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Final Report of the Minor Research Project and Publication out of the project

Annexure -VI

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARL
NEW DELHI –110002.

Final Report of the work done on the Minor Research Project.
(Report to be submitted within 6 weeks after completion of each year)

1. **Project report No. 1st /Final: Final**
2. **UGC Reference No.F. : 47-780/13(WRO)Dt. 20-05-2014**
3. **Period of report: from:20-6-2014 to 19-6-2016**
4. **Title of research project: "Studies on the Effect of Sodium Calcium Ratio on the Germination of Seeds and Growth of Wheat (*Triticum aestivum* L.Variety Lokwan)Cultivar of Rajkot District Region"**

5. **(a) Name of the Principal Investigator: Dr. Viradia Rasiklal Chhaganbhai**
   (b) Dept. :Botany Department
   (c) College where work has progressed :H. & H. B. KOTAK INSTITUTE OF SCIENCE
6. **Effective date of starting of the project: 20-6-2014**
7. **Grant approved and expenditure incurred during the period of the report:**
   a. **Total amount approved Rs. : 3,85,000.00**
   b. **Total expenditure Rs. :2,84,710.00 (approval Rs. 2,85,000.00)**
   c. **Report of the work done: (Please attach a separate sheet) (attached areport copy)**
      i. **Brief objective of the project:**

The objectives of the present research were to assess the following characteristics of the control and salt-stressed plants of wheat (*Triticum aestivum.*L. var.Lokwan) :

(1) Emergence of seedlings.

(2) Fresh weight and dry weight accumulation in plants.

(3) water contain relation

(4) Accumulation of macro nutrients (N,P,K,Na,Ca,Mg) in plant tissues and soil.

ii. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication

PAPER PUBLISHED:IMPLICATIONS OF CALCIUM NUTRITION ON THE RESPONSE OF *Triticum aestivum* L. Var. Lokwan TO SOIL SALINITY (Bioscience Guardian Volum 5,Number 1 , june, 2015 ISSN 2277-9493)

iii. Has the progress been according to original plan of work and towards achieving the objective? ~~if not, state reasons~~ -YES

iv. Please enclose a summary of the findings of the study. One bound copy of the final report of work done may also be sent to the concerned Regional Office of the UGC. – copy attached here with

v. Any other information No.

Signature of the principal investigator

PRINCIPAL
M. & H. B. KOTAK
INST. OF Sc. RAJKOT.
(Seal)

Forward:
(1) **WRO Pune**
(2) **New Delhi**

Summary of project

Greenhouse experiments were conducted to assess the effects of supplemental calcium in salinised soil on the response of germination and plant growth of wheat (Triticum aestivum L. Var.lokwan . NaCl and Ca(NO3)2 × 4H2O were added to soil and 0:0, 1:0, 1:0.25, 1:0.50, 1:0.75, 1:1, 1:1.25 and 1:1.50 Na+ / Ca2+ ratio s were considerable maintained. Salinity significantly retarded the seed germination and plant growth, but the injurious effects of NaCl on seed germination were a little ameliorated and plant growth was restored with calcium supply at the critical level (1:0.25 Na+ / Ca2+ ratio) to salinised soil. Calcium supply above the critical level further retarded the plant growth due to the increased soil salinity. Salt stress reduced N, P, K+ and Ca2+ content in plant tissues, but these nutrients were restored by addition of calcium at the critical level to saline soil. The opposite was true for Na+ . The results are discussed in terms of the beneficial effects of for plant growth under saline conditions.

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Final Report of the work done on the MinorResearch Project. (20-6-2014 to 19-6-2016)

Name:Dr. R.C. Viradia(Subject : Botany)
H. & H. B. KOTAK INSTITUTE OF SCIENCE -RAJKOT (GUJARAT)

Title: "Studies on the Effect of Sodium Calcium Ratio on the Germination of Seeds and Growth of Wheat (*Triticum aestivum*L.Variety Lokwan)Cultivar of Rajkot District Region"

Introduction

Salinity has been an important historical factor and has influenced the life spans of agricultural systems.Although salt tolerance is relatively low in most crop species and cultured woody species, it is encouraging that genetic variability exists not only between species but also between cultivars within a species.Saline soils with an ESP of greater than 15 are termed saline – alkali soils (or saline-sodic soil), have high pH values, and tend to become rather impermeable to both water and aeration when the soluble salts are removed by leaching. These distinctions between saline and alkali (sodic) soils are often insufficiently appreciated in nutrient solution studies by adding high concentrations of single salts (mainly NaCl) but maintaining calcium concentrations low. Such wide Na^+/Ca^{2+} ratios in the substrate are typical for sodic soils but not saline agricultural soils (Maas and Grieve, 1987).One of the major obstacles to increasing food production in arid and semi-arid regions is the lack of fresh water resources. Waters with salinities higher than 3 dSm^{-1} can be used to irrigate salt tolerant crops, but should be used judiciously for salt sensitive crops (FAO, 1992).

Soil salinity is a major abiotic stress to plant growth and development (Slater et al.2003). The high salt content lowers the osmotic potential of soil solution that reduces the soil water potential. In order to absorb water plants have to make osmotic adjustment to maintain internal water potential below that of the soil solution. 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).

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 Na^+/Ca^{2+} 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; Joshi et al. 2012). The spectrum of

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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.

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 *Triticum aestivum* L. Var. Lokwan were determined. This variety of wheat is extensively cultivated in the marginal saline area of kutch (north – west saline desert) and also in coastal saline area and non- saline area of Rajkot district (Saurashtra region) of Gujarat State of India. In the present study experiments were designed to study seed germination, plant growth, water status of plants and acquisition of macro-nutrients by plants. Thus, the present study aims to understand Na^+/Ca^{2+} interactions at the whole plant level for this crop, as such studies are lacking.

Materials and Methods

Study site

The present study was carried out in a greenhouse of the botanical garden of H. & H. B. KOTAK INSTITUTE OF SCIENCE 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.

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 dSm^{-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

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terms of weight. The suspension was shaken and allowed to stand overnight. Thereafter, electrical conductivity was determined with an Eleco conductivity meter CM 183, India.

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

Soil sample will be analyzed for these mineral nutrients.

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 November 2014. Seeds were collected from the saline area of Rajkot district. 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 20 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. Three week after sowing 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 2 months. The experiment was terminated on 15 January 2015. 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 were measured. Leaf area was marked out on graph paper. Fresh and dry weights of leaves, stems, and roots were determined. Water content (%) in plant tissues (leaves, stems, and 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.

Mineral analyses of plant materials

Mineral analyses were performed in triplicate on leaves, stems, and 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, (Shimadju Corporation, Kyoto, Japan) after triacid (HNO_3:

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H_2SO_4: $HClO_4$ in the ratio of 10: 1: 4) digestion. Data were analyzed by t-test and one-way ANOVA.

Results

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

The concentration of available Ca^{2+}, K^+, Mg^{2+} and Na^+ in salinised soil increased linearly with increasing calcium nitrate ($Ca(NO_3)_2 \times 4H_2O$) concentrations (Fig.1).

Seedling emergence

Seedlings began to emerge 3 days after sowing and 95% seed germination was obtained over a period of 14 days under control conditions (0:0 Na^+/Ca^{2+} ratio) (Fig.1). Seed germination was also recorded 3 days after sowing in saline soils, with and without supplemental Ca^{2+}, but for initial 5 or 6 days percentage germination was remarkably poor in these soils as compared to that in control soil. Subsequently, seedlings emerged in large fluxes and percentage germination increased in these soils. Seedling emergence lasted for 11, 11, 16, 15, 14, 15 and 14 days in soils with 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 and corresponding seed germination was 84%, 89.5%, 92.5%, 90%, 90%, 92.5% and 92.5%. Moreover, salinity significantly ($p<0.05$) reduced the percent emergence of seedlings. Extraneously supplied Ca^{2+} to the salinised soil ranging from 1:0.25 to 1:1.50 Na^+/Ca^{2+} enhanced the germination percentage, but beneficial effect of Ca^{2+} was not significant ($p>0.05$).

Plant growth

Salinity significantly retarded ($p <0.01$) elongation of stems and roots (Table 1). Supply of Ca^{2+} to salinity treatment did not significantly reverse the negative effect of NaCl. However, stem height of plants grown in soil with 1:0.25 Na^+/Ca^{2+} ratio was a slightly higher than that of plants grown in salinised (1:0 Na^+/Ca^{2+} ratio) soil. A further increase in supply of external Ca^{2+} where Na^+/Ca^{2+} exceeded the 1:0.25 Na^+/Ca^{2+} ratio caused reduction in stem height and root length. Salinity also significantly reduced ($p<0.01$) the expansion of leaves. A little recovery in leaf expansion was found for plants grown in soil with 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 (Table 1). When compared with control the reduction of dry matter caused by salinity was 40.5%, 23.5% and 32.6%. For leaves, stems

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and roots, respectively. However, dry weight of tissues exhibited a slight increase for the plants grown in soil 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, but decreased with Ca^{2+} supply exceeding 1:0.25 Na^+/Ca^{2+} ratio.

Water status of tissues

Salt stress significantly reduced ($p < 0.01$) water content of leaves, stems and roots (Table 2). Water content of tissues slightly increased in plants grown in soil with 1:0.25 Na^+/Ca^{2+} ratio as compared to that in plants grown in salinised (1:0 Na^+/Ca^{2+} ratio) soil. When Ca^{2+} supply exceeded 1:0.25 Na^+/Ca^{2+} ratio, water content of tissues decreased. Tissues according to their water content can be arranged in the decreasing order of roots, leaves and stems.

Nutrient content of tissues

Na^+ content in the leaf, stem and root tissues of plant significantly increased ($p<0.05$) in response to salinity (Table 3), but increasing the Ca^{2+} in saline soil significantly reduced ($p<0.01$) the Na^+ content in the tissues. Salinity significantly reduced K^+ content in leaves ($p<0.01$), stems ($p<0.05$) and roots ($p<0.01$). 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 significantly decreased in leaves ($p<0.05$), stems ($p<0.01$) and roots ($p<0.01$) in response to salinity, but increasing supply of Ca^{2+} to salinity treatment significantly increased ($p<0.01$) their K^+/Na^+ ratio. There was a significant decrease in N content of tissues ($p<0.01$), Ca^{2+} content of tissues ($p<0.05$) and P content of leaves ($p<0.01$), stems ($p<0.05$) and roots ($p<0.05$) in response to salinity. It was evident that concentrations of these nutrients was completely restored in tissues of plants 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 was not significantly affected by Na^+ /or Ca^{2+} levels in the soil.

Discussion

The deleterious effects of NaCl on seed germination of *T. aestivum* were ameliorated, though not significantly, by increase of Ca^{2+} to a critical level (1:0.25 Na^+/Ca^{2+} ratio) in the

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salinised soil. More ever the beneficial effect of Ca^{2+} persisted when Ca^{2+} supply exceeded the critical level. 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. It has also been reported that salinity reduces protein hydration (Marschner 1995) and induces changes in the activities of many enzymes (Dubey and Rani 1990) in germinating seeds. 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), in *Ricinus communis (*Joshi et al. 2012). 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 reduction in water content of leaves, stems, and 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). Moreover, root elongation for seedlings grown in control and saline soils, with or without Ca^{2+}, was almost double of stem elongation. Result suggests that this wheat variety has a tendency for rapid root extension. It is suggested that rapid root extension ensures existence of plants in dry habitats (Etherington 1987). 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). *T. aestivum* 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 tended to ameliorate the harmful effects of NaCl on *T. aestivum* and plant growth was a little 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*

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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 detrimental effect of Ca^{2+} above 1:0.25 Na^+/Ca^{2+} ratio on plant growth might be due to the decreased osmotic potential of soil solution because soil salinity increased with increase in Ca^{2+} supply.

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 slightly enhanced shoot growth. Calcium supply exceeding the critical level again reduced the shoot and root growth. In the present study, increased nitrate 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^{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 *T. aestivum* in response to NaCl salinity. However, recovery of K^+ content in tissues with external calcium 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; Cramer1997). Likewise, as external Ca^{2+}concentrations increase Na^+ uptake and

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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 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^{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).

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 (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 called nonselective cation 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 (Maathuis & Sanders 1994; Niu et al. 1995). The K^{+} and Na^{+} profiles of *T. aestivum.* suggest that similar mechanism might operate in this species. It is evidenced 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 amount of Na^{+} accumulation in plants. For *T. aestivum*, external supply of calcium reduced Na^{+} content on the whole plant level. Further, high K^{+} content and low Na^{+} content in leaves, stems and roots 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 Davenport2003).

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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 mechanistic explanation for decreased, increased or unchanged P uptake in response to salinisation in different species (Grattan and Grieve1992). 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 calcium 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 recovery of nutrients . The increased salinity (low osmotic potential) can be accounted for decrease of nutrients when calcium supply exceeded the critical level.

Summary

Greenhouse experiments were conducted to assess the effects of supplemental calcium in salinised soil on the response of germination and plant growth of wheat (*Triticum aestivum L. Var.lokwan* . NaCl and $Ca(NO_3)_2 \times 4H_2O$ were added to soil and 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 considerable maintained. Salinity significantly retarded the seed germination and plant growth, but the injurious effects of NaCl on seed germination were a little ameliorated and plant growth was restored with calcium supply at the critical level (1:0.25 Na^{+} / Ca^{2+} ratio) to salinised soil. Calcium supply above the critical level further retarded the 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 calcium at the critical level to saline soil. The opposite was true for Na^{+}. The results are discussed in terms of the beneficial effects of for plant growth under saline conditions.

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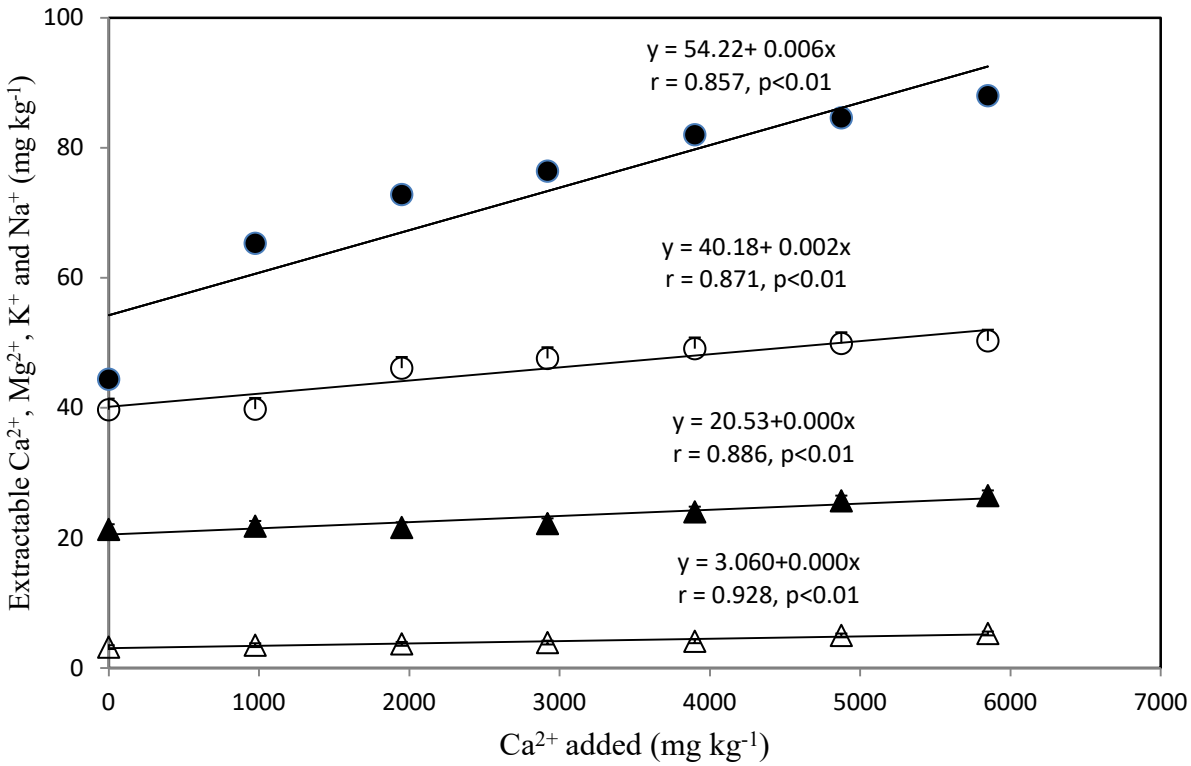

Fig.1. Concentration of available Ca^{2+} (●), Mg^{2+} (o), K^{+} (▲) and Na^{+} (Δ) (mg kg^{-1}) in salinised soil in relation to increasing supply of $Ca(NO_3)_2$ x $4H_2O$). Values are mean ± SE. The data points shown correspond to 1:0, 1:0.25, 1:0.50, 1:0.75, 1:1, 1:1.25 and 1:1.50 Na^{+} / Ca^{2+} ratios respectively, on the X axis.

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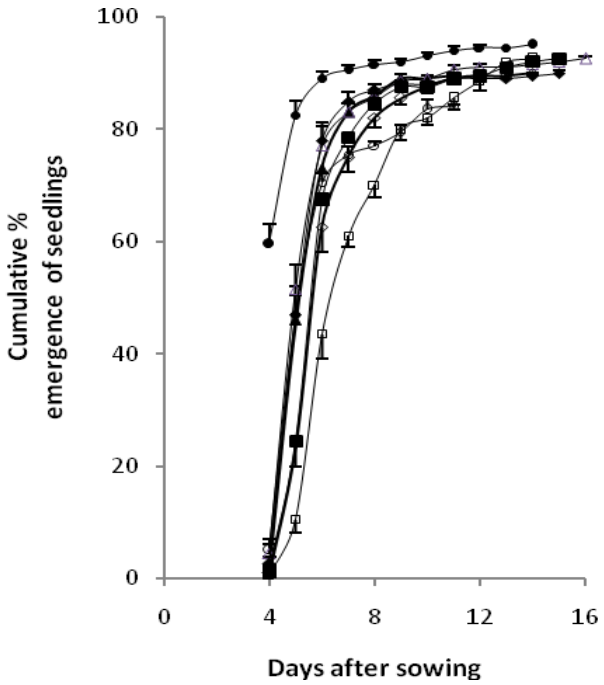

Fig.2. Cumulative emergence of seedlings of *Triticum aestivum* L. Var. Lokwan in soil with 0: 0 (●),1: 0 (○),1: 0.25 (▲),1: 0.50 (△),1: 0.75 (◆),1: 1 (◇),1: 1.25 (■) and 1: 1.50 (□), Na^{+}/Ca^{2+} ratio. Error bars represent SE.

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Table 1. Effect of salinity and Ca^{2+} nutrition on leaf, stem, shoot and root characteristics of *Triticum aestivum* L. Var. Lokwan as indicated by mean ± SE.

Na^{+} / Ca^{2+} ratio	Shoot height (cm)	Root length (cm)	Leaf area (cm^2 $plant^{-1}$)	Leaf dry weight (mg $plant^{-1}$)	Stem dry weight (mg $plant^{-1}$)	Shoot dry weight (Leaf + Stem) (mg $plant^{-1}$)	Root dry weight (mg $plant^{-1}$)	Root/Shoot dry weight ratio
0:0	12.8 ±0.3	33.5±1.8	31.2±0.2	84.8±0.8	74.0±0.6	158.9±0.9	140.3±0.7	0.88±0.01
1:0	8.5±0.2	17.8±0.5	22.7±0.1	50.5±0.8	56.6±1.5	107.2±1.8	94.5±0.8	0.88±0.02
1:0.25	9.0±0.3	16.9±0.6	24.8±0.1	56.2±0.9	58.7±1.0	115.0±1.4	105.5±0.6	0.91±0.01
1:0.50	8.4 ±0.2	16.2±0.5	21.8±0.1	51.4±0.8	52.8±0.6	104.2±1.0	95.4±0.5	0.91±0.01
1:0.75	8.4±0.2	16.2±0.6	20.7±0.1	50.2±0.8	51.6±1.5	101.8±1.8	88.4±0.8	0.87±0.02
1:1	8.3±0.2	16.1±0.5	19.9±0.1	49.1±0.7	49.5±0.7	98.6±1.0	86.9±0.8	0.88±0.01
1:1.25	7.9±0.2	16.1±0.4	18.6±0.1	48.0±0.8	47.8±0.8	95.8±1.0	79.8±0.8	0.83±0.01
1:1.50	7.7±0.1	14.5±0.4	17.8±0.1	45.4±0.9	41.9±0.6	87.3±1.0	74.2±0.6	0.85±0.01
t-values	15.217**	22.184**	14.658**	26.615**	11.304**	23.717**	41.663**	NS
F-values	6.637**	5.498**	40.581**	19.38**	27.018**	41.891**	215.899**	5.464**

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.

Table3: Effect of salinity and Ca^{2+} nutrition on nutrient content (mg g^{-1} DW) of tissues (leaf, stem and root) of *Triticum aestivum* seedlings as indicated by mean ± SE

Tissue	Na^+/Ca^{2+} ratio	N (mg g^{-1} Dw)	P (mg g^{-1} Dw)	K^+ (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	23.7±0.3	1.8±0.0	28.4±0.2	5.9±0.5	11.0±0.5	2.0±0.2	4.8±0.4
	1:0	20.3±0.3	1.5±0.1	24.1±0.1	7.7±0.2	8.9±0.5	1.8±0.2	3.1±0.1
	1:0.25	23.2±0.3	1.8±0.1	27.6±0.3	6.1±0.1	10.8±0.4	2.0±0.2	4.5±0.0
	1:0.50	22.1±0.5	1.7±0.1	26.2±0.1	6.0±0.2	10.8±0.5	2.0±0.2	4.4±0.1
	1:0.75	21.0±0.6	1.7±0.1	25.2±0.1	5.7±0.3	10.2±0.4	2.0±0.2	4.5±0.2
	1:1	20.8±0.1	1.6±0.1	24.5±0.4	4.8±0.1	9.7±0.1	1.8±0.2	5.1±0.03
	1:1.25	20.1±0.2	1.5±0.1	23.1±0.1	4.3±0.2	9.2±0.1	1.7±0.2	5.3±0.3
	1:1.50	19.1±0.6	1.5±0.1	22.5±0.2	4.1±0.1	9.2±0.2	1.7±0.3	5.4±0.2
	t-values	7.474**	5.196**	20.238**	2.954*	3.065*	NS	3.877*
	F-values	13.608**	4.842**	65.514**	45.105**	5.114**	NS	23.838**
Stem	0:0	21.0±0.2	1.7±0.1	25.1±0.6	4.6±0.1	12.4±0.6	1.8±0.2	5.5±0.1
	1:0	19.0±0.2	1.3±0.1	22.2±0.6	5.7±0.4	10.6±0.6	1.5±0.1	3.9±0.1
	1:0.25	21.3±0.9	1.7±0.1	24.5±0.4	4.7±0.1	13.4±0.3	1.8±0.2	5.2±0.1
	1:0.50	21.1±0.1	1.6±0.03	23.4±0.3	4.5±0.3	12.4±0.2	1.8±0.3	5.2±0.2
	1:0.75	20.4±0.2	1.5±0.1	22.4±0.1	4.1±0.1	11.8±0.4	1.7±0.2	5.5±0.1
	1:1	18.0±0.3	1.5±0.1	21.5±0.3	3.9±0.2	11.5±0.3	1.6±0.2	5.5±0.2
	1:1.25	18.0±0.03	1.4±0.1	21.2±0.1	3.8±0.2	11.2±0.1	1.6±0.1	5.6±0.3
	1:1.50	17.4±0.3	1.4±0.1	20.5±0.3	3.8±0.3	10.7±0.2	1.6±0.1	5.5±0.4
	t-values	6.616**	5.500*	3.336*	3.023*	4.588*	NS	9.591**
	F-values	17.257**	3.217*	15.767**	8.448**	17.119**	NS	6.753**
Root	0:0	19.2±0.3	1.5±0.1	22.1±0.2	3.5±0.1	10.4±0.2	1.5±0.1	6.3±0.1
	1:0	15.5±0.3	1.3±0.03	17.5±0.3	4.7±0.3	9.6±0.1	1.2±0.2	3.8±0.2
	1:0.25	19.0±0.3	1.4±0.0	21.7±0.6	4.0±0.3	10.2±0.3	1.5±0.2	5.5±0.2
	1:0.50	19.0±0.2	1.3±0.03	20.7±0.1	3.5±0.3	10.1±0.1	1.5±0.1	5.8±0.4
	1:0.75	18.0±0.1	1.3±0.1	19.6±0.5	3.4±0.3	9.8±0.4	1.4±0.2	5.9±0.4
	1:1	18.0±0.1	1.1±0.1	18.3±0.3	3.1±0.1	9.7±0.4	1.3±0.2	5.9±0.0
	1:1.25	17.2±0.1	1.1±0.1	17.8±0.4	3.0±0.03	9.1±0.1	1.2±0.1	5.9±0.2
	1:1.50	17.0±0.1	1.1±0.03	17.4±0.1	3.0±0.1	8.1±0.2	1.1±0.1	5.8±0.2
	t-values	8.915**	3.500*	12.074**	3.674*	3.098*	NS	11.205**
	F-values	38.895**	5.483**	18.258**	7.254**	7.019**	NS	8.317**

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 $p < 0.05$(*), N.S. = Non significant.

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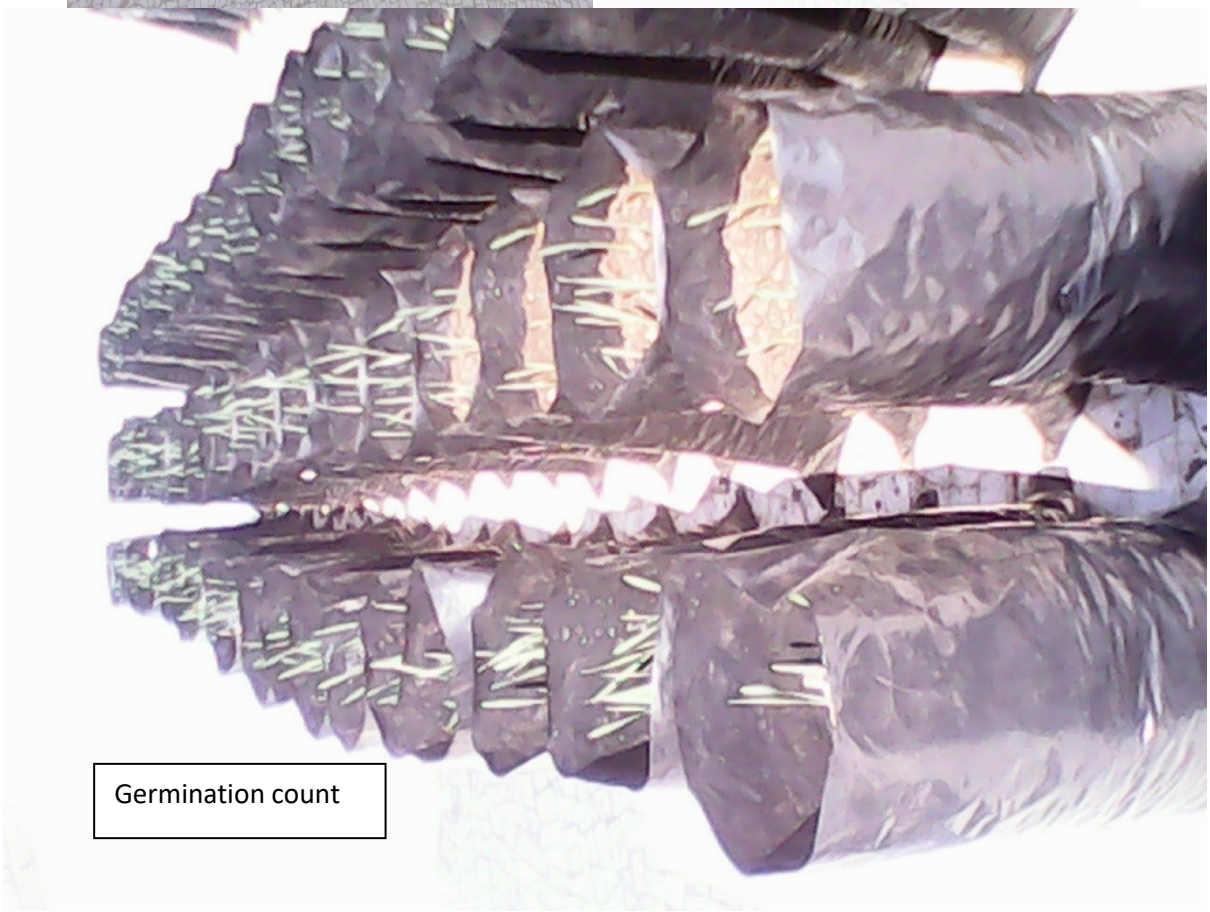
PHOTOGRAPHS

Seed Showing

Germination



Germination count

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Plant Growth 3rd Week


Plant growth 4th Week

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Plant growth 2nd Week

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Plant growth 2nd week

Plant growth

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Plant growth 6th week

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Plant observation

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Plant Harvesting

Plant Harvesting

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Plant Harvesting

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Plant Harvesting

Plant Harvesting and cleaning root

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Plant Harvesting and cleaning root

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Plant length measurement

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Plant length measurement
Plant length measurement

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Plant length, Leaf area and shoot height measurement

Leaf length measurement

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Root length measurement

Plant Fresh Weight measurement

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IMPLICATIONS OF CALCIUM NUTRITION ON THE RESPONSE OF *Triticum aestivum* L. Var. Lokwan TO SOIL SALINITY

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ABSTRACT

Greenhouse experiments were conducted to assess the effects of supplemental calcium in salinised soil on the response of germination and plant growth of wheat (*Triticum aestivum L. Var.lokwan*. NaCl and $Ca(NO_3)_2 \times 4H_2O$ were added to soil and 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 maintained. Salinity significantly retarded the seed germination and plant growth, but the injurious effects of NaCl on seed germination were considerably ameliorated and plant growth was restored to a short extent with calcium supply at the critical level (1:0.25 Na^+ / Ca^{2+} ratio) to salinised soil. Calcium supply above the critical level further retarded the 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 calcium 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 for plant growth under saline conditions.

Key Words: Soil salinity, supplemental calcium, seedling emergence and plant growth

INTRODUCTION

Soil salinity is a major abiotic stress to plant growth and development (Slater et. al.2003). The high salt content lowers the osmotic potential of soil solution that reduces the soil water potential. In order to absorb water, plants have to make osmotic adjustment to maintain internal water potential below that of the soil solution. 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).

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 Na^+/Ca^{2+} 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; Joshi *et al.* 2012). 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.

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 *Triticum aestivum* L. Var. Lokwan were determined. This variety of wheat is extensively cultivated in the marginal saline area of Kutch (north – west saline desert) and also in coastal saline area and non- saline area of Saurashtra region (south to Kutch) of Gujarat State of India. In the present study, experiments were designed to study seed germination, plant growth, water status of plants and acquisition of macro-nutrients by plants. Thus, the present study aims to understand Na^+/Ca^{2+} interactions at the whole plant level for this crop, 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 dSm^{-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 0.3dS 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, Jr. (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 November 2013. Seeds 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 20 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 & the others were uprooted. Three weeks after sowing 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 2 months. The experiment was terminated on 15 January 2014. 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 were measured. Leaf area was marked out on graph paper.

Fresh and dry weights of leaves, stems, and roots were determined. Water content (%) in plant tissues (leaves, stems, and 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.

Mineral analyses of plant materials

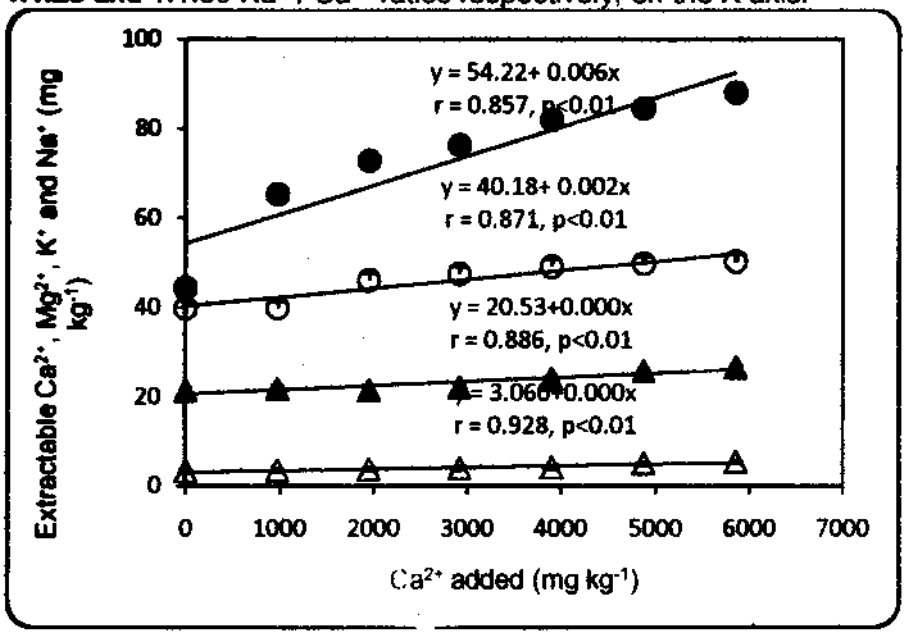
Mineral analyses were performed in triplicate on leaves, stems, and root tissues of seedlings grown at each level of Na^+/Ca^{2+} ratio. Total nitrogen was determined by a micro-Kjeldahl method and phosphorus content was estimated by the chlorostannous molybdophosphoric blue color method in sulphuric acid (Piper 1944). Concentrations of Ca^{2+}, Mg^{2+}, Na^+ and K^+ were determined by Shimadzu double beam atomic absorption spectrophotometer AA-6800, (Shimadju 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 AND DISCUSSION

The concentration of available Ca^{2+}, K^+, Mg^{2+} and Na^+ in salinised soil increased linearly with increasing calcium nitrate ($Ca(NO_3)_2 \times 4H_2O$) concentrations (Figure-1). Seedlings began to emerge 3 days after sowing and 95% seed germination was obtained over a period of 14 days under control conditions (0:0 Na^+/Ca^{2+} ratio) (Figure-1).

Fig.1 Concentration of available Ca^{2+} (•), Mg^{2+} (o), K^+ (▲) and Na^+ (Δ) (mg kg^{-1}) in salinised soil in relation to increasing supply of $Ca(NO_3)_2 \times 4H_2O$. Values are mean ± SE. 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.



Seed germination was also recorded 3 days after sowing in saline soils, with and without supplemental Ca^{2+}, but for initial 5 or 6 days percentage germination was remarkably poor in these soils as compared to that in control soil. Subsequently, seedlings emerged in large fluxes and percentage germination increased in these soils.

Seedling emergence lasted for 11, 11, 16, 15, 14, 15 and 14 days in soils with 1:0, 1:0.25, 1:0.50, 1:0.75, 1:1, 1:1.25 and 1:1.50 Na^+/Ca^{2+} rati os, respectively and corresponding seed germination was 84%, 89.5%, 92.5%, 90%, 90%, 92.5% and 92.5%. Moreover, salinity significantly ($p < 0.05$) reduced the percent emergence of seedlings.

Extraneously supplied Ca^{2+} to the salinised soil ranging from 1:0.25 to 1:1.50 Na^+/Ca^{2+} enhanced the germination percentage, but beneficial effect of Ca^{2+} was not significant ($p>0.05$).

Table 1 Effect of salinity and Ca^{2+} nutrition on leaf, stem, shoot and root characteristics of *Triticum aestivum* L. Var. Lokwan as indicated by mean ± SE.

Na^+ / Ca^{2+} ratio	Total seedling emergence (%)	Shoot height (cm)	Root length (cm)	Leaf area (cm^2 $plant^{-1}$)	Leaf dry Wt. (mg $plant^{-1}$)
0:0	95± 0.5	12.8 ±0.3	33.5±1.8	31.2±0.2	84.8±0.8
1:0	84±0.3	8.5±0.2	17.8±0.5	22.7±0.1	50.5±0.8
1:0.25	89.5±0.4	9.0±0.3	16.9±0.6	24.8±0.1	56.2±0.9
1:0.50	92.5±0.3	8.4 ±0.2	16.2±0.5	21.8±0.1	51.4±0.8
1:0.75	90±0.3	8.4±0.2	16.2±0.6	20.7±0.1	50.2±0.8
1:1	90±0.3	8.3±0.2	16.1±0.5	19.9±0.1	49.1±0.7
1:1.25	92.5±0.4	7.9±0.2	16.1±0.4	18.6±0.1	48.0±0.8
1:1.50	92.5±0.3	7.7±0.1	14.5±0.4	17.8±0.1	45.4±0.9
t-values	2.339*	15.217**	22.184**	14.658**	26.615**
F-values	NS	6.637**	5.498**	40.581**	19.38**

Na^+ / Ca^{2+} ratio	Stem dry Wt. (mg $plant^{-1}$)	Shoot dry Wt. (Leaf + Stem)(mg $plant^{-1}$)	Root dry Wt. (mg $plant^{-1}$)	Root/Shoot dry Wt. ratio
0:0	74.0±0.6	158.9±0.9	140.3±0.7	0.88±0.01
1:0	56.6±1.5	107.2±1.8	94.5±0.8	0.88±0.02
1:0.25	58.7±1.0	115.0±1.4	105.5±0.6	0.91±0.01
1:0.50	52.8±0.6	104.2±1.0	95.4±0.5	0.91±0.01
1:0.75	51.6±1.5	101.8±1.8	88.4±0.8	0.87±0.02
1:1	49.5±0.7	98.6±1.0	86.9±0.8	0.88±0.01
1:1.25	47.8±0.8	95.8±1.0	79.8±0.8	0.83±0.01
1:1.50	41.9±0.6	87.3±1.0	74.2±0.6	0.85±0.01
t-values	11.304**	23.717**	41.663**	NS
F-values	27.018**	41.891**	215.899**	5.464**

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 $p<0.05$ (*), N.S. = Non significant.

Salinity significantly retarded ($p <0.01$) elongation of stems and roots (Table 1). Supply of Ca^{2+} to salinity treatment did not significantly reverse the negative effect of NaCl. However, stem height of plants grown in soil with 1:0.25 Na^+/Ca^{2+} ratio was a little higher than that of plants grown in salinised (1:0 Na^+/Ca^{2+} ratio) soil. A further increase in supply of external Ca^{2+} where Na^+/Ca^{2+} exceeded the 1:0.25 Na^+/Ca^{2+} ratio caused reduction in stem height and root length. Salinity also significantly reduced ($p<0.01$) the expansion of leaves. A little recovery in leaf expansion was found for plants grown in soil with 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 (Table 1). When compared with control the reduction of dry matter caused by salinity was

40.5%, 23.5% and 32.6% for leaves, stems and roots, respectively. However, dry weight of tissues exhibited a little increase for the plants grown in soil 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, but decreased with Ca^{2+} supply exceeding 1:0.25 Na^+/Ca^{2+} ratio.

Table 2 Effect of Salinity Ca^{2+} nutrition on water content in tissues of *Triticum aestivum* L. Var. Lokwan seedlings as indicated by mean ± SE.

Na^+ / Ca^{2+} ratio	Water Content (%)		
	Leaves	Stems	Roots
0 : 0	69.9 ± 0.2	61.1 ± 0.3	81.2 ± 0.1
1: 0	61.6 ± 0.5	56.0 ± 1.2	76.2 ± 0.2
1: 0.25	64.7 ± 0.4	57.2 ± 0.6	79.4± 0.1
1: 0.50	61.4 ± 0.5	55.0 ± 0.5	75.2 ± 0.2
1: 0.75	57.2 ± 0.5	54.3 ± 1.2	74.1 ± 0.3
1:1	56.3 ± 0.6	53.2 ± 0.7	73.2 ± 0.2
1:1.25	55.0 ± 0.8	53.0 ± 0.7	72.0 ± 0.3
1:1.50	54.2 ± 0.9	52.4 ± 0.6	71.2 ± 0.2
t - values	13.44 **	4.049 **	20.993 **
F - values	41.411 **	4.776 **	175.688 **

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 $p < 0.01$

Salt stress significantly reduced ($p < 0.01$) water content of leaves, stems and roots (Table 2). Water content of tissues increased a little in plants grown in soil with 1:0.25 Na^+/Ca^{2+} ratio as compared to that in plants grown in salinised (1:0 Na^+/Ca^{2+} ratio) soil. When Ca^{2+} supply exceeded 1:0.25 Na^+/Ca^{2+} ratio, water content of tissues decreased. Tissues according to their water content can be arranged in the decreasing order of roots, leaves and stems.

Na^+ content in the leaf, stem and root tissues of plant significantly increased ($p<0.05$) in response to salinity (Table 3), but increasing the Ca^{2+} in saline soil significantly reduced ($p<0.01$) the Na^+ content in the tissues. Salinity significantly reduced K^+ content in leaves ($p<0.01$), stems ($p<0.05$) and roots ($p<0.01$). 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 significantly decreased in leaves ($p<0.05$), stems ($p<0.01$) and roots ($p<0.01$) in response to salinity, but increasing supply of Ca^{2+} to salinity treatment significantly increased ($p<0.01$) their K^+/Na^+ ratio. There was a significant decrease in N content of tissues ($p<0.01$),

Ca^{2+} content of tissues ($p<0.05$) and P content of leaves ($p<0.01$), stems ($p<0.05$) and roots ($p<0.05$) in response to salinity. It was evident that concentrations of these nutrients was completely restored in tissues of plants 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 was not significantly affected by Na^+ or Ca^{2+} levels in the soil.

Table 3 Effect of salinity and Ca^{2+} nutrition on nutrient content (mg g^{-1} DW) of tissues (leaf, stem and root) of *Triticum aestivum* seedlings as indicated by mean ± SE

Tissue	Na^+/Ca^{2+} ratio	N (mg g^{-1} Dw)	P (mg g^{-1} Dw)	K^+ (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	23.7±0.3	1.8±0.0	28.4±0.2	5.9±0.5	11.0±0.5	2.0±0.2	4.8±0.4
	1:0	20.3±0.3	1.5±0.1	24.1±0.1	7.7±0.2	8.9±0.5	1.8±0.2	3.1±0.1
	1:0.25	23.2±0.3	1.8±0.1	27.6±0.3	6.1±0.1	10.8±0.4	2.0±0.2	4.5±0.0
	1:0.50	22.1±0.5	1.7±0.1	26.2±0.1	6.0±0.2	10.8±0.5	2.0±0.2	4.4±0.1
	1:0.75	21.0±0.6	1.7±0.1	25.2±0.1	5.7±0.3	10.2±0.4	2.0±0.2	4.5±0.2
	1:1	20.8±0.1	1.6±0.1	24.5±0.4	4.8±0.1	9.7±0.1	1.8±0.2	5.1±0.03
	1:1.25	20.1±0.2	1.5±0.1	23.1±0.1	4.3±0.2	9.2±0.1	1.7±0.2	5.3±0.3
	1:1.50	19.1±0.6	1.5±0.1	22.5±0.2	4.1±0.1	9.2±0.2	1.7±0.3	5.4±0.2
	t-values	7.474**	5.196**	20.238**	2.954*	3.065*	NS	3.877*
	F-values	13.608**	4.842**	65.514**	45.105**	5.114**	NS	23.838**
Stem	0:0	21.0±0.2	1.7±0.1	25.1±0.6	4.6±0.1	12.4±0.6	1.8±0.2	5.5±0.1
	1:0	19.0±0.2	1.3±0.1	22.2±0.6	5.7±0.4	10.6±0.6	1.5±0.1	3.9±0.1
	1:0.25	21.3±0.9	1.7±0.1	24.5±0.4	4.7±0.1	13.4±0.3	1.8±0.2	5.2±0.1
	1:0.50	21.1±0.1	1.6±0.03	23.4±0.3	4.5±0.3	12.4±0.2	1.8±0.3	5.2±0.2
	1:0.75	20.4±0.2	1.5±0.1	22.4±0.1	4.1±0.1	11.8±0.4	1.7±0.2	5.5±0.1
	1:1	18.0±0.3	1.5±0.1	21.5±0.3	3.9±0.2	11.5±0.3	1.6±0.2	5.5±0.2
	1:1.25	18.0±0.03	1.4±0.1	21.2±0.1	3.8±0.2	11.2±0.1	1.6±0.1	5.6±0.3
	1:1.50	17.4±0.3	1.4±0.1	20.5±0.3	3.8±0.3	10.7±0.2	1.6±0.1	5.5±0.4
	t-values	6.616**	5.500*	3.336*	3.023*	4.588*	NS	9.591**
	F-values	17.257**	3.217*	15.767**	8.448**	17.119**	NS	6.753**
Root	0:0	19.2±0.3	1.5±0.1	22.1±0.2	3.5±0.1	10.4±0.2	1.5±0.1	6.3±0.1
	1:0	15.5±0.3	1.3±0.03	17.5±0.3	4.7±0.3	9.6±0.1	1.2±0.2	3.8±0.2
	1:0.25	19.0±0.3	1.4±0.0	21.7±0.6	4.0±0.3	10.2±0.3	1.5±0.2	5.5±0.2
	1:0.50	19.0±0.2	1.3±0.03	20.7±0.1	3.5±0.3	10.1±0.1	1.5±0.1	5.8±0.4
	1:0.75	18.0±0.1	1.3±0.1	19.6±0.5	3.4±0.3	9.8±0.4	1.4±0.2	5.9±0.4
	1:1	18.0±0.1	1.1±0.1	18.3±0.3	3.1±0.1	9.7±0.4	1.3±0.2	5.9±0.0
	1:1.25	17.2±0.1	1.1±0.1	17.8±0.4	3.0±0.03	9.1±0.1	1.2±0.1	5.9±0.2
	1:1.50	17.0±0.1	1.1±0.03	17.4±0.1	3.0±0.1	8.1±0.2	1.1±0.1	5.8±0.2
	t-values	8.915**	3.500*	12.074**	3.674*	3.098*	NS	11.205**
	F-values	38.895**	5.483**	18.258**	7.254**	7.019**	NS	8.317**

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 $p< 0.05$(*), N.S. = Non significant

The deleterious effects of NaCl on seed germination of *T. aestivum* were ameliorated, though not significantly, by increase of Ca^{2+} to a critical level (1:0.25 Na^+/Ca^{2+} ratio) in the salinised soil. Moreover, the beneficial effect of Ca^{2+} persisted when Ca^{2+} supply exceeded the critical level. 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. It has also been reported that salinity reduces protein hydration (Marschner 1995) and induces changes in the activities of many enzymes (Dubey and Rani 1990) in germinating seeds. 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), in *Ricinus communis* (Joshi *et al.* 2012). An insufficient level of Ca^{2+} in the germi na tion 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 reduction in water content of leaves, stems, and 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). Moreover, root elongation for seedlings grown in control and saline soils, with or without Ca^{2+}, was almost double of stem elongation. Result suggests that this wheat variety has a tendency for rapid root extension. It is suggested that rapid root extension ensures existence of plants in dry habitats (Etherington 1987). 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). *T. aestivum* 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 tended to ameliorate the harmful effects of NaCl on *T. aestivum* and plant growth was restored to some extent 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 detrimental effect of Ca^{2+} above 1:0.25 Na^+/Ca^{2+} ratio on plant growth might be due to the decreased osmotic potential of soil solution because soil salinity increased with increase in Ca^{2+} supply.

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 enhanced shoot growth to some extent. Calcium supply exceeding the critical level again reduced the shoot and root growth. In the present study, increased nitrate 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) & 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 *T. aestivum* in response to NaCl salinity. However, recovery of K^+ content in tissues with external calcium 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 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^{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).

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 (Schroeder *et al.* 1994). Na^+ cannot 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 no selective cation 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 (Maathuis & Sanders 1994; Niu *et al.* 1995). The K^+ and Na^+ profiles of *T. aestivum* suggest that similar mechanism might operate in this species. It is evidenced 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 amount of Na^+ accumulation in plants. For *T. aestivum*, external supply of calcium reduced Na^+ content on the whole plant level. Further, high K^+ content and low Na^+ content in leaves, stems and roots 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 mechanistic explanation 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 calcium 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 recovery of nutrients. The increased salinity (low osmotic potential) can be accounted for decrease of nutrients when calcium supply exceeded the critical level.

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