

## GROWTH OF *VACHELLIA NILOTICA* SUBSP. *INDICA* (BENTH.) KYAL & BOATWR. (MIMOSACEAE) SEEDLINGS IN RELATION TO SEA SALT STRESS AND CHANGES IN BIOCHEMICAL AND IONIC COMPOSITION OF LEAVES

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### 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% Sea salt corresponding to ECiw of 0.6, 3.51, 5.23, 9.23, 12.81 and 16.67dS.m<sup>-1</sup>, respectively) on seedling growth and the physiological, biochemical and mineral parameters of growth in *Vachellia nilotica* subsp *indica* (Benth.) Kyal & Boatwr. (Syn. *Acacia nilotica* var. *indica* (Benth.) A.F. Hill). The ECiw of the irrigation medium corresponding to 50% reduction in various growth parameters, varied substantially from 8.16dS.m<sup>-1</sup> (based on number of leaves) to 19.07dS.m<sup>-1</sup> (based on stem length). On average basis 50% reduction in seedling growth performance, when all morphological parameters were taken into consideration corresponded to 10.64 ± 1.71 dS.m<sup>-1</sup>. Excluding the parameter of stem length that gave relatively higher value, 50% reduction in other parameters corresponded to an average value of 8.76 ± 0.338 dS.m<sup>-1</sup>. The salt tolerance index (STI) was high (> 90) in control and low salinity, moderate (50-90) in salinity from 5.23 to 12.81dS.m<sup>-1</sup> and low (< 50) in extreme salinity. STI slightly increased under low salinity and related to ECiw in a quadratic fashion. The response breadths of *V. nilotica* on the basis of different parameters of seedling growth ranged from 0.6198 to 0.9497 (mean niche breadth = 0.7701 ± 0.0469) on salinity gradient of 0.6 to 16.67dS.m<sup>-1</sup>. Foliar concentrations of protein, sugars, proline and phenols increased significantly with the salt stress and the pigments (chlorophylls and carotenoids) concentrations posed a declining behaviour. There was very high increase in foliar Na and Cl contents (317.88 and 253.07% over control, respectively) under extreme salinity of ECiw: 16.67dS.m<sup>-1</sup>. Foliar K concentration although increased substantially with salinity but K/Na ratio declined in treatments of higher salinities (ECiw: ≥ 9.23 dS.m<sup>-1</sup>).

**Key word:** *Vachellia nilotica* subsp. *indica*, growth, biochemical and mineral parameters, salinity tolerance

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### INTRODUCTION

Accounts of salt tolerance of many species of genus *Acacia* have been published (Park, 1995; Marcar *et al.*, 1991, 1995; Singh and Thompson, 1992; Choukr-Allah, 1997; Qureshi and Barrett-Lennard, 1998; Niknam and McComb, 2000; Jadoon and Jha, 2000; Ramoliya and Pandey, 2002; Akhtar *et al.*, 2003; Tewari *et al.*, 2006; Shirazi *et al.*, 2006; Ashraf *et al.*, 2012; Miah, 2013; Azhar *et al.*, 2013). Such studies have generally been undertaken in terms of soil salinity. There are few studies that have been conducted with the use of Seawater dilutions or brackish water for irrigation to investigate salt tolerance of acacias (Marcar *et al.*, 2003; Sahito and Khan, 2013; Sahito *et al.*, 2013; Dhupper *et al.*, 2013). 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 9.5dS.m<sup>-1</sup>. *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). Sahito and Khan (2013) and Sahito *et al.* (2013) have reported on salt tolerance of *Acacia coriacea* ssp. *pendens* and *Acacia stenophylla*, respectively under irrigation with Seawater dilutions. Nsereldeen Adam Ali (Pers. Comm., nsereldeen@gmail.com) on the basis of seedling growth under irrigation with Seawater dilutions (0.4 to 20 dS.m<sup>-1</sup>) reported *A. mellifera*, *A. tortilis* and *A. senegal* to be more tolerant to salinity than *A. nubica* and *A. seyal*. Six species of *Acacia* have been grown in Riyadh under irrigation for four years and compared for their growth and biomass production (Aref *et al.*, 2003). Survival and growth of 24 native species of Australia were tested near Wellington in Central-West-New South Wales on a saline discharge site by Marcar *et al.* (2003).

*A. nilotica* is well-adapted to a variety of soils and prefers well-drained soils. It is reported to show 40% reduction of growth at ECe of about 8 dS.m<sup>-1</sup> (Singh *et al.*, 1991). Fifty percent reduction in growth of *A. nilotica* was reported to be at ECe: 27.9 dS.m<sup>-1</sup> or 1.78% salts in soil (Ashraf *et al.*, 2012). Jadoon *et al.* (2000) reported that *A. nilotica* grew well but with retarded growth at 20 ppm fluorine containing saline water. Singh and Thompson (1992) reported *A. nilotica* to be moderately salt tolerant. Fifty percent reduction in biomass yield corresponded to 20 and 18 ppm fluorine in saline water of 4 and 8 dS.m<sup>-1</sup>. Uptake of fluorine increased with salinity. After nine months in Barisal (Bangla Desh), *A. nilotica* seedlings attained height of 117.01, 129.2, 127.36 and 132.24 cm in control, 10, 15, and 20 ppm salinity irrigations, respectively. Gupta *et al.* (1986) reported that *A. nilotica* withstood salinity as high as 15 dS.m<sup>-1</sup> and it can be grown with less than 50% growth reduction up to 5 dS.m<sup>-1</sup> salinity. Nabil and Coudret (2008) reported that two subspecies of *A. nilotica* (subsp. *cupressiformis* and subsp. *tomentosa*) grew

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healthy up to 100 mM NaCl. Abbas *et al.* (2013) investigated *A. ampliceps* and *A. nilotica* seedling growth under the influence of NaCl (100-400 mM) for 8 weeks. *A. ampliceps* was more tolerant to salinity owing to higher K / Na ratio. *A. nilotica* couldn't survive beyond 300 mM NaCl whereas *A. ampliceps* survived at 400 mM NaCl. Dhupper *et al.* (2013) reported *A. nilotica* to be moderately tolerant in medium level of salinity of irrigation water (4 and 8 dS.m<sup>-1</sup>). Mahmood *et al.* (2012) reported *A. nilotica* to be highly tolerant to salinity up to 80% Arabian Seawater. Dry matter based root / shoot ratio was shown to progressively increase from 1.32 in control to 4.7 in 80% Seawater.

Drought and salinity tolerance of plants and the influence of abiotic stress signals on metabolism of plants have been reviewed by a number of researchers (Yadav *et al.*, 1992; Bartels and Sunkar, 2005; Ramakrishna and Ravishankar, 2011; Mane *et al.*, 2011; Aslam *et al.*, 2011; Rahdari and Hoseini, 2011). The ability of plants to tolerate salts involves multiple biochemical pathways that facilitate retention or acquisition of water, protect chloroplast functions and maintain ion homeostasis and scavenging of oxygen radicals (Parvaiz and Satyawati, 2008; Shahid *et al.*, 2014). 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) and 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).

*Vachellia (Acacia) nilotica* is a widely distributed multi-purpose tree – very important in rural economy. It occurs from sea land to 2000m and can withstand > 50 °C (Bargali and Bargali, 2009). It has long growing period and four peaks of leaf flush (Beniwal *et al.* (1992). Since published data on salt tolerance of this species are inconsistent and varying greatly, we have undertaken experimental investigations related to the growth of a Sindh provenance of *Vachellia nilotica* subsp. *indica* (Benth.) Kyal & Boatwr (Syn. *Acacia nilotica* var. *indica* (Benth.) A.F. Hill) (See nomenclatural details in Ali, 2014) in pots while irrigated with Seawater dilutions to assess its salt tolerance and its possible scope in afforestation under saline irrigation in sandy soils. Besides growth and some important biochemical parameters of growth, foliar contents of Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> are also investigated.

## MATERIALS AND METHODS

The seeds of *V. nilotica* subsp. *indica* collected from its tree growing in Narejo village, District Khairpur Mirs, (riverine forest in Kacche Jo Ilaiqo), Sindh in 2012 were sterilized with sodium Hypochlorite (2%) for two minutes and stored in brown aseptic bottles for around a month.

### 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 collected from sand dunes of Sandspit, Karachi, was passed through a 2 mm sieve to remove gravel and other material. 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 per 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. Each treatment was replicated four times and pots were placed on a bench in the green house in random fashion. The seedlings 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 a crop of *V. nilotica* subsp. *indica* 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, 5.23, 9.23, 12.81 and 16.67 dS.m<sup>-1</sup>, respectively) was prepared by dissolving appropriate amount of Arabian Sea salt in tap water. The irrigation medium of 0.6 dS.m<sup>-1</sup> 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 level. 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 per plant, fresh and dry weight of root, stem and leaves. For dry weights, plant material was dried at 60 °C for 48 h in oven. The salt tolerance index (STI) was estimated by the method of Wu and Lin (1994) as:

$$STI = \frac{\text{Mean value of the salt treated seedlings' height} + \text{mean value of the salt treated seedlings' biomass}}{\text{Mean value of the control seedlings' height} + \text{mean value of the control seedlings' biomass}} \times 100$$

The index magnitude of > 90 indicates high tolerance; 50-90, moderate tolerance and < 50, low tolerance. The measure of the response breadth (RB) was calculated after Levins (1968) as

$$RB = 1 / \left[ \sum_{i=1}^s p_i^2 \right] s,$$

Where  $p_i$  is the proportion of any seedling growth parameter in the  $i$ th state and  $S$  is the total number of states (Treatments). RB ranges from 0 to 1 with 1 being the widest response breadth.  $EC_{50}$  corresponding to 50% reduction in a growth parameter ( $EC_{50}$ ) was determined by simple linear regression technique.

### Biochemical Analyses

Various physiological and biochemical parameters were estimated using standard methods. Foliar concentration of chlorophylls and carotenoids were estimated after Strain *et al.* (1971) and Duxbury and Yentsch (1956), respectively. The protein contents were determined by using Bradford Assay reagent method (Bradford, 1976). The determination of total sugars was determined by the method of Fales (1951), phenolic content by the method of Singleton and Rossi (1965), and the proline contents by the method of Bates *et al.* (1973). The mineral ions in leaf samples were determined according to the method of Chapman and Pratt (1961). The foliar concentration of Cl ion was determined by silver nitrate precipitation with chloridometer (HBI, model No. 4425150). The description of these methods in detail may be seen in Ali, Z. *et al.* (2013).

### RESULTS AND OBSERVATION

The sixty day-old seedlings of *V. nilotica* subsp. *indica* irrigated with various dilutions of un-amended Seawater ( $EC_{iw}$ : 0.60 to 16.67  $dS \cdot m^{-1}$ ) 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 including shoot and root lengths, their dry weights and number of leaves and their weight per seedling generally declined progressively with the rise of salinity of the irrigation medium (Table 1). At any level of salinity, shoot length was larger than root length except under extreme salinity tested when stem length was little smaller than root length (Root / Shoot ratio: 1.102). Dry weight based R/S ratio averaged to  $0.349 \pm 0.02$  under salinity treatments and 0.296 in control. There was 90.98% decline in number of leaves over control in extreme salinity. The dry mass of leaves also exhibited a pattern of decline with salinity, 92.32% over control under extreme salt stress. The decline over control was the minimum in root length (12.5%) in extreme salinity tested. The stem length reduced by 48.6% of the control in the treatment with  $EC_{iw}$ : 16.67  $dS \cdot m^{-1}$ . 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 exhibited initial promotion under low salinity regime but decline thereafter. There was loss of seedling weight with the increase of the salinity of the irrigation medium (Fig.1).

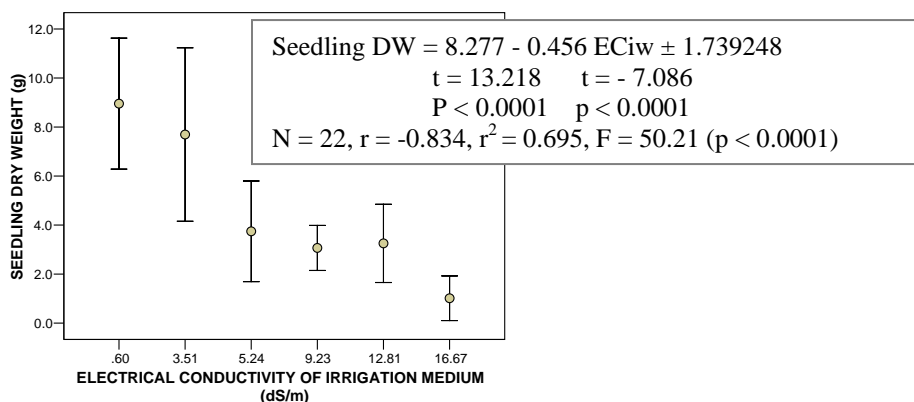


Fig. 1. Relationship of seedling dry weight with the salinity of the irrigation medium.

Table 1. Effects of irrigation with seawater dilutions on the growth of *V. nilotica* subsp. *Indica* seedlings.

Irrigation Medium ECiw	Statistics	Stem Length (cm)	Root Length (cm)	Stem Dry wt. (g)	Root Dry wt (g)	Leaf Dry wt (g)	Seedling Dry wt (g)	Number of Leaves
0.60	Mean	81.750	53.000	5.37950	1.59000	1.98500	8.95450	149.75
	SE	6.032896	10.1735	0.54577	0.200375	.252537	0.8398	11.8066
3.51	Mean	86.000	55.50	4.68750	1.42000	1.58750	7.69500	88.50
	SE	6.09645	4.9413	0.60269	0.201701	0.3309	1.11176	16.1168
	P/R (%)*	5.20	4.72	-12.86	-10.69	-20.03	-14.07	-40.90
5.24	Mean	68.750	39.750	2.3525	0.75050	0.64250	3.74550	49.50
	SE	2.2500	4.0901	.276326	.142257	0.30341	0.64535	7.2629
	P/R (%)	-15.90	-25.00	-56.27	-52.80	-67.63	-58.17	-66.94
9.23	Mean	67.6250	26.50	1.8575	0.67500	0.53500	3.06750	28.250
	SE	3.22345	6.1712	0.2488	0.229946	.237013	0.28854	3.9238
	P/R (%)	-17.41	-50.00	-65.47	-57.55	-53.02	-65.74	-81.14
12.81	Mean	66.750	36.875	2.1125	0.72000	0.4200	3.25250	28.750
	SE	2.1360	9.48766	0.21496	0.16366	.191703	0.50152	10.0778
	P/R (%)	-18.35	-30.42	-60.73	-54.72	-78.84	-63.68	-80.80
16.67	Mean	42.000	46.375	0.60750	0.25500	0.15250	1.01500	13.500
	SE	5.39676	9.0309	0.18291	0.06639	0.05089	0.28614	4.5735
	P/R (%)	-48.62	-12.50	-88.71	-83.96	-92.32	-88.66	-90.98

\*, Percent promotion or reduction over control.

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

Stem Length = $87.11 - 2.284 \text{ ECiw} \pm 10.4778$ (cm) $t = 23.09$ $t = -5.89$ $p < 0.0001$ $p < 0.0003$	$r = -0.782$ ; $r^2 = 0.612$ ; $F = 34.674$ $N = 24$	EQ. # 1
Root Length = $r = -0.258$ ; $r^2 = 0.067$ ; $F = 1.57$ ( $p < 0.277$ ) (NS) (cm)		-
Stem wt. (g) = $5.021 - 0.273 \text{ ECiw} \pm 1.01017$ $t = 13.80$ $t = -7.30$ $p < 0.0001$ $p < 0.0001$	$r = -0.841$ ; $r^2 = 0.708$ ; $F = 53.32$ $N = 24$	EQ. # 2
Root wt. (g) = $1.506 - 0.075 \text{ ECiw} \pm 0.37768$ $t = 11.08$ $t = -5.40$ $p < 0.0001$ $p < 0.0001$	$r = -0.755$ ; $r^2 = 0.570$ $F = 29.13$ $N = 24$	EQ. # 3
Leaf dry wt. (g) = $1.746 - 0.105 \text{ ECiw} \pm 0.5599$ $t = 8.54$ $t = -4.69$ $p < 0.0001$ $p < 0.0001$	$r = -0.724$ ; $R^2 = 0.524$ , $F = 21.99$ $N = 22$	EQ. # 4
Seedling wt. (g) = $8.277 - 0.456 \text{ ECiw} \pm 0.1.7392$ $t = 13.218$ $t = -7.086$ $P < 0.001$ $p < 0.0001$	$r = -0.834$ ; $r^2 = 0.695$ ; $F = 50.21$ $N = 22$	EQ. # 5
leaves / seedling = $119.105 - 7.295 \text{ ECiw} \pm 30.5233$ ; $t = 19.75$ $t = -7.05$ $p < 0.0001$ $P < 0.0001$	$r = -0.800$ ; $r^2 = 0.640$ ; $F = 35.59$ $N = 22$	EQ. # 6

The EC of the irrigation medium corresponding to 50% reduction in various growth parameters, as calculated on the basis of regression equations presented in Table 2 and Fig. 1, varied substantially from 8.16 dS.m<sup>-1</sup> (based on number of leaves) to 19.07 dS.m<sup>-1</sup> (based on stem length). On average basis 50% reduction, when all parameters are included, in seedling growth performance corresponded to 10.64 ± 1.71 dS.m<sup>-1</sup> (Table 3). Excluding the parameter

of stem length, average 50% reduction in other parameters corresponded to  $8.76 \pm 0.338 \text{ dS.m}^{-1}$  (Table 3) – moderate tolerance to salinity. There were two mortalities of seedlings – one in a treatment with 0.9% salt ( $\text{EC}_{\text{iw}}$ :  $12.81 \text{ dS.m}^{-1}$ ) and the other in 1.2% Sea salt ( $\text{EC}_{\text{iw}}$ :  $16.67 \text{ dS.m}^{-1}$ ).

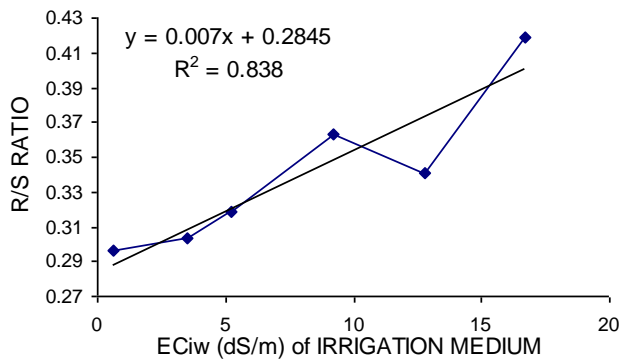


Fig.2. Relationship of dry mass based root / shoot ratio with salinity of the irrigation medium.

Table 3. Salinity of irrigation medium corresponding to 50% reduction in growth of *V. nilotica* subsp. *indica* seedlings as determined by linear regression analysis.

Parameters of Seedling growth	$\text{EC}_{\text{iw}}$ ( $\text{dS.m}^{-1}$ ) (50% reduction in growth)	Mean $\pm$ SE ( $\text{dS.m}^{-1}$ )
Shoot Length	19.07	$10.64 \pm 1.71^*$
Root Length	-	
Stem dry weight	9.196	$8.76 \pm 0.338^{**}$
Root dry weight	10.04	
Weight of Leaves	8.314	
Number of leaves per seedling	8.163	
Seedling dry weight	9.076	

\*, Including shoot declining value; \*\*, excluding shoot length declining value.

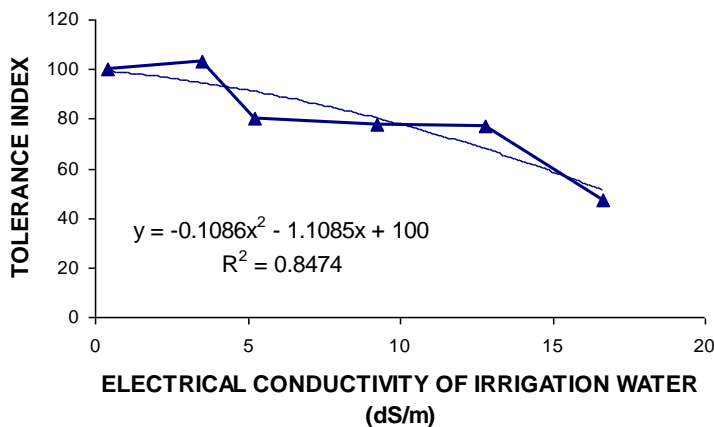


Figure 3. Tolerance index of *V. nilotica* subsp. *indica* seedlings as the function of the irrigation water salinity.

The biomass-based root / shoot ratio increased more or less progressively with the salinity of the irrigation medium ( $r = 0.838$ ) (Fig. 2). The salt tolerance index (STI) calculated according to Wu and Lin (1974), however, declined with salinity in a curvilinear fashion ( $R = 0.8474$ ) (Fig. 3).

The salt tolerance index (STI) was high ( $> 90$ ) in control and low salinity, moderate (50-90) in salinity from 5.23 to 12.81  $\text{dS}\cdot\text{m}^{-1}$  and low ( $< 50$ ) in extreme salinity. STI slightly increased under low salinity and related to  $\text{EC}_{\text{iw}}$  in a quadratic fashion (Fig. 3). The fifty per cent reduction in STI corresponded with  $\text{EC}_{\text{iw}}16.95\text{dS}\cdot\text{m}^{-1}$  indicating good salinity tolerance of this provenance of *V. nilotica* subsp. *indica* from Narejo village, District Khairpur Mirs, (riverine forest in Kacche Jo Ilaiqo), Sindh in pot culture experiment under irrigation with Seawater dilutions. The response breadths on the salinity gradient calculated after Levins (1968) were the widest for stem and root lengths, moderate for stem, root and seedling biomass but low for the number of leaves and leaf biomass (Fig. 4). Electrical conductivities corresponding to 50% reduction ( $\text{EC}_{50}$ ) in various seedling growth parameters had a very close association with the response breadths calculated on the basis of same characteristics (Spearman rho: 0.999) (Fig. 5). However, it comes to mind that determination of  $\text{EC}_{50}$  is more appropriate because it relates to the plant performance in direct terms of salinity.

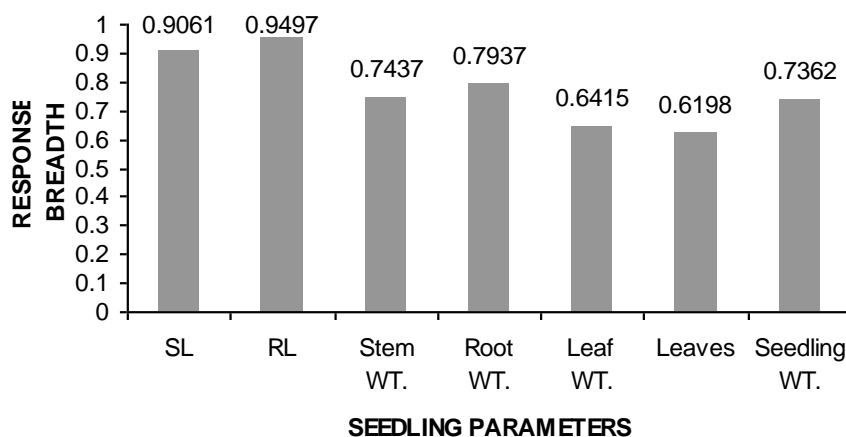


Figure 4. Response breadths based on various seedling parameters of *V. nilotica* subsp. *indica* under salinity.

The concentration of chlorophyll-a, chlorophyll-b and total chlorophyll substantially declined by 92.12, 66.64 and 78.75%, respectively under high salinity (Table 4). Under low salinities of 3.51 and 5.24  $\text{dS}\cdot\text{m}^{-1}$  the inhibition of chlorophylls concentrations was low but very high under extreme salinity. Carotenoids contents behaved in similar manner (Table 4).

Under irrigation with Saline water ( $\text{EC}_{\text{iw}}$ : 0.60 to 16.67  $\text{dS}\cdot\text{m}^{-1}$ ) leaf protein contents increased gradually and progressively up to 110.48% over control in high salinity. Similar trends of increase in leaf total soluble sugars, phenols and proline concentrations were exhibited with promotion over control as high as 48.59, 139.02 and 86.13%, respectively under extreme salt stress (Table 5). Best fit significant regression equations for biochemical parameters against salinity are presented in Table 6.

Sodium concentration in leaves increased with salinity in substantial amounts – around 292.6% over control under salinity treatment of  $\text{EC}_{\text{iw}}$ : 12.81 and 317.88% over control under  $\text{EC}_{\text{iw}}$ : 16.67 $\text{dS}\cdot\text{m}^{-1}$ . K concentration, with some irregularity showed a increasing trend with increase of salt concentration. There was regular increase in chloride concentration along the salinity gradient. It increased by 253.07% over control under saline water irrigation of 16.67 $\text{dS}\cdot\text{m}^{-1}$  (Table 7). The slope of Na increment per unit increase of  $\text{EC}_{\text{iw}}$  ( $b = 0.481 \text{ meq/L}$ ) was substantially higher (Eq. 9, Table 6) than that of K ( $b = 0.134$ ; Eq. 10, Table 6) i.e. Na increment was 3.59 times to that of K. The foliar K/Na ratio increased in lower salinities but decreased higher salinity levels (Table 8). Na / K ratio increased continuously. The rate of Chloride in leaf per unit increase of salinity was higher than that of Na and K. K / Na ratio related with salinity linearly inversely (Fig. 6). Contrary to it, Na / K ratio increased with salinity directly. These relationships were as follows:

$$\text{K / Na Ratio} = 1.005436 - 0.040499 \text{ EC}_{\text{iw}} \pm 0.2855$$

$$t = 10.115 \quad t = -3.54$$

$$p < 0.0001 \quad p < 0.021; N = 22; r = -0.6208; r^2 = 0.3854; F = 12.54(p < 0.02)$$

$$\text{Na / K Ratio} = 1.004209 + 0.086856 \text{ EC}_{\text{iw}} \pm 0.6887$$

$$t = 3.99 \quad t = 3.15$$

$$p < 0.007 \quad p < 0.051; N = 22; r = 0.5756; r^2 = 0.3313; F = 9.91 (p < 0.05)$$

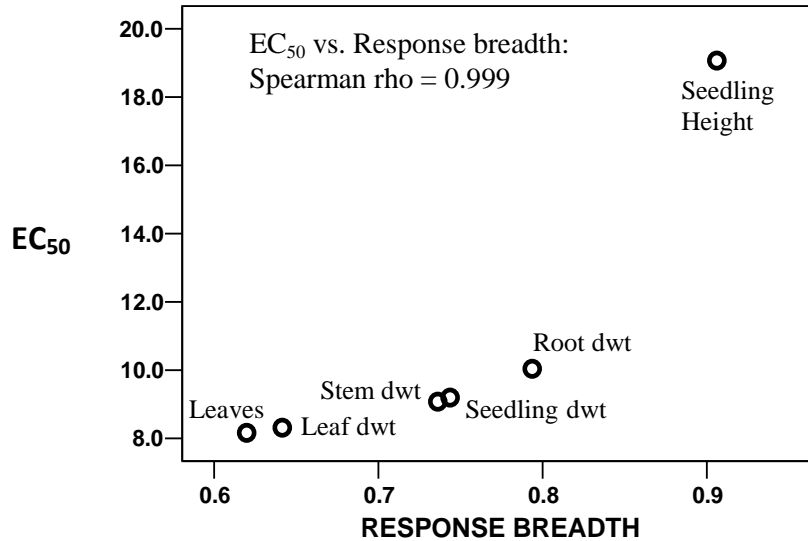


Figure 5. Relationship of EC<sub>50</sub> (dS.m<sup>-1</sup>) for various seedling growth parameters (except root length) and their response breadths.

## DISCUSSION

The experiment was conducted to investigate the influence of Sea salt salinity on the seedling growth and the physiological, biochemical and mineral parameters of growth in *Vachellia nilotica subsp. indica*, a Sindh provenance. Seawater irrigation inhibited the growth parameters significantly as a direct function of Sea salt stress. The salt tolerance index (STI) of Wu and Lin (1994) was high (> 90) in control and low salinity, moderate (50-90) in salinity from 5.23 to 12.81 dS.m<sup>-1</sup> and low (< 50) in extreme salinity. From quadratic relationship of STI and salinity, 50% reduction in STI corresponded with EC<sub>iw</sub>: 16.95 dS.m<sup>-1</sup>. Being based on relative value of height plus biomass in non-saline and saline state, this index seems to overestimate the tolerance in the present case. Taken together the results the provenance in hand may be rated as substantially tolerant to salinity with retarded growth under high salinity. A decrease in plant growth under salinity is a common phenomenon (Ahmad *et al.*, 1985); even in halophytes (Boughalleb and Denden, 2011). The growth suppression in plants under salinity is 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 of growth corresponded to 10.64 ± 1.71 dS.m<sup>-1</sup> was somewhat lower than that for Australian acacia species viz. *Acacia stenophylla* (12.51 ± 1.51) dS.m<sup>-1</sup> (Sahito *et al.*, 2013) and *Acacia coriacea* (14.94 ± 2.18 dS.m<sup>-1</sup>) (Sahito and Khan, 2013). Ashraf *et al.* (2012) reported 50% reduction in *A. nilotica* growth at soil salinity of EC: 27.9 dS.m<sup>-1</sup>. Gupta *et al.* (1986) reported that *A. nilotica* can withstand salinity as high as 15dS.m<sup>-1</sup> and may grow with 50% growth reduction up to 5dS.m<sup>-1</sup> salinity. The two subspecies of *Acacia nilotica* viz. subsp. *cupressiformis* and subsp. *tomentosa* are reported to grew well up to 100mM NaCl (EC: 14.62dS.m<sup>-1</sup>) (Nabil and Coudret (2008). Dhupper *et al.* (2013) have reported *A. nilotica* to be moderately tolerant under medium level of salinity of irrigation medium (8.0 dS.m<sup>-1</sup>; SAR: 8.29). They reported data on *Acacia nilotica* seedling height, stem diameter and total biomass at one year of age. Little calculation by us with their growth equations indicated 50% reduction in above parameters at EC<sub>iw</sub>: 22.03, 29.12, and 28.40 dS.m<sup>-1</sup>, respectively. Ramoliya and Pandey (2002) reported that *A. nilotica* could grow in soil salinity of ECe: 12.2 dS.m<sup>-1</sup> but with retarded elongation of stem and root and leaf tissue exhibiting the maximum reduction at this salinity. Young roots were continuously produced as the old roots died. They suggested that *A. nilotica* removed excessive salts through a phenomenon of "fine-root-turnover" so crucial to the salt tolerance of this species according to them. Miah (2013) has, however, reported increase in *A. nilotica* height in salinity.

Plants that are sensitive to salts tend to have a decrease in growth of 50% at 4-5 dS.m<sup>-1</sup> (7-9% Seawater). Halophytes are highly salt tolerant and have increased growth rate at 4-5 dS.m<sup>-1</sup> and still have a 50% decrease in growth at 50dS.m<sup>-1</sup> (87% Seawater). Salt tolerant non-halophytes maintain growth at low salinity (5-10dS.m<sup>-1</sup>) and gradually reduce growth rate as salinity rises (Barrett-Lennard *et al.*, 2003). As per data of Mahmood *et al.* (2012), 50% reduction in plant height corresponded to EC<sub>iw</sub>: 42.36 dS.m<sup>-1</sup> (calculation is ours - Shoot length (cm) = 18.30 - 0.216 dS.m<sup>-1</sup>, r = 0.931, F = 25.98, p < 0.007). This level of salinity tolerance is comparable to several truly halophytic plants (Aronson, 1987; Masters *et al.*, 2007). The reason of stunting of roots (admeasuring 2.5 cm in

control to 4.5 in 80% Seawater) in Mahmood *et al.* (2012) experiment is not known. However, inoculation with rhizobial nodular extract could have been the possible reason to enhance root growth and elevating biomass-based root / shoot ratio from 1.32 in control to 4.7 in 80% Seawater in 12-week old seedlings treated with salinity for last four weeks of their life.

It was evident that different seedling growth parameters were differentially affected by the salinity as is obvious from the niche breadths of various seedling parameters ranging from 0.6198 to 0.9497 (mean =  $0.7701 \pm 0.0469$ ) on salinity gradient of 0.6 to 16.87 dS.m<sup>-1</sup> (Seawater dilutions). The various parameters of seedling growth of *Acacia nilotica* exhibited somewhat comparable response breadths (0.669 – 0.866) on salinity gradient of ECe: 5-15 dS.m<sup>-1</sup> created by addition of NaCl in soil (Tewari *et al.*, 2006). *Acacia nilotica* is considered to be an early successional species which should have wider habitat ranges (Parrish and Bazzaz, 1982). Tewari *et al.* (2006) have demonstrated *A. nilotica* to have wider niche breadths under salinity and sodicity gradients tolerating higher sodicity (ESP: 40) and salinity up to ECe: 7.5 dS.m<sup>-1</sup>.

There was decline in photosynthetic pigments, chlorophylls and carotenoids under salinity in *V. nilotica*. 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) as common response of plants to salinity which is suggested to be due to inhibition of iron-containing enzymes which activates the biosynthesis of chlorophyll (Rubin and Artiskhovskaya, 1964). Carotenoids were also recorded to decline in *Acacia stenophylla* and *A. coriacea* subsp. *pendens* under irrigation with Seawater dilutions (Sahito *et al.*, 2013; Sahito and Khan, 2013) and in *Sorghum* cultivars (Mr. Burger, Honey Graze and Extra Sweet) under 50 and 100mM NaCl (Ali *et al.*, 2013). 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 been suggested to be related to higher carotenoids accumulation in *N. retusa* (Boughalleb and Denden, 2011). There is more leakage of metabolites from chloroplasts of glycophytes than halophytes (Huchzermeyer *et al.*, 2004)

Table 4. Effects of Irrigation with Seawater dilutions on photosynthetic pigments. \*, Percent promotion or reduction over control.

Irrigation Medium ECiw	Statistics	Chlorophyll-a	Chlorophyll-b	Total Chlorophyll	Carotenoids
0.60	Mean	0.54059	0.38087	0.84920	0.34640
	SE	0.017815	0.017758	0.091547	0.011485
3.51	Mean	0.47014	0.36196	0.77636	0.29732
	SE	0.05222	0.05312	0.142855	0.03027
	P / R (%)*	-13.03	-4.96	-8.58	-14.69
5.24	Mean	0.46239	0.37713	0.83952	0.32900
	SE	0.03906	0.04050	.078685	.025194
	P / R (%)	-14.47	-0.982	-1.14	-5.02
9.23	Mean	0.12357	0.11272	0.23628	0.15655
	SE	0.03204	0.01923	0.013713	0.00646
	P / R (%)	-77.14	-70.40	-61.27	-54.81
12.81	Mean	0.09765	0.11992	0.21757	0.16231
	SE	0.01160	0.01099	0.005450	0.01313
	P / R (%)	-81.95	-68.51	-74.35	-0.5314
16.67	Mean	0.05341	0.12705	0.18046	0.10187
	SE	0.00182	0.01565	0.01738	0.00685
	P / R (%)	-90.12	-66.64	-78.75	-70.59

\*, Percent promotion or reduction over control.

There was increase in proteins, total soluble sugars, proline and phenol contents under salt stress in the leaves of *V. nilotica* subsp. *indica*. This metabolic response resembles to *A. stenophylla* (Sahito *et al.*, 2013) and *A. coriacea*



(Sahito and Khan, 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). 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 (1998) also reported significant promotion in sugar accumulation in salt excretive *Sporobolus arabicus*. Relatively salt tolerant legume, *Indigofera oblongifolia* (reducing growth by 50 % at ECiw:  $12.05 \pm 0.92$  dS.m<sup>-1</sup>) 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 irrigation with seawater dilutions on some biochemical parameters of growth of *V. nilotica* subsp. *indica* seedlings.

Irrigation Medium (ECiw)	Statistics	Protein (mg.g <sup>-1</sup> .FW)	Sugar (mg.g <sup>-1</sup> .FW)	Phenols (mg.g <sup>-1</sup> .FW)	Proline (mg.g <sup>-1</sup> .FW)
0.60	Mean	0.81607	40.9827	1.2048	1.6010
	SE	0.06997	2.57783	.12089	0.0547
3.51	Mean	0.97772	42.5596	1.6819	1.3131
	SE	0.05196	1.34242	.05341	0.14224
	P / R (%)*	19.89	3.85	39.60	-17.98
5.24	Mean	1.28626	46.1499	1.8486	1.6270
	SE	0.07696	2.39249	0.08373	0.02870
	P / R (%)	57.62	12.61	53.09	1.62
9.23	Mean	1.43196	53.6824	2.4162	1.2043
	SE	0.07273	2.91404	0.08822	0.65711
	P / R (%)	75.47	30.99	100.55	-24.77
12.81	Mean	1.76850	61.5721	2.5111	2.9800
	SE	0.02443	0.72253	0.09448	0.17010
	P / R (%)	116.71	50.24	108.42	86.13
16.67	Mean	1.71851	60.8996	2.8797	2.9800
	SE	0.32087	2.91596	0.10671	0.22716
	P / R (%)	110.58	48.59	139.02	86.13

\*, Percent promotion or reduction over control.

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, promotion of protein concentration, proline also increased substantially in *V. nilotica* subsp. *indica*. Proline is reported to increase under water stress (Al-Jebory, 2012) and salt stress (Strogonov, 1964; Rozema, 1978; Rains *et al.*, 1982; Delauney and Verma (1993; Joshi *et al.*, 2005). Its accumulation under stress is considered

beneficial for plant growth (Rozema, 1978; Rains *et al.*, 1982; Nawaz *et al.*, 2012). 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 and stabilization (Schobert and Tschesche, 1978; Yancey *et al.*, 1982), it is compatible in permeability to cytoplasm and maintains turgor and prevents the dehydration of enzymes and other essential structures (Yancey *et al.*, 1982; 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 several factors as temperature, aeration 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 leaves of *V. nilotica* subsp. *indica*. 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 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. Shirazi *et al.* (2006) grew some exotic and native species (including *A. nilotica*) in saline sodic silty clay to clay loam type of soils with salinity (ECe) 15.5 to 60.0 dS.m<sup>-1</sup> in upper soil layer (0-30 cm) and 9.10 to 51.2 dS.m<sup>-1</sup> 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<sup>-1</sup>, respectively and K from 1.41 – 5.0 and 1.03 to 1.92 meq.L<sup>-1</sup>, respectively. The maximum concentration of K recorded in leaves of *A. nilotica* by these workers was 0.76% against Na 0.15% that is K / Na ratio of 5.06 which is much higher than that we found under Seawater irrigation of ECiw: ≥ 9.23 dS.m<sup>-1</sup>. Dhupper *et al.* (2013) under saline irrigation, have reported Na, K and Ca and Mg to increase in leaves of *A. nilotica* with salinity. Na / K ratio increase was from 0.45 in the control to 0.734 under moderate salinity (8.0 dS.m<sup>-1</sup>). There was more K in the leaves than Na. K / Na ratio, however tended to decline under salinity from 2.22 in control to 1.36 under irrigation with water of 8.0 dS.m<sup>-1</sup>. Abbas *et al.* (2013) investigated *A. ampliceps* and *A. nilotica* seedlings growth under the influence of NaCl (100-400 mM NaCl) for 8 weeks. *A. ampliceps* was more tolerant to salinity owing to higher K / Na ratio. In *A. nilotica*, K / Na ratio ranged from 0.3 to 0.4 in root and 0.3 to 0.8 in shoot. Beyond 300 mM NaCl, *A. nilotica* couldn't survive whereas *A. ampliceps* survived at 400 mM NaCl. Singh and Thompson (1992) on the basis of K and Na distribution in root, stem and leaf rated *A. nilotica* to be slightly more tolerant to salinity than *Prosopis juliflora*. In comparison the salt tolerance of *V. nilotica* subsp. *indica* in our studies appeared to be of lesser degree than *P. juliflora*. The EC<sub>50</sub> of 65-day old *P. juliflora* seedlings was found to be 21.3 dS.m<sup>-1</sup> under Seawater irrigation (Khan *et al.*, 1987). Both, *A. nilotica* and *P. juliflora*, however, showed rapid translocation of K from root to stem and from stem to leaf (Khan *et al.*, 1987; Singh and Thompson, 1992).

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<sup>-1</sup>. Such an association of *Glomus* sp. with *V. nilotica* subsp. *indica* may help plant in protecting disruption of K-mediated enzymatic processes under the salt stress which needs further research. Lal and Khanna (1994) have isolated two salinity tolerant strains of *Rhizobium* from *A. nilotica* which tolerated up to 850 mM NaCl. These isolates can, therefore, modulate and fix Nitrogen in saline soils in *A. nilotica* association.

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 two plants under irrigation with water of EC<sub>iw</sub>: 12.81 and 16.67 dS.m<sup>-1</sup> where the pot soil salinity reached to 21.0 and 17.5 dS.m<sup>-1</sup>, respectively after 20 saline irrigations, presumably due to faulty drainage. 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 in foliar phenolic contents in *V. nilotica* up to 139.02 % 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 *V. nilotica subsp. indica*.

Table 6 Best-fit linear equations of regression between salinity (Xi) and biochemical parameters (Yi).

Chlorophyll -a = 0.572 - 0.035EC <sub>iw</sub> ± 0.092018 t = 17.04 t = -9.59 p < 0.0001 p < 0.0001	r = -0.906; R <sup>2</sup> = 0.821; F = 91.96 N = 22	EQ. # 1
Chlorophyll-b = 0.4114 - 0.020758 EC <sub>iw</sub> ± 0.08629 t = 13.06 t = -6.01 p < 0.0001 p < 0.0001	r = - 0.802; R <sup>2</sup> = 0.643; F = 36.06 N = 22	EQ. # 2
Total Chlorophyll = 0.927 - 0.052 EC <sub>iw</sub> ± 0.2000 t = 12.69 t = -6.44 p < 0.0001 p < 0.0001	r = - 0.821; R <sup>2</sup> = 0.674; F = 41.42 N = 22	EQ. # 3
Carotenoids = 0.3630 - 0.0016 EC <sub>iw</sub> ± 0.04801 t = 20.73 t = - 8.59 P < 0.0001 p < 0.0001	r = - 0.887; R <sup>2</sup> = 0.786; F = 73.42 N = 22	EQ. # 4
Protein = 0. 8390 + 0.062 EC <sub>iw</sub> ± 0.23173 (mg.g <sup>-1</sup> .FW) t = 9.91 t = 0.656 p < 0.0001 p < 0.0001	r = 0.830; R <sup>2</sup> = 0.689; F = 44.310 N = 22	EQ. # 5
Total Sugars = 39.212 + 1.464 EC <sub>iw</sub> ± 4.1228 (mg.g <sup>-1</sup> .FW) t = 23.75 t = 8.08 p < 0.0001 p < 0.0001	r = 0.875; R <sup>2</sup> = 0.765; F = 65.28 N = 22	EQ. # 6
Phenols = 1.279 + 0.102 EC <sub>iw</sub> ± 0.1994 (mg.g <sup>-1</sup> .FW) t = 17.58 t = 12.80 p < 0.0001 p < 0.0001	r = 0.944; R <sup>2</sup> = 0.891; F = 163.79 N = 22	EQ. # 7
Proline = 1.120 + 0.110 EC <sub>iw</sub> ± 0.7313 (mg.g <sup>-1</sup> .FW) t = 4.20 t = 3.40 p < 0.0001 p < 0.0001	r = 0.605; R <sup>2</sup> = 0.366; F = 11.57 N = 22	EQ. # 8
Na = 1.209 + 0.481 EC <sub>iw</sub> ± 1.1142 (meq.L <sup>-1</sup> ) t = 2.97 t = 10.77 p < 0.019 p < 0.0001	r = 0.924; R <sup>2</sup> = 0.853; F = 115.95 N = 22	EQ. # 9
K = 1.934 + 0.134 EC <sub>iw</sub> ± 0.87109 (meq.L <sup>-1</sup> ) t = 6.08 t = 3.85 p < 0.019 p < 0.001	r = 0.653; R <sup>2</sup> = 0.426; F = 14.85 N = 22	EQ. # 10
Cl = 3.152 + 0.512 EC <sub>iw</sub> ± 0.9191 (meq.L <sup>-1</sup> ) t = 9.39 t = 13.89 p < 0.0001 p < 0.0001	r = 0.952; R <sup>2</sup> = 0.906; F = 193.05 N = 22	EQ. # 11

The physiological phenomena such as increase of foliar concentration of protein, sugars and proline and secondary metabolites like phenols under saline conditions may play significant role in the salt tolerance of *Vachellia nilotica subsp. indica* which may be rated as moderately salt tolerant legume to multiple salts present in the Arabian Sea salt. The properties of salt tolerance may, however, change with the development of plant and may also change with type of salinity (Strogonov, 1964). The level of growth inhibition under salinity may depend on several genomic, ecological and agronomic factors (Gupta, 1990). It was the contention of Heimann (1958) that within certain limits of salinity, 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. The higher salinities of irrigation water even in sandy stratum (EC<sub>iw</sub>: ≥ 12.81 dS.m<sup>-1</sup>) may, somehow,

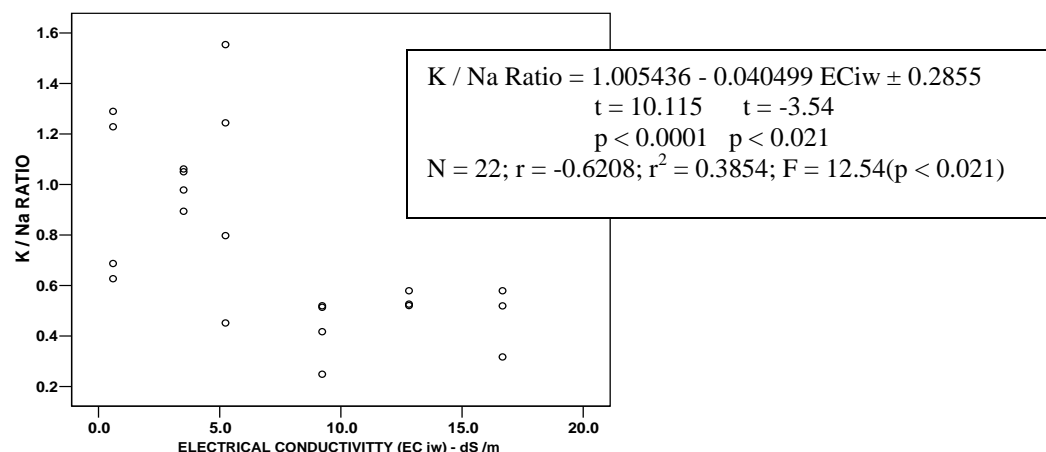


Figure 6. Relationship of K / Na ratio in leaves of *V. nilotica* subsp. *indica* with the salinity of the irrigation water.

Table 7. Foliar concentration of Na, K and Cl ions.

Irrigation Medium (ECiw)	Statistics	Na (meq/L)	K (meq/L)	Cl (meq/L)
0.60	Mean	2.1321	2.0520	3.1250
	SE	0.16651	0.43448	0.58363
3.51	Mean	2.7435	2.7418	4.5750
	SE	0.16162	0.22551	0.26887
	P/R (%)*	29.21	33.62	46.4
5.24	Mean	2.8137	2.5552	6.4500
	SE	.55528	0.43504	0.27234
	P/R (%)	31.97	24.52	106.4
9.23	Mean	5.5549	2.2792	8.1500
	SE	0.52234	0.25438	0.48045
	P/R (%)	160.44	11.07	160.8
12.81	Mean	8.3707	4.5495	10.1000
	SE	0.55046	0.41335	0.17321
	P/R (%)	292.60	121.72	223.2
16.67	Mean	8.9097	4.0889	11.0333
	SE	1.07961	0.59409	0.76884
	P/ R (%)*	317.88	99.26	253.07

\*, Percent promotion or reduction over control.

Table 8. K / Na Ratio in leaves of *V. nilotica* subsp. *indica* seedlings irrigated with seawater dilutions

Irrigation Medium (ECiw)	Mean	SE	P / R (%)*
0.60	0.9580	0.175	-
3.51	0.9660	0.039	3.97
5.24	1.0118	0.243	5.611
9.23	0.4249	0.063	-55.65
12.81	0.3421	0.019	-43.41
16.67	0.4719	0.079	-50.74

prove toxic to the plant and cause substantial mortality. In present studies un-amended seawater dilutions were used. Amendments of the irrigation media to combat toxicity of high sodium concentration may further improve the salt tolerance of the in hand provenance of *V. nilotica* subsp. *indica* (Ahmad *et al.*, 1984).

It is apparent from the above discussion and also in agreement with Marcar *et al.* (1979) that there exists genetic variability in acacias to salinity tolerance. Wardill *et al.* (2006) has pointed out genetic diversity in *Acacia nilotica* subsp. *indica* (Benth.) Brenan which is naturalized in Queensland and the Northern territory of Australia and more than one intraspecific taxon may be present there. The variation in salt tolerance even may exist at species, subspecies and provenance level. Park (1995) has reported that of the two Kenyan provenances of *A. tortilis*, 'Sigor' provenance is more salt tolerant than 'Kitui'. Some provenances of *A. ampliceps* have been reported to survive at 65 dS.m<sup>-1</sup> (Aswathappa *et al.*, 1987). In Pakistan, 2-year old plants of this species had 25-50% reduction in dry weight at 17-20 dS.m<sup>-1</sup> (data of Ansari *et al.*, 1994 as calculated by Qureshi and Barrett-Lennard, 1998). The variation of

salinity tolerance in *Vachellia nilotica* should be investigated at subspecies and provenance levels under a well-defined protocol to facilitate comparison.

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