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Promjena u usvajanju iona kod rajčice (*Lycopersicon esculentum* L.) primjenom kalcija u uvjetima solnog stresa

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# MODULATION OF ION UPTAKE IN TOMATO (*Lycopersicon esculentum* L.) PLANTS WITH EXOGENOUS APPLICATION OF CALCIUM UNDER SALT STRESS CONDITION

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## SUMMARY

**Salinity affects almost every aspect of the physiology and biochemistry of plants due to both osmotic stress and ionic toxicity. We studied the variation of ion uptake in tomato cv. BARI Tomato-5 under different levels of salinity (0, 2, 4, 6 and 8 dS m<sup>-1</sup>) and their mitigation by different concentration of Ca<sup>2+</sup> (0, 5, 10 mM). The results showed that salt stress significantly affects the stomatal conductance of tomato. Salt treatment markedly increased the uptake of Na<sup>+</sup> and decreased both K<sup>+</sup> and Ca<sup>2+</sup> uptake in the leaves of tomato. The uptake of Na<sup>+</sup> decreased and uptake of Ca<sup>2+</sup> and K<sup>+</sup> increased in tomato when salt stressed plants were treated with Ca<sup>2+</sup>. Our results revealed that Ca supplementation can effectively reduce the salt-induced ionic toxicity in tomato plants. Exogenous application of Ca<sup>2+</sup> significantly mitigates the adverse effects of salt induced ionic toxicity.**

**Key-words: calcium, ion selectivity, *Lycopersicon esculentum* L., salinity, stomatal conductance**

## INTRODUCTION

Salinity is one among the several environmental stresses causing drastic changes in the growth, physiology and metabolism of plants and threatening the cultivation of plants around the globe (Jaleel et al., 2007). Much salinity resulted from NaCl cause osmotic pressure of external solution become more than osmotic pressure of plant cells which is required for regulating osmotic pressure to prevent dehydration of plant cells. Uptake and transformation of nutrient ions such as potassium (K<sup>+</sup>) and calcium (Ca<sup>2+</sup>), or surplus of sodium (Na<sup>+</sup>) can cause problems. High Na<sup>+</sup> and Cl<sup>-</sup> rates can cause direct toxic effects on enzymatic and membranous systems (Nazarbeygi et al., 2011). High salinity in soil disturbs intracellular ion homeostasis, leads to cell membrane damage, disrupts the metabolic activity, and thus finally causes growth inhibition and even plant death (Rains and Epstein, 1967). These phe-

nomena were observed in agricultural and horticultural crops, including tomato (*Lycopersicon esculentum* L.) (Juan et al., 2005), which is considered as moderately sensitive or moderately tolerant to salinity depending on cultivar or growth stage (Santa-Cruz et al., 2002; Fernandez-Garcia et al., 2004; Estan et al., 2005).

Once sodium enters the cytoplasm, it inhibits enzyme activity. This inhibition is also dependent on how much K<sup>+</sup> is present: a high Na<sup>+</sup>/K<sup>+</sup> ratio can cause a lot of damage. Ca<sup>2+</sup> is an important factor in the "battle" between Na<sup>+</sup> and K<sup>+</sup> ions. An increased Ca<sup>2+</sup> supply has a protective effect on plants under the Na stress. Calcium sustains K<sup>+</sup> transport and K<sup>+</sup>/Na<sup>+</sup> selectivity in Na-challenged plants. This beneficial effect of Ca is mediated by an intracellular signaling pathway that regulates the expression and activity of K<sup>+</sup> and Na<sup>+</sup> transporters. Calcium may also directly suppress sodium import mediated by nonselective cation channels (Davenport and Tester, 2000; Demidchik and Tester, 2002; Tester and Davenport, 2003; Zhu, 2003; Jouyban, 2012).

Calcium is a crucial regulator of growth and development in plants. It is reported that Ca<sup>2+</sup> can alleviate the negative effects of salinity on root elongation

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(Ashraf and Naqvi, 1991) and shoot growth of plants (Al-Khateeb, 2006; Nedjimi and Daoud, 2009). Besides, the addition of  $\text{Ca}^{2+}$  can not only protect cell membranes from adverse effect of  $\text{Na}^+$ , but also minimize the leakage of cytosolic potassium (Maathuis and Amtmann, 1999). The uptake and transport of  $\text{Na}^+$  can also be decreased by the presence of  $\text{Ca}^{2+}$  in the NaCl solution (Rubio et al., 2003). Furthermore, the application of  $\text{Ca}^{2+}$  in saline medium can prevent  $\text{Na}^+$  from binding to cell walls (Kurth et al., 1986). However, the responses vary depending not only on  $\text{Na}^+$  and  $\text{Ca}^{2+}$  concentrations but also on plant species. Many authors stated that exogenous calcium alleviated stress in *Vigna radiata*, *Glycine max*, *Linum usitatissimum* etc. (Manivannan et al., 2007; Arshi et al., 2010; Khan et al., 2010). The reduction of  $\text{K}^+$  and  $\text{Ca}^{2+}$  ions in plant tissues with a high level of NaCl treatments is a well known fact regarding tomato (Savvas and Lenz, 2000). It is also well known that a high NaCl concentration induces  $\text{Ca}^{2+}$  deficiencies in tomato, where the addition of  $\text{Ca}^{2+}$  to saline solution increases calcium and potassium concentrations in the roots (Lopez and Satti, 1996); it has been associated to a decreased transpiration rate rather than competition effects with  $\text{Na}^+$ . When absorbed and accumulated at large amount in plant,  $\text{Na}^+$  becomes highly toxic at different physiological levels. Physiological impairments caused by  $\text{Na}^+$  toxicity include disruption of  $\text{K}^+$  and  $\text{Ca}^{2+}$  nutrition, development of water stress and induction of oxidative cell damage (Aktas et al., 2006). Therefore, research was conducted in order to determinate the ionic behavior of tomato plants under  $\text{Na}^+$  stress along with the response of  $\text{Ca}^{2+}$  to alleviate the  $\text{Na}^+$  toxicity through enhancing  $\text{K}^+$  and  $\text{Ca}^{2+}$  uptake in leaves over  $\text{Na}^+$ . Hence, we investigated the role of  $\text{Ca}^{2+}$  in mitigating salt stress-induced response in tomato through ion uptake variation.

## MATERIAL AND METHODS

### Experimental site

A pot experiment was conducted at the Sher-e-Bangla Agricultural University, Dhaka, Bangladesh located at 23°74'N latitude and 90°35'E longitude at an altitude of 8.6 meter above the sea level during the period from October 2013 to March 2014. The climate of this area is subtropical. With regard to soil texture of the experimental site, it was silt loam (sand 20.84 %, silt 57.46 % and clay 21.7 %) with pH 6.9, organic matter 0.86%, available potassium 25 mg/kg and available sodium 70 mg/kg.

### Experimental materials and design

A pot experiment was conducted in a vinyl house made of bamboo with a polythene roof. Tomato cv. BARI Tomato-5 was used as a test plant. Thirty day old seedlings were used for the salinity treatment at different levels, i.e. 0, 2, 4, 6, 8 dS  $\text{m}^{-1}$ , combined with three levels of  $\text{Ca}^{2+}$  in the form of  $\text{CaSO}_4 \cdot 0.5\text{H}_2\text{O}$  viz., 0, 5, 10 mM. The

experiment was laid out in Randomized Complete Block Design (RCBD) with four replications.

### Application of sodium and calcium in soil

The experimental treatments of tomato plants included different salinity levels (0, 2, 4, 6 and 8 ds  $\text{m}^{-1}$ ) which were maintained by adding 0, 12, 27, 48 and 58 g of sodium chloride (NaCl) respectively with irrigation water in three intervals (30, 50 and 70 days after transplanting (DAT)), in which each pot contained 10 kg soil. One plant per pot was grown. At same time  $\text{Ca}^{2+}$  was applied at 0, 5 and 10 mM concentration with irrigation water.

### $\text{Na}^+$ , $\text{K}^+$ , $\text{Ca}^{2+}$ content (% DW) in leaves

For the preparation of the plant sample, a dried sub-sample weighing 0.5 g was transferred into a dry, clean 100 ml digestion vessel. 10 ml of di-acid ( $\text{HNO}_3$ :  $\text{HClO}_4$  in the ratio 2:1) mixture was added to the flask. After a while, the flasks were heated at a temperature slowly raised to 200 °C. Heating was stopped when dense white fumes of  $\text{HClO}_4$  occurred. The content of the flasks was boiled until they became clean and colorless. After cooling, the content was taken into a 50 ml volumetric flask and filled up with de-ionized water to the marked spot. Na, K and Ca content was measured from digest sample of tomato leaves by using the flame photometer. The concentrations were measured by using standard curves and expressed as percentage.

### Stomatal conductance

Stomatal conductance was measured using a portable photosynthesis system ADC LC pro+4 (UK), which is a non-destructive method. Mature leaves were measured continuously and expressed in  $\text{mol m}^{-2} \text{s}^{-1}$ .

### Statistical analysis

Collected data were statistically analyzed to determine the level of significance using MSTAT-C computer package program (Russell, 1994). The mean differences were assessed by least significant difference (LSD) at 5% level of probability.

## RESULTS AND DISCUSSION

### $\text{Na}^+$ content in leaves

During study research, a significant ( $p \leq 0.01$ ) difference in  $\text{Na}^+$  was observed in tomato leaves in different levels of salinity (Table 1). The concentration of  $\text{Na}^+$  in leaves increased with increasing levels of salinity. The highest  $\text{Na}^+$  content in leaves (0.61%) was recorded in 8 dS  $\text{m}^{-1}$  salt, whereas the lowest (0.24%) was found in the treatment without salt (control). As higher salinity levels provided more available  $\text{Na}^+$  in soil, the  $\text{Na}^+$  content in leaves tended to increase. Memon et al. (2007) observed that saline water treated plants con-

tained more  $\text{Na}^+$  than plants grown in non-saline water. This result was supported by de Lacerda et al. (2003); Netondo et al. (2004); Bavei et al. (2011); Lolaei et al. (2012) in *Sorghum bicolor*.

Reduced  $\text{Na}^+$  content in tomato leaves was found to be increasing with supplemental  $\text{Ca}^{2+}$  under salt stress conditions (Table 1). The highest  $\text{Na}^+$  content (0.40%) in leaves was recorded in calcium free treatment and the lowest (0.38%) was found from 10 mM Ca. Elevated  $\text{Ca}^{2+}$  concentration in nutrient solution mitigates the adverse effects of NaCl by inhibiting  $\text{Na}^+$  uptake (Kaya et al., 2002) and reducing membrane leakage (Tuna et al., 2007). This was supported by Qadir et al. (2001), Dabuxilatu and Ikeda (2005), Anbu and Sivasankaramoorthy (2014).

The interaction effect between salinity and calcium on  $\text{Na}^+$  content in leaves was statistically significant ( $p \leq 0.01$ ) (Table 1). The highest  $\text{Na}^+$  content (0.61%) in leaves was noted in 8 dS  $\text{m}^{-1}$  Na with 5 and 10 mM Ca which was statistically similar to 6 and 8 dS  $\text{m}^{-1}$  Na with 0 mM Ca treatment combinations. The lowest  $\text{Na}^+$  content in leaves (0.23%) was recorded in combination of 0 dS  $\text{m}^{-1}$  Na with 10 mM Ca, which was statistically equal to 0 dS  $\text{m}^{-1}$  Na and 0 mM Ca, 0 dS  $\text{m}^{-1}$  Na and 5 mM Ca, 2 dS  $\text{m}^{-1}$  Na and 0 mM Ca. The present study revealed that plants accumulated more  $\text{Na}^+$  in leaves with the increasing levels of salinity. However, increasing the amount of calcium significantly decreased the deleterious effect of salinity. Therefore, it might be that the beneficial effects of supplemental  $\text{Ca}^{2+}$  on leaf photochemistry could merely be due to a restriction of  $\text{Na}^+$  uptake by plant roots or by preventing the excessive  $\text{Na}^+$  from being delivered to the shoot (Shabala et al., 2005).

### **$\text{K}^+$ content in leaves**

The result from the Table 1 showed that salinity level had significant ( $p \leq 0.01$ ) effect on  $\text{K}^+$  content in leaves.  $\text{K}^+$  content decreased with the increasing salinity levels. The  $\text{K}^+$  deficiency of salinized plants was inversely correlated to the increased accumulation of  $\text{Na}^+$ , indicating the existence of competition effects between  $\text{Na}^+$  and  $\text{K}^+$  ions which most likely share the same transport system at the root surface (Rus et al., 2001). Netondo et al. (2004) also reported that accumulation of  $\text{K}^+$  in leaves was strongly inhibited by salinity and this similar observation was supported by Thimmaiah (2002), Memon et al. (2007) and Bavei et al. (2011).

$\text{K}^+$  content in tomato leaves under salinity was significantly ( $p \leq 0.05$ ) affected by  $\text{Ca}^{2+}$  treatments. Application of  $\text{Ca}^{2+}$  up to 5 mM increased the  $\text{K}^+$  content in tomato leaves and thereafter decreased slowly at 10 mM Ca (Table 1). So, exogenous application of  $\text{Ca}^{2+}$  increased the  $\text{K}^+$  content in leaf which is strongly supported by Tuna et al. (2007), Arshi et al. (2010) and Soualem et al. (2014).

The interaction between salinity and calcium levels had a significant ( $p \leq 0.01$ ) effect on  $\text{K}^+$  content in tomato leaves (Table 1). The highest  $\text{K}^+$  content (1.21%) was recorded in 4 dS  $\text{m}^{-1}$  Na with a 5 mM Ca treatment, which was statistically similar to 0 dS  $\text{m}^{-1}$  Na and 0 mM Ca (control) treatment and the lowest (0.66%) was obtained from the combination of 8 dS  $\text{m}^{-1}$  Na with 10 mM Ca, which was statistically equal to 6 dS  $\text{m}^{-1}$  Na and 5 mM Ca, 8 dS  $\text{m}^{-1}$  Na and 0 mM Ca, 8 dS  $\text{m}^{-1}$  Na and 5 mM Ca. The application of 5 mM Ca increased the  $\text{K}^+$  accumulation in leaves at 2, 4 and 8 dS  $\text{m}^{-1}$  salinity levels, except in 6 dS  $\text{m}^{-1}$ , where increased  $\text{K}^+$  accumulation was found in 10 mM Ca.

**Table 1. Effect of salinity and/or calcium level on changes of ion uptake (% DW basis) in tomato leaves**

Tablica 1. Utjecaj saliniteta i/ili razine kalcija na promjene usvajanja iona (% DW baza) u listovima rajčice

| Salinity level                 | Na <sup>+</sup> content<br>(% DW basis) | K <sup>+</sup> content<br>(% DW basis) | Ca <sup>2+</sup> content<br>(% DW basis) |          |
|--------------------------------|---|--|--|----------|
| 0 dS m <sup>-1</sup> Na        | 0.24 e                                  | 1.04 a                                 | 0.65 a                                   |          |
| 2 dS m <sup>-1</sup> Na        | 0.28 d                                  | 0.87 b                                 | 0.62 a                                   |          |
| 4 dS m <sup>-1</sup> Na        | 0.34 c                                  | 0.88 b                                 | 0.60 a                                   |          |
| 6 dS m <sup>-1</sup> Na        | 0.49 b                                  | 0.86 b                                 | 0.62 a                                   |          |
| 8 dS m <sup>-1</sup> Na        | 0.61 a                                  | 0.75 c                                 | 0.48 b                                   |          |
| LSD <sub>(0.05)</sub>          | 0.026                                   | 0.086                                  | 0.074                                    |          |
| Level of significance          | **                                      | **                                     | **                                       |          |
| Calcium level                  |   |  |  |          |
| 0 mM Ca                        | 0.40 a                                  | 0.91 ab                                | 0.50 b                                   |          |
| 5 mM Ca                        | 0.40 ab                                 | 0.93 a                                 | 0.66 a                                   |          |
| 10 mM Ca                       | 0.38 b                                  | 0.85 b                                 | 0.63 a                                   |          |
| LSD <sub>(0.05)</sub>          | 0.020                                   | 0.067                                  | 0.057                                    |          |
| Level of significance          | **                                      | *                                      | **                                       |          |
| Salinity level × Calcium level |   |  |  |          |
| 0 dS m <sup>-1</sup> ×         | 0 mM                                    | 0.25 fg                                | 1.18 a                                   | 0.63 a-c |
|                                | 5 mM                                    | 0.25 fg                                | 0.95 bc                                  | 0.71 a   |
|                                | 10 mM                                   | 0.23 g                                 | 0.98 b                                   | 0.63 a-c |
| 2 dS m <sup>-1</sup> ×         | 0 mM                                    | 0.26 e-g                               | 0.84 b-e                                 | 0.50 c-e |
|                                | 5 mM                                    | 0.30 de                                | 0.93 b-d                                 | 0.65 ab  |
|                                | 10 mM                                   | 0.29 d-f                               | 0.81 c-e                                 | 0.71 a   |
| 4 dS m <sup>-1</sup> ×         | 0 mM                                    | 0.32 cd                                | 0.85 b-e                                 | 0.49 de  |
|                                | 5 mM                                    | 0.37 c                                 | 1.21 a                                   | 0.70 a   |
|                                | 10 mM                                   | 0.33 cd                                | 0.86 b-e                                 | 0.63 a-c |
| 6 dS m <sup>-1</sup> ×         | 0 mM                                    | 0.59 a                                 | 0.90 b-e                                 | 0.48 de  |
|                                | 5 mM                                    | 0.46 b                                 | 0.77 ef                                  | 0.68 ab  |
|                                | 10 mM                                   | 0.43 b                                 | 0.91 b-e                                 | 0.70 a   |
| 8 dS m <sup>-1</sup> ×         | 0 mM                                    | 0.60 a                                 | 0.78 ef                                  | 0.40 e   |
|                                | 5 mM                                    | 0.61 a                                 | 0.80 d-f                                 | 0.55 b-d |
|                                | 10 mM                                   | 0.61 a                                 | 0.66 f                                   | 0.50 c-e |
| LSD <sub>(0.05)</sub>          | 0.045                                   | 0.150                                  | 0.128                                    |          |
| Level of significance          | **                                      | **                                     | **                                       |          |

\*\* : significant at  $p \leq 0.01$ , \* : significant at  $p \leq 0.05$ Different lowercase letters beside the mean value indicate significant at  $p \leq 0.05$  or  $p \leq 0.01$ 

### Ca<sup>2+</sup> content in leaves

Different level of salinity stress had significant ( $p \leq 0.01$ ) effect on Ca<sup>2+</sup> content of tomato leaves (Table 1). Tomato leaves accumulated less amount of Ca<sup>2+</sup> at higher salinity level. It was found that Ca<sup>2+</sup> content in leaves was gradually decreased with the decrease of K<sup>+</sup> along with increased levels of salinity.

Different levels of calcium significantly ( $p \leq 0.01$ ) increased the Ca<sup>2+</sup> content in leaves of tomato over the control plant. Ca<sup>2+</sup> content in tomato leaves increased up to 5 mM and thereafter decreased slowly at 10 mM Ca (Table 1). Within certain limits, additional Ca<sup>2+</sup> may ameliorate plant response to salinity (Maggio et al., 2007). Ca<sup>2+</sup> ameliorates the detrimental effects of toxic Na<sup>+</sup> and, the Ca<sup>2+</sup> content will be improved by applying calcium and this result was also supported by Arshi et al. (2010), Lolaei et al. (2012), Anbu and Sivasankaramoorthy (2014).

The interaction of different salinity and calcium levels had significant ( $p \leq 0.01$ ) effect on Ca<sup>2+</sup> content in tomato leaves (Table 1). The highest Ca<sup>2+</sup> content (0.71%) was found from 0 dS m<sup>-1</sup> Na with 5 mM Ca which was statistically equal to treatment combinations of 2 dS m<sup>-1</sup> Na and 10 mM Ca, 4 dS m<sup>-1</sup> Na and 5 mM Ca, 6 dS m<sup>-1</sup> Na and 10 mM Ca. The lowest Ca<sup>2+</sup> content (0.40%) was observed in 8 dS m<sup>-1</sup> Na with 0 mM Ca which was also statistically similar to 6 dS m<sup>-1</sup> Na and 0 mM Ca, 4 dS m<sup>-1</sup> Na and 0 mM Ca, 2 dS m<sup>-1</sup> Na and 0 mM Ca, 8 dS m<sup>-1</sup> Na and 10 mM Ca treatment combinations. Thus calcium increased the Ca<sup>2+</sup> content in leaves in different saline conditions which improved the K<sup>+</sup> content resulting somewhat in salinity tolerance. Therefore, Ca<sup>2+</sup> ions control salt tolerance in different ways: First of all, they maintain Na<sup>+</sup> accumulation in tissues (Rengel, 1992), and prevent Na<sup>+</sup> ions entering into the cell (Maathius et al., 1996). Preservation of or



increase in  $\text{Ca}^{2+}$  concentration could induce maintenance of  $\text{K}^+$ , because the presence of  $\text{Ca}^{2+}$  seems to be necessary for  $\text{K}^+/\text{Na}^+$  selectivity and for the maintenance of an appropriate amount of  $\text{K}^+$  concentration in plant cells.

### $\text{K}^+/\text{Na}^+$ ratio in leaves

The ability of plants to retain  $\text{K}^+$  and to maintain  $\text{K}^+/\text{Na}^+$  selectivity has always been considered a key feature of salt tolerance (Maathuis and Amtmann, 1999; Munns, 2002; Tester and Davenport, 2003). A significant ( $p \leq 0.01$ ) difference in  $\text{K}^+/\text{Na}^+$  ratio was observed in studied tomato leaves under different levels of salinity (Table 2). The highest  $\text{K}^+/\text{Na}^+$  ratio (4.32) was observed in  $0 \text{ dS m}^{-1}$  Na and the lowest value (1.23) was found from  $8 \text{ dS m}^{-1}$  Na. The results showed that the  $\text{K}^+/\text{Na}^+$  content in tomato leaves gradually decreased with the increase of salinity levels. Under saline condition, plants replace more toxic  $\text{Na}^+$  with less non-toxic  $\text{K}^+$ . Increased  $\text{K}^+$  concentrations under saline conditions may help to decrease  $\text{Na}^+$  uptake required for maintaining the osmotic balance (Mahajan and Tuteja, 2005). High  $\text{Na}^+$  in soil solution causes intracellular  $\text{K}^+$  deficiency due to competition and leads to  $\text{K}^+/\text{Na}^+$  disequilibrium (Kronzucker and Britto, 2011; Pardo and Rubio, 2011).

$\text{K}^+/\text{Na}^+$  content in tomato leaves under salinity was significantly ( $p \leq 0.01$ ) affected by calcium treatments (Table 2). Under non-saline conditions, the highest  $\text{K}^+/\text{Na}^+$  ratio (2.80) was observed in  $0 \text{ mM Ca}$  and the lowest value (2.54) was found from  $10 \text{ mM Ca}$ . It was observed that, more  $\text{Na}^+$  ions were found in non-calcium treated plants and calcium reduced the  $\text{Na}^+$  translocation to the shoot portion. In saline conditions, an increase in  $\text{Ca}^{2+}$  can reduce  $\text{Na}^+$  uptake in view of cation competition; therefore,  $\text{K}^+/\text{Na}^+$  ratio rises (Tabatabaei, 2006). Earlier research workers commented that  $\text{Ca}^{2+}$  is thought to improve the  $\text{K}^+/\text{Na}^+$  selectivity of membrane (Marschner, 1995) and prevent the cell from invasion of toxic ions (Cramer et al., 1987).

The interaction between salinity and calcium levels had significant ( $p \leq 0.01$ ) effect on  $\text{K}^+/\text{Na}^+$  ratio in tomato leaves (Table 2). The highest  $\text{K}^+/\text{Na}^+$  ratio (4.90) was found from the  $0 \text{ dS m}^{-1}$  Na with  $0 \text{ mM Ca}$  whereas, the lowest (1.03) was recorded in  $8 \text{ dS m}^{-1}$  Na with  $10 \text{ mM Ca}$  which was statistically equal to  $8 \text{ dS m}^{-1}$  Na and  $0 \text{ mM Ca}$ ,  $8 \text{ dS m}^{-1}$  Na and  $5 \text{ mM Ca}$  treatment combinations. The results showed that supplemental  $\text{Ca}^{2+}$  affected both  $\text{Na}^+$  and  $\text{K}^+$  content, with ameliorative effects on leaf  $\text{K}^+$  being at least as strong as on  $\text{Na}^+$ . Zhong and Lauchli (1994) concluded that one possible mechanism by which supplemental  $\text{Ca}^{2+}$  alleviates the inhibitory effects of  $\text{NaCl}$  on cotton root growth is by maintaining plasma membrane selectivity of  $\text{K}^+$  over  $\text{Na}^+$ . A wheat mutant with enhanced capacity for  $\text{K}^+$  accumulation in leaves was more salt-tolerant than a wild type (Rascio et al., 2001). The recently described salt-tolerant *stl2* mutation in the fern *Ceratopteris*

*richardii* involves an enhanced influx of  $\text{K}^+$  and higher selectivity for  $\text{K}^+$  over  $\text{Na}^+$  (Warne et al., 1995). Thus, it appears that  $\text{K}^+$  transporters may be another target for supplemental  $\text{Ca}^{2+}$  in mediating ameliorating  $\text{Ca}^{2+}$  effects in salinized plants, in addition to the regulation of nonselective cation channels (Demidchik and Tester, 2002; Tester and Davenport, 2003).

### $\text{Ca}^{2+}/\text{Na}^+$ ratio in leaves

The effect of salinity was statistically significant ( $p \leq 0.01$ ) on  $\text{Ca}^{2+}/\text{Na}^+$  ratio in tomato leaves (Table 2). The highest  $\text{Ca}^{2+}/\text{Na}^+$  ratio (2.73) was found in a non-saline condition which was gradually decreased with the increase of salinity levels. The lowest  $\text{Ca}^{2+}/\text{Na}^+$  ratio (0.80) was recorded from  $8 \text{ dS m}^{-1}$  Na, possibly the higher levels of salinity decreased the  $\text{Ca}^{2+}$  uptake, whereas the  $\text{Na}^+$  uptake was increased.  $\text{Ca}^{2+}/\text{Na}^+$  ratio was also decreased with increased salinity concentration which was reported by Shabala et al. (2003); Patel (2010).

Different levels of calcium significantly ( $p \leq 0.01$ ) increased the  $\text{Ca}^{2+}/\text{Na}^+$  ratio in leaves of tomato over the control plant (Table 2). The highest  $\text{Ca}^{2+}/\text{Na}^+$  ratio (1.88) was noted in  $10 \text{ mM Ca}$  which was statistically equal to  $5 \text{ mM Ca}$  while, the lowest ratio (1.55) was found in  $0 \text{ mM Ca}$ . Application of  $\text{Ca}^{2+}$  significantly increased the  $\text{Ca}^{2+}/\text{Na}^+$  ratio (Lolaei et al., 2012; Sivasankaramoorthy, 2013). The results for  $\text{Ca}^{2+}/\text{Na}^+$  ratios suggest that  $\text{Ca}^{2+}$  might have played an important role in maintaining the proper functioning of biological membranes integrity with ion-transport regulation as reported by Maathuis and Amtmann (1999) and their permeability was influenced similarly, as reported by Kent and Lauchli (1985), thereby resulting in relatively normal growth.

Salinity and calcium levels exerted significant ( $p \leq 0.01$ ) effect on  $\text{Ca}^{2+}/\text{Na}^+$  ratio in tomato leaves in case of their interaction effect (Table 2). The maximum  $\text{Ca}^{2+}/\text{Na}^+$  ratio (2.86) was found from  $0 \text{ dS m}^{-1}$  Na with  $5 \text{ mM Ca}$  which was statistically similar with  $0 \text{ dS m}^{-1}$  Na and  $10 \text{ mM Ca}$ ,  $0 \text{ dS m}^{-1}$  Na and  $0 \text{ mM Ca}$ . Calcium increased the  $\text{Ca}^{2+}/\text{Na}^+$  ratio in tomato leaves under saline condition, which was decreased with increased levels of salinity.

**Table 2. Effect of salinity and/or calcium level in ion variation and stomatal conductance of tomato leaves**

Tablica 2. Utjecaj razine saliniteta i/ili kalcija na promjenu sadržaja iona i provodljivost puči kod listova rajčice

| Salinity level                 |       | K <sup>+</sup> / Na <sup>+</sup> | Ca <sup>2+</sup> /Na <sup>+</sup> | Stomatal conductance (mol m <sup>-2</sup> s <sup>-1</sup> ) |
|--------------------------------|-------|----------------------------------|-----------------------------------|---|
| 0 dS m <sup>-1</sup> Na        |       | 4.32 a                           | 2.72 a                            | 0.08 a  |
| 2 dS m <sup>-1</sup> Na        |       | 3.06 b                           | 2.18 b                            | 0.06 b  |
| 4 dS m <sup>-1</sup> Na        |       | 2.88 b                           | 1.79 c                            | 0.05 b  |
| 6 dS m <sup>-1</sup> Na        |       | 1.77 c                           | 1.31 d                            | 0.03 b  |
| 8 dS m <sup>-1</sup> Na        |       | 1.23 d                           | 0.80 e                            | 0.02 b  |
| LSD <sub>(0.05)</sub>          |       | 0.208                            | 0.247                             | 0.026   |
| Level of significance          |       | **                               | **                                | **  |
| Calcium level                  |       |                                  |                                   |   |
| 0 mM Ca                        |       | 2.80 a                           | 1.55 b                            | 0.06 a  |
| 5 mM Ca                        |       | 2.62 b                           | 1.85 a                            | 0.04 b  |
| 10 mM Ca                       |       | 2.54 b                           | 1.88 a                            | 0.03 b  |
| LSD <sub>(0.05)</sub>          |       | 0.161                            | 0.192                             | 0.020   |
| Level of significance          |       | **                               | **                                | **  |
| Salinity level × Calcium level |       |                                  |                                   |   |
| 0 dS m <sup>-1</sup> ×         | 0 mM  | 4.90 a                           | 2.60 ab                           | 0.09 a  |
|                                | 5 mM  | 3.80 c                           | 2.86 a                            | 0.06 b  |
|                                | 10 mM | 4.25 b                           | 2.72 ab                           | 0.05 bc   |
| 2 dS m <sup>-1</sup> ×         | 0 mM  | 3.375 d                          | 2.01 c-e                          | 0.04 bc   |
|                                | 5 mM  | 3.10 de                          | 2.17 cd                           | 0.04 bc   |
|                                | 10 mM | 2.70 f                           | 2.36 bc                           | 0.04 bc   |
| 4 dS m <sup>-1</sup> ×         | 0 mM  | 2.83 ef                          | 1.63 ef                           | 0.05 bc   |
|                                | 5 mM  | 3.20 d                           | 1.84 d-f                          | 0.05 bc   |
|                                | 10 mM | 2.60 f                           | 1.90 d-f                          | 0.04 bc   |
| 6 dS m <sup>-1</sup> ×         | 0 mM  | 1.53 hi                          | 0.81 g                            | 0.04 bc   |
|                                | 5 mM  | 1.68 h                           | 1.49 f                            | 0.03 bc   |
|                                | 10 mM | 2.10 g                           | 1.63 ef                           | 0.02 c  |
| 8 dS m <sup>-1</sup> ×         | 0 mM  | 1.35 h-j                         | 0.70 g                            | 0.03 bc   |
|                                | 5 mM  | 1.30 ij                          | 0.90 g                            | 0.02 bc   |
|                                | 10 mM | 1.03 j                           | 0.79 g                            | 0.01 c-e  |
| LSD <sub>(0.05)</sub>          |       | 0.361                            | 0.428                             | 0.045   |
| Level of significance          |       | **                               | **                                | **  |

\*\*: significant at  $p \leq 0.01$ Different lowercase letters beside the mean value indicate significant at  $p \leq 0.05$  or  $p \leq 0.01$ 

### Stomatal conductance

Stomatal conductance was significantly ( $p \leq 0.01$ ) affected by different levels of salinity (Table 2). Results revealed that stomatal conductance decreased with the increase of salinity levels. The highest stomatal conductivity (0.08 mol m<sup>-2</sup> s<sup>-1</sup>) was recorded in 0 dS m<sup>-1</sup> Na and the lowest value (0.02 mol m<sup>-2</sup> s<sup>-1</sup>) was observed in 8 dS m<sup>-1</sup>. Stomatal conductance decrease was likely caused by the osmotic component of salt stress (Munns, 2002). Zuccarini (2008) argued that salinity decreased growth, stomatal conductance and net photosynthetic rate. It was reported that short term exposure to high salinity leads to an immediate and significant drop in stomatal conductance, due to osmotic stress and local synthesis of ABA in barley (Fricke et al., 2004; 2006). These findings were also supported by Reddy et al. (1992), Netondo et al. (2004).

Different levels of calcium significantly ( $p \leq 0.01$ ) affected the stomatal conductance (Table 2). Stomatal conductance decreased with the increase of calcium levels. The highest stomatal conductance (0.06 mol m<sup>-2</sup> s<sup>-1</sup>) was found in control treatment and the lowest (0.03 mol m<sup>-2</sup> s<sup>-1</sup>) was recorded in 10 mM Ca concentration. It was observed that the stomatal conductance was not increased by the application of calcium which was dissimilar to the findings of Tzortzakis (2010).

Interaction effect of different salinity and calcium levels showed significant ( $p \leq 0.01$ ) difference on stomatal conductance of tomato (Table 2). The highest stomatal conductance (0.09 mol m<sup>-2</sup> s<sup>-1</sup>) was recorded from 0 dS m<sup>-1</sup> Na with 0 mM Ca treatment combination whereas the lowest (0.01 mol m<sup>-2</sup> s<sup>-1</sup>) was recorded in 8 dS m<sup>-1</sup> Na with 10 mM Ca. There are only a few, controversial, reports on the effects of supplemental Ca<sup>2+</sup> on stomatal conductance in response to salinity stress. Perera et al. (1995) reported that elevated Ca<sup>2+</sup>

levels reduced detrimental NaCl effects on stomatal conductance. However, additional  $\text{Ca}^{2+}$  was not able to ameliorate NaCl-induced reduction in stomatal conductance, transpiration, and xylem water potential in blueberry (Wright et al., 1993). Our study showed that higher salinity levels reduced stomatal conductance which were not influenced by application of  $\text{Ca}^{2+}$ .

## CONCLUSION

Considering the above mentioned results, it can be concluded that salt treatment increased  $\text{Na}^+$  concentration significantly in tomato leaves, whereas  $\text{K}^+$  and  $\text{Ca}^{2+}$  concentration was decreased, indicating the toxicity effect of salinity. While exogenous application of calcium reduced the  $\text{Na}^+$  concentration through increasing  $\text{K}^+$  and  $\text{Ca}^{2+}$  concentration as well as saline toxicity, the symptoms were reduced. The  $\text{Na}^+/\text{K}^+$  ratio was also decreased by the application of calcium, which helped the plants to overcome the toxic effects of salinity. Therefore, this experiment suggests that  $\text{Ca}^{2+}$  can effectively mitigate the deleterious effect of  $\text{Na}^+$  stress through up-regulation of the ionic concentration in tomato plant.

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## PROMJENA U USVAJANJU IONA KOD RAJČICE (*Lycopersicon esculentum* L.) PRIMJENOM KALCIJA U UVJETIMA SOLNOG STRESA

### SAŽETAK

*Salinitet utječe na gotovo svaki aspekt fiziologije i biokemije biljaka zbog osmotskoga stresa i ionske toksičnosti. Istraživali smo promjenu usvajanja iona kod rajčice cv. BARI Tomato-5 pri različitim razinama saliniteta (0, 2, 4, 6 i 8 dS m<sup>-1</sup>) u kombinaciji s različitim koncentracijama Ca<sup>2+</sup> (0, 5, 10 mM). Rezultati su pokazali da solni stres značajno utječe na provodljivost puči kod rajčice. Tretman soli izrazito je povećao usvajanje Na<sup>+</sup> i smanjio usvajanje K<sup>+</sup> i Ca<sup>2+</sup> u listovima rajčice. U uvjetima solnoga stresa tretman s Ca<sup>2+</sup> smanjio je usvajanje Na<sup>+</sup> a povećao usvajanje Ca<sup>2+</sup> i K<sup>+</sup>. Naši su rezultati pokazali da kod rajčice aplikacija Ca može učinkovito smanjiti ionsku toksičnost induciranu salinitetom. Vanjska primjena Ca<sup>2+</sup> značajno ublažava negativne učinke ionske toksičnosti inducirane salinitetom.*

*Ključne riječi: kalcij, ionska selektivnost, Lycopersicon esculentum L., salinitet, provodljivost puči*

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