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Growth, water status and nutrient accumulation of seedlings of *Salvadora oleoides* (Decne.) in response to soil salinity

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Abstract: We investigated the response of *Salvadora oleoides* Decne. (Salvadoraceae), a dominant tree in the arid regions of western India and Pakistan, to a range of soil salinities. Greenhouse experiments were conducted to assess the effects of soil salinity on seedling emergence, growth, water status, proline content and mineral accumulation. Sodium chloride was added to the soil and salinity was maintained at 0.2, 3.9, 6.2, 8.1, 10.0, 11.9 and 13.9 dS m^{-1}. Salinity caused reduction in water content and water potential of tissues, which resulted in internal water deficit to plants. Consequently, seedling growth decreased and proline content in tissues increased with increase in salinity. This species has high selectivity for potassium but lacks effective mechanisms to control net uptake of sodium and its transport to shoots. Phosphorus, calcium and magnesium content in tissues significantly decreased while nitrogen content increased as salinity increased. We discuss changes in tissues and whole-plant accumulation patterns of nutrients, as well as possible mechanisms for avoidance of sodium toxicity in this species in response to salinity.

Resumen: Investigamos la respuesta de Salvadora oleoides Decne. (Salvadoraceae), un árbol dominante en las regiones áridas de la India Occidental y de Pakistán, a una gama de valores de salinidad del suelo. Se realizaron experimentos de invernadero para evaluar los efectos de la salinidad del suelo sobre la emergencia, el crecimiento, el estatus hídrico, el contenido de prolina y la acumulación mineral de las plántulas. Se añadió cloruro de sodio al suelo y la salinidad se mantuvo a 0.2, 3.9, 6.2, 8.1, 10.0, 11.9 y 13.9 dS m^{-1}. La salinidad causó la reducción en el contenido de agua y el potencial hídrico de los tejidos, lo cual resultó en déficits internos de agua en las plantas. En consecuencia, decreció el crecimiento de las plántulas y el contenido de prolina en los tejido incrementó con la salinidad. Esta especie tiene una selectividad alta para el potasio pero carece de mecanismos efectivos para controlar la entrada neta de sodio y su transporte a los tallos. Los contenidos de fósforo, calcio y magnesio en los tejidos disminuyeron significativamente, mientras que el contenido de nitrógeno se incrementó conforme aumentó la salinidad. Discutimos los cambios en los patrones de acumulación de nutrientes en los tejidos y en la planta completa, así como posibles mecanismos para evitar la toxicidad por sodio en esta especie en respuesta a la salinidad.

Resumo: Investigou-se a resposta da Salvadora oleoides Decne (Salvadoraceae), uma árvore dominante nas regiões áridas na parte ocidental da Índia e Paquistão, a uma gama de salinidades do solo. Ensaios em estufa foram conduzidos para avaliar os efeitos da salinidade do solo na emergência das plântulas, crescimento, status hídrico, teor em prolina e acumulação de minerais. O cloreto de sódio foi adicionado ao solo e a salinidade foi mantida a 0,2, 3,9, 6,2, 8,1, 10,0, 11,9 e 13,9 dS m^{-1}. A salinidade causou a redução no teor de água e no potencial hídrico dos tecidos, de que resultou um deficit hídrico interno nas plantas. Consequentemente, o crescimento das plântulas decresceu e o teor em prolina nos tecidos aumentou com o aumento da salinidade. Estas espécies apresentam uma elevada selectividade para o potássio mas falta-lhes um mecanismo efectivo para controlar a absorção do sódio e o seu transporte para os

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rebentos. Os teores de fósforo, de cálcio e de magnésio nos tecidos decresceu significativamente enquanto o teor do azoto aumentou com o aumento da salinidade. Discutem-se as mudanças nos tecidos e nos padrões de acumulação dos nutrientes na totalidade da planta bem como os possíveis mecanismos para evitar a toxicidade ao sódio nesta espécie em resposta à salinidade.

Key words: Macro- and micro-nutrients, proline content, salt tolerance, seedling growth, soil salinity, water potential.

Introduction

Soil salinity has detrimental effects on seed germination and plant growth (Bernstein 1962; Patel & Pandey 2008; Patel *et al.* 2010; Ramoliya *et al.* 2006; Taiz & Zeiger 2006). However, plant species differ in their sensitivity or tolerance to salts (Marschner 1995). There is evidence that plant tissues exhibit varying degrees of tolerance to environmental conditions at different developmental stages (Munns 1993). It is reported that soil salinity suppresses shoot growth more than root growth (Maas & Hoffman 1977; Munns 2002; Ramoliya *et al.* 2006). However, there have been fewer studies on the effect of soil salinity on root growth than on shoot growth (Munns 2002). High salinity lowers the osmotic potential of soil water and consequently the availability of soil water to plants. This salt-induced water deficit is one of the main constraints for plant growth in saline soils. In addition to induced water stress, salt-stressed plants also experience other nutrient interactions that may have detrimental consequences for growth and survival. The ionic ratios of saline soils, which differ from those of non-saline soils, affect the net uptake of nutrients by roots and subsequently their transport to different tissues of plants. The uptake of major nutrients by plants growing in saline soils, and the concentrations of these nutrients in plant tissues have been frequently studied (e.g., Cramer *et al.* 1989; Maas & Grieve 1987; Patel & Pandey 2007; Patel *et al.* 2010; Ramoliya *et al.* 2006). However, the relationship between micro-nutrient concentrations and soil salinity is complex and remains poorly understood (Tozlu *et al.* 2000). An investigation of growth and survival of plants on saline substrates can provide insights into the mechanisms that plants use in the avoidance and /or tolerance of salt stress. An understanding of the mechanisms underlying the tolerance of salt stress could enable the screening of plant species for revegetation of saline soils.

We investigated the response of *Salvadora oleoides*, a dominant tree in the arid regions of western India and Pakistan, to a range of soil salinities. The objectives of our study were: (i) to understand the adaptive features of *S. oleoides* that allow it to grow and survive in saline and arid regions and (ii) to assess the pattern of macro- and micro-nutrient accumulation within the tissues of this tree species in response to salt stress.

Materials and methods

Study area

The present study was carried out in a greenhouse of the botanical garden of Saurashtra University at Rajkot (22º 18' N Lat. 70º 56' E Long.) in Gujarat. The Kutch and Saurashtra regions have a tropical monsoon climate and can be classified as arid and semi-arid, respectively. The entire area is markedly affected by the southwest monsoon, which causes the onset of the wet season in mid-June. The retreat of the monsoon at the end of September coincides with a lowering of temperature and the gradual onset of winter. Total annual rainfall is about 395 mm at Bhuj (23º 15' N Lat. 69º 49' E Long.) in Kutch, and about 554 mm at Rajkot in central Saurashtra. Typically, there are three main seasons: summer (April to mid-June), monsoon (mid - June to September) and winter (November to February). The months of October and March are transition periods between the rainy and winter seasons, and between the winter and summer seasons, respectively. Winters are generally mild and summers are hot.

Study species

Salvadora oleoides Decne. (Salvadoraceae) is native to the arid regions of western India and Pakistan and is one of the dominant tree species in the vast area of Kutch, the north-west saline

desert, in the state of Gujarat, India. It also grows abundantly in coastal areas as well as in the non-saline and semi-arid Saurashtra region south of Kutch. The species is found in dry regions of other states in India as well. It has a short trunk, which is quite often twisted. The seeds are greenish-yellow, about 3 mm in diameter, and are spread by birds. The tree regenerates by root suckers and from seeds. This tree species is of multipurpose use because of its oil-yielding potential, pharmaceutical application, and its value as a source of fodder and fuel (Vaghela *et al.* 2009).

Salinization of soil

Seedlings of *S. oleoides* were grown in soil collected from the top 15 cm of an agricultural field. The soil predominant in this Saurashtra region of Gujarat is commonly known as black-cotton soil, which is fertile and fit for intensive agriculture. The soil is a clayey loam containing 19.6 % sand, 20.3 % silt and 60.1 % clay. The available soil water ranges from 18.3 % to 35.0 %, which is between wilting coefficient and field capacity. For the soil used in this study the total organic carbon content was 1.3 % and pH was 7.2. The electrical conductivity of the soil was 0.2 dS m^{-1}. Nitrogen, phosphorus, potassium, calcium and sodium contents were 0.15 %, 0.05 %, 0.03 %, 0.05 % and 0.002 %, respectively.

Surface soil was collected, air dried and passed through a 2 mm mesh screen. Seven lots of soil, each of 100 kg, were separately spread over about 50 mm thick polyethylene sheets. Sodium chloride (NaCl) amounting to 390, 700, 1070, 1275, 1530 and 1800 g was then thoroughly mixed with six of the seven lots of soil, respectively. There was no addition of NaCl to the seventh lot of soil that served as the control.

The electrical conductivity of the soils was measured by preparing a soil suspension in distilled water with a 1:2 soil : water ratio. The suspension was shaken and allowed to stand overnight. Thereafter, electrical conductivity of the supernatant solution was determined with a conductivity meter. The electrical conductivity of control soil was 0.2 dS m^{-1} and this value was approximately equal to 2 mM salinity. Electrical conductivities of the salt-ammended soils – in order of increasing salt addition – were 3.9, 6.2, 8.1, 10.0, 11.9 and 13.9 dS m^{-1}

Seedling emergence

Seeds of *S. oleoides* were collected in the last week of May 2007 from the coastal area of Jamnagar district of Saurashtra. Twenty polyethylene bags were filled with 5 kg of soil for each level of soil salinity and arranged in a completely randomized block design. Tap water was added to each bag to bring the soils to field capacity and soils were allowed to dry for 7 days. Salinity of tap water was 0.18 dS m^{-1}. Soils were then raked using fingers and seeds were sown on 17 July 2007. Ten seeds were sown in each bag at a depth of 8 - 12 mm.

Bags were kept in an uncontrolled greenhouse under natural temperature and light. Immediately after sowing soils were watered (about 300 ml water was added to raise the soil moisture to field capacity) and thereafter about 100 - 150 ml water was added to the soils (just to wet the surface soil) on alternate days. Irrigation of soil with required amount of water was taken as a measure to control the level of soil salinity. Emergence of seedlings was recorded daily over a period of 40 days. A linear model was fitted with cumulative proportion of seed germination as the dependent variable and soil salinity as the independent variable using the expression:

$$\mathrm{Sin}^{-1}\sqrt{p} = \beta_0 + \beta_1 X$$

where, $\mathrm{Sin}^{-1}\sqrt{p}$ is cumulative seed germination (expressed as a proportion), X is soil salinity and β_0 and β_1 are coefficients of linear regression. The salt concentration at which seed germination was reduced to 50 % (SG_{50}) was estimated using the model.

Seedling growth

For investigating growth the two seedlings (of the 10 planted in each bag) that emerged first were retained and the others were uprooted. The growth of these seedlings was then monitored. Seedlings sown in soils at 13.9 dS m^{-1} salinity were excluded from further investigations, because they demonstrated only 12 % seed germination.

Seedlings exhibited emergence of the second leaf after 21 to 27 days. Emergence of the second leaf confirmed the establishment of seedlings. Following emergence of the second leaf the seedling that was more vigorous was left to grow in each bag while the second seedling was uprooted. This yielded a total of 120 bags with one seedling in each – 20 replicate seedlings for each of 6 levels of soil salinity (0.2, 3.9, 6.2, 8.1, 10.0 and 11.9 dS m^{-1}).

Seedlings were watered (to raise the soil moisture to field capacity) on alternate days. The

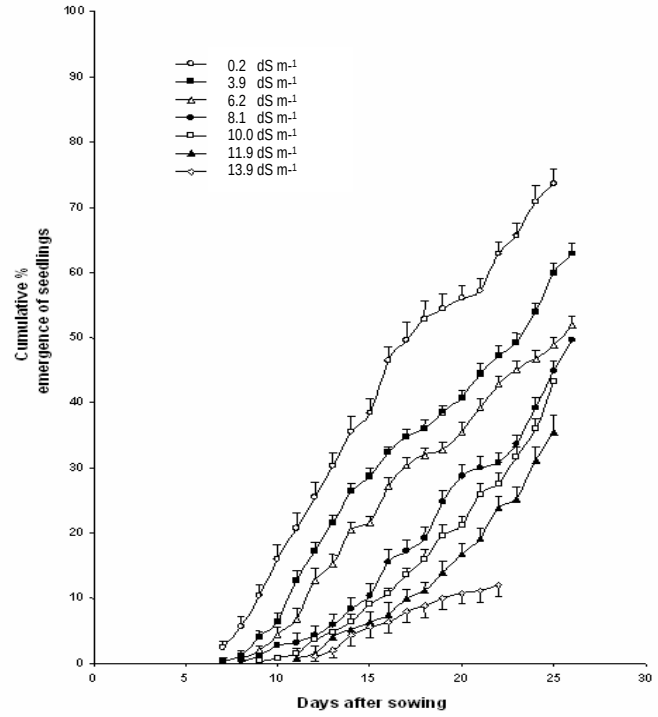

Fig. 1. Cumulative emergence of seedlings of *Salvadora oleoides* in response to soil salinity. One side error bars represent ½SE (n=200).

mean maximum temperature of the greenhouse during the course of the study decreased from 34.3 ± 0.4° C in July to 33.3 ± 1.0° C in September and then increased to 34.5 ± 0.9° C in October 2007. Following this period, mean maximum temperature decreased to 28.1 ± 1.1° C in January 2008. Seedlings were allowed to grow for 6 months till 17 January 2008.

Seedlings were then removed from the bags and were washed to remove soil particles adhered to the roots. Morphological characteristics of each seedling were recorded. Shoot height and length of the tap root were measured. Leaf outlines were traced onto graph paper for determination of leaf area. Fresh and dry weights of leaf, stem, tap root and lateral root were determined. The sum of leaf and stem weight was considered as shoot biomass. Water content (g g^{-1} dry weight) in plant tissues (leaf, stem, tap root and lateral root) was calculated using fresh and dry weight values.

Determination of water potential, proline content, and tissue nutrient concentration

Five additional plants grown in soil at each level of salinity were used for measurement of water potential and for determination of proline in plant tissues. Measurements were taken 15 days before the termination of the growth experiment. Water potential of leaf, stem, tap root and lateral root tissues was measured by Dewpoint Potential Meter WP4 (Decagon Devices, Inc. Pull-man, WA, USA) following Patel *et al.* (2010).

Concentration of proline in plant tissues was determined following Bates *et al.* (1973) using an extract of 0.5 g fresh plant material in aqueous sulphosalicylic acid. The extracted proline was made to react with ninhydrin to form a chromophore and quantified spectrophotometrically at 520 nm.

Mineral analyses were performed in triplicate on leaf, stem, tap root and lateral root tissues of seedlings grown at each salinity level. Total nitrogen (N) was determined by the Kjeldahl method and phosphorus (P) content was estimated by the chlorostannous molybdophosphoric blue colour method in sulphuric acid (Piper 1944). Concentrations of calcium (Ca), magnesium (Mg), sodium (Na), postassium (K), zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) were determined by using a double beam atomic absorption spectrophotometer AA-6800 (Shimadzu Corporation, Kyoto, Japan) after tri-acid digestion (HNO_3 : H_2SO_4 : $HClO_4$ in the ratio 10:1:4).

Differences in morphological characteristics, biomass, water content, proline content, and tissue mineral concentrations among seedlings grown at different levels of soil salinity were analyzed by one way ANOVA. All analyses were performed using SPSS 16.

Results

Effect of salinisation on seedling emergence

Seedlings began to emerge 6 days after sowing and 74 % seed germination was obtained over a period of 25 days, under control conditions (Fig. 1). Seedling emergence in saline soils started 6 - 11 days after sowing. Emergence continued for a maximum of 26 days, except in the case of the highest salinity level, where emergence ceased after 22 days.

Seed germination decreased from 63 % at 3.9 dSm^{-1} to 12 % at 13.9 dSm^{-1} soil salinity. There was a significant reduction in seed germination ($P < 0.001$) with increasing soil salinity. A negative relationship between proportion of cumulative seed germination and concentration of salt was obtained according to the following expression: Y= 62.846 - 2.521X, ($R^2_{Adj} = 0.895$, $P < 0.001$), where, Y

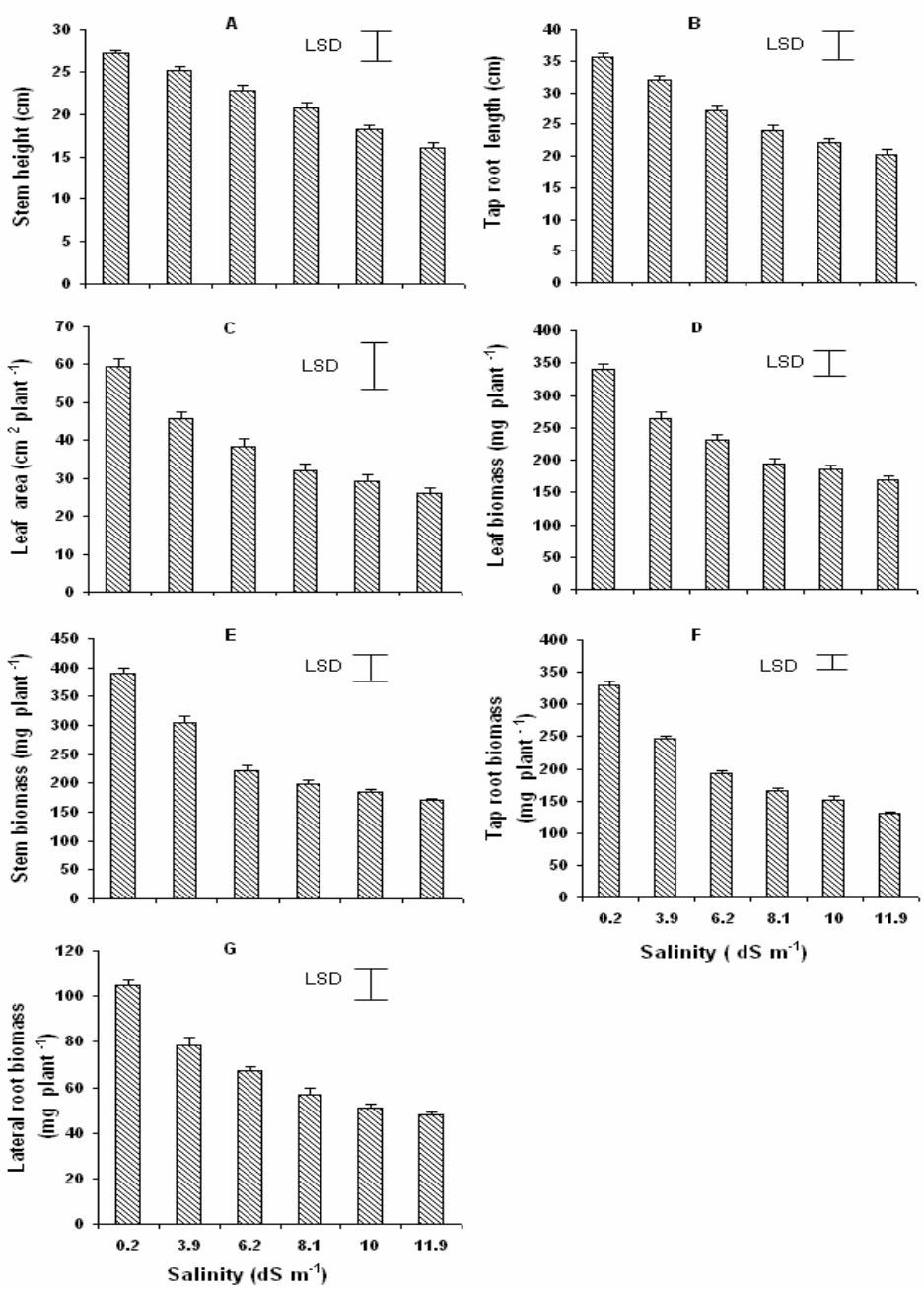

Fig. 2. Effect of soil salinity on elongation of stem (A), and root (B), expansion of leaf (C), and biomass of leaf (D), stem (E), tap root (F) and lateral root (G) of *Salvadora oleoides* seedlings. One side error bars denote one-half standard error; LSD is indicated on each panel.

is arcsine (degrees) of proportion of cumulative seed germination and X is salt concentration.

Effect of salinisation on plant growth

Increasing soil salinity significantly retarded ($P < 0.001$) stem and root elongation (Fig. 2). There was a negative relationship for shoot height ($r = -0.750$, $P < 0.001$) and root length ($r = -0.865$, $P < 0.001$) with increase in salt concentration in soil. Leaf expansion was significantly reduced ($P < 0.001$) by increasing concentration of salt in soil. A negative relationship was obtained between leaf area and salt concentration ($r = -0.811$, $P < 0.001$). However, specific leaf area did not change in response to increased salinity, because leaf weight

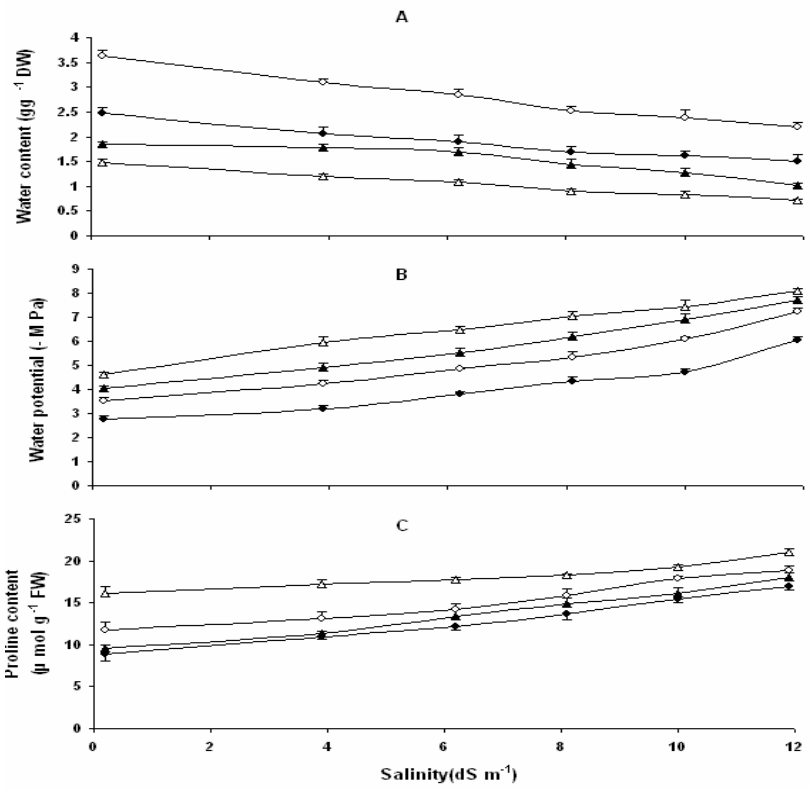

Fig. 3. Effect of soil salinity on water content (A), water potential (B), and proline content (C) of leaf (open circle), stem (open triangle), tap root (closed triangle), and lateral root (solid circle) of *Salvadora oleoides* seedlings. One side error bars represent ½SE.

also decreased with increasing salinity.

Biomass significantly decreased ($P < 0.001$) for leaf, stem, shoot (leaves + stems), tap root, lateral root and total root of seedlings in response to increasing concentration of salt (Fig. 2). A negative relationship was obtained between biomass of tissues and salt concentration (r = - 0.849, - 0.881, -0.910, - 0.942, -0.855 and - 0.944, $P < 0.001$, for leaf, stem, shoot, tap root, lateral root and total root).

Percent relative biomass of tissues of salinised plants compared to those of control plants were computed as: (salinised tissue biomass / control biomass) × 100. Values of percent relative biomass varied from 77.6 to 49.5 % for leaf, from 78.1 to 43.5 % for stem, from 74.6 to 39.6 % for tap root and from 74.8 to 45.9 % for lateral root in response to increasing soil salinity from 3.9 to 11.9 dS m^{-1}. The salt concentration at which biomass will be reduced to 50 % of control plants (B_{50}) was estimated around 10.8, 9.3, 8.9 and 9.8 for leaf, stem, tap root and lateral root tissues, respectively. Root / shoot ratio significantly decreased ($P < 0.05$) as soil salinity increased. There was a negative relationship between root / shoot dry weight ratio and soil salinity (r = -0.302, $P < 0.01$).

Effect of salinisation on tissue water content, water potential, and proline content

Water content in tissues significantly decreased ($P < 0.001$) with increasing concentration of salt in soil (Fig. 3A). Tissues can be arranged in the following decreasing order according to their water content: leaf > lateral root > tap root > stem. There was a negative relationship between water content in different tissues and salt concentration (r = -0.709, -0.717,-0.582 and -0.514, $P < 0.001$, for leaf, stem, tap root and lateral root, respectively).

Water potential significantly became more negative in leaf, stem, tap root ($P < 0.01$) and lateral root ($P < 0.001$) as soil salinity increased (Fig. 3B). Tissues can be arranged in the following decreasing order according to their water potential values (low to high negative values): lateral root > leaf > tap root > stem. There was a negative relationship between water potential of tissues

and salt concentration (r = -0.832, -0.841, -0.855 and -0.889, $P < 0.001$, for leaf, stem, tap root and lateral root, respectively). A positive relationship was obtained between water content and water potential (negative value) (r = 0.946, 0.997, 0.976 and 0.904, $P < 0.001$, for leaf, stem, tap root and lateral root, respectively).

Proline content (μ mol/g FW material) significantly increased for leaf ($P < 0.01$), stem, tap root and lateral root ($P < 0.001$), with increase in soil salinity (Fig. 3C).Tissues can be arranged in the following decreasing order according to their proline content: stem > leaf > tap root > lateral root. There was a positive relationship between salt concentration and proline content of tissues (r = 0.908, 0.891, 0.953, and 0.948, $P < 0.001$, for leaf, stem, tap root and lateral root, respectively). A negative relationship was obtained between water potential and proline content of tissues (r = -0.925, -0.856, -0.963 and -0.919, $P < 0.001$, for leaf, stem, tap root and lateral root, respectively). Similarly, a negative relationship was obtained between water content and proline content of tissues (r = -0.960, -0.975, -0.985 and -0.960, $P < 0.001$, for leaf, stem, tap root and lateral root, respectively).

Effect of salinisation on mineral accumulation

Potassium and Na content (as mg g^{-1} biomass) significantly increased ($P < 0.001$) in tissues in response to increasing soil salinity (Table 1). There was a positive relationship between salt concentration and K content in leaf, stem, tap root and lateral root ($P < 0.001$). Similarly, a positive relationship was obtained between salt concentration and Na content of tissues ($P < 0.001$). The K/Na ratio significantly increased in leaf ($P < 0.001$), stem ($P < 0.01$), tap root ($P < 0.01$) and lateral root ($P < 0.001$) in response to increase in soil salinity. A positive relationship was obtained between K /Na ratio in tissues and soil salinity ($P < 0.001$).

Concentration of N, K and Ca was, in general, greater than that of P, Mg and Na in all tissues under control and salt stress conditions. Nitrogen content exhibited a significant increase, whereas P, Ca and Mg content significantly decreased ($P < 0.001$) in tissues in response to salinity. There was a significant positive relationship between salt concentration and N content in tissues ($P < 0.001$). A negative relationship was obtained between Ca content of tissues and salt concentration ($P < 0.001$).

There was a significant increase in the concentration of the micronutrients Cu, Mn and Fe in response to salinity. On the other hand, Zn content significantly decreased in tissues ($P < 0.001$) in response to salinity.

Discussion

Seedling emergence

Earlier work (Ramoliya *et al.* 2004) indicated that seedling emergence for the salt-tolerant legume tree *Acacia catechu* was reduced to 50 % (SG_{50}) in soil with salinity of 6.0 dS m^{-1}, but for *Salvadora oleoides* SG_{50} was obtained at 6.9 dS m^{-1}. That would suggest that this species, like *Acacia catechu,* is relatively salt tolerant at seed germination. Under field conditions in the coastal region of Saurashtra and the saline desert of Kutch, where this tree species grows, maximum soil salinity is found during the dry season and minimum salinity occurs during the rainy season and in general, salinity for the surface soil (0 - 15 cm depth) varies from 2.0 to 5.0 dS m^{-1}.Thus, seeds of *S. oleoides* can germinate and achieve establishment during the rainy season.

Salinity exceeding 11.9 dS m^{-1} was detrimental to seed germination and can be attributed to decreasing osmotic potential of the soil solution. It was observed that seeds became non-viable within a few days in soil with high concentrations of salt. Although the effects of high salt content on metabolic processes are not fully understood, it has been reported that salinity reduces protein hydration (Slater *et al.* 2003) and induces changes in the activities of many enzymes (Dubey & Rani 1990) in germinating seeds.

Seedling growth and water status

Reduction in water content and water potential of leaf, stem, tap root and lateral root of seedlings grown in saline soil result in internal water deficit to plants, which, in turn, reduces the growth of shoots and roots. It is found that plants subjected to water stress show a general reduction in size and dry matter production (Taiz & Zeiger 2006). In this study, tap root elongation for seedlings grown in control and saline soils, both, was markedly greater than shoot elongation. Result suggests that this species has a tendency for rapid root extension. It is suggested that rapid root extension ensures survival of plants in dry habitats (Etherington 1987) and is

Table 1. Effect of salinisation of soil on nutrient concentration of tissues (leaf, stem, tap root and lateral root) of *Salvadora oleoides*. Values are mean ± SE. Regression equation constants are also provided.

Tissue	Salinity (dSm^{-1})	N ($mg\ g^{-1}$)	P ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)	Na ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	K/Na ratio	Zn ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)
Leaf	0.2	21.6±0.2	2.4±0.2	22.3±0.6	6.8±0.1	34.6±0.4	8.5±0.3	3.3±0.1	53±1.2	49±0.6	44±0.1	251±3.5
	3.9	23.6±0.2	2.3±0.1	26.5±0.3	7.1±0.1	26.4±0.1	7.4±0.2	3.7±0.1	46±0.5	58±1.0	49±2.1	269±0.6
	6.2	24.2±0.1	1.9±0.2	31.1±0.6	7.9±0.1	24.6±0.6	6.7±0.2	3.9±0.1	38±0.3	66±0.8	53±0.2	331±2.3
	8.1	25.1±0.0	1.4±0.1	34.6±0.8	8.6±0.2	20.8±0.1	4.2±0.1	4.0±0.1	30±0.1	73±0.3	61±0.9	335±2.6
	10.0	25.7±0.1	1.2±0.1	39.8±0.1	9.7±0.2	18.4±0.2	3.1±0.1	4.1±0.1	27±0.3	81±1.4	68±2.1	389±1.2
	11.9	27.6±0.1	1.1±0.1	42.9±0.2	10.3±0.1	16.2±0.5	3.0±0.1	4.2±0.0	23±0.3	88±0.6	71±1.2	460±1.5
	α	21.69	2.55	20.6	6.24	33.91	9.06	3.38	54.28	46.44	40.83	223.00
	ß	0.41	-0.12	1.82	0.32	-1.54	-0.53	0.07	-2.69	3.42	2.49	17.29
	r	0.989	-0.906	0.985	0.952	-0.986	-0.956	0.878	-0.988	0.991	0.961	0.949
	F value	199.392**	20.640**	242.312**	89.586**	289.379**	198.794**	11.889**	407.723**	283.128**	65.027**	1247.111**
	$LSD_{0.05}$	0.4	0.4	1.5	0.4	1.1	0.5	0.3	1.7	2.5	3.9	6.4
Stem	0.2	14.5±0.3	1.7±0.1	31.9±0.3	10.8±0.2	36.3±0.1	7.6±0.2	3.0±0.0	68±0.7	29±0.9	37±0.9	186±2.1
	3.9	15.2±0.4	1.2±0.1	37.5±0.6	12.2±0.2	30.0±0.6	6.2±0.2	3.1±0.1	65±1.1	34±1.0	38±0.9	197±0.6
	6.2	16.2±0.2	1.1±0.1	40.4±0.5	13.0±0.1	28.9±0.3	5.0±0.2	3.1±0.0	59±0.5	42±0.6	39±0.1	255±1.7
	8.1	17.2±0.1	0.9±0.2	43.9±0.5	13.6±0.2	26.8±0.9	3.3±0.1	3.2±0.1	52±1.9	56±1.0	41±1.5	277±2.3
	10.0	18.3±0.2	0.7±0.2	46.5±0.3	14.0±0.1	25.8±0.6	2.9±0.1	3.3±0.0	48±1.1	63±0.3	43±0.3	286±1.0
	11.9	19.1±0.1	0.4±0.0	54.2±0.3	16.1±0.4	18.8±0.1	2.8±0.3	3.4±0.1	46±1.0	66±1.1	44±0.3	298±0.7
	α	13.97	1.17	30.53	10.54	36.46	7.68	2.93	70.57	24.51	35.78	177.78
	ß	0.41	-0.10	1.76	0.41	-1.29	-0.45	0.04	-2.14	3.54	0.66	10.73
	r	0.958	-0.898	0.973	0.952	-0.945	-0.966	0.823	-0.961	0.966	0.885	0.959
	F value	48.201**	11.268**	299.543**	70.104**	88.686**	119.186**	5. 539*	65.736**	317.425**	12.964**	930.119**
	$LSD_{0.05}$	0.8	0.4	1.3	0.6	1.8	1.7	0.2	3.4	2.6	2.4	4.6
Tap root	0.2	15.6±0.3	1.9±0.1	28.6±0.3	6.6±0.1	26.4±0.3	5.7±0.2	4.3±0.1	47±0.6	42±1.5	36±0.6	199±1.3
	3.9	16.3±0.4	1.7±0.1	37.7±0.3	8.5±0.1	24.7±0.5	4.7±0.3	4.4±0.1	44±0.5	52±1.0	37±1.2	219±3.8
	6.2	17.2±0.6	1.3±0.1	42.8±0.8	9.3±0.1	20.8±0.1	4.1±0.2	4.6±0.0	35±0.5	61±1.8	39±0.3	264±1.0
	8.1	18.0±0.6	1.1±0.1	46.6±0.5	9.8±0.2	19.4±0.2	3.3±0.1	4.8±0.1	32±0.4	67±1.7	40±0.1	289±1.5
	10.0	18.8±0.6	0.9±0.1	49.8±0.8	10.0±0.1	17.7±0.3	3.0±0.6	5.0±0.1	30±0.2	72±1.1	42±0.5	320±1.6
	11.9	19.8±0.3	0.7±0.0	52.6±0.5	10.2±0.3	14.3±0.4	2.2±0.0	5.2±0.2	22±0.9	83±1.9	43±0.4	333±1.9

Contd...

Table 1. Continued.

Tissue	Salinity (dSm^{-1})	N ($mg\ g^{-1}$)	P ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)	Na ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	K/Na ratio	Zn ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)
Lateral root	α	15.20	2.01	29.18	7.04	27.51	5.81	4.21	49.27	39.93	35.43	186.53
	ß	0.36	-0.11	2.06	0.30	-1.03	-0.29	0.07	-2.12	3.41	0.64	12.52
	r	0.884	-0.923	0.990	0.935	-0.976	-0.941	0.844	-0.972	0.980	0.954	0.983
	F value	10.102**	16.654**	231.859**	59.531**	183.242**	20.105**	7.822*	288.100**	90.775**	41.033**	685.952**
	$LSD_{0.05}$	1.4	0.3	1.7	0.4	1.0	0.8	0.3	1.6	4.5	1.3	6.0
	0.2	18.4±0.3	2.8±0.1	25.9±1.1	10.4±0.5	20.7±0.2	7.8±0.2	2.5±0.2	59±0.4	23±0.3	57±0.3	829±1.2
	3.9	19.0±0.3	2.7±0.1	28.7±0.2	10.8±0.2	16.5±0.5	6.5±0.5	2.7±0.2	52±0.4	34±1.0	60±1.5	869±1.7
	6.2	19.2±0.2	2.3±0.1	32.4±0.0	11.2±0.1	15.6±0.2	6.4±0.4	2.9±0.0	45±1.6	42±1.5	66±1.0	908±1.5
	8.1	20.4±0.2	2.1±0.1	37.8±0.4	12.4±0.2	13.8±0.2	4.9±0.3	3.0±0.0	34±1.0	46±0.3	77±1.7	934±2.6
	10.0	21.3±1.0	1.9±0.1	42.2±0.4	13.5±0.3	12±0.5	3.9±0.0	3.1±0.1	33±0.4	53±1.1	106±2.1	978±1.3
	11.9	23.2±0.3	1.7±0.1	45.3±0.1	14.2±0.0	10.2±0.3	2.9±0.5	3.2±0.1	32±0.1	57±0.4	114±1.7	1049±0.6
	α	17.67	2.95	23.54	9.76	20.63	8.21	2.47	59.88	22.71	44.67	807.14
	ß	0.38	-0.10	1.76	0.34	-0.87	-0.42	0.06	-2.59	2.94	5.22	17.97
	r	0.854	-0.921	0.972	0.924	-0.984	-0.931	0.890	-0.960	0.992	0.906	0.971
	F value	14.247**	21.067**	233.938**	41.715**	121.174**	25.916**	10.875**	192.778**	195.560**	357.884**	2415.039**
	$LSD_{0.05}$	1.4	0.3	1.5	0.7	1.0	1.1	0.2	2.4	2.6	3.8	4.7

Relationship is significant at $P < 0.001$; * *F* values are significant at $P < 0.01$; ** *F* values are significant at $P < 0.001$.

considered an adaptation to dry habitats. Root / shoot dry weight ratio of *S. oleoides* was 0.60 under control conditions, which is greater than root/shoot dry weight ratio measured for aridity- and salt-tolerant seedlings of *Acacia catechu* (0.47) growing abundantly in saline desert of Kutch (Ramoliya *et al.* 2004).

In general, salinity can reduce plant growth or damage the plants through: (i) osmotic effect (causing water deficit), (ii) toxic effect of ions and (iii) imbalance of the uptake of essential nutrients. These modes of action may operate on the cellular as well as on higher organizational levels and influence all aspects of plant metabolism (Garg & Gupta 1997; Kramer 1983). Reduction in growth of seedlings of *S. oleoides* with increasing salt concentration can further be explained by reduction in leaf area (photosynthetic area). Curtis & Lauchli (1986) reported that growth in Kenaf (*Hibiscus cannabinus*) under moderate salt stress was affected primarily through a reduction in elongation of stem and leaf area development. Garg & Gupta (1997) reported that salinity causes reduction in leaf area as well as in rate of photosynthesis, which together result in reduced crop growth and yield. Also high concentration of salt tends to slow down or stop root elongation (Kramer 1983; Patel & Pandey 2008) and causes reduction in root production (Garg & Gupta 1997).

Results for biomass and relative biomass of tissues in response to increasing salinity suggest that there was maximum reduction in biomass of tap roots while there was minimum reduction in biomass of leaves. The maximum reduction in tap root biomass and minimum in leaf biomass resulted in decreased root/shoot dry weight ratio for seedlings with increase in salinity.

Proline accumulation

Proline accumulation is a common response to salinity and related stresses (Stewart & Lee 1974). At high concentrations, it leads to osmotic adjustment, stabilization of macromolecules and regulation of cellular redox status (Storey *et al.* 1977). The increase of proline concentrations in *S. oleoides* with increasing Na concentration indicates that higher proline accumulation may help alleviate NaCl stress in *S. oleoides* Proline accumulation was greater in shoots than that in roots. This confirms the observation of Munns (2002) that organic solutes are often lower in roots than shoots.

Mineral accumulation

In general, Na^+ depresses K^+ uptake (Fox & Guerinot 1998). In the present study, we observed significant increase of K^+ in all tissues of seedlings with increasing soil salinity. Our results indicate high selectivity of *S. oleoides* for K^+ over Na^+. Gorham (1990) reported that in wheat, salt tolerance is associated with low rates of transport of Na^+ to shoots with high selectivity for K^+ over Na^+. Further, the exchange of K^+ for Na^+ by the cells in the stele of the roots or in the vascular bundles in stems is considered as one type of control on the transport of salt to leaves or growing tissues. The significant increase of Na^+ in leaves and stems suggests that this mechanism of blocking Na^+ transfer to growing tissues was not effective in *S. oleoides* at high salt concentration. It seems that there were no effective mechanisms to control net uptake of Na^+ on root plasma membrane and subsequently its transport to shoot tissues. Significant increase in K^+/Na^+ ratio in all the tissues with increase in salinity can be explained by greater accumulation of K^+ than that of Na^+.

Uptake mechanisms for both K^+ and Na^+ are similar (Schroeder *et al.* 1994; Watad *et al.* 1991). Sodium cannot move through the plasma membrane lipid bilayer, but the ion is transported through both low and high affinity transport systems, which are necessary for K^+ acquisition. As a consequence, Na^+ could enter the cell through high affinity K^+ carriers or through the low affinity channels called non selective cation channels that are strongly influenced by Ca^{2+}. These cation channels could allow entry of large amount of Na^+ from a highly saline soil if not adequately regulated (Amtmann & Sanders 1999). Low affinity K^+ uptake is not inhibited by Na^+ but the high affinity process is restricted (Schroeder *et al.* 1994; Watad *et al.* 1991). Similarly Na^+ toxicity in plants is correlated with two proposed Na^+ uptake pathways (Maathuis & Sanders 1994; Niu *et al.* 1995). The K^+ and Na^+ profiles of *S. oleoides* suggest that similar mechanism might operate in this species. It is demonstrated that Ca^{2+} causes closure of non-selective cation channels and restricts Na^+ uptake (Rus *et al.* 2001). As a result, calcium fertilizers may mitigate Na^+ toxicity to plants. Tolerence of non-halophytes to salinity further depends upon their ability to sequester Na^+ that enters the tissues, into vacuoles (Munns 2005; Munns & Tester 2008) and salt - resistant tissues (Ramoliya *et al.* 2006). In *S. oleoides,* Na^+ accumulation was greater in stem tissues than in leaves. As the lateral roots and stem tissues are salt-resistant, plants of this species may sequester

salts that they absorb in the lateral roots and stems, thus minimizing the exposure of leaf cells and hence the photosynthetic apparatus to salt. "Integration in the whole plant" is an important aspect of salt tolerance in glycophytes (Garg & Gupta 1997). Considering that stem tissues will be reinforced by growth with time, it can be predicted that after seedling stage Na^+ tolerance of plants may increase above 11.9 dS m^{-1} salinity which is maximum salt concentration in this experiment.

In general, salinity reduces N accumulation in plants (Feigin 1985), but in this plant nitrogen increased with increase in salinity. Dubey & Rani (1989) reported that protein level in several crops under salinisation increases due to the increased synthesis of pre-existing and certain new sets of proteins. It is evidenced that P concentration is related to the rate of photosynthesis, since it decreases the conversion of fixed carbon in to starch (Overlach *et al.* 1993) and, therefore, decrease of P in leaves may reduce plant growth.

Salinity also decreased Ca^{2+}, suggesting that Na^+ reduced internal concentration in roots and shoots that might have slowed growth of seedlings. Uptake of Ca^{2+} may decrease because of ion interactions, precipitation and increase in ionic strength (Janzen & Chang 1987). Besides the role of Mg^{2+} in chlorophyll structure and as an enzyme cofactor, another important role of Mg^{2+} in plants is in the export of photosynthates, which when impaired leads to enhanced degradation of chlorophyll in Mg^{2+} deficient source leaves, resulting in increased oxygenase activity of RuBP carboxylase (Marschner & Cakmak 1989).

It is reported that salinity generates an increase in reactive oxygen species (ROS) which have deleterious effects on cell metabolism (Borsani *et al.* 2001). Super oxide dismutases (SODs) detoxify ROS and may contain Cu, Zn, Mn and Fe as metal components (Slater *et al.* 2003). Increase in Cu, Mn and Fe content at the whole plant level might be a requirement of the plant for survival and growth in response to salinity.

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