Research Article

Foliar Calcium Application Ameliorates Salinity-Induced Changes of Tomato Crop Grown in Saline Conditions

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Abstract | The influence of foliar calcium application on tomato crop grown in saline conditions was investigated by exposing tomato plants to 0, 75 and 150 mM salinity; and foliar application of 0.0, 0.25, 0.50, 0.75, 1.0, 1.25% calcium solutions. Salinity stress increased leaf Na^+ and Na^+/K^+, fruit firmness and blossom end rot (BER) incidence but significantly decreased the leaf K^+ and Ca content of the fruit and yield. The foliar calcium application decreased the Na^+ accumulation, Na^+/K^+ ratio and BER incidence as well as increased the leaf K^+ and Ca content of tomato fruit, yield and fruit firmness. The interaction of salinity and calcium significantly affected the yield and BER incidence of tomato fruit. Whereas, the yield of tomato decreased with increasing salinity levels, the decrease in yield was comparatively less with foliar calcium application. By contrast, salinity increased the BER incidence but the salinity-induced increase in BER incidence was lower with calcium application as compared to control plants.

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Introduction

Tomato is a major vegetable crop. The tomato fruit is consumed either as fresh produce or is processed into many different products. Tomato is a sub-tropical vegetable crop that grows and yields best at 25 - 30 °C during the day and 21 °C during the night (Camejo et al., 2005; El-Aidy et al., 2007). Abiotic stresses adversely affect the growth and yield of tomato (Cuartero et al., 2006). The soil salinity is one of the most common and serious problems limiting crop production (Pervez et al., 2009; Shrivastava and Kumar, 2015). The soil salinity problem may emerge due to less precipitation or use of saline water for irrigation (Hussain et al., 2006). Hot dry conditions that do not allow leaching of excess soluble salts out of the root zone may also increase soil salinity (Yokas et al., 2008). The excess sodium, in saline soils, decreases the uptake of K^+, Ca^{+2}, Mg^{+2} and NO^-_3, the mineral elements essential for growth (Ahmad and Jabeen, 2005). Salinity also increases the concentration of Na^+ in the cells and causes Na^+ toxicity (Tester and Davenport, 2003). While tomato genotypes may vary in their salts sensitivity, high salinity may decrease biomass production by 50-60% (Albacete et al., 2008).

The salinity stress initially develops as osmotic stress due to low water uptake and high salts accumulation that disrupts the cell membrane and nutrients balance (Shabala and Pottosin, 2014). The plants may exhibit decrease Na^+ toxicity by synthesizing compatible solutes such as proline, glycine, betaine and sugar (Ashraf and Harris, 2004). The compatible solutes or osmolytes are organic compounds that are inert in cellular metabolism and protect the cells against stress-induced damage (Ashraf and Foolad, 2007).

Foliar application of nutrients increase the nutrients concentration in the tissue and, thus, decrease the adverse effects of salinity (El-Fouly et al., 2011). Calcium is a major plant nutrient that constitutes 0.5 to 3% of the dry matter (Del-Amor and Marcelis, 2003). The calcium ions strengthen cell walls and stabilize cell membranes (Kadir, 2004). Thus, optimum supply of calcium is needed for leaf, root and canopy growth. Calcium deficiency results in several physiological disorders, including blossom end rot (BER) of tomato (Del-Amor and Marcelis, 2003; Combrink, 2013). Since, salinity decreases the calcium uptake (Ahmad and Jabeen, 2005); its foliar application may decrease the salinity-induced calcium deficiency in the leaves and fruits and minimize the harmful effects of salinity (Arshi et al., 2010). The calcium is also involved in regulating the salinity response of the plants (Kader and Sylvia, 2010), foliar calcium application may add in adaptation to salinity (Parida and Das, 2005). The influence of foliar calcium application on salinity stress responses of tomato plants was, therefore, investigated to understand the mechanism of calcium-induced beneficial effects on tomato grown in saline conditions.

Materials and Methods

Experimental site

The experiment was conducted at government research farm at Peshawar, located at (Lat 34° 0' 28" North, 71° 34' 24 East, Altitude 359 m) with a sub-humid climate. The annual average temperature is 22.7 °C, with the maximum summer temperature ranged from 45-49 °C. The mean maximum photoperiod during the summer months was 14.16 hours. The region is characterized by dry early summer followed by mild moon rains.

Plant material and transplanting

The seeds of tomato cultivar Rio-Grande were obtained from Agricultural Research Institute, Tarnab, Peshawar and sown in 2nd week of January in nursery beds containing a mixture of silt, garden soil, and compost (1:1:1 ratio). Seedlings, at 4-5 leaf stage, were transplanted to earthen pots of 30 cm diameter and 45 cm height filled with the growing medium. The transplants were allowed to establish for 20 days before starting the salinity treatment and foliar calcium application.

Salinity and foliar Ca application treatments

The tomato plants were irrigated, when required, with saline water 0, 75 and 150 mM NaCl strengths. For foliar Ca application, $CaSO_4$ was used as Ca source. Calcium solutions of 0, 0.25. 0.50, 0.75, 1.0 and 1.25% strength were prepared. The control treatment (0.0% Ca) was sprayed with tap water only. The salinity and foliar Ca application treatments were initiated 20 days after transplanting. The plants were irrigated, when required, with saline water of specified strengths. Foliar Ca application was carried out at 15 days interval.

The Na, K and Ca analysis

The sodium content of the fruit tissue was determined by oven drying of tissue samples at 80 °C for 48 hours. For wet digestion, 5 ml of concentrated nitric acid was added to 0.2 g of sample. The samples were, then, kept at room temperature for 48 hours. Finally, the samples were placed on a hot-block set to 90 °C for two hours. The sample was removed from the hot block after persistent color was observed and sample particulates were no longer visible. The samples were allowed to cool and the volume of extract was made to 50 ml by adding double distal water. The samples were then analyzed for sodium content by flame photometer (JENWAY PFP7) and converted to sodium content (μ*M*/g D.wt) of the tissue (Watad et al.,1986). The same solution (as for sodium content) was used for the determination of potassium content of the leaf (Watad et al.,1986). The Na/K ratio was calculated by dividing sodium content over the potassium content of the leaf samples.

The Calcium content of fruit was determined at the time of final harvest. For this purpose, the tomato fruit were picked at full size with no signs of BER development. The pericarp discs were made from the blossom end of tomato fruit. The locular tissue was removed from the discs and the discs were washed with distilled water. The pericarp discs were, then, incubated in an oven at 50 °C and periodically weighed. When the weight got stable, the samples were ground into a fine powder for wet digestion. The drying process was terminated and dry weight recorded when no further change in weight was observed. After oven drying, the leaf and fruit tissue samples were ground using a Tema mill, cleaned thoroughly with a brush and acetone for each treatment. The ground leaf and fruit samples were dry ashed and mineralized by adding 4 ml of 65% aqueous nitric acid solution and then heating. The calcium content of the samples was approximated with Atomic Absorption Spectrophotometer (GBC AA 932), calibrated with a standard solution of 5 µg-ml^{-1} (Isaac and Kerber, 1971).

The yield (t ha^{-1}) was estimated by regularly harvesting the fruits at the pink mature stage. The yield from each plant was recorded and added at final harvest. The yield per plant was converted to yield per hectare. The fruit firmness was determined by the method used by Pocharski et al. (2000) with Effigi, FT-011 Penetrometer. The fruit firmness was determined at the area surrounding the blossom end of the fruit harvested at the physiologically mature (mature green) stage before the visible symptoms of BER development. The Blossom End Rot (BER) incidence was estimated by visual observation of the fruits at each harvest. Fruits with BER symptoms were counted and presented as percent of total fruit harvested from each treatment.

Statistical analysis

The experiment was laid out in two factorials Randomized Complete Block Design (RCBD) with split plot arrangement and three replications. The data recorded on various parameters analyzed by Analysis of Variance (ANOVA) method to determine the difference between different treatment and their interactions. The treatment means were separated by Least Significant Difference (LSD) test 5% level of significance (Steel and Torrie, 1997).

Results and Discussion

Leaf Na^+ content (µM/g D wt)

Salinity and foliar calcium application significantly affected the Na^+ content of the leaf, but the interaction of salinity and calcium application was not significant. The Na^+ content of the leaf was 3491 µM/g D.wt in control plants that increased to 4059 and 4363 µM/g D wt with increasing salinity to 75 and 150 mM, respectively. Foliar calcium application decreased the Na^+ content of the leaf from 4528 µM/g D.wt in control plants to the minimum of 3140 µM/g D.wt in plants exposed to foliar application of 1.25% calcium solution (Table 1). The Na^+ content of the leaf increased by 13.99 and 19.97% with 75 and 150 m*M* salinity respectively over the control. The salinity stress results in accumulation of sodium ions in the roots and leaves (Sudhir and Murthy, 2004; Roy and Mishra 2014). The Na^+ uptake is accomplished by a non-selective system (Tester and Davenport, 2003). Hence, the high Na^+ in the growing medium increased its uptake and build up in the cytoplasm of leaf cells (Jha et al., 2010). The application of 1.25% calcium as foliar spray decreased the Na^+ content of the leaf by 30.66% as compared to control. Since, the calcium improves the metabolism of other nutrients and regulates enzymatic and hormonal functions as well as acts as a secondary messenger in the stress responses of the plants (White and Broadley, 2003), it may reduce excessive Na^+ uptake of the plants grown in saline conditions.

Leaf K+ content (µM/g D.wt)

The leaf K+ content decreased significantly from 8759 µM/g D.wt in control plants to 7726 and 6719 µM/g D.wt with increased salinity levels of 75 and 150 mM, respectively. The foliar calcium application and salinity x calcium interaction, however, had no significant effect on the K+ content of the leaf of tomato plants grown at different salinity levels (Table 1). The leaf K^+ content decreased by 6.8 and 12.24% with 75 and 150 mM salinity, stress respectively. The high Na^+ concentration decreases the intracellular K^+ influx (Alleva et al., 2006; Akram et al., 2007). The K^+ beside a major nutrient, is a major solute used for osmotic adjustment, maintenance of turgor and, thus, minimizing the adverse effects of salinity stress (Wang et al., 2013). The minimum K^+ content with 150 m*M* NaCl indicates that potassium ions uptake and its transport to the leaves is inhibited by Na^+ (Tester and Davenport, 2003) by decreasing the activity of K^+ transporter genes (Su et al., 2002). However, the decreased K^+ may also be attributed to decreased uptakes due to competition with Na^+ for Na^+- K^+ co-transporters (Zhu, 2002). It is interesting to observe that the foliar application of calcium also decreased the K^+ of the leaf. Thus, K^+ of the leaf was 13.37% lower under 1.25% foliar Ca application than the control plants.

Na+/K+ Ratio

The Na+/K+ ratio of the leaf increased significantly with increasing salinity levels but declined with increasing calcium concentration in foliar spray. The least Na+/K+ ratio (0.40) of control leaves increased to 0.53 and 0.65 in plants exposed 75 and 150 mM salinity, respectively. By contrast, the foliar calcium application decreased the Na+/K+ ratio of the leaf from 0.59 in control plants to the minimum (0.42) in plants sprayed with 1.25% calcium solution (Table 1). Since salinity increased the Na^+ and decreased the K^+ content, it increased the Na^+/K^+ ratio. Salinity, generally, increases Na^+/K^+ due to K^+ displacement by Na^+ in the plant cell (Wakeel et al., 2011). It is clear that whereas, salinity increase the Na^+/K^+ ratio, foliar calcium application decrease it by inhibiting Na^+ accumulation. It may explain less salinity-induced damage with foliar application of calcium (Yildirim et al., 2009).

The calcium content of tomato fruit

Salinity and foliar calcium application significantly affected the calcium content of tomato fruit. However, the interaction of salinity and foliar calcium application was not significant. The calcium content of tomato fruit in control plants was 0.77 mg/100 g, that declined to 0.70 and 0.65 mg/100 g in plants exposed to 75 and 150 mM salinity, respectively. By contrast, calcium application increased the calcium content of tomato fruit significantly. The calcium content of control fruit (0.61 mg/100 g) increased significantly with increasing calcium concentration to the maximum of 0.81 mg/100 g in plants treated with 1.25% calcium solution (Table 1). The foliar application of calcium significantly increased the calcium content of the fruit. Calcium is a major plant nutrient required for cell wall structure and function (Kadir, 2004). Calcium, despite its abundance in soils, may be deficient in plants due to its poor mobility in the soil and plants (Hepler, 2005). Thus, a regular supply is essential to avoid calcium deficiency in the plants (Del-Amor and Marcelis, 2003). Exposure of plants to salinity also decreases the calcium contents of the plant (Arshi et al., 2010). The calcium content of the fruit declined by 15.58% with 150 mM NaCl (Table 1). The decline in calcium can be attributed to the competition of Na^+ ion and Ca^{+2} ions for binding sites (Kaya and Higgs, 2003). In contrast, the foliar calcium application increases the calcium content of the plant (Arshi et al., 2010). The calcium content of tomato fruit increased by 24.69% over the control plants. Since, calcium helps in adaptation to salinity stress by reducing the toxic effects of NaCl (Parida and Das, 2005); its application may decrease the adverse effects of salinity (Munns, 2002).

Yield (t ha^{-1})

The yield of tomato crop was significantly affected by salinity levels, calcium concentration and salinity x calcium interaction. The mean yield of tomato decreased from a maximum of 10.69 t. ha^{-1} in control plants to 7.86 and 4.46 t.ha^{-1}. In contrast, foliar calcium application increased the mean yield from the minimum (6.83 t.ha^{-1}) of control plants to the maximum of 8. 82 t.ha^{-1} with 1.25% calcium applied as foliar spray (Table 2). The interaction of salinity and foliar calcium application significantly affected the yield of tomato crop. Whereas the yield of tomato decreased significantly with increasing salinity levels, but the decline was less with calcium application. The yield of control (0 mM NaCl + 0.0% Ca) plants (9.83 t.ha^{-1}) declined to 6.68 and 3.99 t.ha^{-1} with no Ca application and exposure to 75 and 150 mM salinity stress respectively.

Table 1: *The influence of salinity and foliar calcium application on the Na, K and Ca content of tomato leaves. Means in a column with different letters are significant at $p \leq 0.05$.*

Salinity Levels (mM NaCl)	Leaf Na^+ Content (µM/g D wt)	Leaf K^+ Content (µM/g D wt)	Na^+ / K^+ Ratio	Fruit Ca^{+2} Content (mg/100 g DW)
0	3491 b	8759 a	0.40c	0.77 a
75	4059 a	7726 b	0.53 b	0.70 b
150	4363 a	6719 c	0.65 a	0.65 c
Significance	*	*	*	*
Calcium (%)				
0	4528 a	7789	0.59 a	0.61 c
0.25	4344 a	7777	0.57 b	0.66 bc
0.50	4165 ab	7706	0.55 b	0.69 b
0.75	3956 b	7679	0.53 c	0.72 b
1.0	3694 b	7766	0.48 d	0.76 ab
1.25	3140 c	7691	0.42 e	0.81 a
Significance	*	ns	*	*
Salinity × Calcium	ns	ns	ns	ns

Links Researchers

Table 2: *The influence of salinity and foliar calcium application on the fruit yield, fruit firmness and BER incidence of tomato. Means in a column with different letters are significant at p ≤ 0.05*

Salinity Levels (mM NaCl)	Yield (t ha-1)	Fruit Firmness (kg cm-2)	BER Incidence (%)
0	10.69 a	3.92 a	9.28 c
75	7.86 b	4.54 b	12.17 b
150	4.46 c	5.17 b	14.39 a
Significance	*	*	*
Calcium (%)			
0	6.83 b	3.93 c	15.33 a
0.25	6.96 b	4.19 bc	13.67 ab
0.50	7.47 b	4.45 b	12.56 b
0.75	7.72 ab	4.67 b	10.78 bc
1.0	8.24 ab	4.96 ab	10.33 bc
1.25	8.82 a	5.06 a	9.00 c
Significance	*	*	*
Salinity × Calcium	*	ns	ns

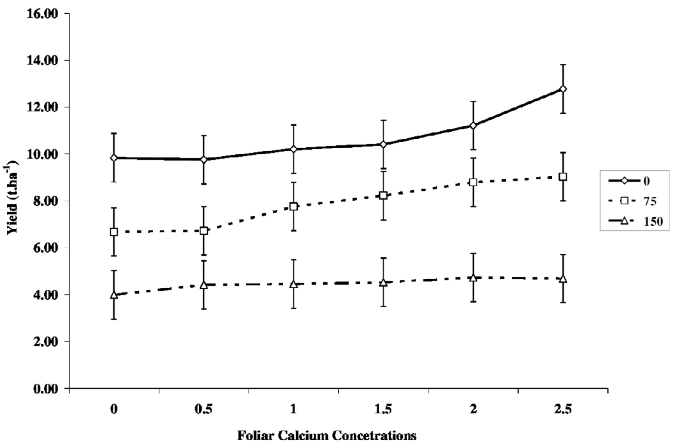

Figure 1: *The influence of salinity levels and calcium concentration applied as a foliar spray on the yield of tomato crop.*
The vertical error bars represent LSD at p ≤ 0.05

However, at each level of salinity, the yield was higher with increased calcium concentrations. The yield of plants exposed to 75 and 150 mM NaCl + 1.25% Ca was 9.02 and 4.68 t.ha^{-1} respectively, compared to 12.77 t.ha^{-1} with 0 mM NaCl stress + 1.25% Ca applications (Figure 1). The yield of tomato plants exposed to 150 mM NaCl stress decreased by 59.41%. The salinity stress decreases the rate of photosynthesis (Pervez et al., 2009; Tsonev et al., 2011) and the transport of photosynthates within the plants (Hajiboland et al., 2010), that may decrease the fruit size and yield (Juan et al., 2005; Rubio et al., 2009). The optimum yield of a crop also depend on a balanced supply of nutrients (Akhtar et al., 2010), but salinity decreases the uptake K^+, Ca^{+2}, Mg^{+2} and NO^-_3 (Ahmad and Jabeen, 2005; Wang et al., 2013). Thus, the yield decline can also be attributed to salinity-induced nutrients deficiency in the plants. In contrast, the foliar application of calcium decreased the decline in yield due to salinity. The yield of plants exposed to 150 mM NaCl + 1.25% calcium was 17.29% higher than plants grown at the same salinity level and no calcium (0.0%) treatment (Figure 2). The adverse effects of salinity on the yield can be reduced by foliar application of nutrients and other chemicals (Kaya et al., 2009). The promotion of yield by foliar calcium application can be attributed to increased calcium levels (Table 2), less membrane damage (Figure 1) and enhanced potassium accumulation (Khayyat et al., 2009), that are essential for optimum yield (Chapagain and Wiesman, 2004).

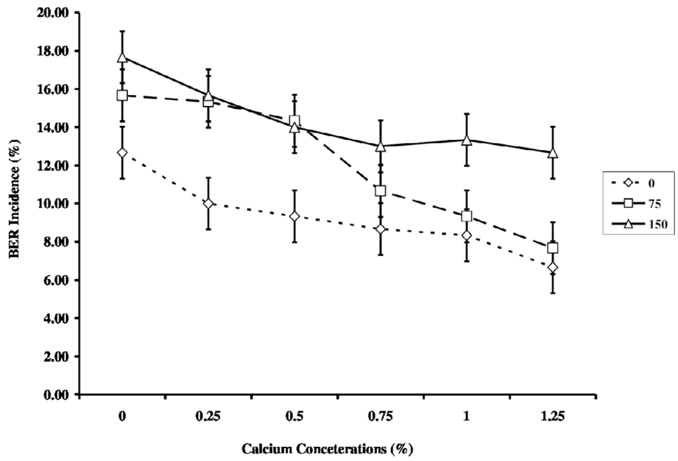

Figure 2: *The Blossom End Root (BER) incidence in relation to salinity levels and calcium concentration applied as a foliar spray.*
The vertical error bars represent LSD at p ≤ 0.05

Fruit Firmness (kg cm^{-2})

The firmness of tomato fruit increased significantly with increasing salinity and the concentration of calcium in foliar spray. The salinity x calcium interaction was, however, not significant (Table 1). The fruit firmness of control (0 mM NaCl) fruit (3.92 kg.cm^{-2}) increased to 4.54 and 5.17 kg.cm^{-2} with plant exposure to 75 and 150 mM salinity stress respectively. The foliar application also increased the tomato fruit firmness from 3.93 Kg.cm^{-2} in control fruits to the maximum (5.06 kg.cm^{-2}) with 1.25% foliar calcium application to the plants (Table 2). The fruit firmness is an important quality attribute of tomato fruit (Rab et al., 2013). The fruit firmness, generally, declines with senescence (Mostofi and Tolvoen, 2006). However, the fruit firmness increased by 59.41% with increasing salinity to 150 mM NaCl (Table 1). Since the fruit firmness is a sign of quality, it seems unlikely to observe increased fruit firmness in plant exposed to salinity. Yet, similar observations were also made by Del-Amor and Marcelis (2003) and Flores et al. (2003). The increased fruit firmness of plants grown in saline conditions may be due to small and thick walled cells induced by salinity (Flores et al., 2003). However, the increased firmness of tomato fruit harvested from plants exposed to salinity could also be due to greater flaccidity of the tissue under study. Calcium application also increased the fruit firmness in both control and salinity stress conditions (Table 1). Since calcium is a structural component of the cell wall and it delays the degradation of cell wall polymers, it is commonly used to promote the fruit firmness, quality and storage performance (Kadir, 2004; Madani et al., 2015). A critical concentration of calcium is essential for the mechanical strength of cell wall (Huang et al., 2005). Thus, calcium application seems to increase the calcium concentration in the cell wall and, thus, enhances the fruit firmness (Ho and White, 2005).

Blossom End Rot incidence (%)

The Blossom End Rot (BER) incidence was significantly affected by salinity levels, calcium concentration and salinity x calcium interaction. The least blossom end rot across salinity levels (9.28%) increased to 12.17 and 14.39% with increasing salinity stress to 75 and 150 m*M* NaCl stress respectively. In contrast, the BER incidence was the highest (15.33%) in control plants that declined to the minimum of 9.00 with foliar application of 1.25% calcium solution. The interaction of salinity x calcium revealed that BER incidence of control fruits (12.67%) increased to 15.67 and 17.67% with increasing salinity levels to 75 and 150 mM NaCl + no (0.0%) calcium application (Table 2). The calcium application, however, decreased the BER incidence in control and salinity stressed plants. The BER incidence was the minimum (6.67%) with no salinity stress and 1.25% calcium application. In contrast, the BER incidence with 1.25% calcium application and 75 and 150 mM NaCl stress was 7.67 and 12.67% (Figure 2). The blossom end rot (BER) is a physiological disorder of tomato, caused the calcium deficiency (Taylor et al., 2004). The BER develops due to collapsed cells in the epidermis and sub-epidermal parenchyma that results in the appearance of a watery and discolored, necrotic tissue (Suzuki et al., 2003). Since, salinity declines nutrients uptake (Magan et al., 2008), it enhanced the blossom end rot incidence (Rubio et al., 2009). Salinity is also found to disrupt the cell membrane, especially at the blossom end. Thus, it is likely to observe increased BER incidence with increasing salinity (Yoshida et al., 2014). The application of 1.25% calcium as folair spray decreased the BER incidence of fruit by 50, 54 and 26% in plants exposed to 75 and 150 mM NaCl stress accordingly. The BER disorder of tomato fruit is, generally, attributed to Ca^{2+} deficiency (Saure, 2005). Calcium application may increase the water soluble calcium at the blossom end of the fruit, thereby declines the BER incidence (Yoshida et al., 2014).

Conclusions

It can be concluded that salinity resulted in increased Na^+ and decreased K^+ accumulation and hence increased the Na^+/K^+ ratio of the leaf. Salinity also decreased the Ca^{+2} of the fruit as well as yield but increased the fruit firmness and BER incidence. By contrast, the foliar calcium application decreased the salinity-induced damage by decreasing Na^+ and increasing K^+ accumulation that decreased the Na^+/K^+ ratio of the leaf with a concomitant increase Ca^{+2} content of the fruit, yield, fruit firmness and lower BER incidence.

Author's Contributions

All the authors contributed to this research study presented in this paper. Abdur Rab developed the concept of the research. Naveed Ahmad and Syed Ghias Ali were responsible for field work and recording the data. Muhammad Sajid conducted the Lab work and chemical analysis. Khalid Nawab helped in write up and statistical analysis of the data.

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