

# Changes in oxalic acid and mineral contents of tomato plant under salinity stress

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## **Abstract**

The synthesis of oxalic acid in the plant and its relations with other minerals in plant biochemistry were investigated under saline growing conditions. The effects of NaCl salt applied to the nutrient solution at concentrations of 0, 25, 50 and 75 mM on the mineral nutrients and oxalic acid contents of the leaves, roots and fruits of the tomato plant were examined. NaCl applied to the soil significantly decreased the vegetative, root and fruit dry matter amounts of the tomato plant and the N, P, K, Ca, Mg, Fe, Mn, Zn and Cu contents in these parts of the plant. The oxalic acid content of the plant also decreased with increasing salt concentration. Ca and oxalic acid contents of the plant were determined at different levels in the vegetative, root and fruit organs of the plant. Depending on the applied NaCl levels, significant decreases were recorded in the total and soluble oxalic acid and Ca levels of the plant. Physiologically effective oxalic acid in tomato plant was recorded only in fruits with low Ca content. The data confirm the effect of salinity on Ca nutrition and show the effects of ion interaction on oxalic acid synthesis in plants.

**Keywords:** Oxalic acid, Plant nutrients, Tomato

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## **1 Introduction**

It can be seen from the literature that studies on oxalic acids and their functions in plants, which have attracted the attention of researchers in various subjects for nearly half a century, have gained momentum in certain periods. Concerns about oxalic acids in food products

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include issues related to reduced strength in canned products, reductions in Fe and Ca bioavailability in human metabolism caused by foods with high oxalic acid content, as well as chronic health problems such as kidney stone formation. In terms of plant biochemistry, the relationship between oxalic acid and mineral metabolism has attracted more attention. Tomato is a plant that is widely used in the world, is demanded as a staple food in the diet of many cultures, and contains relatively more oxalic than other vegetable plants.

It has been known for many years that the nitrogen and phosphorus nutrition level of the plant has an important effect on the formation of oxalic acid in the plant. It is stated that nitrate, which is one of the nitrogen forms, generally increases the formation of oxalic acid, while ammonium nitrogen decreases it. In addition, various stress factors that the plant is exposed also significantly affect the nutritional metabolism and development of the plant.

In salty soil conditions, significant changes occur in the metabolic functions of cultivated plants sensitive to soil salinity and the plants do not develop healthily and thus salinity is a major factor in reducing plant growth and productivity. Oxalic acid, synthesized in plant cells, has an important function in maintaining cation and anion balance in plant metabolism and in the mobility of calcium ions in cells. Oxalic acid synthesis in plants varies significantly depending on mineral nutrition, cultural practices and various stress conditions (Topcuoğlu and Kütük, 2000).

Na and Cl are the dominant ions in saline soil conditions. Exposure to NaCl may affect plant growth through osmotic effects, by a specific ion effect or by inadequate uptake of an essential nutrient (Greenway and Muns, 1980). In soil conditions where Na ions are dominant, there is a significant decrease in Ca uptake in plants due to ion antagonism. In many cultivated plants the rate of Ca uptake is decreased linearly by increasing salinity (Adams and Ho, 1993). Ca deficiency is a fundamental cause of blossom-end rot physiological disorder of tomato plant. The prevalence and severity of blossom-end rot has been consistently associated with factors causing a low Ca ratio (Ca/soil solution salts) in the soil or substrate medium. Ca is an essential and very immobile macro element once deposited in a plant tissue. Ca immobility may result from ion complexes as oxalate (oxalic acid salts) or other insoluble forms and from binding to the cell wall making it unavailable for transport (Ferguson, 1979). Ca may be precipitated in the form of calcium oxalate in plant cells and thus oxalic acid may limit Ca availability for shoot growth (Behling et al., 1989). This is accepted as very important phenomenon in plant nutrition.

Observing metabolic changes in extreme growing conditions can help to better understand the metabolism of oxalic acid, which is a product of anion and cation balance in plants. In this paper, oxalic acid formation and uptake of plant nutrients were investigated in tomato plants under salinity conditions.

## 2 Materials and Methods

The experiment was carried out in greenhouse, in plastic pots contained 20 L distilled water washed perlite + peat mixture (1:1) and received basal nutrient solution plus the treatments of NaCl. Each pot had a hole at the bottom to facilitate drainage when renewing the nutrient solution. The composition of basal nutrient solution was given in Table 1. NaCl was applied to

basal nutrient solution at 0 (control), 25, 50 and 75 mM. The four NaCl treatments was replicated four times in a randomised plot design.

Table 1. Composition of the Basal Nutrient Solutions

	mM L <sup>-1</sup>		µM L <sup>-1</sup>
KH <sub>2</sub> PO <sub>4</sub>	1.25	Fe-EDDHA	40
Ca(NO <sub>3</sub> ) <sub>2</sub>	4.20	Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> .H <sub>2</sub> O	30
KNO <sub>3</sub>	3,60	MnSO <sub>4</sub> .H <sub>2</sub> O	5
MgSO <sub>4</sub>	2.00	ZnSO <sub>4</sub> .7H <sub>2</sub> O	4
K <sub>2</sub> SO <sub>4</sub>	1.87	CuSO <sub>4</sub> .5H <sub>2</sub> O	0,75
(NH <sub>4</sub> ) <sub>2</sub> NO <sub>3</sub>	1.50	Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	0,5

Tomato seedlings were planted as one plant per pot. Pots were irrigated every day with nutrient solutions contained above mentioned NaCl levels. Pots were leached with distilled water every three irrigation to prevent salt accumulation and then nutrient solution was added as previously mentioned. Fresh weights of tomato fruits obtained at 60-90 days of development period were recorded and sampling was done for analysis. After 90-days of vegetative growth period, all tomato plants were cut from the pot surface. The roots of the tomato plant were removed by carefully draining the substrate medium in the harvested pots. Plant above ground (leaves+stems) and root samples were washed with distilled water. Fresh plant material was dried at 65 °C, weighed, blended and stored in screwcap glass bottles.

In dried plant samples, total chloride content was determined by ion chromatography (EPA, 1984), total (HCl extracted) and soluble (water extracted) oxalic acids were determined by gas chromatographic method (Ohkawa, 1985). Total N was determined by Kjeldahl method. Total P was determined by spectrophotometrically. In wet ashed (with HNO<sub>3</sub>) plant samples total Na, K, Ca, Mg, Fe, Mn, Zn, Cu and soluble (water extracted) Ca (Behling et al., 1989) were analysed by using ICP-MS under optimised measurement conditions, and values were adjusted for oven dried (12 h at 105 °C) material according to the international standard. Physiologically active oxalic acid that is a stoichiometric calculation, calculated as the excess of equivalent amount of total oxalic acid than that of calcium (Allison, 1966).

Analysis of variance was used to evaluate the effects of different factors. Statistical analyses were performed by using SPSS-16 for Windows program.

## 3 Results and Discussions

### 3.1. Plant Dry Matter Yields

NaCl applications on vegetative biomass, root biomass and fruit yield (as dry weight) of tomato plant were found significant (Figure 1). NaCl applications significantly decreased the vegetative and root biomass and fruit yield of tomato plants. Decreases were observed in the parameters examined with increasing NaCl applications, and phytotoxic effects were observed at the highest NaCl application level. Depending on the increasing salt concentration, the dry matter losses observed in the tomato plant differed in the vegetative and root organs. At the 50 and 75 mM levels of NaCl applications, the losses in the vegetative organs were determined to

be relatively higher than the losses in the root organs. The reduction in plant dry matter with increasing salinity was of the same order as that reported previously for NFT (Ehret and Ho, 1986; Lopez and Satti, 1996).

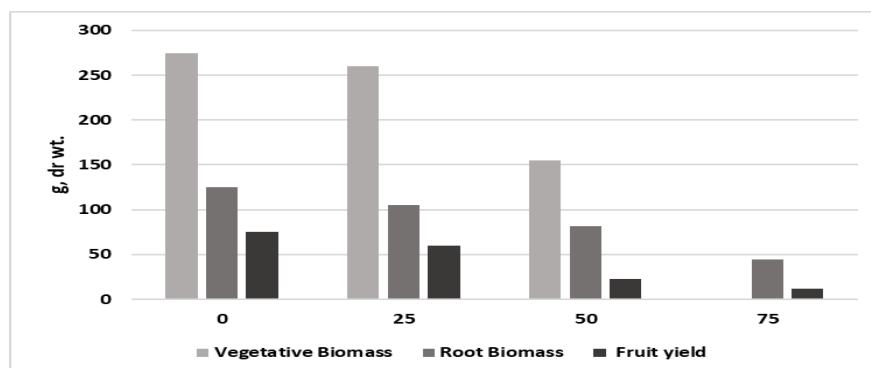


Fig. 1. The Effect NaCl applications on the dry matter of tomato plant.

### 3. 2. Plant Mineral Nutrition

It was determined that the N, P, K, Ca, Mg, Fe, Mn and Zn contents of the vegetative organs of the tomato plant decreased, while the Na and Cl contents increased, depending on the increasing salt concentration (Table 1). The increase in Na and Cl contents is expected with respect to NaCl applied to the plant. NaCl applications significantly limited the mineral substance intake of the plant with its antagonist effect. According to the nutrient sufficiency levels of the tomato plant, it is seen that the plant nutrition is at normal sufficiency levels in the control process. However, it is observed that plant nutrition is impaired due to NaCl applications and the nutrition of plants is insufficient.

Table 2. The Effect NaCl applications on the mineral concentrations in tomato leaves

NaCl Levels (mM)	N	P	K	Ca	Mg	Na	Cl	Fe	Mn	Zn	Cu
	%							mg kg <sup>-1</sup>			
0 (Control)	4,5	0,62	4,5	3	0,7	0,05	0,5	180	85	75	8
25	4,2	0,55	4,0	2,2	0,6	0,12	1,6	165	74	66	8
50	3,8	0,33	3,8	2,1	0,3	0,23	2,2	125	66	56	7
75	3,2	0,3	2,5	1,8	0,2	0,39	3,6	85	55	25	3
Significance	**	**	**	**	*	**	**	*	**	**	**
Sufficiency ranges	4-5,5	0,4-0,65	3-6	3-4	0,35-0,8	0,05-0,1	0,5-2,5	60-300	40-100	30-80	6-12

Data represent means of four replications, \*, \*\*: Significant at 5 %, 1 % probability levels, respectively.

A similar trend is observed in the fruit and root organs of tomato plants with respect to NaCl applications (Table 2 and Table 3). It was determined that the nutrient contents of the vegetative organs of the tomato plant were higher than the root and fruit organs, and the nutrient contents of the fruit were generally higher than the roots with the exception of Ca, Na and Cl elements. Ca, Na and Cl contents of tomato plant roots were determined higher than

vegetative and fruit organs. Regarding the NaCl applied to the tomato plant, the Cl contents determined in the vegetative, root and fruit organs were generally higher than the Na content with exceptions. These results indicate that Na is more restricted than Cl from entering the leaves. Previous results confirm the experimental findings that the chloride content of plant tissue increased as the external NaCl concentration was increasing (Al-Rawahy et al., 1992; Topcuoğlu, 2000).

Table 3. The Effect NaCl applications on the mineral concentrations in tomato fruits

NaCl Levels (mM)	N	P	K	Ca	Mg	Na	Cl	Fe	Mn	Zn	Cu
	%							mg kg <sup>-1</sup>			
0 (Control)	2,5	0,3	3	0,3	0,2	0,012	0,012	50	20	35	4
25	2,02	0,25	2,8	0,28	0,18	0,016	0,026	45	19	33	3,8
50	1,85	0,22	2,2	0,22	0,16	0,039	0,089	40	16	25	3,3
75	1,5	0,1	2	0,1	0,1	0,089	0,156	25	10	15	2
Significance	**	**	**	**	*			*	**	**	**

Data represent means of four replications, \*, \*\*: Significant at 5 %, 1 % probability levels, respectively.

Table 4. The Effect NaCl applications on the mineral concentrations in tomato roots

NaCl Levels (mM)	N	P	K	Ca	Mg	Na	Cl	Fe	Mn	Zn	Cu
	%							mg kg <sup>-1</sup>			
0 (Control)	1,2	0,18	1,3	1,4	0,20	0,08	0,6	45	35	25	7
25	1,1	0,2	1,6	1,2	0,14	0,15	1,7	39	28	30	8
50	1,1	0,22	0,8	0,9	0,16	0,28	3,2	23	33	15	6
75	0,8	0,14	1,5	0,9	0,14	0,54	3,5	25	18	22	3
Significance	*	-	-	*	-			*	*		*

Data represent means of four replications, \*, \*\*: Significant at 5 %, 1 % probability levels, respectively.

### 3. 3. Oxalic Acid Calcium Relations

Regarding NaCl applied to tomato plant, total and water-soluble oxalic acid and total and water-soluble Ca contents in vegetative organs decreased (Figure 2). Total and soluble Ca contents were determined higher than total and water-soluble oxalic acid in all treatments. Soluble oxalic acid content was determined lower than soluble Ca content.

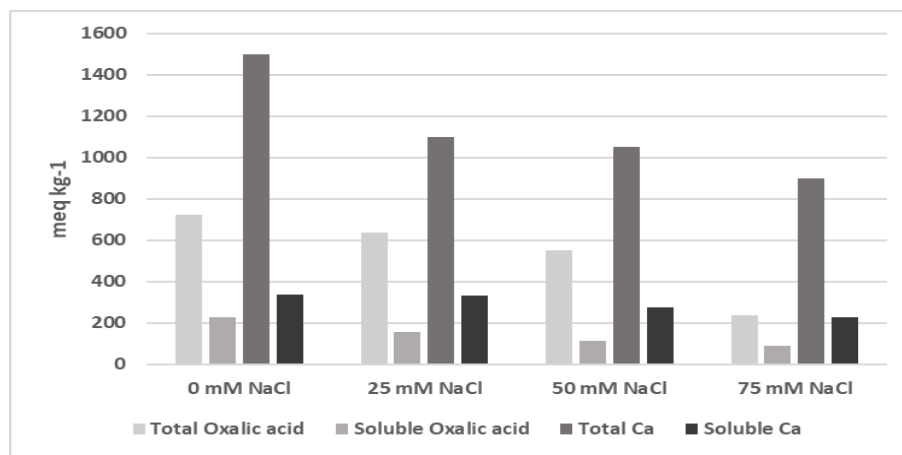


Fig. 2. The Effect NaCl applications on the total and soluble oxalic acids, total and soluble Ca contents (meq kg<sup>-1</sup>) in tomato leaves.

Although total and soluble oxalic acid and Ca values in the fruits of tomato plant decreased depending on NaCl applications, it was determined that total and soluble Ca contents were lower than oxalic acid contents (Figure 3). This may be related to the low level of Ca transport in tomato plant, which has poor mobility to fruit. In contrast to vegetative organs, soluble oxalic acid content was determined higher than soluble Ca content.

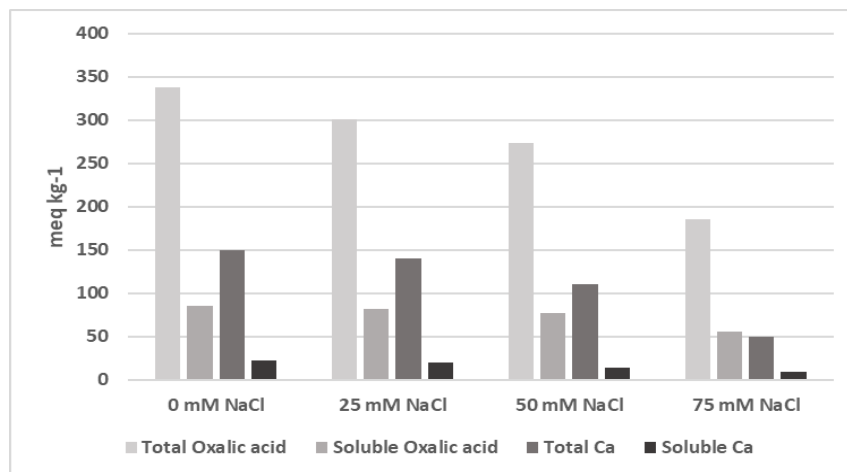


Fig. 3. The Effect NaCl applications on the total and soluble oxalic acids, total and soluble Ca contents (meq kg<sup>-1</sup>) in tomato fruits.

Total and soluble oxalic acid and Ca values in root organs of tomato plant are similar to the distribution in vegetative organs. Total and soluble Ca values in root organs were higher than total and soluble oxalic acid (Figure 4). However, oxalic acid and Ca concentrations determined in root organs were found to be lower than vegetative organs. This indicates that the synthesis site of oxalic acid in plant metabolism is largely vegetative organs. As in vegetative organs, soluble oxalic acid content was determined lower than soluble Ca content.

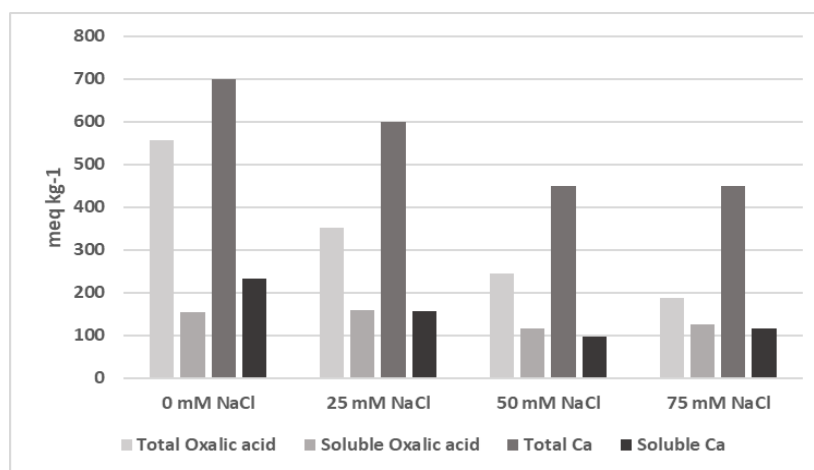


Fig. 4. The Effect NaCl applications on the total and soluble oxalic acids, total and soluble Ca contents (meq kg<sup>-1</sup>) in tomato roots

Increasing NaCl applications decreased total oxalic acid content. Higher Cl accumulation at NaCl applications may be cause of lower total oxalic acid concentration. These findings supported by researchers (Epstein, 1988) whose findings showed that an increase in inorganic anions (Cl<sup>-</sup>, PO<sub>4</sub><sup>3-</sup>) in proportion to cations can cause a reduction in oxalic acid content.

Salinity reduced the total Ca concentration in tomato. The data that decreasing Ca content was related with the salt applications confirm the previous findings (Adams and Ho, 1993; Lopez and Satti, 1996). Oxalic acid has a significant effect on Ca mobility in plant metabolism due to the strong oxalate salts it forms with Ca ions. Ca element, which is taken from the soil by passive absorption, is transported in the xylem due to transpiration and reaches the necessary tissues, which limits its mobility. The presence of soluble oxalic acids produced in plant metabolism immediately binds soluble Ca ions and converted it into insoluble calcium oxalate which metabolically inert in plant tissues. It is reported that highly significant relationships were found between insoluble Ca and oxalic acid contents (Hall, 1977). High concentration of oxalic acid may result Ca immobility (Ferguson, 1979) and stoichiometrically limits Ca availability (Behling et al., 1989).

Physiologically effective oxalic acid contents of tomato plant under salinity stress are given in Figure 5. Physiologically active oxalic acid content was determined in the fruits of tomato plant. Physiologically active oxalic acid contents, which are considered as oxalic acid values higher than total Ca content, decreased under the effect of salinity. This is thought to be due to the anionic antagonism effect of high levels of Cl ions.

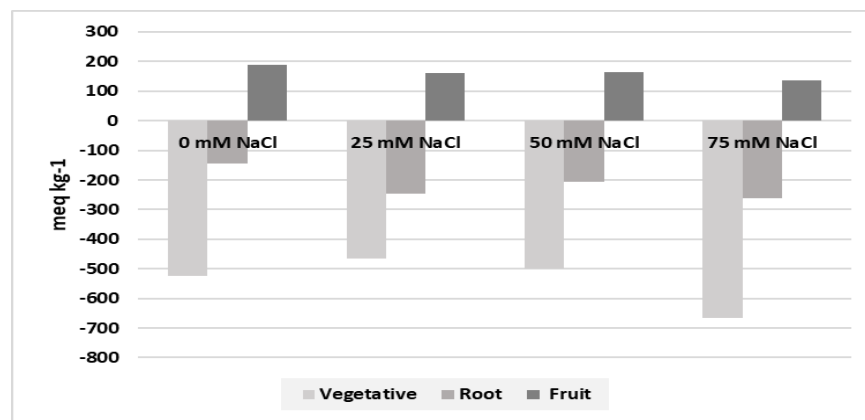


Fig. 5. Physiologically active oxalic acid content of tomato (represented by positive values in the fruit)

## 4 Conclusion

The data confirm the effect of salinity on Ca nutrition and show the effects of salinity on the oxalic acid formation which may affect Ca mobility. In addition to Ca deficiency induced by low Ca uptake due to high salinity, high oxalic acid synthesis in tomato may be thought as a limiting factor on Ca availability and plant productivity. Especially in the edible fruit tissue of tomato plant, low Ca and high oxalic acid values increase the physiologically active oxalic acid content. Due to the synthesis of oxalic acid depending on the anion and cation balance in the plant, it is considered that applications that encourage Ca uptake and increase Ca availability in the soil are important in plant nutrition practices.

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## Conflict of interests

The author declares that there are no competing interests.

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