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# *Article* Salt stress mitigation by calcium nitrate in tomato plant

Rezowana Nizam<sup>1</sup>, Md. Tofail Hosain<sup>1\*</sup>, Md. Elias Hossain<sup>1</sup>, Md. Meftaul Islam<sup>2</sup> and Md. Ariful Haque<sup>3</sup>

<sup>1</sup>Department of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh <sup>2</sup>Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh <sup>3</sup>Department of Medicine and Public Health, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh

<sup>\*</sup>Corresponding authors: Md. Tofail Hosain, Department of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. Phone: +8801815382270; E-mail: tofailhossain@gmail.com

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**Abstract:** Salt stress is one of the most subversive abiotic stress which severely affects the agricultural productivity in various ways. The pot experiment was conducted at the Horticulture Farm of Sher-e-Bangla Agricultural University, Dhaka during the period from November 2017 to April 2018. BARI Tomato-5 was used as planting material. The two factors experiment was laid out in RCBD with four replications. Five levels of salinity induced by sodium (Na+) viz., 0, 2, 4, 6 and 8 dS m<sup>-1</sup> and three levels of Ca<sup>2+</sup> viz., 0, 5 and 10 mM were used as treatment variables. The results of this experiment showed that, the salt stress reduced the yield parameters and yield of tomato with the increase of salinity. The lowest data was recorded from 8 dS m<sup>-1</sup> and highest value was observed at control. The present results also showed that, Ca<sup>2+</sup> significantly increased the yield contributing characters as well as yield of tomato in both saline and non-saline conditions. However, for combined effect, highest number of fruits plant<sup>-1</sup> (50.8) and the highest yield plant<sup>-1</sup> (3.88 kg) was produced from 0 dS m<sup>-1</sup> Na x 10 mM Ca<sup>2+</sup>; whereas the lowest from 8 dS m<sup>-1</sup> x 0 mM Ca<sup>2+</sup>. This result suggests that, exogenous Ca<sup>2+</sup> can effectively mitigate the deleterious effect of salt stress in tomato.

Keywords: calcium; yield; Lycopersicon esculentum; salt stress; salt tolerant

#### **1. Introduction**

Tomato (*Solanum lycopersicum* L.) is one of the most important and popular vegetable crop. Food value of tomato is very rich because of higher contents of vitamins A, B and C including calcium and carotene (Bose and Som, 1990). Tomato adds flavor to the foods and it is also rich in medicinal value. Nowadays, tomatoes are grown round the year. In Bangladesh, the yield of tomato is not enough satisfactory in comparison with other tomato growing countries of the World (Aditya *et al.*, 1997). The low yield of tomato may be attributed to a number of reasons, viz. unavailability of quality seeds of high yielding varieties, biotic stress as well as production in abiotic stress conditions. The environmental stresses resulting from drought, temperature, salinity, air pollution, heavy metals, pesticides and soil pH are major limiting factors in crop production (Alqudah *et al.*, 2011).

Salinity is the major stress factor, (Rueda-Puente *et al.* 2007) and is one of the most serious environmental problems influencing crop growth and production (Lopez *et al.* 2002). Salinity disturbs the physiology of plants by changing the metabolism of plants (Garg *et al.*, 2002), causing cell injury in transpiring leaves, thus reducing growth of plant (Munns, 2005). Salinity badly reduces leaf area, accumulation of dry matter content and also reduces net rate of  $CO_2$  assimilation (Murillo-Amador *et al.*, 2000) as well as fruit production. Salinity reduced tomato yield (Sonnenveld and Welles, 1988). Separately, plants have developed a well-organized defense mechanism of biochemical and physiological processes to protect themselves from the salinity-induced damages including antioxidant responses, ionic homoeostasis and/or osmoregulation (Parida and Das, 2005).

Calcium (Ca) is a signaling molecule and acts as a second messenger which is increased in the cytosol by activating influx channel both in the plasma membrane and tonoplast and plays a significant role in mediating mechanisms involved in recognition and response to abiotic stresses in plants (Kader and Lindberg, 2010). In addition, Hussain *et al.* (2010) reported that Ca2+ restrict the entry of Na+ into the plant cells under sodium stress. Calcium is associated with the middle lamella of cell walls playing a role in support and growth of cell (Wu *et al.*, 2002). The Ca<sup>2+</sup> has a pivotal participation in salt stress signaling that controls ion homeostasis pathways (Yokoi *et al.*, 2002). It was confirmed by Ca<sup>2+</sup> dependent activation of phosphatase leading to transcription of the ENA<sub>1</sub> gene, which encodes the P-type ATPase (Mendoza *et al.*, 1994). These findings suggest that calcium can mitigate the sodium toxicity of plant. Many authors stated that exogenous calcium alleviates stress in *Vigna radiata*, *Glycine max*, *Linum usitatissimum* (Manivannan *et al.*, 2007; Arshi *et al.*, 2010; Khan *et al.*, 2010).

In addition, the calcium nitrate {Ca(NO<sub>3</sub>).  $4H_2O$ } has been exogenously applied to the saline soil for improving the soil chemical, leading to enhance the crop productivity (Cha-Um *et al.*, 2012). However, the studies investigating the role of calcium in response to salinity induced stress in tomato are largely lacking. With this view of objectives, a study has been carried out to investigate the role of Ca<sup>2+</sup> in mitigating salt stress-induced response in tomato.

### 2. Materials and Methods

#### 2.1. Experimental site

A pot experiment was conducted at Sher-e-Bangla Agricultural University, Dhaka, Bangladesh situated at  $23^{\circ}74$ 'N latitude and  $90^{\circ}35$ 'E longitude at an altitude of 8.6 m above the sea level during the period from November 2017 to April 2018. The soil of the experimental site was silt loam in texture (sand 20.84%, silt 57.46% and clay 21.7%) with pH 6.9, organic matter 0.86%, available potassium 25 mg kg<sup>-1</sup> and available sodium 70 mg kg<sup>-1</sup>. The climate of this area is subtropical.

### 2.2. Experimental treatment and design

The experiment was placed under venyl house which was made by bamboo with polythene roof and pots were kept on the bamboo made frame of 70 cm height. Seedlings of 30 days of BARI Tomato-5 were used as planting materials in this experiment. Five levels of salinity induced by sodium (Na+) viz., 0, 2, 4, 6 and 8 dS m<sup>-1</sup> and three levels of Ca<sup>2+</sup> viz., 0, 5 and 10 mM were used as treatment variables. The experiment was laid out in Randomized Complete Block Design (RCBD) with four replications and comprised of 60 pots. Each pot was 35 cm in diameter and 30 cm in height. The distance maintained between two blocks and two plots were 1.0 m and 0.5 m, respectively.

#### 2.3. Preparation of the pot

The experimental pots were first filled at 10 December, 2017 where per pot containing 10 kg soil. Potted soil was brought into desirable fine tilth by hand mixing. The stubble and weeds were removed from the soil. The final pot preparation was done on 15 December. The soil was treated with insecticides (cinocarb 3G @ 4 kg/ha) at the time of final pot preparation to protect young plants from the attack of soil inhibiting insects such as cutworm and mole cricket.

#### 2.4. Crop husbandry

The seeds were sown in the seedbed on 11 November 2017 and after sowing, seeds were covered with light soil to a depth of about 0.6 cm. Necessary shading, weeding, mulching and irrigation were done from time to time as and when required and no chemical fertilizer was used in the seedbed. The fertilizers i.e. urea, TSP, MoP and manures i.e. cowdung were applied @ 250 kg, 200 kg, 175 kg and 20 tons ha<sup>-1</sup> (BARI, 2015). The entire amounts of TSP, MP and cowdung were applied during the final land preparation. Urea was applied in three equal installments at 15, 30 and 45 days after seedling transplanting. Healthy and uniform 30 days old seedlings were transplanted in the experimental pots in the afternoon of 10 December, 2017. This allowed an accommodation of 1 plant in each pots. Intercultural operations were done to ensure normal growth of the crop. Plant protection measures were followed as and when necessary.

# 2.5. Application of NaCl and Ca<sup>2+</sup>

NaCl was applied in the pot during application of water. The tray was used in the bottom of each pot to collect the water and different nutrient. The  $Ca^{2+}$  [calcium ( $Ca^{2+}$ ) which used as a form of calcium nitrate { $Ca(NO_3)$ .

 $4H_2O$ ] also applied with irrigation in the pot according treatment combination. NaCl solution and Ca<sup>2+</sup> applied in the pot soil at 25, 55and 85 days after transplanting.

### 2.6. Data collection

Different data were collected from plant of each unit pot. Data on the following parameters were recorded during the course of the experiment such as - No. of flowers cluster<sup>-1</sup>, No. of flowers plant<sup>-1</sup>, No. of fruits cluster<sup>-1</sup>, Length of fruit, Diameter of fruit, Dry matter of fruit and Yield plant<sup>-1</sup> (kg).

# 2.7. Statistical package

All the collected data were tabulated and analyzed statistically using analysis of variance technique and subsequently, Least Significance Difference (LSD at 5%) for comparing the treatment means, by MSTAT-C software (Gomez and Gomez, 1984).

### 3. Results and Discussion

### 3.1. Number of flowers cluster<sup>-1</sup>

Different levels of salt stress varied significantly in terms of number of flowers per cluster of tomato (Table 1). The highest number of flowers per cluster (7.80) was recorded from 0 dS m<sup>-1</sup> Na. On the other hand, the lowest number (5.78) was recorded from 8 dS m<sup>-1</sup> Na. Luo *et al.* (2013) reported that salt stress of NaCl, stronger inhibitory effect on tomato growth.

Number of flowers per cluster of tomato showed significant differences for different levels of calcium nitrate (Table 1). The highest number of flowers per cluster (7.56) was found from 10 mM Ca, while the lowest number (6.53) was recorded from 0 mM Ca.

Statistically significant variation was recorded for the combined effect of different levels of salt stress and calcium nitrate on number of flowers per cluster (Table 2). The highest number of flowers per cluster (8.70) was recorded from the treatment combination of 0 dS  $m^{-1}$  Na with 10 mM Ca, while the lowest number (5.75) was found from 8 dS  $m^{-1}$  Na with 0 mM Ca treatment combination.

## **3.2.** Number of flowers plant<sup>-1</sup>

Number of flowers per plant of tomato varied significantly due to different levels of salt stress (Table 1). The highest number of flowers per plant (67.3) was found from 0 dS  $m^{-1}$  Na, while the lowest number (38.4) was observed from 8 dS  $m^{-1}$  Na.

Statistically significant variation was recorded for different levels of calcium nitrate on number of flowers per plant of tomato (Table 1). The highest number of flowers per plant (62.2) was recorded from 10 mM Ca, again the lowest number (47.0) was observed from 0 mM Ca.

Different levels of salt stress and calcium nitrate showed significant differences on number of flowers per plant due to combined effect (Table 2). The highest number of flowers per plant (76.1) was found from 0 dS m<sup>-1</sup> Na with 10 mM Ca treatment combination and the lowest number (35.1) was observed from 8 dS m<sup>-1</sup> Na with 0 mM Ca treatment combination.

Table 1. Effect of salt stress and calcium (Ca) on yield contributing characters of tomato.

Treatments	Number of flower Cluster <sup>-1</sup>	Number of flowers plant <sup>-1</sup>	Number of fruits cluster <sup>-1</sup>	Length of fruit (cm)	Diameter of fruit (cm)	Dry matter content in fruit (%)	Yield plant <sup>-1</sup> (kg)
0 (dS m <sup>-1</sup> Na)	7.80 a	67.3 a	5.10 a	8.96 a	5.83 a	8.97 a	3.19 a
$2 (dS m^{-1} Na)$	7.55 a	62.0 b	4.93 a	8.46 ab	5.58 ab	8.83 a	2.86 b
4 (dS m <sup>-1</sup> Na)	7.48 a	59.0 b	4.85 a	8.04 bc	5.31 bc	8.42 b	2.63 b
6 (dS m <sup>-1</sup> Na)	6.68 b	48.0 c	4.30 b	7.59 с	5.18 c	8.04 c	2.07 c
8 (dS m <sup>-1</sup> Na)	5.78 с	38.4 d	3.88 c	6.06 d	4.41 d	7.32 d	1.42 d
LSD(0.05)	0.38	3.57	0.31	0.60	0.37	0.36	0.26
0 (mM Ca)	6.53 c	47.0 c	4.18 c	6.99 c	4.80 b	7.88 b	1.93 c
5 (mM Ca)	7.09 b	55.6 b	4.62 b	7.97 b	5.37 a	8.41 a	2.48 b
10 (mM Ca)	7.56 a	62.2 a	5.03 a	8.51 a	5.61 a	8.65 a	2.89 a
LSD <sub>(0.05)</sub>	0.30	2.76	0.24	0.46	0.28	0.28	0.20
CV(%)	6.57	7.88	8.22	9.27	8.41	5.31	12.87

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability.

Salinity and	Number of	Number of	Number of	Length of	Diameter of	Dry matter	Yield
calcium level	flower	flowers	fruits	fruit (cm)	fruit (cm)	content in fruit	plant <sup>-1</sup> (kg)
( <b>mM</b> )	cluster <sup>-1</sup>	plant <sup>-1</sup>	cluster <sup>-1</sup>			(%)	
$0 \text{ dS m}^{-1} \text{ Na} \times$							
0 (mM Ca)	6.50 f-h	54.0 d-f	4.40 d-f	7.79 c	5.03 d-g	8.16 cd	2.37 ef
5 (mM Ca)	8.20 ab	71.7 a	5.10 bc	9.37 ab	5.98 ab	9.18 a	3.33 bc
10 (mM Ca)	8.70 a	76.1 a	5.80 a	9.71 a	6.49 a	9.56 a	3.88 a
$2 \text{ dS m}^{-1} \text{ Na} \times$							
0 (mM Ca)	6.80 d-f	50.9 ef	4.45 d-f	7.51 cd	5.02 d-g	8.10 cd	2.09 fg
5 (mM Ca)	7.50 b-d	61.1 bc	4.95 b-d	8.33 bc	5.75 bc	9.01 a	2.91 cd
10 (mM Ca)	8.35 a	74.0 a	5.38 ab	9.53 a	5.97 ab	9.37 a	3.58 ab
4 dS m <sup>-1</sup> Na $\times$							
0 (mM Ca)	7.55 bc	55.1 с-е	4.60 c-f	8.15 c	4.79 e-h	8.02 cd	2.28 ef
5 (mM Ca)	7.35 с-е	58.8 b-d	4.80 b-e	7.94 c	5.42 b-e	8.34 bc	2.72 de
10 (mM Ca)	7.55 bc	63.0 b	5.15 bc	8.03 c	5.72 b-d	8.90 ab	2.90 cd
$6 \text{ dS m}^{-1} \text{ Na} \times$							
0 (mM Ca)	6.05 g-i	39.9 gh	4.05 f	6.27 e	5.01 d-g	8.21 cd	1.71 gh
5 (mM Ca)	6.65 e-g	48.2 f	4.25 ef	7.87 c	5.26 c-f	8.02 cd	2.06 fg
10 (mM Ca)	7.35 с-е	55.8 с-е	4.60 c-f	8.64 a-c	5.26 c-f	7.88 cd	2.44 d-f
8 dS m <sup>-1</sup> Na $\times$							
0 (mM Ca)	5.75 i	35.1 h	3.40 g	5.21 f	4.14 h	6.92 e	1.20 i
5 (mM Ca)	5.75 i	38.3 gh	4.00 f	6.34 e	4.45 gh	7.53 de	1.40 hi
10 (mM Ca)	5.85 hi	41.8 g	4.25 ef	6.64 de	4.63 f-g	7.52 de	1.67 gh
LSD(0.05)	0.66	6.18	0.54	1.04	0.63	0.63	0.45
CV(%)	6.57	7.88	8.22	9.27	8.41	5.31	12.87

Table 2. Combined effect of salt stress and calcium (Ca) on yield contributing characters of tomato.

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability.

# **3.3.** Number of fruits cluster<sup>-1</sup>

Number of fruits per cluster of tomato varied significantly for different levels of salt stress (Table 1). The highest number of fruits per cluster (5.10) was recorded from 0 dS  $m^{-1}$  Na. On the other hand, the lowest number (3.88) was recorded from 8 dS  $m^{-1}$  Na. Lolaei (2012) showed that fruit number per plant decreased by the NaCl treatment.

Different levels of calcium nitrate showed significant differences on number of fruit per cluster of tomato (Table 1). The highest number of fruits per cluster (5.03) was found from 10 mM Ca, whereas the lowest number (4.18) was found from 0 mM Ca. Hao and Papadopoulos (2004) reported that at 300 mg·L<sup>-1</sup> Ca, on total fruit number. Lolaei (2012) observed that increasing Ca<sup>2+</sup> concentration in the nutrient solution increased the fruit production. Rubio *et al.* (2009) found that increasing of Ca<sup>2+</sup> concentration in the plant medium under saline condition increased the number of fruits per plant.

Combined effect of different levels of salt stress and calcium nitrate showed significant differences on number of fruits per cluster (Table 2). The highest number of fruits per cluster (5.80) was attained from 0 dS m<sup>-1</sup> Na with 10 mM Ca treatment combination, while the lowest number (3.40) was recorded from 8 dS m<sup>-1</sup> Na with 0 mM Ca treatment combination.

#### 3.4. Length of fruit

Length of fruit of tomato varied significantly for different levels of salt stress (Table 1). The highest length of fruit (8.96 cm) was recorded from 0 dS m<sup>-1</sup> Na. On the other hand, the lowest length (6.06 cm) was recorded from 8 dS m<sup>-1</sup> Na. Hao and Papadopoulos (2004) reported that at 300 mg L<sup>-1</sup> Ca, total fruit length increased linearly.

Different levels of calcium nitrate showed significant differences on length of fruit of tomato (Table 1). The highest length of fruit (8.51 cm) was attained from 10 mM Ca, whereas the lowest length (6.99 cm) was recorded from 0 mM Ca. Ahmad (2014) showed that exogenous application of silicon and potassium nitrate reduced sodium uptake, increased potassium and consequently improved ear length.

Combined effect of different levels of salt stress and calcium nitrate showed significant differences on length of fruit (Table 2). The highest length of fruit (9.71 cm) was recorded from 0 dS m<sup>-1</sup> Na with 10 mM Ca treatment

combination, again the lowest length (5.21 cm) was observed from 8 dS  $m^{-1}$  Na with 0 mM Ca treatment combination.

#### 3.5. Diameter of fruit

Different levels of salt stress varied significantly for diameter of fruit of tomato (Table 1). The highest diameter of fruit (5.83 cm) was recorded from 0 dS  $m^{-1}$  Na, while the lowest diameter (4.41 cm) was found from 8 dS  $m^{-1}$  Na. Posada and Rodriguez (2009) reported that fruits of salt-stressed plants had reduced diameter.

Statistically significant variation was recorded due to different levels of calcium nitrate on diameter of fruit of tomato (Table 1). Data revealed that the highest diameter of fruit (5.61 cm) was recorded from 10 mM Ca, whereas the lowest diameter (4.80 cm) was found from 10 mM Ca. Jafari *et al.* (2009) observed that application of supplemental calcium resulted in partially restoring the adverse effects of high salinity on plant growth.

Diameter of fruit showed significant differences due to combined effect of different levels of salt stress and calcium nitrate (Table 2). The highest diameter of fruit (6.49 cm) was observed from 0 dS m<sup>-1</sup> Na with 10 mM Ca treatment combination and the lowest diameter (4.14 cm) was recorded from 8 dS m<sup>-1</sup> Na with 0 mM Ca treatment combination.

#### **3.6.** Dry matter content in fruit

Statistically significant variation was observed in terms of dry matter content in fruit of tomato for different levels of salt stress (Table 1). The highest dry matter content in fruit (8.97%) was recorded from 0 dS  $m^{-1}$  Na, while the lowest (7.32%) was recorded from 8 dS  $m^{-1}$  Na. Posada and Rodriguez (2009) reported that fruits of salt-stressed plants had reduced total dry matter.

Dry matter content in fruit of tomato showed significant differences due to different levels of calcium nitrate (Table 1). The highest dry matter content in fruit (8.65%) was found from 10 mM Ca and the lowest (7.88%) was recorded from 0 mM Ca. Hao and Papadopoulos (2004) reported that at 300 mg L<sup>-1</sup> Ca, total fruit yield and fruit dry matter increased linearly. Kaya *et al.* (2006) showed that application of silicon increased corn dry weight and improved grain yield.

Combined effect of different levels of salt stress and calcium nitrate showed significant differences on dry matter content in fruit (Table 2). The highest dry matter content in fruit (9.56%) was recorded from 0 dS m<sup>-1</sup> Na with 10 mM Ca treatment combination, whereas the lowest (6.92%) was found from 8 dS m<sup>-1</sup> Na with 0 mM Ca treatment combination.

#### 3.7. Yield plant<sup>-1</sup>

Different levels of salt stress varied significantly in terms of yield per plant of tomato (Table 1). The highest yield per plant (3.19 kg) was recorded from 0 dS m<sup>-1</sup> Na, while the lowest yield (1.42 kg) was found from 8 dS m<sup>-1</sup> Na. Most crops tolerate salinity up to a threshold level, above which yields decrease as salinity increases (Maas, 1986). Tomato yield were subjected to 75 and 150 mM NaCl stress in order to study the effect of salt stress on its antioxidant response and stress indicators by Slathia and Choudhary (2013). Rubio *et al.* (2009) found that total fruit yield was increased because the fruit weight was increased. NaCl to nutrient solution, significantly reduced fruit yield in terms of number of fruits per plant from 8.13 (control) to 3.83 (Lolaei, 2012). Different levels of calcium nitrate showed significant differences on yield per plant of tomato (Table 1). The highest yield per plant (2.89 kg) was recorded from 10 mM Ca, whereas the lowest yield (1.93 kg) was observed from 0 mM Ca. Hao and Papadopoulos (2004) reported that at 300 mg·L<sup>-1</sup> Ca, total fruit yield increased linearly. Ahmad (2014) showed that exogenous application of silicon and potassium nitrate reduced sodium uptake, increased potassium and consequently improved seed yield. Howladar and Rady (2012) found that seed coating with calcium paste reduced the toxic effects of NaCl on plant growth and yield by increasing leaf pigments, ascorbic acid, proline contents and enzymatic activities.

Yield per plant varied significantly due to the combined effect of different levels of salt stress and calcium nitrate (Table 2). The highest yield per plant (3.88 kg) was recorded from 0 dS  $m^{-1}$  Na with 10 mM Ca treatment combination and the lowest yield (1.20 kg) was observed from 8 dS  $m^{-1}$  Na with 0 mM Ca treatment combination.

#### 4. Conclusions

Considering the above mentioned results, it may be concluded that, the fruit yield of tomato gradually decreased by the increase of salinity levels and this reduction rate was decreased by exogenous supply of calcium. Among the calcium levels, 10 mM showed the highest result in fruit production. Therefore, this experiment suggests

that  $Ca^{2+}$  can effectively mitigate the deleterious effect of  $Na^{+}$  stress in tomato cultivation. Calcium can be recommended for farmers to use in their fields for alleviating salt stress.

#### **Conflict of interest**

None to declare.

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