Calcium supplementation ameliorates salinity stress in *Lactuca sativa* plants

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**Abstract**

Salinity represents an increasing threat to agricultural production in every region of the world. The objective of this investigation was to determine ameliorative effects of calcium chloride (CaCl$_2$) on salt stressed lettuce (*Lactuca sativa*) in terms of growth, pigments and biochemical contents. The treatments were divided into 4 sections: control, 80mM NaCl, 80mM NaCl +5mM CaCl$_2$, and 5mM CaCl$_2$ alone. NaCl and CaCl$_2$ stress decreased lettuce plant root and stem length, number of leaves and fresh weight versus the control. NaCl combined with CaCl$_2$ increased these parameters versus treatments with NaCl or CaCl$_2$ alone. Salt stress reduced the shoot and root fresh weight. The roots showed slightly increased growth under salinity, but after the treatment with CaCl$_2$, the plants were normal. The pigment chlorophyll showed a diminishing trend in NaCl stressed plants, but it increased with CaCl$_2$ application. The chlorophyll content increased in all plants with age. There was a slight decrease in carotenoid and anthocyanin contents with NaCl treated plants. CaCl$_2$ also showed decrease in carotenoid and anthocyanin contents, but it was still higher than that of NaCl treated plants. Proline and phenol contents increased in lettuce plants under treatment with NaCl and CaCl$_2$ when compared to the control. From these results, it can be concluded that the addition of CaCl$_2$ to NaCl-stressed lettuce plants have a significant role in partial alleviation of salinity stress. Our results indicated that the cultivation of vegetable plants like lettuce in saline areas would be possible with supplemental calcium application.

**Key words:** Lettuce (*Lactuca sativa*), salinity, morphology, pigments, physiology

**Introduction**

The usage of low quality water for irrigation results in soil salinity which leads major abiotic stress and limit plant growth and productivity in many areas of the world. Worldwide, nearly more than 20% of cultivated land is affected by soil salinity and the amount is increasing continuously (Gupta and Huang, 2014). Salinity is the main environmental stress to plants throughout the world (Munns and Gilliham, 2015). This soil salinity increases the stress factor that accumulates in the plant cells and can alters a wide array of metabolic pathways inside the plant organs (Munns and Tester, 2008). It is well demonstrated that, soil salinity reduce the growth of plants because of ion toxicity due to osmotic stress (Sedghi *et al.*, 2010; James *et al.*, 2011; Gupta and Huang, 2014).

Water shortage and salinity problems are the major limiting factors in the UAE Agriculture since long time. To overcome the scarcity of irrigation water, saline/brackish ground water and desalinated water has been used for irrigating plants and the mismanagement of these resources leads to soil salinization in the agriculture regions (Abdelfattah and Shahid, 2014). Poor irrigation water is increasingly threatening agriculture in humid regions.

Calcium is required as a essential plant nutrient for various structural roles in the cell wall and membranes of plants and in coordinating responses to numerous environmental challenges (Duca, 2015). Campo *et al.* (2014) explained the overexpression of a calcium-dependent protein kinase during salt and drought tolerance in rice. There are reports that calcium can alleviates the adverse of salinity in some crop plants like peas, wheat, sunflower, tomato (Bonilla *et al.*, 2004; Daowei and Moxin, 2010). Zehra *et al.* (2012) reported the role of calcium in alleviating effect of salinity on germination of *Phragmites karka* seeds. Lettuce (*Lactuca sativa*) belongs to the family Asteraceae and is an annual plant. Being an important leafy vegetable, it is cultivated in considerable scale in the United Arab Emirates (Al Muhairi *et al.*, 2015). There are lots of research concerning the effects of NaCl on lettuce plants (Tesi *et al.*, 2003; Eraslan *et al.*, 2007). Also salt stress mitigation through methods like silicon application (Milne *et al.*, 2012) and arbuscular mycorrhizal symbiosis (Aroca *et al.*, 2013) have been reported. In our previous studies we reported methods of water conservation (Salem *et al.*, 2010) and deficit irrigation treatments (Al Muhairi *et al.*, 2015) in lettuce cultivation, relatively little is known about the ameliorative effects of calcium chloride on the salinity stress in lettuce plants. The objective of this study was to determine calcium chloride effects on salt stressed lettuce in terms of growth, pigments and biochemical contents.

**Materials and methods**

The lettuce seeds were locally purchased. The experimental part of this work was carried out in Al-Foah Experimental Station of College of Food and Agriculture, UAEU. The methodologies adopted are described below.

**Cultivation methods:** The plants were raised in Al-Foah Experimental Station of College of Food and Agriculture, UAEU. In order to get maximum germination, the seeds were sown separately in raised seedbeds by broadcasting method and covered with fine soil. The nursery beds were irrigated twice a day and weeded regularly in order to ensure healthy growth of the seedlings until transplantation.
Treatments and samplings: The treatments were divided into 4 sections. They were, control, 80mM NaCl, 80mM NaCl + 5mM CaCl₂ and 5mM CaCl₂. Ten pots were used for each treatment. Before transplating, the pots were irrigated with the respective treatment solutions and the electrical conductivity (EC) of the soil mixture was measured. Control plants were irrigated with well water. Three plants were planted per pot and the pots were watered to the field capacity with deionized water up to 90 days after planting (DAP), and every care was taken to avoid leaching. The initial EC level of the soil was maintained by flushing each pot with the required volume of corresponding treatment solution on 45, 60 and 75 DAP. The position of each pot was randomized at four-day intervals to minimize spatial effects in the greenhouse. The seedlings were thinned to one per pot on 20 DAP. Plants were uprooted randomly on 90 DAP and used for determining growth, pigment composition and biochemical constituents.

Morphological parameters: The plant height was measured from the soil level to the tip of the shoot and expressed in cm. The plant root length was measured from the point of first cotyledonary node to the tip of longest root and expressed in cm. The number of fully developed leaves were counted and expressed as number of leaves per plant. After washing the plants in the tap water, fresh weight was determined by using an electronic balance and the values were expressed in grams. Plants were dried at 60°C in hot air oven for 24 hours. After drying, the weight was measured and the values were expressed in g.

Chlorophyll, carotenoid and anthocyanin contents: Chlorophyll and carotenoid were extracted from the leaves and estimated by the method of Arnon (1949). Carotenoid content was estimated using the formula of Kirk and Allen (1965) and expressed in milligrams per gram fresh weight. Anthocyanin was extracted and estimated from the flowers by the method of Beggs and Wellmann (1985).

Proline and total phenols: Proline content was estimated following the method of Bates et al. (1973). Total phenol was estimated by the method of Malick and Singh (1980).

Results and discussion

NaCl and CaCl₂ stress decreased lettuce plant root and stem length versus the control. NaCl combined with CaCl₂ increased root and stem length versus treatments with NaCl or CaCl₂ alone. The number of leaves decreased with NaCl treatment when compared to control. The calcium supplementation increased the number of leaves, but still it was less when compared with untreated control plants (Table 1). Salt stress reduced the shoot and root fresh weight. The reduction in shoot growth was 40 per cent over control on 90 DAP in NaCl stressed plants. But the application of CaCl₂ to the stressed plants reduced the stress effects and increased the shoot fresh weights. The roots showed slightly increased growth under salinity, but after the treatment with CaCl₂, the plants were normal (Table 1). Salinity can inhibit root growth by altering the external water potential, increasing ion toxicity, or causing an ion imbalance as shown under alkaline salts stress, where, the growth of G. gracilis seedlings was more intensely inhibited (Shi et al., 2015). Taifouo et al. (2015) reported that CaCl₂ treatment significantly increase the growth parameters in V. ungiculata plants.

The pigment chlorophyll showed a diminishing trend in NaCl stressed plants, but it increased with CaCl₂ application. The chlorophyll content increased in all plants with age (Table 2). The NaCl with CaCl₂ increased the chlorophyll content when compared to NaCl stressed plants. There was a slight decrease in carotenoid contents with NaCl treated plants. CaCl₂ also showed decrease in carotenoid contents, but it was still higher than that of NaCl treated plants. The combinations of NaCl and CaCl₂ showed a partial recovery in terms of increased carotenoid contents. The anthocyanin content of the leaves of lettuce plants decreased with the NaCl treatments, but significantly increased with the CaCl₂ treatments (Table 2). Even though it was less than control plants, the amount was higher when compared to NaCl treated plants.

Our results are supported by earlier findings on Brassica juncea by Yousuf et al. (2015) and Vigna radiata by Sharma and Dhanda (2015). Photosynthetic pigments increased when the salt stressed cowpea plants were treated with calcium chloride (Mohamed and Basalah, 2015). The chlorophyll reduction under salinity has been attributed to the destruction of the pigments and the instability of the pigment protein complex (Levitt, 1980).

Proline content increased in lettuce plants under treatment with NaCl and CaCl₂ when compared to the control. Phenol content slightly increased in lettuce plants under treatment with NaCl and CaCl₂ when compared to the control (Table 3). Addition of CaCl₂ together with NaCl increased the proline content further, to confer stress protection. Our results are in accordance with the findings of

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>T1 [80mM NaCl]</th>
<th>T2 [80mM NaCl + 5mM CaCl₂]</th>
<th>T3 [5mM CaCl₂]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root length</td>
<td>25±1.2</td>
<td>20±1.8</td>
<td>22±1.6</td>
<td>24±1.2</td>
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<tr>
<td>Shoot length</td>
<td>30±0.9</td>
<td>19±1.4</td>
<td>24±1.2</td>
<td>25±1.4</td>
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<tr>
<td>Number of leaves</td>
<td>19±0.8</td>
<td>14±0.7</td>
<td>16±0.9</td>
<td>17±0.8</td>
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<tr>
<td>Shoot fresh weight</td>
<td>64.12±3.4</td>
<td>55.24±3.6</td>
<td>57.5±1.9</td>
<td>60.3±3.9</td>
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<td>Root fresh weight</td>
<td>6.01±0.5</td>
<td>3.2±0.2</td>
<td>4.02±0.1</td>
<td>5.04±0.2</td>
</tr>
<tr>
<td>Shoot dry weight</td>
<td>5.1±0.3</td>
<td>3.2±0.2</td>
<td>4.02±0.1</td>
<td>4.5±0.3</td>
</tr>
<tr>
<td>Root dry weight</td>
<td>0.45±0.1</td>
<td>0.32±0.01</td>
<td>0.4±0.03</td>
<td>0.43±0.02</td>
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</table>

Data shows results from 90 DAP; values are means ± SD of 3 replicates

<table>
<thead>
<tr>
<th>Pigments</th>
<th>Control</th>
<th>T1 [80mM NaCl]</th>
<th>T2 [80mM NaCl + 5mM CaCl₂]</th>
<th>T3 [5mM CaCl₂]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll ‘a’</td>
<td>3.43±0.20</td>
<td>3.12±0.12</td>
<td>3.13±0.2</td>
<td>4.3±0.3</td>
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<tr>
<td>Chlorophyll ‘b’</td>
<td>1.21±0.08</td>
<td>1.6±0.05</td>
<td>1.23±0.01</td>
<td>1.48±0.11</td>
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<tr>
<td>Total Chlorophyll</td>
<td>4.65±0.40</td>
<td>4.18±0.3</td>
<td>4.36±0.3</td>
<td>5.78±0.2</td>
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<tr>
<td>Carotenoid</td>
<td>0.581±0.03</td>
<td>0.514±0.01</td>
<td>0.541±0.04</td>
<td>0.697±0.04</td>
</tr>
<tr>
<td>Anthocyanin</td>
<td>1.158±0.09</td>
<td>1.916±0.08</td>
<td>1.1±0.01</td>
<td>1.2±0.07</td>
</tr>
</tbody>
</table>

Data shows results from 90 DAP; values are means ± SD of 3 replicates
Table 3. Proline and phenol contents of lettuce under treatment with NaCl, CaCl₂, and their combination

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>T1 [80mM NaCl]</th>
<th>T2 [80mM NaCl]</th>
<th>T3 [5mM CaCl₂]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proline</td>
<td>0.028±0.001</td>
<td>0.053±0.004</td>
<td>0.041±0.002</td>
<td>0.049±0.002</td>
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<tr>
<td>Phenol</td>
<td>0.431±0.03</td>
<td>0.431±0.003</td>
<td>0.339±0.03</td>
<td>0.653±0.05</td>
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</tbody>
</table>

Data shows results from 90 DAP; values are means ± SD of 3 replicates.

References


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