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# Ameliorative effect of $CaCl_2$ on growth, membrane permeability and nutrient uptake in *Oryza sativa* grown at high NaCl salinity

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**ABSTRACT**

A pot culture was carried out with *Oryza sativa L.* vari-Co-39, to investigate the effects of supplementary calcium chloride on plants grown at NaCl (50mM) concentration. Treatments were: (1) Control: nutrient solution alone (C); (2) nutrient solution plus 50mM sodium chloride (NaCl); (3) nutrient solution plus 10mM calcium chloride ($CaCl_2$); (4) nutrient solution plus 15mM calcium chloride ($CaCl_2$); (5) nutrient solution and 50 mM NaCl plus supplementary 10 mM $CaCl_2$ (NaCl + $CaCl_2$); and (6) 50 mM NaCl plus additional mixture of 15 mM $CaCl_2$ in nutrient solution (NaCl + $CaCl_2$). The plants grown under salt stress produced low dry weight and relative water content than those grown in standard nutrient solution and in $CaCl_2$ alone. Supplemental calcium chloride added to nutrient solution containing salt significantly improved growth and relative water content. Membrane permeability increased with high NaCl application and these increases in root membrane permeability were decreased with supplementary Ca. The concentration of chloride (Cl) increases highly for all treatments. Sodium (Na) concentration in plant tissues increased in both shoots and roots at high NaCl treatment. Application of supplementary Ca lowered Na concentration. Concentrations of Ca. K and N were at deficient ranges in the plants grown at high NaCl levels and these deficiencies were corrected by supplementary Ca. The ameliorating effect of Ca on growth and physiological variables could reduce the negative effect of salinity of *Oryza sativa L.*, plants.

***Keywords*:** Ameliorative; nutrient; *Oryza sativa*; permeability

## 1. INTRODUCTION

The problem of salinity is enormous in arid and semi-arid regions of Algeria. Many curative and management practices have been adopted by soil scientists to overcome the salinity problem but most of these methods are highly expensive. One of the possible solutions is development of crop cultivars tolerant to higher concentrations of salinity. The biological approach, to overcome the salinity problem, has received considerable attention in the last few decades (Weber et al., 2007). The deleterious effect of salinity on plant growth area associated with (i) low osmotic potential of the soil solution (water stress), (ii)

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nutritional imbalance, (iii), specific ion effects (salt stress) or (iv), a combination of these factors (Shannon, 1998). All of these cause adverse pleiotropic effects on plant growth and development at physiological and biochemical levels (Parida and Das, 2005) and at the molecular level (Winicov, 1998).

Calcium is an essential plant nutrient and has a role in metabolic activities, like stabilization of membranes, signal transduction through second messenger, and control of enzyme activity in *Cassia angustifolia* (Arshi et al., 2006). $Ca^{2+}$ can help to remediate the adverse effect of salinity on plants. It helps in maintaining membrane integrity and ion-transport regulation and is essential for $K^+/Na^+$ and $Ca^{2+}/Na^+$ selectivity (Maathuis and Amtmann, 1999); in *Cornus stolonifera* (Renault, 2005). Elevated $Ca^{2+}$ concentration in nutrient solution mitigates the adverse effects of NaCl by inhibiting Na+ uptake (Kaya et al., 2002) and reducing membrane leakage (Tuna et al., 2007). $K^+$ concentrations reduced by salinity, can be restored to adequate levels by an additional supply of calcium, as it protects cell membranes from adverse effect of $Na^+$ and minimizes the leakage of cytosolic potassium. Calcium plays a vital role in the regulation of ionic relations in plants and in improving the soil physical conditions (Qadir et al., 2001).

*Oryza sativa* spp. are among the most salt-tolerant higher plants. They have adapted to salinity by tolerating salts internally and/or by excreting salt. However, the presence of high salt levels does not seem to be required for optimal growth. Although the mitigating effect of $Ca^{2+}$ on the adverse NaCl effects has been reported in many plant species (Tuna et al., 2007, Mellgar et al., 2006); in *Cassia angustifolia* (Arshi et al., 2006).

For overcoming the negative impact of high salinity, addition of supplemental $Ca^{2+}$ to the growth medium as an ameliorative agent could be necessary. An experiment was conducted with *Oryza sativa* plants in pot culture conditions to assess the effectiveness of supplemental calcium on mitigating the effect of salinity stress. The aim was to determine if this would correct $Ca^{2+}$ deficiencies in the presence of high NaCl and also to assess effects of supplemental $Ca^{2+}$ on some key growth and physiological parameters (e.g. dry weigh and membrane permeability).

## 2. MATERIALS AND METHODS

### 2. 1. Plant material and growth conditions

Certified seeds of *Oryza sativa* var. co-39 seeds were collected from Tamilnadu Rice Research Institute, Aduthurai. The seeds were surface sterilized for two minutes in 0.2 % Mercuric chloride ($HgCl_2$) solution. The surface sterilized seeds were thoroughly washed with tap water. The sterilized seeds were used in the pots on the garden. The pots were filled with homogenous mixture of garden soil it comprising of red earth, sand and farmyard manure in the ratio of 1:2:1. Each pots contain 10 seeds and they were treated with water. After 7 days, the seedlings were treated with nutrient solution modified: 2.5 mM Ca $(NO_3)_2$, 2.5 mM KCL, 1.0 mM $MgSO_4$ 0.25 mM Ca $(H_2PO_4)_2$, 12.5 µM $H_3BO_3$, 1.0 µM $MnSO_4$, 1.0 µM $ZnSO_4$, 0.25 µM $CuSO_4$. 0.2 µM $(NH_4)_6 M_{o7}O_{24}$ and 10 µM Fe-EDDHA (Feethylenedia-mine-di-o-hydroxphenylacetic acid). After 13 days, plants were treated with control(C); 50 mM NaCl; 10 mM $CaCl_2$; 15 mM $CaCl_2$; 50 mM NaCl + 10 mM $CaCl_2$ and 50 mM NaCl + 15mM $CaCl_2$.

Dry weight, relative water content, cations, chloride and membrane permeability were measured after 30 days of the treatments.

### 2. 2. Dry weight determinations and chemical analysis

Plants were divided into shoots and roots, and dried in an oven at 65 °C for 72 h to determine dry weights and mineral contents. Chemical analyses were carried out on dry weight basis. Total N was determined in samples of 1 g dry weight using the Kjeldahl method. Chloride ion content (Cl) was measured with a DX-100 ion chromatograph. Sodium (Na) and potassium (K) and calcium (Ca) concentration was determined from dry, powdered plant tissue after extraction in HCl using an atomic absorption spectrophotometer (905AA, GBC, Australia).

### 2. 3. Relative water content (RWC).

Leaf relative water content (RWC) was estimated by recording the turgid weight which represents fully hydrated leaf weight by keeping then in water for 4 h, followed by their drying equation was used for determining RWC:

$$RWC\ (\%) = [FW - DW/\ TW - DW] \times 100$$

where TW stands for turgid weight, FW fresh weight and DW dry weight.

### 2. 4. Electrolyte leakage

Electrolyte leakage was used to assess the membrane permeability and thereby on the relative ion content in the apoplastic space. Electrolyte leakage was assessed as described by (Lutts et al., 1996), using nine young leaf discs for each treatment. Samples were washed three time with deionised water to remove surface-adhered electrolytes. Leaf discs were placed in closed vials containing 10 ml of deionised water and incubated at 25 °C on a rotary shaker for 24 h; subsequently electrical conductivity of the solution ($CE_o$) was determined. Samples were then autoclaved at 120 °C for 20min and the final electrical conductivity ($CE_t$) was obtained after equilibration at 25 °C. The electrolyte leakage was defined as follows:

$$\text{Electrolyte leakage } (\%) = [CE_O / CE_t] \times 100$$

### 2. 5. Data analysis

The experiment was set up as a completely randomized design, with five replication of each treatment. Data were analyses statistically, using the SPSS 7.5 software package, by ANOVA.

## 3. RESULTS AND DISCUSSION

The results obtained from this experiment show that salt stress (NaCl) caused a significant reduction in plant dry weight and leaf relative water content (Tables 1 and 2). However, both supplementary calcium chloride increased these parameters compared to plants stressed with NaCl. There was a slight, but not significant, increase in the dry weight and leaf relative water in plants treated with $CaCl_2$ alone compared with the control (Table 1 and 2). At high salinities, growth reduction might be caused by a reduced ability to make osmotic adjustments as a result of saturation of solute uptake system (Munns, 2002). Other factors, such as nutrient deficiencies may also play an important role (Munns, 2005).

**Table 1.** Dry weight (DW) and root: shoot ration of *Oryza sativa* grown in nutrient solution containing high concentration of NaCl (50 mM) with or without application of supplementary Ca.

| Treatment | Total DW (mg/plant) | Shoot DW (mg/plant) | Root DW (mg/plant) | Root: Shoot Ratio |
|---|---|---|---|---|
| C | 232.54ab | 191.33ab | 41.21b | 0.215c |
| 10mM$CaCl_2$ | 248.36a | 201.13ab | 47.23b | 0.234c |
| 15Mm $CaCl_2$ | 278.42a | 225.21a | 53.27b | 0.236c |
| NaCl | 201.11c | 162.19c | 38.92c | 0.239b |
| NaCl+10mM$CaCl_2$ | 220.97b | 182.43b | 43.90b | 0.240a |
| NaCl+15mM$CaCl_2$ | 231.63b | 187.62b | 45.35b | 0.241a |

Different letters in the same column indicate significant difference at $P < 0.01$ lend, C: control, nutrient solution alone; NaCl: 50 mM $CaCl_2$ supplemented in nutrient solution (n = 5).

**Table 2.** Leaf relative water content (RWC) and electrolyte leakage of *Oryza sativa* grown in nutrient solution containing high concentration of NaCl (50 mM) with or without application of supplementary Ca.

| Treatments | Relative water content (%) | Electrolyte leakage (%) |
|---|---|---|
| C | 52.41ab | 9.23c |
| 10mM$CaCl_2$ | 56.12a | 10.32c |
| 15Mm $CaCl_2$ | 59.24a | 11.61c |
| NaCl | 41.76c | 21.89a |
| NaCl+10mM$CaCl_2$ | 46.24b | 13.53b |
| NaCl+15mM$CaCl_2$ | 49.31b | 14.79b |

Different letters in the same column indicate significant difference at $P < 0.01$ lend, C: control, nutrient solution alone; NaCl: 50 mM sodium chloride added to nutrient solution; $CaCl_2$; 10 mM; $CaCl_2$; 15 mM $CaCl_2$ supplemented in nutrient solution (n = 5).

Present study showed that dry weights of shoots and roots were significantly reduced when NaCl is applied alone in comparison to the control. However, the reduction was less when NaCl and $CaCl_2$ were added together (Table 1). The reduction in biomass production that is shown in this paper could be due to the high concentration of $Na^+$ and $Cl^-$ in *Vigna unguiculata* (Murillo-Amador et al., 2006). External application of calcium can ameliorate the growth of salinised plants. This beneficial effect of calcium on the development of many species grown under saline conditions has been reported previously in *Cornus stolonifera* (Renault, 2005); in *Cassia angustifolia* (Arshi et al., 2006); in *Gossypium hirsetum* (Amuthavalli et al., 2012); in *Arachis hypogaea* (Sivasankaramoorthy, 2013); in *Cajanus cajan* (Sivasankaramoorthy, 2013). There was a conductive, but not significantly increase in dry growth in plants treated with $CaCl_2$ alone compared with the control and when NaCl was applied with $CaCl_2$ together.

Membrane permeability was determined by electrolyte leakage. Electrolyte leakage increased in the shoots of *Oryza sativa* plant grown under salt stress (NaCl) compared to the control plants, but supplementary calcium chloride (10 and 15 mM $CaCl_2$) ameliorated this electrolyte leakage (Table 2). However, the values were still higher compared to the control plants and when plants were treated with $CaCl_2$ alone (Table 2).

The cellular membrane dysfunction due to salt stress is well expressed in its increased permeability for ions and electrolytes, which can be readily measured by the efflux of electrolyte *Oryza sativa* (Lutts et al., 1996). Addition of calcium to the saline nutrient solution reduces the permeability of plasma membrane to $Na^+$. The reduction in membrane permeability to $Na^+$ by $Ca^{2+}$ reduces the accumulation of $Na^+$ by passive influx (Melgar, 2006). In the present study, membrane permeability was determined by measuring electrolyte leakage. Salt treatment (50 mM NaCl) induced a significant increase in electrolyte leakage compared to the control treatment (Table 2); however supplementary calcium chloride resulted in a decrease in membrane permeability. Similar results were obtained by Kaya et al., (2002) and Tuna et al., (2007) and who reported that high salt concentration increased the membrane permeability of strawberry cultivars and tomato plants, respectively.

Leaf relative water content generally tends to decline with increasing rhizospheric salinity *Crithmum maritimum* (Ben Amor et al., 2005). This may be due to the possibility that lowered water potential in the roots can trigger a signal from roots to shoot, such as abscisic acid (Sibole et al., 2003). Sodium concentration ($Na^+$) in plant tissues increased in the high NaCl treatment, but it was reduced by supplementary calcium chloride (Table 3). The concentration chloride ($Cl^-$) increased highly for all treatment compared to the control plant, and the greatest increase was obtained when NaCl was applied with $CaCl_2$ together (Table 3).

Concentration of $Ca^{2+}$, $K^+$ and N in the shoots and roots were lower in plants grown at high NaCl than those in unstressed treatment (Table 3). Application of both supplementary calcium corrected $Ca^{2+}$ concentration was obtained when $CaCl_2$ was applied alone.

**Table 3.** Sodium (Na), K, Ca, and N concentration (% dry weight) in shoots and roots of *Oryza sativa* grown in nutrient solution containing high concentration of NaCl (50 mM) with or without application of supplementary Ca.

| Treatments | Na | Ca | K | N | Cl |
|---|---|---|---|---|---|
| **Shoots** | | | | | |
| C | 1.20d | 1.75b | 5.63a | 2.23ab | 0.23c |
| 10mM$CaCl_2$ | 0.97e | 2.50a | 5.70a | 2.47ab | 6.54b |
| 15Mm $CaCl_2$ | 0.49e | 2.98a | 5.84a | 2.29ab | 7.65b |
| NaCl | 11.98a | 0.64c | 3.67b | 1.42c | 6.42b |
| NaCl+10mM$CaCl_2$ | 6.30b | 1.80b | 5.54a | 2.21b | 8.32a |
| NaCl+15mM$CaCl_2$ | 5.43c | 1.95b | 5.20a | 2.46a | 10.75a |
| **Roots** | | | | | |
| C | 0.20c | 0.70bc | 4.53b | 0.29a | 0.13c |
| 10mM$CaCl_2$ | 0.17d | 1.45a | 4.67b | 0.27a | 5.25b |
| 15Mm $CaCl_2$ | 0.14d | 1.58a | 5.14a | 0.20a | 6.36b |
| NaCl | 2.18a | 0.64c | 1.11d | 0.12b | 6.12b |
| NaCl+10mM$CaCl_2$ | 2.13b | 1.80b | 2.15c | 0.18b | 7.23a |
| NaCl+15mM$CaCl_2$ | 1.53b | 1.85b | 2.24c | 0.24ab | 7.5a |

Different letters in the same column indicate significant difference at $P < 0.01$ lend, C: control, nutrient solution alone; NaCl:50 mM sodium chloride added to nutrient solution; $CaCl_2$; 10 mM; $CaCl_2$; 15 mM $CaCl_2$ supplemented in nutrient solution (n = 5).

Ratios of $K^+/Na^+$ and $Ca^{2+}/N^+$ were lower in the salt treatment (NaCl) and compared to the control treatment, but supplementary calcium chloride ameliorate partially these ratios in

both shoots and roots (Table 4). A great increase of $K^+$ /$Na^+$ and $Ca^{2+}$ /$N^+$ ratios was observed when $CaCl_2$ was applied alone (Table 4). Similar results were observed in *Chickpea* (Sivasankaramoorthy, 2013).

**Table 4.** Ca/Na and K/Na ration in shoots and roots of *Oryza sativa* grown in nutrient solution containing high concentration of NaCl (50 mM) with or without application of supplementary Ca.

| Treatments | Shoots | | Roots | |
|---|---|---|---|---|
| | Ca/Na | K/Na | Ca/Na | K/Na |
| | | | | |
| C | 1.45b | 4.69b | 3.05b | 22.65b |
| 10mM$CaCl_2$ | 2.57a | 5.87a | 8.52a | 27.47a |
| 15Mm $CaCl_2$ | 6.08a | 11.91a | 11.28a | 36.71a |
| NaCl | 0.05e | 0.30b | 0.29c | 0.50d |
| NaCl+10mM$CaCl_2$ | 0.28 | 0.87d | 0.84c | 1.00c |
| NaCl+15mM$CaCl_2$ | 0.35c | 0.95c | 1.20c | 1.46c |

Different letters in the same column indicate significant difference at $P < 0.01$ lend, C: control, nutrient solution alone; NaCl:50 mM sodium chloride added to nutrient solution; $CaCl_2$; 10 mM; $CaCl_2$; 15 mM $CaCl_2$ supplemented in nutrient solution (n = 5).

The $K^+$ and $Ca^{2+}$ contents decreased under NaCl stress; but NaCl + $CaCl_2$ treatment reduced the extent of decrease caused by NaCl (Arshi et al., 2010). High $Na^+$ concentration in root zone inhibits uptake and transport of $Ca^{2+}$ and thus subsequently, salt stressed plants have lower $Ca^{2+}$ /$Na^+$ ratios (Ashraf and Akhtar, 2004). Calcium has been show to ameliorate the adverse effects of salinity on plants (White and Broadley, 2003). Calcium is well known to have regulatory roles in metabolism (Epstein, 1998) and sodium ions may compete with calcium ions for membrane binding sites. Therefore, it has been suggested that high calcium levels can protect the cell membrane from the adverse effects of salinity (Rengel, 1992).

Addition of NaCl to growth medium, significantly reduced potassium concentration (Table 3). One of the primary plant responses to salinity is the decrease in $K^+$ concentration in plant tissues in maize (Hua et al., 2008); in *Oryza sativa* (Tanveer er al., 2009), and thus the substitution of $K^+$ by $N^+$ may lead to nutritional imbalances. Both these ions might compete for entry into plant root cells. This competition can have significant negative effects on plant growth, where concentrations of sodium often exceed those of potassium (Tester and Devenport, 2003). This can result in low $K^+$ / $Na^+$ ratios (as shown in Table 4) that reduce plant growth and eventually become toxic (Maathuis and Amtmann, 1999; Schactman and Liu, 1999). In the present study, addition of $Ca^{2+}$ increased $K^+$ / $Na^+$ ratios (Table 4). Similar results were observed in tomato (Levent et al., 2007). The presence of adequate $Ca^{2+}$ in the substrate improves the $K^+$ / $Na^+$ selectivity by shifting the uptake ratio in favour of $K^+$ at the expense of $Na^+$ (Tuna et al., 2007). At all treatments in this experiment, a large percentage of $Cl^-$ was sequestered in the roots and shoots of *Oryza sativa*. Result in *Cornus stolonifera* (Renault, 2005) reported that chloride could have also causes a reduction in growth due to competition with $NO_3^-$ and $PO_4^{3-}$.

Reduced nitrogen content of plants grown under salt stress was reported in a range of crops such as Cucumber and tomato (Cerda and Martinez, 1988), in barley (Shen et al., 1994), *Salvadora persica* (Ramoliya et al., 2004) and *Plantago coronopus* (Koyro, 2006). Inhibition of nitrogen uptake may occur by $NO_3^-$ / $Cl^-$ interaction at the sites for ion transport in *Nicotiana tabacum* (Ruiz et al., 1999) and (Mansour, 2000).

## 4. CONCLUSION

In conclusion, salt stress significantly decreased plant growth and relative water content, but increased membrane permeability and $Na^+$ concentration in *Oryza sativa*. It also reduced $K^+$, $Ca^{2+}$ and N contents in shoots and roots. However, calcium chloride added to salinity nutrient solution significantly improved the variables affected by high salinity (e.g. plant growth and membrane permeability) and also increased $K^+$, $Ca^{2+}$ and N. The addition of calcium chloride offer an economical and simple solution to *Oryza sativa* production problems caused by high salinity.

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