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Effect of supplemental Ca^{2+} on NaCl-stressed castor plants (*Ricinus communis* L.)

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Abstract – Greenhouse experiments were conducted to assess the effects of supplemental Ca^{2+} in salinised soil on germination and plant growth response of castor plant (*Ricinus communis* L. Var. Avani-31, Euphorbiaceae). NaCl amounting to 390 g was thoroughly mixed with soil of seven lots, of 100 kg each, to give electrical conductivity of 4.1 dS m^{-1}. Further, $Ca(NO_3)_2 \times 4H_20$ to the quantity of 97.5, 195, 292.5, 390, 487.5, and 585 g 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^{2+} ratios, respectively. The soil of the seventh lot contained only NaCl and its Na^+/Ca^{2+} ratio was 1:0. Soil without addition of NaCl and Ca $(NO_3)_2 \times 4H_20$ served as control, with a 0:0 Na^+/Ca^{2+} ratio. Salinity significantly retarded seed germination and plant growth, but the deleterious effects of NaCl on seed germination were ameliorated and plant growth was restored with Ca^{2+} supply at the critical level (1:0.25 Na^+/Ca^{2+} ratio) to salinised soil. Supply of Ca^{2+} above the critical level further retarded seed germination and plant growth due to the increased soil salinity. Salt stress reduced N, P, K^+ and Ca^{2+} content in plant tissues, but these nutrients were restored by addition of Ca^{2+} at the critical level to saline soil. In contrast, Na^+ content in plant tissues significantly increased in response to salinity, but significantly decreased with increasing Ca^{2+} supply to saline soil. The results are discussed in terms of the beneficial effects of Ca^{2+} supply on the plant growth of *Ricinus communis* grown under saline conditions.

Keywords: Na^+/Ca^{2+} ratio, *Ricinus communis*, seedling growth, salt tolerance, salt stress

Introduction

Soil salinity is a major abiotic stress to plant growth and development (SLATER et al. 2003). A high salt content lowers the osmotic potential of soil solution that reduces the soil water potential. Plants can absorb water only as long as the water potential of roots is lower (more negative) than that of the soil solution. In saline soils, plant cells have to decrease their water potential below that of the soil solution by lowering their solute potential

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through accumulation of solutes. This osmotic adjustment causes water stress to plants. In addition, ionic toxicity and many nutrient interactions in salt-stressed plants can reduce plant growth or damage the plants (MARSCHNER 1995, TAIZ and ZEIGER 2006). Salt tolerance of plants requires compartmentalization of potentially toxic ions in the vacuole and accumulation of compatible solutes, (organic solutes) in the cytosol where they function in osmotic adjustment and osmoprotection. The osmoprotectants that accumulate most commonly are proline and glycine betaine, although other molecules can accumulate to high concentrations in certain species (HASEGAWA et al. 2000)

Application of gypsum has long been considered a common practice in reclamation of saline-sodic and sodic soils (MARSCHNER 1995). The addition of Ca^{2+} to the soil (as gypsum, lime or other soluble calcium salts) 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. Thus exogenously supplied Ca^{2+} not only improves soil structure, but also alters soil properties in various ways (SHABALA et al. 2003) that benefit the plant growth. Moreover, an improved Ca^{2+}/Na^+ ratio in the soil solution enhances the capacity of roots to restrict Na^+ influx (MARSCHNER 1995). The importance of interaction between Na^+ and Ca^{2+} was recognized after LAHAYE and EPSTEIN (1969) reported that exogenously supplied Ca^{2+} may significantly alleviate detrimental effects of Na^+ on the physiological performance of hydroponically grown plants. Since that time, many investigators have become interested in understanding the effects of divalent cations, specifically the effects of Ca^{2+} on various physiological processes in plants (CRAMER et al. 1985; LAUCHLI 1990; RENGEL 1992; SHABALA et al. 2003, 2006; CHEN et al. 2007; VAGHELA et al. 2009). The spectrum of Na^+/Ca^{2+} 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, the interaction of Na^+ with Ca^{2+} in plants still remains unclear.

Castor plant (*Ricinus communis* L.), an oil yielding crop, is native to India, the South Eastern Mediterranean region and Eastern Africa. It is cultivated in tropical regions of India. Moreover, it is extensively cultivated in the marginal saline area of Kutch (north – west saline desert) of Gujarat State of India. This plant is the source of castor beans (used in ornamentation) and castor oil, which is extracted from seeds. The seed cake, which is left over after pressing contains a protein toxin known as ricin.

There is evidence that Na^+ induces Ca^{2+} deficiency in plant tissues (CRAMER 1997; PATEL et al. 2010). Consequently, it is assumed that Ca^{2+} supply to saline soils may mitigate Na^+ toxicity to plants. An understanding of how and how far Ca^{2+} supply modifies responses of plant species to salinity may be of practical significance. In the present investigation calcium nitrate $Ca(NO_3)_2 \times 4H_2O$, which is a nitrogenous fertilizer, was supplied to saline soil and the remedial effects of Ca^{2+} on salt stressed plants of *R. communis* were determined by studying germination, growth, water status and acquisition of macro-nutrients. Thus, the present study was designed to improve understanding of Na^+/Ca^{2+} interactions at the whole plant level for this crop species, as such studies are lacking.

Materials and methods

Study site

The present study was carried out in a greenhouse of the botanical garden of Saurashtra University at Rajkot (22°18' N Latitude, 70°56' E Longitude) in Gujarat. For seedling emergence and plant growth the top 15 cm of black-cotton soil, which is predominant in the Saurashtra region of Gujarat, was collected from an agricultural field. This soil is a clayey loam containing 19.6% sand, 20.3% silt and 60.1% clay. The available soil water between wilting coefficient and field capacity ranged from 18.3% to 35.0%, respectively. The total organic carbon content was 1.3% and pH was 7.2. The electrical conductivity of soil was 0.3 dS m^{-1}. N, P, K, Ca and Na 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^{2+} ratios

Surface soil was collected, air dried and passed through a 2 mm mesh screen. Eight lots of soil, of 100 kg each, were separately spread, about 50 mm thick, over polyethylene sheets. Sodium chloride (NaCl) amounting to 390 g was thoroughly mixed with soil of 7 lots to give electrical conductivity of 4.1 dS m^{-1}. The soil was salinised to this level because this plant is cultivated on marginal saline lands in Kutch. Further, calcium nitrate ($Ca(NO_3)_2 \times 4H_2O$) in quantities of 97.5, 195, 292.5, 390, 487.5 and 585 g 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^{2+} ratios, respectively, and then soil salinity for the corresponding lots was 4.3, 4.6, 4.9, 5.0, 5.1 and 5.2 dS m^{-1}. The soil of seventh lot containing only NaCl was considered saline soil and its Na^+/Ca^{2+} ratio was 1:0. There was no addition of NaCl and $Ca(NO_3)_2 \times 4H_2O$ to the eighth lot of soil, which served as control with 0:0 Na^+/Ca^{2+} ratio. The electrical conductivity of control soil was 0.3 dS m^{-1} and this value was approximately equal to 3.0 mM salinity. A total of eight grades of soil, defined according to their Na^+/Ca^{2+} ratios, 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 Systronics conductivity meter 304, India.

Available Ca^{2+}, K^+, Na^+ and Mg^{2+} in soil

For all grades of soil, Ca^{2+}, K^+, Na^+ and Mg^{2+} were extracted with 1N CH_3COONH_4 adjusted to pH 7.0 and measured by Shimadzu double beam atomic absorption spectrophotometer AA-6800, Japan following JONES, J_R. (2001).

Seedling emergence

Twenty polyethylene bags for each grade of soil were each filled with 5 kg of soil. Tap water was added to each bag to bring the soils to field capacity and soils were allowed to dry for 7 days. Soils were then raked using fingers and seeds were sown on 15 August 2008. Seeds of *R. communis* Var. Avani-31 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 8–12 mm. Immediately after sowing soils were watered (300 mL water was added to raise the soil moisture to field capacity) and thereafter about 100–150 mL water was added to soil (just to wet the surface soil) on alternate days. Irrigation of soil with the required amount of water was taken as a measure to control the Na^+/Ca^{2+} ratio. Emergence of seedlings was recorded daily over a period of 30 days and data of cumulative emergence of seedlings were analysed by t-test (compared 0:0 and 1:0 Na^+/Ca^{2+} treatments) and one-way ANOVA (compared treatments ranging from 1:0 to 1:1.50 Na^+/Ca^{2+}).

Plant growth

For the growth studies, the two seedlings that emerged first were left in each of the 20 bags for each grade of soil and the others were uprooted. Seedlings grown in soils at 0:0 (control), 1:0 (saline), 1:0.25, 1:0.50, 1:0.75, 1:1, 1:1.25 and 1:1.50 Na^+/Ca^{2+} ratios exhibited emergence of the second leaf after 7, 9, 8, 8, 8, 9, 9 and 9 days, respectively. Emergence of the second leaf confirmed the establishment of seedlings. Following the emergence of the second leaf, the more vigorous of the two seedlings was allowed to grow in each bag and the other was uprooted. Thus twenty replicates for each of 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^{2+} ratios) were prepared. This gave a total of 160 bags, which were arranged in twenty randomized blocks. Plants were watered (to raise the soil moisture to field capacity) on alternate days and allowed to grow for 4 months. The experiment was terminated on 15 December 2008. The mean maximum temperature of the greenhouse increased from 31.7 ± 0.6 °C in August to 35.9 ± 0.8 °C in October and thereafter consistently decreased to 30.5 ± 0.6 °C in December 2008. Plants contained in 20 bags at each grade of soil were washed to remove soil particles adhered to roots. Morphological characteristics of each plant were recorded. Shoot height and root length (tap root) were measured. Leaf area was marked out on graph paper. Fresh and dry weights of leaves, stems, tap roots and lateral roots were determined. Water content (g g^{-1} dry weight) in plant tissues (leaves, stems, tap roots and lateral roots) was calculated using fresh and dry weight values. Data recorded for morphological characteristics, dry weight and water content of 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 calcium nitrate treatment on the growth of salinised plants.

Determination of water potential and proline content

Ten additional plants grown in soil at each grade of soil were used for the measurement of water potential and proline determination in plant tissues. Water potential of leaves, stems, tap roots and lateral root tissues was measured by Dewpoint Potential Meter WP4 (Decagon Devices, Inc.Pullman, WA, USA) following PATEL et al. (2010). All the measurements were taken between 8 to 10.30 a.m. Concentration of proline in plant tissues was determined 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. Toluene was added to terminate the reaction. Optical density of chromophores was measured at 520 nm by a Systronics UV-VIS spectrophotometer 118, India. A stock solution of proline was used to prepare a standard curve for proline concentration and optical density. Data were analyzed by t-test and one-way ANOVA.

Mineral analyses of plant materials

Mineral analyses were performed in triplicate on leaves, stems, tap roots and lateral root tissues of seedlings grown at each level of Na^+/Ca^{2+} ratio. Total nitrogen was determined by a micro-Kjeldahl method and phosphorus content was estimated by the chlorostannous molybdophosphoric blue color method in sulphuric acid (PIPER 1944). Concentrations of Ca^{2+}, Mg^{2+}, Na^+ and K^+ were determined by Shimadzu double beam atomic absorption spectrophotometer AA-6800, (Shimadzu Corporation, Kyoto, Japan) after triacid (HNO_3: H_2SO_4: $HClO_4$ in the ratio of 10: 1: 4) digestion. Data were analyzed by t-test and one-way ANOVA.

Results

The concentration of available Ca^{2+}, K^+, Mg^{2+} and Na^+ in salinised soil increased with increasing calcium nitrate ($Ca(NO_3)_2 \times 4H_2O$) concentrations (Fig. 1). Salt stress significantly ($p<0.01$) reduced the percent emergence of seedlings (Tab. 1). Supply of external Ca^{2+} to the salinity treatment significantly enhanced the germination percentage ($p<0.01$) and the process was stimulated. These effects were evident until Na^+/Ca^{2+} ratio in soil increased to 1:0.25 and 1:0.50. Seed germination again decreased with further supply of Ca^{2+} to salinised soil.

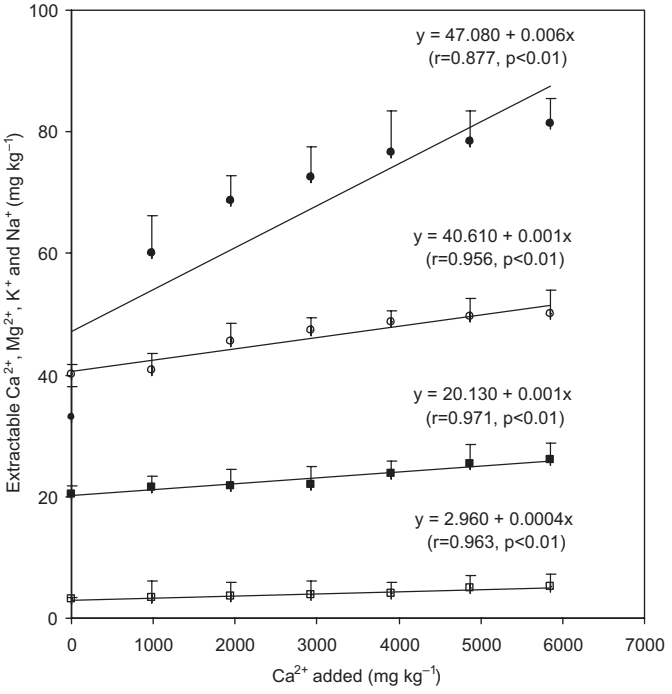

Fig. 1. Concentrations of available Ca^{2+} (●), Mg^{2+} (○), K^+ (■) and Na^+ (□)(mg kg^{-1}) in salinised soil in relation to increasing supply of $Ca(NO_3) \times 4H_2O$. Valus are mean ±SEM. The data points shown correspond to 1:0, 1:0.25, 1:0.50, 1:0.75, 1:1, 1:1.25 and 1:1.50 Na^+/Ca^{2+} ratios respectively, on the X axis.

Tab. 1. Effect of salinity and Ca^{2+} nutrition on leaf, stem, shoot and root characteristics of *Ricinus communis* seedlings as indicated by mean ± SEM.

Na^+/Ca^{2+} ratio	Total seedling emergence (%)	Shoot height (cm)	Root length (cm)	Leaf area (cm^2)	Leaf dry weight (mg)	Stem dry weight (mg)	Shoot dry weight (leaf+stem) (mg)	Tap root dry weight (mg)	Lateral root dry weight (mg)	Total root dry weight (mg)	Root/Shoot dry weight ratio
0:0	93 ± 2	42 ± 1	27 ± 1	198 ± 18	660 ± 64	758 ±39	1419 ± 92	132 ± 5	106 ± 12	238 ± 12	0.17 ± 0.1
1:0	84 ± 2	36 ± 1	19 ± 1	144 ± 3	546 ± 24	556 ± 36	1103 ± 51	97 ± 5	72 ± 6	170 ± 7	0.16 ± 0.1
1:0.25	88 ± 2	41 ± 1	25 ± 1	208 ± 9	651 ± 38	762 ± 21	1413 ± 43	134 ± 12	110± 11	244 ± 22	0.17 ± 0.1
1:0.50	92 ± 2	37 ± 0.4	18 ± 1	165 ± 8	616 ± 51	661 ± 43	1277 ± 72	127 ± 11	96 ± 11	223 ± 15	0.18 ± 0.1
1:0.75	80 ± 2	35 ± 1	16 ± 1	152 ± 5	564 ± 31	542 ± 27	1106 ± 29	112 ± 12	81 ± 12	193 ± 18	0.17 ± 0.1
1:1	77 ± 2	31 ± 1	14 ± 1	137 ± 4	520 ± 23	525 ± 35	1045 ± 52	96 ± 5	70 ± 8	167 ± 8	0.16 ± 0.1
1:1.25	67 ± 2	29 ± 1	13 ± 1	128 ± 3	498 ± 20	476 ± 35	974 ± 47	89 ±7	56 ± 6	146 ± 11	0.15 ± 0.1
1:1.50	52 ± 1	27 ± 1	11 ± 0.3	114 ± 4	402 ± 36	404 ± 29	806 ± 50	68± 8	46 ± 6	114 ± 14	0.14 ± 0.1
t – values	3.48**	4.59**	7.29**	3.07**	3.11**	4.06**	4.52**	3.54**	3.12**	4.07**	NS
F – values	29.82**	39.45**	36.89**	28.16**	5.05**	12.80**	13.17**	4.48**	5.67**	8.00**	NS
$LSD_{0.05}$	8.3	3.2	2.0	16.0	106.4	91.5	151.8	30.9	26.2	44.9	NS

Results of 1:0 and 0:0 Na^+/Ca^{2+} 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 $p<0.01$, N.S. = Non significant.

Salinity significantly retarded ($p<0.01$) elongation of stems and roots (Tab. 1). Supply of Ca^{2+} to salinity treatment reversed the negative effect of NaCl. For example, stem height and root length of plants grown in soil at 1:0.25 Na^+/Ca^{2+} ratio were almost equal to those of plants grown under control conditions. A further increase in supply of external Ca^{2+} where Na^+/Ca^{2+} exceeded the 1:0.25 ratio caused reduction in stem height and root length. In addition, salinity significantly reduced ($p<0.01$) the expansion of leaves. There was recovery in leaf expansion with increase of Ca^{2+} supply to salinised soil until 1:0.25 Na^+/Ca^{2+} ratio. Following this Na^+/Ca^{2+} ratio in soil, leaf expansion exhibited a decreasing trend.

The dry weight of leaves, stems, shoots (leaves + stems), and roots significantly decreased ($p<0.01$) in response to salinity (Tab. 1). When compared with the control, the reduction of dry matter caused by salinity was 17.2%, 26.6%, 26.3% and 31.4% 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 plants grown with 1:0.25 Na^+/Ca^{2+} ratio. Ca^{2+} supplies to the saline soil exceeding 1:0.25 Na^+/Ca^{2+} ratio caused significant decreases in the dry weight of all tissues. Root/shoot dry weight ratio of plants did not change with salinity and Ca^{2+} treatments.

Salt stress significantly reduced ($p<0.01$) the water content in leaves, stems, tap roots and lateral roots (Tab. 2). Supply of Ca^{2+} to salinity treatment resulted in a significant recovery ($p<0.01$) of water content in tissues. The results suggested that water content in the tissues of seedlings increased up to 1:0.25 Na^+/Ca^{2+} ratio and was almost equal to that in control plant tissues. Moreover, water content in tissues exhibited a decreasing trend when Na^+/Ca^{2+} exceeded the 1:0.25 ratio. Tissues according to their water content can be arranged in the decreasing order of lateral roots, tap roots, leaves and stems. Water potential of leaves, stems, tap roots and lateral roots of plants grown in saline soil became significantly ($p<0.05$) more negative than that in tissues of control plants. It is evident that water potential of tissues of plants grown in soil at 1:0.25 Na^+/Ca^{2+} ratio was significantly ($p<0.01$) restored. Further increase in the supply of external Ca^{2+} to salinity treatment again reduced water potential of tissues. According to their water potential (low to high negative values), tissues can be arranged in decreasing order of lateral roots, tap roots, leaves and stems.

Proline content significantly increased ($p<0.05$) in leaves, stems, tap roots and lateral root tissues in response to salinity (Tab. 2). Results suggested that proline content in tissues decreased to minimum level with 1:0.25 Na^+/Ca^{2+} treatments, but it further increased as the external supply of Ca^{2+} to saline soil increased. According to their proline content tissues can be arranged in decreasing order of leaves, stems, tap roots and lateral roots.

Na^+ content in the leaf, stem and root tissues of plants significantly increased ($p<0.05$) in response to salinity (Tab. 3), but increasing the Ca^{2+} in saline soil significantly reduced ($p<0.01$) the Na^+ content in the tissues. Salinity significantly reduced ($p<0.05$) K^+ content in the tissues. There was a complete recovery in K^+ content of plants grown under the 1:0.25 Na^+/Ca^{2+} ratio. Reduction in K^+ content in tissues was again recorded when Na^+/Ca^{2+} in soil exceeded the 1:0.25 ratio. The K^+/Na^+ ratio of tissues significantly decreased ($P<0.05$) in response to salinity, but increasing supply of Ca^{2+} to salinity treatment significantly increased ($p<0.01$) their K^+/Na^+ ratio. Concentrations of N, P and Ca^{2+} in the tissue of plants significantly decreased ($p<0.05$) in response to salinity. It is evident that concentrations of these nutrients were completely restored in tissues of plants

Tab. 2. Effect of salinity and Ca^{2+} nutrition on water content, water potential and proline content in tissues of *Ricinus communis* seedlings as indicated by mean ± SEM.

Na^+/Ca^{2+} ratio	Water Content (g g^{-1} DW)				Water Potential (-MPa)				Proline Content (μ mol g^{-1} FW)			
	Leaves	Stems	Tap Roots	Lateral Roots	Leaves	Stems	Tap Roots	Lateral Roots	Leaves	Stems	Tap Roots	Lateral Roots
0:0	3.4 ± 0.1	2.6 ± 0.1	3.8 ± 0.1	4.3 ± 0.1	2.9 ± 0.3	4.1 ± 0.1	2.2 ± 0.1	1.6 ± 0.2	26 ± 2	26 ± 1	22 ± 1	18 ± 1
1:0	2.9 ± 0.1	2.0 ± 0.1	3.4 ± 0.0	3.9 ± 0.1	3.8 ± 0.2	4.7 ± 0.1	2.9 ± 0.1	2.4 ± 0.1	34 ± 1	29 ± 1	26 ± 1	22 ± 1
1:0.25	3.3 ± 0.1	2.6 ± 0.1	3.8 ± 0.1	4.1 ± 0.1	3.1 ± 0.1	4.5 ± 0.1	2.4 ± 0.1	1.9 ± 0.2	27 ± 2	25 ± 1	23 ± 1	19 ± 1
1:0.50	3.1 ± 0.1	2.3 ± 0.1	3.6 ± 0.1	3.9 ± 0.1	3.5 ± 0.1	4.6 ± 0.1	2.5 ± 0.1	2.1 ± 0.2	29 ± 1	26 ± 1	24 ± 1	20 ± 1
1:0.75	3.0 ± 0.1	2.1 ± 0.1	3.4 ± 0.1	3.8 ± 0.1	3.7 ± 0.1	4.6 ± 0.0	2.6 ± 0.0	2.3 ± 0.2	32 ± 1	28 ± 2	25 ± 1	21 ± 1
1:1	2.8 ± 0.0	2.0 ± 0.1	3.2 ± 0.1	3.7± 0.0	3.9 ± 0.2	4.7 ± 0.2	2.8 ± 0.3	2.4 ± 0.1	34 ± 0.4	29 ± 1	26 ± 1	21 ± 0
1:1.25	2.7 ± 0.0	1.9 ± 0.1	3.0 ± 0.1	3.5 ± 0.1	4.1 ± 0.1	4.9 ± 0.1	3.0 ± 0.1	2.7 ± 0.2	35 ± 1	30 ± 2	27± 1	22 ± 1
1:1.50	2.6 ± 0.0	1.7 ± 0.1	2.9 ± 0.0	3.3 ± 0.1	4.5 ± 0.1	5.3 ± 0.2	3.9 ± 0.2	3.1 ± 0.1	38 ± 0.3	31 ± 1	27 ± 1	22 ± 2
t - values	4.311**	3.795**	6.165**	4.701**	6.584*	7.235*	6.913*	6.235*	6.547*	6.333*	7.216*	6.621*
F - values	9.572**	7.578**	22.605**	32.176**	8.473**	8.672**	10.752**	6.637**	12.060**	5.683**	6.265**	5.625**
LSD 0.05	0.2	0.3	0.2	0.1	0.2	0.2	0.2	0.2	1.4	1.5	1.3	1.2

Results of 1:0 and 0:0 Na^+/Ca^{2+} 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 $p<0.01$ (**) and $p<0.05$ (*).

Tab. 3. Effect of salinity and Ca^{2+} nutrition on nutrient content (mg g^{-1} DW) of tissues (leaf, stem, tap root and lateral root) of *Ricinus communis* seedlings as indicated by mean ± SEM.

Tissue	Na^+/Ca^{2+} Ratio	N (mg g^{-1} DW)	K^+ (mg g^{-1} DW)	P (mg g^{-1} DW)	Na^+ (mg g^{-1} DW)	Ca^{2+} (mg g^{-1} DW)	Mg^{2+} (mg g^{-1} DW)	K^+/Na^+ ratio
Leaf	0:0	23.0 ± 0.7	27.3 ± 0.7	2.4 ± 0.0	8.8 ± 0.1	12.6 ± 0.8	1.1 ± 0.3	3.1 ± 0.1
	1:0	20.0 ± 1.2	23.4 ± 0.3	2.0 ± 0.1	10.0 ± 0.3	10.1 ± 0.4	0.9 ± 0.2	2.3 ± 0.1
	1:0.25	23.0 ± 0.5	26.6± 0.4	2.5 ± 0.2	9.0 ± 0.3	13.6 ± 0.5	1.1 ± 0.3	3.0 ± 0.1
	1:0.50	22.0 ± 0.3	26.1 ± 0.4	2.5 ± 0.2	8.6 ± 0.3	12.6 ± 0.5	1.1 ± 0.2	3.0 ± 0.1
	1:0.75	21.0 ± 0.5	24.8 ± 0.8	2.4 ± 0.1	8.2 ± 0.3	12.1 ± 0.2	1.1 ± 0.1	3.0 ± 0.2
	1:1	21.0± 0.6	24.0 ± 0.3	1.9 ± 0.2	7.4 ± 0.3	11.8 ± 0.4	1.1 ± 0.2	3.3 ± 0.1
	1:1.25	20.0 ± 0.2	22.3 ± 0.2	1.7 ± 0.1	6.9 ± 0.2	11.2 ± 0.6	1.0 ± 0.2	3.2 ± 0.1
	1:1.50	19.0 ± 0.6	21.9 ± 0.3	1.5 ± 0.2	6.3 ± 0.3	10.9 ± 0.7	1.0 ± 0.2	3.5 ± 0.2
	t – values	5.002*	7.986*	5.292*	6.928*	5.339*	NS	11.003*
	F – values	4.679**	18.810**	6.369**	19.805**	5.581**	NS	6.650**
	$LSD_{0.05}$	0.8	0.5	0.2	0.4	0.6	NS	0.1
Stem	0:0	21.0 ± 1.0	21.6± 0.3	2.2 ± 0.1	9.1 ± 0.1	12.5 ± 0.7	1.0 ± 0.3	2.4 ± 0.1
	1:0	19.0 ± 1.2	18.5 ± 0.3	1.9± 0.0	10.8 ± 0.3	10.6± 0.4	0.8 ± 0.2	1.7 ± 0.1
	1:0.25	22.0 ± 0.6	21.2 ± 0.8	2.2 ± 0.1	9.4 ± 0.3	13.4 ± 0.4	1.0 ± 0.3	2.3 ± 0.1
	1:0.50	21.0 ± 0.5	19.5 ± 0.5	2.1 ± 0.1	8.6 ± 0.2	12.4 ± 0.3	1.0 ± 0.4	2.3 ± 0.1
	1:0.75	20.0 ± 0.7	18.5 ± 0.8	2.0 ± 0.1	8.2 ± 0.4	11.8 ± 0.4	1.0 ± 0.2	2.3 ± 0.1
	1:1	18.0 ± 0.7	16.3 ± 0.7	1.8 ± 0.1	7.2 ± 0.2	11.6 ± 0.3	0.9 ± 0.2	2.3 ± 0.1
	1:1.25	18.0 ± 0.7	15.2 ± 0.7	1.7 ± 0.1	7.0 ± 0.4	11.1 ± 0.2	0.9 ± 0.1	2.2 ± 0.1
	1:1.50	18.0 ± 0.4	14.1 ± 0.8	1.7 ± 0.1	6.7 ± 0.2	10.9 ± 0.7	0.9 ± 0.2	2.1 ± 0.1
	t – values	5.774*	5.529*	5.196*	4.348*	7.208*	NS	5.232*
	F – values	4.996**	13.976**	4.807**	24.982**	5.067**	NS	5.677**
	$LSD_{0.05}$	0.9	0.8	0.1	0.4	0.5	NS	0.1

Tab. 3. – continued

Tissue	Na^+/Ca^{2+} Ratio	N (mg g^{-1} DW)	K^+ (mg g^{-1} DW)	P (mg g^{-1} DW)	Na^+ (mg g^{-1} DW)	Ca^{2+} (mg g^{-1} DW)	Mg^{2+} (mg g^{-1} DW)	K^+/Na^+ ratio
Tap root	0:0	20.0 ± 1.0	18.4 ± 0.6	2.0 ± 0.1	9.7± 0.6	11.8 ± 0.1	0.9 ± 0.3	1.9 ± 0.1
	1:0	16.0 ± 1.0	13.9 ± 0.4	1.7 ± 0.1	10.8 ± 0.8	10.0 ± 0.3	0.7 ± 0.3	1.3 ± 0.1
	1:0.25	20.0 ± 0.4	18.2 ± 0.5	1.9 ± 0.1	9.9 ± 0.7	12.1 ± 0.3	0.9 ± 0.2	1.9 ± 0.2
	1:0.50	19.0 ± 0.6	16.9 ± 0.7	1.9 ± 0.1	8.6 ± 0.4	12.1 ± 0.3	0.9 ± 0.2	2.0 ± 0.2
	1:0.75	18.0 ± 0.6	16.7 ± 0.3	1.8 ± 0.1	8.4 ± 0.4	11.9 ± 0.3	0.8 ± 0.2	2.0 ± 0.1
	1:1	18.0 ± 0.5	15.2 ± 0.2	1.5 ± 0.1	7.6 ± 0.2	11.8 ± 0.5	0.8 ± 0.2	2.0 ± 0.0
	1:1.25	17.0 ± 0.6	14.6 ± 0.6	1.4 ± 0.2	7.3 ± 0.2	11.7 ± 0.4	0.8 ± 0.2	2.0 ± 0.1
	1:1.50	16.0 ± 0.9	13.9 ± 0.9	1.3 ± 0.2	7.0 ± 0.3	10.7 ± 0.3	0.8 ± 0.1	2.0 ± 0.1
	t – values	4.703*	4.666*	4.645*	4.715*	4.754*	NS	5.871*
	F – values	4.870**	8.897**	4.508**	8.520**	4.723**	NS	4.798**
	$LSD_{0.05}$	0.8	0.7	0.1	0.6	0.5	NS	0.2
Lateral root	0:0	19.0 ± 1.3	13.2 ± 0.5	1.7 ± 0.0	10.2 ± 0.5	14.4 ± 0.6	0.8 ± 0.0	1.3 ± 0.1
	1:0	14.0 ± 0.9	8.9 ± 0.4	1.4± 0.0	11.6 ± 0.5	12.6 ± 0.3	0.7 ± 0.1	0.8 ± 0.0
	1:0.25	19.0 ± 0.5	13.6 ± 0.7	1.6 ± 0.1	10.5 ± 0.4	15 ± 0.5	1.0 ± 0.1	1.3 ± 0.1
	1:0.50	19.0 ± 0.6	13.4 ± 0.5	1.6 ± 0.1	9.7 ± 0.3	14.8 ± 0.4	1.0 ± 0.2	1.4 ± 0.1
	1:0.75	18.0 ± 1.0	13.1 ± 0.5	1.6 ± 0.1	8.8 ± 0.3	13.5 ± 0.7	1.0 ± 0.2	1.5 ± 0.0
	1:1	18.0 ± 0.6	12.5 ± 0.4	1.4 ± 0.1	7.9 ± 0.5	13.1 ± 0.5	0.9 ± 0.0	1.6 ± 0.1
	1:1.25	17.0 ± 1.0	11.7 ± 0.5	1.2 ± 0.0	7.7 ± 0.5	12.8 ± 0.3	0.9 ± 0.3	1.5 ± 0.1
	1:1.50	16.0 ± 0.9	11.3 ± 0.6	1.2 ± 0.1	7.3 ± 0.7	12.1 ± 0.1	0.9 ± 0.3	1.6 ± 0.1
	t – values	5.879*	5.000*	5.090*	11.094*	6.079*	NS	5.120*
	F – values	4.948**	10.296**	4.963**	10.778**	6.063**	NS	8.830**
	$LSD_{0.05}$	1.0	0.6	0.1	0.6	0.5	NS	0.1

Results of 1:0 and 0:0 Na^+/Ca^{2+} 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 $p<0.01$ (**), and $p<0.05$ (*), NS = Non significant.

grown in soil with a 1:0.25 Na^+/Ca^{2+} ratio. Moreover, high Ca^{2+} in saline soil reduced the concentration of these nutrients in the tissues. Concentrations of Mg^{2+} in plants were not significantly affected by Na^+ and / or Ca^{2+} levels in the soil.

Discussion

The deleterious effects of NaCl on germination of *R. communis* were ameliorated by increase of Ca^{2+} to a critical level (1:0.25 Na^+/Ca^{2+} ratio) in the salinised soil. The detrimental effect of NaCl salinity on germination is associated with an accumulation of toxic ions (Mohammad and Sen 1990), a decrease of available water to the seeds (Pujol et al. 2000) or both. The beneficial effect of Ca^{2+} did not persist when Ca^{2+} supply exceeded the critical level. In the present study, the concentration of available Na^+ and soil salinity increased with increase in the external supply of Ca^{2+} to the saline soil. Secondly, the water uptake by the germinated seeds decreased with both salinity ($20.2 \pm 0.5\%$) and increased Ca^{2+} levels ($13.6 \pm 0.6\%$). Therefore, the beneficial effect of Ca^{2+} on *R. communis* seed germination appears due to counteraction of the toxic effect of Na^+. An insufficient level of Ca^{2+} in the germination medium could result in a general deterioration and loss of selectivity of the plasma membrane (Whittington and 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 (Marcar 1986), in barley (Bliss et al. 1986), in *Salvadora oleoides* (Vaghela et al. 2009). The detrimental effect of Ca^{2+}, above 1:0.50 Na^+/Ca^{2+} ratio, on seed germination might be due to the decreased osmotic potential of soil solution because soil salinity increased with increase in Ca^{2+} supply.

A reduction in water content and water potential of leaves, stems, tap roots and lateral roots of plants grown in saline soil might have resulted in internal water deficit to plants, which in turn, reduced the elongation of stems and 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 and Zeiger 2006). In general, salinity can reduce plant growth or damage to the plants 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 cellular as well as on higher organizational levels and influence all the aspects of plant metabolism (Kramer 1983, Garg and Gupta 1997). *R. Communis* exhibited a reduction in leaf area (photosynthetic area) in response to salinity treatment. Garg and Gupta (1997) reported that salinity causes reduction in leaf area as well as in rate of photosynthesis, which together result in reduced crop growth and yield. Also, a high concentration of salt tends to slow down or stop root elongation (Kramer 1983) and causes reduction in root production (Garg and Gupta 1997). Supply of Ca^{2+} to the salinised soil ameliorated the harmful effects of NaCl on *R. communis* and plant growth was restored at the 1:0.25 Na^+/Ca^{2+} ratio. It has been reported that supplemental Ca^{2+} in salinised growth media alleviated inhibition of barley root growth (Shabala et al. 2003), shoot growth of *Phaseolus vulgaris* (Cachorro et al. 1994), shoot and root growth both in *Salvadora oleoides* (Vaghela et al. 2009). In maize plants grown with a high Na^+:Ca^{2+} ratio, the hydraulic conductance was reduced; supplemental Ca^{2+} (10 mM) improved growth by restoring hydraulic conductance back to that of the control plants (Cramer 1992).

The inhibiting effect of salinity on plant growth was lowest in leaves and highest for stems, tap roots and lateral roots. Consequently, leaves were more resistant and other tissues were sensitive to soil salinity. Likewise, the recovery of dry matter at 1:0.25 Na^+/Ca^{2+} ratio was 98.6%, 100.4%, 101.5% and 103.8% for leaves, stems, tap roots and lateral roots, respectively. Results suggested that there was a resemblance in the shoot and root growth of plants and their root/shoot dry weight ratio did not change with salinity and Ca^{2+} treatments.

Salt tolerance in plants is associated with the accumulation of organic solutes in cytoplasm to balance the osmotic pressure of ions in the vacuoles. Proline accumulates in the cytoplasm without having any detrimental effects on cytosolic enzymes activities (STEWART and LEE 1974, HASEGAWA et al. 2000). In *R. communis*, osmotic adjustment was achieved by K^+ (as evidenced by higher K^+ than Na^+ content in tissues) and increase in the quantity of proline in tissues when water content decreased because of salinity. In addition to its conventional osmoprotective role, proline prevents NaCl-induced K^+ efflux from roots and may operate as ion channel regulators (CUIN and SHABALA 2005) or reactive oxygen species (ROS) scavengers (BOHNERT et al. 1995). Such a regulatory role does not require significant amounts of proline to be accumulated and is, therefore, of low carbon cost to the plant. Results further indicated that increase in water content and water potential of tissues with Ca^{2+} treatment was related to decrease in 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 and 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. Supply of Ca^{2+} exceeding the critical level again reduced the shoot and root growth. In the present study, increased nitrate content together with chloride content caused an increase in soil salinity with Ca^{2+} treatment. The increased soil salinity, in other words, the decreased osmotic potential, might be responsible for retardation of growth at high supply of Ca^{2+}.

K^+ 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^{2+}, which was believed to be primarily located at the plasma membrane (CRAMER et al. 1985). In addition, NaCl-salinity displaced membrane-associated Ca^{2+} on protoplasts of corn (LYNCH and LAUCHLI 1988) and 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^{2+} 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 *R. communis* in response to NaCl salinity. However, recovery of K^+ content in tissues with external Ca^{2+} supply at the critical level (1:0.25 Na^+/Ca^{2+} 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^{2+} uptake and

concentrations decrease in plant cells and tissues as the external Na^+ concentration increases (RENGEL 1992, CRAMER 1997). Likewise, as external Ca^{2+} concentrations increase Na^+ uptake and concentrations decrease and Ca^{2+} uptake and concentrations increase. One consequence of these Na^+:Ca^{2+} interactions is the reduction of K^+ content in salinised plants, which can be prevented with supplemental Ca^{2+}. SHABALA et al. (2006) reported that supplemental Ca^{2+} may prevent K^+ efflux from the cell by blocking the depolarization – activated outward – rectifying K^+ channels. In addition, salinity generates reactive oxygen species (SLATER et al. 2003) which activates non-selective cation channels (NSCC) inducing further K^+ leak (DEMIDCHIK et al. 2002). This leak is additional to one caused by membrane depolarization (CHEN et al. 2007). As a result supplemental Ca^{2+} may prevent such ROS – induced NSCC activation and associated K^+ leak. However, increase in soil salinity with high Ca^{2+} 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^{2+} (10 mM) indicating that K^+ efflux is affected by osmotic factors in these solutions and not associated with Na^+-specific displacement of membrane-associated Ca^{2+} (CRAMER et al. 1985).

Na^+ content significantly increased in tissues of salt-stressed plants, but decreased with increase in Ca^{2+} supply to saline soil. It is reported that uptake mechanisms of both K^+ and Na^+ are similar (SCHROEDER et al. 1994). 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 (NSCC) 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 and SANDERS 1999). Low affinity K^+ uptake is not inhibited by Na^+ but the high affinity process is restricted (SCHROEDER et al. 1994). Similarly Na^+ toxicity in plants is correlated with two proposed Na^+ uptake pathways (NIU et al. 1995). The K^+ and Na^+ profiles of *R. communis* suggest that a similar mechanism might operate in this species. It has been shown that Ca^{2+} is an efficient blocker of NSCC, a major route for Na^+ uptake into the cell (DEMIDCHIK and TESTER 2002, DEMIDCHIK and MAATHUIS 2007) and, thus, may directly reduce the amount of Na^+ accumulation in plants. For *R. communis*, external supply of Ca^{2+} reduced Na^+ content on the whole plant level. Further, the high K^+ content and low Na^+ content in leaves, stems and tap roots tissues suggest that this plant has the characteristic for rapid transport of K^+ to shoot tissues. Intracellular K^+/Na^+ homeostasis is a key component of salinity tolerance in plants (TESTER and DAVENPORT 2003).

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 and BINGHAM 1973, GARG and GUPTA 1997). The interaction between salinity and P is very complex and there is no clear cut mechanism for decreased, increased or unchanged P uptake in response to salinisation in different species (GRATTAN and GRIEVE 1992). However, it is known that P concentration is related to the rate of photosynthesis, but it decreases the conversion of fixed carbon into starch (OVERLACH et al. 1993) and therefore decrease of P in leaves will reduce shoot growth. 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 (MARSCHNER

1995). External Ca^{2+} supply reversed the effects of Na^+ and concentrations of N and P were restored in tissues of seedlings grown at 1:0.25 Na^+/Ca^{2+} ratio. The high influx or low efflux of nutrients might be responsible for restoration or recovery of nutrients. The increased salinity (low osmotic potential) can be accounted for decrease of nutrients when Ca^{2+} supply exceeded the critical level.

In the present study, available Ca^{2+} in salinised soil with supplemental Ca^{2+} at the critical level (1:0.25 Na^+/Ca^{2+} ratio) was two 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 two times higher than that in normal soil in order to ameliorate the injurious effects of NaCl on seed germination and growth of *Ricinus communis*.

Conclusions

Results of the present investigation show that germination and growth of *R. communis* plants were dependent upon external supply of Ca^{2+} up to the critical level (1:0.25 Na^+/Ca^{2+} ratio) to the salinised soil. Our results are in accordance with the assumption that external Ca^{2+} supply to the saline soil may alleviate Na^+ toxicity to castor plants. The beneficial effects of high Ca^{2+} concentration are reflected in: (a) the almost complete recovery in germination percentage; (b) the negative effect of soil salinity on elongation of stems and roots, leaf area development and dry matter accumulation in tissues can be reduced by additional supply of Ca^{2+}; (c) water content and water potential of leaves, stems, tap roots and lateral root tissues increased with increase in Ca^{2+} up to the critical level in salinised soil; (d) it seems that much of growth reduction associated with 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; (e) a decrease in the efflux of K^+ and probably other mineral nutrients resulted in the restoration of nutrients. Moreover, the beneficial effects of Ca^{2+} did not persist when the external supply of this element exceeded the critical level because further Ca^{2+} supply increased soil salinity.

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