European Journal of Medicinal Plants

32(3): 41-47, 2021; Article no.EJMP.63013
ISSN: 2231-0894, NLM ID: 101583475

Assessment of NaCl-Induced Stress in Tomato (*Lycopersicon esculentum* L.)

Abhishek Kumar[1], Khushbu Jain[1], Mahesh Kumar[2], Md. Shamim[2], Jitesh Kumar[3*], Anil Kumar[1] and Abhishek Prasad Sahu[4]

[1]Department of Plant Breeding and Genetics, BAC, Sabour, Bhagalpur, Bihar-813210, India.
[2]Department of Molecular Biology and Genetic Engineering, Dr. Kalam Agricultural College, BAU Sabour, Bihar-855107, India.
[3]Department of Molecular Biology and Genetic Engineering, BAU, Sabour, Bhagalpur, Bihar- 813210, India.
[4]Department of Botany, Ranchi University, Ranchi-834002, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author AK designed the study, collection and preparation of plant material, done all the laboratory works and managed the literature searches. Author JK supervised the work and contributed in drafting the paper. Author KJ contributed in statistical analysis. Authors MK and MS contributed in providing the laboratory facilities. Authors AK and APS contributed to several reading of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2021/v32i330380
Editor(s):
(1) Dr. Daniela Rigano, University Federico II of Naples, Italy.
(2) Prof. Marcello Iriti, University of Milan, Italy.
Reviewers:
(1) Mayara Fávero Cotrim, Sao Paulo State University, Brazil.
(2) Işılay Lavkor, Biological Control Research Institute, Turkey.
Complete Peer review History: http://www.sdiarticle4.com/review-history/63013

Original Research Article

Received 16 September 2020
Accepted 21 November 2020
Published 01 June 2021

ABSTRACT

Comparative study about the salt-induced oxidative stress and lipid peroxidation has been realised in primary root tissues for Tomato (*Lycopersicon esculantum* L.) in order to evaluate their responses to salt stress. Salinity impacts in terms of root growth, H_2O_2 generation, lipid peroxidation and membrane destabilisation were more pronounced in roots. Salt treatment in form of NaCl was given to the roots of the tomato plants in hydroponics culture. Root length was measured by centimetre scale, H_2O_2 and lipid peroxidation was confirmed by spectrophotometer. Absorbance for H_2O_2 estimation was recorded at 480 nm whereas for Lipid peroxidation was done at 600nm. When the tomato plants were treated with different

**Corresponding author: E-mail: jitesh1jan@gmail.com;*

concentrations of NaCl, it was observed that as the concentration of NaCl was increasing, there was decreased root growth resulting in reduced root length and proportionate increase in the amount of H_2O_2 production level with increase in the concentrations of NaCl treatment upto 300mM Concentration and Significant increase in Lipid peroxidation was observed with the increase in NaCl concentrations upto 500mM Concentration. Comparative response may be helpful in developing a better understanding of tolerance mechanisms to salt stress in Tomato.

Keywords: Tomato; salinity; hydroponics; hydrogen peroxide; lipid peroxidation.

1. INTRODUCTION

Stress is usually defined as an external factor that exerts a disadvantageous influence on plants. In both natural & agriculture conditions, plants are frequently exposed to environmental stresses. Stress can be environmentally induced (abiotic) or can be induced by weeds, pathogens and insects predation (biotic). Salinity is considered as a significant factor affecting crop production and agricultural sustainability in many regions of the world as it is also associated with ecological balance of the area. Salinity is the concentration of dissolved mineral salts present in the soil (soil solution) and water. Crop species show a spectrum of responses to salt, although all have their growth, and eventually, their yield reduced by salt. Salt effects are the combined result of the complex interaction among different morphological, physiological and biochemical processes [1].

Salinity may directly or indirectly inhibit cell division and enlargement in the plant's growing point. Reduced shoot growth caused by salinity originates in growing tissues, not in mature photosynthetic tissues. As a result, leaves and stems of the affected plants appear stunted [1]. Chloride induces elongation of the palisade cells, which leads to leaves becoming succulent. Salt stress reduces dry matter content, increases root: shoot ratio, and diminishes leaf size; as a result grain yield is reduced [1]. Salt stress affects many aspects of plant metabolism and, as a result, growth is reduced. Among various salts influencing soil salinity, NaCl is the most abundant and powerful one due to its ability to compete essential nutrients resulting to cause nutrient deficiency and certain toxic symptoms in the plants [2] (Tester & Davenport, 2003).

In addition to be generated from saline soil exposure, the ROS can also be inevitably byproducts from ordinary cellular metabolisms [3] even under good regulation and under present of ROS removal systems [4]. Under harsh conditions, ROS production rate in plant tissues will overcome ROS scavenging rate and the oxidative stress symptom will be incarnated since the generated ROSs attack any vital biomolecules and disturb cellular metabolism which ultimately cause cell death [5]. In addition, plants that are exposed to high salinity condition can also be stressed with reactive oxygen species (ROS) such as superoxide ($O2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH). These existed ROSs have much ability to harm plant tissues due to their highly reactive properties [6,7]. Similar studies was done in Maize crop (*Zea Mays* L.) [8].

Naturally, some plants can develop several protective mechanisms that can effectively eliminate or reduce the ROSs at different stress induced deterioration levels [9] and the ability has been known to be varied in species and varieties. In addition to ionic and osmotic components, salt stress like other abiotic stresses also leads to oxidative stress through an increase in reactive oxygen species (ROS), such as super oxide (O^{2-}) hydrogen peroxide (H_2O_2) & hydroxyl radical (OH) [4,10]. These ROS are highly reactive and can alter normal cellular metabolism through oxidative damage to lipids, proteins and nucleic acid.

The present work deals with the NaCl-induced stress. For this the model plant tomato had been selected and the effects of high salinity had been studied in its root system under hydroponic culture. Tomato is the most consumed berry fruit worldwide as well as one of the most important constituents of the Mediterranean diet representing a key source of minerals, vitamins and antioxidants [11].

2. MATERIALS AND METHODS

2.1 Plant Material and Seed Germination

Petri plates were washed, dried and autoclaved along with filter paper discs. The filter papers were moistened with distilled water and 8-10 seeds were placed in each peri plates. After this the filter papers were moistened with distilled water. Tomato **(***Lycopersicon esculentum* L.)

seeds were placed on soaked filter paper. These procedures were done in a laminar flow to avoid contamination in seeds. These petri plates were kept in a controlled growth chamber at 25-28^{o}C with 10 hours light. Observations were done so that the filter paper must be moist till the seedling germination. After this, the seedlings were transferred in pots containing balanced Knop's media. after J. A. L. W. Knop (1891) .

2.2 Preparation of Knop's Media

For preparation of 2000 ml of Knop's media, a container of 2 L was taken and surface sterilized with alcohol. Then 5 ml each of the autoclaved stock solution was added with the help of sterilized pipette and the final volume of the mixture was maintained to 2000 ml with distilled water. Before maintaining volume to 2000ml, pH was maintained to 5.5 - 5.8.

The chemical composition & working concentration of Knop's media for 2L was divided into Macronutrients as KNO3(6mM) and KH2PO4(4mM) whereas micronutrients as MgSO4.7H2O(500μM),Na3C6H5O7.2H2O(500μM), FeCl3 (100μM), CaCl2.2H2O(1mM), H3BO3 (50μM), MnCl2.4H2O (10μM), ZnSO4.7H2O (0.9μM), Na2MoO4.2H2O (2μM), CuSO4.5H2O (0.4μM) and CoCl2.6H2O (0.2μM) .

The whole procedure was done in laminar flow. Containers were then kept in plant growth chamber. Temperature was adjusted to 28±2^{0}C and 75% relative humidity was maintained with 14 hours of light period. The plants were grown hydroponically in these containers. After two weeks, plants were taken for experimental progress Noren et al. [12].

2.3 Transfer of Seedlings

A thermocol plate was cut according to the size of container and 15 to 20 pores were made on it. This plate was surface sterilized and fitted in the container containing 2L media 15-20 seedlings were transferred with the help of brush Noren et al. [12].

2.4 Salt Treatment

Salt treatment in form of NaCl was given to the roots of the hydroponics culture of tomato plants. For NaCl treatment plants were transferred in containers containing fresh Knop's medium supplemented with various concentrations of NaCl. Concentrations used were 100mM, 200mM, 300mM, 400mM and 500mM. Plants grown without any NaCl treatment were used as control.

2.5 Root Growth

After 96 hr treatment of tomato plants with different concentrations of NaCl the growth of roots in terms of root length was measured by using a centimeter scale.

2.6 Estimation of Hydrogen Peroxide

H_2O_2 estimation was done by following the method of Sagisaka, 1976. For H_2O_2 estimation, 0.2g of root tissue was homogenised in 3ml of 5% TCA, and centrifuged at 17,000g at 0^{0}C for 10 minutes. Then 1.6ml of the supernatant was taken, and to it was added 0.4 ml TCA (50%), 0.4 ml ferrous ammonium sulphate (10mM) and 0.2 ml Potassium thiocyanate (2.5M). Absorbance of the reaction mixture was recorded at 480 nm on spectrophotometer. H_2O_2 estimation was done 6 hr after NaCl treatment.

2.7 Quantification of Lipid Peroxidation

For lipid peroxidation, 0.2g of root tissue was taken and homogenized in 0.1% (m/v) cold TCA. Homogenate was centrifuged at 10000g for 20 minutes. 1 ml of the supernatant was taken and 1 ml 20% TCA containing 0.5% TBA and 0.01 ml BHT (4% solution in ethanol) was added, the reaction mixture was heated in a water bath at 95^{0}C for 30 minutes followed by cooling in ice. The sample was centrifuged at 10000g for 15 minutes and the absorbance of the supernatant was recorded at 532nm & correction was done at 600nm. Quantification of lipid peroxidation was done 48 hr after NaCl treatment.

3. RESULTS

3.1 Effect of NaCl Treatment on Root Length

When the tomato plants were treated with different concentrations of NaCl, it was observed that as the concentration of NaCl was increasing, there was decreased growth of roots resulting in reduced root length. Maximum root length was observed at 0 mM NaCl whereas shortest root length was observed at 500mM NaCl.

3.2 Change in H_2O_2 Level

There was a proportionate increase in the amount of H_2O_2 production level with increase in the concentrations of NaCl treatment using different concentrations. Maximum production of H_2O_2 was observed at 300mM NaCl concentration whereas least production was observed at 0mM.

3.3 Effect of NaCl Treatment on Lipid Peroxidation

Significant increase in Lipid peroxidation was observed with the increase in NaCl concentrations. It was observed that highest peak at 500mM concentration and lowest with value at 0mM NaCl.

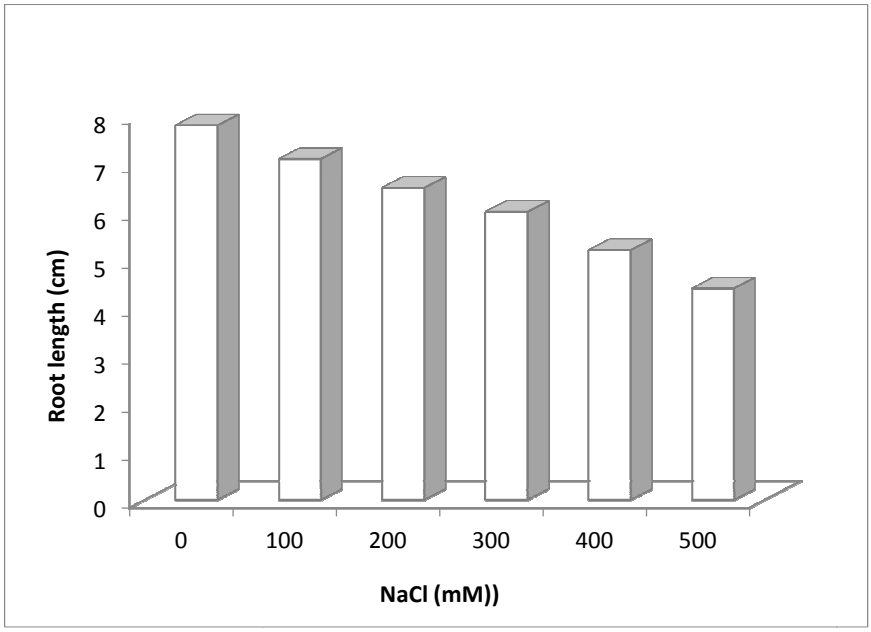

Fig. 1. Effect of NaCl treatment on root length

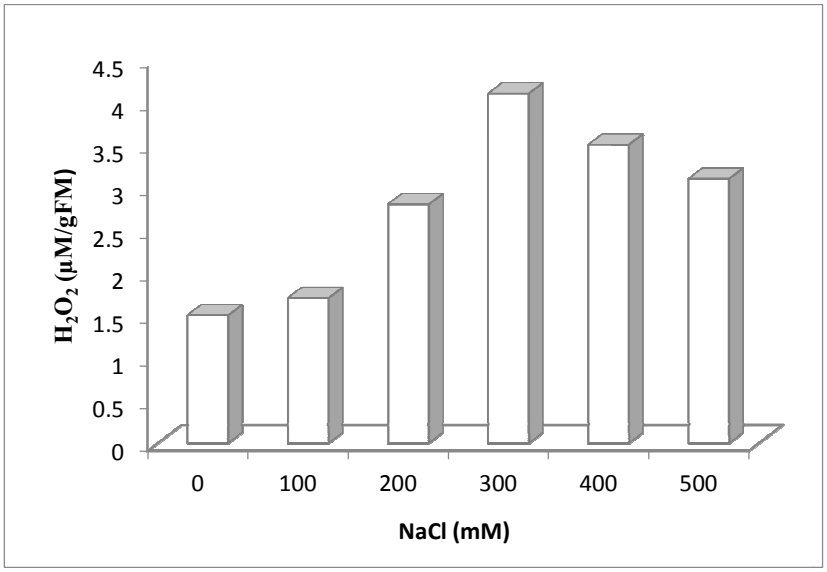

Fig. 2. Changes in H_2O_2 production level after treatment with NaCl after 6 hour

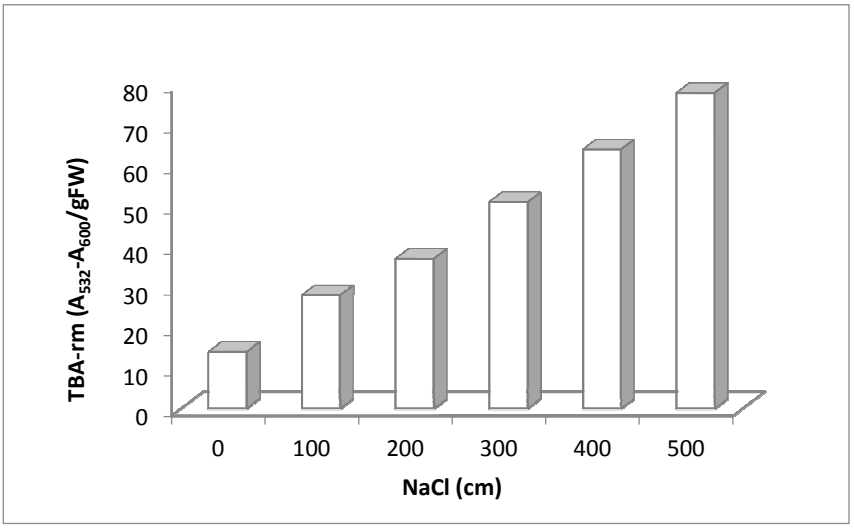

Fig. 3. Effect of NaCl treatment on Lipid peroxidation after 48 hours

4. DISCUSSION

In the present study, we determined the salt stress responses of roots. Salinity impacts were investigated in terms of root growth, hydrogen peroxide (H_2O_2) generation and lipid peroxidation. Comparative response may be helpful in developing a better understanding of tolerance mechanisms to salt stress in Tomato.

The decrease in the fresh and dry weight of the roots as observed in the results showed that the effect of salt stress on the performance and growth are mediated through decrease in stimulating conduction and photosynthesis. Reductions in growth with increased salinity might be due to the adjustment of osmotic potential [13-15]. Salinity (NaCl) stress can cause ionic toxicity and imbalance, membrane damage, reduced uptake of CO_2 as a result of stomatal closure, decreased hydrolytic enzyme activity and increased lipid peroxidation level, it may stimulate formation of ROS such as superoxide, hydrogen peroxide, and hydroxyl radicals.

High concentrations of Na^+ in the soil solution may depress nutrient-ion activities and produce extreme ratios of Na^+/Ca^{2+} or Na^+/K^+ [16]. Increase in cations and their salts, NaCl in particular, in the soil generates external osmotic potential that can prevent or reduce the influx of water into the root. High salinity affects plants in two main ways: High concentrations of salts in the soil disturb the capacity of roots to extract water and high concentrations of salts within the plant itself can be toxic, resulting in an inhibition of many physiological and biochemical processes such as nutrient uptake and assimilation [17-20].

The production of reactive oxygen species (ROS) such as O_2^- and H_2O_2 in plant tissues is the earliest event following the salt stress. During oxidative stress, H_2O_2 is a strong toxic oxidant that can easily damage membrane and other cellular components [21]. The H_2O_2 generation and consequently the increase of lipid peroxidation with salt stress conditions corroborate of already reported results for rice [22,23] and maize [2].

Changes in lipid peroxidation serve as an indicator of the extent of oxidative damage under stress, with an unchanged lipid peroxidation level seeming to be a characteristic of tolerant plants coping with elevated salinity [24]. Lipid peroxidation measured as the amount of thiobarbituric acid reactive substance which is produced when polyunsaturated fatty acids in the membrane undergo oxidation by the accumulation of free oxygen radicals.

5. CONCLUSION

In the present study, effects of high salinity had been studied in its root system under hydroponic culture. Among various salts influencing soil salinity, NaCl is the most abundant and powerful one due to its ability to compete essential nutrients resulting to cause nutrient deficiency and certain toxicity symptoms to the plants. The decrease in the length of roots as observed in

the results showed the effect of salt stress. NaCl salinity leads to an increase in ROS generation, including H_2O_2 which is a strong toxic oxidant responsible for a secondary oxidative stress that can easily damage membrane and other cellular components. The induced effects of NaCl are reflected in the lipid composition of the roots. lipid peroxidation was found to be high in roots of salt sensitive tomato cultivar. At 300mM NaCl concentration,H_2O_2 production reaches at highest level and then decereases.Whereas lipid peroxidation continuously increases with increase in NaCl concentrations upto 500mM which is responsible for the decrease in growth of root and loss of biomass of root.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Singh KN, Chatrath R. Salinity tolerance pp.101-110. In Eds., M. P. Reynolds, J.J. Ortiz-Monasterio & A. McNab (Eds.), Application of physiology in wheat breeding.Mexico: CIMMYT; 2001.
2. Azevedo Neto AD, Prisco JT, Eneas-Filho J, de Abreu CEB, Gomes-Filho E. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salttolerant and salt-sensitive maize genotypes. Environ Exp Bot. 2006;56:87–94.
3. Martinez CA, Loureiro ME, Oliva MA, Maestri M. Differential responses of superoxide dismutase in freezing resistant Solanum tuberosum subjected to oxidative and water stress. Plant Sci. 2001;160:505-515.
4. Mitter R. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 2002;7:405-410.
5. Sakihama Y, Cohen MF, Grace SC, Yamasaki H. Plant phenolic antioxidant and prooxidant activities: Phenolics-induced oxidative damage mediated by metals in plants. Toxicology. 2002;177:67-80.
6. McKersie BD, Leslem YY. Stress and stress coping in cultivated plants. Kluwer Academic Publishers, Dordrecht, The Netherlands. 1994;256.
7. Implay JA. Pathways of oxidative damage. Annu. Rev. Microbiol. 2003;57:395-418.
8. Hajlaoui H, Denden M, Ayeb El N. Changes in fatty acids composition, hydrogen peroxide generation and lipid peroxidation of salt-stressed corn (*Zea mays* L.) roots. Acta Physiol Plant. 2009;31:787–796.
9. Baek KH, Skinner DZ. Alteration of antioxidant enzyme gene expression during cold acclimation of nearisogenic wheat lines. Plant Sci. 2003;165:1221-1227.
10. Alscher RG, Donahue JL, Cramer C. Reactive oxygen species and antioxidants: Relatioships in green cells. Pysiol Plant. 1997;100:224-287. DOI:10.1111/j.1399-3054.1997. Tb04778.x
11. Canene-Adams K, Campbell JK, Zaripheh S, Jeffery EH, Erdman JW. The tomato as a functional food. J. Nutr. 2005;135:1226–1230. DOI: 10.1093/ jn/135.5.1226.
12. Norén H, Svensson P, Andersson B. A convenient and versatile hydroponic cultivation system for Arabidopsis thaliana. Physiol Plant. 2004;121:343-348. DOI: 10.1111/j.0031-9317.2004.00350.x.
13. Gangopadhyay G, Basu S, Mukherjee BB, Gupta S. Effects of salt and osmotic shocks on unadapted and adapted callus lines of tobacco. Plant Cell, Tissue, and Organ Culture. 1997;49:45–52.
14. Arndt SK, Wanek W, Clifford SC, Popp M. Contrasting adaptations to drought stress in field-grown *Ziziphus Mauritiana* and *Prunus Persica* trees: Water relations, osmotic adjustment and carbon isotope composition. Australian Journal of Plant Physiology. 2000;27:985–996.
15. Kerepesi H, Galiba G. Osmotic and salt stress induced alteration in soluble carbohydrate content in wheat seedling. Crop Science. 2002;40:482–487.
16. Grattana SR, Grieveb CM. Salinity-mineral nutrient relations in horticultural crops. Scientia Horticulturae. 1999;78:127-157.
17. Hasegawa PM, Bressan RA, Zhu JK, Bohnert H. Plant cellular and molecular

responses to high salinity. Annu. Rev. Plant Mol. Biol. 2000;51:463-499.
18. Munns R. Comparative physiology of salt and water stress. Plant, Cell and Environment. 2002;25(2):239-250.
19. Munns R, Schachtman D, Condon A. The significance of a two-phase growth response to salinity in wheat and Barley. Functional Plant Biology. 1995;22(4):561-569.
20. Munns R, Tester M. Mechanisms of salinity tolerance. Annual Review of Plant Biology. 2008;59:651-681.
21. Hung SH, Yu CW, Lin CH. Hydrogen peroxide function as a stress signal in plants. Bot Bull Acad Sin. 2005;46:1–10.
22. Vaidyanathan H, Sivakumar P, Chakrabarty R, Thomas G. Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.)-differential response in salt-tolerant and sensitive varieties. Plant Sci. 2003;165:1411–1418.
23. Khan MH, Singha Ksh LB, Panda SK. Changes in antioxidant levels in *Oryza sativa* L. roots subjected to NaCl-salinity stress. Acta Physiol Plant. 2002;24:145–148.
24. Masood A, Ahmad SN, Zeeshan M, Abraham G. Differential response of antioxidant enzymes to salinity stress in two varieties of Azolla (*Azolla pinnata* and *Azolla filiculoides*). Environ Exp Bot. 2006;58:216–222.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/63013