Implications of calcium nutrition on the response of *Butea monosperma* (Fabaceae) to Soil Salinity

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Resumen

Implicaciones de la nutrición del calcio en la respuesta de Butea monosperma (Fabaceae) a la salinidad del suelo.

Se investigaron los efectos de los niveles de Ca^{2+} sobre la respuesta de germinación y el crecimiento en los semilleros de *Butea monosperma* (Lam.) Taub. (Fabaceae) a la salinidad del suelo. Aunque la salinidad retrasó significativamente ambos aspectos, los efectos perjudiciales de NaCl sobre la germinación se aliviaron y el crecimeinto de los plantones se restauró al añadir calcio al suelo al nivel crítico (proporción Na/Ca 1:0,50). El suplemento de calcio por encima del nivel crítico retrasó más ambos procesos, debido al incremento de la salinidad. El estrés salino redujo en contenido de N, P, K y Ca en los tejidos de la planta, pero dicho contenido fue recuperado al añadir Ca^{2+} hasta el nivel crítico. Al añadir Na^{+} ocurrió lo contrario. Se discuten los resultados respecto a los efectos benéficos del suplemento de calcio sobre el crecimiento de *B. monosperma* en semilleros bajo condiciones de salinidad.

Palabras clave: *Butea monosperma*, Nutrición del calcio, Recuperación, Estrés salino, Tolerancia a la sal, Crecimiento de plántula, Estado hídrico.

Abstract

Effects of Ca^{2+} level on the response of germination and seedling growth of *Butea monosperma* (Lam.) Taub. (Fabaceae) to NaCl salinity in soil were investigated. Salinity significantly retarded the seed germination and seedling growth, but the injurious effects of NaCl on seed germination were ameliorated and seedling growth was restored with calcium supply at the critical level (1:0.50 Na/Ca ratio) to salinised soil. Calcium supply above the critical level further retarded the seed germination and seedling growth due to the increased soil salinity. Salt stress reduced N, P, K and Ca content in plant tissues, but these nutrients were restored by addition of Ca^{2+} at the critical level to saline soil. The opposite was true for Na^{+}. The results are discussed in terms of the beneficial effects of calcium supply on the seedling growth of *B. monosperma* grown under saline conditions.

Key words: *Butea monosperma,* Calcium nutrition, Recovery, Salt stress, Salt tolerance, Seedling growth, Water status.

Introduction

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. (Patel & Pandey 2008). The high salt content lowers osmotic potential of soil water and consequently the availability of soil water to plants (Ramoliya et al. 2004). The salt-induced water deficit is one of the major constraints for plant growth in saline soils (Ramoliya et al. 2004). In addition, ionic toxicity and many nutrient interactions in salt stressed plants can reduce or damage the plant growth (Marschner 1995, Taiz & Zeiger 2006).

The application of gypsum has long been considered a common practice in reclamation of saline-sodic and sodic soils (Marschner 1995). The addition of calcium to the soil (as gypsum or lime) displaces Na^+ from clay particles. This prevents the clay from swelling and dispersing (Sumner 1993) and also makes it possible for Na^+ to be leached deeper into the soil. By improving the soil structure, exogenously supplied calcium may also alter soil properties in various ways (Shabala et al. 2003) that benefit the plant growth. Moreover, an improved Ca/Na ratio in the soil solution enhances the capacity of roots to restrict Na^+ influx (Marschner 1995). Importance of interaction between Na and Ca was recognized after LaHaye & Epstein (1969) reported that exogenously supplied calcium may significantly alleviate detrimental effects of Na^+ on the physiological performance of hydroponically grown plants. Since that time, many investigators became interested in understanding the effects of divalent cations, specifically the effects of Ca^{2+} on various physiological processes in plants (Cramer et al. 1985, 1989, Lauchli 1990, Rengel 1992, Reid & Smith 2000, Shabala 2000, Elphick et al. 2001, Shabala et al. 2003, Vaghela et al. 2009). The spectrum of Na/Ca interactions in plants seems to be extremely broad, ranging from those at the molecular level, such as reduced binding of Na^+ to cell wall or plasma membrane, to those manifested at the whole-plant level, such as effects on root and shoot elongation growth, increased uptake and transport of K^+ or reduced Na^+ accumulation in plants (Lauchli 1990, Rengel 1992). Despite the impressive bulk of literature, interaction of Na with Ca in plants still remains unclear.

Butea monosperma (Lam.) Taub. (Fabaceae), a deciduous tree species, grows on marginal saline lands in Kutch (north-west saline desert) of Gujarat State in India. It also grows successfully in the non-saline and semi-arid area of the Saurashtra region, south to the Kutch. *B. monosperma* is a forest tree species in Gujarat as well as in several other states in India. This tree species is of multiple uses to the local people. When in fully flower this tree is a conspicuous and handsome object in the forests. A gum called "Bengal Kino" is obtained from the bark and flowers furnish a brilliant but fleeting dye. Leaves are useful for packing materials and making plates. The wood is used mainly for fuel. Earlier study (Hirpara et al. 2005) suggested that NaCl reduced the growth of *B. monosperma* and this tree species has no effective mechanism to block the sodium transfer to shoot tissues. Na^+ induced Ca^{2+} deficiency in plant tissues implicating that Ca fertilizer may mitigate Na^+ toxicity to this plant. In addition, increasing salt stress impaired succulent feature of tap roots and seedlings died when soil salinity exceeded 6.2 dSm^{-1}. In the present investigation, seedlings of *B. monosperma* were grown in soil containing different Na/Ca ratios and the study aimed to answer some questions. Can exogenous calcium alleviate the effect of Na^+ on the whole-plant level? Can succulent feature of tap roots be restored by Ca^{2+}? How does excess supply of external Ca^{2+} (above the critical or optimal level) influence the growth of *B. monosperma*?

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 Lat, 70º56' E Long) in Gujarat. For the emergence and growth of seedlings, the top 15 cm black-cotton soil (Vertisol), which is predominant in Saurashtra region of Gujarat, was collected from an agricultural field. This soil is a clayey loam containing 19.6% sand, 20.3% silt and 60.1% clay. The available soil water between wilting coefficient and field capacity ranged from 18.3% to 35.0%, respectively. The total organic carbon content was 1.3% and pH was 7.2. The electrical conductivity of soil was 0.3 dSm^{-1}. Ni-

trogen, 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).

Na/Ca ratios

Surface soil was collected, air dried and passed through a 2mm mesh screen. Eight lots of soil, of 100kg each, were separately spread, about 50mm thick over polyethylene sheets. Sodium chloride (NaCl) amounting to 390g was thoroughly mixed with soil of 7 lots to give electrical conductivity of 3.7 dSm^{-1}. Soil was salinised to this level because in our earlier study (Hirpara et al. 2005) seedlings of B. monosperma did not survive when soil salinity exceeded 6.2 dSm^{-1}. Further, gypsum ($CaSO_4$ $2H_2O$) to the quantity of 97.5, 195, 292.5, 390, 487.5 and 585g was separately mixed with soil of six lots to give 1:0.25, 1:0.50, 1:0.75, 1:1, 1:1.25 and 1:1.50 Na/Ca ratios, respectively, and then soil salinity for corresponding lots was 3.9, 4.2, 4.8, 5.1, 5.3 and 5.6 dSm^{-1}. The soil of seventh lot containing only NaCl was considered as the saline soil and its Na/Ca ratio was 1:0. There was no addition of NaCl and $CaSO_4$ $2H_2O$ to eighth lot of soil that served as control with a 0:0 Na/Ca ratio. The electrical conductivity of control soil was 0.3 dSm^{-1} and this value was approximately equal to 3.0mM salinity. Total eight grades of soils were used in this study. 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 was determined with a conductivity meter.

Available calcium, potassium and sodium in soil

For all grades of soil, calcium, potassium, sodium and magnesium were extracted with 1N CH_3OONH_4 adjusted to pH 7.0 and measured using flame atomic absorption spectrophotometer.

Seedling emergence

Twenty polyethylene bags for each grade of soil were each filled with 5kg of soil. Tap water was added to each bag to bring the soil to field capacity and soil was allowed to dry for 7 days. The soil was then raked using fingers and seeds were sown on 11 October 2006. Seeds of *B. monosperma* were collected from the saline desert of Kutch. Bags were kept in an uncontrolled greenhouse under natural temperature and light. Ten seeds were sown in each bag at a depth of 8 – 12mm. Immediately after sowing soils were watered (300mL water was added to raise the soil moisture to field capacity) and thereafter about 100-150mL water was added to the soils (just to wet the surface soil) on alternate days. Irrigation of soil with required amount of water was taken as a measure to control the Na/Ca ratio. Emergence of seedlings was recorded daily over a period of 30 days and data of cumulative emergence of seedlings were analyzed by t-test (compared 0:0 and 1:0 Na/Ca treatments) and one-way ANOVA. (compared treatments ranging from 1:0 to 1:1.50 Na/Ca).

Seedling growth

For the growth studies, two seedlings that emerged first were left in each of 20 bags for each grade of soil and others were uprooted. Seedlings grown in soils at 0:0 (control), 1:0 (saline), 1:0.25 and 1:0.50 Na/Ca ratios exhibited emergence of the second leaf after 25, 30, 28 and 28 days, respectively. Emergence of the second leaf was recorded after 32 days on seedlings grown in soil where Na/Ca exceeded 1:0.50 ratio. Emergence of the second leaf confirmed the establishment of seedlings. Following emergence of the second leaf, one seedling having better vigor was allowed to grow in each bag and another seedling was further uprooted. Thus twenty replicates factorialzed with eight grades of soil (0:0, 1:0, 1:0.25, 1:0.50, 1:0.75, 1:1, 1:1.25 and 1:1.50 Na/Ca ratios) were prepared. This gave a total of 160 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 6 months. Experiment was terminated on 11April 2007. The mean maximum temperature of the greenhouse during the course of study decreased from 34.3 ± 0.4ºC in October to 27.8 ± 0.6ºC in December 2006 and increased thereafter to 39.2 ± 0.6ºC in April 2007. Five seedlings at 1:1.50 Na/Ca ratio died during the course of experiment. Therefore, seedlings contained in 15 bags at each grade of soil were washed to remove soil particles adhered to roots. Morphological characteristics of each seedling were recorded.

Shoot height and root length (tap root) were measured. A characteristic feature of seedlings was that their tap roots were succulent and markedly thick. Maximum thickness (circumference) of tap roots was measured. A thin wire was twisted around the tap root at the thickest part and the length of wire was measured. Similarly, stem thickness above the soil surface was 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 tissues were analyzed by t-test to assess the effect of salinity on plant growth and by one way ANOVA to assess the effect of gypsum treatment on the growth of salinied plants.

Determination of water potential and proline content

Ten additional plants grown in soil at each grade of soil 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, USA, following Patel et al. (2009). All the measurements were taken between 8 to 10.30 A.M. Concentration of proline in plant tissues was estimated following Bates et al. (1973). Extract of 0.5g fresh plant material with aqueous sulphosalicylic acid was prepared. The extracted proline was made to react with ninhydrin to form chromophore and read at 520nm. Data were analyzed by t-test and 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 Na/Ca ratio were pooled separately. Plant samples were ground using mortar and pestle. Three subsamples of plant tissues were analyzed. Total nitrogen was determined by Kjeldahl method and phosphorus content estimated by the chlorostannous molybdophosphoric blue colour method in sulphuric acid (Piper 1944). Concentrations of calcium, magnesium, sodium and potassium were determined by Shimadzu double beam atomic absorption spectrophotometer AA-6800, Japan, after triacid (HNO_3: H_2SO_4: $HClO_4$ in the ratio of 10:1:4) digestion. Mineral data were analyzed by t-test and one way ANOVA.

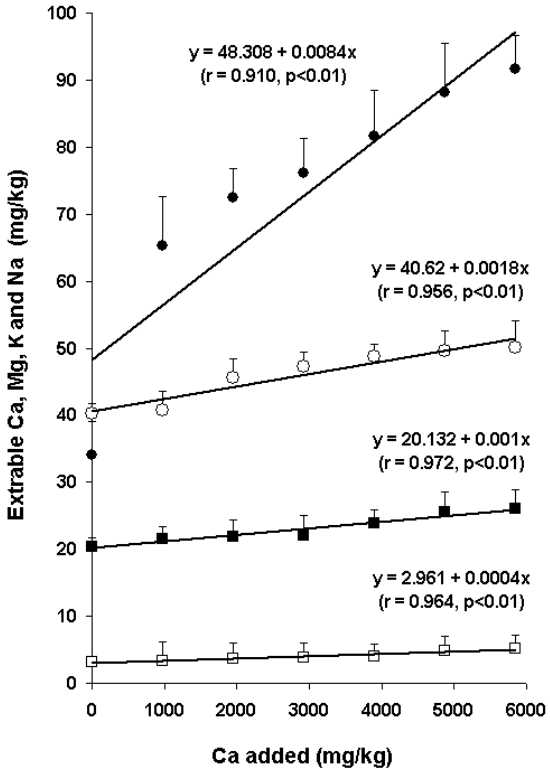

Figura 1. Concentraciones de CH_3COONH_4 extraibles (mg/kg) de Ca (●), Mg (○), K (■) y Na (□) en suelo salinizado en relación a una adición creciente de $CaSO_4.2H_2O$. Los conjuntos de datos y sus rectas de regresión correspondes a las siguientes proporciones Na/Ca: 1:0, 1:0,25; 1:0,50; 1:0,75; 1:1; 1:1,25. Las barras representan el SE.

Figure 1. CH_3COONH_4 extractable concentrations (mg/kg) of Ca (●), Mg (○), K (■) and Na (□) in salinised soil in relation to increasing supply of $CaSO_4.2H_2O$. The data points shown correspond to 1:0, 1:0.25, 1:0.50, 1:0.75,1:1, 1:1.25 and 1:1.50 Na/Ca ratios, respectively, on the X axis. Bars on data points represent SE.

Results

The concentrations of available calcium, potassium, magnesium, and sodium in salinised soil increased linearly with increasing gypsum (CaO_4) treatment (Fig. 1). Salt stress significantly ($p<0.01$) reduced the total emergence of seedlings (Table 1). Calcium supply to the salinity treatment significantly enhanced the germination percentage ($p<0.01$) and the process was stimulated. These effects were evident until Na/Ca ratio in soil increased to 1:0.50 and 1:0.75. Seed germination again decreased with further supply of calcium to

Na/Ca ratio	Total seedling emergence (%)	Shoot height (cm)	Root length (cm)	Leaf area (cm^2 $plant^{-1}$)	Stem thickness (cm)	Tap root thickness (cm)	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 $plant^{-1}$)	Root/Shoot dry weight ratio
0:0	74.9 ± 0.5	45.4 ± 1.6	40.1 ± 2.4	229.7 ± 21.5	2.4 ± 0.1	6.5 ± 0.3	584.0 ± 81.6	562.0 ± 72.7	1146.0 ± 137.7	3308.0 ± 313.1	453.3 ± 52.4	3761.3 ± 333.9	3.9 ± 0.5
1:0	51.8 ± 0.4	35.9 ± 1.9	33.7 ± 4.0	135.5 ± 15.0	1.9 ± 0.1	4.5 ± 0.3	385.3 ± 51.8	479.3 ± 45.5	864.7 ± 86.1	2290.7 ± 244.4	353.3 ± 62.4	2644.0 ± 259.3	3.4 ± 0.5
1:0.25	61.9 ± 0.5	37.7 ± 1.2	35.1 ± 2.1	156.1 ± 16.8	2.5 ± 0.2	5.4 ± 0.3	426.0 ± 51.2	483.6 ± 39.9	909.3 ± 77.1	2498.0 ± 189.3	393.3 ± 46.3	2891.3 ± 190.2	3.4 ± 0.3
1:0.50	68.8 ± 0.5	40.6 ± 1.9	46.5 ± 2.9	211.4 ± 7.1	3.1 ± 0.2	5.8 ± 0.3	534.3 ± 49.5	581.0 ± 41.5	1115.3 ± 37.2	2803.3 ± 359.6	446.7 ± 27.4	3250.0 ± 364.2	3.0 ± 0.3
1:0.75	70.5 ± 0.4	34.6 ± 1.2	32.7 ± 1.8	159.5 ± 16.6	2.2 ± 0.1	4.5 ± 0.2	395.3 ± 44.6	541.0 ± 20.5	936.3 ± 61.1	1794.5 ± 236.8	360.0 ± 40.0	2154.5 ± 248.6	2.7 ± 0.5
1:1	42.7 ± 0.3	34.2 ± 3.0	31.9 ± 2.0	140.2 ± 14.3	2.1 ± 0.1	4.4 ± 0.2	374.0 ± 51.4	478.0 ± 47.1	852.0 ± 52.1	1717.3 ± 282.3	306.7 ± 28.4	2024.0 ± 297.9	2.5 ± 0.4
1:1.25	36.9 ± 0.4	32.1 ± 1.1	26.7 ± 1.5	125.5 ± 11.7	1.9 ± 0.1	3.7 ± 0.2	266.7 ± 63.2	355.3 ± 47.5	622.0 ± 81.6	1553.6 ± 120.3	226.7 ± 26.7	1780.3 ± 129.7	3.8 ± 0.6
1:1.50	28.8 ± 0.2	28.3 ± 2.1	25.7 ± 1.9	103.2 ± 10.4	1.5 ± 0.1	1.5 ± 0.1	181.3 ± 33.5	326.0 ± 42.2	507.3 ± 52.9	1458.0 ± 108.8	168.7 ± 15.1	1626.7 ± 101.4	4.1 ± 0.8
t - values	5.169**	3.880**	3.000*	3.136*	3.506**	5.296**	1.972*	2.456*	2.612*	3.264**	2.341**	3.460**	NS
F-values	4.285**	5.060**	6.722**	8.613**	15.325**	41.588**	5.086**	3.483**	6.936**	6.613**	6.134**	7.972**	NS
$LSD_{0.05}$	11.555	5.124	6.600	40.948	0.337	0.628	156.217	131.732	242.521	675.244	112.037	707.199	NS

Results of 1:0 and 0:0 Na/Ca treatments were compared by t-test.
Results of treatments ranging from 1:0 to 1:1.50 were compared by F- test.
** Values are significant at p< 0.01; * values are significant at p< 0.05; NS = Non significant.

Tabla 1. Efecto de la salinidad y la nutrición del cacio en las características de la hoja, tallo, brote y raíz de *Butea monosperma*, indicado como media ± SEM.
Table 1. Effect of salinity and calcium nutrition on leaf, stem, shoot and root characteristics of *Butea monosperma* as indicated by mean ± SEM.

salinised soil.

Salinity significantly retarded elongation of stems ($p<0.01$) and roots ($p<0.05$). Increasing supply of calcium to salinity treatment reversed the negative effect of NaCl (Table 1). For example, stem height and root length of seedlings grown in soil at 1:0.50 Na/Ca ratio were almost equal to those of seedlings grown under control conditions. A further increase in calcium to salinised soil where Na/Ca exceeded the 1:0.50 ratio caused reduction in stem height and root length. In addition, salinity significantly reduced ($p<0.05$) the expansion of leaves. There was recovery in leaf expansion with increasing calcium supply to salinised soil until 1:0.50 Na/Ca ratio in soil. Following this Na/Ca ratio in soil, leaf expansion exhibited a decreasing trend. Thickness of stems and tap roots was significantly reduced ($p<0.01$) by salt stress, but calcium supply to salinised soil resulted in recovery of stem and tap root thickness. It is evident that thickness of stems and tap roots of seedlings grown in soil at 1:0.50 Na/Ca ratio was nearly equal to that of seedlings grown in control soil. Further increase in calcium in saline soil caused reduction in thickness of stems and tap roots of seedlings.

Dry weight significantly decreased for leaves ($p<0.05$), stems ($p<0.05$), shoots (leaves + stems) ($p<0.01$), tap roots ($p<0.01$), lateral ($p<0.01$), roots and total roots (tap roots + lateral roots) ($p<0.01$) in response to salinity (Table 1). When it is compared with control, the reduction of dry matter caused by salinity was 34.7%, 14.7%, 30.7% and 22.1% for leaves, stems, tap roots and lateral roots, respectively. However dry weight of tissues exhibited either a complete or a significant recovery ($p<0.01$) in the seedlings grown with1:0.50 Na/Ca ratio. Calcium supplies to the saline soil exceeding 1:0.50 Na/Ca ratio caused significant reduction in dry weight of tissues. Root/shoot dry weight ratio of seedlings did not change in response to salinity, and calcium treatments.

Salt stress significantly reduced the water content in leaves ($p<0.01$), stems ($p<0.05$), tap roots ($p<0.01$) and lateral roots ($p<0.05$) (Table 2). Increase in calcium supply to salinity treatment resulted in a significant recovery ($p<0.01$) of water content in tissues. Results suggested that water content in tissues of seedlings grown in soil at 1:0.50 Na/Ca ratio was equal to that in tissues of control plants. Moreover, water content in tissues exhibited a decreasing trend when Na/Ca exceeded the 1:0.50 ratio. Tissues according to their water content can be arranged in the following decreasing order: lateral roots>tap roots=stems> leaves. Water potential of leaves, stems, tap roots and lateral roots of seedlings grown in saline soil became significantly ($p<0.05$) more negative than that in tissues of control plants. (Table 2). Recovery in water potential of tissues was obtained with increase in calcium supply to salinity treatment. It is evident that water potential of tissues of seedlings grown in soil at 1:0.50 Na/Ca ratio was equal to that in tissues of control plants. Further increase in supply of external calcium to salinity treatment again reduced water potential of tissues. Tissues according to their water potential (low to high negative values) can be arranged in the following decreasing order: lateral roots>leaves> stems=tap roots.

Proline content exhibited a significant increase ($p<0.05$) in leaves, stems, tap roots and lateral root tissues in response to salinity (Table 2). Results suggested that proline content in tissues decreased to minimum level at 1:0.50 Na/Ca ratio in soil, but it further increased as the external supply of calcium to saline soil increased. Tissues according to their proline content can be arranged in the following decreasing order: leaves>stems> tap roots>lateral roots.

Sodium content in tissues significantly increased ($p<0.05$) in response to salinity (Table 3); but increasing supply of calcium to salinity treatment significantly reduced ($p<0.01$) the Na content in tissues. Salinity significantly reduced potassium content in leaves ($p<0.05$), stems ($p<0.05$), tap roots ($p<0.01$) and lateral roots ($p<0.05$), but increasing calcium in saline soil resulted in a complete or a significant ($p<0.01$) recovery of K content in tissues of seedlings grown in soil at 1:0.50 Na/Ca ratio. Reduction in K content in tissues was again recorded when Na/Ca in soil exceeded the 1:0.50 ratio. The K/Na ratio significantly decreased for leaves ($p<0.05$), stems ($p<0.01$), tap roots ($p<0.01$) and lateral roots ($p<0.05$) in response to salinity but an increasing supply of calcium to salinity treatment significantly increased ($p<0.01$) their K/Na ratio. Concentration of nitrogen significantly decreased in leaves ($p<0.01$), stems, tap roots and lateral roots ($p<0.05$) in response to salinity. However, a significant recovery ($p<0.05$) was recorded in nitro-

Na/Ca ratio	Water Content (gg^{-1} DW)				Water Potential (-MPa)				Proline Content (m mol / g FW)			
	Leaves	Stems	Tap Roots	Lateral Roots	Leaves	Stems	Tap Roots	Lateral Roots	Leaves	Stems	Tap Roots	Lateral Roots
0:0	3.3 ± 0.2	3.5 ± 0.2	3.7 ± 0.2	4.1 ± 0.4	3.0 ± 0.2	4.1 ± 0.2	4.3 ± 0.2	1.8 ± 0.1	11.6 ± 0.3	9.5 ± 0.1	8.9 ± 0.2	7.7 ± 0.2
1:0	2.3 ± 0.2	2.9 ± 0.7	2.9 ± 0.2	3.3 ± 0.3	3.9 ± 0.1	5.2 ± 0.2	5.3 ± 0.1	2.3 ± 0.1	12.9 ± 0.1	10.2 ± 0.3	9.9 ± 0.4	8.6 ± 0.2
1:0.25	2.5 ± 0.4	3.1 ± 0.2	3.3 ± 0.3	3.6 ± 0.2	3.4 ± 0.1	4.8 ± 0.3	5.0 ± 0.1	2.0 ± 0.1	10.4 ± 0.1	9.2 ± 0.1	9.2 ± 0.1	7.7 ± 0.1
1:0.50	3.2 ± 0.3	3.5 ± 0.4	3.6 ± 0.3	3.9 ± 0.3	3.1 ± 0.2	4.4 ± 0.2	4.5 ± 0.2	2.0 ± 0.1	8.9 ± 0.3	8.7 ± 0.2	8.6 ± 0.2	7.4 ± 0.2
1:0.75	2.4 ± 0.2	3.0 ± 0.4	3.3 ± 0.3	3.3 ± 0.2	3.5 ± 0.2	4.7 ± 0.2	4.9 ± 0.1	2.0 ± 0.1	8.9 ± 0.2	8.9 ± 0.2	8.8 ± 0.1	7.8 ± 0.1
1:1	2.3 ± 0.2	2.9 ± 0.4	3.0 ± 0.2	3.1 ± 0.4	3.8 ± 0.3	5.2 ± 0.1	5.2 ± 0.1	2.4 ± 0.2	9.9 ± 0.2	9.4 ± 0.2	9.2 ± 0.2	8.3 ± 0.1
1:1.25	2.1 ± 0.3	2.4 ± 0.3	2.4 ± 0.2	3.0 ± 0.3	4.4 ± 0.2	5.4 ± 0.2	5.5 ± 0.2	2.5 ± 0.2	11.4 ± 0.2	10.6 ± 0.2	9.9 ± 0.1	8.6 ± 0.2
1:1.50	1.6 ± 0.2	1.8 ± 0.4	1.8 ± 0.14	2.4 ± 0.2	4.6 ± 0.3	5.6 ± 0.3	5.6 ± 0.3	2.6 ± 0.1	12.6 ± 0.1	10.9 ± 0.2	10.1 ± 0.1	8.8 ± 0.1
t - values	3.291 **	2.821 *	3.264 **	1.820 *	3.891 *	3.564 *	3.811 *	4.100 *	4.412 *	4.400 *	5.632 *	4.545 *
F-values	3.965**	2.176**	6.616**	3.582**	3.911*	3.569*	4.467*	4.338*	29.611**	7.467**	7.285**	9.633**
$LSD_{0.05}$	0.730	1.089	0.649	0.784	0.306	0.309	0.252	0.183	0.270	0.282	0.186	0.183

Results of 1:0 and 0:0 Na/Ca treatments were compared by t-test.
Results of treatments ranging from 1:0 to 1:1.50 were compared by F- test.
** Values are significant at $p< 0.01$; * values are significant at $p< 0.05$.

Tabla2. Efecto de la salinidad y la nutrición del cacio en el contenido hídrico, potencial hídrico y contenido de prolina de los tejidos de *Butea monosperma*, indicado como media ± SEM.
Table 2. Effect of salinity and calcium nutrition on water content, water potential and proline content in tissues of *Butea monosperma* as indicated by mean ± SEM.

Tissue	Na/Ca ratio	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
Leaf	0:0	26.0 ± 0.6	2.6 ± 0.1	16.2 ± 0.3	2.9 ± 0.1	18.4 ± 0.2	3.9 ± 0.1	5.5 ± 0.1
	1:0	21.7 ± 0.9	2.0 ± 0.1	15.1 ± 0.3	3.6 ± 0.1	16.8 ± 0.2	3.1 ± 0.1	4.3 ± 0.2
	1:0.25	24.0 ± 1.2	2.4 ± 0.2	18.2 ± 0.3	3.3 ± 0.1	18.2 ± 0.4	4.0 ± 0.1	5.6 ± 0.1
	1:0.50	29.7 ± 0.9	2.6 ± 0.2	18.6 ± 0.2	3.1 ± 0.1	19.0 ± 0.3	4.0 ± 0.1	6.0 ± 0.2
	1:0.75	25.7 ± 1.9	2.1 ± 0.2	11.2 ± 0.3	3.0 ± 0.1	18.1 ± 0.3	3.1 ± 0.1	3.7 ± 0.1
	1:1	22.0 ± 2.1	1.8 ± 0.1	8.5 ± 0.2	2.8 ± 0.1	17.8 ± 0.2	2.8 ± 0.1	3.0 ± 0.1
	1:1.25	20.0 ± 1.2	1.6 ± 0.1	8.4 ± 0.2	2.6 ± 0.1	17.6 ± 0.2	2.8 ± 0.1	3.3 ± 0.1
	1:1.50	19.3 ± 1.5	1.5 ± 0.1	8.1 ± 0.2	2.6 ± 0.1	15.8 ± 0.3	2.6 ± 0.1	3.1 ± 0.1
	t - values	13.000**	7.559*	6.047*	4.750*	4.618*	6.933*	4.450*
	F-values	3.756*	9.805**	206.973**	6.400**	9.225**	29.476**	32.888**
	$LSD_{0.05}$	1.951	0.195	0.316	0.138	0.281	0.111	0.211
Stem	0:0	24.0 ± 0.6	1.8 ± 0.1	10.4 ± 0.3	4.4 ± 0.1	10.4 ± 0.3	3.7 ± 0.1	2.4 ± 0.1
	1:0	20.0 ± 0.6	1.6 ± 0.1	7.9 ± 0.4	5.2 ± 0.1	9.1 ± 0.1	3.0 ± 0.2	1.5 ± 0.1
	1:0.25	21.0 ± 1.2	1.7 ± 0.1	8.4 ± 0.2	4.7 ± 0.3	10.0 ± 0.2	3.7 ± 0.1	1.8 ± 0.1
	1:0.50	24.0 ± 1.2	1.9 ± 0.1	9.8 ± 0.3	3.5 ± 0.1	11.8 ± 0.1	3.8 ± 0.1	2.8 ± 0.2
	1:0.75	20.0 ± 0.6	1.8 ± 0.1	6.2 ± 0.2	3.5 ± 0.2	9.8 ± 0.2	2.8 ± 0.1	1.8 ± 0.1
	1:1	19.3 ± 0.7	1.5 ± 0.2	5.8 ± 0.2	3.3 ± 0.1	8.4 ± 0.3	2.7 ± 0.1	1.8 ± 0.1
	1:1.25	19.0 ± 1.2	1.3 ± 0.1	5.1 ± 0.4	3.2 ± 0.1	8.1 ± 0.1	2.7 ± 0.1	1.6 ± 0.1
	1:1.50	19.2 ± 1.8	1.3 ± 0.2	4.9 ± 0.2	2.5 ± 0.2	8.1 ± 0.2	2.7 ± 0.1	2.0 ± 0.1
	t - values	4.620*	4.630*	7.757*	6.933*	4.850*	5.547*	36.440**
	F-values	5.064*	7.390**	29.905**	44.561**	29.213**	13.330**	7.937**
	$LSD_{0.05}$	0.838	0.096	0.381	0.429	0.243	0.134	0.148
Tap root	0:0	20.3 ± 0.9	1.6 ± 0.1	7.2 ± 0.2	4.5 ± 0.1	11.2 ± 0.2	3.5 ± 0.1	1.6 ± 0.1
	1:0	16.1 ± 0.7	1.4 ± 0.1	6.4 ± 0.2	5.1 ± 0.2	9.0 ± 0.1	3.0 ± 0.1	1.3 ± 0.1
	1:0.25	18.0 ± 0.6	1.6 ± 0.2	7.0 ± 0.3	4.5 ± 0.1	10.3 ± 0.2	3.5 ± 0.1	1.5 ± 0.1
	1:0.50	20.7 ± 0.9	1.6 ± 0.1	7.4 ± 0.2	3.1 ± 0.1	11.6 ± 0.1	3.5 ± 0.1	2.4 ± 0.1
	1:0.75	16.3 ± 0.9	1.6 ± 0.1	5.8 ± 0.2	2.9 ± 0.1	9.9 ± 0.3	2.5 ± 0.1	2.0 ± 0.1
	1:1	14.1 ± 1.2	1.4 ± 0.2	5.0 ± 0.1	2.7 ± 0.2	8.6 ± 0.1	2.6 ± 0.1	1.9 ± 0.2
	1:1.25	11.8 ± 1.2	1.3 ± 0.1	4.8 ± 0.4	2.4 ± 0.2	7.8 ± 0.2	2.6 ± 0.1	2.0 ± 0.1
	1:1.50	12.0 ± 0.6	1.3 ± 0.2	4.7 ± 0.2	2.5 ± 0.1	7.8 ± 0.1	2.6 ± 0.1	1.9 ± 0.2
	t - values	4.230*	4.850*	13.856**	4.358*	11.000**	4.620*	10.604**
	F-values	8.342*	6.945**	28.434**	38.221**	53.050**	16.514**	18.895**
	$LSD_{0.05}$	1.172	0.069	0.214	0.559	0.193	0.113	0.085
Lateral root	0:0	20.0 ± 0.6	1.4 ± 0.1	5.0 ± 0.2	4.6 ± 0.1	12.2 ± 0.3	3.8 ± 0.1	1.3 ± 0.1
	1:0	15.2 ± 1.2	1.0 ± 0.2	5.2 ± 0.1	5.3 ± 0.3	11.2 ± 0.1	3.2 ± 0.1	1.1 ± 0.1
	1:0.25	19.1 ± 0.6	1.1 ± 0.1	5.6 ± 0.1	4.6 ± 0.1	11.6 ± 0.2	3.6 ± 0.1	1.2 ± 0.1
	1:0.50	23.3 ± 1.2	1.4 ± 0.1	5.9 ± 0.2	3.0 ± 0.1	12.8 ± 0.2	3.7 ± 0.1	1.9 ± 0.1
	1:0.75	21.2 ± 1.2	1.4 ± 0.2	5.4 ± 0.2	2.9 ± 0.2	12.0 ± 0.2	2.9 ± 0.1	1.9 ± 0.1
	1:1	20.4 ± 1.8	1.4 ± 0.1	4.7 ± 0.3	3.0 ± 0.1	11.2 ± 0.1	2.4 ± 0.1	1.6 ± 0.1
	1:1.25	17.7 ± 0.9	1.2 ± 0.1	4.2 ± 0.2	3.1 ± 0.1	11.2 ± 0.2	2.3 ± 0.2	1.4 ± 0.2
	1:1.50	15.6 ± 0.3	1.2 ± 0.2	4.0 ± 0.2	3.1 ± 0.1	11.2 ± 0.2	2.3 ± 0.1	1.3 ± 0.1
	t - values	4.191*	14.000**	4.530**	7.000*	4.650*	6.000*	4.989*
	F-values	3.698*	6.235**	15.746**	30.698**	8.645**	16.903**	13.668**
	$LSD_{0.05}$	1.408	0.075	0.185	0.160	0.227	0.157	0.088

Results of 1:0 and 0:0 Na/Ca treatments were compared by t-test.
Results of treatments ranging from 1:0 to 1:1.50 were compared by F- test.
**** Values are significant at p< 0.01; * values are significant at p< 0.05.**

Tabla 3. Efecto de la salinidad y la nutrición del calcio en el contenido de nutrientes en los tejidos de *Butea monosperma* indicado como media ± SEM.

Table 3. Effect of salinity and calcium nutrition on nutrient content of tissues of *Butea monosperma* as indicated by mean ± SEM.

gen content of tissues of seedlings grown with 1:0.50 Na/Ca ratio. Salinity significantly ($p<0.05$) reduced the phosphorus content of tissues. Moreover, there was a significant recovery in phosphorus content of leaves ($p<0.01$), stems ($p<0.01$), tap roots ($p<0.01$) and lateral roots ($p<0.05$) of seedlings grown with 1:0.50 Na/Ca ratio. Concentrations of calcium and magnesium significantly decreased ($p<0.05$) in tissues in response to salinity. Concentration of these nutrients significantly increased ($p<0.01$) in response to calcium supply to salinity treatment. It is evident that concentrations of these nutrients were completely restored in tissues of seedling grown in soil at 1:0.50 Na/Ca ratio. Moreover, high calcium in saline soil reduced the concentration of N, P, Ca, and Mg in tissues.

Discussion

The injurious effects of NaCl on germination of *B. monosperma* were ameliorated by increase of calcium to a critical level (1:0.50 and 1:0.75 Na/Ca ratio) in the salinised soil. The detrimental effect of NaCl salinity on germination is associated with an accumulation of toxic ions (Uhvits 1946, Mohammad & Sen 1990), a decrease of available water to the seed (Bernstein & Hayward 1958, Pujol *et al*. 2000) or both. The beneficial effect of Ca^{2+} did not persist when calcium supply exceeded the critical level. In the present study,concentration of available sodium and soil salinity increased with increase in external supply of calcium to the saline soil. Secondly, the water uptake by the germinated seeds decreased with both salinity (20.2 ± 0.5%) and increased Ca^{2+} levels (13.6 ± 0.6%). Therefore, the beneficial effect of Ca^{2+} on B. monosperma seed germination appears due to counteraction of the toxic effect of Na+. The absence of sufficient level of Ca^{2+} in the germination medium could result in a general deterioration and loss of selectivity of the plasma membrane (Whittington & Smith 1992). This aggravates salt effects, probably by increasing membrane permeability and leads to a higher accumulation of toxic ions and/or leakage of solutes (Cramer et al. 1987, Lauchli 1990). A positive response to Ca^{2+} application on germination rate under saline conditions has also been reported in *Phaseolus vulgaris* (Cachorro et al. 1994), in wimmera ryegrass (Lolium rigidum) (Marcar 1986) and in barley (Bliss et al. 1986). Detrimental effect of calcium, above the critical 1:0.50 Na/Ca ratio, on seed germination might be due to the decreased osmotic potential of soil solution because soil salinity increased with increase in calcium supply.

A reduction in water content and water potential of leaves, stems, tap roots and lateral roots of seedlings grown in saline soil might have resulted in internal water deficit to plants, which in turn, reduced the elongation of stems and roots, thickness of stems and tap roots and dry matter accumulation in tissues. It is found that plants subjected to water stress show a general reduction in size and dry matter production (Taiz & Zeiger 2006). In general, salinity can reduce plant growth or damage the plant through (i) osmotic effect (causing water deficit), (ii) toxic effect of ions and (iii) imbalance of the uptake of essential nutrients. (Ramoliya et al. 2004). These modes of action may operate on the celluar as well as on higher organizational levels and influence all the aspect of plant metabolism (Kramer 1983, Garg & Gupta 1997). *B. monosperma* exhibited a reduction in leaf area (photosynthetic area) in response to salinity treatment. 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). Calcium supply to the salinised soil ameliorated the injurious effects of NaCl on *B. monosperma* and plant growth was restored at 1:0.50 Na/Ca ratio. It has been reported that supplemental Ca^{2+} in salinised growth media alleviated inhibition of root growth of barley (Shabala et al. 2003), shoot growth of *Ph. vulgaris* (Cachorro et al. 1994), shoot and root growth of *Salvadora oleoides* (Vaghela et al. 2009).

The inhibiting effect of salinity on seedling growth was more striking in leaves and tap roots than in the other plant parts. Because of concurrent and differential reduction in dry weight of tissues, root/shoot dry weight ratio did not change in response to salinity. Likewise, the recovery of dry weight at the 1:0.50 Na/Ca ratio was maximum for stems and minimum for tap roots. According to rapidity of recovery, tissues can be arranged in the following decreasing order: stems>leaves>

lateral roots>tap roots.

In *B. monosperma*, osmotic adjustment was achieved by K^+ (as evidenced by high K and low Na content in tissues) and increased proline content in tissues when water content decreased because of salinity. In some plant species, salt tolerance is associated with accumulation of organic solutes in cytoplasm to balance the osmotic pressure of ions in the vacuoles. The compounds that accumulate most commonly are proline and glycine betaine, although other molecules can accumulate to high concentration in certain species (Hasegawa et al. 2000). Proline accumulates in the cytoplasm without having any detrimental effects on cytosolic enzyme activities (Stewart & Lee 1974). Conversely, increase in water content and water potential of tissues with calcium treatment was related with decrease of proline content.

In the present study, there was a significant decrease of Ca^{2+} content in all the tissues with salinity treatment. 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 interaction, precipitation and increase in ionic strength that reduce the activity of Ca^{2+} (Janzen & Chang 1987). It is found that salinity can alter Ca^{2+} uptake and transport leading to Ca^{2+} deficiency in plants (Cramer et al. 1987). Consequently, addition of Ca^{2+} to salinised soil to the critical level resulted in recovery of shoot and root growth. In addition, decrease in thickness of succulent tap roots and their milky juice content (for milky juice only visual observation was made by cutting tap roots) were restored by external calcium supply to the critical level. Though succulence is primarily an adaptation to water stress, it provides salt resistance to plants because it temporarily puts off the setting of severe water deficit induced by salt stress. Calcium supply exceeding the critical level again reduced the shoot and root growth and impaired succulence of tap roots. In the present study, the increased sulfate content together with chloride content caused increase in soil salinity with calcium treatment. The increased soil salinity, in other words, decreased osmotic potential might be responsible for retardation of growth at high supply of calcium.

Potassium is a major osmoticum in plant cells (Marschner 1995) and, therefore is essential for all extension growth. It is evidenced that in salt stressed roots of cotton, Na displaced membrane-associated Ca, which was believed to be primarily located at the plasma membrane (Cramer et al. 1987). In addition, NaCl-salinity displaced membrane-associated Ca on protoplasts of corn (Lynch et al. 1987, Lynch & Lauchli 1988), barley (Bittisnich et al. 1989), and on plasma membrane vesicles of melon (Yermiyahu et al. 1994). One consequence of the displacement of membrane-associated Ca by Na is the immediate increase of K efflux across the plasma membrane of salt-stressed cotton roots (Cramer et al. 1985). This effect may be related to the rapid depolarization of the membrane potential upon salinisation (Cramer 1997). In the present study, the increased efflux of K^+ might be one of the reasons for the significant decrease of K content in tissues of *B. monosperma* in response to NaCl salinity. However, recovery of K content in tissues with external calcium supply to the critical level (1:0.50 Na/Ca ratio) may be the result of repolarization of membrane. There is abundant evidence that salinity alters the ion transport and contents of plants (Cramer 1997). In general, Na uptake and concentrations increase and Ca uptake and concentrations decrease in plant cells and tissues as the external Na concentration increases (Rengel 1992, Cramer 1997, Lazof & Bernstein 1999). Likewise, as external Ca concentrations increase Na uptake and concentrations decrease and Ca uptake and concentrations increase. One consequence of these Na:Ca interactions is the reduction of K content in salinised plants, which can be prevented with supplemental Ca. Moreover, increase in soil salinity with high calcium supply caused a decrease in K content in tissues and it can be accounted for low osmotic potential of soil solution. Isosmotic concentrations of mannitol have similar effects as saline treatments with supplemental Ca (10mM) indicating that K efflux is affected by osmotic factors in these solutions and not associated with Na-specific displacement of membrane-associated Ca (Cramer et al. 1987).

Sodium content significantly increased in tissues of salt-stressed plants, but decreased with increase in calcium supply to saline soil. 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. High-affinity K^+ uptake is mediated by K^+ transporter and low-affinity by inwardly-rectifying K^+ channels.

There is no specific Na^+ transporter, Na^+ entry being gained by competition with other cations, in particular K^+. Thus, 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 *B. monosperma* 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). Consequently, Ca^{2+} supply may reduce Na^+ effect on plants. For *B. monosperma*, external supply of calcium reduced Na content on the whole plant level. Results further suggest that sodium accumulation was greater in stems, tap roots and lateral roots, than that in leaves. It can be attributed to cell types in stems and roots that are better able to retain Na^+.

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, 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. 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). External calcium supply reversed the effects of Na^+ and concentrations of N, P and Mg were restored in tissues of seedlings grown at a 1:0.50 Na/Ca ratio. The high influx or low efflux of nutrients might be responsible for recovery of nutrients. The increased salinity (low osmotic potential) can account for a decrease of nutrients when calcium supply exceeds the critical level.

In the present study, available Ca^{2+} in salinised soil with supplemental calcium at the critical level (1:0.50 Na/Ca ratio) was three times higher than that in non-saline control soil. Thus, it can be suggested that available Ca^{2+} in saline soil should be maintained nearly three times higher than that in normal soil in order to ameliorate the injurious effects of NaCl on seed germination and growth of *B. monosperma*.

Conclusions

Results of the present investigation show that germination and growth of *Butea monosperma* seedlings were dependent upon external supply of calcium up to the critical level (1:0.50 Na/Ca ratio) to the salinised soil. The beneficial effects of high Ca^{2+} concentration are reflected in: (a) an almost complete recovery in germination percentage. From an agronomical point of view this result may be advantageous; (b) the negative effect of soil salinity on elongation of stems and roots, thickness of stems and tap roots, leaf area development and dry matter accumulation in tissues can be reduced by additional supply of calcium; (c) succulent feature of tap roots was restored at the critical concentration of calcium; (d) water content and water potential of leaves, stems, tap roots and lateral root tissues increased with increase in calcium to the critical level in salinised soil; (e) it seems that growth reduction caused by salinity is due to high Na^+ and low Ca^{2+} levels in tissues. Thus increasing Ca^{2+} concentration reduces the uptake of Na^+ and increases Ca^{2+} uptake, consequently decreasing Na^+ toxicity; (f) a partial preservation of membrane integrity from NaCl damage takes place decreasing the efflux of K^+ and probably of other mineral nutrients. Moreover, beneficial effects of calcium did not persist when external supply of this element exceeded the critical level because further calcium supply increased soil salinity.

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