Growth, water status and nutrient accumulation of seedlings of *Cassia fistula* L. in response to soil salinity

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Resumen

Crecimiento, estado hídrico y acumulación de nutrientes en plántulas de Cassia fistula *L. en respuesta a la salinidad*

Se realizaron experimentos en invernaderos para estimar el efecto de la salinidad sobre la emergencia, crecimiento, estado hídrico, contenido de prolina y acumulación mineral en plántulas de *Cassia fistula* L. (Fabaceae). Se añadió NaCl al suelo, manteniendo salinidades de 0,2; 2,1; 3,9; 6,2; 8.1; 10,0 y 11,9 dSm^{-1}. La salinidad redujo el contenido de agua y el potencial hídrico de los tejidos lo que resultó en déficit hídrico de las plantas. Consecuentemente, el crecimiento de las plántulas se redujo con el incremento de salinidad. En contenido de prolina aumentó con la salinidad. No hubo mecanismos efectivos para controlar la absorción de Na^+, por lo que fue transportado a los brotes y el contenido de Na aumentó significativamente con la salinidad. Los contenidos de N, K y Ca en los tejidos disminuyeron significativamente con la salinidad. Se discuten los cambios en los tejidos y los patrones de acumulación de otros nutrientes

Palabras clave: Macro y micronutrientes, contenido de prolina, tolerancia a la sal, crecimiento de plántulas, salinidad del suelo, potencial hídrico.

Abstract

Greenhouse experiments were conducted to assess the effect of soil salinity on emergence, growth, water status, proline content and mineral accumulation of seedlings of *Cassia fistula* L. (Fabaceae). NaCl was added to the soil and salinity was maintained at 0.2, 2.1, 3.9, 6.2, 8.1, 10.0 and 11.9 dSm^{-1}. Salinity caused reduction in water content and water potential of tissues that resulted in internal water deficit to plants. Consequently, seedling growth significantly decreased as salinity increased. Proline content in tissues increased as salinity increased. There were no effective mechanisms to control net uptake of Na^+ and subsequently its transport to shoot tissues. Na content significantly increased in tissues as salinity increased. N, K and Ca content in tissues significantly decreased as salinity increased. Changes in tissues and whole-plant accumulation pattern of other nutrients, as well as possible mechanisms for avoidance of Na toxicity in this species in response to salinity, are discussed.

Key words: Macro- and micro-nutrients, Proline content, Salt tolerance, Seedling growth, Soil salinity, Water potential.

Introduction

Salinisation of soil is common in arid and semi arid regions where the amount of rainfall is insufficient for substantial leaching. High concentrations of salts have detrimental effects on plant growth (Bernstein 1962, Taiz & Zeiger 2006, Ramoliya et al. 2004). However, plant species differ in their sensitivity or tolerance to salts (Marschner 1995). There are evidences 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 the root growth (Maas & Hoffman 1977, Munns 2002, Ramoliya et al. 2004). However, fewer studies on the effect of soil salinity on root growth have been conducted (Munns 2002). The high salt content lowers osmotic potential of soil water and consequently the availability of soil water to plants. The salt-induced water deficit is one of the major constraints for plant growth in saline soils. In addition, many nutrient interactions in salt–stressed plants can occur that may have important consequences for growth. Internal concentrations of major nutrients and their uptake have been frequently studied (e.g., Maas & Grieve 1987, Cramer et al. 1989, Ramoliya et al. 2004), but the relationship between micro-nutrient concentrations and soil salinity is rather complex and remains poorly understood (Tozlu et al. 2000). An understanding of growth and survival of plants under saline habitat conditions is needed for (i) screening the plant species for the afforestation of saline deserts and (ii) understanding the mechanism that plants use in the avoidance and /or tolerance of salt stress.

Cassia fistula L. (Fabaceae) is an ornamental small tree and is native to tropical Asia. This tree species is found abundantly in marginal-saline area of Kutch (north-west saline desert) in Gujarat State of India. It also grows successfully in coastal area as well as in non saline and semi-arid central area of Saurashtra region, to the north of Kutch. This tree is considered as a firewood source. The reddish wood, strong and durable, is suited for farm implements. The drug “cassia fistula”, a mild laxative, is obtained from the sweetish pulp around the seed. In addition medicines are extracted from fruits for the treatment of abdominal pain, fever, heart disease and leprosy. 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 performed with the following objectives: (i) to understand the adaptive features of *C. fistula* which allow it to grow and survive in saline and arid regions and (ii) to assess the pattern of macro– and micro– nutrient accumulation within the tissues of this tree species in response to salt stress.

Material and methods

Study area

The present study was carried out in a greenhouse of the botanical garden of Saurashtra University at Rajkot (22º 18’ N Lat, 70º56’ 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 0.2dSm^{-1}, Nitrogen, phosphorus, potassium, calcium and sodium contents were 0.15%, 0.05%, 0.03%, 0.05% and 0.002%, respectively. This soil is fertile and fit for intensive agriculture. Physical and chemical properties of soil are given earlier (Pandya et al. 2004). 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˚15’ N Lat, 69˚49’ E Long) in Kutch and about 554mm at Rajkot in central Saurashtra which occurs totally during the rainy season. Typically, there are three main seasons: summer (April to mid June), monsoon (mid June to September) and winter (November to February). The months of October and March are transition periods between rainy (monsoon) and winter and between winter and summer seasons, respectively. Winters are generally mild and summers are hot.

Salinisation of soil

Surface soil was collected, air dried and passed through a 2 mm mesh screen. Seven lots of soil of 100 kg each, were separately spread over about 50 mm thick polyethylene sheets. Sodium chloride (NaCl) amounting to 210, 390, 700, 1070, 1275 and 1530 g was then thoroughly mixed with soil of six lots, respectively to give electrical conductivities of 2.1, 3.9, 6.2, 8.1, 10.0 and 11.9 dSm^{-1}. There was no addition of NaCl to seventh lot of soil that served as control. The electrical conductivity of control soil was 0.2 dSm^{-1} and this value was approximately equal to 2 mM salinity. For the measurement of electrical conductivity a soil suspension was prepared in distilled water at a ratio of 1:2 in terms of weight. The suspension was shaken and allowed to stand overnight. Thereafter, electrical conductivity of the supernatant solution was determined with a conductivity meter.

Seedling emergence

Twenty polyethylene bags for each level of soil salinity were each filled with 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 7 February 2008. Seeds of *C. fistula* were collected from the coastal area of Arabian Sea at Jamnagar district 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 similar amount of water was added to the soil on alternate days. Irrigation of soil with required amount of water was taken as a measure to control the level of soil salinity. Emergence of seedlings was recorded daily over a period of 40 days. A linear model was fitted to cumulative proportion of seed germination and increasing soil salinity, using the expression:

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

where, $Sin^{-1}\sqrt{p}$ 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 0.2, 2.1, 3.9 and 6.2 dSm^{-1} salinity exhibited emergence of the second leaf after 19 days, whereas the second leaf on seedlings grown in soils at 8.1, 10.0, and 11.9 dSm^{-1} began to emerge after 26 days. Emergence of the second leaf indicated the probable establishment of seedlings. However, only 16.4 and 10% seed germination was recorded respectively in soils at 10.0 and 11.9 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. Plants grown in soil at 8.1 dSm^{-1} salinity died during the course of experiment. Thus twenty replicates factorized with four grades of soil (0.2, 2.1, 3.9 and 6.2 dSm^{-1}) were prepared. This gave a total of 80 bags, which were arranged in 20 randomized blocks. Seedlings were watered (to raise the soil moisture to field capacity) on alternate days and allowed to grow for six months. Experiment was terminated on 7 August 2008. 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. Sum of leaf and stem weight was considered as shoot weight. 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 tissues were analyzed by one way ANOVA to assess the effect of salinity on plant growth.

Determination of water potential and proline content

Five 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. 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. Water potential and proline content of tissues were estimated in triplicate. 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 sub samples of plant tissues were analyzed. Total nitrogen was determined by 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 after triacid (HNO3:-H2SO4:HClO4 in the ratio of 10:1:4) digestion. Mineral data were analyzed by one way ANOVA.

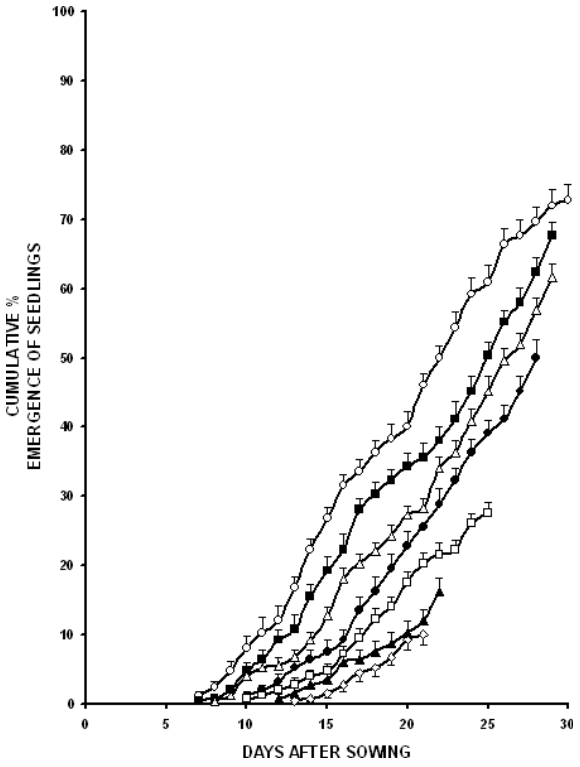

Figura 1. Emergencia acumulada de plántulas de *Cassia fistula* en respuesta a la salinidad del suelo. 0,2 dSm^{-1} (○), 2,1 dSm^{-1} (■), 3,9 dSm^{-1} (Δ), 6,2 dSm^{-1} (●), 8,1 dSm^{-1} (□), 10,0 dSm^{-1} (▲) and 11,9 dSm^{-1} (◊). Las barras de error representan ES.

Figure 1. Cumulative emergence of seedlings of *Cassia fistula* in response to soil salinity. 0.2 dSm^{-1} (○), 2.1 dSm^{-1} (■), 3.9 dSm^{-1} (Δ), 6.2 dSm^{-1} (●), 8.1 dSm^{-1} (□) , 10.0dSm^{-1} (▲) and 11.9 dSm^{-1} (◊). Error bars represent SE.

Correlations and linear regression equations between mineral content and salt concentrations were determined.

Results

Effect of salinisation on seedling emergence

Seedlings began to emerge 6 days after sowing and 72.8% seed germination was obtained over a period of 30 days, under control (0.2 dSm^{-1} salinity) conditions (Fig. 1). Seedling emergence in saline soils was recorded 6-12days after sowing. Seedling emergence lasted for 29, 29, 28, 25, 23 and 21 days in soils with 2.1,3.9, 6.2, 8.1, 10.0 and 11.9 dSm^{-1} salinities, respectively and corresponding seed germination was 67.6%, 61.6%, 50%, 27.6%, 16.4% and 10%. 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=66.328-3.225X, ($R^2_{adj}=0.918$; $p<0.01$), where Y is arcsine (degrees) of proportion of cumulative seed germination and X is salt concentration.

Effect of salinisation on stem and root elongation and leaf expansion

Increasing soil salinity significantly retarded ($p<0.01$) stem and root elongation (Table 1). Root length was almost equal to shoot height for seedlings grown in control and saline soils. There was a negative relationship ($p<0.01$) for shoot height and root length with increase in salt concentration in soil. Leaf expansion was significantly reduced ($p<0.01$) by increasing concentration of salt in soil. A negative relationship was obtained between leaf area and salt concentration ($p<0.01$).

Effect of salinisation on 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 tissues (leaves, stems, shoots, tap roots, lateral roots and total roots) and salt concentration ($p < 0.01$).

Percent relative weight of tissues of salinised

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 $plant^{-1}$)
0.2	32.3 ± 0.7	30.0±0.6	185.8±4.0	578.4±11.1	478.1±13.4	1056.4±19.5	418.5±9.0	197.2±2.2	615.7±9.0
2.1	26.9±0.7	25.7±0.7	107.7±3.1	357.7±10.9	302.1±10.1	659.8±17.6	269.5±7.2	126.4±3.4	395.9±6.2
3.9	21.7±0.7	18.0±0.7	82.0±0.8	295.3±6.3	246.8±5.3	543.0±7.0	222.2±8.2	109.7±1.9	331.8±8.2
6.2	18.1±0.6	15.6±0.6	67.0±1.1	206.2±6.5	201.2±4.0	407.3±6.9	159.4±5.3	94.4±2.8	253.7±6.4
α	32.16	30.16	169.5	542.2	443.92	986.12	395.29	182.03	577.32
β	-2.39	-2.53	-18.99	-58.98	-44.08	-103.07	-41.27	-16.18	-57.44
r	-0.865	-0.970	-0.892	-0.914	-0.872	-0.914	-0.905	-0.874	-0.918
$LSD_{0.05}$	5.2	5.0	19.8	68.1	68.3	106.0	57.0	19.9	57.0

Relationship is significant at $p < 0.01$.

Tabla 1. Efecto de la salinización del suelo en las características de la hoja, tallo, brote y raíz *Cassia fistula*, indicado como media ± ESM y las contantes de la ecuación de regresión. En todos los casos se refiere a peso seco.

Table 1. Effect of salinisation of soil on leaf , stem , shoot and root characteristics of *Cassia fistula* as indicated by mean ± SEM and regression equation constants. Weight refers to dry weight.

plants compared to those of control plants was computed as: (salinised tissue dry weight/control dry weight)×100. Dry weight values of tissues given in Table 1 were used for the calculation of percentage relative weight of tissues. Values of percent relative weight varied from 61.8 to 35.7% for leaves, from 63.2 to 42.1% for stems, from 64.4 to 38.1% for tap roots and from 64.1 to 47.9% for lateral roots in response to increasing soil salinity from 2.1 to 6.2 dSm^{-1}. As has been estimated using regression equations given in results, the salt concentration at which dry weight will be reduced to 50% of control plants (DW_{50}) were around 4.3, 4.6, 4.5 and 5.2 for leaves, stems, tap roots and lateral root tissues, respectively. Root/shoot dry weight ratio was 0.59 under control conditions and did not change as soil salinity increased.

Effect of salinisation 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 leaves and minimum in tap roots. Tissues according to their water content can be arranged in following decreasing order: leaves>stems>lateral roots>tap roots. There was a negative relationship between water content in different tissues and salt concentration (r=-0.520, -0.565, -0.374 and -0.612; $p<0.01$, for leaves, stems, tap roots and lateral roots, respectively).

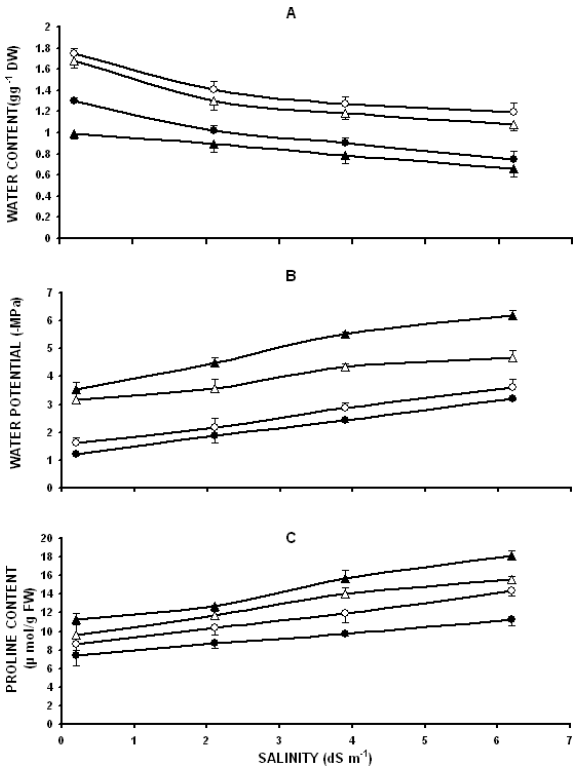

Figura 2. Efecto de la salinidad del suleo en: **A.**n Contenido de agua (gg^{-1} DW); **B.** potencial hídrico (-MPa); **C.** contenido de prolina (µmol/g FW) de hojas (○), tallo (Δ), raíz primaria (▲) y raíces secundaria (●) de plántulas de *Cassia fistula*.

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 (○), stem (Δ), tap root (▲) and lateral roots (●) of *Cassia fistula* seedlings. Error bars represent SE.

Effect of salinisation 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 decreasing order: lateral roots>leaves> stems>tap roots. There was a negative relationship between water potential of tissues and salt concentration (r=-0.895, -0.826, -0.958 and -0.967; $p<0.01$, for leaves, stems, tap roots and lateral roots, respectively). A positive relationship was obtained between water content and water potential (negative value) (r=0.921, 0.923, 0.992 and 0.979; $p<0.01$, for leaves, stems, tap roots and lateral roots, respectively) .

Effect of salinisation on proline content of tissues

Proline content (µmol/g fresh weight 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: tap roots>stems>leaves>lateral roots. There was a positive relationship between salt concentration and proline content of tissues (r=0.882, 0.833, 0.938 and 0.803; $p<0.01$, for leaves, stems, tap roots and lateral roots, respectively). A negative relationship was obtained between water potential and proline content (r= -0.933, -0.772, -0.940 and -0.736; $p<0.01$, for leaves, stems, tap roots and lateral roots, respectively). Similarly, a negative relationship was obtained between water content and proline content (r=-0.921, -0.954, -0.994 and -0.977; $p<0.01$, for leaves, stems, tap roots and lateral roots, respectively).

Effect of salinisation on mineral accumulation

Potassium and sodium content and K/Na ratio

Potassium content (as mg g^{-1} dry weight) significantly decreased ($p<0.01$) in leaves, stems, tap roots and lateral root tissues in response to increasing soil salinity (Table 2). There was a negative relationship between potassium content in tissues and increase in salt concentration in soil ($p<0.01$). Sodium content significantly increased ($p<0.01$) in leaves, stems, tap roots and lateral root tissues with increasing soil salinity. A positive relationship was obtained between Na content in tissues and increase in salt stress ($p<0.01$). K/Na ratio significantly decreased ($p<0.01$) in leaves, stems, tap roots and lateral roots in response to increase in soil salinity. A negative relationship was obtained between K/Na ratio in tissues and increase in salt stress ($p<0.01$).

Nitrogen, phosphorus, calcium and magnesium

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, phosphorus, calcium and Magnesium content significantly decreased in leaves, stems, tap roots and lateral root tissues ($p<0.01$), as the salinity increased (Table 2). A negative relationship was obtained between N, P, Ca and Mg content of tissues and salt concentration ($p<0.01$).

Micro-elements

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

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 dSm^{-1},but for *Cassia fistula* SG_{50} was obtained at 4.9 dSm^{-1}.That would suggest that this plant species is relatively salt tolerant at seed germination. Under field conditions in coastal region of Saurashtra and in saline desert of Kutch, where this tree species grows, 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 2.0 to 5.0 dSm^{-1}. Eventually, seeds of *C. fistula* can germinate and achieve establishment during the rainy season. However, salt concentration exceeding 8.1 dSm^{-1} was detrimental to seed germination that can be

Tissue	Salinity (dSm^{-1})	N ($mg\ g^{-1}$)	P ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)	Na ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	K/Na ratio	Zn ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)
Leaf	0.2	29.3±0.2	1.7±0.1	36±0.3	2.0±0.1	32.3±0.6	2.7±0.1	18±0.4	45±0.2	10±0.6	54±0.2	420±2.3
	2.1	26.2±0.5	1.6±0.1	33.6±1.1	2.7±0.2	28.0±0.0	2.2±0.1	12.5±0.3	46±0.6	12±0.0	59±2.1	326±2.6
	3.9	24.4±0.1	1.3±0.1	28.8±0.3	4.3±0.2	26.6±0.6	2.0±0.0	6.8±0.3	49±0.5	19±0.3	67±0.5	239±0.6
	6.2	19.1±0.1	1.2±0.1	26.7±0.1	4.6±0.1	24.1±0.1	1.8±0.1	5.8±0.2	50±0.1	24±0.8	72±0.6	196±1.5
	α	29.87	1.72	36.37	1.94	31.78	2.62	17.29	45.71	8.6	53.35	412.75
	β	-1.65	-0.88	-1.64	0.46	-1.3	-0.15	-2.1	0.9	2.47	3.11	-37.9
	r	-0.983	-0.971	-0.958	0.942	-0.953	-0.902	-0.946	0.934	0.973	0.966	-0.977
	$\mathbf{LSD_{0.05}}$	0.8	0.2	1.7	0.4	1.3	0.3	0.9	1.2	1.5	3.3	5.7
Stem	0.2	21.2±0.3	1.4±0.0	33.8±0.2	3.1±0.1	33.0±0.5	2.2±0.1	10.9±0.2	47±0.3	8.0±0.1	44±1.0	297±0.6
	2.1	16.3±0.3	1.2±0.1	28.6±0.2	3.9±0.4	29±0.6	1.8±0.1	7.3±0.1	49±0.3	9.0±0.3	45±0.6	184±1.5
	3.9	13.9±0.1	1.1±0.1	24.8±0.4	4.6±0.1	27±0.0	1.5±0.1	5.4±0.2	55±0.4	10±0.6	55±0.4	146±1.2
	6.2	11.9±0.	1.0±0.1	22.4±0.5	6.7±0.2	24.8±0.4	1.4±0.1	3.3±0.0	56±0.5	13±1.0	61±1.5	127±2.3
	α	20.52	1.36	33.3	2.76	32.59	2.14	10.56	46.66	7.46	41.71	272.85
	β	-1.54	-0.06	-1.9	0.58	-1.33	-0.14	-1.23	1.64	0.81	3.07	-27.2
	r	-0.963	-0.872	-0.97	0.966	-0.959	-0.889	-0.978	0.94	0.879	0.945	0.914
	$\mathbf{LSD_{0.05}}$	0.6	0.2	1.1	0.4	1.3	0.3	0.5	1.1	1.8	2.9	4.5
Tap roots	0.2	18.0±0.1	1.0±0.1	19.0±0.1	3.7±0.3	21.5±0.3	1.8±0.1	5.2±0.4	35±0.5	9±0.2	54±0.2	527±2.0
	2.1	14.0±0.1	0.9±0.0	17.1±0.1	5.3±0.3	20.8±0.1	1.5±0.1	3.2±0.1	37±0.3	11±0.5	59±2.1	402±3.8
	3.9	12.0±0.2	0.8±0.1	15.7±0.9	6.3±0.1	20.2±0.1	1.3±0.1	2.5±0.1	40±0.1	13±0.1	67±0.5	316±2.5
	6.2	11.0±0.1	0.7±0.0	14.6±0.5	8.7±0.1	19.0±0.3	1.2±0.1	1.7±0.1	45±0.2	15±0.6	72±0.6	308±1.5
	α	17.27	1.03	18.86	3.48	21.68	1.77	4.84	34.3	8.88	53.35	502.21
	β	-1.14	-0.06	-0.72	0.81	-0.41	-0.1	-0.56	1.65	1.01	3.11	-36.76
	r	-0.944	-0.897	-0.899	0.981	-0.941	-0.852	-0.938	0.986	0.966	0.966	-0.923
	$\mathbf{LSD_{0.05}}$	1.4	0.1	1.5	0.6	0.6	0.3	0.6	0.9	1.2	2.1	7.6
Lateral roots	0.2	17.0±0.3	1.2±0.0	20.6±0.5	6.5±0.1	30±0.2	5.4±0.2	3.2±0.1	52±0.6	11±0.5	44±1.0	1074±2.3
	2.1	15.2±0.1	1.1±0.0	17.0±0.1	6.6±0.1	27.0±0.0	4.9±0.3	2.6±0.0	55±0.3	17±0.5	45±0.6	1022±1.5
	3.9	13.5±0.1	1.0±0.0	16.0±0.2	7.1±0.1	26.0±0.6	4.7±0.3	2.3±0.0	64±1.0	22±0.4	55±0.4	1014±1.2
	6.2	10.7±0.2	0.9±0.0	15.0±0.6	10.1±0.3	23.7±0.0	3.9±0.1	1.5±0.0	73±0.2	28±0.5	61±1.5	986±2.1
	α	17.36	1.19	19.88	5.75	29.81	5.46	3.21	49.69	10.75	41.71	1066.6
	β	-1.04	-0.05	-0.88	0.58	-1.01	-0.24	-0.27	3.64	2.82	3.07	-13.72
	r	-0.991	-0.834	-0.899	0.87	-0.968	-0.858	-0.984	0.978	0.993	0.945	-0.949
	$\mathbf{LSD_{0.05}}$	1.6	0.1	1.2	0.5	0.9	0.6	0.2	1.8	1.4	1.8	5.4

Relationship is significant at $p < 0.01$

Tabla 2.Efecto de la salinización del suelo en el contenido de nutrinetes de los tejidos de *Cassia fistula* indicado como la media ± ESM y las constantes de la ecuación de regresión
Table 2.Effect of salinisation of soil on nutrient content of tissues (leaf and stem) of *Cassia fistula* as indicated by mean ± SEM and regression equation constants.

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 concentrations 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 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). Root / shoot dry weight ratio of *C. fistula* was 0.59 under control conditions and was greater than that for aridity and salt tolerant seedlings of *Acacia catechu* (0.47) growing abundantly in saline desert of Kutch (Ramoliya et al. 2004).

In general, salinity can reduce plant growth or damage the plants through: (i) osmotic effect (causing water deficit), (ii) toxic effect of ions and (iii) imbalance of the uptake of essential nutrients. These modes of action may operate on the cellular as well as on higher organizational levels and influence all the aspects of plant metabolism (Kramer 1983, Garg & Gupta 1997). Results for reduction of shoot growth and leaf area development of *C. fistula* with increasing salt concentration are in conformity with the finding of Curtis & 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 & 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 & Gupta 1997).

Results for dry weight and relative dry weight of tissues in response to increasing salinity suggest that there was maximum reduction in dry weight of leaves and tap roots while lowest in lateral roots. Consequently, lateral roots were most resistant and leaves were sensitive to increasing soil salinity. Tissues can be arranged in decreasing order of salt tolerance as: lateral roots>stems>tap roots>leaves. Root/shoot dry weight ratio did not change with increase in salinity due to the concurrent and differential reduction in dry weight of tissues. 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 potassium ions 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 *C. fistula* survived up to the soil salinity of 6.2 dSm^{-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 associates 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 addition, the primary role of proline may not be solely as an osmolyte, but it also helps the cells to overcome oxidative stress in salt stressed plants (Rajendrakumar et al. 1994).

The cation K^+ is essential for cell expansion, osmoregulation and cellular and whole–plant

homeostasis (Schachtman et al. 1997). High stomatal K^+ requirement is reported for photosynthesis (Chow et al. 1990). The role of K^+ in response to salt stress is also well documented, where Na^+ depresses K^+ uptake (Fox & Guerinot 1998). In the present study, significant decrease of K^+ content in all the tissues of seedlings with increasing soil salinity suggests that Na inhibited K^+ uptake. 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 salts to leaves or growing tissues. Moreover, the significant increase of Na to leaves and stem tissues of *C. fistula* suggests that this mechanism to block Na^+ transfer to growing shoot tissues was not effective at high salt concentration. The decrease in K^+/Na^+ ratio in all the tissues with increase in salinity can be accounted for relatively lower accumulation of K^+ than that of Na^+. As a consequence 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 *C. fistula* conforms to group C and / or group D plants in Marschner's (1995) classification of the ability of plants to substitute Na^+ with K^+. In this classification Marschner divided plants into four groups, A, B, C and D depending upon whether K^+ is mostly exchangeable with Na^+. Sodium has a positive effect on growth in A and B plants (mostly salt tolerant plants). Group C plants contain very little K^+ that can be substituted with Na^+ without a negative effect on growth, and group D plants exhibit no K^+/Na^+ substitution (salt-sensitive plants).

It is reported that uptake mechanisms of both K^+ and Na^+ are similar (Watad et al. 1991, Schroeder et al. 1994). Plants utilize two systems for K^+ acquisition, low- and high-affinity uptake mechanisms. Na^+ can not move through the plasma membrane lipid bilayer, but the ion is transported through both low- and high–affinity transport systems, which are necessary for K^+ acquisition. As a consequence, Na^+ could enter the cell through high affinity K^+ carriers or through the low affinity channels called non selective cation channels that are strongly influenced by Ca^{2+}. These cation channels could allow entry of large amount of Na^+ from a highly saline soil if not adequately regulated (Amtmann & 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 *C. fistula* suggest that similar mechanism might operate in this species. It is evidenced that Ca^{2+} causes closure of nonselective cation channels and restricts Na^+ uptake (Rus et al. 2001). As a result, calcium fertilizers may mitigate Na^+ toxicity to this plant.

In general, salinity reduces N accumulation in plants (Feigin 1985). This is due to the fact that an increase in chloride uptake and accumulation is mostly accompanied by a decrease in shoot nitrate concentration (Torres & Bingham 1973, Garg & Gupta 1997). The interaction between salinity and P is very complex and there is no clear cut mechanistic explanation for decreased, increased or unchanged P uptake in response to salinisation in different species (Champagnol 1979, Grattan & Grieve 1992). However it is known that P concentration is related to the rate of photosynthesis, since it decreases the conversion of fixed carbon in to starch (Overlach et al. 1993) and therefore decrease of P in leaves 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^{2+} content in all the tissues with salinisation of soil. As a result, Na^+ induced Ca^{2+} deficiency in tissues. 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 & Chang 1987, Garg & Gupta 1997). Besides the role of Mg^{2+} in chlorophyll structure and as an enzyme cofactor, another important role of Mg^{2+} in plants is in the export of photosynthates, which when impaired leads to enhanced degradation of chlorophyll in Mg^{2+} deficient source leaves, resulting in increased oxygenase activity of RuBP carboxylase (Marschner & Cakmak 1989).

It is 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

enhanced Zn, Cu and Mn accumulation, while reduced Fe accumulation at the whole plant level. Besides, cofactors for enzymes, Fe and Cu are essential for biological redox system, Mn for photolysis of water in photosynthesis 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. Salinity generates an increase in reactive oxygen species (ROS) which have deleterious effects on cell metabolism (Borsani et al. 2001). Super oxide dismutases (SODs) detoxify ROS and may contain Cu, Zn, Mn and Fe as metal components (Slater et al. 2003). Increase in Cu, Zn and Mn content at the whole plant level might be the requirement of this plant for survival and growth in response to salinity.

Conclusions

As the salt concentration increased during soil salinisation, seed germination decreased in a linear fashion. The SG_{50} concentration was estimated to be 4.9 dSm^{-1} suggesting that this plant species is comparatively salt tolerant at seed germination. Expansion of leaves and elongation of stems and roots also decreased, exhibiting a linear trend in response to increasing soil salinity. Furthermore, dry weight of leaves, stems, tap roots and lateral roots of seedlings decreased following a linear pattern, with increasing concentration of salt in soil. Dry-weight reduction was lowest for lateral roots, while it was maximum for leaves and tap roots in response to increasing salinity. Water content and water potential of tissues of salt- stressed plants decreased and might have resulted internal water deficit to plants, which in turn, reduced the growth of shoots and roots. Root/shoot dry weight ratio did not change as soil salinity increased. Proline content in tissues increased, exhibiting a linear trend in response to increasing soil salinity.

Sodium content increased, while potassium content decreased in tissues in a linear fashion in response to increase in salinity. Results further suggested that Na inhibited K uptake and there were no effective mechanisms to block subsequent Na^+ transfer to shoot tissues. A significant decrease of calcium content in all the tissues with increase in salinity suggested that Na^+ induced Ca^{2+} deficiency in tissues. This result implicates that Ca fertilizers may mitigate Na^+ toxicity to plants. Concentration of N, P and Mg decreased with increase in soil salinity. There was a significant increase in the concentration of Zn, Cu and Mn in tissues in response to increase in salt stress. Increase in concentration of these micro-elements at the whole- plant level might be an advantage to this plant for survival and growth in saline soils.

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