Int. Res. J. of Science & Engineering, Special Issue A9, July, 2020
ISSN: 2322-0015
SJIF Impact Factor 6.70
RESEARCH ARTICLE
OPEN ACCESS

Effect of salinity stress on seed germination and growth parameters of two Tomato cultivars (*Solanum lycopersicum*) with calcium chloride treatment

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Manuscript Details

Available online on https://www.irjse.in
ISSN: 2322-0015

Editor: Dr. Arvind Chavhan

Cite this article as:
Tomar Pooja, Karande Sucheta, Sharma Asha and Patel Saloni. Effect of salinity stress on seed germination and growth parameters of two Tomato cultivars (*Solanum lycopersicum*) with calcium chloride treatment, *Int. Res. Journal of Science & Engineering,* 2020, Special Issue A9: 215-222.

Article published in Special issue of International e-Conference on "Emerging trends and Challenges In life sciences" organized by Department of Botany, Indraraj Arts, Commerce & Science College, Sillod-431112, Dist Aurangabad, Maharashtra, India date, June 18-19, 2020.

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Abstract

The world's agricultural production is facing a lot of challenges. Abiotic stress conditions are identified to cause huge losses to the production of agriculture worldwide. Among all the abiotic stresses, high level of salinity stress is most harsh environmental stress. High salinity exerts its negative impact mainly by disturbing the ionic and osmotic equilibrium of the cell. In the saline soils, high levels of sodium ions lead to plant growth inhibition and even the death of plant. The main objective of this work was to understand the different physiological and biochemical features in responses to salinity in the horticulture crop i.e. tomato. This work assessed to check the effect of calcium chloride on the NaCl stressed two cultivars of tomato (C 21 and Pusa hybrid). Both the cultivars were treated with solution of 0mM, 60mM, 90mM, 120mM and 150 mM NaCl with 0.5mM, 1.0mM, 1.5mM and 2.0mM of $CaCl_2$. The plants under the salinity stress had evident adverse effect on their growth. Different physiological parameters like germinability of seed, plant growth, fresh weight and dry weight selected cultivars were checked.

Keywords-Salinity, Toxicity, inhibition, treated, Stress

dSm-1……………………….. DeciSiemens per meter
ECe…………………………….Electrical conductivity
mmol/l………………………...millimole per litre
Cl^- ……………………….….Chloride ions
μMS^{-1}…………………………micromolar per second
µM ……………………………micromolar
mg g^{-1}…………………………milligram per gram
AR Grade…………………….Analytical Reagent grade
rpm…………………………...revolution per minute

Introduction

The world's agricultural production is facing a lot of challenges. Approximately 70% additional food will be needed for additional 2.3 billion population projected by 2050. However, crop yield is not growing as par with the rising demand of food. This matter of food uncertainty is added mainly by the degradation of the fertility of soil, reduced crop productivity and random climate change and this situation is getting worse in the near future. Salinization of land may result from the flooding of land by seawater, weathering of rocks and minerals, poor agricultural practices and improper on farm water drainage (secondary salinization). Irrigation practices in large part play a fundamental role in meeting the world's demand of food supply. Both the salinity and drought stress are most vital factors which are generally manifested as osmotic stress [1]. Most of the plants can bear up to 200mM NaCl [2]. Currently about 40% of the global food harvest is contributed by irrigated land [3]. Salinity has dramatically affected the agriculture potential of land in many areas of the world and affected the productivity of a large number of crops [4]. The NaCl and magnesium dichloride are readily soluble salt and releases ions upon dissolving, while the production of the ions was relatively lesser in the calcium compounds viz., calcium sulphates, calcium carbonates and magnesium sulphates. Several Na^+ ion transporters exist in plants. A sodium transporter called SKC1 is also a sodium ion eclectic carrier present in resistant cultivar of *Oryza sativa* partially culpable for endurance of Na^+ [5]. After the absorption of Na^+ by the roots, it travels through the plant trunk with the transpiration flow by decreasing the potassium/sodium fraction. The ion equilibrium gets disturbed which finally leads to the procreation of active oxygen species causing cell bereavement [6]. Some hormones also act as regulator during the plant salt and metal stress. The NaCl stress causes the deleterious action in the plants but to avoid all these, some solutes gets distributed throughout the cytosol of the cell in massive concentration by unaffecting and interrupting cell metabolism. In plants and vegetables the stress is induced by so many diverse natural and anthropogenic stress factors. The deterioration of the environment has generated an increase of stress in all the forms of life. Salinity poses major threat to the growth of plants [7]. Salt stress directly affects productivity of major cash crops of the globe [8]. Approximately one-fifth of the world productivity is influenced by saline soil [9]. The hypertonic microenvironment of roots due to high salt concentration in soil deters the uptake of water by the roots of the plant [10], thereby slashing down the net productivity of the crop [11]. The salinity stress negatively impacts the intracellular Ca^{2+} levels of plants [12]. Moreover, the $CaCl_2$ imparts the ability to tolerate the salinity stress [13-15]. The study is thus designed to understand the role of calcium chloride salt in imparting tolerance to salinity stress.

Methodology

Plant material and Experimental pots

The soil samples were collected from the fields that are in use for cultivation purpose in villages adjoining Rohtak. The soil Nitrogen, phosphorus and potassium (NPK) contents were estimated and the soil pH was also taken at Krishi Vigyan Kendra, Rohtak. Soil was systematically mixed with natural manure and laden into the pots (Figure 3.1). The soil laden pots were irrigated with normal water for 2 days to maintain the moisture content.

The seeds of two tomato varieties, viz., cv. C 21 and cv. Pusa hybrid were purchased from IARI, Pusa, New Delhi. The seeds were checked for their viability at Agro-mall, Rohtak, before setting up the experiments. All the experiments were conducted thrice for two seasons in the consecutive years, 2015-16 and 2016-17. The plants were maintained in university herbal garden to provide the optimum and identical growth conditions throughout the experiment to the plants of both the cultivars.

Five different concentrations of NaCl i.e., 0mM, 60mM, 90mM, 120mM and 150 mM were prepared in Hoagland's solution to provide salinity stress to the plants. The pots were irrigated with the different concentrations of the saline solution. To check the effect of Calcium ions, five different concentrations viz., 0mM, 0.5mM, 1.0mM, 1.5mM and 2.0 mM of $CaCl_2$ were prepared. The $CaCl_2$ was supplemented with NaCl salt solution in various permutations and combinations.

Growth parameters

Effect on seed germination and growth rate

Germination percentage

The seeds were surface sterilized with dilute solution of 1% sodium hypochlorite (NaOCl) for 12 minutes to prevent any fungal contamination and then rinsed thrice with deionized water. The surface sterilized seeds were sowed in pots (10 seeds per pot) at a depth of 1 cm along the side of margins at spacing of 15-20 cm. The soil in the pots was irrigated with different combinations of the $CaCl_2$ and NaCl solution. The seed germination was recorded after 14 days of sowing and the germination percentage was calculated as per the following formula:

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} X100$$

Determination of Physical Parameters

Root and Shoot Length

The tomato plants were uprooted on 45th day of sowing and its roots were washed with sterile distilled water. Excess water was wiped off using a paper towel. The lengths of roots and shoots were measured for each plant with the help of a transparent one feet scale and the reading was noted down in cm.

Results and Discussions

Seed Germination in two cultivars of tomato upon salinity stress in the presence of calcium salt

83$\pm$5.77% of the control seeds of C21 cultivar were germinated while 96$\pm$5.67% of Pusa hybrid seeds were germinated (Table 4.1 a, b, Figure 4.1). The germination percents of the two cultivars were significantly different from each other with p value >0.005 when analysed by Student's T Test. Upon application of NaCl to the plants the germination percent decreased in accordance with the increase in the NaCl concentration and reached 49.17$\pm$10.00 % in C21and 34.52$\pm$15.28% in Pusa Hyrid under the stress of 150mM NaCl (Table 4.1 a,b, Figure 4.1).

Although NaCl stress had pronounced effect on the percent seed germination (ANOVA; P value<0.0001 for C21 and P value<0.0001 for Pusa hybrid), the $CaCl_2$ treatment could not reverse the effect of the salinity stress on the seed germination of both the cultivars of tomato (ANOVA; P value>0.05; nonsignificant for both the cultivars).

Root and Shoot length of the two cultivars of tomato upon salinity stress in the presence of calcium salt.

The plant height was recorded separately for the roots and shoots of a plant. Root length was almost same in the C21 and Pusa hybrid cultivars of tomato with root length ~9.00 cm (Table 4.2). On the other hand, the lengths of the shoot of the two cultivars were different. The shoot length of C21 was 12.97$\pm$0.83 cm while that of Pusa hybrid was 14.62+1.04 cm (Student's T test: p value> 0.05) (Table 4.3). The differences in the growth were visually evident in the plants of both the cultivars (Figure 4.2.).

The plants under the salinity stress had evident adverse effect on their growth (Figure 4.3, 4.4; Table 4.2, 4.3). The plant heights of both the C 21 and Pusa Hybrid cultivars of tomato were reduced with the increase in the salinity stress and were stunted to nearly one-third of the control when grown in presence of 150mM NaCl (ANOVA; P value<0.0001 for both the cultivars) . The salinity equally affected both the root as well as the shoot length of both the plants. The negative effects of salinity on the growth of tomato plants are well studied (Dumbroff and Cooper, 1974; Snapp and Shennan, 1992). The effect of salinity on the shoot growth was reported to be more pronounced in the younger seedlings as compared with the older ones (Dumbroff and Cooper, 1974).

One-to-one comparison of the two cultivars using the Student's T test revealed significant difference between the root lengths when 90mM of NaCl stress was countered with 0.5mM or 1.5mM of CaCl2. Similarly, differences between the shoots lengths were recorded when 120mM of NaCl was countered with 1.0 mM of $CaCl_2$.

Figure 3.1. Mixing soil with natural manure

Table: 4.1(a). Effect of NaCl stress and $CaCl_2$ supplementation on the % germination of C21 cultivar of Tomato. Data are mean value of (n=6)± SD. Data was analyzed by two-way ANOVA. Data are significant at P value<0.0001

C21	Germination	Percent	$CaCl_2$		
NaCl	0mM	0.5mM	1.0mM	1.5mM	2.0mM
0mM	83.33±5.77	88.33±11.69	88.33±11.69	81.67±7.53	85.00±10.49
60 mM	76.67±5.77	71.67±7.53	73.33±10.33	76.67±15.06	76.67±15.05
90mM	60.00±5.77	65.00±10.49	61.67±14.71	58.33±14.72	61.67±7.53
120mM	59.17±10.00	56.67±8.16	56.67±5.16	60.00±10.95	58.33±7.53
150mM	49.17±10.00	45.00±8.37	51.67±9.83	48.33±9.83	50.00±8.94

Table: 4.2 (a).Effect of NaCl stress and CaCl2 supplementation on the root length (cm) of C21 cultivar of Tomato. Values are mean of six replicates + SD.Data was analyzed with two-way ANOVA. Data are significant at P value<0.0001,

C21	Root	Length (cm)	$CaCl_2$		
NaCl ↓	0mM	0.5mM	1.0mM	1.5mM	2.0mM
0mM	9.00±0.54	8.99±0.22	8.88±0.69	8.47±0.93	8.53±0.74
60 mM	8.75±0.80	8.65±1.17	8.30±1.20	9.08±1.53	10.65±2.01
90mM	5.02±0.43	6.88±0.56	7.05±1.12	6.89±1.15	6.84±0.90
120mM	3.49±0.14	6.84±0.90	6.13±0.82	5.71±1.00	5.74±0.85
150mM	2.58±0.24	4.37±0.79	4.90±0.58	4.85±0.44	4.51±0.46

Table: 4.2 (b). Effect of NaCl stress and CaCl2 supplementation on the root length (centimeters) of Pusa hybrid cultivar of Tomato. Values are mean of six replicates + SD. Data was analyzed with two-way ANOVA. Data are significant at P value<0.0001

Pusa Hybrid	Root	Length (cm)	$CaCl_2$		
NaCl ↓	0mM	0.5mM	1.0mM	1.5mM	2.0mM
0mM	9.43±0.33	9.47±0.67	9.16±1.18	9.00±0.50	8.46±0.76
60 mM	9.60±0.68	9.12±0.84	8.54±1.06	9.59±0.76	10.31±1.46
90mM	7.53±0.82	8.60±1.03	8.02±1.34	8.13±0.55	7.86±0.96
120mM	4.55±0.43	7.40±0.58	6.70±0.55	5.83±0.47	5.87±0.95
150mM	3.49±0.54	4.18±0.79	4.70±0.79	4.23±0.68	4.03±0.70

Table: 4.3(a) Effect of NaCl stress and CaCl2 supplementation on the shoot length (cm) of C21 cultivar of Tomato. Values are mean of six replicates + SD.Data was analyzed with two-way ANOVA. Data are significant at P value<0.0001 for NaCl and P<0.05 for CaCl2

C21	Shoot	Length (cm)	$CaCl_2$		
NaCl ↓	0mM	0.5mM	1.0mM	1.5mM	2.0mM
0mM	12.97±0.83	13.15±1.41	13.65±0.72	12.98±1.47	12.16±1.74
60 mM	12.15±0.73	11.83±1.63	11.46±1.05	11.63±1.60	12.04±1.50
90mM	7.72±0.43	10.19±2.12	10.55±1.43	9.78±1.97	9.78±1.76
120mM	6.17±0.14	8.60±1.44	7.70±0.80	6.91±1.14	6.50±2.01
150mM	4.34±0.24	6.39±2.26	7.46±1.438	6.81±1.52	6.61±1.17

Table: 4.3 (b). Effect of NaCl stress and CaCl2 supplementation on the shoot length (cm) of Pusa hybrid cultivar of Tomato. Values are mean of six replicates + SD. Data was analyzed with two-way ANOVA. Data are significant at P value<0.0001 for NaCl

Pusa Hybrid	Shoot	Length (cm)	$CaCl_2$		
NaCl ↓	0mM	0.5mM	1.0mM	1.5mM	2.0mM
0mM	14.62±1.04	13.75±1.94	14.66±3.30	13.44±4.00	13.13±4.14
60 mM	13.65±0.57	13.53±2.77	12.62±1.97	12.33±2.18	14.24±2.17
90mM	9.16±0.70	11.03±1.96	10.62±2.29	10.95±1.99	10.62±2.09
120mM	6.33±0.95	9.89±0.79	9.76±0.94	7.58±3.66	8.09±1.28
150mM	5.93±0.32	7.59±1.53	7.73±1.52	7.09±1.19	6.30±1.23

Table: 4.1(b). Effect of NaCl stress and CaCl2 supplementation on the % germination of Pusa Hybrid cultivar of Tomato. Data are mean value of (n=6)+ SD. Data was analyzed by two-way ANOVA. Data are significant at P value<0.0001

Pusa Hybrid	Germination	Percent	$CaCl_2$		
NaCl ↓	0mM	0.5mM	1.0mM	1.5mM	2.0mM
0mM	96.67±5.16	86.67±5.16	90.00±8.94	90.00±8.94	85.00±15.16
60 mM	82.86±17.32	76.67±5.16	73.33±5.16	71.67±8.94	76.67±5.16
90mM	75.95±11.55	60.00±6.32	61.67±4.08	65.00±14.72	66.67±5.16
120mM	51.79±10.00	56.67±8.16	58.33±11.69	56.67±15.06	48.33±14.72
150mM	34.52±15.28	53.33±10.32	46.67±8.16	45.00±5.48	48.33±9.83

Conclusion

Tomato is globally cultivated for its fleshy fruits and known as protective food for the reason that of its special nutritive value and its wide spread production. *Solanum lycopersicum* is the plant and it belongs to the Solanaceae family. Tomato is measured as a crop of significant value worldwide. The world's agriculture produce is posed with a lot of challenges.

In case of any environmental factor surpasses the optimal range of the plant it possess stress on the plant and in turn manipulate its structural, physiological, developmental and biochemical processes (Jaleel *et al*., 2007a). Out of all these stresses, the salt stress is most stubborn. The salinity stress is known to negatively impact the intracellular Ca^{2+} levels of plants [12]. Moreover, the CaCl2 imparts the ability to tolerate the salinity stress [13-15]. The study is thus designed to understand the role of chloride salt of calcium in imparting tolerance to salinity stress.

The seedlings of Pusa hybrid and C21 cultivars of tomato were selected for the work. The plants were grown in presence of the NaCl stress and the stress was countered by added Ca salt. The treatment of Calcium chloride and salinity were given by adding 60, 90,120,150 mM NaCl and 0.5, 1.0, 1.5, 2.0 mM of $CaCl_2$ in Hoagland solution right from germination stage. Plant growth and development of the two cultivars was studied under these conditions by assessing the seed germination, seedling growth, fresh weight and dry weight of selected cultivars.

- The Pusa hybrid had higher ability to germinate as compared with the C21 cultivar. Eighty three percent ($\pm$5.77%) of the control seeds of C21 cultivar were germinated against 96$\pm$5.67% of Pusa hybrid seeds. Although NaCl stress had pronounced effect on the percent seed germination (ANOVA; P value<0.0001 for C21 and P value<0.0001 for Pusa hybrid), the $CaCl_2$ treatment could not reverse the effect of the salinity stress on the seed germination of both the cultivars of tomato (ANOVA; P value>0.05; nonsignificant for both the cultivars).
- The plants under the salinity stress had evident adverse effect on their growth. The plant heights of both the C 21 and Pusa Hybrid cultivars of tomato were reduced with the increase in the salinity stress and were stunted to nearly one-third of the control when grown in presence of 150mM NaCl. The salinity equally affected both the root as well as the shoot length of both the plants. Plant growth is directly related to the healthy metabolism including protein synthesis in plants that need efficient hormonal and enzymes system. The Na^{+} ions accumulate in leaves when the plant is grown at high salt concentrations.

Conflicts of interest: The authors stated that no conflicts of interest.

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