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EFFECT OF SOIL SALINITY ON GROWTH, WATER STATUS AND NUTRIENT ACCUMULATION IN SEEDLINGS OF *Ziziphus mauritiana* (RHAMNACEAE)

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ABSTRACT

Greenhouse experiments were conducted to assess the effects of soil salinity on emergence, growth, water content, proline content and mineral accumulation of seedlings of *Ziziphus mauritiana* Lam. (Rhamnaceae). Sodium chloride (NaCl) was added to the soil to maintain electric conductivity at 0.3, 3.9, 6.0, 7.9, 10.0 and 11.9 dSm^{-1}. Salinity caused reduction in seedling emergence, water content and water potential of seedling organs (leaves, stems, tap roots and lateral roots). Consequently, shoot and root elongation, leaf expansion and dry matter accumulation in seedling organs significantly decreased while proline content increased with increasing soil salinity. A significant increase of K content in all organs of the seedlings with increasing soil salinity evinced high selectivity of this tree species for K^+. There were no effective mechanisms to control net uptake of Na on root plasma membrane and subsequently its transport to shoot tissue. Nitrogen, phosphorus, calcium and magnesium content in seedling organs significantly decreased as soil salinity increased. Changes in both organs and whole-plant accumulation patterns of nutrients, as well as possible mechanisms for avoidance of Na toxicity in this tree species in response to salinity, are discussed.

Key words: Soil salinity, seedling growth, proline content, water potential, macro- and micro-nutrients, salt tolerance

INTRODUCTION

Most of plants do not grow in saline soils. Globally, soil salinisation is more common in arid and semi-arid regions than in humid regions. High concentrations of salts have detrimental effects on seed germination and plant growth (Taiz and Zeiger, 2006; Ramoliya et al., 2006 Bernstein, 1962; Garg and Gupta, 1997) and eventually kill growing plants (Garg and Gupta, 1997). However, plant species differ in their sensitivity or tolerance to salts (Marschner, 1995). There are evidences that plant organs, tissues and cells at different developmental stages exhibit varying degrees of tolerance to environmental conditions (Munns, 1993). It was found that shoot growth is often suppressed more than the root growth by soil salinity (Maas and Hoffman, 1977; Munns, 2002; Ramoliya et al., 2006). However, fewer studies on the effect of soil salinity on root growth have been conducted (Munns, 2002).

The high salt content lowers osmotic potential of soil water and consequently the availability of soil water to plants. The salt–induced water deficit is one of the major constraints for plant growth in saline soils. In addition, many nutrient interactions in salt–stressed plants can occur that may have important consequences for growth. Internal concentrations of major nutrients and their uptake have been frequently studied (e.g. Cramer et al., 1989; Maas and Grieve, 1987; Ramoliya et al., 2006; Patel and Pandey, 2007), but the relationship between micro-nutrient concentrations and soil salinity is rather complex and remains poorly understood (Tozlu et al., 2000). An understanding of growth and survival of plants under saline habitat conditions is needed for (i) screening the plant species for the afforestation of saline deserts and (ii) understanding the mechanisms that plants use in the avoidance and/or tolerance of salt stress.

Ziziphus mauritiana Lam. (Rhamnaceae), a horticulture tree about 10 to 30 feet tall, is cultivated on marginal saline lands of Kutch (north west saline desert) in Gujarat state of India. It is also cultivated in coastal area of Saurashtra adjacent south to Kutch. Its centre of origin is Yunnan in southern China. This plant is remarkable in its ability to tolerate drought. Trees remain leafless for several weeks in hot summers. Fruits may reach 6 cm in length and 4-5 cm in width. The aroma of fruit is apple-like and pleasant. However, the potential of this tree species to grow and survive in coastal area of Saurashtra and in marginal saline desert of Kutch is not known. The present investigation was conducted with the following objectives: (i) to understand -adaptive features of *Z. mauritiana* which 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 organs of this tree species in response to salt stress.

MATERIAL AND METHODS

Study area

The study presented was carried out in a greenhouse of the botanical

garden of Saurashtra University at Rajkot (22°18' N Lat, 70°56' E Long) in Gujarat. For the emergence and growth of seedlings the top 15 cm of black-cotton soil, which is predominant in Saurashtra region of Gujarat, was collected from an agricultural field. This soil is a clayey loam containing 19.6% sand, 20.3% silt and 60.1% clay. The available soil water content between wilting coefficient and field capacity ranged from 18.3% to 35.0%, respectively. The total organic carbon content was 1.3% and pH was 7.2. The electrical conductivity of the soil was 0.3 dSm^{-1}. Total nitrogen, phosphorus, potassium, calcium and sodium contents were 0.15, 0.05, 0.03, 0.05, and 0.002%, respectively. This soil is fertile and fit for intensive agriculture. Physical and chemical properties of soil were given earlier (Patel and Pandey, 2007).

The Kutch and Saurashtra regions are tropical monsoonic and can be ecoclimatically classified as arid and semi-arid, respectively. Total annual rainfall is 362 mm at Bhuj (23°15' N Lat, 69°49' E Long) in Kutch and 551 mm at Rajkot in central Saurashtra, which occurs totally during the rainy season. Typically, there are three main seasons: summer (April – mid June), monsoon (mid June – September) and winter (November – February). The months of October and March are transition periods between rainy (monsoon) and winter and between winter and summer seasons, respectively. Winters are generally mild and summers hot.

Salinisation of soil

Surface soil was collected, air dried and passed through a 2 mm mesh screen. Six lots of soil, of 100 kg each, were separately spread, about 50 mm thick, over polyethylene sheets. Sodium chloride (NaCl) amounting to 280, 590, 690, 1090 and 1410 g was then thoroughly mixed with soil of five lots, respectively to give electrical conductivities of 3.9, 6.0, 7.9, 10.0 and 11.9 dSm^{-1}. There was no addition of NaCl to sixth lot of soil that served as a control. The electrical conductivity of control soil was 0.3 dSm^{-1} and this value was approximately equal to 3 mM salinity. For the measurement of electrical conductivity a soil suspension was prepared in distilled water at a ratio of 1:2 (w/w). The suspension was shaken and allowed to stand overnight. Thereafter, electrical conductivity of the supernatant solution was determined with a conductivity meter.

Seedling emergence

Twenty polyethylene bags for each level of soil salinity were each filled with 5 kg of soil. Tap water was added to each bag to bring the soils to field water capacity and soils were allowed to dry for 7 days. Bags were kept in a greenhouse. Soils in the bags were then raked using fingers and ten seeds were sown in each bag at a depth of 8-12 mm on 7 September 2006. Immediately after sowing soils were watered and thereafter watering was carried out on alternate days. Emergence of seedlings was recorded daily over a period of 30

days. A linear model was fitted to cumulative proportion of seed germination and increasing soil salinity using the expression:

$Sin^{-1} \sqrt{P} = ß0 + ß1X$

where:

$Sin^{-1}\sqrt{P}$ is the proportion of cumulative seed germination,

X is soil salinity

ß0 and ß1 are constants.

Salt concentration at which seed germination was reduced to 50% (SG_{50}) was estimated using the model.

Seedling growth

For the growth studies, two seedlings that emerged first were left in each of 20 bags at each level of salinity and others were uprooted. Emergence of the second leaf indicated the initiation of establishment of seedlings. The second leaf emerged after 7 days on seedlings grown in soils at 0.3, 3.9 and 6.0 dSm^{-1} and after 9 days on seedlings grown in soils at 7.9 and 10.0 dSm^{-1}. Only 15% seed germination was recorded in soil with EC 11.9 dSm^{-1} and further experiments were not conducted on those seedlings. Both the seedlings in each bag were allowed to grow and achieve establishment for one month following their emergence. Thereafter, one seedling having better vigour was allowed to grow in each bag and another seedling was further uprooted. Thus twenty replicates factorialized with five grades of soil (0.3, 3.9, 6.0, 7.9 and 10.0 dSm^{-1}) were prepared. This gave a total of 100 bags, which were arranged in twenty randomized blocks. Seedlings were watered (about 250 ml water was added to raise the soil moisture to field capacity) at alternate days and allowed to grow for 6 months. Experiment was terminated on 7 March 2006. Seedlings grown in 20 bags at each salinity level were washed to remove soil particles adhered to roots. Shoot height and root length (tap root) were measured. Leaf area was marked out on graph paper. Fresh and dry weights of leaves, stems, tap roots and lateral roots were determined. Water content (g g^{-1} dry weight) in plant organs (leaves, stems, tap roots and lateral roots) was calculated using fresh and dry weight values. Data recorded for morphological characteristics, dry weight and water content of different components were analyzed by one way ANOVA to assess the effect of salinity on plant growth.

Determination of water potential and proline content

Ten additional plants grown in soil at each level of salinity were used for measurement of water potential and proline determination in plant organs. Water potential of leaves, stems, tap roots and lateral root tissues was measured by Dewpoint Potential Meter WP4. Concentration of proline in plant tissues was determined following Bates et al. (1973). Extract of 0.5 g fresh plant material with aqueous sulphosalicylic acid was prepared. The extracted proline was reacted with ninhydrin to form chromophore and quantified spectrofotometrically at 520 nm. Data were analyzed by one way ANOVA.

Mineral analyses of plant materials

Mineral analyses were performed on leaves, stems, tap roots and lateral root tissues. Plant parts of the seedlings grown in soil at same level of salinity were pooled separately and ground using mortar and pestle. Three subsamples of plant tissues were analyzed. Total nitrogen was determined by Kjeldahl method and phosphorus content estimated by the chlorostannous molybdophosphoric blue colorimetric method in sulphuric acid (Piper, 1944). Concentrations of Ca, Mg, Na, K, Zn, Fe, Mn and Cu were determined by Shimadzu double beam atomic absorption spectrophotometer AA-6800 after triacid (HNO_3: H_2SO_4: $HClO_4$, 10: 1: 4, v/v) digestion. Data were analyzed by one way ANOVA. Correlations and linear regression equations between mineral content and salt concentrations were determined.

RESULTS

Effect of soil salinisation on seedling emergence

Seedlings began to emerge 3 days after sowing and 94% of seeds germinated over a period of 12 days under control (0.3 dSm^{-1} salinity) conditions (Fig. 1). Seedling emergence in saline soils was recorded 4-6 days after sowing. Emergence lasted for 13, 13, 13, 13 and 10 days in soils with 3.9, 6.0, 7.9, 10.0 and 11.9 dSm^{-1} salinities, respectively, and corresponding seed germination rates were 82, 71, 60, 40 and 15%. There was a significant reduction in seed germination ($p < 0.01$) with increasing salt stress. A negative relationship between proportion of cumulative seed germination and concentration of salt was obtained according to the following expression: $Y = 83.421 – 5.147X$ ($R^2_{Adj} = 0.881$, $p < 0.01$), where Y is arcsine (degrees) of proportion of cumulative seed germination and X is salt concentration.

Effect of salinisation on stem and root elongation and leaf expansion

Increasing soil salinity significantly retarded ($p < 0.01$) stem and root elongation (Tab. 1). A negative relationship was obtained for shoot height and root length with salt concentration in soil ($p < 0.01$). Root length was approximately – 1.5 times higher than shoot height in control as well as in saline soils. Leaf expansion was significantly reduced ($p < 0.01$) by increasing concentration of salt in soil. A negative relationship was obtained between leaf area and salt concentration ($p < 0.01$).

Effect of salinisation on seedling dry weight

Dry weight in leaves, stems, shoots (leaves + stems), tap roots, lateral roots and total roots significantly decreased ($p < 0.01$) in response to increasing concentration of salt (Tab. 1). A negative relationship was obtained between dry weight of seedling organs (leaves, stems, shoots, tap roots, lateral roots and total roots) and salt concentration ($p < 0.01$).

Percent relative weights of tissues of salinised plants compared with those of control plants were

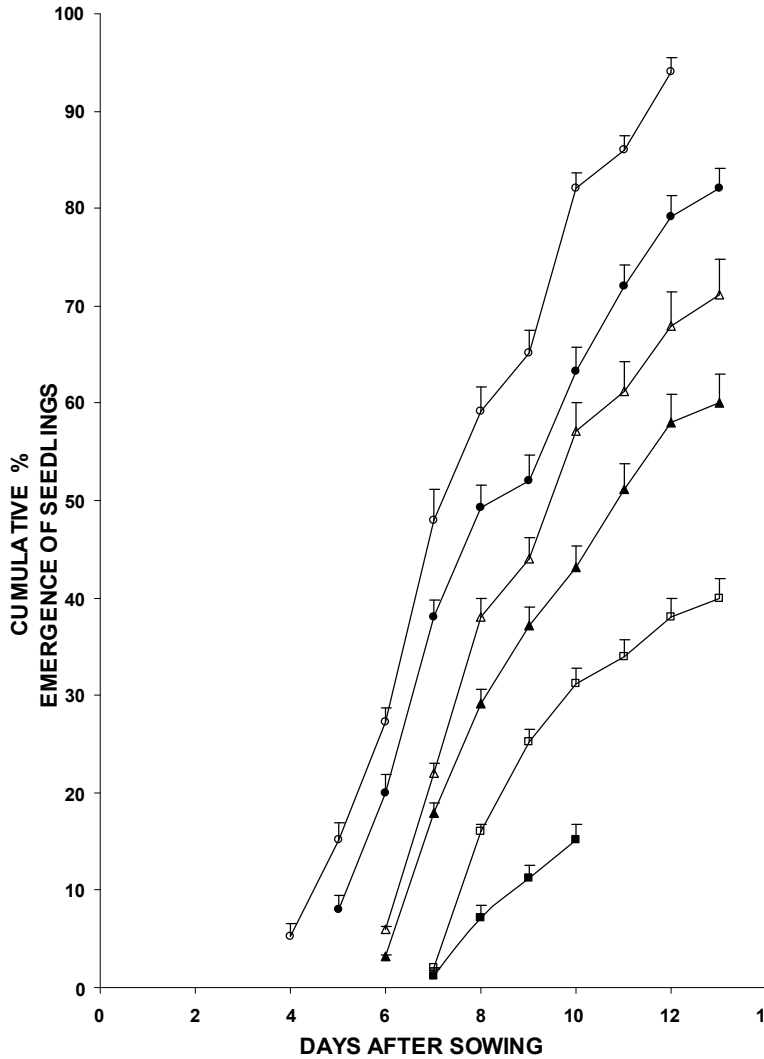

Figure 1. Cumulative emergence of seedlings of *Ziziphus mauritiana* in response to soil salinity. 0.3 dSm^{-1}(○), 3.9 dSm^{-1} (●), 6.0 dSm^{-1} (Δ), 7.9 dSm^{-1} and (▲), 10.0 dSm^{-1} (□). Error bars represent SE

computed as: (salinised organ dry weight/control organ dry weight) x 100. Dry weight values of seedling organs given in (Tab. 1) were used for the calculation of percent relative weight. Values of percent relative weight varied from 79.6 to 43.6 for leaves, from 73.9 to 30.1 for stems, from 80.1 to 42.8 for taproots and from 85 to 43.6 for lateral roots in

Table 1. Effect of soil salinity on leaf, stem, soot and root characteristics of *Ziziphus muritiana*

Salinity [dSm^{-1}]	Shoot height [cm]	Root length [cm]	Leaf area [cm^2 $plant^{-1}$]	Leaf weight [mg $plant^{-1}$]	Stem weight [mg $plant^{-1}$]	Shoot weight (leaf+stem) [mg $plant^{-1}$]	Tap root weight [mg $plant^{-1}$]	Lateral roots weight [mg $plant^{-1}$]	Total roots weight [mg $plant^{-1}$]
0.3	21.1 ± 1.3	34.7 ± 1.2	220.0 ± 5.0	462.0 ± 23.4	280.7 ± 15.0	742.7 ± 28.6	254.7 ± 20.6	91.0 ± 8.4	345.7 ± 25.5
3.9	18.7 ± 1.0	27.6 ± 1.0	173.0 ± 3.0	316.0 ± 22.0	207.3 ± 9.8	523.3 ± 17.7	204.0 ± 15.7	77.3 ± 7.7	264.0 ± 20.1
6.0	16.2 ± 1.1	22.5 ± 1.2	155.0 ± 2.0	248.0 ± 14.5	164.7 ± 7.2	412.7 ± 16.7	182.7 ± 13.9	61.7 ± 6.5	229.4 ± 18.0
7.9	13.1 ± 0.7	17.6 ± 0.9	123.0 ± 3.0	162.7 ± 9.0	112.7 ± 6.9	275.3 ± 12.8	152.0 ± 11.6	44.7 ± 4.1	184.9 ± 14.0
10	9.3 ± 0.8	13.4 ± 0.8	96.0 ± 3.0	104.0 ± 5.2	84.3 ± 7.3	173.3 ± 6.9	109.7 ± 8.1	39.7 ± 3.9	140.6 ± 10.6
α	22.52	35.64	224.60	459.30	286.88	758.64	262.14	94.91	357.05
β	-1.22	-2.23	-12.67	-34.42	-20.81	-59.28	-14.51	-5.70	-20.21
r	-0.726	-0.853	-0.961	-0.871	-0.884	-0.946	-0.796	-0.675	-0.862
$LSD_{0.05}$	6.60	8.10	18.90	106.90	60.90	113.20	63.20	35.80	68.70

r – values are significant at $p < 0.01$

response to increasing soil salinity from 3.9 to 10.0 dSm^{-1}. As has been estimated using regression equations given in results, the salt concentration at which dry weight will be reduced to 50% of control plants (DW_{50}) were 6.6, 6.8, 9.3 and 8.7 dSm^{-1} for leaves, stems, tap roots and lateral roots, respectively. Root/shoot dry weight ratio was 0.47 under control conditions and it significantly ($p < 0.01$) increased in response to increasing soil salinity. There was a positive relationship between root/shoot dry weight ratio and soil salinity ($r = 0.681$, $p < 0.01$).

Effect of salinisation on water content in seedling organs

Water content in leaves, stems, tap roots and lateral roots significantly decreased ($p < 0.01$) with increasing concentration of salt in soil (Fig. 2). There was maximum water content in leaves and minimum in lateral roots. The organs can be arranged according to their water content in the following decreasing order: leaves >stems > tap roots > lateral roots. There was a negative relationship between water content in different organs and salt concentration ($r = -0.887, -0.853, -0.773$ and -0.630, $p < 0.01$ for leaves, stems, tap roots and lateral roots, respectively).

Effect of salinisation on water potential of seedling organs

Water potential significantly became more negative in leaves, stems, tap roots and lateral roots ($p < 0.01$) as soil salinity increased (Fig. 2). Seedling organs can be arranged according to their water potential values (low to high negative) in the following order: leaves > stems > tap roots > lateral roots. There was a negative relationship between water potential of tissues and salt concentration ($r = -0.959, -0.964, -0.916$ and -0.957, $p < 0.01$ for leaves, stems, tap roots and lateral roots, respectively). A positive relationship was obtained between water content and water potential (negative value) ($r = 0.980, 0.952, 0.838$ and 0.990, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively).

Effect of salinisation on proline content in seedling organs

Proline content (μmol/g FW) significantly increased ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues with increase in soil salinity (Fig. 2). Seedling organs can be arranged according to their proline content in the following decreasing order: leaves > stems > tap roots > lateral roots. There was a positive relationship between salt concentration and proline content in seedling organs ($r = 0.957, 0.973, 0.989$ and 0.964, $p < 0.01$ for leaves, stems, tap roots and lateral roots, respectively). A negative relationship was obtained between water potential and proline content ($r = -0.872, -0.959, -0.922$ and -0.892, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively. Similarly, a negative relationship was obtained between water content and proline content ($r = -0.871, -0.976, -0.961$ and -0.917, $p < 0.01$, for leaves, stems, tap roots and lateral roots, respectively).

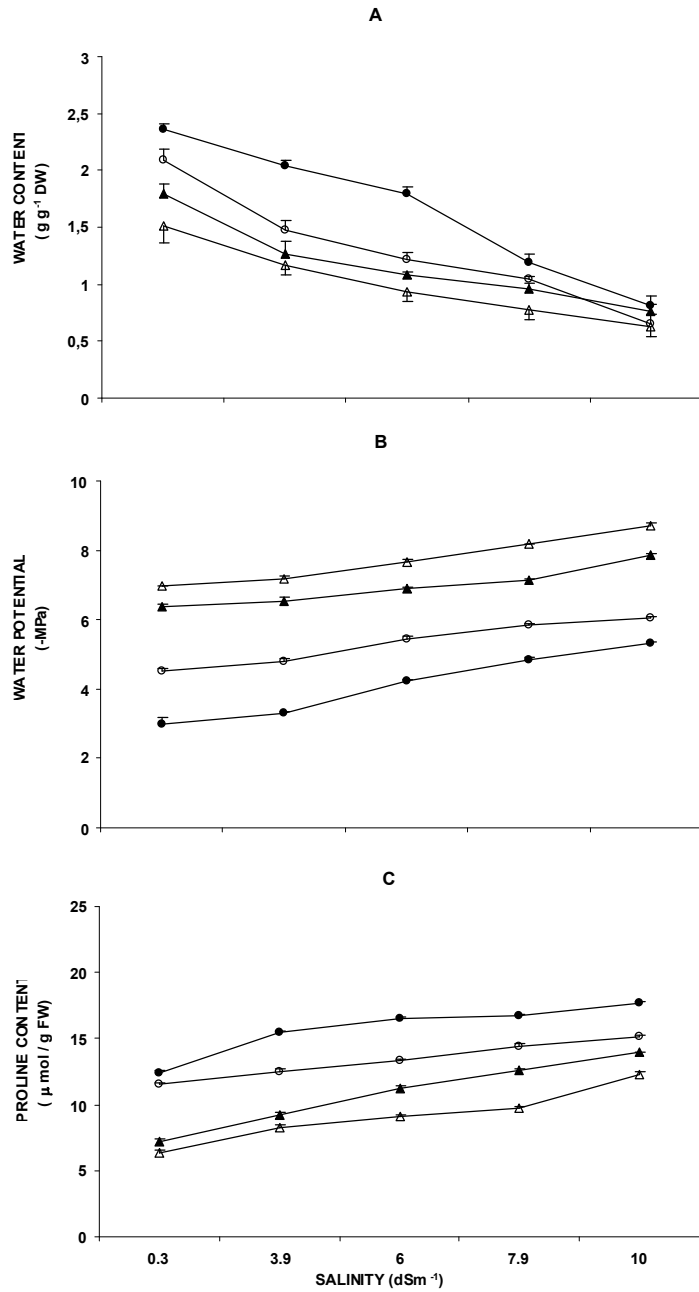

Figure 2. Effect of soil salinity on A. water content (g g $^{-1}$ DW) and B. water potential (-MPa) and C. proline content (µ mol/g FW) of leaves (●), stems (○), tap roots (▲) and lateral roots (Δ) of *Ziziphus mauritiana* seedlings. Error bars represent SE

Table 2a. Effect of soil salinity on nutrient content in leaf, stem, tap root and lateral root of *Ziziphus mauritiana*

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	0.3	34.2±2.3	1.8±0.1	7.9±0.2	5.8±0.2	15.9±0.7	7.9±0.3	1.4±0.1	4.2±0.1	6.1±0.6	15±0.7	299±5.5
	3.9	31.4±1.1	1.7±0.1	8.1±0.2	6.4±0.2	14.2±0.6	6.9±0.2	1.3±0.0	4.0±0.3	5.8±0.6	17±1.5	284±6.4
	6.0	25.5±0.6	1.3±0.3	9.4±0.1	7.9±0.2	11.3±1.2	5.5±0.2	1.2±0.0	3.9±0.2	3.9±0.6	24±1.2	256±6.4
	7.9	21.5±1.2	1.0±0.1	9.6±0.2	9.5±0.1	08.8±0.9	3.8±0.2	1.0±0.0	3.6±0.2	3.2±0.1	29±1.5	233±5.3
	10	19.3±0.6	0.8±0.1	10.6±0.2	10.6±0.3	06.7±0.6	3.2±0.3	1.0±0.0	2.9±0.3	3.1±0.6	34±1.2	189±5.2
	a	35.75	1.94	7.48	5.13	16.98	8.34	1.38	4.39	6.43	12.08	314.72
	b	-1.66	-0.11	0.29	0.52	-0.99	-0.51	-0.04	-0.12	-0.36	2.09	-11.16
	r	-0.929	-0.845	0.934	0.952	-0.947	-0.961	-0.936	-0.721	-0.814	0.945	-0.937
	$LSD_{0.05}$	2.70	0.40	0.50	0.80	2.00	0.50	0.10	0.50	1.10	4.50	12.30
Stem	0.3	26.6±1.2	1.4±0.1	6.2±0.1	7.3±0.3	17.43±0.6	8.6±0.1	0.9±0.0	3.8±0.1	4.9±0.6	21±0.6	272±6.1
	3.9	22.3±1.2	1.3±0.2	6.8±0.4	7.7±0.2	16.73±2.2	7.9±0.3	0.9±0.0	3.3±0.1	3.6±0.2	27±0.9	242±5.8
	6.0	20.6±1.2	1.0±0.1	8.5±0.3	8.0±0.1	15.57±0.6	6.2±0.2	1.1±0.1	2.8±0.2	2.7±0.3	32±1.7	213±6.4
	7.9	18.7±1.8	0.8±0.1	8.9±0.2	10.0±0.2	11.50±1.5	4.3±0.1	0.9±0.0	2.3±0.1	2.4±0.1	35±1.2	198±6.4
	10	16.4±0.6	0.6±0.1	9.3±0.1	11.5±0.4	07.50±0.9	3.7±0.3	0.8±0.0	2.0±0.5	2.1±0.1	39±0.7	174±5.3
	a	26.76	1.51	5.98	6.46	19.44	9.30	NS	3.91	4.86	20.22	276.96
	b	-1.03	-0.08	0.35	0.43	-1.01	-0.56	NS	-0.19	-0.30	1.86	-10.17
	r	-0.893	-0.868	0.956	0.895	-0.881	0.950	NS	-0.838	-0.903	0.968	-0.967
	$LSD_{0.05}$	2.50	0.30	0.50	0.80	1.30	0.70	NS	0.60	0.60	3.70	14.00

r – values are significant at $p < 0.01$, NS = Non significant

Table 2b. Effect of soil salinity on nutrient content in leaf, stem, tap root and lateral root of *Ziziphus mauritiana*

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 [$\mu g\ g^{-1}$]
Tap root	0.3	25.1±1.7	1.2±0.1	5.3±1.7	4.7±0.1	11.3±1.2	5.3±0.2	1.1±0.0	2.9±0.3	4.6±0.2	26±0.9	331±5.8
	3.9	21.6±0.6	1.2±0.1	5.8±0.7	4.9±0.2	10.5±0.6	4.5±0.2	1.2±0.0	2.6±0.1	3.6±0.6	33±1.2	315±5.5
	6.0	18.1±1.1	0.9±0.2	6.2±0.8	5.4±0.2	09.2±0.8	3.9±0.2	1.2±0.0	2.2±0.1	2.8±0.2	40±0.6	286±5.0
	7.9	16.3±0.6	0.7±0.1	6.9±0.4	7.2±0.3	07.6±0.6	2.9±0.2	1.0±0.0	2.0±0.1	2.3±0.1	44±1.7	263±6.4
	10	11.7±1.7	0.5±0.1	7.8±0.6	8.3±0.2	05.3±1.6	2.7±0.1	0.9±0.0	1.8±0.1	2.1±0.2	47±1.2	241±6.4
	a	26.20	1.33	4.98	3.95	12.24	5.49	NS	2.91	4.57	24.91	341.18
	b	-1.35	-0.08	0.25	0.37	-0.61	-0.29	NS	-0.11	-0.26	2.31	-9.63
	r	-0.921	-0.864	0.951	0.892	-0.894	-0.948	NS	-0.828	-0.893	0.970	-0.950
	$LSD_{0.05}$	2.50	0.30	0.30	0.70	1.00	0.60	NS	0.30	0.60	4.30	13.70
Lateral root	0.3	21.8±0.6	1.0±0.1	4.9±0.1	4.3±0.1	6.9±0.1	4.6±0.2	1.2±0.1	1.7±0.1	3.9±0.3	31±1.2	415±5.0
	3.9	18.2±1.7	0.9±0.2	4.7±0.2	4.5±0.1	6.2±0.8	3.8±0.1	1.1±0.0	1.6±0.1	3.1±0.3	39±1.2	409±6.6
	6.0	16.6±0.6	0.8±0.1	5.6±0.1	5.1±0.2	5.8±0.8	3.6±0.1	1.1±0.0	1.2±0.1	2.7±0.1	43±0.9	398±5.5
	7.9	14.5±1.7	0.6±0.1	6.2±0.3	6.6±0.1	4.9±0.3	2.9±0.1	0.9±0.0	1.0±0.1	2.1±0.2	49±1.2	381±6.4
	10	10.6±0.6	0.5±0.1	7.3±0.1	7.3±0.3	3.9±0.9	2.5±0.1	1.0±0.0	0.7±0.1	1.6±0.2	54±1.7	363±6.1
	a	22.58	1.06	4.57	3.70	7.24	4.72	NS	1.84	4.08	29.92	423.25
	b	-1.11	-0.05	0.17	0.33	-0.30	-0.23	NS	-0.11	-0.24	2.36	-5.36
	r	-0.901	-0.831	0.885	0.918	-0.827	-0.976	NS	-0.863	-0.946	0.972	-0.865
	$LSD_{0.05}$	2.50	0.40	0.30	0.60	0.60	0.60	NS	0.20	0.50	5.00	13.00

r – values are significant at $p < 0.01$, NS = Non significant

Effect of salinisation on accumulation of minerals

Potassium and sodium content and K/Na ratio

Potassium and sodium content (mg g^{-1} DW) significantly increased ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues in response to increasing soil salinity (Tab. 2a, 2b). There was a positive relationship for increase in K and Na content in leaves, stems, tap roots and lateral roots with increase in salt concentration in soil ($p < 0.01$). The K/Na ratio significantly decreased ($p < 0.05$) in leaves whereas it did not change in stems, tap roots and lateral root tissues. A negative relationship was obtained between K/Na ratio of leaves and salt concentration ($p < 0.01$).

Nitrogen, phosphorus, calcium and magnesium

The concentration of N, K, Na, Ca and Mg was, in general, greater than that of P in all tissues under control and salt stress conditions. Nitrogen, phosphorus, calcium and magnesium content significantly ($p < 0.01$) decreased in leaves, stems, tap roots and lateral roots in response to increase in soil salinity (Tab. 2a, 2b). A negative relationship ($p < 0.01$) was obtained between N, P, Ca and Mg content in seedling organs and salt concentration in soil ($p < 0.01$).

Microelements

There was a significant decrease in the concentration of Zn ($p < 0.05$), Cu and Fe ($p < 0.01$) in leaves, stems, tap roots and lateral root tissues in response to increase in salt stress (Tab. 2a, 2b). A negative relationship ($p < 0.01$) was obtained between Zn, Cu and Fe content in seedling organs and salt concentration ($p < 0.01$). The concentration of Mn significantly increased ($p < 0.01$) in leaves, stems, tap roots and lateral roots with the increase in soil salinity. A positive relationship was obtained between salt concentration and Mn content in seedling organs ($p < 0.01$).

DISCUSSION

Earlier work of Ramoliya et al. (2004) indicated that seedling emergence for salt–tolerant legume tree *Acacia catechu* was reduced to 50% (SG_{50}) in soil with salinity of 6.0 dSm^{-1}, but for *Z. mauritiana* SG_{50} was obtained at 6.5 dSm^{-1}. That would suggest that this plant species is relatively salt tolerant at seed germination stage. Under field conditions in coastal region of Saurashtra and in saline desert of Kutch, where this tree species is cultivated, maximum soil salinity is found during the dry period and minimum during the rainy season (wet period). In general, salinity for the surface soil (0-15 cm depth) varies from 2.0 to 6.0 dSm^{-1}. Eventually, seeds of *Z. mauritiana* can germinate and achieve establishment during the rainy season. However, salt concentration exceeding 10.0 dSm^{-1} was detrimental to seed germination that can be attributed to decreasing osmotic potential of the soil solution. It was observed that seeds became non-viable within a few days in the soil

with high concentration of salt. Although the effects of high salt content on metabolic processes are yet to be fully elucidated, it has been reported that salinity reduces protein hydration (Marschner, 1995) and induces changes in the activities of many enzymes (Dubey and Rani, 1990) in germinating seeds.

Reduction in water content and water potential of leaves, stems, tap roots and lateral roots of seedlings grown in saline soil might have resulted in internal water deficit to plants, which in turn reduced 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 and Zeiger, 2006). Moreover, tap root elongation for seedlings grown in control and saline soils both was markedly greater than that of shoot. Result suggests that this species has a tendency for rapid root extension. It is suggested that rapid root extension ensures existence of plants in dry habitats (Etherington, 1987) and is considered an adaptation to survive in dry habitats. Root/shoot dry weight ratios of seedlings grown in control and saline soils indicate that salinity reduced root growth less than the shoot growth. This type of root growth is an adaptation for plants that grow in arid and saline soils. Root/shoot dry weight ratio of *Z. mauritiana* was 0.47 under control conditions and was equal to that 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 the aspects of plant metabolism (Kramer, 1983; Garg and Gupta, 1997). Results for reduction of shoot growth and leaf area development of *Z. mauritiana* with increasing salt concentration are in conformity with the finding of Curtis and Lauchli (1986), who reported that growth of Kenaf (*Hibiscus cannabinus*) under moderate salt stress was affected primarily through a reduction in elongation of stem and leaf area development. Garg and 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) and causes reduction in root production (Garg and Gupta, 1997).

Results for dry weight and relative dry weight of seedling organs in response to increasing salinity suggest that reduction in dry weight was the lowest in lateral roots and the highest in stems. Consequently, lateral roots were most resistant, and stems were sensitive to increasing soil salinity. Seedling organs can be arranged in decreasing order of salt tolerance as: lateral roots > tap roots = leaves > stems. The concurrent and differential reduction in dry weight

of organs resulted in increased root/shoot dry weight ratio for seedlings with increase in salinity. In principle, salt tolerance can be achieved by salt exclusion or salt inclusion (Marschner, 1995). The salt excluders exhibit water deficit which reduces the plant growth. Adaptation by exclusion requires mechanisms for avoidance of an internal water deficit. Adaptation by salt inclusion requires either high tissue tolerance to Na^+ and Cl^- or avoidance of high tissue concentration. The includers (halophytes) utilize inorganic salts (K^+, Na^+) for turgour maintenance or for the replacement of K^+ in various metabolic functions by Na^+ (Marschner, 1995). Consequently, growth of these plants does not decline under natural conditions and plants are salt tolerant. In the present study, seedlings of *Z. mauritiana* survived up to 10.0 dSm^{-1} of soil salinity and, therefore, this tree species is moderately salt tolerant. In addition, salinity caused reduction in growth of seedlings primarily through lowering the water status (or causing water deficit) in tissues. It is reported that salt excluders suffer from adverse effects on water balance and exhibit much reduced growth rates on saline substrates (Greenway and Munns, 1980). As a result, this tree species can be grouped among salt excluders. Further, salt exclusion is a predominant salt avoidance mechanism in glycophytes (Greenway and Munns, 1980). Considering selectivity of ion uptake by root cells, it is still unclear which cell types control this process.

In some plant species, salt tolerance is associated with accumulation of organic solutes in cytoplasm to balance the osmotic pressure of ions in the vacuoles. The compounds that accumulate most commonly are proline and glycine betaine, although other molecules can accumulate to high concentration in certain species (Hasegawa et al., 2000). Proline accumulates in the cytoplasm without having any detrimental effects on cytosolic enzyme activities (Stewart and Lee, 1974). In the present study, osmotic adjustment was achieved by increase in concentration of proline and K^+ in tissues when water content decreased with increase in salinity. In addition, the primary role of proline may not be solely as an osmolyte, but it also helps the cells to overcome oxidative stress in salt-stressed plants (Rajendrakumar et al., 1994).

The cation K^+ is essential for cell expansion, osmoregulation and cellular and whole-plant homeostasis (Schachtman et al., 1997). High stomatal K requirement is reported for photosynthesis (Chow et al., 1990). The role of K in response to salt stress is also well documented, where Na depresses K uptake (Fox and Guerinot, 1998). In the present study, significant increase of K content in all organs of seedlings with increasing soil salinity might be due to high selectivity of *Z. mauritiana* for K^+. 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 to transport of salt to leaves or growing tissues. Moreover, the significant increase of Na to leaves and stem tissues suggests that this mechanism of blocking Na transfer to growing tissues was not effective in *Z. mauritiana* at high salt concentration. Decrease in K/Na ratio in leaves with increase in salinity suggests that Na was transported in greater proportion than K to these organs. There was no change in K/Na ratio in stems, tap roots and lateral roots because of proportionate increase of K and Na both in these organs as salinity increased. Results suggest that there were no effective mechanisms to control net uptake of Na on root plasma membrane and subsequently its transport to leaf tissue. The pattern of accumulation of K and Na in *Z. mauritiana* conforms to group C and/or group D plants in Marschner's (1995) classification of the ability of plants to substitute Na with K. In this classification Marschner divided plants into four groups, A, B, C and D depending upon whether K is mostly exchangeable with Na. Sodium has a positive effect on growth in A and B plants (mostly salt tolerant plants). Group C plants contain very little K that can be substituted with Na without a negative effect on growth, and group D plants exhibit no K/Na substitution (salt-sensitive plants).

It is reported that uptake mechanisms of both K and Na are similar (Watad et al., 1991; Schroeder et al., 1994). Plants utilize two systems for K acquisition, low- and high-affinity uptake mechanisms. Na^+ can not 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 and Sanders, 1999). Low affinity K uptake is not inhibited by Na but the high affinity process is restricted (Watad et al., 1991; Schroeder et al., 1994). Similarly, Na toxicity in plants is correlated with two proposed Na uptake pathways (Maathuis and Sanders, 1994; Niu et al., 1995). The K and Na profiles of *Z. mauritiana* suggest that similar mechanism might operate in this species. It is evidenced that Ca^{2+} causes closure of nonselective cation channels and restricts Na^+ uptake (Rus et al., 2001). As a result, calcium fertilizers may mitigate Na toxicity to this plant.

In general, salinity reduces N accumulation in plants (Feigin, 1985). This is due to the fact that an increase in chloride uptake and accumulation is mostly accompanied by a decrease in shoot nitrate concentration (Torres and Bingham, 1973; Garg and Gupta, 1997). The interaction between salinity and P is very complex and there is no clear cut mechanistic explanation for decreased, increased or unchanged P

uptake in response to salinisation in different species (Grattan and Grieve, 1992). However, it is known that P concentration is related to the rate of photosynthesis, but it decreases the conversion of fixed carbon into starch (Overlach et al., 1993) and therefore decrease of P in leaves will reduce shoot growth.

Calcium is important during salt stress, e.g., in preserving membrane integrity (Rengel, 1992), signalling in osmoregulation (Mansfield et al., 1990) and influencing K/Na selectivity (Cramer et al., 1987). In the present study, there was a significant decrease of Ca content in all the tissues with salinisation of soil. As a result Na induced Ca deficiency in tissues. It is reported that uptake of Ca^{2+} from the soil solution may decrease because of ion interaction, precipitation and increase in ionic strength that reduce the activity of Ca^{2+} (Janzen and Chang, 1997; Garg and Gupta, 1997). Besides the role of Mg in chlorophyll structure and as an enzyme cofactor, another important role of Mg in plants is in the export of photosynthates, which when impaired leads to enhanced degradation of chlorophyll in Mg deficient source leaves, resulting in increased oxygenase activity of RuBP carboxylase (Marschner and Cakmak, 1989).

It is difficult to suggest mechanistic explanations of salinity influence on microelement concentration due to relatively smaller differences between control and salinised tissues (Tozlu et al., 2000). In the present study, it appears that salinity reduced Zn, Cu and Fe accumulation, but it increased Mn accumulation, at the whole-plant level. Besides, cofactors for enzymes Fe and Cu are essential for biological redox systems (Marschner, 1995), Mn for photosynthetic reaction as part of water-splitting enzyme of photosystem II (Cheniae, 1970), and Zn for DNA replication, regulation of gene expression and integrity of biomembranes (Marschner, 1995). In addition, high concentration of iron is required for structural and functional integrity of the thylakoid membranes and synthesis of ferredoxin and chlorophyll (Marschner, 1995). Pushnik and Miller (1989) reported that iron is involved in photosystem I (PSI) development and assembling the subunits in the thylakoid membranes. The simultaneous decrease of Zn, Cu and Fe in leaves of *Z. mauritiana* might limit the growth of plants. Salinity generates an increase in reactive oxygen species (ROS) which have deleterious effects on cell metabolism (Borsani et al., 2001). Superoxide dismutases (SOD_S) detoxify ROS and may contain Cu, Zn, Mn or Fe as metal components (Slater et al., 2003). Increase in Mn content at the whole-plant level might be the requirement of this plant for survival in saline soils.

REFERENCES

Amtmann A., Sanders D. 1999. Mechanisms of Na^+ uptake by plant cells. ADV. BOT. RES. 29: 76-112.

Bates L.S., Waldren R.P., Teare F.D. 1973. Rapid determination of free proline from water stress studies. PLANT AND SOIL. 39: 205-207.

Bernstein L. 1962. Salt affected soils and plants. Proceedings of the Paris Symposium, UNESCO, May 1960. ARID ZONE RES. 18: 139-174.

Borsani O., Valpuesta V., Botella M.A. 2001. Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in *Arabidopsis* seedlings. PLANT. PHYSIOL. 126: 1024-1030.

Cheniae G.M. 1970. Photosystem II and O_2 evolution. ANNU. REV. PLANT PHYSIOL. 21: 467-498.

Chow W.S., Ball M.C., Anderson J.M. 1990. Growth and photosynthetic responses of spinach to salinity, implication of K nutrition for salt tolerance. AUST. J. PLANT PHYSIOL. 17: 563-578.

Cramer G.R., Lynch J., Lauchli A., Polito U.S. 1987. Influx of Na, K^+ and Ca into roots of salt-stressed cotton seedlings. Effects of supplemental Ca. PLANT PHYSIOL. 83: 510-516.

Cramer G.R., Epstein E., Lauchli A. 1989. Na-Ca interactions in barley seedlings, relationship to ion transport and growth. PLANT CELL AND ENVIRON. 12: 551-558.

Curtis P.S., Lauchli A. 1986. The role of leaf area development and photosynthetic capacity in determining growth of Kenaf under moderate salt stress. AUST. J. PLANT PHYSIOL. 13: 553-565.

Dubey R.S., Rani M. 1990. Influence of NaCl salinity on the behavior of protease, aminopeptidase and carboxylpeptidase in rice seedlings in relation to salt tolerance. AUST. J. PLANT PHYSIOL. 17: 215-224.

Etherington J.R. 1987. Penetration of dry soil by roots of *Dactylis glomerata* L. clones derived from well drained and poorly drained soils. FUNCT. ECOL. 1: 19-23.

Feigin A. 1985. Fertilization management of crops irrigated with saline water. PLANT AND SOIL. 89: 285-299.

Fox T.C., Guerinot M.L. 1998. Molecular biology of cation transport in plants. ANNU. REV. PLANT PHYSIOL. PLANT MOL. BIOL. 49: 669-696.

Garg B.K., Gupta I.C. 1997. Saline Wastelands Environment and Plant Growth. Scientific Publishers, Jodhpur, India, 283 p.

Gorham J. 1990. Salt tolerance in the Triticeae: K/Na discrimination in synthetic hexaploid wheats. J. EXP. BOT. 41: 623-627.

Grattan S.R., Grieve C.M. 1992. Mineral element acquisition and growth response of plants grown in saline environments, AGRIC. ECOSYST. ENVIRON. 38: 275-300.

Greenway H., Munns R. 1980. Mechanisms of salt tolerance in non-halophytes. ANNU. REV. PLANT. PHYSIOL. 31: 149-190.

Hasegawa P.M., Bressan R.A., Zhu J.K., Bohnert H.J. 2000. Plant cellular and molecular responses to high salinity. ANNU. REV. PLANT PHYSIOL. PLANT MOL. BIOL. 51: 463-499.

Janzen H.H., Chang C. 1987. Cation nutrition of barley as influenced by soil solution composition in a saline soil. CANAD. J. SOI. SCI. 67: 619-629.

Kramer P.J. 1983. Water Relations of Plants. Academic Press, New York. 489 p.

Maas E.V., Grieve C.M. 1987. Sodium induced calcium deficiency in salt-stressed corn. PLANT CELL AND ENVIRON. 10: 559-564.

Maas E.V., Hoffman G.J. 1977. Crop salt tolerance – current assessment. J. IRRIG. DRAIN. DIV. ASCE. 103: 115-134.

Maathuis F.J.M., Sanders D. 1994. Mechanism of high-affinity potas-

sium uptake in roots of *Arabidopsis thaliana*. PROC. NAT. ACAD. SCI, USA. 91: 9272-9276.

Mansfield T.A., Hetherington A.M., Atkinson C.J. 1990. Some aspects of stomatal physiology. ANNU. REV. PLANT PHYSIOL. PLANT MOL. BIOL. 41: 55-75.

Marschner H. 1995. Mineral Nutrition of Higher Plants. Academic Press, London 889 p.

Marschner H., Cakmak I. 1989. High light intensity enhances chlorosis and necrosis in leaves of zinc, potassium and magnesium deficient bean *Phaseolus vulgaris* plants. J. PLANT PHYSIOL. 134: 308-315.

Munns R. 1993. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. PLANT CELL AND ENVIRON. 16: 15-24.

Munns R. 2002. Comparative physiology of salt and water stress. PLANT CELL AND ENVIRON. 25: 239-250.

Niu X., Bressan R.A., Hasegawa P.M., Pardo J.M. 1995. Ion homeostasis in NaCl stress environments. PLANT PHYSIOL. 109: 735-742.

Overlach S., Diekmann W., Raschke K. 1993. Phosphate translocator of isolated guard-cell chloroplasts from *Pisum sativum* L. transport glucose-6-phosphate. PLANT PHYSIOL. 101: 1201-1207.

Patel A.D., Pandey A.N. 2007. Effect of salinisation of soil on growth, water status and nutrient accumulation in seedlings of *Jatropha curcas* (Euphorbiaceae). BASIC. APP. DRYL. RES. (in press).

Piper C.S. 1944. Soil and Plant Analysis. Interscience, New York 368 pp.

Pushnik J.C., Miller G.W. 1989. Iron regulation of chloroplast photosynthetic function: mediation of PS I development. J. PLANT NUTRI. 12: 407-421.

Ramoliya P.J., Patel H.M., Pandey A.N. 2004. Effect of salinization of soil on growth and macro- and micro-nutrient accumulation in seedlings of *Acacia catechu* (Mimosaceae). ANN. APPL. BIOL. 144: 321-332.

Ramoliya P.J., Patel H.M., Pandey A.N. 2006. Effect of salinization of soil on growth and nutrient accumulation in seedlings of *Prosopis cineraria*. J. PLANT NUTR. 29: 283-303.

Rajendrakumar C.S., Reddy B.V., Reddy A.R. 1994. Proline-protein interaction: Protection of structural and functional integrity of M_4 lactate dyhydrogenase. BIOCHEM. BIOPHYS. RES. COMM. 201: 957-963.

Rengel Z. 1992. The role of calcium in salt toxicity. PLANT CELL AND ENVIRON. 15: 625-632.

Rus A., Yokoi S., Sharkhuu A., Reddy M., Lee B.H., Matsumoto T.K., Koiwa H., Zhu J.K., Bressan R.A., Hasegawa P.M. 2001. AtHKT1 is a salt tolerance determinant that controls Na^+ entry into plant roots. PROC. NATL. ACAD. SCI. USA, 98: 14150-14155.

Schachtman D.P., Kumar R., Schroeder J.I., Marsh E.L. 1997. Molecular and functional characterization of a novel low-affinity cation transporter (LCTI) in higher plants. PROC. NATL. ACAD. SCI. USA, 94: 11079-11084.

Schroeder J.I., Ward J.M., Gassmann W. 1994. Perspectives on the physiology and structure of inward-rectifying K channels in higher plants, biophysical implications for K uptake. ANNU. REV. BIOPHYS. BIOMOL. STRUCT. 23: 441-471.

Slater A., Scott N., Fowler M. 2003. Plant Biotechnology. The genetic manipulation of plants. Oxford

University Press. Inc. New York 364 p.
Stewart G.R., Lee J.A. 1974. The role of proline accumulation in halophytes. PLANTA. 120: 279-289.
Taiz L., Zeiger E. 2006. Plant physiology (Fourth Edition). Sinauer Associates, Inc., Publishers, Sunderland, USA 764 p.
Torres B.C., Bingham F.T. 1973. Salt tolerance of Mexican wheat. I. Effect of NO_3 and NaCl on mineral nutrition, growth and grain produc-tion of four wheats. SOI. SCI. SOC. AM. PROC. 37: 711-715
Tozlu I., Moore G.A., Guy C.L. 2000. Effect of increasing NaCl concentration on stem elongation, dry mass production, and macro- and micronutrient accumulation in *Poncirus trifoliata*. AUST. J. PLANT PHYSIOL. 27: 35-42.
Watad A.A., Reuveni M., Bressan R.A., Hasegawa P.M. 1991. Enhanced net K uptake capacity of Nacl-adapted cells. PLANT PHYSIOL. 95: 1265-1269.

WPŁYW ZASOLENIA GLEBY NA WZROST, GOSPODARKĘ WODNĄ I AKUMULACJĘ SKŁADNIKÓW MINERALNYCH W SIEWKACH *Ziziphus mauritiana* (RHAMNACEAE)

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S T R E S Z C Z E N I E

W warunkach szklarniowych badano wpływ zasolenia na kiełkowanie, wzrost siewek, zawartość wody, zawartość proliny i składników mineralnych w siewkach *Ziziphus mauritiana* Lam. NaCl dodano w takich ilościach, aby otrzymać przewodnictwo elektryczne 0,3; 3,9; 6,0; 7,9; 10,0 i 11,9 dSm^{-1}. Zasolenie powodowało hamowanie wzrostu siewek, obniżało zawartość wody i akumulację suchej masy tkanek, ale następował wzrost poziomu proliny w siewkach. Poziom azotu, fosforu, wapnia i magnezu ulegał obniżeniu proporcjonalnie do stężenia zasolenia w glebie, natomiast zawartość potasu była wyższa w warunkach zasolenia gleby. W pracy dyskutowane są możliwe mechanizmy działania zasolenia na badane parametry w siewkach *Ziziphus mauritiana*.

Słowa kluczowe: zasolenie gleby, wzrost siewek, zawartość proliny, potencjał wodny, makro- i mikro-składniki, tolerancja na zasolenie