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Mitigation of Salinity Stress Effects in Tomato (Lycopersicon esculentum Mill.) by Using Calcium Chloride

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Abstract. *. In the world, salinity has become an increasing danger for agricultural production. A factorial pot experiment using a completely randomized design (CRD) was conducted in the present research, aimed to look at how growth, yield parameters, and biochemical contents were influenced by the exogenous application of calcium chloride (CaCl2, 0, 5, and 10 mM) of tomato exposed to salt stress (NaCl, 0, 25, and 75 mM), Growth was slowed and Both the sodium percentage and proline content increased under salt stress. However, the growth, yield, and biochemical content characteristics were improved by the application of Ca, enhanced plant height, dry weight of the shoots and roots, the number of fruits per plant, fruit weight, the percentage of nitrogen, calcium, magnesium, phosphorus, potassium, relative water content (RWC), and increased proline content, moreover a significant reduction in the sodium percentages, when compared to the control. Lastly, exogenous Ca use improves resistance to salt stress. Moreover, the use of 10 mM CaCl2 caused the beneficial effects of Ca, which has been suggested to enhance tomato performance in salinized condition.*

Keywords: *Salinity; $CaCl_2$; Tomato.*

INTRODUCTION

Tomato are the second-most important solanaceous crop grown and consumed worldwide, after potatoes [1,2]. In 2020, 86.8 million tons were produced internationally [3]. it is rich in nutrients, 37 minerals, carbohydrates, calories, water, proteins, fibers, and vitamins [4]. It has antioxidant properties, lowers blood pressure, guards against cancer and eye diseases, and lessens the risk of kidney stones forming [5]. Tomato is used in cooking, fresh food, and canning, which also turns it into juice, pulp, paste, and other sauces [6]. Tomato can be eaten raw or cooked, and they are a crucial crop for processing into sauces, purees, pastes, ketchup, juices, and powders. It gives cuisine a range of flavors and colors. Tomato are widely recognized for their lycopene pigment, pigments called anthocyanin and beta-carotene are responsible for the colors pink and yellow. The ascorbic acid content in tomato is high, ranging from 15 to 31 mg per 100 g of fresh weight [7]. Plants are regularly subjected to a range of biotic and abiotic stressors, which can have a

50%, detrimental effect on agricultural productivity. These interconnected stresses have the potential to alter a plant's morphology, physiology, and biochemistry, which may result in plant mortality [8-10]. Salinity is a major worldwide problem that represents a threat to the agricultural industry, particularly in regions where water is scarce. Ionic toxicity, osmotic stress, and oxidative stress are highly inhibitive to plant performance and crop productivity [11]. Many plants have been shown to experience growth inhibition as an effect of salinity, and these plants develop many defensive mechanisms. The effects of salinity increase with increasing concentrations in many plants [12-14]. exhibiting a reduction in plant growth overall, as well as in the growth of roots and shoots and leaf area [15]. reduced levels of chlorophyll, delayed flowering, and decreased enzyme activity [16,17], as well as nutritional deficiencies such as N, P, K, Ca, and Mg deficiency in plants [18]. In many plants, salt stress also leads to the accumulation of toxic active oxygen species in the cells, which can later result in injury to proteins, membrane lipids, and nucleic acids [19,20]. In addition, salinity can change lipid metabolism and lipid peroxidation, which can lead to membrane deterioration [21]. It has also been shown that some plants accumulate proline in response to salt accumulation [22,23]. Tomato seeds can be severely affected by even low levels of salt, which may inhibit their germination and vigor. Also, at tomato seedlings' early growth stage, salinity has been shown to slow and inhibit their growth [24]. It causes the two important characteristics of producers of tomatoes to decrease: fruit size and total yield per plant. In addition, it has been found that tomato plants growing in salt stress have reduced leaf area and shorter plant heights. Also, under salinized conditions, the relative chlorophyll content of leaves, which indicates the amount of nitrogen a key factor for improving photosynthesis may also be reduced [25].

To improve plants' ability to tolerate abiotic stress, calcium is required [26,27]. Ca regulates and improves the structural integrity of membrane organelles in plants under stress by regulating the structure, signaling, and function of membranes [28]. To mitigate the effects of salt stress, exogenous Ca management is vital. Exogenous calcium has also been shown in several previous studies to mitigate salt stress in tomato by osmotic adjustment, an increase in antioxidant enzyme activities, the accumulation of sodium, potassium, and proline, as well as through enhancing the formation of roots and shoots [29-31]. It increases tolerance to salt stress by modifying growth performance, photosynthetic effectiveness, and stress-induced ROS metabolism [32,33]. Some crop plants, such as peas, wheat, sunflower, and tomato, may benefit from calcium to lessen the negative effects of salt, according to some results [34,35]. This research aimed to investigate how foliar calcium chloride treatment affects tomato quality and yield in salinity stress.

METHODS

Tomato seeds were surface-sterilized for 15 minutes using 2.5% sodium hypochlorite (NaOCl) and then thoroughly washed with distilled water several times. During the 2021–

2022 growing season, a factorial experiment with three replications was designed according to the Completely Randomized Design (CRD). Six seeds were sown on pots, each pot containing 4 kg of a mixture of dry soil and peat moss (1:1) , NPK (17:17:17) fertilizers were added before sowing. Plants were thinned to three plants per pot after 20 days of germination. The treatment with salt stress (0, 25, and 75 mM NaCl) was started 20 days following seeding. Calcium in the form of $CaCl_2$ (0, 5, and 10 mM) was sprayed on shoots three times during the growing season after the appearance of 4-6 true leaves for the first spray and at an interval of 20 days for the second and third sprays, respectively.

A. Assessment of Growth and Yielding Parameters

Nine weeks after the commencement of the treatment, the crops were harvested. After harvesting, plant samples were separated into parts for the fruit, roots, shoots, and leaves. the height of the plants was measured using a scale. Samples of shoots and roots were oven-dried for 72 hours at 70°C to be weighed. Data on yield components, such as the weight and number of fruits per plant, were recorded at the time of final harvest.

B. Calculating the Relative Water Content

The relative water content (RWC) of the leaves was determined three weeks after the treatment was given as already explained by [36]. Twenty leaf discs with a diameter of 0.5 cm were taken from the topmost, completely grown young leaves, and their initial fresh weight was noted. After soaking the discs in double-distilled water for 60 minutes, turgid weights were measured. Following 72 hours of oven drying at 70 °C, dry weights were measured. The RWC was calculated using the following formula:

$$\text{RWC} = \frac{Fresh\ weight - Dry\ weight}{Turgid\ weight - Dry\ weight} \times 100$$

C. Determination of N, P, K, Na, Ca, and Mg concentrations

The plant shoot material with leaves was dried in an oven at 70°C. for 72 hours, the dried materials of 0.5 g were cut and digested by HNO_3:$HClO_4$ (5:1) acid mixture according to [37], The Kjeldahl apparatus determined nitrogen according to the method [38]. A spectrophotometer was used to measure phosphorus using the method [39]. Potassium and sodium were calculated using the flame photometer by the procedure [40]. Magnesium and calcium concentrations have been measured using [41].

D. Determination of Proline Content

The proline content was determined 50 days post-transplant using the methodology outlined by [42].

E. Statistical Analysis

The data analysis for inquiry into the effect of treatments was conducted by the SAS application [43]. The means of the treatments have been compared using the Least Significant Difference (LSD) Testing at a level of probability of 0.05.

RESULT AND DISCUSSIONS

A. Growth and Yielding Parameters

Tabel 1. The effect of NaCl, CaCl2, and their interaction on some growth, and yield characteristics of tomato plants.

NaCl (mM)	$CaCl_2$ (mM)	Plant height (cm)	Shoot dry weight (g)	Root dry weight (g)	No. fruit/plant	Fruit weight (g)
0	0	100.67	26.93	1.33	8.33	46.70
	5	106.00	31.27	1.53	10.67	48.77
	10	107.67	29.33	1.67	12.67	52.27
Mean		104.78	29.18	1.51	10.56	49.24
25	0	90.67	22.93	1.10	5.67	39.27
	5	96.33	26.20	1.33	8.67	40.67
	10	100.33	26.90	1.47	9.67	46.06
Mean		95.78	25.34	1.30	8.00	42.00
75	0	70.67	19.40	0.83	3.67	32.08
	5	76.33	22.33	1.17	8.33	35.56
	10	79.67	25.33	1.00	9.33	41.50
Mean		75.56	22.35	1.00	7.11	36.38
Average	0	87.34	23.09	1.09	5.89	39.35
	5	92.89	26.60	1.34	9.22	41.66

	10	95.89	27.19	1.38	10.56	46.61
LSD	NaCl	1.176	0.259	0.083	0.577	0.260
0.05	$CaCl_2$	1.176	0.259	0.083	0.577	0.260
	Interaction	2.036	0.450	0.143	0.999	0.451

Table 1 indicates that when sodium chloride concentration increased from 0 to 75 mM, salinity significantly decreased the growth and yield traits of tomato plants. Compared to the control plants, the plant height was reduced by 276.8%, from 104.78 cm to 75.78 cm, the shoot dry weight was reduced by 23.41%, from 29.18 g to 22.35 g, the root dry weight was reduced by 33.77%, from 1.51 g to 1.00 g, number of fruits reduced by 32.67%, from 10.56 to 7.11, and fruit weight was reduced by 26.12% from 49.24 g to 42.38 g. Exogenous application of 5, and 10 mM Ca to tomato plants under salt stress, however, enhanced growth and yield traits. The 10 mM Ca-treated sample showed the most amount of these traits, plant height by 9.79%, root dry weight by 26.60%, number of fruits by 79.29%, and fruit weight by 18.45% Compared to the control plants respectively. The 5 mM Ca-treated sample showed the most amount of shoot dry weight by 17.76%.

The study found that the two factors had a significant interaction with a maximum value at 0 mM NaCl and 10 mM CaCl2 on plant height, root dry weight, fruit weight, and number of fruits, which reached (107.67cm, 1.67g, 52.27g, and 12.67) respectively. While 0 mM NaCl and 5 mM CaCl2 showed the highest value in shoot dry weight (31.27g).

B. Relative Water Content

The findings in Table 2 showed that increasing the sodium chloride concentration also significantly reduced the relative water content, which was 9.85%. Calcium enhanced RWC performance by reducing the negative effects of salinity stress. The maximum RWC (85%) was recorded at 5 mM Ca. When the two factors interacted, significant effects were found; the highest RWC (88%) was reported at 0 mM NaCl and 5 mM $CaCl_2$.

C. Mineral Concentrations

From the results of Table (3), with an increase in NaCl concentration, there is a significant decrease in the percentage of nitrogen, phosphorus, potassium, calcium, and magnesium, and a significant increase in sodium; Exogenous application of Ca had a significant effect on the average percentage of nitrogen, phosphorus, potassium, calcium, and magnesium with the superiority of treatment 10mM, as it gave the highest rate of nitrogen and phosphorus by 42.04%, and 35.35% respectively. and the superiority of treatment 5mM, as it gave the highest rate of potassium, calcium, and magnesium, by

62.41%, 18.16%, and 18.13% respectively. the treatment with calcium resulted in significant differences in the percentage of sodium, as shown in the table, the lowest rate of Na accumulation was o.272% at a concentration of 10mM.

D. Proline Content

Table 4 shows that salinity significantly increased the proline content in tomato plants when the concentration of sodium chloride increased from 0 to 75 mM. In comparison with the control plants, the highest rates of proline content at a 75 mM concentration were found in the leaves and fruits, at 1.187 and 0.866, respectively, whereas the control treatment had the lowest rates, which were 0.541 and 0.508 respectively.

There were significant differences in the proline content after the Ca treatment. According to Table(4), at a concentration of 10 mM, the highest rates of proline content were found in leaves and fruits at 1.343 and 0.875 respectively. however, the control treatment's lowest proline content rate was 0.569 and 0.538 respectively. The interaction effect between the two factors was significant and the highest proline content rates in leaves and fruits at 75 mM sodium chloride and 10 mM calcium were 2.056 and 1.203 respectively.

Table 2. The effect of NaCl, $CaCl_2$, and their interaction on the relative water content of tomato plants.

NaCl (mM)	$CaCl_2$ (mM)	Relative water content (%)
0	0	84.67
	5	88.00
	10	84.67
	Mean	85.78
25	0	80.33
	5	84.67
	10	79.33
	Mean	81.44
75	0	74.33
	5	82.33
	10	75.33

	Mean	77.33
Average	0	79.78
	5	85.00
	10	79.78
LSD	NaCl	0.813
0.05	$CaCl_2$	0.813
	Interaction	1.408

Table 3. The effect of NaCl, $CaCl_2$, and their interaction on leaves' nutrient concentrations of tomato plants.

NaCl (mM)	$CaCl_2$ (mM)	N (%)	P (%)	K (%)	Na (%)	Ca (%)	Mg (%)
	0	1.287	0.413	1.813	0.250	2.360	0.780
0	5	1.430	0.480	2.750	0.227	3.030	0.917
	10	1.957	0.607	2.723	0.213	2.840	0.877
Mean		1.558	0.500	2.429	0.230	2.743	0.858
	0	1.153	0.377	1.333	0.390	2.180	0.700
25	5	1.347	0.407	2.090	0.303	2.287	0.853
	10	1.607	0.500	2.167	0.257	2.487	0.800
Mean		1.369	0.428	1.863	0.317	2.318	0.784
	0	1.123	0.210	1.130	0.537	2.067	0.637
75	5	1.190	0.290	2.107	0.440	2.490	0.733
	10	1.260	0.313	1.713	0.347	2.103	0.757
Mean		1.191	0.271	1.650	0.441	2.220	0.709
Average	0	1.188	0.333	1.426	0.392	2.202	0.706
	5	1.322	0.392	2.316	0.323	2.602	0.834

	10	1.608	0.473	2.201	0.272	2.477	0.811
LSD	NaCl	0.021	0.010	0.026	0.009	0.023	0.009
0.05	$CaCl_2$	0.021	0.010	0.026	0.009	0.023	0.009
	Interaction	0.036	0.018	0.045	0.016	0.039	0.017

Table 4. The effect of NaCl, $CaCl_2$, and their interaction on leaves and fruits' proline content of tomato plants.

NaCl (mM)	$CaCl_2$ (mM)	Proline in leaves (μmol g^{-1})	Proline in fruits (μmol g^{-1})
	0	0.514	0.503
0	5	0.520	0.509
	10	0.589	0.513
Mean		0.541	0.508
	0	0.589	0.518
25	5	0.728	0.610
	10	1.383	0.908
Mean		0.900	0.679
	0	0.603	0.594
75	5	0.900	0.801
	10	2.056	1.203
Mean		1.186	0.866
Average	0	0.569	0.538
	5	0.716	0.640
	10	1.343	0.875
LSD	NaCl	0.011	0.012

0.05	$CaCl_2$	0.011	0.012
	Interaction	0.019	0.020

E. Disscussions

Growth and Yielding Parameters

Salinity stress resulted in a significant decrease in tomato plant growth and yield, including plant height, shoot and root dry weight, fruit number, and fruit weight (Table 1). Reduction in plant growth and yield is a common impact of salinity in several plant species [13,14,44]. These reductions might be caused by stress-induced decreases in cell division, elongation, and plant nutrient absorption inhibition. They might also be the result of an ionic imbalance caused by a high level of salt. Growth is inhibited by oxidative and osmotic stress, as well as ion homeostasis disturbance due to saline [45]. Salt stress ultimately influences reproductive development by causing an increase in ovule abortion and fertilized embryo senescence [46]. It was found that applying calcium exogenously decreased the inhibition of crop growth at salt stress [47,48]. Also, exogenous calcium treatment may improve mineral element absorption and reduce the effect of stress on crop growth and roduction[49].

Relative Water Content

As compared to the control, the relative water content (RWC) of tomato decreased by salinity stress (Table 2). Salt stress, which decreases the root water potential by depositing salt in root zones and inhibits the uptake of water, can lead to osmotic imbalances [50,51]. These findings correspond to those of [44]. Under salt stress, the external application of calcium improved tomato RWC, which probably is caused by decreasing damage to membranes and improving water balance. Calcium could maintain the turgid guard cells, which may have a beneficial effect on stomatal functioning [52].

Mineral Concentrations

The uptake of N, P, K, Ca, and Mg was significantly decreased by salt stress, whereas the uptake of Na increased significantly (Table 3). Because the rhizosphere is surrounded by Na ions, which causes an influx of Na ions via membrane-located channels and transporters on the plasma membrane, salt stress creates a significant electrochemical gradient of ions [53,54]. Na has an antithetical relationship with the ions of other essential minerals, which ultimately leads to an ion imbalance when salt stress is applied. The external application of calcium decreases sodium concentration while other element concentrations increase. It may be due to calcium's role in regulating the plasma membrane's selective permeability, decreasing sodium ion concentration while other essential element concentrations increase [55,56].

Proline Content

The proline content increased significantly with salinity stress. and the rate of proline accumulation suggests that it is helping a variety of plants with their osmoregulation and reduction of salt stress [47,57]. And when Ca was applied exogenously, these levels increased further. Ca is a vital messenger molecule that has a role in proline production [58].

CONCLUSION

Stress from salinity affected plant growth, yield, and other physiological traits. Calcium treatment improves tomato plants and reduces the negative impacts of sodium chloride salt stress. When plants were stressed by NaCl, Ca (10 mM) was shown to be beneficial for both growth and physiological response. This indicates that plants can be protected from the damaging effects of salt stress if calcium is provided to plants at an appropriate rate.

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