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Effect of Calcium/Sodium Ratio on Growth and Ion Relations of Alfalfa (*Medicago sativa* L.) Seedling Grown under Saline Condition

S.A. Al-Khateeb
Department of Crops and Range, King Faisal University, Al-Hassa, Saudia Arabia

Abstract: Growth and ionic concentrations of alfalfa (*Medicago sativa* L.) seedlings in response to interactive effects of different concentrations of Na^+ (10, 50, 100 and 200 mol m^{-3}) and Ca^{2+} (0, 5 and 10 mM) in growth media were studied. Dry weights of roots, stems and leaves were substantially declined with NaCl concentration increase, however, Ca^{2+} supplementation to the growth media alleviated the deteriorative effects of NaCl. Na^+ and Cl^- were significantly increased with increasing NaCl concentrations in all plant organs, but addition of Ca^{2+} had markedly reduced Na^+ and Cl^- content, particularly in roots. K^+ was significantly reduced with increasing salinity level in roots and no clear trend was noticed in shoots. Ca^{2+} supplementation significantly increased K^+ contents, particularly in roots. Ca^{2+} content was unchanged or slightly increased with increasing NaCl. However, K/Na and Ca/Na ratios were significantly decreased with increasing NaCl, but addition of Ca^{2+} had clearly altered the ratios to a positive increasing trend.

Key words: Salinity, Ca/Na ratio, alfalfa, *Medicago sativa*, root, stem, leaf

INTRODUCTION

More than 30% of the irrigated land world wide is affected by salinity (Carter, 1975) and new areas are increasingly affected in many regions of the world (Chauhan, 1987). Increasing demands for quality water for both industrial development and population is likely to entail the use of low quality, brackish water (Epstein and Rains, 1987). High salt concentration in root medium affects the growth and economic yield of many important crops (Maas and Hoffman, I977). Glycophytes, in order to overcome high salt concentration must be able to withstand potential water deficit, ion toxicity and nutrient imbalances (Greenway and Munns, 1980).

Alfalfa is moderately sensitive to salinity (Maas and Hoffman, I977) and is considered as the most important forage crop in the Kingdom of Saudi Arabia. Ion toxicity was usually associated with either excessive Cl^- or Na^+ intake (Groham *et al*., 1985; Munns and Termaat, 1986). Addition of Ca^{2+} has been reported to ameliorate the adverse effects of salinity on root elongation of plants (Ashraf and Naqvi, 1991; Cramer *et al*., 1989; Nakamura *et al*., 1990; Zidan *et al*., 1990) and shoot growth (Ashraf and Naqvi, 1991; Cramer *et al*., 1989; Zidan *et al*., 1990; Kent and Lauchli, 1985; Grieve and Fujiyama, 1987; Grieve and Mass, 1988; Subbarro *et al*., I990; Yeo *et al*., I991). Elevated Ca^{2+} levels in NaCl medium inhibited Na^+ binding to cell walls (Stassart *et al*., 1981) and the plasma membrane (Cramer *et al*., 1985; Lynch and Cramer, 1987) and hence reduced leakiness of membranes (Cramer *et al*., 1985; Van Steveninck, 1985; Picchioni *et al*., 1991) however, Lynch and Lauchli (1985) did not observe this in salt stressed corn root protoplasts. Ca^{2+} also increased the uptake and transport of K^+ (Nakamura *et al*., 1990; Cramer *et al*., 1985) and reduced Na^+ accumulation in plants (Subbarro *et al*., I990; Cramer *et al*., 1987; Ehret *et al*., 1990; Zidan *et al*., 1991). The growth stimulation effect of Ca^+ addition under saline conditions was attributed not only to the function of membranes, but also to cell elongation and cell division (Zidan *et al*., 1990; Kurth *et al*., 1986).

The present investigation was aimed to study the interactive effects of NaCl salinity and Ca/Na ratio on growth, ion uptake and translocation of alfalfa seedlings.

MATERIALS AND METHODS

Seeds of alfalfa (*Medicago sativa* L.) were sterilized with 0.5% sodium hypochlorite solution for one minute and then washed twice with distilled water. Seeds were germinated in petri-dishes with filter paper Watman No. 1. Three days after germination, seedlings developed their first pair of leaves. At this stage, five seedlings were transferred to several 100 mL conical flasks filled with 25 cm^3 L^{-1}. Twenty milli Liter of NaCl were pipetted into the conical flasks, which were then sealed with polyethylene sheets. The flasks were incubated in a programmed refrigerated incubation using 16 h light: 8 h dark at 25 to 15°C day: night. Seedlings were grown at combined concentrations of 10, 50, 100 and 200 mM NaCl and 0, 5 and 10 mM $CaCl_2$. The twelve combined salt treatments were added to one-tenth strength modified Hoagland's solution. The concentrations of Na^+, K^+ and Ca^{2+} were 0.005, 0.6 and 0.4 mM, respectively. A completely randomized design with factorial combinations of 4 levels of NaCl and 3 levels of $CaCl_2$ replicated six times was used to conduct the experiment. Plant were harvested 21 days after treatment application. Plants were separated into leaves, stems and root for the determination of fresh and dry weights. Stem and leaves of alfalfa seedings were washed two times in distilled water while ions were removed from the free space of roots by washing 2 min in sorbitol solutions isotonic with the treatments concentration in which the plants had grown. Leaves, stems and roots were dried at 85°C for 48 h to determine their dry weights.

For the analysis of K^+, Na^+, Ca^{2+} and Cl^-, three samples each of ≈ 200 mg of fresh material of roots, stems and leaves were homogenized using a pestle and mortar and extracted in 25 mL of deionized water at 90°C for 4 h. The Na^+, K^+ and Ca^{2+} were determined with a GBS 905 Atomic Absorption Spectrophotometer. Cl^- was determined using a chloride meter (Jenway, PCLLM3).

The data were statistically analyzed according to the technique of analysis of variance (ANOVA) for the factorial experiment in the completely randomized design as published by Gomez and Gomez (1984). The treatment means were compared using Baysian Least Significant Difference (BLSD) at 5% level of probability (Waller and Duncan, 1969). All statistical analysis were performed using the facility of computer and SAS software package (SAS Institute, 1996).

RESULTS AND DISCUSSION

Increasing NaCl concentration in the rooting media significantly reduced dry weights of root, stem and leaves (Fig. 1). This is consistent with the results obtained by other researchers (Chow *et al.*, 1990; Bar-Tal *et al.*, 1991; Khan *et al.*, 1998; Kinraide, 1999; Al-Khateeb, 2005a). Adverse effects of increasing NaCl concentration were more pronounced on leaves than on stem and root, indicating that root growth was less affected by salinity (Greenway and Munns, 1980; Delane *et al.*, 1982).

Ca^{2+} supplementation in the rooting media particularly at the highest NaCl concentration, significantly increased dry weight of root, stem and leaves. This is consistent with the results obtained by other researchers (Ashraf and Naqvi, 1991; Kent and Lauchli, 1985; Reid and Smith, 2000; Bonilla *et al.*, 2004). This may indicate that under NaCl salinity Ca^{2+} supplementation decreases the inhibitory effects of salinity in stressed plant

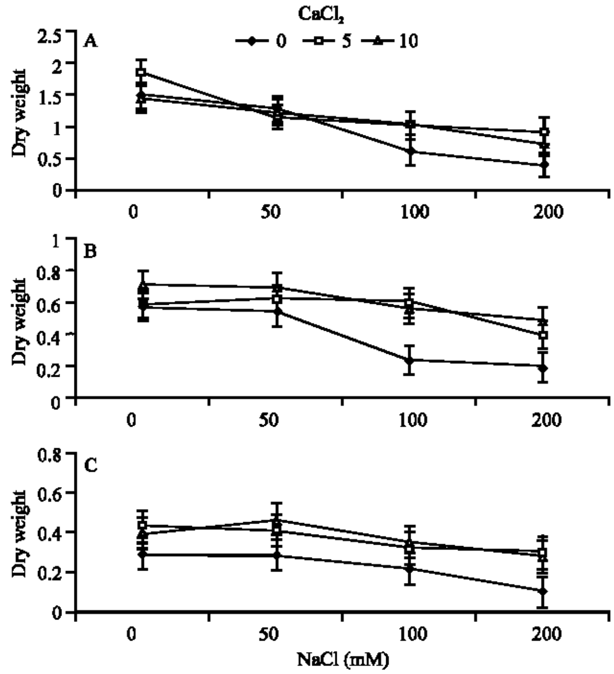

Fig. 1: Effects of Ca^{2+} supplementation (mM) on dry weights of leaves (A), stem (B) and root (C) (mg/plant) of alfalfa seedlings grown under saline conditions (Bars = BLSD 0.05)

(Khavari-Nejad, 1988). Supplemental Ca^{2+} alleviates deleterious salt effects probably through mitigating the toxic effects of Na^+ ions (Bliss *et al.*, 1986; Qadir *et al.*, 2002). However, no significant differences were detected between 5 and 10 mM $CaCl_2$. Insignificant differences may be attributed to the ability of alfalfa seedlings to show almost constant growth pattern when Ca^{2+} is abundant to plants.

The Na^+ content increased significantly in root, stem and leaves as NaCl concentration increased (Fig. 2). Ca^{+2} supply significantly decreased Na^+ accumulation in root and leaves, particularly under high salinity levels. However, no significant differences were detected between 5 and 10 mM $CaCl_2$. The reduction in Na^+ concentration with the addition of Ca^{2+} was much pronounced on root.

The reduction in the accumulation of Na^+ by alfalfa seedlings with the addition of Ca^{2+} supports the findings of others (Ashraf and Naqvi, 1991; Subbarro *et al.*, I990; Cramer *et al.*, 1987; Zidan *et al.*, 1991; Muhammad *et al.*, 1987; Rengel, 1992) who observed a decrease in Na^+ uptake with Ca^{2+} supplementation. The reduction in Na^+accumulation may be associated with the decrease of membrane permeability due to the reduction of Na^+ binding to cell walls and the plasma membrane which

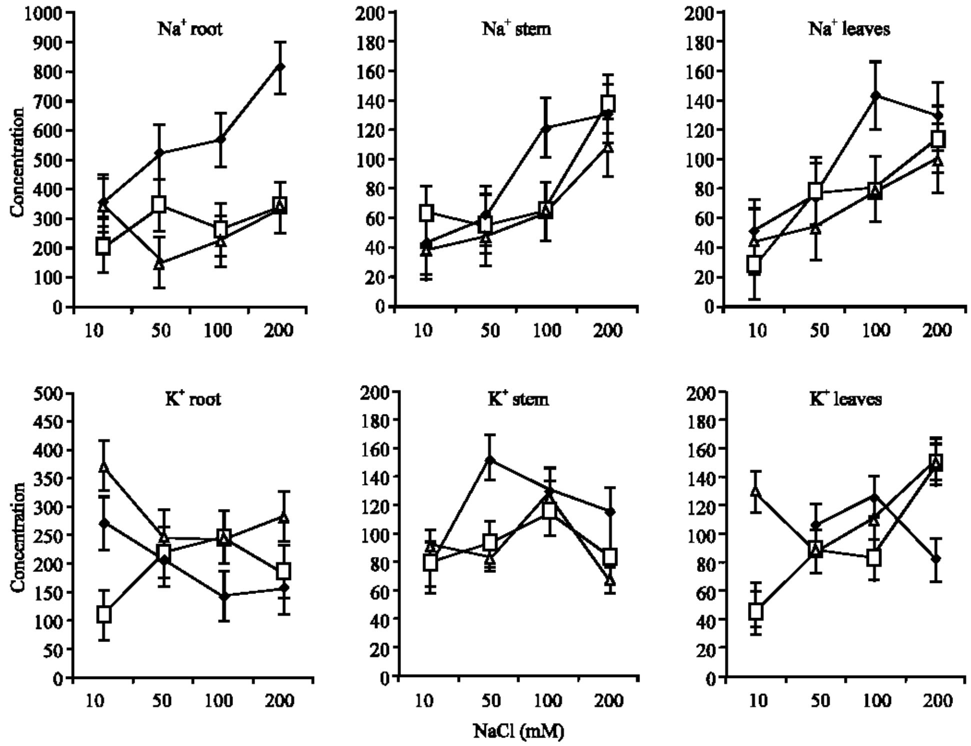

Fig. 2: Effects of Ca^{2+} supplementation (mM) on Na^+ and K^+ concentrations (μM) in root, stem and leaves of alfalfa seedlings grown under saline conditions (Bars = BLSD 0.05)

alleviates membrane leakage (Cramer *et al*., 1985; Picchioni *et al*., 1991; Rengel, 1992).

Na^+concentration was higher in root compared to stem and leaves. Translocation of $Na^+$from root to stem was most probable with Ca^{2+} supplementation while a mechanism that retains Na^+in root might be operating under absence of Ca^{2+} .

Supplemental Ca^{2+} at 200 mM NaCl had no significant effect in decreasing Na^+in stem and to some extend in leaves. It is quite probable that the role of Ca^{2+} in maintenance of plasma membrane integrity and functioning are much disturbed under high level of salinity.

Root K^+ content under no Ca^{2+} addition was decreased significantly as NaCl concentration increased, while with Ca^{2+} supplementation K^+ concentration was significantly increased, particularly under high Ca^{2+} concentrations. Stem K^+ contents seems to be less affected by high level NaCl in root media, since K^+ contents was highest under Ca^{2+} deficient. In leaves, K^+ content was also higher with no Ca^{2+} supplementation except under 200 mM where Ca^{2+} addition increased K^+ contents (Fig. 2). Moreover, addition of 10 mol m^{-3} of Ca^{2+} significantly increased K^+ concentration under the lowest NaCl concentration, however, a trend of increasing K^+ concentration under Ca^{2+} supplementation with increasing NaCl concentration was clearly evident.

The lower internal K^+ concentration with an increase in external Na^+concentration in the absence of Ca^{2+} supply is normally expected (Esechie and Rodrignez, 1998). This could be attributed to the competition of $Na^+$with the uptake of K^+ (Salisbury and Ross, 1994).

There was higher K^+ accumulation in root while leaves had generally the lowest concentration. Wolf and Jeschke (1987) reported that in shoot of salt stressed barley there was recirculation of K^+ via phloem from shoot to root. Part of this recirculated K^+ was transferred from phloem to xylem within the root and returned to the shoot via xylem. Similar results were reported in *Lupinus albus* (Jeschke *et al*., 1986). The lower K^+ concentration with Ca^{2+} addition in stem and leaves may be attributed to the role of Ca^{2+} in decreasing membrane selectivity which may generate a selective ion transport mechanism similar to that reported for Na^+. Similar results were also reported by Groham *et al*. (1985), while contradicting results were obtained by Kawasaki and Moritsuger (1979) and Ashraf and Naqvi (1991).

Interestingly, Ca^{2+} concentration in roots under Ca^{2+} deficient remained almost unchanged with increasing NaCl concentration, while its concentration at 50 and 100 mM NaCl was significantly lower with 10 mM $CaCl_2$ (Fig. 3).

Stem Ca^{2+} concentration was generally higher with Ca^{2+} supplementation. This trend was distinctly obvious

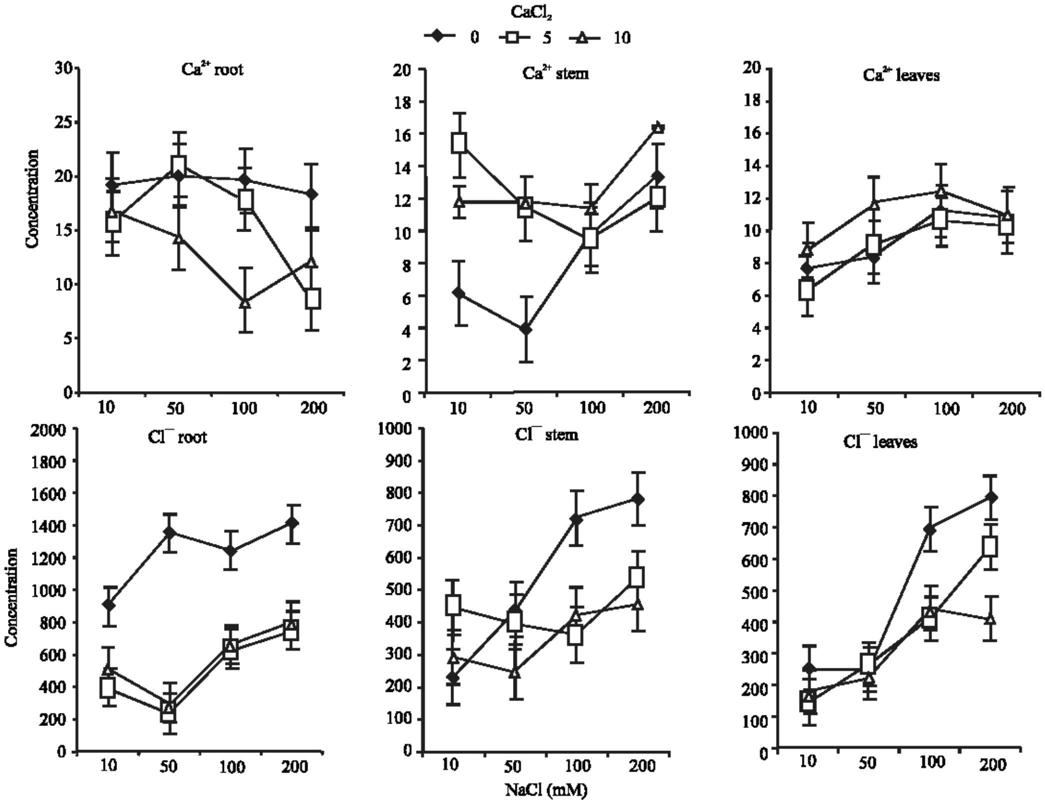

Fig. 3: Effects of Ca^{2+} supplementation (mM) on Ca^{2+} and Cl^{-} concentrations (μM) in root, stem and leaves of alfalfa seedlings grown under saline conditions (Bars = BLSD 0.05)

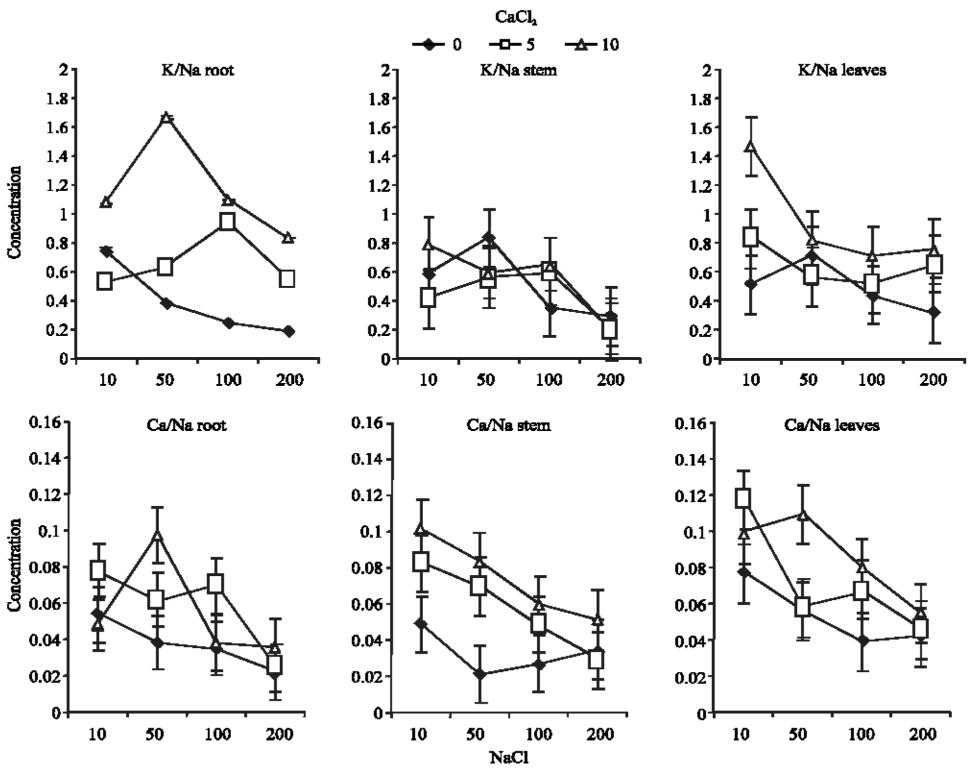

Fig. 4: Effects of Ca^{2+} supplementation (mM) on K/Na and Ca/Na ratio in root, stem and leaves of alfalfa seedlings grown under saline conditions (Bars = BLSD 0.05)

at the lowest two NaCl concentrations. Under Ca^{2+} supplementation, Ca^{2+} concentration was a slightly changed with increasing NaCl concentration in the rooting media.

The concentration of Ca^{2+} in leaves was gradually increased with increasing NaCl concentration. However, the increase was only significant under 50 mM NaCl and 10 mM $CaCl_2$.

Lower Ca^{2+} concentrations in plant tissues with increasing salinity levels have been reported in barley (Lynch and Lauchli 1985; Cramer *et al*., 1990; Lynch *et al*., 1988), Cotton (Cramer *et al*., 1987) and Alfalfa (Al-Khateeb, 2005b), but this was not true in *Spergularia* sp. (Olubukanla *et al*., 1984). Lower Ca^{2+} concentration particularly in roots even with Ca^{2+} supplementation could be attributed to the role of Na^+in reducing adsorption of Ca^{2+} to root cell walls in competition for the same binding sites (Stassart *et al*., 1981). However, the role of Ca^{2+} in plant under saline conditions had been widely reported to be related to both membrane permeability which might reduce Na^+accumulation in plant tissue as well as increasing the uptake and transport of K^+ which was clearly evident in the present study. It is worth mentioning that the slight change in concentration of Ca^{2+} in leaves with Ca^{2+} addition could be attributed to the reduction of Ca^{2+} transport to the shoot particularly under the highest NaCl concentration (Lynch and Lauchli 1985; Wolf *et al*., 1991). However, a mechanism that retained Ca^{2+} in stem is highly operative under these circumstances. Davenport *et al.* (I997) reported that in salt sensitive wheat cultivars, Na^+ significantly inhibited Ca^{2+} translocation, reflecting probably differences in Ca^{2+} transport processes, which may be partially due to greater salt sensitivity of alfalfa seedings.

Cl^- concentration in roots was significantly higher as NaCl concentration increased. Supplementation of Ca^{2+} significantly reduced Cl^- uptake even under the highest NaCl concentrations. Similar trend was also reported in stem, except under the lowest NaCl concentration where Ca^{2+} addition increased Cl^- concentration. Concentration of Cl^- in Stem was much lower than those observed in roots. In leaves, Cl^- concentration was higher under the highest NaCl concentration, while Ca^{2+} supplementation reduced Cl^- concentration in leaf tissues of alfalfa seedlings (Fig. 3).

The higher Cl^- concentration in plant organs with increasing NaCl concentration is normally expected (Esechie and Rodrignez, 1998; Al-Khateeb, 2005b) Olubukanla *et al.* (1984) reported that increasing both salinity and Ca^{2+} concentration had positively affected Cl^- absorption, while this phenomenon was not observed by Ashraf and Oleary (1997). Cl^- seems to have a significant role in decreasing membrane selectivity that may generate a selective ion transport mechanism similar to that reported for $Na^+$and K^+. Moreover, Cl^- translocation from root to shoot seems to be much inhibited with Ca^{2+} supplementation. It is much likely that when external Cl^- concentration are high, the tonoplast rather than plasmalemma becomes a barrier to Cl^- absorption (Cram, 1973) which may explain the higher Cl^- concentration in the present study with increasing Cl^- concentration in the media.

K/Na ratio was significantly decreased as NaCl concentration increased in all plant organs. Increasing Ca^{2+} concentration in the media showed significant increase in K/Na ratio in root and lower increase in leaves. Roots were able to accumulate one fold higher $Na^+$and K^+ concentration, compared to that of stem and leaves (Fig. 4). It was clearly shown that alfalfa seedlings were able to maintain relatively constant K/Na ratio between 0.2 to 1.4 for all plant organs with the lower values in stems. It seems that the ability to maintain higher K/Na ratio particularly in roots are much related to salt tolerance.

Ca/Na ratio decreased significantly with increasing NaCl concentration in all plant organs. Ca^{2+} supplementation significantly increased Ca/Na ratio, particularly under the lowest NaCl concentration (Fig. 4). Alfalfa seedlings maintained higher Ca/Na ratio in all plant organs, but not under 200 mM NaCl salinity. This trend may indicate that alfalfa seedlings can readly tolerate higher NaCl concentration less than 200 mM if sufficient Ca^{2+} concentration is available for plant. K^+ and Ca^{2+} concentrations in alfalfa seedling organs seem to be much related to their K/Na and Ca/Na ratios, respectively, rather than their concentrations in plant organs or media. However Ca^{2+} appeared to play a significant role in mitigating the deleterious effects of salinity (i.e., $Na^+$and Cl^-) probably by controlling Na^+, K^+ and Cl^- absorption through a generating ion selective mechanism.

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