
THE INFLUENCE OF SALINITY AND DROUGHT STRESS ON SODIUM, POTASSIUM AND PROLINE CONTENT OF SOLANUM LYCopersicum L. cv. RIO GRANDE

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Abstract

The influence of salinity and drought stress on sodium (Na+), potassium (K+) and proline content of Solanum lycopersicum L. (tomato) cv. Rio Grande was investigated by exposing the plants to five salinity levels i.e., 0 (Control), 50, 100, 150 and 200 mM NaCl and four drought regimes i.e. 0 (Control), 2, 4 and 6 days, applied from seedling (4-5 true leaves) to the harvesting stage. The means across salinity levels showed an increase in proline content and Na+ concentration but a reduced K+ concentrations, resulting in high Na+/K+ ratios in shoot and root tissue. In contrast, drought stress decreased the Na+ and K+ content, Na+/K+ ratio but increased the proline content in both the root and shoot tissue. The interaction of salinity and drought significantly affected the sodium (Na+) and potassium (K+) contents, Na+/K+ and proline content of the shoot but K+ content and proline accumulation were not significant. The root and shoot tissue of control plants (0 mMNaCl + 0 Days drought stress) had the minimum Na+ content (2316 and 3490 µMg D.wt.), Na+/K+ ratio (0.399 and 0.364) and proline content (0.72 and 1.91 µMg F.wt.) but the highest K+ content (6399 and 9603 µMg D.wt.). Whereas, the Na+ content increased with salinity, the K+ content declined. It resulted in the maximum Na+/K+ ratio of the root (1.26) and shoot (0.76) with 200 mMNaCl + 0 Days drought stress. The drought stress also increased the Na+/K+ ratio. Thus, the highest Na+/K+ ratio of root (0.78) and shoot (0.77) was recorded in plants grown under 200 mMNaCl + 6 Days drought stress. The proline content of the root and shoot were 0.462 and 1.904 µMg F.wt. respectively in control plants which increased with increasing salinity and drought stress duration. Thus, the maximum proline content of root (10.61 µMg F.wt.) and shoot (28.05 µMg F.wt.) was recorded in plants exposed to 200 mMNaCl + 6 days drought stress combination.

Key words: Tomato, Salinity, Drought, Proline, Na+ and K+

Introduction

Tomato is a major vegetable crop in Pakistan, but the yield is lower than international average (Imran et al., 2012). Soil salinity and limited irrigation are among the serious limitations in increasing tomato crop productivity (Araus et al., 2002). Soil salinity adversely affects crop production throughout the world (Pervez et al., 2009). Salinity problem may emerge when precipitation is not enough to leach the excess soluble salts from the root zone (Hug & Shoaih, 2013) or where salt rich water is used for irrigation (Marcum, 2006). Water deficit and subsequent drought stress is most common in countries of the arid and semi arid regions (Oliveira et al., 2013). The global climate changes may further increase the severity of droughts, especially during the summer months of the year (Hamdy et al., 2003; Munns, 2005). Due to limited water availability, the farmers are forced to use poor quality water and suboptimum irrigation (Dayal & Chauhan, 2010). The salinity and drought stress are present at the same time in the arid and semiarid regions (Gonzalez et al., 2012) and constitute the major abiotic stresses causing decreased plant growth and crop productivity. High soluble salts in the soil solution increase the osmotic pressure resulting ion toxicity (Teakle & Tyerman, 2010) and low water potential and nutrient uptake by the plants (Tavakkoli et al., 2011). Soil salinity and water deficit also decline the rate of photosynthesis, transpiration and other biochemical processes associated with plant growth and productivity (Tiwari et al., 2010). While different crops may vary in their sensitivity to salinity (Farooq et al., 2008) and drought, they require normal functioning despite high salts concentration in the tissues (Rajendran et al., 2009). The toxic effect of salts can be reduced by compartmentalization of ions at cellular and whole-plant level, synthesis of compatible solutes, change in photosynthetic pathway, alteration in membrane structure, induction of antioxidative enzymes (Ashraf & Harris, 2004; Parida & Das, 2005; Turkan & Demiral, 2009; Flowers et al., 2010). Ions uptake at the optimum levels is crucial for growth (Tavakkoli et al., 2011) and the excess salts is compartmentalized in the vacuole (Zhu, 2003). In saline conditions, excessive Na+ in the rhizosphere and subsequent uptake adversely affect metabolism and causes physiological droughts (Giannakoula & Ilias, 2013). Excess sodium inhibits uptake of K+, Ca2+, Mg2+ and NO3, the mineral elements essential for growth (Ahmad & Jabeen, 2005). The decline in uptake of K+, Ca2+, Mg2+ and NO3 results in lower Na+/K+, Na+/Ca2+ and Na/Mg2+ ratios (Hakim et al., 2014). Since access sodium ions are sequestered in vacuoles (Brini & Masmoudi, 2012), it is balanced osmotically by the synthesis of compatible solutes, such as proline (Shahid et al., 2013).

Drought stress is another serious problem that may, sometime, accompany the salinity stress (Giannakoula & Ilias, 2013). Tomato is drought sensitive plant and a short period of drought can cause significant decrease in yield. It requires 70 mm of water per week during hot and dry season (Shankara et al., 2005). An optimum water supply decreases the incidence of blossom end rot in tomato fruit (Vossen et al., 2004). Drought stress decreases the growth and reproduction of tomato plants (Pervez et al., 2009; Vijitha & Mehendran, 2010) by adversely affecting the availability, transport and partitioning of nutrients.
(Makela et al., 2002). The drought stress may further aggravate the salinity induced damage (Leogrande et al., 2012). The present research was therefore conducted to investigate the influence of salinity and drought stress on the sodium and potassium contents, alterations in Na⁺/K⁺ and proline synthesis in root and shoot of tomato plants.

Materials and Methods

The influence of salinity and drought stress on sodium, potassium and proline content of tomato cv. Rio Grande plants was investigated at Center of Plant Biodiversity and Botanical Garden, Nowshera during the crop years 2011-2012. The experimental site is located about 271 meters above the sea level with a sub-humid climate and average annual rainfall of 550 mm (Harris et al., 2002). The maximum temperature during the summer may be as high as 45-49°C. The roots and shoot of tomato were examined for sodium, potassium contents, sodium/potassium ratio and proline content at different levels of salinity and drought. The experiment was conducted according to two factorial randomize complete block design (RCBD) with five salinity levels i.e. control, 50, 100, 150 and 200mM of NaCl, applied with first irrigation and drought regimes i.e., 0, 2, 4, 6, days. There were three replications of each treatment and 3 plants in each replication.

For planting tomato seedlings, a circular structure of 54 cm diameter and 72 cm depth was made. The surface of the hole was lined with thick plastic sheet. Three tubes (54 × 36 cm, containing 9 kg media) were placed in each hole. The tubes were perforated at the bottom to allow absorption of salt solution by the rooting medium. Equal amounts of saline solution were applied to each whole to moisten the whole media. A 3.6 meter high lath house structure with G.I. pipe was constructed over the experimental plot and covered with transparent plastic sheet when needed to avoid rain water to the experimental plot.

The following parameters were studied during the course of experiments:

Sodium and potassium contents: Both the roots and shoot sample were collected after harvesting the fruits. For analysis of Na⁺ and K⁺ content, young shoots bearing 4-5 leaves were taken. For root analysis, plants were carefully removed from the growth container. The roots system was thoroughly washed with water to remove the soil particles. Clean roots were used for estimating Na⁺ and K⁺ content.

Sodium content of the tissue was determined using the method of Watad et al. (1986). The tissue samples were oven dried at 80°C till a constant weight was achieved. The dried samples of shoots and roots were ground into a fine powder for wet digestion. For wet digestion, 5 ml of concentrated nitric acid were added to 0.2g of each ground sample. The samples were then kept at room temperature for 48 hours. On the next day the samples were placed in a hot-block set to 90°C for approximately two hours. When no further color change was seen and sample particulates were no longer visible, the sample was removed from the hot block and allowed to cool and raised the volume of extract up to 50 ml by adding double distilled water. Samples were then analyzed for sodium content by flame photometer (JENWAY PFP7). The values obtained from flame photometer were then used to calculate the sodium content (µM/g D.wt).

Potassium content of the tissue was determined using the method of Watad et al. (1986). The same solution (as for sodium content) was used for the determination of potassium content in roots and shoots by flame photometer (JENWAY PFP7).

Sodium – Potassium ratio: After determining the Na⁺ and K⁺ content, the Na⁺/K⁺ ratios in the root and shoots were calculated.

Proline content in root and shoot tips and in leaves: Proline was determined by the method of Bates et al. (1973). For this purpose 0.2g of fresh and young tips from each sample of shoot and root were taken and dip into liquid nitrogen for 2-3 minutes immediately after harvest. The tissues were then crushed with a tissue miser and then homogenized with 4 ml of 3% sulfoalicylic acid (C₆H₆O₃ S·2H₂O). The homogenate was then centrifuged at 3000 rpm for five minutes at room temperature. The supernatant were filtered through Whatman No. 2 filter paper and again mix a 4 ml of 3% sulfoalicylic acid. The filtrates were then reacted with 2cm³ acid ninhydrin in a test tube in boiling water bath for one hour. Reaction was terminated in an ice bath. Reaction mixture was extracted with 4cm³ toluene and tubes were cool down to room temperature. Absorbance was measured at 520 nanometer against toluene blank. The values obtained were then used for calculating the proline content (µM/g F.wt).

Results and Discussion

Shoot sodium content: Salinity and drought significantly affected the sodium concentration in shoots of tomato plants (Table 1). The sodium content of tomato shoots was the least (3870 µM/g D.wt.) in the plants treated with 0 mMNaCl solution that increased significantly to 4448, 4724 and 4880 µM/g D.wt. with 50, 100 and 150 mMNaCl treatment respectively. The difference in 100 and 150 mMNaCl stress was, however, non-significant. The shoot sodium content increased significantly with increase in levels of NaCl stress to 200 mM. The sodium content of non stressed plants was 4293 µM/g D.wt. and was non significant with 4346 µM/g D.wt. after 2 days drought stress but, thereafter, increased significantly to 5241 µM/g D.wt. with increasing drought stress duration to 4 days. The shoot sodium content, however, declined to 4421 µM/g D.wt. with increasing drought stress to 6 days. The interaction between salinity and drought stress revealed the highest sodium content (5846 µM/g D.wt.) in the shoots of plants exposed to 200 mMNaCl stress and 4 days drought stress, that was non significantly different from 200 mMNaCl and 2 days drought stress and 100 mMNaCl and 4 days drought stress. Whereas 200 mMNaCl and 6 days drought stress resulted in the least sodium content in tomato shoots (Fig. 1).
Table 1. Effects of salinity and drought on sodium and potassium contents of tomato plant root and shoot.

<table>
<thead>
<tr>
<th>Salinity levels</th>
<th>Sodium content (µM/g D.wt)</th>
<th>Potassium content (µM/g D.wt)</th>
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<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>0 mM</td>
<td>3870 d</td>
<td>2337 e</td>
</tr>
<tr>
<td>50 mM</td>
<td>4448 c</td>
<td>2766 d</td>
</tr>
<tr>
<td>100 mM</td>
<td>4724 b</td>
<td>3180 c</td>
</tr>
<tr>
<td>150 mM</td>
<td>4880 ab</td>
<td>3376 b</td>
</tr>
<tr>
<td>200 mM</td>
<td>4955 a</td>
<td>3577 a</td>
</tr>
<tr>
<td>LSD at 0.05</td>
<td>206.7</td>
<td>114.3</td>
</tr>
<tr>
<td>Percent change</td>
<td>28.04%</td>
<td>53.06%</td>
</tr>
</tbody>
</table>

Drought

- 0 days: 4293 b, 3748 a, 7779 a, 4925 a
- 2 days: 4346 b, 3328 b, 6599 b, 4892 a
- 4 days: 5241 a, 2664 c, 6461 b, 4588 b
- 6 days: 4421 b, 2450 d, 6814 b, 4333 c
- LSD at 0.05: 184.9, 102.2, 363.2, 211.8
- Percent change: 22.1%, 34.63%, 16.9%, 12.02%

Salinity × Drought

- LSD at 0.05: 412.4, 228.5, 812.2, Ns

Means followed by similar letters in a column are non significantly different from each other at α 0.05

The plants accumulate excessive Na⁺ and Cl⁻ ions in the leaves under salinity stress (Roy & Mishra, 2014). Thus, the build-up of the sodium ion (Na⁺) in the cytoplasm of leaf cells is a major effect of salinity stress (Jha et al., 2010). The mean increase in Na⁺ ions in the shoot system was 34.67% higher with 200 mM NaCl treatment (Blaha et al., 2000; Tester & Davenport, 2003; Munns et al., 2006; Munns & Tester, 2008). Thus, the control of Na⁺ transport at both the tissue and cellular level is major mechanism of salinity tolerance (Tester & Davenport, 2003; Apsé & Blumwald, 2007; Munns & Tester, 2008). Drought stress decreases the nutrient uptake by the roots and its transportation to the shoots due to decrease in rate of transpiration, impaired active transport and membrane permeability (Yuncal & Schmidhalter, 2005). The decline in nutrient uptake may also be due to low soil moisture, which hinders the diffusion rate of nutrients in the soil to the absorbing root surface (Raynaud & Leadlay, 2004), thus low sodium content of shoot system was observed with increasing drought stress duration.

Root sodium content: Salinity, drought and their interaction significantly affected the root sodium content. The mean root sodium content increased with increasing levels of salinity from the least (2337 µM/g D.wt) in the control condition (0 mMNaCl stress) to 2766, 3180 and 3376 µM/g D.wt with 50, 100 and 150 mMNaCl treatment respectively. The maximum sodium content (3577 µM/g D.wt) was observed in plants exposed to 200 mMNaCl. By contrast, the sodium content of the plant’s root decreases significantly with longer duration of drought stress. The highest Na⁺ level (3748 µM/g D.wt) with 0 days drought stress (control) declined to the least (2450 µM/g D.wt) with 6 days drought stress treatment. Sodium content of the root was 3328 and 2664 µM/g D.wt with 2 and 4 days drought stress treatments respectively (Table 1). As the sodium content increase with salinity and decrease with drought, therefore the interaction effect was also significant (Fig. 2). The plants exposed to 0 mMNaCl and 6 days drought stress had the least (2042 µM/g D.wt) sodium ions in the root, while 200 mMNaCl and 0 days drought stress had the highest sodium content (4925 µM/g D.wt). Salinity stress increases the uptake and accumulation of sodium and chloride ions that reduces the uptake of other mineral nutrients, such as potassium and calcium (Sudhir & Murthy, 2004). The Na⁺ uptake at the root/soil boundary is achieved by less selective system than other cations (Tester & Davenport, 2003). The sodium can enter plant cells through several types of channels: low-affinity inward-rectifying K⁺ channels (Kronzucker et al., 2013), voltage independent channels (Maathuis & Sanders, 2001) and non-selective cation channels (Demidchik & Tester, 2002). Hence increase Na⁺ in the growing medium increase its uptake. The drought stress increased the Na⁺ concentration in roots but severe drought decreased its uptake (Raza et al., 2013). Thus, it is suggested that the rate of Na⁺ translocation from root to shoot was more limited than that of other cations i.e., K⁺ in water stresses (Raza et al., 2013). While concentration of Na⁺ in roots decreased under severe water deficit, that is independent from leaf Na⁺ content, due to positive correlation with root relative water content (Fayyaz et al., 2013).

Shoot potassium content: The potassium content of the tomato shoots decreased significantly with increasing salinity levels. The highest potassium content (8906 µM/g D.wt) in shoots in control plants (0 mMNaCl) decreased significantly to 7596, 7074 and 5841 µM/g D.wt. with increasing salinity levels to 50, 100 and 150 mMNaCl respectively. The least potassium content (5149 µM/g D.wt.) was recorded in the shoots of the plants treated with 200 mMNaCl solution. Drought stress also reduced the potassium concentration in the shoots of tomato plants. The highest mean potassium content was 7779 µM/g D.wt. in control plants (0 days drought stress) that decreased significantly to 6500 µM/g D.wt. in plants exposed to 2 days drought stress duration. Further increase in drought stress to 4 or 6 days, however, did not have any significant effects (Table 1). The interaction between salinity and drought stress revealed the highest potassium content (9603 µM/g D.wt.) in shoots of tomato plants grown in control conditions (0 mM salinity and 0 days drought stress), that gradually decreased with increasing levels of salinity or drought stress duration. The potassium content of the shoot was the least (4393 µM/g D.wt.) in plants grown under 200 mMNaCl stress and 4 days drought stressed (Fig. 3).
Potassium nutrition is known to be disturbed under salt stress (Akram et al., 2007). Beside a macronutrient required for plant growth and development (Amjad et al., 2014), potassium ion (K⁺) is a prominent inorganic plant solute that contribute to lower the osmotic potential in the stele of roots resulting in turger pressure development and solute transport in xylem (Shabalal et al., 2010). Thus, an optimum level of potassium content may help in osmotic adjustment, maintenance of turgor at low leaf water potentials and, thus, minimize the adverse effects of drought and salinity stress (Wang et al., 2013). The maximum potassium content in the shoots of the non-stressed (control) plants and the minimum with plant stressed with 200mM NaCl reveals a clear inhibition of potassium ions uptake by the roots (Tester & Davenport, 2003) and/or its transportation from the roots to the plant shoot (Garcia & Medina, 2013). It has been observed that transcript level of several K⁺ transporter genes decline under salinity stress (Su et al., 2002) and the decline in K⁺ contents of the xylem and shoot (Moshiae et al., 2014) and the expanding leaf tissue (Su et al., 2001) indicate a decrease in the transport of K⁺ (Wang et al., 2013). Yet another reason for low K⁺ uptake may be due its competition with Na⁺ uptake through Na⁺- K⁺ co-transporters, which may also block K⁺ specific transporters of root cell under salinity (Zhu, 2002). In the present study, the K⁺ uptake was reduced in all treatments under salinity stress that could be due to excessive Na⁺ that is known to antagonize K⁺ uptake (Sarwar & Ashraf, 2003). While, K⁺ through its osmotic adjustment effect may decrease the adverse effects of salinity on the plants (Jabeen & Ahmad, 2012) because high K⁺ content are commonly observed in salt tolerant species (Ashraf & Sarwar, 2002; Ashraf et al., 2005). Similarly, plant species expressing low reduction in potassium despite saline conditions, are generally more tolerant to salinity (Wang et al., 2013).

**Root potassium content:** Significant differences of potassium content were observed in roots of tomato plants treated with different salinity levels and irrigation regimes while the interaction effect was non significant (Table 1). Plants exposed to 0 mMNaCl had the highest mean root potassium content (5639 µM/g D.wt.) followed by 5200 µM/g D.wt. in plants exposed to 50 mMNaCl stress. The root potassium content decreased to 4661 and 4098 µM/g D.wt. with 100 and 150 mMNaCl treatments respectively and finally to the least (3826 µM/g D.wt.) in the plants exposed to 200 mMNaCl. Drought stress duration of 4 and 6 days also decreased the potassium content of tomato roots from 4925 µM/g D.wt. in control plants to 4588 and 4333 µM/g D.wt. with 4 and 6 days drought stress respectively (Table 1). The interaction of salinity and drought stress was, however, non significant.

Generally there is an increased uptake of sodium and chloride ions with a decline in the uptake of other mineral nutrients, such as potassium in plant grown under salinity stress (Sudhir & Murthy, 2004). It is observed that high concentration of external Na⁺ ions decrease the intracellular potassium (K⁺) influx by affecting the transport of ions across plasmalemma of root cells through rupturing of the cellular membranes (Alleva et al. 2006). Thus, it increases the accumulation of Na⁺ and Cl⁻ ions while decreases K⁺ accumulation (Al-Karaki, 2000). Limited water availability also adversely affects the nutrient uptake capability of root (Ge et al., 2013), due to decreased rate of transpiration, impaired active transport and membrane permeability (Akinci & Losel, 2012). Thus, K⁺ uptake by roots is diminished (Nahar & Gretzmacher, 2002).

**Shoot sodium potassium ratio:** The Na⁺/K⁺ ratio in shoots increased significantly with increasing salinity levels, drought stress duration and the interaction of both the stresses (Table 2). The means across salinity revealed the least (0.439) Na⁺/K⁺ ratio, which increased to the maximum (0.992) in the shoots of plants exposed 200 mMNaCl.

Drought stress also increased the Na⁺/K⁺ ratio from the minimum of 0.572 in control plants to 0.708 with 2 days drought stress and finally to the maximum of 0.879, when tomato plants were exposed to 4 days drought stress. However, increasing drought stress to 6 days decreased sodium/potassium ratio of tomato shoots to 0.676 (Table 2). This might be due to less absorption of saline water in drought stress condition.

The interaction between salinity and drought had an additive effect of the Na⁺/K⁺ ratio of the shoot. It was the least (0.346) in control plants that increased with increasing salinity or drought stress duration. While the increase in Na⁺/K⁺ ratio of tomato shoots was relative less pronounced as a function of drought stress, it increased drastically to 0.760 with 200 mMNaCl and 0 days drought. At the same salinity levels and 4 days drought stress Na⁺/K⁺ ratio was the maximum (1.351) and declined with further increase in drought stress to 6 days (Fig. 4).

Increasing salinity stress levels resulted in a significant increase in Na⁺ content and a considerable decrease in K⁺ content, resulting in a significant increase in the sodium potassium ratio (Table 2). Increasing Na⁺ concentration leads to toxic effects on plant growth due to increased sodium/potassium ratio and K⁺ displacement by Na⁺ in the plant cell that may affect the plasma membrane associated H⁺-ATPase (Wakeel et al., 2011). According to Blumwald et al. (2000), the decrease in K⁺ concentration in salinity stress is caused by high external Na⁺ concentration. It is well evident from the observations that high Na⁺ and low K⁺ accumulation occur in tomato leaves with increase salt concentration (Al-Karaki, 2000) whereas adding K⁺ to NaCl and water deficit stressed plants ultimately decreased Na⁺, increased K⁺ content and thus, decreased the Na⁺/K⁺ ratio (Wakeel et al., 2011). Since, the Na⁺ content increased in tomato shoot with increasing NaCl doses and decreased with water deficit condition, and the K⁺ content decreased with increasing NaCl doses and drought stress.

**Root sodium potassium ratio:** The Na⁺/K⁺ ratio of tomato root increased with increasing salinity levels but decreased with increase in drought stress duration (Table 2). The Na⁺/K⁺ ratio of the root was the lowest (0.416) with 0 mMNaCl (control) that increased significantly to 0.530, 0.683 and 0.820 with increasing salinity stress to 50, 100 and 150 mMNaCl respectively and finally to the maximum of 0.930 when the tomato plants were exposed to 200 mMNaCl (Table 2). The drought stress treatments had the opposite effect on the Na⁺/K⁺ ratio of tomato root. The highest root Na⁺/K⁺ ratio (0.804) in control plants decreased significantly to 0.703 with 2 days drought stress. Further, the Na⁺/K⁺ ratio of tomato root decreased significantly to 0.599 and 0.597 in plants exposed to 4 and 6 days drought stress respectively.
Fig. 1. Effect of salinity and drought on sodium content of tomato shoots. The vertical error bars represents LSD (412.4) at $\alpha = 0.05$.

Fig. 2. Effect of salinity and drought on sodium content of tomato roots. The vertical error bars represents LSD (228.5) at $\alpha = 0.05$.

Fig. 3. Effect of salinity and drought on potassium content of tomato shoots. The vertical error bars represents LSD (812.2) at $\alpha = 0.05$.

Fig. 4. Effect of salinity and drought on sodium potassium ratio of tomato shoots. The vertical error bars represents LSD (0.1280) at $\alpha = 0.05$.

Fig. 5. Effect of salinity and drought on sodium potassium ratio of tomato roots. The vertical error bars represents LSD (0.0739) at $\alpha = 0.05$.

Fig. 6. Effect of salinity and drought on proline content of tomato shoots. The vertical error bars represents LSD (3.095) at $\alpha = 0.05$. 
The interaction between salinity and drought stress also significantly affected the Na⁺/K⁺ ratio in tomato plants and was in conformity of the influence of both the stresses applied independently. The maximum (1.262) Na⁺/K⁺ ratio in the root of tomato plants was recorded with the combinations of 200 mMNaCl and 0 days drought stress treatment. While increasing drought stress to 2 days at the same saline stress levels (200 mMNaCl) decreased Na⁺/K⁺ ratio to 0.958 but decreasing salinity stress to 150 mMNaCl and drought stress to 0 days resulted in Na⁺/K⁺ ratio of 0.939. The minimum Na⁺/K⁺ ratio 0.399 was recorded in root of control plants (0 mMNaCl + 0 days drought stress), that was statistically at par with 2, 4 and 6 days drought stress treatments and a sodium potassium ratio of 0.461, 0.440 and 0.362 respectively (Fig. 5).

Since the plants grown in saline condition, accumulates more sodium than potassium ions (Sudhir & Murthy, 2004). Generally the increased accumulation of sodium causes potassium deficiency in salt stressed plants, indicating the existence of competition effects between sodium and potassium ions (Maggio et al., 2007). Because of the similarity between Na⁺ and K⁺ in their hydrated ionic radii (Munns, 2005), Na⁺ competes with K⁺ at the sites of entry and ultimately decrease the K⁺ uptake (Shabala et al., 2003).

During drought stress, root growth and the rates of K⁺ diffusion in the soil towards the roots are restricted, that limit K acquisition and absorption (Wang et al., 2013). Mineral elements uptake by crop plants is generally decreased under water stress conditions (Ashraf et al., 2013), but it is also evident from the present study that in severe drought stress (6 days drought) the Na⁺ uptake was more affected than K⁺, that resulted in decreased Na⁺/K⁺ ratio with 6 days drought stress.

**Shoot proline content:** Salinity, drought and their interaction significantly affected the shoot proline content of tomato plants (Table 2). At 0 mMNaCl level, the proline content in shoot was 3.713 µM/g F.wt. which increased to 6.150, 8.227, 15.698 µM/g F.wt. with increasing salinity stress to 50, 100 and 150 mMNaCl respectively. The highest proline content (22.867 µM/g F.wt.) was in plants exposed to 200 mMNaCl stress.

The proline content of tomato shoots increased slowly with increasing drought stress. The lowest proline content (8.933 µM/g F.wt.) in control plants increased significantly to 10.936 and 11.039 µM/g F.wt. with 2 and 4 days drought stress respectively. The difference in proline content of 2 and 4 days drought stressed plants was, however, non significant. Increasing drought stress to 6 days resulted in further increase in proline content to 14.415 µM/g F.wt. of tomato shoot (Table 2).

The interaction of salinity levels and drought stress duration also significantly affected the proline accumulation in shoots of tomato plants. The minimum proline content (1.904 µM/g F.wt.) was recorded in control condition, which increased significantly to 5.704 µM/g F.wt. after 6 days drought stress. In contrast, the minimum proline content (1.904 µM/g F.wt.) observed with 0 mMNaCl and 0 days drought stress increased to 16.494 µM/g F.wt. when salinity stress was increased to 200 mMNaCl despite 0 days drought stress. On the same level of salinity (200 mMNaCl), the proline content increased to 28.051 µM/g F.wt. with increasing drought stress condition to 6 days (Fig. 6).

### Table 2. Effects of salinity and drought on sodium/potassium ratio and proline contents of tomato plant root and shoot.

<table>
<thead>
<tr>
<th>Salinity levels</th>
<th>Sodium potassium ratio</th>
<th>Proline content (µM/g F.wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>0 mM</td>
<td>0.439 e</td>
<td>0.416 e</td>
</tr>
<tr>
<td>50 mM</td>
<td>0.589 d</td>
<td>0.530 d</td>
</tr>
<tr>
<td>100 mM</td>
<td>0.669 c</td>
<td>0.683 c</td>
</tr>
<tr>
<td>150 mM</td>
<td>0.856 b</td>
<td>0.820 b</td>
</tr>
<tr>
<td>200 mM</td>
<td>0.992 a</td>
<td>0.930 a</td>
</tr>
<tr>
<td>LSD at α 0.05</td>
<td>0.06402</td>
<td>0.3696</td>
</tr>
<tr>
<td>Percent change</td>
<td>125.97%</td>
<td>123.56%</td>
</tr>
<tr>
<td><strong>Drought</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 days</td>
<td>0.572 c</td>
<td>0.804 a</td>
</tr>
<tr>
<td>2 days</td>
<td>0.708 b</td>
<td>0.703 b</td>
</tr>
<tr>
<td>4 days</td>
<td>0.879 a</td>
<td>0.599 c</td>
</tr>
<tr>
<td>6 days</td>
<td>0.676 b</td>
<td>0.597 c</td>
</tr>
<tr>
<td>LSD at α 0.05</td>
<td>0.05726</td>
<td>0.03306</td>
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<tr>
<td>Percent change</td>
<td>53.67%</td>
<td>25.75%</td>
</tr>
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<td><strong>Salinity × Drought</strong></td>
<td></td>
<td></td>
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<tr>
<td>Fig. 4.</td>
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<td>Fig. 5.</td>
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<tr>
<td>Fig. 6.</td>
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<tr>
<td>LSD at α 0.05</td>
<td>0.1280</td>
<td>0.07392</td>
</tr>
</tbody>
</table>

Means followed by similar letters in a column are non-significantly different from each other at α 0.05.
Proline is a well known amino acid that generally accumulates when plants are exposed to environmental stresses (Kavi-Kishor et al., 2005). The accumulation of proline in plants grown under salinity stress is a common stress indicator and is associated with salt stress tolerance of different plant species (Demiral & Turkan, 2005). Enhanced proline synthesis is a common response of tomato plants to salinity and may determine the stress tolerance (Ali et al., 2011). Proline is believed to acts as a signaling molecule that initiates adaptation to the stress (Maggio et al., 2002), acts as osmolyte for osmotic adjustment (Hayat et al., 2012), helps in stabilizing membranes/proteins and scavenges free radicals (Ashraf & Foolad, 2007). Thus, it decreases the adverse effects of cytoplasmic acidosis and maintains proper NADP+/NADPH ratios (Liang et al., 2013). In plants grown under saline conditions, proline induces the expression of salt stress responsive genes and, thus, decreases the damage due to excessive Na⁺ ions accumulation (Chinnusamy et al., 2005). Proline act as a compatible solute in the plants (Mansour, 2000) and, generally, increases with increase in both the salinity stress and drought stress duration (Kishor & Sreenivasulu, 2014). Thus, it is likely to observe enhanced proline synthesis with increasing salinity levels or with drought stress duration.

**Root proline content:** The proline content in roots varied significantly with different levels of salinity and drought stress but the interaction of salinity and drought stress was not significant (Table 2). The root proline content at 0 mMNaCl stress was 2.010 µM/g F.wt. that increased to 2.917, 4.202 and 6.218 µM/g F.wt. with increasing salinity stress to 50, 100 and 150 mMNaCl respectively. The highest proline accumulation in the roots (9.530 µM/g F.wt.) was observed, when tomato plants were exposed to 200 mMNaCl treatment.

Drought also increased the proline content in roots of tomato plants. The concentration of proline in roots was 3.526 µM/g F.wt. in control plants that increased to 4.737 µM/g F.wt. with 2 days drought stress. The proline content of the roots increased further to 5.935 µM/g F.wt. with 4 days drought stress treatment but declined non-significantly to 5.704 µM/g F.wt. when the drought stress was extended to 6 days.

The accumulation of proline under stressful conditions especially salinity stress has been correlated with salt stress tolerance (Ali et al., 2011). The proline content in roots of alfalfa is found to increase eight fold when the plants are exposed to salt stress conditions (Trinchant et al., 2004). Similarly, salt-tolerant plants are known to accumulate high levels of proline in response to salinity (Demiral & Turkan, 2005). Increased proline synthesis is not associated with salinity stress alone but its accumulation is also commonly observed in plants subjected to drought stress. For example, in rice plants subjected to water deficit have higher proline concentration in the leaves (Hsu et al., 2003) and the rate of proline accumulation and utilization is significantly higher in the drought-tolerant cultivars (Nayyar & Walia, 2003). The protective role of enhanced proline synthesis is also evident from the fact that exogenous application of proline enhances the stress tolerance of the plant, probably due to its role as osmoprotectants (Noreen et al., 2013). For example, the adverse effects of salinity can be decreased (Bakht et al., 2012) and plant growth can be enhanced by exogenous application of proline in plants grown under saline conditions (Patade et al., 2014).

It can be concluded that salinity increase the Na⁺ of the root and shoot with concomitant decrease in K⁺ of the root and shoot, leading to increased Na⁺/K⁺ ratio. In contrast, extended drought stress (6 days) decreased the accumulation of Na⁺. As a result the Na⁺/K⁺ ratio increased in roots but showed a mixed trend in the shoot system with increasing drought stress duration. Salinity and drought stress also increased the synthesis and accumulation of proline in both the root and shoots system. However, the proline accumulation was greater in the shoot than the root system.

**References**


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