*ejpmr*, 2017,4(9), 639-642

**SJIF Impact Factor 4.161**

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH
www.ejpmr.com

Research Article
ISSN 2394-3211
EJPMR

# ENHANCED ROS- SCAVENGING ENZYME ACTIVITY BY SALICYLIC ACID ELICITATION OF SALT TREATED SEEDS OF *VIGNA RADIATA* (L.) WILCZEK CULTIVARS

**Shweta Dhariwal*, Laxman Nagar, Ishwar Singh and Y. Vimala**

Department of Botany, CCS University Campus, Meerut.

***Corresponding Author: Shweta Dhariwal**
Department of Botany, CCS University Campus, Meerut.

**Article Received on 18/07/2017** **Article Revised on 07/08/2017** **Article Accepted on 28/08/2017**

## ABSTRACT
In the present investigation analyses of different concentrations of salt, i.e., NaCl (50mM,100mM and 150mM) and salicylic acid (0.01mM, 0.025mM, 0.050mM) were carried out *in vitro*, individually and in combinations, to find out their effect on ROS scavenging enzymes, Catalase and Peroxidase, on two cultivars of *Vigna radiata* -Pusa Vishal (P.V.) and SML 668. A separate set in distilled water was also analysed as control. Enzymatic activity was analysed on 3$^{rd}$, 6$^{th}$ and 9$^{th}$ day of sowing after the radicle emergence. Peroxidase activity was found to be maximum in 0.01mM concentration of Salicylic Acid on 3$^{rd}$ day after sowing in both the cultivars. Maximum catalase activity was observed in the combination treatment of 0.025mM S.A. + 100 mM NaCl on 6$^{th}$ day after sowing in the cultivar Pusa Vishal. However, in case of second cultivar i.e. SML 668 maximum catalase activity was observed in the combination treatment of 0.050mM + 150mM NaCl on the 6$^{th}$ day after sowing. All the results indicate that use of salicylic acid led to increase in ROS scavenging enzymes and help the seeds to combat with the salt stress.

**KEYWORDS:** Salicylic Acid (S.A.), Sodium Chloride (NaCl), Cultivars (c.v.), Reactive Oxygen Species (ROS).

## INTRODUCTION
*Vigna radiata* is among the most important food crop plant in India. Apart from all the essential nutrients it also contains flavonoids, phenolic acids and organic acids as well.[1] Importance of *Vigna radiata* has been recognized since time immemorial. In Ayurveda, it is known as Mudga, which means “that which brings joy, delight and gladness”.

Soil salinity is among one of the major constrains limiting the agricultural productivity in nearly 20% of cultivated are and half of the irrigated area worldwide.[2,3,4] Salt stress leads to several problems like cellular dehydration, reduction in rate of leaf expansion followed by cessation of expansion as the stress intensifies but the growth resumes when the stress is relieved.[5]

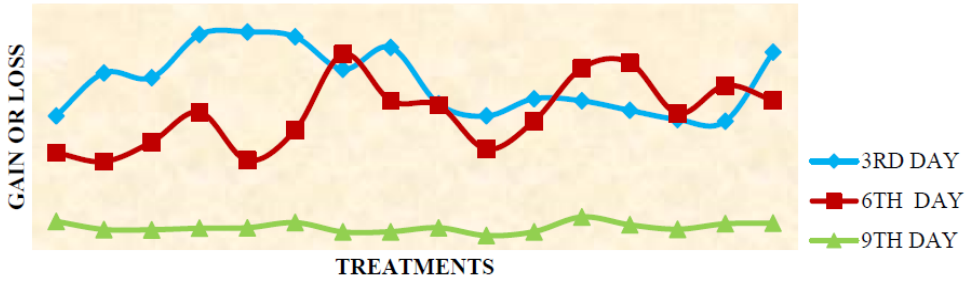
S.A. has been recognized as a regulatory signal mediating plant response to abiotic stresses such as drought[6,7], chilling[8,9] and heavy metal tolerance.[10,11,12] It also regulates many physiological functions like photosynthesis and seed germination.

## MATERIAL AND METHODS
The present investigation was carried out to identify various changes occurring in ROS scavenging enzymes in seeds treated with salt and salicylic acid individually as well as in combinations of both as compared to distilled water set used as control. Seeds of both cultivars were procured from Seed Distribution Center, IARI, New Delhi. Experimental design used is completely randomized design. Enzymatic analysis was conducted on seeds treated with NaCl (50mM,100mM, 150mM), S.A. (0.01mM, 0.025mM, 0.050mM) and the combinations of both (50mM NaCl+0.01mM S.A., 50mM NaCl+0.025mM S.A., 50mM NaCl+0.050mM S.A., 100mM NaCl +0.01mM S.A., 100mM NaCl+0.025mM S.A., 100mM NaCl+0.050mM S.A., 150mM NaCl+0.01mM S.A., 150mM NaCl+0.025mM S.A., 150mM NaCl+0.050mM S.A.). The seeds were surface sterilized with 0.01% solution of sodium hypochlorite, and 20 seeds of each cultivar of *Vigna radiata* (Pusa Vishal and SML 668) were put in the petri plates with different concentrations of each treatment. This was done in the Plant Physiology and Tissue Culture Laboratory, Department of Botany, C.C.S. University, Meerut. Total Peroxidase Activity[13] and Total Catalase Activity[14] was estimated on 3$^{rd}$, 6$^{th}$ and 9$^{th}$ day after sowing.

## RESULT AND DISCUSSION
Peroxidase activity was observed to decrease from 3$^{rd}$ to 9$^{th}$ day after sowing in *Vigna radiata* cv. Pusa Vishal, in

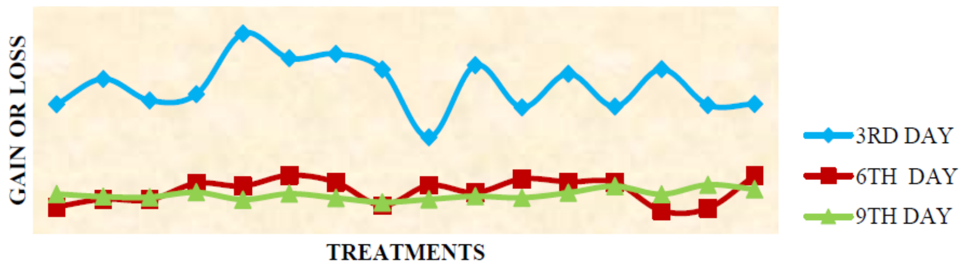
control as well as in all the three concentrations of NaCl and S.A. individually and in combination treatment of 50mM NaCl concentration with all the three concentrations of S.A. However, the peroxidase activity increased from 3rd to 6th day up to 100mM NaCl with 0.025mM and 0.050mM S.A. It also increased in combination of 150mM NaCl with 0.01mM and 0.025mM S.A. concentrations. However, peroxidase activity decreased among control and treated seedlings after 9 days after sowing. Maximum peroxidase activity was recorded in 0.01mM S.A. on 3 days after sowing (Fig.1).

**Fig. 1: Gain or loss in peroxidase activity of lab grown 3, 6 and 9 days old *Vigna radiata* cv. Pusa Vishal seeds with varying concentrations of abiotic elicitors as compared to respective control**




In cv. SML 668, the maximum peroxidase activity, like previous cv. Pusa Vishal, was also observed in 0.01mM S.A. Among control as well as treated seedlings the peroxidase activity decreased from 3rd to 9th days after sowing (Fig.2).

**Fig. 2: Gain or loss in peroxidase activity of lab grown 3, 6 and 9 days old *Vigna radiata* cv. SML 668 seeds treated with varying concentrations of abiotic elicitors as compared to respective control**

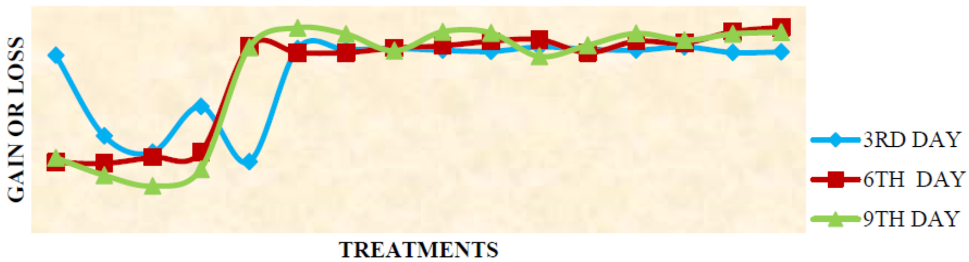



In case of catalase activity in the cv. Pusa Vishal, the catalase activity was noted to be decreasing from 3rd to 9th day after sowing in case of control set and NaCl treated seedlings. While among the individual concentrations of S.A. and all the combination sets, the catalase activity was noted to be increasing. Maximum activity was observed in 100mM NaCl + 0.025mM S.A.(Fig.3).

**Fig.3: Gain or loss in catalase activity of lab grown 3, 6 and 9 days old *Vigna radiata* cv. Pusa Vishal seeds treated with varying concentrations of abiotic elicitors as compared to respective control**


The same response was seen with the second cv. SML 668, the catalase activity decreased from 3rd to 9th days after sowing in control sets and NaCl treated seedlings, while increased in case of all concentrations of S.A. and combined treatments of both the elicitors. This indicates synergistic response of both the elicitors on catalase activity in both the cvs. of *Vigna radiata* L. Wilczek (Fig.4).

**Fig. 4: Gain or loss in catalase activity of lab grown 3, 6 and 9 days old *Vigna radiata* cv. SML 668 seeds treated with varying concentrations of abiotic elicitors as compared to repective control**




The results indicate and reiterate the previous findings of involvement of Salicylic acid in modifying abiotic stress response.[15] Cultivar specific modification of salt stress response by salicylic acid is very starkly evident. Results indicate that maximum peroxidase activity was shown with the lowest concentration of SA i.e. 0.01mM irrespective of the cv. and this activity decreased from 3rd to 9th day after sowing of seeds. Accumulation of ROS in cells might activate the ROS scavenging pathways leading to increase in superoxide dismutase and peroxidase activity and results in suppression of ROS.[16] In the case of catalase the maximum activity is recorded with a combination treatment of both the elicitor molecules. Catalase and a variety of peroxidases are known to catalyze the breakdown of hydrogen peroxide.[17] Increasing NaCl concentration leads to increased leakage of electrolytes and Malondialdehye content in *Brassica napus* seedlings and although SA is not essential for germination under normal conditions, it plays a promotive role in seed germination under high salinity by reducing oxidative damage.[18]

## CONCLUSION

In the light of results, we can conclude that, S.A., which itself is a ROS generating elicitor under biotic stress, is increasing the activity of ROS scavenging enzymes, peroxidase and catalase. Thereby, showing completely antagonistic response under salt stress. This leads to a new vision of finding some common factors through genetic engineering which can work in increasing tolerance capacity of plants against both biotic as well as abiotic stress.

**REFERENCES**


1. Kavya S, Padmalatha H, Evaluation of physicochemical and Phytochemical Study of Polyherbal Formulation. World Journal of Pharmacy and Pharmaceutical Sciences, 2014; 3(5): 759-764.
2. Ghassemi F, Jakeman AJ, Nix HA, Salinisation of Land and Water Resources: Human Causes, Extent, Management and Case Studies. *Unsw. Press*, Sydney, Australia and Cab International, Wallingford, U.K, 1995.
3. Cushman JC, Bohnert HJ, Genomic Approaches to Plant Stress Tolerance. Curr. Opin. Plant Biol, 2000. 3(2): 117-124.
4. Zhu JK, Plant Salt Tolerance. Trends in Plant Science, 2001; 6: 66-71.
5. Parida AK, Das AB, Salt Tolerance and Salinity Effects on Plants: A Review. Ecotoxicology and Environmental Safety, 2005; 60(3): 324-349.
6. Munne-Bosch S, Penuelas J, Photo- and Antioxidative Protection, and a Role for Salicylic Acid during Drought and Recovery in field Grown *Phillyrea angustifolia* Plants. Planta, 2003; 217: 758-766.
7. Chini A, Grant JJ, Seki M, Shinozaki K, Loake GJ, Drought Tolerance established by Enhanced Expression of the *CCI-NBS-LRR* Gene, *ADRI*, requires Salicylic Acid, EDSI and AB11. The Plant Journal, 2004; 38: 810-822.
8. Janda T, Szalai G, Tari I, Paldi E, Hydropronic Treatment with Salicylic Acid decreases the Effects of Chilling Injury in Maize (*Zea mays* L.) Plants. Planta, 1999; 208: 175-180.
9. Kang HM, Saltveit ME, Chilling Tolerance of Maize, Cucumber and Rice Seedling, Leaves and Roots are differentially affected by Salicylic Acid. Physiologia Plantarum, 2002; 115: 571-576.
10. Metwally A, Finkemeier I, Georgi M, Dietz KJ, Salicylic Acid alleviates the Cadmium Toxicity in Barley Seedlings. Plant Physiology, 2003; 132: 272-281.
11. Yang ZM, Wang J, Wang SH, Xu LL, Salicylic Acid- Induced Aluminium Tolerance by Modulation of Citrate Efflux from Roots of *Cassia tora* L. Planta, 2003; 217: 168-174.
12. Freeman JL, Garcia D, Kim D, Hopf AM, Salt DE, Constitutively Elevated Salicylic Acid Signals Glutathione- Mediated Nickel Tolerance in *Thlapsi* Nickel Hyperaccumulators. Plant Physiology, 2005; 137: 1082-1091.
13. Maehly AC, Chance B, The assay of catalases and peroxidase. In: *Methods of Biochemical Analysis*, ed. Glick D, Inter science publications Inc. N.Y, 1967; 1: 357-422.
14. Aebi H, Catalase *in vitro*. Methods of Enzymology, 1984; 105: 121-126.
15. Vimala Y, Gupta MK, A physiochemical approach towards understanding of salicylic acid-mediated modification in salinity response of *Brassica campestris*, Proceedings of ICPEP-2, Plant Response to Environmental Stress, International Book Distributing Co. Lucknow, 2006; 295-302.
16. Devi S, Angrish R, Datta KS Kumar B, Antioxidant defence system in wheat seedlings under sodium chloride stress: An inductive role of hydrogen peroxide. Indian J. Plant Physiol, 2008; 13: 118-124.
17. Chang H, Siegel BZ, Siegel SM, Salinity induced changes in isoperoxidase in taro, *Colocasia esculenta*. Phytochemistry, 1984; 23: 233-235.
18. Salarizdah M, Baghizadeh A, Abasi F, Mozaferi H, Salarizdah D, Response of *Brassica napus* L. grains to the interactive effect of salinity and salicylic acid. Journal of Stress Physiology and Biochemistry, 2002; 8(2): 159-166.