Growth, water status and nutrient accumulation of seedlings of *Jatropha curcas* L. (Euphorbiaceae) in response to soil salinity

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Resumen

Crecimiento, estado hídrico y acumulación de nutrientes en plántulas de Jatropha curcas *L. (Euphorbiaceae) en respuesta a la salinidad.*

Se ha valorado el efecto de la salinidad sobre la emergencia, crecimiento, estado hídrico, contenido de prolina y acumulación mineral de plántulas de *Jatropha curcas* L. (Euphorbiaceae). Se añadió NaCl al suelo y se mantuvo la salinidad 0,3; 3,9; 6,0; 7,9 y 10,0 dSm^{-1}. La salinidad produjo una reducción del contenido y potencial hídricos de los tejidos, lo que resultó en un déficit hídrico interno para las plantas. Consecuentemente, disminuyó significativamente el alargamiento de los tallos y raíces, la expansión de las hojas y la materia seca acumulada. También se observó que disminuyó la suculencia de tallos y raíces. El contenido de prolina aumentó en consonancia con la salinidad, lo mismo que el K, Na y N, mientras que P, Ca y Mg disminuyeron. Se discute sobre los cambios en los patrones de acumulación de nutrientes en los tejidos y la planta completa, así como los posibles mecanismos para evitar la toxicidad del sodio en respuesta a la salinidad.

Palabras clave: Acumulación mineral, Contenido de prolina, Crecimeinto de plántulas, Salinidad del suelo, Potencial hídrico.

Abstract

Effects of soil salinity on emergence, growth, water status, proline content and mineral accumulation of seedlings of *Jatropha curcas* L. (Euphorbiaceae) were assessed. NaCl was added to the soil and salinity was maintained at 0.3, 3.9, 6.0, 7.9 and 10.0 dSm^{-1}. Salinity caused reduction in water content and water potential of tissues, which resulted in internal water deficit to plants. Consequently, stem and root elongation, leaf expansion and dry matter accumulation in seedlings significantly decreased. Salinity impaired succulence of stems and tap roots. Proline content in tissues increased as salinity increased. Likewise K, Na and N content significantly increased in tissues as salinity increased, while P, Ca and Mg content in tissues significantly decreased. We discuss changes in tissues and whole-plant accumulation patterns of nutrients, as well as possible mechanisms for avoidance of sodium toxicity in this tree species in response to salinity.

Key words: Mineral accumulation, Proline content, Seedling growth, Soil salinity, Water potential.

Introduction

Soil salinity has detrimental effects on seed germination and plant growth (Bernstein 1962, Garg & Gupta 1997, Ramoliya et al. 2006, Patel & Pandey 2008, Patel et al. 2010). There are evidences that organs, tissues and cells at different developmental stages of plants exhibit varying degrees of tolerance to adverse environmental conditions (Munns 1993). However, plant species differ in their sensitivity or tolerance to salts (Marschner 1995). It is reported that soil salinity suppresses shoot growth more than the root growth (Maas & 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 detrimental consequences for growth. Internal concentrations of major nutrients and their uptake have been frequently studied (Cramer et al. 1989, Maas & Grieve 1987, Ramoliya et al. 2006, Patel & Pandey 2007, Malik et al. 2009, Patel et al. 2010), 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 of plants to combat desertification and (ii) understanding the mechanisms that plants use in the avoidance and/or tolerance of salt stress.

Jatropha curcas L. (Euphorbiaceae) is a small tree and its probable centre of origin is Mexico and Central America. It is now naturalized and widespread throughout the tropics. Plants are succulent and grow on the poor and dry habitats. Its oil is an environmentally safe and cost-effective renewable source of non-conventional energy and promising substitute for diesel, kerosene and other fuels. This plant is also a source of poisons and medicines. Young plants can be used as green manure. There is much emphasis for the cultivation of this tree species on wastelands in western region of Gujarat State in India. The western region of Gujarat can be divided into two zones: (i) the Kutch, a northern saline desert and (ii) the Saurashtra, to the south of Kutch. Saurashtra zone comprises of a peripheral coastal area along the shore of the Arabian Sea and a central area. The intensive agriculture is restricted only to central area of Saurashtra and a vast area in Western Gujarat is dry and saline with varying intensity. Cultivation of this tree species is viewed with two purposes: (i) it will help combating desertification in Western Gujarat and (ii) it will also yield biofuel as an additional benefit. However, the potential of this tree species to grow and survive in dry and saline regions of Western Gujarat is not known. The present investigation was carried out to evince what adaptive features *J. curcas* has evolved that may allow it to grow and survive in dry and saline habitats.

Material and Methods

Study area

The present study was carried out in a greenhouse of the botanical garden of Saurashtra University at Rajkot (22º18' N, 70º56' E) 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 0.3dSm^{-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. 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 362mm at Bhuj (23º15' N, 69º49' E) in Kutch and 551mm at Rajkot in central Saurashtra. Of the total rainfall, 96% at Bhuj and 99.7% at Rajkot occurs during the rainy (monsoon) 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 peri-

ods 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 2mm mesh screen. Five lots of soil, of 100kg each, were separately spread, about 50mm thick, over polyethylene sheets. Sodium chloride (NaCl) amounting to 280, 590, 690 and 1090g was then thoroughly mixed with soil of four lots, respectively to give electrical conductivities of 3.9, 6.0, 7.9 and 10.0dSm^{-1}. There was no addition of NaCl to fifth lot of soil that served as control. The electrical conductivity of control soil was 0.3dSm^{-1} and this value was approximately equal to 3mM 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 following Ramoliya et al. (2004).

Seedling emergence

Twenty polyethylene bags for each level of soil salinity were each filled with 5kg of soil. Tap water was added to each bag to bring the soils to field capacity and soils were allowed to dry for 7 days. Soils were then raked using fingers and seeds were sown on 18 July 2005. Bags were kept in an uncontrolled greenhouse under natural temperature and light. Ten seeds were sown in each bag at a depth of 8-12 mm. Immediately after sowing soils were watered (about 300mL water was added to raise the soil moisture to field capacity) and thereafter about 100-150mL water was added to 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 expression:

$$\mathrm{Sin}^{-1}\sqrt{P} = ß0 + ß1X$$

where, $\mathrm{Sin}^{-1}\sqrt{P}$ is cumulative proportion of seed germination, X is soil salinity and 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. Seedlings grown in soils at 0.3, 3.9, 6.0 and 7.9dSm^{-1} salinity exhibited emergence of the second leaf after 9 days. Emergence of the second leaf indicated the initiation of establishment of seedlings. Moreover, only 5% seed germination was recorded in soil at 10.0dSm^{-1} salinity 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 vigor was allowed to grow in each bag and another seedling was further uprooted. Thus twenty replicates factorialzed with four grades of soil (0.3, 3.9, 6.0 and 7.9dSm^{-1}) were prepared. This gave a total of 80 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 9 months. Experiment was terminated on 18 April 2006. The mean maximum temperature of the greenhouse during the course of study decreased from 36.1±0.6ºC in July to 33.2 ±0.4ºC in August and then increased to 36.7 ±0.5ºC in October 2005. Following this period, mean maximum temperature decreased to 30.8 ±0.4ºC in January 2006 and again increased to 40.9±0.3ºC in April. 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 at the end of the experimental period. 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 (gg^{-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 and linear regression.

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. Wa-

ter potential of leaves, stems, tap roots and lateral root tissues was measured by Dewpoint Potential Meter WP4 (Decagon Devices Inc., Pullman, WA, USA). All the measurements were taken between 7.30 and 10 AM. Concentration of proline in plant tissues was estimated following Bates et al. (1973) using an extract of 0.5g fresh plant material in aqueous sulphosalicylic acid. The extracted proline was made to react with ninhydrin to form chromophore and read at 520nm. Data were analyzed by one-way ANOVA and linear regression.

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 the Kjeldahl method and phosphorus content estimated by the chlorostannous molybdophosphoric blue colour 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 (Shimadzu Corporation, Kyoto, Japan) after triacid (HNO_3: H_2SO_4: $HClO_4$ at a ratio of 10:1:4) digestion. Mineral data were analyzed by one-way ANOVA and linear regression.

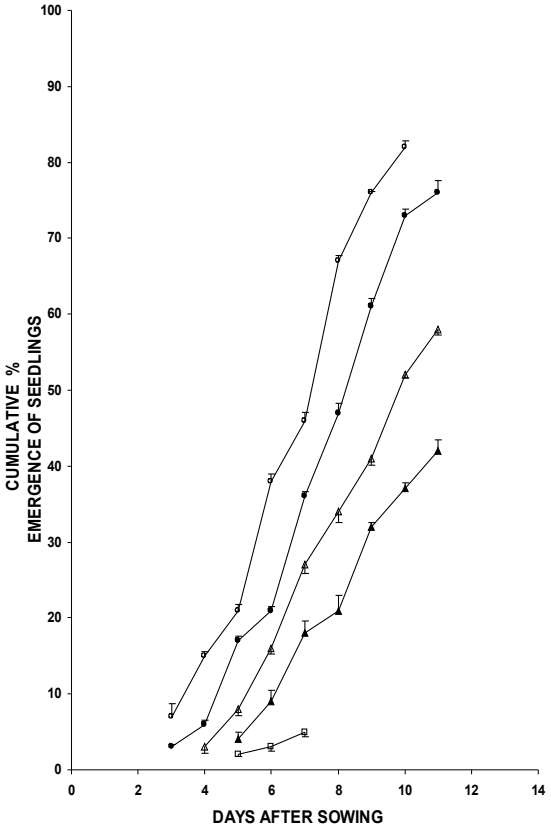

Figura 1. Emergencia acumulada de plántulas de Jatropha curcas en respuesta a la salinidad del suelo. 0,3dSm^{-1} (○), 3,9dSm^{-1} (●), 6,0dSm^{-1} (Δ), 7,9dSm^{-1} and (▲), 10,0 dSm^{-1} (□).

Figure 1. Cumulative emergence of seedlings of Jatropha curcas 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 (n=200).

Results

Effect of salinity on seedling emergence

Seedlings began to emerge 2 days after sowing and 82% seed germination was obtained over a period of 10 days under control (0.3 dSm^{-1} salinity) conditions (Fig. 1). Seedling emergence in saline soils was recorded 2-4 days after sowing. Emergence continued for 11, 11, 11 and 7 days in soils with 3.9, 6.0, 7.9 and 10dSm^{-1} salinities, respectively. Seed germination decreased from 76% at 3.9dSm^{-1} salinity to 5% at 10dSm^{-1} salinity. There was a significant reduction in seed germination ($p<0.001$) with increasing salt stress. A negative relationship between proportion of cumulative seed germination and concentration of salt was obtained according to the following expression: $Y = 74.3 - 5.1X$ ($R^2_{Adj} = 0.818$, $p<0.001$), where Y is arcsine (degrees) of proportion of cumulative seed germination and X is salt concentration.

Effect of salinity on stem and root elongation and leaf expansion

Increasing soil salinity significantly retarded ($p<0.01$) elongation of stems and roots (Table 1). A negative relationship was obtained for shoot height and root length with increasing salt concentration ($p<0.001$). 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 ($p<0.001$).

Effect of salinity on dry weight

Dry weight significantly decreased ($p<0.01$) for leaves, stems, shoots (leaves+stems), tap roots, lateral roots and total roots in response to increas-

Salinity (dSm^{-1})	Shoot height (cm)	Root length (cm)	Leaf area (cm^2 $plant^{-1}$)	Leaf dry weight (mg $plant^{-1}$)	Stem dry weight (mg $plant^{-1}$)	Shoot dry weight (leaf+stem) (mg $plant^{-1}$)	Tap root dry weight (mg $plant^{-1}$)	Lateral root dry weight (mg $plant^{-1}$)	Total root dry weight (mg $plant^{-1}$)
0.3	33.7 ± 1.6	32.22 ± 1.3	124 ± 2.0	951 ± 28.5	2708 ± 91.8	3658.5 ± 100.3	1658 ± 29.0	772.5 ± 25.6	2430.5 ± 44.7
3.9	29.2 ± 1.7	30.72 ± 1.1	108 ± 1.7	686 ± 16.4	1942 ± 81.1	2627.5 ± 85.5	1284 ± 20.8	573.0 ± 28.6	1856.5 ± 25.6
6.0	27.3 ± 1.6	27.2 ± 1.5	87 ± 1.1	521 ± 24.8	1309 ± 99.5	1829.5 ± 103.4	836 ± 29.4	506 ± 34.0	1341 ± 50.5
7.9	23.3 ± 1.2	26.8 ± 1.1	70 ± 1.3	375 ± 12.6	798 ± 92.7	1172.0 ± 98.0	472 ± 14.0	314 ± 13.5	785 ± 19.6
α	34.28	25.84	129.43	976.57	1768.60	3807.90	1771.10	799.79	2570.90
β	-1.3	-0.75	-7.080	-75.920	-156.290	-328.390	-156.680	-57.160	-213.840
r	-0.481	-0.357	-0.777	-0.915	-0.957	-0.908	-0.959	-0.803	-0.955
$LSD_{0.05}$	4.9	3.9	11.1	68.10	289.3	306.9	76.5	83.9	118.2

r values are significant at $p<0.001$

Tabla 1. Efecto de la salinización del suelo en las características de las hojas, tallos y raíces de plantas de *Jatropha curcas* de nueve meses de edad, indicadas media± SEM (n=20) y ecuación de la regresión lineal, siendo α la intersección y β la pendiente.

Table 1. Effect of salinisation of soil on leaf, stem, shoot and root characteristics of nine-month old *Jatropha curcas* plants as indicated by mean ± SEM (n=20) and linear regression equation, where α is Y intercept and β is slope of regression line.

ing concentration of salt (Table 1). A negative relationship was obtained between dry weight of different tissues and salt concentration ($p<0.001$).

Percent relative weights of tissues of salinised plants compared with those of control plants were computed as (salinised tissue dry weight / control dry weight) x 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 74.2 to 40.6 for lateral roots, from 72.1 to 39.4 for leaves, from 71.7 to 29.5 for stems and from 77.4 to 28.4 for tap roots in response to increasing soil salinity from 3.9 to 7.9dSm^{-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, 6.0, 6.0 and 7.2dSm^{-1} for leaves, stems, tap roots and lateral root tissues, respectively. Root/shoot dry weight ratio was 0.67 under control conditions and it did not change with increase in soil salinity.

Effect of salinity on water content of tissues

Water content in leaves, stems, tap roots and lateral root tissues significantly decreased ($p<0.01$) with increasing concentration of salt in soil (Fig. 2A). There was maximum water content in lateral roots and minimum in leaves. Tissues according to their water content can be arranged in the following decreasing order: lateral roots>tap roots> stems> leaves. There was a negative relationship between water content in tissues and salt concentration (r= -0.735, -0.818, -0.787 and -0.771, $p<0.001$, for leaves, stems, tap roots and lateral roots, respectively).

Effect of salinity 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 (Fig. 2B). Tissues according to their water potential values (low to high negative) can be arranged in the following order: lateral roots>tap roots>stems> leaves. There was a negative relationship between water potential of tissues and salt concentration (r= -0.955, -0.945, -0.958 and -0.980, $p<0.001$, for leaves, stems, tap roots and lateral roots, respectively). A positive relationship was obtained between water content and water potential (negative value) (r=0.998, 0.995, 0.997 and 0.997, $p<0.001$, for leaves, stems, tap roots and lateral roots, respectively).

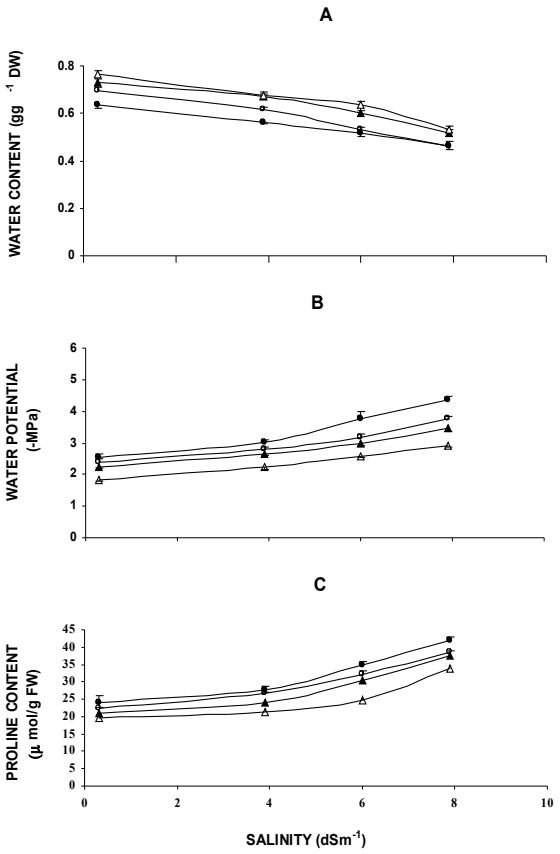

Figura 2. Efecto de la salinidad del suelo sobre: A: contenido hídrico (gg^{-1} DW). B: potencial hídrico (-MPa). Contenido de prolina (µmol/g FW) de las hojas (●), tallos (○), raíz primaria (▲) y raíces laterales (Δ). de plántulas de *Jatropha curcas* de nueve meses de edad. Las barras de error representan ES (n=20 para el contenido hídrico y n=3 para el potencial hídrico y el contenido de prolina en los tejidos).

Figure 2. Effect of soil salinity on: A: water content (gg^{-1} DW). B: water potential (-MPa). C: proline content (µmol/g FW) of leaves (●), stems (○), tap roots (▲) and lateral roots (Δ) of nine-month old *Jatropha curcas* seedlings. Error bars represent SE (n=20 for water content and n=3 for water potential and proline content of tissues).

Effect of salinity on proline content of tissues

Proline content (µmol/g FW material) significantly increased ($p<0.01$) in leaves, stems, tap roots and lateral root tissues with increase in soil salinity (Fig. 2C). 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=0.925, 0.963, 0.943 and 0.870, $p<0.001$, for

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 ($\mu g\ g^{-1}$)
	0.3	24.1±1.8	2.8±0.3	12.4±0.4	12.6±0.5	34.0±0.3	9.0±0.6	1.0±0.0	8.6±0.4	12.0±0.4	174.7±4.4	665.0±18.2
	3.9	26.3±1.2	2.6±0.3	14.5±0.4	13.6±0.3	30.4±0.2	8.2±0.3	1.1±.0.0	9.5±0.4	13.5±0.4	164.7±3.8	617.0±9.8
	6.0	29.4±1.3	2.2±0.2	17.1±0.8	14.5±0.6	28.3±0.5	7.7±0.3	1.2±0.1	10.8±0.4	14.9±0.2	155.3±3.8	567.0±7.2
Leaf	7.9	30.7±2.1	1.8±0.1	19.5±0.6	15.6±0.2	23.5±0.3	7.2±0.2	1.3±.0.0	11.3±0.3	15.0±0.7	146.3±4.3	546.3±14.0
	α	17.07	2.95	11.88	12.36	23.01	9.04	0.97	8.35	11.98	177.0	672.1
	β	0.96	-0.13	0.80	0.38	-1.33	-0.23	0.03	0.37	0.41	-3.71	-16.19
	r	**0.761***	**-0.800***	**0.931****	**0.867****	**-0.978****	**-0.779***	**0.719***	**0.880****	**0.861****	**-0.868****	**-0.921****
	$LSD_{0.05}$	3.90	1.70	1.80	1.30	1.50	1.10	1.20	1.40	1.40	12.40	39.50
	0.3	32.0±3.5	2.4±0.3	22.3±0.6	29.7±0.6	41.6±0.2	12.7±0.3	0.8±0.0	14.2±0.2	19.9±0.8	211.3±9.3	739.3±8.8
	3.9	33.0±1.2	2.1±0.3	23.8±0.4	32.1±0.3	38.8±0.3	10.4±0.7	0.7±0.0	16.2±0.4	21.9±0.2	185.7±6.5	709.3±7.0
	6.0	35.0±2.3	1.8±0.4	25.1±.05	36.3±0.3	35.8±0.3	9.7±0.5	0.7±0.0	17.2±0.5	22.4±0.8	167.0±7.1	666.0±4.2
Stem	7.9	37.0±2.7	1.6±0.3	27.8±0.3	38.5±0.7	31.6±0.3	8.0±0.2	0.7±0.0	18.3±0.3	24.6±0.6	145.0±4.7	633.7±7.8
	α	31.01	2.45	21.66	28.74	31.03	12.90	-	14.10	19.57	216.1	750.5
	β	0.68	-0.11	0.68	1.20	-1.31	-0.60	-	0.53	0.58	-8.59	-14.02
	r	**0.791***	**-0.773***	**0.908****	**0.957****	**-0.978****	**-0.929****	**-**	**0.941****	**0.852****	**-0.919****	**-0.950****
	$LSD_{0.05}$	3.60	0.50	1.40	1.50	1.60	1.40	N.S.	1.10	2.00	21.50	21.70
	0.3	27.0±1.9	2.9±0.3	14.9±0.4	26.1±1.0	32.3±0.1	10.2±0.3	0.6±0.0	10.0±0.3	16.5±0.5	172.0±3.5	597.0±3.8
	3.9	32.0±2.6	2.5±0.2	17.2±0.2	28.8±0.6	29.7±0.2	9.1±0.4	0.6±0.0	11.2±0.4	18.3±0.4	159.7±4.8	563.3±6.8
	6.0	34.0±1.2	2.3±0.2	18.4±0.6	30.7±0.6	27.2±0.4	8.3±0.2	0.6±0.0	13.0±0.3	19.0±0.4	149.0±6.5	539.0±5.2
Tap root	7.9	36.0±3.0	2.1±0.3	20.6±0.4	33.0±1.2	26.4±0.1	7.8±0.2	0.6±0.0	13.6±0.5	19.9±0.2	138.0±.4.4	513.7±4.1
	α	26.81	2.89	14.47	25.62	25.42	10.31	-	9.70	16.39	174.70	602.40
	β	1.18	-0.10	0.72	0.89	-0.77	-0.32	-	0.50	0.45	-4.43	-10.87
	r	**0.911****	**-0.778***	**0.945****	**0.892****	**-0.860****	**-0.905****	-	**0.920****	**0.912****	**-0.868****	**-0.978****
	$LSD_{0.05}$	3.40	0.50	1.30	2.60	2.10	0.90	N.S.	1.20	1.20	14.90	12.60
	0.3	21.0±0.6	2.7±0.2	11.9±0.4	18.9±0.3	29.4±0.1	8.3±0.3	0.6±0.0	12.7±0.3	14.8±0.5	192.3±4.5	703.7±8.5
	3.9	23.0±2.3	2.4±0.2	13.5±0.6	22.6±0.5	27.0±0.3	7.4±0.3	0.6±0.0	14.0±0.8	15.5±0.5	176.0±5.1	685.0±7.5
	6.0	26.0±1.7	2.2±0.2	14.9±0.4	24.6±1.3	25.0±0.2	6.7±0.3	0.6±0.0	15.4±0.6	16.9±0.4	166.0±5.3	663.7±4.1
Lateral root	7.9	27.0±0.6	2.0±0.3	16.4±0.4	27.5±0.3	21.6±0.2	6.0±0.1	0.6±0.0	16.0±0.1	17.7±0.5	152.7±4.9	634.7±8.7
	α	21.22	2.75	11.53	18.42	21.16	8.50	-	12.51	14.44	194.8	711.6
	β	0.69	-0.10	0.59	1.10	-1.01	-0.31	-	0.44	0.39	-5.11	-8.81
	r	**0.780***	**-0.719***	**0.916****	**0.945****	**-0.924****	**-0.905****	-	**0.858****	**0.843****	**-0.895****	**-0.932****
	$LSD_{0.05}$	4.00	0.40	1.40	2.20	2.50	0.80	N.S.	1.50	1.40	15.10	15.50

* r values are significant at $p<0.01$; ** r value are significant at $p< 0.001$

Tabla 2. Efecto de la salinización del suelo sobre el contenido de nutrientes de los tejidos (hojas, tallos, raíz primaria y secundarias) de plántulas de nueve meses de edad de *Jatropha curcas* indicado como media ± SEM (n=3) y ecuación de la regresión lineal, siendo α la intersección y β la pendiente.

Table 2. Effect of salinisation of soil on nutrient content of tissues (leaf, stem, tap root and lateral root) of nine - month old *Jatropha curcas* plants as indicated by mean ± SEM (n=3) and linear regression equation, where α is Y intercept and β is slope of regression line.

leaves, stems, tap roots and lateral roots, respectively). A negative relationship was obtained between water potential and proline content (r=- 0.941, -0.906, -0.959 and -0.892, p<0.001, for leaves, stems, tap roots and lateral roots, respectively).Similarly, a negative relationship was obtained between water content and proline content (r= -0.988, -0.987, -0.984 and -0.904, p<0.01, for leaves, stems, tap roots and lateral roots, respectively).

Effect of salinity 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 tissues with increase in salt concentration in soil (p<0.001). The K/Na ratio significantly increased in leaves (p<0.05) in response to increase in soil salinity, while it did not change in stems, tap roots and lateral root tissues. There was a positive relationship between K/Na ratio in leaves and salt concentration (p<0.01).

Nitrogen, phosphorus, calcium and magnesium

The concentration of N, K, Na and Ca was, in general, greater than that of P and Mg in all tissues under control and saline conditions. Nitrogen content significantly increased in leaves, stems, tap roots and lateral root tissues (p<0.05) in response to increase in soil salinity (Table 2). A positive relationship was obtained between salt concentration and N content of leaves (p<0.01, stems (p<0.01), tap roots (p<0.001), and lateral roots (p<0.01). Phosphorus content significantly decreased in leaves (p<0.01), stems, tap roots and lateral roots (p<0.05) with increase in soil salinity. There was a negative relationship for P content of tissues with increase in soil salinity (p<0.01). Concentration of calcium decreased (p<0.01) in leaves, stems, tap roots and lateral root tissues in response to increase in soil salinity. A negative relationship was obtained between Ca content of tissues and salt concentration (p<0.001). Magnesium content exhibited a significant decrease in leaves (p<0.05), stems, tap roots and lateral root tissues (p<0.01) in response to increase in soil salinity. There was a significant negative relationship between salt concentration in soil and Mg content of leaves (p<0.01), stems, tap roots and lateral roots (p<0.001).

Micro-elements

There was a significant increase in the concentration of Zn and Cu (p<0.01) in leaves, stems, tap roots and lateral root tissues in response to increase in soil salinity (Table 2). A positive relationship was obtained for Zn and Cu content in tissues with soil salinity (p<0.001). The concentration of Mn and Fe significantly decreased (p<0.01) in leaves, stems, tap roots and lateral roots with the increase in soil salinity. A negative relationship was obtained for salt concentration and Mn and Fe content in tissues with salt concentration (p<0.001).

Discussion

Earlier work (Ramoliya et al. 2004) indicated that seedling emergence for salt-tolerant legume tree *Acacia catechu* was reduced to 50% (SG50) in soil with salinity of 6.0dSm^{-1}, but for *J. curcas* SG50 was obtained at 4.8dSm^{-1}. This result would suggest that this plant species is relatively salt tolerant at seed germination. Under field conditions in coastal region of Saurashtra and in Kutch, where this tree species is being grown, 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 (0-15cm depth) varies from 2.0 to 5.0dSm^{-1}. Eventually, seeds of *J. curcas* can germinate and achieve establishment during the rainy season. However, salt concentration exceeding 7.9dSm^{-1} was detrimental to seed germination (Fig. 1) that can be attributed to decreasing osmotic potential of the soil solution. It was observed that seeds became nonviable 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 (Slater et al. 2003) and induces changes in the activities of many enzymes (Dubey & Rani 1990) in germinating seeds.

Reduction in water content and water potential of leaves, stems, tap roots and lateral roots of seedlings (Fig. 2) 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 & Zeiger 2006). Stems and tap roots of

J. curcas are succulent (genetically controlled) and contain milky juice. Reduction in concentration of milky juice was found (only visual observation was made by cutting stems and tap roots) with increase in soil salinity. As a result, salinity impaired succulence of stems and tap roots. The maximum salt concentration for tolerance of seedlings was 7.9dSm^{-1} because in this experiment seedlings did not survive when salinity exceeded this concentration. Though succulence is primarily an adaptation to water stress, it provides salt resistance to plants because it dilutes the ionic concentration and temporarily puts off the setting of severe water-deficit induced by salt stress. Root/shoot dry weight ratio of *J. curcas* was 0.67 under control conditions and was greater than that for aridity and salt tolerant seedlings of *A. 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 & Gupta 1997). Reduction in shoot growth of *J. curcas* with increasing salt concentration can be accounted for reduction in leaf area (photosynthetic area). Curtis & Lauchli (1986) reported that growth in Kenaf (*Hibiscus cannabinus*) under moderate salt stress was affected primarily through a reduction in elongation of stem and leaf area development. Garg & Gupta (1997) reported that salinity causes reduction in leaf area as well as in rate of photosynthesis, which together result in reduced crop growth and yield. Also, high concentration of salt tends to slow down or stop root elongation (Kramer 1983, Patel & Pandey 2008) and causes reduction in root production (Garg & Gupta 1997).

Results for dry weight (Table 1) and relative dry weight of tissues in response to increasing salinity suggest that there was lowest reduction in dry weight of lateral roots and leaves, while reduction was maximum for stems and tap roots. Consequently, lateral roots and leaves were less sensitive, and stems and tap roots were more sensitive to increasing soil salinity. Tissues can be arranged in decreasing order of salt tolerance as: lateral roots=leaves>stems=tap roots. The concurrent and almost equal rate of reduction in dry weight of leaves and lateral roots on the one hand and that of stems and tap roots on the other resulted in equal root/shoot dry weight ratios for seedlings grown in control and saline conditions. 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 turgor 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 *J. curcas* survived up to the soil salinity of 7.9dSm^{-1} and, therefore, this tree species is moderate 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 & 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 & Munns 1980). 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 & 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 maximum in leaves and stems than that in tap roots and lateral roots as salinity increased (Fig. 2). Result corroborates the conclusion of Munns (2002) that organic solutes are often lower in roots than in 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 & Guerinot 1998). In the present study, significant increase of K content in all tissues of seedlings (Table 2) with increasing soil salinity might be due to high selectivity of *J. curcas* 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^+. 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 *J. curcas* suggests that this mechanism to block Na transfer to growing tissues was not effective at high salt concentration. A significant increase in K/Na ratio in leaves with increase in salinity suggests that K was transported to leaves in greater amount than Na in order to protect this tissue. There was no change in K/Na ratio in stems, tap roots and lateral roots because Na and K both increased in these tissues 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 shoot tissues. The pattern of accumulation of K and Na in *J. curcas* 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 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 (Amtmann & 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 & Sanders 1994, Niu et al. 1995). The K and Na profiles of *J. curcas* 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. Tolerence of non-halophytes to salinity further depends upon their ability to sequester Na^+ , that enter the tissues, into vacuoles (Munns 2005, Munns & Tester 2008) and salt resistant tissues (Ramoliya et al. 2004). In *J. curcas*, sodium accumulation was greater in stems and tap roots than in leaves. Plants of this species sequester salts, that they absorb, in stems and tap roots and thus minimize the exposure of leaf cells (photosynthetic apparatus) to salt. "Integration in the whole plant" is an important aspect of salt tolerance in glycophytes (Garg & Gupta 1997). Considering that stem and tap root tissues will be reinforced by growth in time, it can be predicted that after seedling stage Na tolerance of plant may improve above $7.9 dSm^{-1}$, 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 & 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. The interaction between salinity and P is very complex and there is no clear cut mechanis-

tic explanation for decreased, increased or unchanged P uptake in response to salinisation in different species (Grattan & 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 increase in soil salinity. 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 1987, 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 & 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 and Cu accumulation, whereas reduced Mn and 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 & 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 and Mn in leaves of J. curcas might limit photosynthesis and 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 Zn and Cu content at the whole-plant level might be the requirement of this plant for survival in saline soils.

Conclusion

Results of the present investigation show that *Jatropha curcas* L. is relatively salt tolerant at seed germination stage, though the percent germination linearly decreased with increase in soil salinity. The stems and tap roots of the plants are succulent and contain high concentration of Na^+. It might be that these tissues sequester Na^+ in their large vacuoles. Succulence is responsible for diluting the salt concentration of the cells of salt-stressed plants. Salt tolerance of this plant is further ascribed to its high selectivity for K^+ and low rate of transport of Na^+ to leaves. The osmotic adjustment by the plants was achieved by increased quantity of K^+ and proline in tissues when water content decreased because of salinity. The increase of proline content with increasing Na^+ concentration indicates that higher proline accumulation may alleviate NaCl stress in *J. curcas*. The plants of this species sequester Na^+, that they absorb, in stems and tap root tissues and thus minimize the exposure of leaf cells (photosynthetic apparatus) to salt. Thus, salt tolerance of *J. curcas* at the whole-plant level is dependent on integration of different attributes that may help alleviate NaCl stress.

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