mean	3.61788	2.29614	8.55000
5.24	SE	0.25655	0.421127	0.45735
	P / R (%)	279.50	- 0.586	146.04
	Mean	3.88390	1.55261	9.60
9.23	SE	0.39540	.195110	0.18257
	P / R (%)	307.41	- 32.78	176.26
	Mean	3.15687	1.81236	10.0250
12.81	SE	0.35855	0.45099	0.41908
	P / R (%)	230.08	- 21.53	188.49
	Mean	3.87608	2.66578	10.90
16.67	SE	0.330510	.842993	0.56125
	P / R (%)	306.59	15.42	213.67

*, Percent promotion or reduction over control.

ECiw	Mean	SE	P/R (%)
0.60	3.44814	1.55082	-
3.51	0.65446	0.14951	- 81.92
5.24	0.62788	0.09582	- 81.78
9.23	0.42744	0.09112	- 87.60
12.81	0.61028	0.18164	- 82.30
16.67	0.68861	0.19117	- 80.03

Table 7. K / Na ratio in leaves of *A. Coriacea* irrigated with Seawater dilutions.

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. 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 other Acacias could be suspected to help plant in protecting disruption of K-mediated enzymatic processes under the salt stress.

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). Obviously, At higher salinities the effects of sodium accumulation

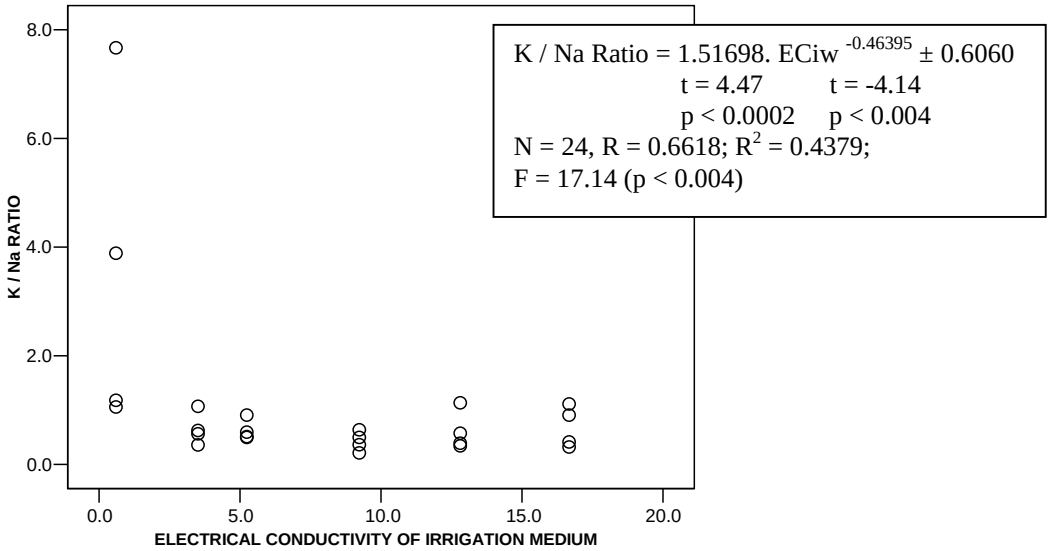

Fig. 1. Relationship of K / Na ratio with ECiw (dS.m^{-1}).

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

Chlorophyll-a = 0.4019 + 0.0118 ECiw ± 0.0766 (mg.g^{-1}.FW) t = 14.58 t = 4.17 p < 0.0001 p < 0.0004	r = 0.665; R^2 = 0.442; F = 17.41 N = 24	EQ. # 1
Chlorophyll-b = 0.220 + 0.008 ECiw ± 0.0985 (mg.g^{-1}.FW) t = 6.20 t = 2.19 p < 0.0001 p < 0.039	r = 0.424; R^2 = 0.180; F = 4.81 N = 24	EQ. # 2
Total Chlorophyll = 0.622 + 0.020 ECiw ± 0.1223 (mg.g^{-1}.FW) t = 14.12 t = 4.38 p < 0.0001 p < 0.0001	r = 0.682; R^2 = 0.466; F = 19.170 N = 24	EQ. # 3
Carotenoids = r = 0.194; R^2 = 0.038; F = 0.86 (NS) (mg.g^{-1}.FW)		EQ. # 4
Protein = 0.2694 + 0.08065 ECiw ± 0.10677 (mg.g^{-1}.FW) t = 20.40 t = 7.01 p < 0.0001 p < 0.0001	r = 0.975; R^2 = 0..834; F = 110.77 N = 24	EQ. # 5
Total Sugars = 34.634 + 0.1.246 ECiw ± 3.19843 (mg.g^{-1}.FW) t = 30.10 t = 10.53 p < 0.0001 p < 0.0001	r = 0.913; R^2 = 0.834; F = 110.27 N = 24	EQ. # 6
Phenols = 1.040 + 0.0.087 ECiw ± 0.326610 (mg.g^{-1}.FW) t = 8.84 t = 7.16 p < 0.0001 p < 0.0001	r = 0.836; R^2 = 0.699; F = 51.19 N = 24	EQ. # 7
Proline = 0.9860 + 0.0420 ECiw ± 0.27367 (mg.g^{-1}.FW) t = 10.01 t = 4.15 p < 0.0001 p < 0.0001	r = 0.662; R^2 = 0.439; F = 17.18 N = 24	EQ. # 8
Na = 1.692 + 0.151 ECiw ± 0..91712 (meq.L^{-1}) t = 5.12 t = 4.46 p < 0.0001 p < 0.0001	r = 0.689; R^2 = 0.476; F = 19.90 N = 24	EQ. # 9
K : r = 0.140; R^2 = 0.020; F = 0.442 (NS)		-

$Cl = 4.2268.\ ECiw^{0.354118} \pm 0.1263$ (meq.L^{-1}) t = 21.44 t = 15.17 p < 0.0001 p < 0.0001	r = 0.955; R^2 = 0.913; F = 229.94 N = 24	EQ. # 10

in the treated plants should result in mortality of plants as observed in case of few plants *A. stenophylla* under irrigation with water of ECiw: 12.81 and 16.67 $dS.m^{-1}$ (Sahito et al, 2013). No such mortality was observed in *A. coriacea* under such level of salinities. 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 135.32 % 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. coriacea subsp. pendens.*

The physiological phenomena such as increase of phyllode concentration of protein, total soluble sugars and proline and secondary metabolite like phenols under saline conditions may play significant role in the salt tolerance of *Acacia coriacea subsp. pendens* which may be rated as moderately salt tolerant taxon to the stress of multiple salts present in the Sea salt. The level of growth inhibition in salinity may depend upon 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). It was the contention of Heimann (1958) 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. There was no mortality of seedlings of the taxon in the given range of salinity.

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