Growth, water status and nutrient accumulation of seedlings of *Holoptelea integrifolia* (Roxb.) Planch in response to soil salinity

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ABSTRACT

Greenhouse experiments were conducted to assess the effects of soil salinity on emergence, growth, water status, proline content and mineral accumulation of seedlings of *Holoptelea integrifolia* (Roxb.) Planch (*Ulmaceae*). 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. 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 soil salinity. There were no effective mechanisms to control net uptake of Na transport to shoot tissue. Potassium content increased in leaves to avoid Na toxicity to this tissue. Nitrogen content significantly increased in tissues in response to salinity. Phosphorus, calcium and magnesium content in tissues significantly decreased as salinity increased. Changes in tissues and whole-plant accumulation patterns of other nutrients, as well as possible mechanisms to avoid Na toxicity in this species in response to salinity, are discussed.

Keywords: soil salinity; seedling growth; proline content; water potential; macro- and micro-nutrients; salt tolerance

Saline soils are abundant in semi-arid and arid regions, where the amount of rainfall is insufficient for substantial leaching (Marschner 1995). Salinity is a scourge for agriculture, forestry, pasture development and other similar practices. 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 to avoid and/or tolerate salt stress. *Holoptelea integrifolia* (Roxb.) Planch (*Ulmaceae*), a deciduous tree species, grows in coastal forests of Saurashtra in the Gujarat State of India. It also grows successfully on marginalsaline lands of Kutch (north-west saline desert) contiguous to Saurashtra. H. integrifolia yields a good timber. Seeds are eaten and used to treat ring worms locally. Bark yields fiber. The juice of bark is applied on rheumatic swellings. However, the potential of this tree species to grow and survive in dry coastal area of Saurashtra and in marginal saline desert of Kutch is not known. The present investigation was performed to understand the adaptive features of *H. integrifolia* that allow it to grow and survive in saline and arid regions and to assess the pattern of macro- and micro-nutrient accumulation within the tissues of this tree species in response to salt stress.

MATERIAL AND METHODS

The present study was carried out in a greenhouse of the botanical garden of Saurashtra University at Rajkot (22°18′ N, 70°56′ E) in Gujarat. For the emergence and growth of seedlings the top 15 cm black-cotton soil, which is predominant in Saurashtra, was used. Physical and chemical

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properties of the soil were given earlier Pandya et al. (2004). Soil was air dried and passed through a 2 mm mesh screen. Seven 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, 1410 and 1690 g was then thoroughly mixed with soil of six lots to give electrical conductivities of 3.9, 6.0, 7.9, 10.0, 12.1 and 13.9 dS/m, respectively. There was no addition of NaCl to the seventh lot of soil that served as control. The electrical conductivity of control soil was 0.3 dS/m, which is equal to 3 mmol/l salinity. Measurement of electrical conductivity of soil followed Ramoliya et al. (2004). Twenty polyethylene bags for each level of soil salinity were filled with 5 kg of soil each. Ten seeds were sown in each bag at a depth of 8-12 mm on 15 August 2005. 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:

$$\sin^{-1} \sqrt{P} = 60 + 61X$$

where: $\sin^{-1}\sqrt{P}$ is the cumulative proportion of seed germination; X is soil salinity; ß0 and ß1 are constants

Salt concentration at which seed germination was reduced to 50% (${\rm SG}_{50}$) was estimated using the model.

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 and 7.9 dS/m salinity exhibited emergence of the second leaf after 24 days. Emergence of the second leaf confirmed the establishment of seedlings. Moreover, only 5% seed germination was recorded in soil at 10.0 dS/m salinity and further experiments were not conducted on those seedlings. Seedlings did not emerge in soils where salinity exceeded 10.0 dS/m. Following emergence of the second leaf, one seedling having better vigor was allowed to grow in each bag and the rest of them were further uprooted. Seedlings were watered (about 300 ml water was added to raise the soil moisture to field capacity) at alternate days and the experiment was terminated after 6 months. The mean maximum temperature of the greenhouse in the course of study increased from 33.3 ± 1.2 °C in August to 37.8 ± 0.8 °C in October and declined thereafter to 33.8 ± 0.3°C

in February. Seedlings 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. Sum of leaf and stem weight was considered as shoot weight. Water content (g/g dw) in plant tissues (leaves, stems, tap roots and lateral roots) was calculated using fresh and dry weight values.

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 tissues was measured by the Dewpoint Potential Meter WP4. Concentration of proline in plant tissues was estimated following Bates et al. (1973). Mineral analyses were performed on the tissues. Total nitrogen was determined by the Kjeldahl method and phosphorus content was estimated by the chlorostannous molybdophosphoric blue colour method (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 $(HNO_3:H_2SO_4:HClO_4$ in the ratio of 10:1:4) digestion. Data for plant responses to salinity were analyzed by one-way ANOVA.

RESULTS AND DISCUSSION

Effect of salinity on seedling emergence

Seedlings began to emerge 2 days after sowing and 85% seed germination was obtained over a period of 12 days under control conditions (Figure 1). Seedling emergence in saline soils was recorded 2–4 days after sowing. Emergence lasted for 11, 11, 13 and 8 days in soils with 3.9, 6.0, 7.9 and 10.0 dS/m salinities, respectively, and corresponding seed germination was 76, 68, 32 and 5%. 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:

$$Y = 76.814 - 5.406X$$
, $(R^2_{Adj} = 0.835, P < 0.01)$

where: Y is arcsine (°) of proportion of cumulative seed germination and X is salt concentration

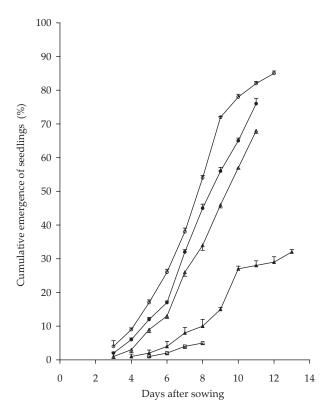


Figure 1. Cumulative emergence of seedlings of *Holoptelea integrifolia* in response to soil salinity: 0.3 dS/m (\bigcirc), 3.9 dS/m (\bigcirc), 6.0 dS/m (\triangle), 7.9 dS/m (\triangle), 10.0 dS/m (\square); error bars represent SE

Effect of salinity 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 shoot height for both control and salt stressed seedlings. There was a negative relationship for shoot height and root length with increasing salt concentration in soil (P < 0.01). In addition, 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 salinity on dry weight

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

Table 1. Effect of salinisation of soil on leaf, stem, shoot and root characteristics of Holoptelea integrifolia as indicated by mean ± SEM and regression equation

constants

Salinity (dS/m)	Shoot height (cm)	Root length (cm)	Leaf area (cm²/plant)	Leaf weight (mg/plant)	Stem weight (mg/plant)	Shoot weight (leaf + stem) (mg/plant)	Tap root weight (mg/plant)	Lateral root weight (mg/plant)	Total root weight (mg/plant)
0.3	25.7 ± 0.7	48.4 ± 0.9	165.4 ± 2.3	659 ± 8.2	880 ± 23.1	1538.6 ± 26.7	558 ± 7.9	255.8 ± 5.7	814.2 ± 8.4
3.9	23.2 ± 0.7	43.2 ± 1.2	148.2 ± 2.4	580 ± 5.7	679 ± 24.0	1259.2 ± 24.2	479 ± 5.5	189.8 ± 3.4	669.1 ± 6.1
0.9	21.0 ± 0.9	38.4 ± 0.9	95.3 ± 2.5	523 ± 7.1	566 ± 15.7	1088.6 ± 17.7	415 ± 6.3	115.4 ± 4.1	530 ± 8.7
7.9	18.1 ± 0.8	31.8 ± 0.9	74.9 ± 2.2	471 ± 6.6	452 ± 9.5	923.0 ± 14.5	362 ± 3.9	85.1 ± 1.9	447.5 ± 4.5
α	26.46	50.05	177.57	669.65	897.85	1567.50	570.89	267.09	837.99
β	-0.98	-2.12	-12.51	-24.66	-56.03	-80.69	-25.90	-23.33	-49.23
r	-0.628	-0.803	-0.801	-0.915	-0.887	-0.925	-0.937	-0.957	-0.971
$LSD_{0.05}$	2.5	3.1	16.9	22.0	60.1	2.5	19.3	12.7	22.6

Relationship was significant at P < 0.01

pared to those of control plants was computed as: (salinised tissues dry weight/control dry weight) \times 100. Values of relative weight varied from 88 to 71.5% for leaves, from 85.8 to 64.9% for tap roots, from 77 to 51% for stems and from 74 to 33% for lateral roots in response to increasing soil salinity from 3.9 to 7.9 dS/m. As has been estimated using regression equations given in results, the salt concentration at which dry weight would be reduced to 50% of control plants (DW $_{50}$) were around 13.8, 8.2, 11.3 and 6.0 for leaves, stems, tap roots and lateral root tissues, respectively. Root/shoot dry weight ratio was 0.53 under control conditions, and significantly decreased (P < 0.01) as soil salinity increased.

Effect of salinity on water content, water potential and proline content of tissues

Water content in tissues significantly (P < 0.01) decreased with increasing concentration of salt in soil (Figure 2). There was a negative relationship between water content in different tissues and salt concentration (r = -0.820, -0.756, -0.866 and

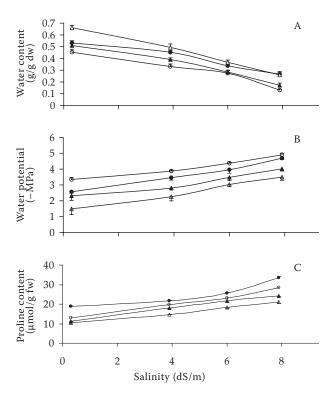


Figure 2. Effect of salinisation of soil on: (A) water content (g/g dw), (B) water potential (-MPa), (C) proline content (μ mol/g fw) of leaves (\bullet), stems (\circ), tap roots (\triangle) and lateral roots (\triangle) of *Holoptelea integrifolia* seedlings; error bars represent SE

-0.810, P < 0.01 for leaves, stems, tap roots and lateral roots, respectively). Water potential significantly became more negative in tissues (P < 0.01) as soil salinity increased. There was a negative relationship between water potential of tissues and salt concentration (r = -0.921, -0.951, -0.950 and -0.978, P < 0.01 for leaves, stems, tap roots and lateral roots, respectively). Proline content significantly increased (P < 0.01) in tissues with increase in soil salinity. There was a positive relationship between salt concentration and proline content of tissues (r = 0.917, 0.989, 0.996 and 0.979, P < 0.01 for leaves, stems, tap roots and lateral roots, respectively).

Effect of salinity on mineral accumulation

Potassium content significantly increased (P < 0.01) in leaves, whereas it did not change in other tissues in response to increasing soil salinity (Table 2). There was a positive relationship (P < 0.01) between K content in leaves and salt concentration. Sodium content significantly increased (P < 0.01) in tissues with increasing soil salinity. A positive relationship was obtained between Na content in tissues and increase in salt stress (P < 0.01). The K/Na ratio did not change in tissues in response to increase in soil salinity. N content significantly increased in tissues (P < 0.01), as the salinity increased. A positive relationship was obtained in N content of tissues and salt concentration (P < 0.01). Concentration of phosphorus, calcium and magnesium significantly decreased (P < 0.01) in tissues in response to increase in soil salinity. A negative relationship (P < 0.01) was obtained between P, Ca and Mg content of tissues and salt concentration. There was a significant increase in concentration of Zn, Cu, Mn and Fe (P < 0.01) in tissues in response to increase in salt-stress. A positive relationship (P < 0.01) was obtained between Zn, Cu, Mn and Fe content of tissues and salt concentration.

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, but for *Holoptelea integrifolia* SG $_{50}$ was obtained at 5.0 dS/m. That would suggest that this plant species is relatively salt tolerant at seed germination. However, salt concentration exceeding 10 dS/m was detrimental to seed germination that can be attributed to decreasing osmotic potential of the soil solution. It is reported that salinity reduces protein hydra-

Table 2. Effect of salinisation of soil on nutrient content of tissues (leaf, stem, tap root and lateral root) of Holoptelea integrifolia as indicated by mean ± SEM and regression equation constants

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Tissue	Salinity (dS/m)	N (mg/g)	P (mg/g)	K (mg/g)	Na (mg/g)	Ca (mg/g)	Mg (mg/g)	K/Na ratio	Zn (µg/g)	Cu (µg/g)	Mn (µg/g)	Fe (µg/g)
	0.3	14.0 ± 0.6	3.7 ± 0.1	18.7 ± 0.6	3.3 ± 0.3	29.0 ± 0.3	11.3 ± 0.6	5.6 ± 0.4	9.2 ± 0.2	10.5 ± 0.2	53.7 ± 1.8	757 ± 14.5
	3.9	17.0 ± 2.3	3.4 ± 0.1	20.3 ± 0.9	3.5 ± 0.1	27.5 ± 0.5	9.7 ± 0.7	5.8 ± 0.3	10.0 ± 0.1	11.0 ± 0.1	58.7 ± 1.5	820 ± 3.5
	0.9	17.7 ± 1.2	3.2 ± 0.3	22.5 ± 0.6	4.1 ± 0.1	26.2 ± 0.2	9.0 ± 0.2	5.5 ± 0.3	11.1 ± 0.2	12.7 ± 0.1	61.7 ± 1.5	863 ± 4.9
J == 1	7.9	21.3 ± 0.9	2.5 ± 0.1	24.1 ± 0.8	5.0 ± 0.2	25.3 ± 0.3	8.4 ± 0.1	4.8 ± 0.3	12.3 ± 0.2	13.5 ± 0.2	66.0 ± 0.6	923 ± 6.4
Lear	α	13.49	3.82	18.11	2.97	29.24	11.30	ı	8.80	10.01	52.84	744.06
	β	0.88	-0.14	0.72	0.22	-0.49	-0.38	I	0.40	0.41	1.58	21.39
		0.761	0.801	0.875	0.826	0.935	0.836	NS	0.951	0.934	0.910	0.971
	$\mathrm{LSD}_{0.05}$	4.60	1.70	2.30	0.70	1.10	1.50	1	0.50	0.40	4.20	25.70
	0.3	15.7 ± 0.9	3.4 ± 0.1	13.2 ± 0.4	3.6 ± 0.1	23.4 ± 0.3	9.1 ± 0.1	3.7 ± 0.0	10.5 ± 0.2	14.5 ± 0.2	66.0 ± 1.5	855 ± 4.7
	3.9	18.7 ± 1.5	3.3 ± 0.2	12.6 ± 0.2	4.1 ± 0.2	21.4 ± 0.3	8.6 ± 0.1	3.1 ± 0.2	11.6 ± 0.2	15.6 ± 0.2	69.7 ± 0.7	867 ± 4.9
	0.9	19.3 ± 1.5	3.0 ± 0.1	12.0 ± 0.2	4.7 ± 0.1	19.9 ± 0.3	8.0 ± 0.1	2.5 ± 0.0	12.3 ± 0.3	16.2 ± 0.2	73.0 ± 1.2	905 ± 6.7
Ctoss	7.9	22.3 ± 0.9	2.4 ± 0.2	11.6 ± 0.5	5.5 ± 0.2	18.3 ± 0.2	7.1 ± 0.1	2.1 ± 0.1	13.1 ± 0.2	18.4 ± 0.1	76.0 ± 1.7	1024 ± 24.6
Stem	α	15.30	3.69	ı	3.40	23.79	9.35	ı	10.33	14.06	65.22	822.56
	β	0.82	-0.10	ı	0.24	-0.67	-0.25	ı	0.34	0.47	1.31	19.95
	i.	0.791	0.773	NS	0.922	0.974	0.943	NS	0.957	0.924	0.886	0.813
	$LSD_{0.05}$	3.60	0.50	ı	0.40	0.80	1.10	-	09.0	0.50	4.00	40.00
	0.3	19.0 ± 1.2	3.4 ± 0.1	8.9 ± 0.3	4.6 ± 0.1	18.6 ± 0.1	7.1 ± 0.1	1.9 ± 0.0	16.4 ± 0.1	16.3 ± 0.2	76.0 ± 1.5	975 ± 6.7
	3.9	24.3 ± 1.5	3.3 ± 0.2	8.3 ± 0.2	5.0 ± 0.2	17.4 ± 0.4	6.4 ± 0.1	1.4 ± 0.1	17.2 ± 0.2	17.0 ± 0.2	83.0 ± 0.6	1027 ± 6.4
	0.9	27.7 ± 0.9	3.0 ± 0.1	8.0 ± 0.2	5.6 ± 0.1	16.8 ± 0.2	5.9 ± 0.1	1.3 ± 0.1	17.7 ± 0.1	18.6 ± 0.1	85.3 ± 1.2	1060 ± 5.7
Tap	7.9	28.7 ± 0.9	2.4 ± 0.2	7.9 ± 0.2	6.0 ± 0.2	15.7 ± 0.1	5.3 ± 0.1	1.1 ± 0.1	18.6 ± 0.2	19.3 ± 0.2	88.0 ± 2.1	1093 ± 14.9
root	α	18.93	3.61	I	4.44	18.18	7.21	I	16.23	15.97	75.99	80.696
	β	1.32	-0.13	I	0.19	-0.37	-0.23	I	0.28	0.40	1.57	15.41
	7	0.911	0.733	SN	0.911	0.947	696.0	NS	0.949	0.946	0.901	0.957
	$LSD_{0.05}$	3.40	0.52	1	0.50	0.70	0.30	ı	0.50	0.50	4.40	27.90
	0.3	28.0 ± 1.5	2.1 ± 0.1	7.1 ± 0.2	5.7 ± 0.2	15.5 ± 0.2	5.8 ± 0.1	1.2 ± 0.1	18.9 ± 0.1	18.7 ± 0.1	86.3 ± 1.5	1052 ± 8.5
	3.9	30.3 ± 1.8	2.1 ± 0.1	6.8 ± 0.2	6.3 ± 0.1	14.6 ± 0.2	5.3 ± 0.2	1.1 ± 0.0	19.5 ± 0.1	19.4 ± 0.1	87.7 ± 0.9	1077 ± 7.5
	6.0	32.0 ± 1.0	1.8 ± 0.2	6.6 ± 0.1	6.7 ± 0.2	13.7 ± 0.3	4.9 ± 0.2	1.0 ± 0.0	20.1 ± 0.1	20.3 ± 0.1	91.0 ± 1.2	1105 ± 4.1
Lateral	7.9	34.7 ± 0.7	1.2 ± 0.2	6.3 ± 0.3	7.1 ± 0.1	12.7 ± 0.1	4.2 ± 0.2	1.0 ± 0.0	21.3 ± 0.1	22.1 ± 0.3	95.7 ± 0.9	1121 ± 8.7
root	α	27.42	2.26	I	2.60	15.80	5.92	I	18.55	18.21	84.83	1046.60
	β	0.85	-0.11	I	0.19	-0.37	-0.20	I	0.31	0.42	1.18	9.31
	7	0.780	0.719	SN	0.935	0.953	968.0	NS	0.928	0.922	0.846	0.920
	$\mathrm{LSD}_{0.05}$	4.00	0.40		0.40	09:0	0.50		0.40	0.50	3.40	22.60

r values are significant at P < 0.01; NS – not significant

tion (Slater et al. 2003) and induces changes in the activities of many enzymes (Dubey and Rani 1990) in germinating seeds.

Reduction in water content and water potential of tissues of seedlings in response to soil salinity might have resulted in internal water deficit to plants, which in turn, reduced growth of shoots and roots. 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 (Marschner 1995). Reduction in shoot growth of H. integrifolia with increasing salt concentration can further be accounted for reduction in leaf area (photosynthetic area). Curtis and 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. Moreover, seedlings exhibited a rapid root extension that is considered a valuable adaptation to exploit moisture in dry habitats (Etherington 1987). Results for dry weight and relative dry weight of tissues in response to increasing salinity suggest that there was the lowest reduction in dry weight of leaves while reduction was maximum for lateral roots. Consequently, leaves were most resistant, and lateral roots were sensitive to increasing soil salinity. Moreover, there was concurrent and differential reduction in dry weight of tissues. The maximum dry weight reduction in lateral roots and minimum reduction in dry weight of leaves caused reduction in root/shoot dry weight ratio with increasing salt stress. In some plant species, salt tolerance associates with accumulation of organic solutes in cytoplasm to balance the osmotic pressure of ions in the vacuoles (Hasegawa et al. 2000). The increase in proline content of the tissues in response to salinity indicates that proline accumulation may contribute to the alleviation of NaCl stress in the plant. 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). Proline accumulation was greater in leaves and stems than that in tap roots and lateral roots as salinity increased. This result is in accordance with the conclusion of Munns (2002) that organic solutes are often lower in roots than shoots.

Significant increase of Na in leaves and stem tissues of *H. integrifolia* suggests its two traits:

(i) high Na⁺ influx and/or low Na⁺ efflux on root plasma membrane and (ii) lack of effective exchange of K⁺ for Na⁺ by the cells in the stele of roots or in the vascular bundles in stems - a mechanism of salt tolerance - to block Na transfer to growing tissues at high salt concentration. Also, lateral root tissues are the least resistant to salt stress and therefore do not have ability to accumulate high concentration of Na. An increase of K content in leaves in response to salinity evinces an enhanced transport of K from stem tissues to protect leaves. It is reported that uptake mechanisms of both K and Na are similar (Watad et al. 1991). Plants utilize two systems for K acquisition, low- and high-affinity uptake mechanisms. Low affinity of K uptake is not inhibited by Na but the high affinity process is restricted (Watad et al. 1991). Similarly Na toxicity in plants is correlated with two proposed Na uptake pathways (Niu et al. 1995). The K and Na profiles of H. integrifolia suggest that similar mechanism might operate in this species. It is reported that Ca²⁺ causes closure of nonselective cation channels (low-affinity transport system) 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), but in this plant nitrogen increased with increase in salinity. Dubey and 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. However, P, Ca and Mg were the limiting factors for growth of seedlings in saline soil. Salinity generates an increase in reactive oxygen species (ROS) that have deleterious effects on cell metabolism. Superoxide dismutases (SODs) detoxify ROS and may contain Cu, Zn, Mn or Fe as metal components (Slater et al. 2003). An increase in Zn, Cu, Mn and Fe content at the whole-plant level might be the requirement of this plant for survival in saline soils.

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