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EFFECT OF SALINIZATION OF SOIL ON GROWTH, WATER STATUS AND NUTRIENT ACCUMULATION IN SEEDLINGS OF *ACACIA AURICULIFORMIS* (FABACEAE)

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□ Greenhouse experiments were conducted to assess the effects of salinization of soil on emergence, growth, water status, proline content, and mineral accumulation of seedlings of *Acacia auriculiformis* A. Cunn. ex Benth. (Fabaceae). Sodium chloride (NaCl) was added to the soil and salinity was maintained at 0.3, 3.9, 6.0, 7.9, 10.0, 12.1, and 13.9 dS m⁻¹. Salinity caused reduction in water potential of tissues, which resulted in internal water deficit to plants. Consequently, seedling growth significantly decreased with increase in soil salinity. Proline content in tissues increased with increase in salinity. Potassium and sodium content significantly increased in tissues as salinity increased. Nitrogen content significantly increased in tissues with salinization of soil. Phosphorus, calcium and magnesium content significantly decreased as salinity increased. Changes in tissues and whole-plant accumulation patterns of other nutrients, as well as possible mechanisms for avoidance of sodium toxicity in this tree species in response to salinity, are discussed.

Keywords: salinization of soil, seedling growth, proline content, water potential, macro- and micronutrients, salt tolerance

INTRODUCTION

Soil salinity has detrimental effects on seed germination and plant growth (Bernstein, 1962; Garg and Gupta, 1997; Ramoliya et al., 2006). However, plant species differ in their sensitivity or tolerance to salts (Marschner, 1995). There is evidence that organs, tissues and cells at different developmental stages of plants exhibit varying degrees of tolerance to environmental conditions (Munns, 1993). It is reported that soil salinity suppresses shoot growth more than root growth (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

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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, 2008), but the relationship between micro-nutrient concentrations and soil salinity is rather complex and remains poorly understood (Tozlu et al., 2000). The knowledge acquired regarding the growth and survival of plants in saline habitat conditions could be useful for (i) screening the plant species for the afforestation of saline regions and also (ii) for understanding the mechanisms that plants use in the avoidance and/or tolerance of salt stress.

Acacia auriculiformis A. Cunn. ex Benth. (Fabaceae) is a tree species and is native to Australia, Indonesia and Papua New Guinea. Plants of this species have been transplanted in coastal area of Arabian Sea in Saurashtra region and at lowlands in Kutch (north-west saline desert) in Gujarat State of India. Kutch is located adjacent and to the north of Saurashtra region. Arabian Sea water enters and spreads to a considerable distance in the lowlands in Kutch. The transplanted plants grow successfully in these saline areas and are locally known as Australian teak. This tree species shows a high degree of salt tolerance and is considered to be valuable for utilizing saline soils. Its bark is a valuable material for tannin and wood is used as fuel. However, the potential of this tree species to grow and survive in coastal area of Saurashtra and in saline desert of Kutch is not known. The present investigation was carried out (i) to understand the adaptive features of *A. auriculiformis* which allow it to grow and survive in saline and arid regions and (ii) to assess the pattern of macro- and micronutrient accumulation within the tissues of this tree species in response to salt stress.

Study Area

The present study 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 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 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 soil was 0.3 dS m⁻¹. Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and sodium (Na) contents were 0.15%, 0.05%, 0.03%, 0.05%, and 0.002%, respectively. This soil is fertile and fit for intensive agriculture. The Kutch and Saurashtra regions are tropical monsoonic and can be

ecoclimatically classified as arid and semi-arid, respectively. The entire area is markedly affected by south-western monsoon which causes the onset of wet season in mid-June, and its retreat by the end of September coincides with a lowering of temperature and gradual onset of winter. Total annual rainfall is about 395 mm at Bhuj ($23^{\circ}16'$ N. Lat., $69^{\circ}40'$ E. Long.) in Kutch and about 554 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.

MATERIALS AND METHODS

Salinization of Soil

Surface soil was collected air dried and passed through a 2 mm mesh screen. Seven lots of soil, 100 kg each, were separately spread, about 50 mm thick, over polyethylene sheets. Sodium chloride (NaCl) amounting to 280, 590, 690, 1090, 1410, and 1690 g was then thoroughly mixed with soil of six lots, respectively to give electrical conductivities of 3.9, 6.0, 7.9, 10.0, 12.1, and 13.9 dS m^{-1} . There was no addition of NaCl to seventh lot of soil that served as control. The electrical conductivity of control soil was 0.3 dS m^{-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 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.

Seedling Emergence

Twenty polyethylene bags for each 3.9, 6.0, 7.9, 10.0, 12.1, and 13.9 dS m^{-1} soil salinity were each filled with 5 kg of soil. Tap water was added to each bag to bring the soils to field capacity and soils were allowed to dry for seven days. Salinity of tap water was 0.17 dS m^{-1} . Soils were then raked using fingers and seeds were sown on 22 July 2005. Seeds of *A. auriculiformis* were collected from the coastal area of Saurashtra. Bags were kept in a greenhouse. Ten seeds were sown in each bag at a depth of 8–12 mm. 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 30 days. A linear model was fitted to cumulative proportion of seed germination and

increasing soil salinity using the equation:

$$\text{Sin}^{-1}\sqrt{P} = \beta_0 + \beta_1 X$$

where, $\text{Sin}^{-1}\sqrt{P}$ is cumulative proportion of seed germination, X is soil salinity and β_0 and β_1 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. Seedlings grown in soils at 0.3, 3.9, 6.0, 7.9, 10.0, and 12.1 dS m^{-1} salinity exhibited emergence of the second leaf after 14 days. Emergence of the second leaf confirmed the establishment of seedlings. Moreover, only 8% seed germination was recorded in soil at 13.9 dSm^{-1} salinity and further experiments were not conducted on those seedlings. Following emergence of the second leaf, one seedling having better vigor was allowed to grow in each bag and another seedling was further uprooted. Thus twenty replicates factorialized with six grades of soil (0.3, 3.9, 6.0, 7.9, 10.0, and 12.1 dS m^{-1}) were prepared. This gave a total of 120 bags, which were arranged in twenty randomized blocks. Seedlings were watered (to raise the soil moisture to field capacity) at alternate days and allowed to grow for six months. For each salinity level, about 50 g surface soil was carefully taken out from three bags at the intervals of one month during the course of experiment and salinity was measured. Salinity of salinized soil became insignificantly lower (only by 0.3 to 0.5 dS m^{-1}) until the experiment was terminated on 22 January 2006. Seedlings contained in 20 bags at each salinity level were washed to remove soil particles adhered with roots. Morphological characteristics of each seedling were recorded. 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 tissues (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 estimation in plant tissues. Water potential of leaves, stems, tap roots and lateral root tissues was measured by Dewpoint Potential Meter WP4 (Decagon Devices, Inc., Pullman, WA, USA). Preparation of plant samples followed Ehlig (1962). Seedlings

were washed only prior to the measurement and water particles over roots and shoots were dried by blotting paper. Small pieces of the tissue were cut and weighed to 500 mg. Thereafter, sample was spread at the bottom of a disposable cup and it was placed into the chamber of the instrument for reading. All the measurements were taken between 8 to 10.30 AM. Concentration of proline in plant tissues was estimated following Bates et al. (1973). Extract of 0.5 g fresh plant material with aqueous sulphosalicylic acid was prepared. The extracted proline was made to react with ninhydrin to form chromophore and read 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. Plant samples were 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 colour method in sulfuric acid (Piper, 1944). Concentrations of Ca, magnesium (Mg), Na, K, zinc (Zn), iron (Fe), manganese (Mn), and copper (Cu) were determined by atomic absorption spectroscopy after triacid [nitric (HNO_3): sulfuric (H_2SO_4): perchloric (HClO_4) acids in the ratio of 10: 1: 4] digestion. Mineral data were analyzed by one way ANOVA. Correlations and linear regression equations between mineral content and salt concentrations were determined.

RESULTS

Effect of Salinization on Seedling Emergence

Seedlings began to emerge two days after sowing and 87% seed germination was obtained over a period of 12 days under control (0.3 dSm^{-1} salinity) conditions (Figure 1). Seedling emergence in saline soils was recorded two to four days after sowing. Emergence lasted for 13, 13, 12, 13, 12, and 8 days in soils with 3.9, 6.0, 7.9, 10.0, 12.1, and 13.9 dS m^{-1} salinities, respectively, and corresponding seed germination was 77%, 68%, 55%, 49%, 41%, and 8%. 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: $Y = 79.325 - 7.745X$, ($R_{\text{Adj}}^2 = 0.919$, $p < 0.01$), where Y is arcsine (degrees) of proportion of cumulative seed germination and X is salt concentration.

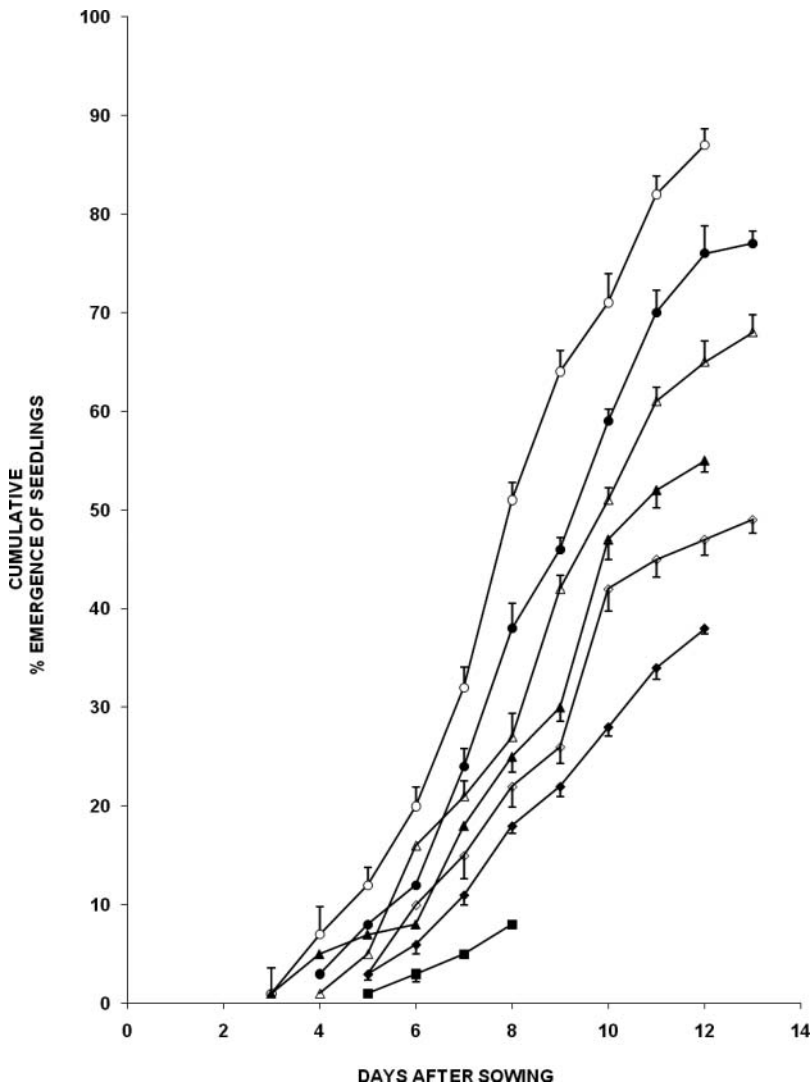


FIGURE 1 Cumulative emergence of seedlings of *Acacia auriculiformis* in response to soil salinity. 0.3 dSm⁻¹ (○), 3.9 dS m⁻¹ (●), 6.0 dS m⁻¹ (△), 7.9 dS m⁻¹ (▲), 10.0 dSm⁻¹ (◇), 12.1 dSm⁻¹ (◆) and 13.9 dS m⁻¹ (■). Error bars represent SE.

Effect of Salinization on Stem and Root Elongation and Leaf Expansion

Increasing concentration of salt in soils significantly retarded ($P < 0.01$) elongation of stems and roots (Table 1). Nevertheless, root length was nearly double than stem height for seedlings grown in control and saline soils. There was a negative relationship for shoot height and root length with increasing salt concentration in soil ($P < 0.01$). Leaf expansion was

TABLE 1 Effect of salinization of soil on leaf, stem, shoot and root characteristics of *Acacia auriculiformis* as indicated by mean \pm SEM

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 Root weight (mg plant ⁻¹)	Total Root weight (mg plant ⁻¹)
0.3	21.4 \pm 0.8	39.9 \pm 1.1	135.8 \pm 1.8	695.1 \pm 75.4	387.2 \pm 36.5	1082.3 \pm 93.7	207.6 \pm 21.2	233.7 \pm 23.9	441.3 \pm 42.0
3.9	17.1 \pm 0.5	37.3 \pm 1.2	104.4 \pm 2.5	499.8 \pm 46.2	314.6 \pm 32.8	814.4 \pm 70.7	124.7 \pm 13.5	146.0 \pm 17.5	270.7 \pm 24.0
6.0	15.5 \pm 0.8	29.0 \pm 1.7	92.8 \pm 3.4	443.3 \pm 30.3	250.2 \pm 10.2	693.5 \pm 29.8	82.2 \pm 7.4	127.5 \pm 10.9	209.7 \pm 12.8
7.9	15.0 \pm 0.9	27.0 \pm 1.6	68.6 \pm 2.1	353.2 \pm 31.8	179.4 \pm 11.2	532.6 \pm 34.1	59.6 \pm 5.4	98.8 \pm 13.2	158.4 \pm 17.4
10.0	14.2 \pm 0.4	23.4 \pm 1.7	42.2 \pm 2.3	281.8 \pm 30.9	151.3 \pm 13.8	433.1 \pm 42.7	50.3 \pm 3.5	95.4 \pm 11.8	145.7 \pm 14.5
12.1	13.5 \pm 0.7	21.8 \pm 2.0	39.3 \pm 2.9	237.2 \pm 36.5	131.3 \pm 21.25	368.5 \pm 58.6	41.3 \pm 5.9	62.1 \pm 11.4	103.4 \pm 16.6
α	20.8	43.4	149.8	721.8	419.3	1170.2	202.0	231.1	436.9
β	-1.29	-3.93	-19.81	-86.670	-52.57	-141.7	-30.780	-29.67	-62.11
R	0.882	0.977	0.984	0.971	0.977	0.979	0.914	0.932	0.934
LSD _{0.05}	3.2	7.3	11.2	85.2	89.3	96.1	45.3	56.8	95.4

Relationship is significant at $p < 0.01$.

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 Salinization on Dry Weight

Dry weight significantly decreased ($P < 0.01$) for leaves, stems, shoots (leaves + stems), tap roots, lateral roots and total roots of seedlings in response to increasing concentration of salt (Table 1). A negative relationship was obtained between dry weight of different tissues and salt concentration ($P < 0.01$).

Percentage relative weights of tissues of salinized plants compared to those of control plants were computed as: (salinized tissues dry weight/control dry weight) \times 100. Dry weight values of tissues given in Table 1 were used for the calculation of percent relative weight of tissues. Values of percent relative weight varied from 80 to 34% for stems, from 72 to 34% for leaves, from 62 to 22% for lateral roots and from 60 to 19% for tap roots in response to increasing soil salinity from 3.9 to 12.1 dS m⁻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 (DW₅₀) were around 7.8, 7.0, 4.9, and 5.9 for leaves, stems, tap roots and lateral root tissues, respectively. Root/shoot dry weight ratio was 0.44 under control conditions, while it was obtained 0.37, 0.30, 0.28, 0.26, and 0.24 for seedlings grown in soils at 3.9, 6.0, 7.9, 10.0, and 12.1 dS m⁻¹ salinity, respectively.

Effect of Salinization on Water Content of Tissues

Water content in leaves, stems, tap roots and lateral roots significantly decreased ($P < 0.01$) with increasing concentration of salt in soil (Figure 2). There was maximum water content in lateral roots and minimum in tap roots. Tissues, according to their water content, can be arranged in the following decreasing order: lateral roots > leaves > stems = tap roots. There was a negative relationship between water content in different tissues and salt concentration ($r = -0.275, -0.451, -0.331$ and $-0.311, P < 0.01$ for leaves, stems, tap roots and lateral roots, respectively.).

Effect of Salinization on Water Potential of Tissues

Water potential significantly became more negative in leaves, stems, tap roots and lateral root tissues ($P < 0.01$) as soil salinity increased (Figure 2). Further, water potential significantly differed ($P < 0.01$) for tissues of control and salt-stressed seedlings. Tissues according to their water potential values (less negative) can be arranged in the following order: lateral roots > leaves = tap roots > stems. There was a negative relationship between water

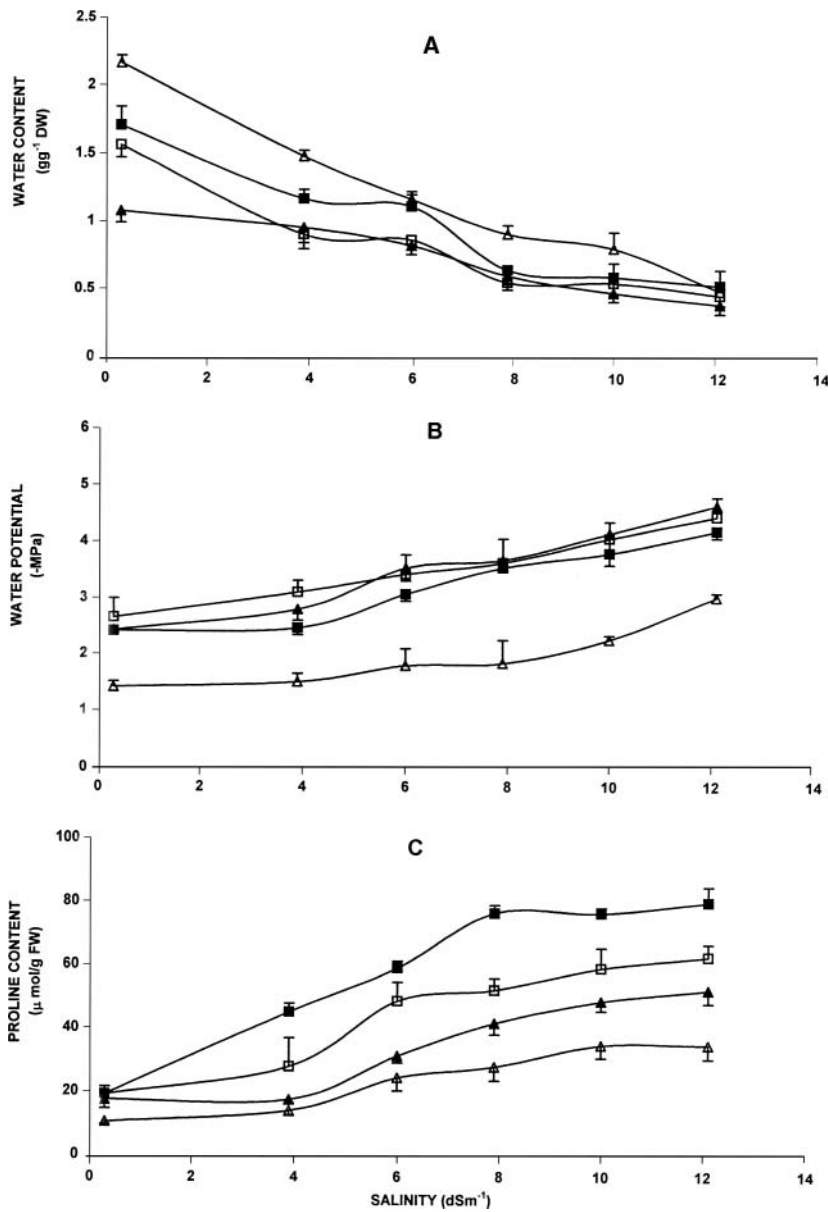


FIGURE 2 Effect of soil salinity on A. water content (gg⁻¹ DW). B. water potential (-MPa) and C. proline content (μmol/g FW) of leaves (■), stem (□), tap root (▲) and lateral roots (Δ) of *Acacia auriculiformis* seedlings. Error bars represent SE.

potential of tissues and salt concentration ($r = -0.967, -0.994, -0.987$ and -0.906 , $P < 0.01$ for leaves, stems, tap roots and lateral roots, respectively.). A positive relationship was obtained between water content and water potential (negative value) ($r = 0.888, 0.935, 0.962$ and 0.884 , $P < 0.01$, for leaves, stems, tap roots and lateral roots, respectively.).

Effect of Salinization on Proline Content of Tissues

Proline content ($\mu\text{mol/g}$ FW material) significantly increased ($P < 0.01$) in leaves, stems, tap roots and lateral root tissues with increase in soil salinity (Figure 2). Tissues, according to their proline content can be arranged in following decreasing order: leaves > stems > tap roots > lateral roots. There was a positive relationship between salt concentration and proline content of tissues ($r = 0.935, 0.964, 0.956$ and $0.956, P < 0.01$ for leaves, stems, tap roots and lateral roots, respectively.). A negative relationship was obtained between water potential and proline content ($r = -0.890, -0.754, -0.872$ and $-0.617, P < 0.01$, for leaves, stems, tap roots and lateral roots, respectively). Similarly, a negative relationship was obtained between water content and proline content ($r = -0.707, -0.835, -0.943$ and $-0.988, P < 0.01$, for leaves, stems, tap roots and lateral roots, respectively).

Effect of Salinization on Mineral Accumulation

Potassium and Sodium Content and K/Na Ratio

Potassium and sodium content (as mg g^{-1} dry weight) significantly increased ($P < 0.01$) in leaves, stems, tap roots and lateral root tissues in response to increasing soil salinity (Table 2). There was a positive relationship for K and Na content in leaves, stems, tap roots and lateral roots with increase in salt concentration ($P < 0.01$). The K/Na ratio significantly increased in leaves and tap roots ($P < 0.01$) in response to increase in soil salinity, while it did not change for stems and lateral root tissues. There was a positive relationship between increase in soil salinity and increase in K/Na ratio of leaves and tap roots ($P < 0.01$).

Nitrogen, Phosphorus, Calcium, and Magnesium

The 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 significantly increased ($P < 0.01$) in leaves, stems, tap roots and lateral root tissues as the salinity increased (Table 2). A positive relationship was obtained in N content of tissues and salt concentration ($P < 0.01$). Phosphorus, calcium and magnesium content significantly decreased ($P < 0.01$) in leaves, stems, tap roots and lateral root tissues in response to increase in soil salinity. There was a negative relationship for P, Ca, and Mg content in tissues with salt concentration ($P < 0.01$).

Microelements

There was a significant increase in the concentration of Zn, Mn, and Cu ($P < 0.01$) in leaves, stems, tap roots and lateral root tissues in response to increase in salt-stress (Table 2). There was a positive relationship between salt concentration and Zn, Mn, and Cu content in tissues ($P < 0.01$). The

TABLE 2 Effect of salinisation of soil on nutrient content of tissues (leaf, stem, tap root and lateral root) of *Acacia auriculiformis* as indicated by mean \pm SEM

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 (μ g g ⁻¹)	Cu (μ g g ⁻¹)	Mn (μ g g ⁻¹)	Fe (μ g g ⁻¹)	
Leaf	0.3	18.0 \pm 1.1	2.3 \pm 0.2	10.3 \pm 0.3	3.8 \pm 0.0	14.8 \pm 1.1	4.8 \pm 0.3	2.7 \pm 0.1	11.0 \pm 0.4	6.2 \pm 0.4	107 \pm 7.2	510 \pm 15.6	
	3.9	21.0 \pm 1.0	2.1 \pm 0.2	12.2 \pm 0.2	3.9 \pm 0.1	12.4 \pm 0.9	3.8 \pm 0.2	3.1 \pm 0.1	11.7 \pm 0.5	7.2 \pm 0.5	119 \pm 8.5	460 \pm 27.0	
	6.0	23.0 \pm 1.1	1.9 \pm 0.2	15.1 \pm 0.9	4.3 \pm 0.4	10.1 \pm 0.9	3.1 \pm 0.1	3.5 \pm 0.2	12.9 \pm 0.8	9.2 \pm 0.7	137 \pm 6.9	420 \pm 16.3	
	7.9	26.0 \pm 1.3	1.8 \pm 0.3	17.3 \pm 0.5	4.8 \pm 0.2	8.2 \pm 0.9	2.4 \pm 0.3	3.6 \pm 0.1	14.0 \pm 0.3	10.9 \pm 0.6	146 \pm 9.3	390 \pm 19.9	
	10.0	28.0 \pm 0.8	1.5 \pm 0.2	20.1 \pm 0.8	5.6 \pm 0.2	7.2 \pm 0.7	1.4 \pm 0.2	3.6 \pm 0.1	16.2 \pm 0.7	12.2 \pm 0.3	161 \pm 6.9	370 \pm 27.0	
	12.1	31.0 \pm 0.9	0.9 \pm 0.2	23.6 \pm 0.5	6.4 \pm 0.3	6.8 \pm 0.6	1.2 \pm 0.1	3.7 \pm 0.2	18.4 \pm 0.4	15.1 \pm 0.5	174 \pm 11.5	330 \pm 8.6	
	α	17.05	2.47	8.80	3.32	14.79	4.94	2.80	9.82	5.06	101.66	515.07	
	β	1.11	-0.11	1.14	0.22	-0.72	-0.32	0.08	0.63	0.75	5.84	-15.17	
	r	0.938	-0.773	0.966	0.81	-0.900	-0.962	0.842	0.917	0.948	0.878	-0.898	
	LSD _{0.05}	3.25	0.65	1.79	0.72	2.69	0.67	0.37	1.63	1.55	25.91	61.16	
	Stem	0.3	10.0 \pm 0.5	1.2 \pm 0.1	8.4 \pm 0.1	4.0 \pm 0.1	13.5 \pm 1.2	3.8 \pm 0.2	2.1 \pm 0.0	11.4 \pm 0.3	9.2 \pm 0.4	68 \pm 1.9	995 \pm 22.3
		3.9	11.4 \pm 0.6	1.1 \pm 0.2	8.9 \pm 0.1	4.3 \pm 0.1	11.2 \pm 0.8	3.5 \pm 0.2	2.1 \pm 0.1	12.1 \pm 0.8	10.9 \pm 0.4	77 \pm 4.3	970 \pm 17.6
6.0		13.0 \pm 1.1	0.8 \pm 0.1	12.2 \pm 0.1	5.7 \pm 0.1	8.6 \pm 0.3	2.8 \pm 0.2	2.1 \pm 0.0	13.3 \pm 0.9	12.2 \pm 0.5	89 \pm 3.7	940 \pm 23.6	
7.9		15.1 \pm 0.6	0.7 \pm 0.1	14.6 \pm 0.3	6.6 \pm 0.2	7.9 \pm 0.9	2.1 \pm 0.2	2.2 \pm 0.0	14.2 \pm 0.8	13.6 \pm 0.3	94 \pm 2.2	900 \pm 16.7	
10.0		18.0 \pm 0.6	0.6 \pm 0.0	16.2 \pm 0.2	7.4 \pm 0.2	7.1 \pm 0.7	1.9 \pm 0.2	2.2 \pm 0.1	15.4 \pm 0.7	15.2 \pm 0.5	101 \pm 5.1	860 \pm 27.1	
12.1		16.0 \pm 0.7	0.6 \pm 0.1	19.2 \pm 0.1	8.9 \pm 0.1	6.4 \pm 0.4	1.4 \pm 0.2	2.2 \pm 0.0	17.6 \pm 0.7	16.8 \pm 0.7	109 \pm 4.1	830 \pm 17.7	
α		9.27	1.25	6.81	3.30	13.25	4.07	—	10.59	8.60	65.62	1014.80	
β		0.69	-0.06	0.96	0.42	-0.61	-0.21	—	0.51	0.65	3.56	-14.80	
r		0.928	-0.824	0.967	0.964	-0.878	-0.932	NS	0.864	0.959	0.930	-0.875	
LSD _{0.05}		2.14	0.34	0.48	0.45	2.53	0.59	—	2.21	1.48	11.27	64.29	

Tap root	0.3	8.0±0.8	1.0±0.1	9.1±0.1	3.8±0.2	9.2±0.7	3.2±0.2	2.4±0.1	9.2±0.4	9.6±0.4	58±1.2	1330±32.7
	3.9	10.0±0.6	0.9±0.1	10.3±0.5	3.9±0.2	8.2±0.7	2.5±0.3	2.6±0.0	10.3±0.5	10.1±0.5	59±1.8	1250±36.1
	6.0	11.1±0.7	0.9±0.1	12.2±0.2	4.1±0.1	6.7±0.7	2.2±0.1	3.0±0.1	12.1±1.0	10.9±0.3	61±2.1	1180±38.4
	7.9	12.0±1.0	0.8±0.0	14.8±0.2	4.6±0.2	5.8±0.6	1.8±0.2	3.2±0.1	14.4±0.6	11.6±0.6	63±1.8	1130±24.1
	10.0	12.8±0.9	0.7±0.1	16.9±0.4	5.5±0.2	4.6±0.3	1.4±0.1	3.1±0.1	15.8±0.7	12.3±0.5	67±2.6	1090±51.1
	12.1	14.6±0.7	0.6±0.1	20.0±0.6	6.2±0.2	4.4±0.4	1.1±0.1	3.2±0.1	17.1±0.6	13.2±0.7	74±2.9	1010±34.8
α		7.84	1.03	7.52	3.30	9.47	3.22	2.42	8.34	9.20	55.00	1344.10
β		0.53	-0.03	0.95	0.20	-0.44	-0.17	0.07	0.71	0.31	1.29	-26.73
r		0.878	-0.759	0.963	0.884	-0.888	-0.937	0.832	0.933	0.850	0.793	-0.890
LSD _{0.05}		2.38	0.24	1.14	0.54	1.79	0.53	0.33	2.03	1.55	6.49	112.56
Lateral root	0.3	12.0±0.6	0.8±0.0	8.3±0.1	4.6±0.3	8.4±0.5	5.2±0.2	1.8±0.2	16.8±0.5	11.1±0.8	138±5.6	1640±76.3
	3.9	14.0±1.0	0.7±0.1	8.7±0.3	5.2±0.1	7.8±0.6	4.8±0.2	1.7±0.1	18.1±1.1	12.4±0.4	157±2.7	1480±35.2
	6.0	15.0±1.1	0.7±0.1	11.0±0.2	6.4±0.3	6.0±0.4	3.8±0.2	1.7±0.1	19.3±0.7	13.1±0.7	163±4.4	1419±47.4
	7.9	17.0±0.8	0.5±0.1	14.7±0.1	7.7±0.2	5.3±0.5	3.1±0.1	1.9±0.1	21.1±1.2	14.1±0.7	177±5.4	1360±32.7
	10.0	20.0±0.6	0.5±0.0	16.2±0.1	8.8±0.2	4.2±0.3	2.5±0.1	1.9±0.0	22.6±1.2	16.1±0.6	183±4.1	1280±42.3
	12.1	21.0±1.0	0.3±0.1	18.2±0.1	10.13±0.1	3.8±0.4	2.2±0.1	1.8±0.0	24.8±0.8	19.8±0.7	195±6.3	1210±22.6
α		11.09	0.89	6.64	3.50	8.77	5.52	—	15.9	9.88	136.96	1637.30
β		0.80	-0.04	0.92	0.52	-0.42	-0.28	—	0.68	0.68	4.74	-35.70
r		0.918	-0.864	0.958	0.980	-0.914	-0.959	NS	0.875	0.884	0.933	-0.903
LSD _{0.05}		2.58	0.17	0.45	0.65	1.41	0.53	—	2.93	2.05	14.84	139.43

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

concentration of Fe significantly decreased in leaves, stems, tap roots and lateral roots ($P < 0.01$) with increase in soil salinity. There was a negative relationship between salt concentration and Fe content in tissues ($P < 0.01$).

DISCUSSION

Earlier work (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 dS m^{-1} , but for *Acacia auriculiformis* SG_{50} was obtained at 6.4 dS m^{-1} . That would suggest that this plant species is salt-tolerant at seed germination. Under field conditions in coastal area of Saurashtra and in saline desert of Kutch, where this tree species has been transplanted, maximum soil salinity is found during the dry period and minimum during the rainy season (wet period) in the year. In general, salinity for the surface soil (0–15 cm depth) varies from 4.0 to 6.0 dS m^{-1} . Eventually, seeds of *A. auriculiformis* plants which have been transplanted in coastal area of Saurashtra and in saline desert of Kutch can germinate and achieve establishment during the rainy season that may result in expansion of population. However, further increase in salt concentration 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 concentration 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 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, elongation of tap roots was double than that of stems for control and salt stressed seedlings. Results suggest 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 an adaptation to survive in dry habitats. Leaves (phyllodes) of this tree species are thick and succulent to a little extent and store water. Though succulence is primarily an adaptation to water stress, it provides salt resistance to plants because it temporarily puts off the setting of severe water-deficit induced by salt stress. Root/shoot dry weight ratio of *A. auriculiformis* was 0.44 under control conditions and was almost 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). Results for reduction of shoot growth and leaf area development of *A. auriculiformis* with increasing salt concentration are in conformity with the finding of Curtis and Lauchli (1986), who 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 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 tissues in response to increasing salinity suggest that the lowest reduction occurred for the dry weight of stems and leaves while the largest reduction occurred for tap roots. Consequently, stems and leaves were most resistant, and tap roots were highly sensitive to increasing soil salinity. Tissues can be arranged in decreasing order of salt tolerance as: stems = leaves > lateral roots > tap roots. Moreover, there was concurrent and differential reduction in dry weight of tissues. The rapid dry weight reduction in tap roots and lateral roots caused reduction in root/shoot dry weight ratio with increasing salt stress. 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 utilize inorganic salts in metabolic processes. Consequently, growth of these plants does not decline under natural conditions and plants are salt tolerant. However, moderate tolerance for salinity (12.1 dS m^{-1}) and reduction in growth of leaves, stems and root tissues suggest that this tree species can be grouped among salt excluders. Greenway and Munns (1980) reported that in glycophytes salt exclusion is predominant salt avoidance mechanism. Considering selectivity of ions by root cells, it is still unclear which cell types control the selectivity of ions from the soil solution.

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 enzymes activities (Stewart and Lee, 1974). In the present study osmotic adjustment

was achieved by increase in quantity 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). In the present study, proline accumulation was greater in leaves and stems than that in tap roots and lateral roots as salinity increased. Result corroborates the conclusion of Munns (2002) that organic solutes are often lower in roots than shoots.

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 tissues of seedlings with increasing soil salinity might be due to high selectivity of *A. auriculiformis* 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 of *A. auriculiformis* suggests that this mechanism to block Na transfer to growing tissues was not effective at high salt concentration. Significant increase in K/Na ratio in leaves with increase in salinity suggests that K was transported in greater proportion than Na to this tissue. There was no change in K/Na ratio in stems and lateral roots because there was rapid increase of Na in these tissues along with K as salinity increased. The increase in K/Na ratio in tap roots with increase in salinity can be accounted for relatively less accumulation of Na in this tissue. The pattern of accumulation of K and Na in *A. auriculiformis* 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^+ 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 nonselective 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 (Ammann and Sanders, 1999). Low affinity K uptake is not inhibited by Na but the high affinity process is restricted (Wataid 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 *A. auriculiformis* 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. Further, high K content and low Na content in tissues suggest two distinct traits: (i) low Na^+ influx and/or high Na efflux by Na^+/H^+ antiporter on root plasma membrane and (ii) high K^+/Na^+ discrimination to select K from soil with high Na concentration. Results further suggest that sodium accumulation was greater in stem tissues than in leaves. It can be attributed to cell types in stems that are better able to retain Na^+ . 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 12.1 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. Increase in nitrogen content in tissues was in conformity with increase in proline content. Dubey and Rani (1989) reported that protein level in several crops under salinization increases due to the increased synthesis of pre-existing and certain new sets of proteins. 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. A decrease of P concentration in root tissues, on the other hand, strongly stimulates the formation of root hairs and lateral roots in leguminous trees, rape, spinach, tomato, and white lupin (Racette et al., 1990).

Calcium is important during salt stress, e.g., in preserving membrane integrity (Rengel, 1992), signaling in osmoregulation (Mansfield et al., 1990) and influencing K/Na selectivity (Cramer et al., 1987). In the present study, Na induced Ca deficiency in tissues of salt-stressed seedlings. It is reported that uptake of Ca^{2+} from the soil solution may decrease because of ion interactions, precipitation and increase in ionic strength that reduce the activity of Ca^{2+} (Janzen and Chang, 1987). 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 micro-element concentration due to relatively smaller differences between control and salinised tissues (Tozlu et al., 2000). In the present study, it appears that salinity increased Zn, Mn, and Cu accumulation, while reduced Fe 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 Fe in leaves of *A. auriculiformis* might limit photosynthesis and growth of plants. Salinity generates an increase in reactive oxygen species (ROS) which may have deleterious effect on cell metabolisms (Borsani et al., 2001). Superoxide dismutases (SODs) detoxify ROS and may contain Cu, Zn, Mn or Fe as metal components (Slater et al., 2003). Increase in Zn, Mn, and Cu content at the whole plant level might be the requirement of this plant for survival in saline soils.

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