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Effect of Calcium Nutrition on Reducing the Effects of Salinity on Tomato Plant

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ABSTRACT
Through the positive and negative impacts of calcium chloride on the dry weight of shoot and root and growth conditions of plant to reduce the destructive effects of salinity stress, research on tomato was followed. By maintaining the proper amount of calcium ions in the soil, toxicity of sodium ions is controlled. In this study the effects of three different nutrient solutions, including the sodium chloride concentration with 0, 30, 60 and 90 mmol, calcium chloride concentrations with 0, 10, 20, 30 mmol and sodium chloride+calcium chloride respectively to the same concentration were reviewed. This test investigated on two cultivated tomato varieties in a hydroponic form and pots containing Coco Peat. In the vegetative growth stage, relative water content of tissue and cytoplasmic membrane stability and leaf chlorophyll concentration after removal of the root and shoot dry weight were measured. Results showed that increase of salinity caused a significant reduction in relative water content of tissues, cytoplasmic membrane stability and chlorophyll concentration in leaves. Dry weight yield of roots and shoots also decreased with increasing the salinity so that all the characters were lowest in 90 mmol of sodium chloride concentration. The results showed that the growth terms of calcium chloride and chloride+calcium chloride solutions were better, as the 10 mmol concentration of calcium chloride has a significant impact on improving the damage caused by the salinity.

Key words: Salinity stress, tomato, sodium chloride, calcium chloride

INTRODUCTION
The water and soil salinity is one of the important preventives in development of agriculture in most places of the world such that after dryness, the salinity is the important and most common environmental stress across the world such as Iran. According to the studies which have been done, the extent of saline lands in Iran is about 25,000,000 ha that about 65 ha of it includes the agricultural lands (Movahhedy-Dehnavy *et al.*, 2009). Irregular use of chemical fertilizers, inaccurate management of culture and irrigation caused to increase the development of saline lands.

Selection of salt tolerant plants, breeding varieties for increasing the salt tolerance, proper nutrition management in saline conditions are actions that are considered for increasing the efficiency of plant physiology in plant salt stress conditions (Iqbal *et al.*, 2006). On the other hand, the proper management of the planting the product includes bed-making, the positioning of seeds in the soil, planting systems, irrigation and leaching management and other aspects of crop management that is necessary in reducing the effects of salinity on crop production and minimizing the stress intensity in plant to achieve economic yield and sustainable production in saline circumstances. Sholi (2012) showed that in saline soils, use of high amounts of Ca^{2+} eads to increase the plant tolerance to salinity and its yield. Alizade (2005) considers the salinity stress in all stages of plant growth. Of course, it is likely that the plant sensitivity to be different in one stage of growth with other one.

Different studies showed that use of Ca caused to decrease the negative effects of salinity in plants. Grattan and Grieve (1994) stated that increasing Ca level of plants will protect them from sodium chloride toxicity. Girija *et al.* (2002) indicated that in the internal environment of cell, Calcium act as a secondary messenger and by affecting the stability and enzyme activities can ease the stress.

Accessing to an optimum concentration of Calcium ion to increase the plant resistance is very important. Calcium is one of the elements which has the protective effects on the plants root and causes the plant to be survived in the high saline conditions. Jaleel *et al.* (2007) reported that tomato growth in saline conditions leads to change the combination and concentration of plant shoot elements and these terms, the sodium and chlorine concentration to be highly increased and Potassium, Calcium, Magnesium and Nitrate to be decreased.

According to the model of Maas and Hoffman (1977), salt tolerance threshold of tomato was between 2 to 2.5 dS m^{-1} and decrease of yield was between 9 to 10% per each unit of salinity increase above the salinity threshold. Mirabdulbaghi and Pishbeen (2012) stated that the amount of yield decrease in crops by the effect of salinity is variable according to the species. These researchers also indicated that yield decrease percentage of tomato in 2.5, 3.5 and 7.6 dS m^{-1} is respectively 0, 10 and 50.

In this study, the effect of calcium ion in membrane stability and cell wall of tomato to the salinity stress, investigating the positive and negative impacts of calcium chloride on the dry weight of shoot and root and growth conditions of plant and changes in concentration of sodium chloride via the use of calcium chloride to decrease the destructive effects of salinity stress will be addressed.

MATERIALS AND METHODS

This study was conducted in a period of 70 days and in a cultivation without soil using the factorial experiment in a the basic randomize complete block design with three solutions (sodium chloride, calcium chloride and sodium chloride+calcium chloride), four concentrations (0, 30, 60 and 90 mmol for sodium chloride and 0, 10, 20 and 30 mmol for calcium chloride), two tomato varieties (Erli flat and American Moral) and three replications in Isfahan 2011.

This experiment was conducted in two parts. In the first part, three solutions of salinity (sodium chloride, calcium chloride and calcium chloride+sodium chloride) with different concentrations (Table 1) were used in the plant growth stage and yield of root dry weight, shoots, relative water content of tissues, membrane stability and chlorophyll concentration of leaves growth until the end of growth period was measured.

Table 1: Different levels of calcium chloride and sodium chloride in a nutritional solution

Treatment	S1	S2	S3	S3	S3
Salt concentration	$NaCl_{30}$	$CaCl_{2,10}$	$NaCl_{30}+CaCl_{2,10}$	$NaCl_{60}+CaCl_{2,10}$	$NaCl_{90}+CaCl_{2,10}$
	$NaCl_{60}$	$CaCl_{2,20}$	$NaCl_{30}+CaCl_{2,20}$	$NaCl_{60}+CaCl_{2,20}$	$NaCl_{90}+CaCl_{2,20}$
	$NaCl_{90}$	$CaCl_{2,30}$	$NaCl_{30}+CaCl_{2,30}$	$NaCl_{60}+CaCl_{2,30}$	$NaCl_{90}+CaCl_{2,30}$

S_1: Indicating the sodium chloride and S_2: Indicating the calcium chloride and S_3: Indicating the calcium chloride+sodium chloride

In the second part, was sampled using the dry matter of the shoots in the vases which were fed from 0, 30, 60 and 90 mmol of NaCl solutions and $NaCl_{30}$+$CaCl_2$,10 '$NaCl_{60}$+$CaCl_2$, 20, $NaCl_{90}$+$CaCl_2$, 30 solutions. The value of the mineral elements such as chlorine, sodium and calcium available in the samples was measured via the atomic absorption device.

Index of each solution indicates the amount of salinity in terms of mmol. In different treatments, the different levels of sodium and calcium chloride were added to the basic nutritional solution (Johnson standard).

Determination of the relative content of water texture: In order to measure the relative content of water, was used of the following equation. In this regard, Mf was the wet tissue weight of terminal leaflets in each bush (g) and Mt was the swollen tissue weight of leaflets (g) after being placed 20 h in the distilled water and temperature of 5°C in the fridge and Md was the dry tissue weight of plant (g) after 24 h being placed at the temperatures of 70°C in the oven:

$$\text{RWC (\%)} = \frac{\text{Mf-Md}}{\text{Mt-Md}} \times 100$$

Identifying the stability of the cytoplasm membrane: In order to measure the stability of the cytoplasmic membrane, 10 disk in the diameter of 7 mm in a circle form was prepared from the sample leaf and placed inside the glasses with cap containing 10 cc distilled water for 20 h at a temperature of 5°C. At last, the stability of the cytoplasmic membrane by the electrical conductivity was measured (Eman and Ryad, 2013).

Identifying the chlorophyll concentration: To measure the tissue chlorophyll content was used of SPAD device. In the end of growth period, the root and shoots were separated and then placed for 48 h at 70°C in the oven and at last the dry weight of them was determined.

RESULTS AND DISCUSSION

Yield of shoots dry weight: Shoot dry weight in sodium chloride solution was lower compared with the solutions of calcium chloride and calcium chloride+sodium chloride. So that the shoot dry weight (30 g) was the lowest amount of itself in the concentration of 90 mmol sodium chloride (Table 2). Cuartero and Fernandez-Munoz (1998) have reported the reduction of leaf and stem growth in different cultivars of tomato under the salinity stress. Of course, the rate of stem growth reduction compared to the leaf was less. By increasing the salt concentration in each of three solutions, the dry weight of shoot showed a significant reduction. In this condition dry weight of it in Erli flat cultivar was more in high concentration of salinity (Table 2). Therefore, we can see that Erli flat cultivar by having more dry weight than American Moral cultivar showed more consistency to the salinity stress. Also the results indicated that the maximum dry weight of shoot (85 g) was observed in the calcium chloride concentration of 10 mmol. So, this result indicates that the existence of calcium in the nutritional solution causes that to reduce the salinity stress intensity. Grattan and Grieve (1994) stated that increasing Ca level of plants will protect them from sodium chloride toxicity.

Yield of root dry weight: The yield of root dry weight was different in a variety of cultivars such that root dry weight in the cultivar of Erli flat in comparison with American Moral was more in the

Table 2: Effect of salinity different concentrations on the dry root weight, shoot dry weight, relative water content, membrane stability and leaf chlorophyll in different cultivars of tomato

Treatment*		American moral cultivar				Erli flat cultivar			
		Calcium chloride (mmol)				Calcium chloride (mmol)			
Characteristics	Sodium chloride (mmol)	0	10	20	30	0	10	20	30
Shoot dry weight	0	70^{bc}	85^{a}	50^{a}	42^{a}	65^{b}	80^{a}	42^{e}	36^{ef}
(gr)	30	66^{b}	82^{a}	41^{a}	35^{f}	62^{b}	75^{ab}	35^{a}	20^{g}
	60	43^{a}	45^{e}	25^{g}	20^{gh}	30^{f}	40^{e}	36^{a}	30^{f}
	90	25^{g}	30^{fg}	22^{gh}	20^{gh}	27^{fg}	35^{a}	30^{f}	29^{f}
Dry root weight	0	30^{b}	40^{a}	25^{bc}	10^{d}	30^{bc}	45^{a}	28^{bc}	12de
(gr)	30	18^{c}	30^{b}	22^{bc}	12^{d}	30^{bc}	40^{ab}	25^{c}	17^{d}
	60	13^{d}	20^{c}	15^{cd}	10^{d}	17^{d}	35^{b}	15^{d}	10de
	90	4^{e}	15^{cd}	10^{d}	13^{d}	10^{a}	19^{cd}	13^{d}	10de
Relative water	0	85^{ab}	85^{ab}	88^{a}	91^{a}	85a^{b}	86^{ab}	91^{a}	92^{a}
content (%)	30	78^{b}	80^{b}	75^{bc}	70^{c}	82^{b}	83^{b}	80^{b}	77^{bc}
	60	75^{bc}	76^{bc}	76^{bc}	69^{c}	81^{b}	84^{ab}	77^{bc}	80^{b}
	90	67^{cd}	70^{c}	72^{c}	73^{c}	68^{c}	75^{bc}	73^{c}	74^{bc}
Membrane	0	450^{e}	366^{g}	416^{f}	486^{d}	450^{g}	486^{f}	683^{bc}	627^{cd}
stability (ds cm^{-1})	30	495^{d}	371^{fg}	400^{f}	495^{d}	564^{e}	505^{f}	585^{d}	630^{cd}
	60	609^{b}	500^{d}	404^{f}	520^{cd}	654^{c}	630^{cd}	620^{cd}	670^{bc}
	90	652^{a}	550^{c}	580^{bc}	585^{bc}	744^{a}	650^{c}	670^{bc}	680^{bc}
Leaf chlorophyll	0	55^{a}	55^{a}	48^{b}	43^{c}	50^{a}	52^{a}	47^{b}	38^{c}
(%)	30	45^{c}	47^{c}	42^{d}	40^{d}	46^{b}	48^{ab}	45^{b}	40^{c}
	60	40^{d}	42^{d}	44^{c}	39^{d}	40^{c}	42^{c}	44^{b}	39^{c}
	90	30^{f}	35^{e}	38^{d}	42^{d}	36^{d}	40^{c}	42^{c}	30^{e}

*Means followed by same letter do not differ statistically at $p = 0.05$ according the Duncan's multiple range test

different concentrations of sodium chloride and calcium chloride (Table 2). The different concentrations of sodium chloride affected the root dry weight more than the calcium chloride and calcium chloride+sodium chloride and showed more reduction too. The high tolerance to the salinity was calculated between 4 to 6 dS m^{-1} regarding the reduction of root biomass (Yang *et al.*, 2003).

In this experiment, the concentration of 90 mmol sodium chloride has the salinity of 10 dS. The root dry weight in all levels of sodium chloride that was mixed with the concentration of 10 mmol calcium chloride was more in comparison with other concentration and calcium chloride caused the salinity of sodium chloride to be adjusted. Calcium is one of the elements that if has a desired concentration will show protective effects on the roots and shoot of the plants from itself and causes the plant to be survived in the saline conditions (Caines and Shennan, 1999).

Water relative content: Water relative content of tissue in Erli flat cultivar in all the solution densities containing the calcium chloride was more in comparison with other solutions, while the water relative content of tissue in American Moral cultivar were least in solutions containing the sodium chloride (Table 2). The Erli flat cultivar by having the maximum relative water content showed more consistency to the salinity stress. The minimum relative water content was observed in the nutritional solutions containing calcium chloride. Increasing the salt concentration in the cells of plant caused the water potential of leaf and relative water content of tissue to be reduced and there is a relationship between the reduction of tissue relative water content and increase of the carbohydrate concentration (Caines and Shennan, 1999).

By increasing the concentration of calcium chloride, the relative water content was increased. Such that in 0 mmol of sodium chloride and calcium chloride (control) the control solution the relative water content of tissue had a minimum rate (85%) and in 0 mmol of sodium chloride and 30 mmol calcium chloride, the amount of water content in Erli flat and American Moral, respectively were 91 and 92% (Table 2). Although by increasing the calcium chloride to the nutritional solution containing the sodium chloride at different concentrations, the negative effect of sodium on the relative water content of tissue was adjusted partly. Such that in the concentration of 10 mmol sodium chloride, the relative water content of tissue in all levels of sodium chloride was more (Table 2). Therefore, we can see that calcium in the nutritional solutions is able to increase the relative water content of tissue.

Cytoplasmic membrane stability: Comparing the average of cytoplasmic membrane stability of diverse cultivars showed that in all nutritional solutions the membrane stability was more in American Moral cultivar in compare to Erli flat cultivar cellular. Therefore, the resistance of American Moral cultivar was further to the increase of osmosis potential because leakage in Erli flat cultivar was more than American Moral cultivar. Also most cellular leakages in nutrient solution containing the sodium chloride with density of 90 mmol and amount of 744 μ mos cm^{-1} in the cultivar of Erli flat were observed (Table 2). Increasing the salt concentration in the cells of plant caused the cellular leakages to be increased and membrane stability of tissue to be reduced. The results showed that the least amount of cellular leakages (366 μ mos cm^{-1}) was related to the calcium chloride nutritional solution with density of 10 mmol in the American Moral cultivar. Therefore, we can see that the cellular leakages in the nutritional solution containing the calcium chloride were significantly less than other solutions. In this regard, Calcium acts as a secondary messenger and by effecting the membrane stability and activity of enzymes causes to protect the cells in stress terms. Thus, calcium plays an important role in the membrane stability under the salinity stress (Girija *et al*., 2002).

Leaf chlorophyll: The leaf chlorophyll concentration showed a significant difference between the different concentrations of nutritional solutions. The maximum amount of leaf chlorophyll (55) was related to the nutritional solution of control and the minimum amount of it (30) observed in the nutritional solution containing the 90 mmol of sodium chloride and 0 mmol calcium chloride in the American Moral cultivar (Table 2). The results showed that with nutritional solutions containing salt, sodium chloride especially is able to decrease the leaf chlorophyll. The salinity stress caused the leaf chlorophyll concentration and plant photosynthesis to be reduced (Yang *et al*., 2003).

The results showed that application of 10 mmol calcium chloride in comparison with nutritional solutions containing sodium chloride caused the leaf chlorophyll amount in each of two studied cultivars to be increased (Table 2). Therefore, use of calcium chloride especially in the low concentrations caused the leaf chlorophyll in comparison with the solutions containing the sodium chloride to be increased. This result indicates that the existence of calcium in the nutritional solution causes that the chlorophyll pigments of plant cells for better light absorption in the plant, to be formed. Omami (2005) reported that applied Ca^{2+} ameliorated the negative effects of NaCl on leaf area and leaf chlorophyll amount.

Mineral elements in herbaceous tissues: Comparison of the sodium, calcium and chloride average percent in tissue of plant shoot showed that by increasing the density of nutritional

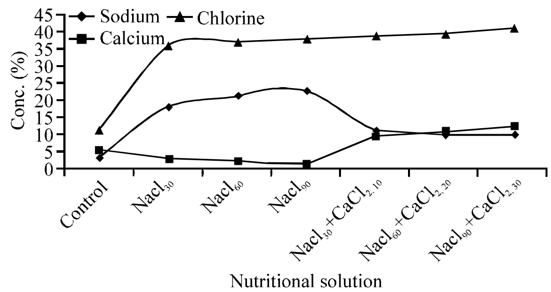

Fig. 1: Comparing the concentration of sodium, calcium and chlorine in shoot tissues of plant

solutions, mineral density of plant tissues has increased too. Such that sodium and chloride in the plant tissues in the nutritional solution containing 90 mmol of sodium chloride, respectively was 23 and 38%. Whereas in the grown plant tissues, the amount of sodium and chloride, respectively was 3.5 and 11.4% in the nutritional solution of control (Fig. 1). But in the nutritional solution containing calcium chloride+sodium chloride in the other levels was significantly decreased in shoots such that in the nutritional solution containing 90 mmol of sodium chloride with 30 mmol of calcium chloride (NaCl90+$CaCl_2$,30), sodium and calcium concentration in the tissues of shoots, respectively was 10.2 and 12.7 that in comparison with the amount of sodium in the nutritional solution containing 90 mmol of Sodium chloride (23%) was reduced more than 50% in the concentration of sodium (Fig. 1).

In these conditions, by increasing the concentrations of calcium, calcium content was not more than plants need to be able to cause some problems in the plant. Calcium concentration required in the nutrient solution formulas is about 200 mg L^{-1} (Arzani, 2007). Chlorine percentage of shoo tissues in the nutritional solutions containing sodium chloride and calcium chloride+sodium chloride at different concentrations showed no significant difference (Fig. 1).

Therefore, using the calcium chloride to reduce the damaging effects of sodium was not observed any significant effect in the increase of chlorine and risks due to its high concentration. Chlorine concentration of leaf is ranging from lower levels i.e., 0.02% of dry weight to the concentration of 0.15% (Arzani, 2007).

CONCLUSION

Calcium chloride causes to adjust the salinity stress such that traits affected by calcium chloride in saline terms were improved. Also by adding the calcium chloride to the sodium chloride, the amount of sodium was reduced and calcium amount was increased in dry shoot tissues. The dry materials weight of root at all salinity levels compared to the shoot dry weight shows more decrease that indicates the growth and development of root compared to the plant shoot can show more damages in stress terms. In this experiment, the stability characteristics of membrane and relative water content of tissue were significant in comparison with the measured traits in all the salinity treatments.

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