Evaluation of salt (NaCl) tolerance in tomato (*Lycopersicon esculentum*) cultivars

Seyedeh Laleh Alavi¹ and Nasser Abbaspour¹*

¹, Department of Biology, Faculty of Science, Urmia University, Urmia, Iran

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*Corresponding author:
Department of Biology, Faculty of Science, Urmia University, Urmia, Iran.
Email: n.abbaspour@urmia.ac.ir

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**ABSTRACT**

**Purpose:** Soil salinity is a prevalent abiotic stress that adversely affects crop productivity worldwide. Salinity is an environmental stress that limits growth and development in fruits and vegetables due to increasing osmotic pressure, imbalancing of nutrients and toxicity of some special ions. Tomato (*Lycopersicon esculentum*) is one of the most important fruits and vegetables.

**Research method:** In this study, effect of salt stress (NaCl) on eight cultivars of tomato (king stone, Caligen, Super Strain B, Primo Early, Early Urbana VF, Early Urbana 111, Cal-j-N3 and Peto Early CH) were investigated. Plants were cultured in hydroponic conditions with five treatments of NaCl including 0, 30, 60, 90 and 120 mM. Each test has repeated three times. The growth indices, Ions, chlorophyll, soluble sugar and proline contents, were measured in roots, stems, petioles and leaves.

**Main findings:** The results showed that, sodium and chloride contents increased in all parts of the cultivars with increasing NaCl concentration. However, nitrate and potassium contents decreased. Proline and soluble sugars contents increased in leaves under salt stress too. In Primo- Early, Early Urbana 111, Cal-j-N3 and Petoearly CH, soluble sugar content increased with increasing NaCl concentration up to 90 mM. In all varieties, high salinity reduced chlorophyll a and b, total chlorophyll, carotenoids contents and growth indices. As far as the measured factors are concerned, it seems that Early Urbana VF and Super Strain B showed a high capacity to tolerate salinity stress.

**Limitations:** No limitations were founded.

**Originality/Value:** This is an opportunity to identify salinity-tolerant tomato cultivars that play an important role in the cultivation and production of quality crops.
INTRODUCTION

Salinity is one of the most serious environmental stresses. It affects 19.5% of the irrigated lands and 2.1% of the dry lands agriculture worldwide (FAO, 2000). All soils have a low amount of soluble salts that are essential for plant growth, but if the amount of salt increases, it causes problems for the plants (Teymouri et al., 2005). NaCl is the main salt that induces salt stress. Na⁺ is toxic for wide range of plants and some plants are affected by high concentration of chloride. When sodium enters into cytoplasm, plants activate their high affinity system for uptaking potassium. They can absorb enough amount of this ion because the low amount of K⁺ caused by absorbing more sodium limits growth in plants (Jian Kang, 2002). Sodium and chloride are toxic at high concentrations, so the effect of both ions should be the same (Teakle & Tyerman, 2010).

Salinity causes two stresses in plants: (1) a water shortage stress resulting from high solute concentrations in the soil and (2) ion-specific stress resulting from changed K⁺/Na⁺ and NO₃⁻/Cl⁻ ratios and Na⁺ and Cl⁻ ion concentrations. The modification of ion ratios in the plant is due to the influx Na⁺ and Cl⁻ through pathways involved in the acquisition of potassium and nitrate (Blumwald et al., 2000). Salinity tolerance includes two mechanisms: avoidance and resistance. NaCl resistance includes producing compatible solutes, sodium compartmentation and ions exchange (Garg & Gupta, 1997). Potassium has various functions including balancing membrane potential and turgor, regulating osmotic pressure, activating enzymes, tropisms and stomatal movement (Cherel, 2004).

Plants have many adaptive strategies in response to environmental stresses that include changes in physiological and biochemical processes. Accumulation of solutions such as soluble sugars and proline correlates with adaptation of these stresses to metabolic compatibility (Yancey et al., 1982). Proline accumulation is a plant response to environmental stresses and has a clear contribution as an osmoticum and a compatible solute (Yancey et al., 1982). Accumulation of sugars in different parts is enhanced in response to the variety of environmental stresses (Prado et al., 2000). The duty of sugars is the production of energy, the synthesis of other compounds and the stabilization of membranes (Hoekstra et al., 2001).

Tomato (Lycopersicon esculentum) is one of the most important fruits and vegetables and is a herbaceous plant, from the family of Solanaceae. It is relatively resistant to salinity (Maas, 1986). Tomato is a vegetable crop which is consumed fresh, cooked, canning, sauce and other. This crop distributed a different climates ranging. Despite its high adaptability, its production is mostly in warm and dry regions: more than 30% of world production comes from countries around the Mediterranean Sea and about 20% from California (FAO, 1995). The aim of this study was the investigation and evaluation of eight Lycopersicon esculentum cultivars responses under salt stress and analyzing their abilities for salt tolerance or resistance.

MATERIALS AND METHODS

Plant Material
The seeds with the same size of eight tomato cultivars (Lycopersicon esculentum): "King stone", "Caligen", "Super Strain B", "Primo Early", "Early Urbana VF", "Early Urbana 111", "Cal-j-N3" and "Peto Early CH" were provided from Falat company, Iran. The seeds were disinfected with benomyl (1% w/v). In the next step, the disinfected seeds were shed on a plastic net inside bottles containing distilled water then were covered with a damp cloth. On the eighth day, seedlings from each variety were selected and transferred to plastic pots with the aerated Hoagland solution containing 0.125 mM KNO₃, 0.125 mM Ca (NO₃)₂, 0.05 mM
MgSO$_4\cdot$7H$_2$O, 0.0125 mM KH$_2$PO$_4$, 5.75 µM H$_3$BO$_3$, 1.34 µM MnCl$_2\cdot$4H$_2$O, 0.1 µM ZnSO$_4\cdot$7H$_2$O, 0.038 µM CuSO$_4\cdot$5H$_2$O, 0.025 µM Na$_2$MoO$_4\cdot$2H$_2$O and 8.88 µM Fe-EDTA (1/8 strength). After eight days, the plants were transferred to a new Hoagland solution of 1/4 strength. Light period was 16/8 hr of light and dark, respectively. On the 25$^{th}$ day of growth, the plants were treated with NaCl (0, 30, 60, 90 and 120 mM) in Hoagland solution for 2 weeks (relative humidity 80% and temperature of 20-30 ºC).

**Growth indices**

After two weeks, plants were harvested and growth indices, chlorophyll a, b, total chlorophyll and carotenoids were analyzed.

**Chlorophyll and carotenoids assay**

Chlorophyll a, b, total chlorophyll and carotenoids contents were analyzed following the method of Lichtenthaler and Wellburn (1983). Fresh leaves (0.1g) were used for photosynthetic pigment extraction and immersed in 5 ml of 100% acetone. The absorbance of the samples were measured in 662,645 and 470 nm by spectrophotometer PD-303 UV (Range of measurement is 190 - 1000 nanometers. Dimensions 460 x 350 x 180 mm and Weight is 12.0 kg, Ukraine).

The following equations (1-4) were used to calculate chlorophyll a, b, total and carotenoids contents:

\[
\begin{align*}
    \text{Chl}_a &= 11.75A_{662} - 2.350A_{645} \\
    \text{Chl}_b &= 18.61A_{645} - 3.960A_{662} \\
    \text{Chl}_t &= \text{Chl}_a + \text{Chl}_b \\
    C &= 1000A_{470} - 2.270 \text{Chl}_a - 81.4 \text{Chl}_b/227
\end{align*}
\]

**Ion analysis**

100 mg of the powdered dry matter of shoot and root was put in glass tubes. Then 10 mL distilled water was added. The samples were put in the boiling water for an hour and after cooling in the room temperature were centrifuged for 20 min (4000 rpm) and the supernatant was transferred to a new tube. The volume of the new solution was reached to 10 ml with the distilled water. Sodium and potassium contents were determined using flame photometer (Fater electronic 405, Iran). 0.5 ml of this solution was analyzed to measure tissue Cl$^-$ concentration using chloride analyzer (Model 926, sherwood scientific,UK). Nitrate (NO$_3^-$) was measured using salicylic sulfuric acid. The absorbance of the samples was read in 410 nm by spectrophotometer (Cataldo et al., 1975).

**Soluble sugars assay**

Soluble sugars were measured according to Dubois et al. (1956) method with minor modification. 0.05 g of leaf and root dry matter was poured in 10 mL 70% ethanol. The samples were kept in 5ºC for a week. The samples were then centrifuged (4000 rpm) and 1 ml phenol 5% and 5 ml pure sulfuric acid added to 1 ml of each upper extract. The absorbance of the samples was read in 485 nm by spectrophotometer (Biochrom WPA S1200, UK).

**Proline assay**

Proline content was measured using the method of Bates et al. (1973). Briefly, 0.05 g leaf and root dry matter was homogenized with 15 ml of 3% sulfoisalicylic acid and were kept in 4ºC for 72 hours. The samples were centrifuged for a 10 minutes. 2 ml acetic acid glacial and 2 ml nin-hydryn (20 ml phosphoric acid 6M, 30 ml acetic acid glacial, 1.25 g nin-hydryn) were
added to 2 ml of each sample and then were incubated in 95°C for 1 hour. The samples were cooled by ice and were mixed with 4 ml toluene. The absorbance of the upper phase was read at 520 nm by spectrophotometer.

**Statistical analysis**
Data analysis was performed by SPSS (version 16.00). The difference between the means was calculated with the one-way ANOVA. GLM (General Linear Model) was used to determine the significant between different treatments and then Tukey’s multiple range tests ($P \leq 0.05$) was used.

**RESULTS AND DISCUSSION**

The results obtained from the data analysis showed that shoot and root longitudinal growth ($P < 0.05$) decreases significantly with increasing NaCl concentration in all the cultivars. The maximum reduction of shoot longitudinal growth viewed in "Cal-j-N3" variety. Maximum reduction of root longitudinal growth viewed in "Early Urbana VF" cultivar (Fig. 1). Excessive amounts of salts causes salinity of water and soil. Usually, if sodium and chloride contents are high, salt stress occurs (Kumar & Bandhu, 2005). The reducing growth is the most important response of the plant to salinity stress. Growth prevention happens because of the high accumulation of NaCl in shoot and root, and then osmosis and ionic effects on plants (Nedjimi, 2011). Decreasing the plant length can be due to metabolic activities such as meiosis, enzyme activities, and plant hormones (Amira & Qados, 2011). In the present study, salinity had a significant effect ($P < 0.05$) on decreasing the fresh and dry weights and shoot length in all varieties.

![Fig. 1] Changes in shoot (A) and root (B) longitudinal growth of the plants treated with different concentrations of NaCl. Bars are ± SE of the means (n=3) $p < 0.05$. 
According to the GLM analysis, fresh and dry weight in shoot and root (p<0.05) decreased significantly with increasing sodium chloride concentration in all the cultivars. The maximum reduction of fresh and dry weight in shoot and root viewed in "Early Urbana VF" cultivar (Fig. 2). Salt stress results in a considerable decrease in the fresh and dry weights of shoot and root (Chartzoulakis & Klapaki, 2000; Azarafshan & Abbaspour, 2014). Also, increased salinity leads to a significant decrease in shoot and root growth and increase in root/shoot ratio in cotton (Meloni et al., 2001). Tester and Davenport (2003) reported that the osmotic stress in the salinity treatment causes decreasing in cellular water content and shoot lengthening. The salinity reduced the growth parameters such as fresh and dry weights in shoot and root and preharvesting growth stages of the three tomato cultivars (Hajer et al., 2006).

**Fig. 2.** Fresh and dry weight changes in shoot and root of the plants treated with different concentrations of NaCl. Bars are ±SE of the means (n=3) p<0.05.
According to the results, the chlorophyll a and b, the total chlorophyll and carotenoids content in leaves (p<0.05) decreased significantly with increasing NaCl concentration in all the cultivars (Fig. 3). The chlorophyll a, b, total Chlorophyll and carotenoids content of tomato leaves decreased with increasing sea water salinity (Hajer et al., 2006). The chlorophyll content in tomato leaves decreased under salinity stress (Khavarinejad & Mostofi, 1998). The decreased chlorophyll content under salinity has adverse effects on membrane stability, which has been reported in various studies (Ashraf & Bhatti, 2000; Al-Sobhi et al., 2006). In the present study, chlorophyll a, b, total and carotenoids contents of leaves decreased with increasing salt concentration. Maximum decrease in total chlorophyll observed in "Caligen" cultivar. A significant correlation was between growth and photosynthesis in sunflower and wheat cultivars under salt stress (Ashraf, 1999; El-Hendaway et al., 2005). A decrease in chlorophyll content of rice leaves under salinity was reported by Mitsuya et al. (2003). Chlorophyll content reduction was observed with increasing salinity in four grape cultivars (Fozouni et al., 2012). Parida and Das (2005) suggested that a similar decrease in chlorophyll content in response to salt stress.

Fig. 3. Changes of the chlorophyll a (A), b (B), total chlorophyll (C) and carotenoids (D) content in leaves of the plants treated with different concentrations of NaCl. Bars are ±SE of the means (n=3) p<0.05.
The decrease of chlorophyll content in plants under salinity resulted in functions such as increased chlorophyllase activity and change in the lipid protein ratio of pigment-protein complexes (Parida et al., 2004). The low photosynthesis ability in plants under salt stress is attributed to the closure of stomata, the prevention of chlorophyll synthesis, effects on carotenoids and photosynthetic enzymes, reduction of carboxylase activity and high chlorophyllase activity (Stepien & Klobus, 2006; Abd El-Aziz et al., 2006).

Salinity had obvious effects on Na+ and Cl− accumulation in shoot and root in different concentrations of NaCl. In the shoot, maximum increase of Na+ viewed in "Cal-j-Ni" cultivar and maximum increase of Cl− viewed in "Peto Early CH". In the root, maximum increase of Na+ and Cl− viewed in "Caligen" cultivar (Fig. 4). Symptoms seen in plants included decrease growth, ethylation and drying.

Based on the obtained results, potassium and nitrate contents (p<0.05) decreased significantly with increasing external NaCl concentrations in the shoot and root. Maximum K+ reduction showed in "Peto Early CH". In the shoot, maximum NO3− reduction observed in "King Stone" and in the root, maximum NO3− reduction observed in "Primo Eearly". There was a significant difference among all salinity levels (Fig. 5). Increasing sodium accumulation in root, osmotic potential decreases and causes the plasmolysis and reduces nutrient absorption on the root surface. Sodium absorption increases with increasing the salt stress. High sodium content in cytoplasm causes depolarization of cell membrane (Bor et al., 2003).

Fig. 4. Changes of sodium and chloride content in shoot and root of the plants treated with different concentrations of NaCl. Bars are ±SE of the means (n=3) p<0.05.
Fig. 5. Changes of K⁺ and NO₃⁻ content in shoot and of the plants treated with different concentrations of NaCl. Bars are ±SE of the means (n=3) p<0.05.

This is study, with increasing NaCl concentration, Na⁺ content in the shoot and root increased in all cultivars. Teakel et al. (2006) reported that NaCl stress causes the accumulation of sodium in shoot and root and this increase in Lotus cornicolatus was higher than Lotus tenuis. The difference between salt tolerant and salt sensitive plants is low sodium levels. The resistance of plants to salinity appears to depend on the capacity of the ions accumulate. It was seem that resistant species accumulated lower Na⁺, and the decrease of K⁺ was lower in the sensitive than tolerant species (Essa, 2002; Yasar et al., 2006; Kusvuran et al., 2007). Potassium content of the eight cultivars was affected by NaCl treatment. Salt stress caused significant decreases in K⁺ content of all cultivars. Potassium concentrations decreased under salinity of all cultivars. Potassium reduction can be due to sodium competition on the sites of binding to plasma membrane carriers or potassium leakage due to instability of the plasma membrane (Ferreira-Silva et al., 2008).

Chloride concentration may cause nitrogen and phosphorus deficiencies. Chloride accumulation decreases nitrate reductase enzyme activity and low nitrate ion absorption causes the growth decreasing in the plant. Meanwhile, Cl⁻ and Na⁺ ions accumulation is always simultaneous and their toxic effects on the plant are complementary (Teakel & Tyerman, 2006). In this study, shoot and root Cl⁻ content increased in all cultivars with increasing salinity. The lowest chloride uptake was in the "Early Urbana VF" cultivar, indicating that has more capacity to tolerate high salinity. The competition of chloride with nitrate leads to a decrease in the absorption of nitrate ions. So, plants that are exposed to high amounts of sodium and chloride develop a backwardness due to lack of nitrate as a result of
the high chloride content. In salinity conditions, by decreasing the absorption of calcium the permeability of the membrane is reduced and chloride ion is easily and without spending energy accumulated in the tissues of the plant (Fisarakis et al., 2001). According to Massa et al. (2009), there is a positive correlation between sodium, chloride and salt concentration in rose plants. Savvas et al. (2007) also showed the same pattern in bean plants. Our results showed this correlation between ions uptake and external salt concentration, too. Nitrogen accumulation reduction can be due to the antagonism of chloride and nitrate absorption, nitrogen metabolism reduction by decreasing the activity of leaf nitrate reductase enzyme and reducing water consumption due to decreased water absorption by plant (Sotiropoulos et al., 2006). The high concentration of N in reduced form, is observed in xylem sap in tomato plants under salinity (Cramer et al., 1995). In the present study, salinity had a significant effect on the shoot and root nitrate content of the cultivars. In fact, the lowest amount of root nitrate in the "Early Urbana VF" cultivar could be due to the more tolerance during salinization and preventing chloride entry to the shoot. In the present study, based on the results of data analysis, the K⁺/Na⁺ and NO₃⁻/Cl⁻ ratios in each of the eight cultivars showed a decrease in the shoot and root with increasing salt concentration. For the selection of salt resistant varieties the ratio K⁺/Na⁺ can be used (Salam et al., 1999; Chen et al., 2005). The suitable K⁺/Na⁺ ratio in the cytoplasm under salt stress is due to an efficient partitioning of both ions (Munns et al., 2006). Our results are consistent with the above data, as well as of the findings of Leonova et al. (2005), Carden et al. (2003), Eker et al. (2006), and Chen et al. (2007). The interaction of NO₃⁻/Cl⁻ might cause inhibition nitrate absorption at the sites for ion transport (Cram, 1983) and Na⁺ ions cause severe membrane depolarisation in tomato (Suhayda et al., 1990). The K⁺/Na⁺ and NO₃⁻/Cl⁻ ratio showed reduction with increasing NaCl concentration in all the cultivars (Table 1).

The proline content increased in the shoot and root under salinity stress in the studied cultivars. The maximum shoot and root proline content viewed in "Early Urbana 111" cultivar (Fig. 6).

The soluble sugars content increased in both shoot and root under salinity stress. However, some fluctuations were observed among the cultivars. Maximum soluble sugars content in shoot was observed in "Super Strain B" and maximum soluble sugars content in root was observed in "Caligen" cultivar (Fig. 7). Plants are able to tolerate salt stress through the osmotic mechanisms to maintain their water status by accumulating metabolites such as proline and soluble sugars and other ions. Under the salinity stress, soluble sugars and proline can act as osmotic protectants (Bray, 2003). Increasing the soluble sugars in response to salinity may cause their less displacement from leaves. Slow consumption in leaves is resulted from growth decreasing and other changes like hydrolysis of starch (Azarafshan & Abbaspour, 2014). In this study, with increasing salinity levels, the osmolitic concentrations included soluble sugars (except 4 cultivars) and proline in all eight cultivars. Sugars major functions including: osmotic adjustment, osmoprotection, radical scavenging and carbon storage. Salinity increases sugars in some of plants (Kerepesi & Galiba, 2000; Khatkar & Kuhad, 2000; Singh et al., 2000). In this study, the proline amounts increased under the NaCl stress and this increase was observed in all the cultivars and was more in "EarlyUrbana 111". Proline biosynthesis of glutamate is considered to be the main proline pathway for biosynthesis and its accumulation especially under stress conditions. Proline, along with osmotic regulation, has other tasks, such as protecting the plasma membrane, removing hydroxyl radicals and active oxygen, and can be a source of carbon and nitrogen (Bartels & Sunkar, 2005). Our results were matched with Azarafshan and Abbaspour (2014).
Table 1. The ratio of $K^+$/Na$^+$ and NO$_3$/$Cl^-$ in shoot and root

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Salinity (mM NaCl)</th>
<th>(K$^+$/Na$^+$) Shoot</th>
<th>(K$^+$/Na$^+$) Root</th>
<th>(NO$_3$/$Cl^-$) Shoot</th>
<th>(NO$_3$/$Cl^-$) Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>King stone</td>
<td>0</td>
<td>48.94±7.13 b</td>
<td>501±11.92 b</td>
<td>1.86±0.303 b</td>
<td>8.58±1.04 b</td>
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<td></td>
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</tr>
<tr>
<td></td>
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<td>0.02±0.001 a</td>
</tr>
<tr>
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<td>0</td>
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<td>Super strain B</td>
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<tr>
<td>Cal-j-N3</td>
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<td>0.15±0.037 b</td>
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<td>0.025±0.002 c</td>
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<td>Peto Early CH</td>
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<td>35.15±3.67 b</td>
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<td>0.006±0.001 a</td>
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</table>

CONCLUSION

Based on the results, among the eight cultivars of the studied tomatoes, Super strain B and Early Urbana VF were better than other cultivars against salt stress, which were identified as tolerant cultivars. While, two cultivars called King stone and Caligen did not perform as well as other cultivars against salinity stress and were identified as sensitive to salinity. The other cultivars including Primo Early, Early Urbana 111, Cal-j-N3 and Peto Early CH are between these two ranges.
Fig. 6. Changes of proline content in shoot (A) and root (B) of the plants treated with different concentrations of NaCl. Bars are ±SE of the means (n=3) p<0.05.

Fig. 7. Changes of soluble sugars content in shoot (A) and root (B) of the plants treated with different concentrations of NaCl. Bars are ±SE of the means (n=3) p<0.05.

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Conflict of Interest
The authors have no conflict of interest to report.

REFERENCES


