



Journal of
Plant Sciences

ISSN 1816-4951



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Short Communication

Enhancing Salinity Tolerance in Brinjal Plants by Application of Salicylic Acid

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Abstract

Background and Objective: The plant hormone, Salicylic Acid (SA) plays an important role in induction of plant defense against various biotic and abiotic stresses. The objective of this study was to investigate the protective role of salicylic acid in alleviating salt stress on brinjal plants. **Materials and Methods:** Greenhouse experiment was conducted in brinjal plants irrigated with tap water, 100 mM NaCl, marine water-1 and marine water-2 with and without foliar spraying of 0.1 mM SA for every 24 h interval up to 168 h. Plants treated with water alone served as control for analyzing the chlorophyll content, lipid peroxidation and electrolyte leakage. The values are presented in the Mean \pm Standard deviation and the data was analyzed using two-way analysis of variance (ANOVA) using SAS software (version 9.2). **Results:** The saline stressed brinjal plants showed decreased chlorophyll content and increased lipid peroxidation and electrolyte leakage levels when compared with the control plants, but the foliar application of SA showed ameliorative effect in the plants with saline stress. **Conclusion:** This study concluded that salicylic acid treated plants showed protective role and prevented the decrease in chlorophyll content and increase in lipid peroxidation and electrolyte leakage levels caused due to salinity stress.

Key words: Chlorophyll, electrolyte leakage, eggplant, lipid peroxidation, plant hormone, salt stress

Received: October 18, 2016

Accepted: November 21, 2016

Published: December 15, 2016

Citation : Vijayakumar Rajeshwari and Veluswamy Bhuvaneshwari, 2017. Enhancing salinity tolerance in brinjal plants by application of salicylic acid. J. Plant Sci., 12: 46-51.

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Competing Interest: The authors has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Salt stress is one of the major abiotic stress factor that limit plant growth and productivity and alters various biochemical and physiological responses in plants, thereby affecting almost all plant processes^{1,2}. The major effects of salinity show reduction in plant growth and restrict photosynthesis by decreasing the green pigments and causing stomatal closure and oxidative stress, resulting in the formation of Reactive Oxygen Species (ROS)³. Increasing level of ROS can enhance membrane lipid peroxidation, cause electrolyte leakage and damage the chloroplast, thus inhibiting photochemical reaction and decreasing photosynthesis⁴.

Brinjal (*Solanum melongena* L.), known as eggplant, aubergine or Guinea squash is cultivated on more than 1.5 Mha in the world. Brinjal is moderately sensitive to salinity⁵. Extensive research has been carried out to examine salt-induced morphological, biochemical and physiological changes in eggplant⁵. Conflicting writing exists on brinjal plant tolerance to soil salinity; brinjal is delicate to water stress caused by salinity and the salt tolerance of the various sorts of the brinjal plant is varied from selection to selection^{6,7}. Salicylic acid, a naturally present plant hormone act as a vital signaling molecule and thus contributes to the tolerance against abiotic stresses. It plays a significant role in plant growth, photosynthesis, water relation, ion uptake and transport⁸. It is frightening that salt affected areas of worldwide are increasing rapidly. So it's important to produce stress tolerant plants by providing external treatment to plants. Thus plants can be grown under salt stress conditions and also that can be used in future land improvement projects. To foremost of our insight, no study has been conducted related to SA application in brinjal plant. The objective of this study was to investigate the effect of exogenous foliar application of SA on brinjal plants under salt stressed conditions by analyzing the chlorophyll content, lipid peroxidation and electrolyte leakage.

MATERIALS AND METHODS

The experiment was done at the laboratory of the Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore during the period February, 2015-January, 2016.

Seeds and water collection and growth condition: Marine Water-1 (MW-1) was collected from Marina beach, Chennai, Tamil Nadu, India and Marine Water-2 (MW-2) was collected from Kanyakumari beach, Kanyakumari, Tamil Nadu, India. Brinjal seeds (CO₂ variety) were collected from Tamilnadu

Agriculture University, Coimbatore. Seeds were sown in sterilized soil rite in green house. Plants were watered daily and were sprayed weekly with 100% Hoagland's nutrient solution⁹. About 60 days old brinjal seedlings were separated into 8 groups each group containing ~150 plants and they were treated in 160 cm² plot daily for 7 days.

SA treatment: Salicylic Acid (SA; 2-hydroxybenzoic acid) was initially dissolved in 100 µL dimethyl sulfoxide and concentration of 0.1 mM was made up with 1 L of distilled water containing 0.02% Tween 20.

Set 1: Control plants watered with tap water

Set 2: Plants watered with 2 L of 100 mM NaCl (NaCl)

Set 3: Plants watered with 2 L of MW-1

Set 4: Plants watered with 2 L of MW-2

Set 5: Control plants watered with tap water+foliar spraying with SA

Set 6: Watered with 2 L of 100 mM NaCl+Foliar spraying of SA (Na+SA)

Set 7: Watered with 2 L of 100 mM MW-1+Foliar spraying of SA (MW-1+SA)

Set 8: Watered with 2 L of 100 mM MW-2 (MW-2+SA)

The root and leaf samples were collected randomly at 24 h interval from each group consisting of 3 replicates from 0th to 7th day of the treatment. Leaves of 4th and 5th node from the base were sampled at 0, 24, 48, 72, 96, 120, 144 and 168 h stored at -20°C for further analysis.

Total chlorophyll: Leaf sample of about 0.3 g was extracted with 2 mL of 80% acetone and centrifuged at 5000 rpm for 5 min and the supernatant was collected. The absorbance of the supernatant was read at 645 and 663 nm and the amount of chlorophyll was estimated by method of Witham *et al.*¹⁰.

Lipid peroxidation: The level of lipid peroxidation of leaf and root tissues collected was measured as far as malondialdehyde (MDA) content, a result of lipid peroxidation. The MDA was measured according to modified method of Heath and Packer¹¹.

Electrolyte leakage percentage: The percentage electrolyte leakage from fresh leaf tissues was determined using an electrical conductivity meter (aquapro digital water tester). The procedure followed was according to the method of Sairam *et al.*¹². This was used to assess the changes in cell membrane permeability.

Statistical analysis: The results presented are the Mean±Standard deviation and the data was analyzed using two-way analysis of variance (ANOVA) using the general linear model procedure and the least squares means test of the statistical software SAS (version 9.2 developed by SAS institute Inc., Cary, NC). The level of significance was 5%. Multiple pairwise-comparison tests using least-square means were performed for post-hoc comparisons after two-way ANOVA with treatment and time as two factor with replications. The corrections used for multiple comparisons were Tukey's honest significantly differences (HSD) test, Dunnett and Bonferroni procedure. Vertical bars in the graph indicate the standard deviation¹³.

RESULTS

Salt treated (100 mM NaCl, MW-1 and MW-2) plants showed decreasing amount of chlorophyll content when compared with control plants (Fig. 1). Treatment with 0.1 mM SA increased the chlorophyll content in all salinity induced brinjal plants. Total chlorophyll content significantly ($p < 0.0001$) decreases after 120 h of NaCl, MW-1 and MW-2 treatments when compared with respective controls. Whereas decrease after 120 h was prevented by the SA combined with NaCl, MW-1 and MW-2. In 168 h of treatment of SA treated plants showed significant increase in chlorophyll content ($p < 0.0001$).

For control plants (leaf and root), level of MDA was low, but under salt condition MDA level was increased. When application of 0.1 mM SA on salt stressed plants significantly ($p < 0.0001$) decrease the level of MAD in both leaf and root

tissues (Fig. 2a, b). The result of MDA concentration showed significant decrease ($p < 0.0001$) in 168 h of treated plants against non treated plants (Fig. 2a, b). Increasing level of MDA causes the membrane damage in plants.

The electrolyte leakage is a useful parameter when evaluating the physiological water status of plants. Control plants showed the lower level of electrolyte leakage percentage (Fig. 2c). In salt stressed plants, electrolyte leakage percentage was significantly increases ($p < 0.0001$). Foliar application of SA on salinity induced plants significantly ($p < 0.0001$) decrease the electrolyte leakage percentage in brinjal plants. Comparing to all the hours, 168 h of SA treated plants showed significant decreases ($p < 0.0001$) in the electrolyte leakage percentage.

DISCUSSION

The chlorophyll plays an important role in light absorption and energy transduction and is necessary for photosynthesis. The reduction of leaf chlorophyll under high salinity has been attributed to the destruction of pigments and the instability of the pigment protein complex¹⁴. In this study, salt treatment diminished the chlorophyll content of brinjal plants and the addition of SA to salt stressed plants especially increased the chlorophyll content. Addition of SA to salt stressed plants greatly increased the chlorophyll content. because SA treatment increase the activity of certain enzymes through stimulating chlorophyll biosynthesis or reducing chlorophyll degradation. This is leading to increased net photosynthesis under salt stress tolerance. The increase in chlorophyll content with SA confirmed the

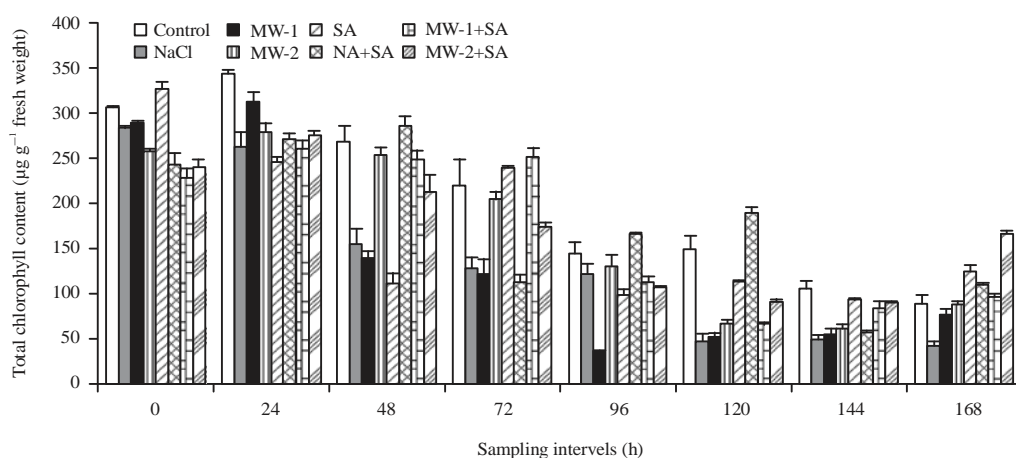


Fig. 1: Effect of salt and SA on chlorophyll in brinjal over a time period of 0-168 h

MW: Marine water, NA: NaCl, SA: Salicylic acid, Values expressed as Mean±Standard deviation (n = 3) Significant level 5% ($p < 0.0001$)

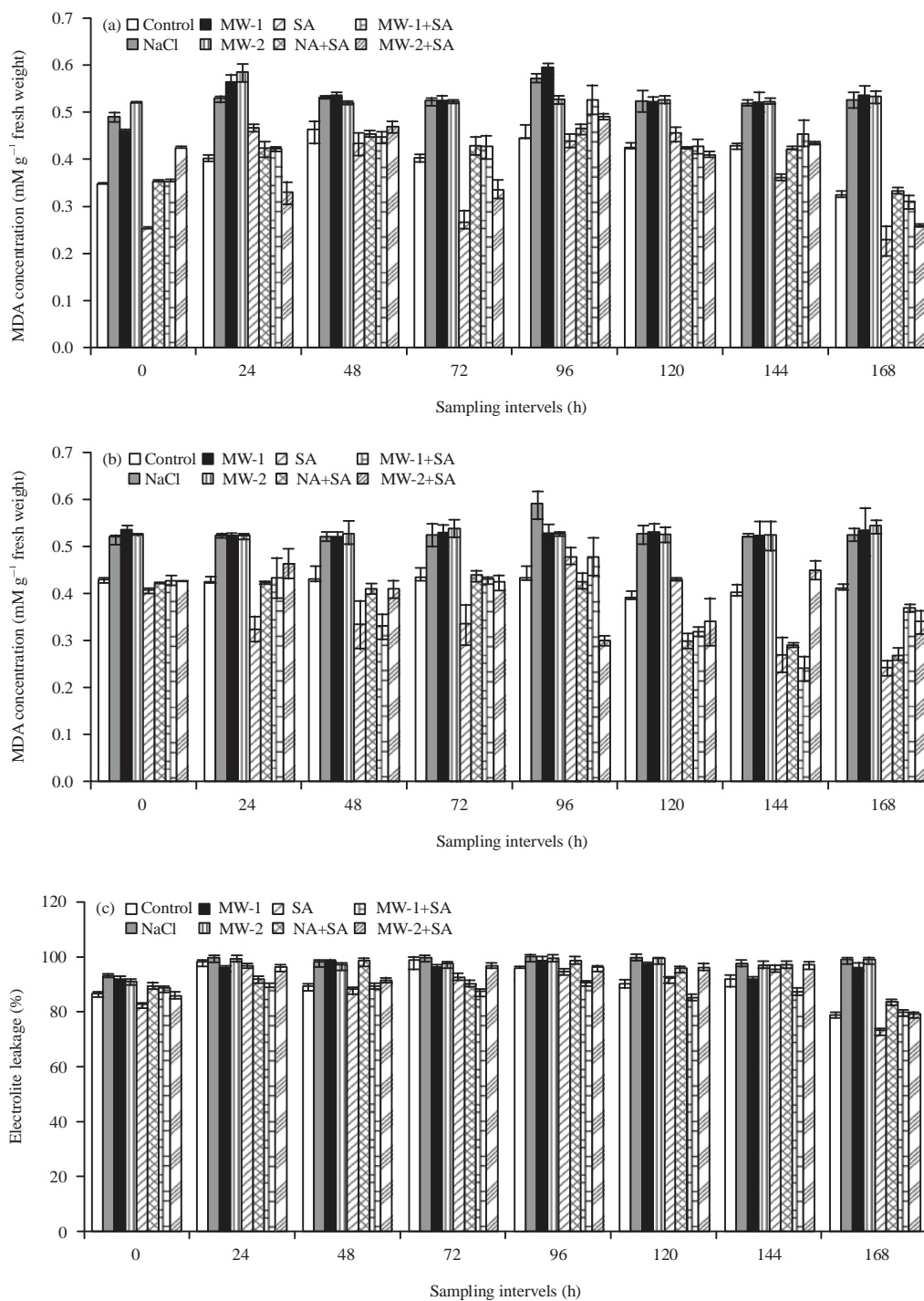


Fig. 2(a-c): Effect of salt and SA on MDA and electrolyte leakage in brinjal over a time period of 0-168 h (a) Leaf MDA content, (b) Root MDA content, (c) %Electrolyte leakage
 MW: Marine water, NA: NaCl, SA: Salicylic acid, Values expressed as Mean \pm Standard deviation (n = 3), Significant level 5% (p<0.0001)

reports of Gunes *et al.*¹⁵ for maize, El-Tayeb¹⁶ and Pirasteh-Anosheh *et al.*¹⁷ for barley and Yildirim *et al.*¹⁸ for cucumber. Similar results was reported by Li *et al.*¹⁹ who found that NaCl treatment reduced the chlorophyll content of

T. grandis seedlings and spraying of SA to NaCl treated plants increase the chlorophyll content. In white bean 0.1 mM SA treatment increased the chlorophyll content under salt stress²⁰. Arfan *et al.*⁸ in spring wheat and Azooz²¹ in bean

revealed that pretreatment with SA, significantly increased 5% the total chlorophyll content.

Salinity stress causes the damage of plant cell membranes, which can impair their ion selectivity. This deficiency results in the exosmosis of intracellular ions and infiltration of extracellular toxic ions, ultimately disrupting plant physiological and biochemical processes. The maintenance of cellular membrane integrity under salt stress is considered to be an essential part of the salinity tolerance mechanism²². The membrane damage caused by different abiotic stresses, including salinity is largely mediated through the end product of lipid peroxidation (MDA)¹⁹. In this study, SA application significantly 5% reduced the increasing MDA concentration and membrane permeability in brinjal plants under salt stress. An increase in the electrical conductivity with increasing salt stress indicated an elevated leakiness of ions due to a loss of membrane integrity, which is in agreement with the results described by Parida and Das²³. Similar results was found in *T. grandis* seedlings. The SA treatment prevented the lipid peroxidation and alleviated the membrane damage in *T. grandis* under salinity stress. Dong *et al.*²⁴ reported that SA treatment lower the level of MDA and electrolyte leakage in *Gossypium hirsutum* plants under salt stress. NaCl treated plants supplement with salicylic acid showed significant 5% decrease of MDA in leaf and roots of tomato plants²⁵. The application of exogenous SA could alleviate the membrane deterioration in plants under salt stress and facilitate the maintenance of membrane functions^{15,22}.

CONCLUSION

The foliar application of SA (0.1 mM) for of 168 h has improved plant tolerance to salinity as compared to the non treated plants. It was also found that the SA treatment significantly increased the chlorophyll content, reduced the electrolyte leakage rate and MDA content in brinjal plants under salt stress. About 17% area of the irrigated land in India is salt-affected, however based on the present findings, the foliar treatment of SA may ameliorate the negative effect of salinity on the growth of brinjal. In future, the tsunami and flood affected areas need to be utilized for growing plants.

SIGNIFICANCE STATEMENT

This study discover the protective role of a plant inducer (salicylic acid) to fight against the salt stress in brinjal plants. This study will help the researcher to uncover the critical area of signal transduction mechanism induced by the plant hormone to protect against salt stress in brinjal plants.

ACKNOWLEDGMENTS

Authors are thankful to University Grant Commission, Govt. of India, President, Secretary and Director, Principal and Department of Biotechnology of Kongunadu Arts and Science College for providing facilities and encouragement to carry out this research work. This work was financially (Grant no: MRP-4998/14 (SERO/UGC) Dt. March 2014) supported and sponsored by University Grant Commission, Govt. of India under the Minor Project Scheme.

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