Journal of Applied Horticulture, 18(2): 138-140, 2016

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 lattuce 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 $CaCl_2$ and 5mM $CaCl_2$. 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 $CaCl_2$ stress decreased lettuce plant root and stem length versus the control. NaCl combined with $CaCl_2$ increased root and stem length versus treatments with NaCl or $CaCl_2$ 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 $CaCl_2$ 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 $CaCl_2$ 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 alkali salts stress, where, the growth of *G. gracilis* seedlings was more intensely inhibited (Shi *et al.,* 2015). Taffouo *et al.* (2015) reported that $CaCl_2$ treatment significantly increase the growth parameters in *V. unguiculata* plants.

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 (Table 2). The NaCl with $CaCl_2$ increased the chlorophyll content when compared to NaCl stressed plants. There was a slight decrease in carotenoid contents with NaCl treated plants. $CaCl_2$ also showed decrease in carotenoid contents, but it was still higher than that of NaCl treated plants. The combinations of NaCl and $CaCl_2$ 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 $CaCl_2$ 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 $CaCl_2$ when compared to the control. Phenol content slightly increased in lettuce plants under treatment with NaCl and $CaCl_2$ when compared to the control (Table 3). Addition of $CaCl_2$ together with NaCl increased the proline content further, to confer stress protection. Our results are in accordance with the findings of

Table 1. Morphological parameters of lettuce under treatment with NaCl, $CaCl_2$ and their combination

Parameters	Control	T1 [80mM NaCl]	T2 [80mM NaCl +5mM $CaCl_2$]	T3 [5mM $CaCl_2$]
Root length	25±1.2	20±1.8	22±1.6	24±1.2
Shoot length	30±0.9	19±1.4	24±1.2	25±1.4
Number of leaves	19±0.8	14±0.7	16±0.9	17±0.8
Shoot fresh weight	64.12±3.4	55.24±3.6	57.5±1.9	60.3±3.9
Root fresh weight	6.01±0.5	3.2±0.2	4.02±0.1	5.04±0.2
Shoot dry weight	5.1±0.3	3.2±0.2	4.02±0.1	4.5±0.3
Root dry weight	0.45±0.1	0.32±0.01	0.4±0.03	0.43±0.02

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

Table 2. Photosynthetic pigment contents of lettuce under treatment with NaCl, $CaCl_2$ and their combination

Pigments	Control	T1 [80mM NaCl]	T2 [80mM NaCl +5mM $CaCl_2$]	T3 [5mM $CaCl_2$]
Chlorophyll 'a'	3.43±0.20	3.12±0.12	3.13±0.2	4.3±0.3
Chlorophyll 'b'	1.21±0.08	1.6±0.05	1.23±0.01	1.48±0.11
Total Chlorophyll	4.65±0.40	4.18±0.3	4.36±0.3	5.78±0.2
Carotenoid	0.581±0.03	0.514±0.01	0.541±0.04	0.697±0.04
Anthocyanin	1.158±0.09	1.916±0.08	1.1±0.01	1.2±0.07

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, $CaCl_2$ and their combination

Parameters	Control	T1 [80mM NaCl]	T2 [80mM NaCl +5mM $CaCl_2$]	T3 [5mM $CaCl_2$]
Proline	0.028±0.001	0.053±0.004	0.041±0.002	0.049±0.002
Phenol	0.431±0.03	0.431±0.003	0.339±0.03	0.653±0.05

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

Cheng *et al.* (2013), where they reported calcium-induced proline accumulation can enhance amelioration of NaCl injury in greater duckweed. Salinity treatment increased the proline contents of all parts of the plants to a larger extent as observed as a result of NaCl-treated soybean (Wei *et al.,* 2015) and sorghum (Kaneko *et al.*, 2015). Treatment with calcium on NaCl-stressed plants markedly reduced the accumulation of proline content when compared with NaCl-stressed plants. However, NaCl-stressed plants had a higher content of proline than the control.

From these results, it can be concluded that the cultivation of vegetable plants like lettuce in saline areas would be possible with supplemental calcium application. To ascertain this conclusion field experiments and further investigations are required.

References

Abdelfattah, M.A. and S.A. Shahid, 2014. Spatial distribution of soil salinity and management aspects in the Northern United Arab Emirates. In: *Sabkha Ecosystems: Volume IV: Cash Crop Halophyte and Biodiversity Conservation.* Springer, Netherlands. pp. 1-22.

Al Muhairi, M.A., A.J. Cheruth, S.S. Kurup, G.A. Rabert and M.S. Al-Yafei, 2015. Effect of abscisic acid on biochemical constituents, enzymatic and non enzymatic antioxidant status of lettuce (*Lactuca sativa* L.) under varied irrigation regimes. *Cogent Food Agri*., 1(1): 1080-1088.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts, ployphenol oxidase in *Beta vulgaris* L. *Plant Physiol.,* 24: 1-15.

Aroca, R., M.R.L. Juan, M.Z. Ángel, A.P. José, M.G.M. José, J.P María and A.L.R. Juan, 2013. Arbuscularmycorrhizal symbiosis influences strigolactone production under salinity and alleviates salt stress in lettuce plants. *J. Plant Physiol*., 170(1): 47-55.

Bates L.S., R.P. Waldern and I.D. Teare, 1973. Rapid determination of free proline for water stress studies. *Plant Soil.,* 39: 205-207.

Beggs, C.J. and E. Wellmann, 1985. Analysis of light controlled anthocyanin synthesis in coleoptiles of *Zea mays* L.: The role of UV-B blue red and far red light. *Photochem. Photobiol*., 41: 401-406.

Bonilla, I., A. El-Hamdaoui and L. Bolanos, 2004. Boron and calcium increase *Pisum sativum* seed germination and seedling development under salt stress. *Plant and Soil*., 267: 97-107.

Campo, S., P. Baldrich, J. Messeguer, E. Lalanne, M. Coca and B. San Segundo, 2014. Overexpression of a calcium-dependent protein kinase confers salt and drought tolerance in rice by preventing membrane lipid peroxidation. *Plant physiol.,* 165(2): 688-704.

Cheng, T.S., M.J. Hung, Y.I. Cheng and L.J. Cheng, 2013. Calcium-induced proline accumulation contributes to amelioration of NaCl injury and expression of glutamine synthetase in greater duckweed (*Spirodela polyrhiza* L.). *Aquatic Toxicol.*, 144: 265-274.

Daowei, Z. and X. Moxin, 2010. Specific ion effects on the seed germination of sunflower. *J. Plant Nutr.,* 33: 255-266.

Duca, M. 2015. Mineral nutrition of plants. In: *Plant Physiology,* Springer International Publishing. pp. 149-185.

Eraslan, F., A. Inal, O. Savasturk and A. Gunes, 2007. Changes in antioxidative system and membrane damage of lettuce in response to salinity and boron toxicity. *Sci. Hort.*, 114: 5-10.

Gupta, B. and B. Huang, 2014. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *Int. J. Genom.,* Article ID 701596, 18 pages, doi:10.1155/2014/701596.

James, R.A., C. Blake, C.S. Byrt and R. Munns, 2011. Major genes for Na+ exclusion, Nax1 and Nax2 (wheat HKT1;4 and HKT1;5), decrease Na+ accumulation in bread wheat leaves under saline and waterlogged conditions. *J. Exp. Bot*., 62(8): 2939-2947.

Kaneko, T., T. Horie, Y. Nakahara, N. Tsuji, M. Shibasaka and M. Katsuhara, 2015. Dynamic regulation of the root hydraulic conductivity of barley plants in response to salinity/osmotic stress. *Plant and Cell Physiol.,* 56(5): 875-882.

Kirk J.T.O. and R.L. Allen, 1965. Dependence of chloroplast pigment synthesis on protein synthesis: Effect of acsidione. *Biochem. Biophys. Res. Commun*., 21: 530-532.

Levitt, J. 1980. *Responses of Plants to Environmental Stresses*. Vol. 2, Academic Press, New York.

Malick, C.P. and M.B. Singh, 1980. *Plant Enzymology and Histo Enzymology*. Kalyani Publishers, New Delhi, p. 286.

Milne, C.J., C.P. Laubscher, P.A. Ndakidemi, J. L. Marnewick and F. Rautenbach, 2012. Salinity induced changes in oxidative stress and antioxidant status as affected by applications of silicon in lettuce (*Lactuca sativa*). *Int. J. Agric. Biol.,* 14: 763-768

Mohamed, A.K. and M.O. Basalah, 2015. The active role of calcium chloride on growth and photosynthetic pigments of cowpea *Vigna unguiculata* L. (Walp) under salinity stress conditions. *American-Eurasian J. Agric. Environ. Sci.,* 15(10): 2011-2020.

Munns, R. and M. Tester, 2008. Mechanisms of salinity tolerance. *Ann. Rev. Plant Biol*., 59: 651-681.

Munns, R. and M. Gilliham, 2015. Salinity tolerance of crops–what is the cost?. *New Phytol.*, 208(3): 668-673.

Salem, M.A., W. Al-Zayadneh and A.J. Cheruth, 2010. Water conservation and management with hydrophobic encapsulation of sand. *Water Res. Manage.,* 24(10): 2237-2246.

Sedghi, M., A. Nemati and B. Esmaielpour, 2010. Effect of seed priming on germination and seedling growth of two medicinal plants under salinity. *Emir. J. Food Agric.,* 22(2): 130-139.

Sharma, A. and S. Dhanda, 2015. Application of calcium chloride to mitigate salt stress in *Vigna radiata* L. cultivars. *Int. J. Curr. Microbiol. App. Sci*, 4(2): 764-769.

Shi, L., S. Ma, Y. Fang and J. Xu, 2015. Crucial variations in growth and ion homeostasis of *Glycine gracilis* seedlings under two types of salt stresses. *J. Soil Science Plant Nutr.*, (AHEAD), 0-0. http://dx.doi.org/10.4067/S0718-95162015005000070.

Taffouo, V.D., S.E. Sondi, L.T. Meguekam, A.E. Nouck, O.F. Wamba and E. Youmbi, 2015. Changes in growth and nutrient uptake in response to foliar application of sodium and calcium chloride in cowpea cultivars (*Vigna unguiculata* L. Walp). *African J. Biotechnol.*, 13(47): 4382-4389

Tesi, R., A. Lenzi and P. Lombardi, 2003. Effect of salinity and oxygen level on lettuce grown in a floating system. *Acta Hort.*, 609: 383-387.

Wei, P., D. Chen, R. Jing, C. Zhao and B. Yu, 2015. Ameliorative effects of foliar methanol spraying on salt injury to soybean seedlings differing in salt tolerance. *Plant Growth Regul.*, 75(1): 133-141.

Yousuf, P.Y., A. Ahmad, A.H.G. Hemant, I.M. Aref and M. Iqbal, 2015. Potassium and calcium application ameliorates growth and oxidative homeostasis in salt-stressed Indian mustard (*Brassica juncea*) plants. *Pak. J. Bot.*, 47(5): 1629-1639.

Zehra, A., B. Gul, R. Ansari and M.A. Khan, 2012. Role of calcium in alleviating effect of salinity on germination of *Phragmites karka* seeds. *South African J. Bot*., 78: 122-128.

Received: May, 2015 ; Revised: August, 2015 ; Accepted: August, 2015