Advances in Bioresearch
Adv. Biores., Special Issue 1 -2025: 48-52

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL: http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.SPL1.4852

Advances in Bioresearch

Effect of salt stress on production of "ROS" scavenging enzymes, grown *In vitro* condition in *Indigofera tinctoria*

Sarita P Shinde*, Pratik Shinde
Department of Botany, JET's Zulal Bhilajirao Patil College Dhule – 424002
***Corresponding Author:** Sarita P Shinde
Email ID: orshidssaru1995@gmail.com

ABSTRACT
Several metabolic processes, including crucial ones like photosynthesis and respiration, contribute to the generation of oxygen species that are reactive (ROS). Oxidative stress, sometimes referred to as ROS stress, arises when there is an imbalance between the generation of ROSs and their removal by associated enzymes. Examples of these enzymes include catalase, peroxidase, superoxide dismutase, and many more. Exogenous factors, including stress, possible thirst, salt, and heavy metal stressors, can also contribute to the generation of ROS. The subject matter under consideration pertains to the salt stress in vitro culture case study. The host's response to stress involves the activation of a defensive mechanism, leading to the upregulation of ROS scavenging enzymes in plants, known as callus. This heightened production of ROS-scavenger enzymes is essential for the occurrence of beneficial effects. Initially, the presence of elevated salt levels in the soil leads to a decrease in its osmotic potential, hence causing drought stress in plants. The initial phase of salinity stress manifests in a sequential manner. Moreover, it leads to toxicity due to the difficulty in encapsulating sodium ions within the vacuoles. One additional element to consider is that the exchange of salts and other nutrients leads to an imbalance between macronutrients and micronutrients. The combined impact of these three stress stages leads to substantial tissue damage in plants, ultimately culminating in their demise.
Kyewords: *oxidative stress, defense, Reactive Oxygen Species, invitro, callus, toxicity*

Received 14.10.2024 Revised 30.11.2024 Accepted 29.12.2024

How to cite this article:
Sarita P Shinde, Pratik Shinde. Effect of salt stress on production of "ROS" scavenging enzymes, grown in vitro condition in *Indigofera tinctoria*. Adv. Biores., Special Issue 1 -2025: 48-52

INTRODUCTION

This study has been done with an objective to determine the effects of phytohormones and salt stress on the production of ROS (peroxides, superoxide, hydroxyl radical and singlet oxygen scavenging enzymes in Indigofera tinctoria plant. The pleiotropic effects observed in this study, along with the lack of significant impact on water loss from detached leaves due to trehalose accumulation, indicate that the synthesis of this sugar did not result in an osmoprotectant effect. Instead, it appears to have influenced sugar metabolism and regulatory pathways that impact plant development and stress tolerance [1]. The study examined the response of maize grains to pre-soaking treatment with 10 mM trehalose under salinity-induced stress conditions. Results showed that trehalose treatment improved the metabolic activity of maize seedlings, increased hill-reaction activity, and increased organic solutes. Trehalose treatment also stabilized plasma membranes, decreased ion leakage and lipid peroxidation, and increased K/Na ion ratio in leaves [4, 5]. ROS generation poses a significant threat to plant health and food security, necessitating the use of antioxidants to maintain a balance between generation and quenching [2]. Sweet potato's salt tolerance traits, including glutathione reductase, sodium, chlorine, and potassium uptake, are not affected by salt stress, requiring further research [3]. The osmoprotectants and lipid peroxidation in *S. caseolaris* seedlings were influenced by salt treatment. The examination of the transcriptome revealed significant pathways and potential genes implicated in the regulation of salt tolerance [11]. PGPR can improve plant resilience to salinity stress by regulating levels of reactive oxygen species (ROS). The action of ACC deaminase in rhizobacteria aids in the regulation of ethylene levels in plants impacted by salt.(Bharti & Barnawal, 2019). The growth characteristics of *Moringa oleifera* were efficiently maintained in the presence of moderate salt. The therapeutic properties of moringa were increased by salt stress through an increase in antioxidant molecules [6]. The 'NL895' poplar plantlets exhibit distinct adaptation mechanisms in their roots and

leaves to effectively manage salt stress. The salt tolerance is influenced by the coordination of sodium (Na+) and potassium (K+) transport, as well as antioxidant activities [8]. Salt stress hampers the growth of shoots and reduces the amount of chlorophyll present. Elevated salinity during periods of stress has a beneficial impact on root growth [7]. Reactive carbonyl species (RCS) are known to play a role in the inhibition of plant growth when subjected to salt stress. Dipeptides containing histidine can alleviate salt stress by scavenging reactive carbon species (RCS) [9]. Electromagnetic (EM) application mitigated the adverse impacts of salt stress. Electromagnetic (EM) use has great potential for increasing crop yields in salty croplands [10].

MATERIALS AND METHODS

Plant material: *Indigofera tinctoria* L. Seeds germination using invitro technique.

The seeds were ordered from FRI Dehradun, seeds were grown with *In vitro* facilities (plant tissue culture) in M.S. Media, antioxidant property during salt stress was observed in presence of NaCl at different concentrations.

Gemination percentage: Germinated seeds/non-germinated seeds x 100 Germination of the seeds sown invitro showed its germination up to cotyledonary stage after 15 days of inoculation. The average germination rate observed in invitro was 34%.

Salinity treatment: Salinity treatment was given to the sub-cultured callus after one month into the same media having same concentration of the treatment of growth hormones. Following concentration were used to for salinity stress:

The media composition was half M.S. media and the growth hormones with NAA and kinetin.

Sterilization of Glassware's: Beakers, test tubes, culture bottles, pipettes, measuring cylinder, petri plates and conical flasks, tissue papers were used or all the processes. All the glassware and forceps blades were cleaned properly and then autoclaved and used.

Surface sterilization of the seeds:

The process involves washing with liquid detergent for 30 minutes, then 3-4 times with D/W, then adding 1g/100ml Bavistin solution wash for 10 minutes, then sterile D/W for 5 minutes, then 0.1%HgCl2 for 10 minutes, and finally alcohol.

Table 1 Salt treatment

Treatment (PGR'S)	NAA mg/ml	KINETIN (mg/ml)	Treatments (Salt treatment)	Concentration of salt (mM)
D	1.0	1.0	D	20mM
				50mM
				100mM
E	2.0	2.0	E	20mM
				50mM
				100mM
F	3.0	3.0	E	20mM
				50mM
				100mM
S	1.5	0.5	S	20mM
				50mM
				100mM
U	1.5	1.5	D	20mM
				50mM
				100mM

Enzyme assay and methods

Catalase (EC1.11.1.6) [13]

Catalase catalyzes the breakdown of H_2O_2 to H_2O and O_2.

The rate of by decomposition of H2O2 by catalase is measured spectrophotometrically at 240nm.

SOD: (EC.1.15.1.1) [12]

Superoxide dismutase) superoxide ions convert NBT to NBT diformazan.

Which is a coloured compound and this absorbs light at 560 nm.

SOD reduces the superoxide's formed, and this is measured by the decrease in NBT diformazan formation.

Guaiacol Peroxidase (EC1.11.1.7) [11]

The rate of decomposition of hydrogen peroxide by peroxidase, with guaiacol as hydrogen donor, is determined by measuring the rate if color spectrophotometrically at 460nm.

Protein estimation in seed done by method of Bradford's Method [14].

ble 2: Protein Estimation: Bradford method

	Absorbance	Protain (100ul extract)	Protein (in 1 g callus)
0.2 (control)	0.198	0.278	27.88 mg
0.4	0.301	0.429	42.98 mg
0.2 (Treated)	0.187	0.262	26.27 mg
0.4	0.326	0.466	46.65 mg

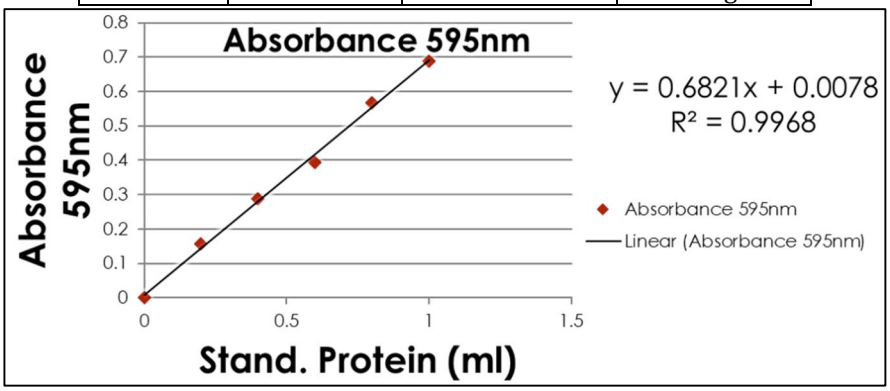

Figure 1 Protein Estimation: Bradford method

RESULTS AND DISCUSSION

The above-mentioned results were carried out from the callus extract using the plant tissue culture mechanism. Where half M. S. media was prepared, and the seeds (*Indigofera tinctoria*) were inoculated. After 10 days of inoculation. There was the growth of seedling up to the cotyledonary stage and the different parts (cotyledons , root, shoot apex) were inoculated as a explant in the M. S. media containing the PGR'S (NAA and Kinetin) at different concentration and named as(A,B,C,D,E……U.) these name suggests different concentration of NAA and Kinetin .After 20 days a undifferentiated mass of cells "callus" was observed .

Table 3 Enzyme Activity

Treatments	Dos-es of NaCl (mM)	Protein mg/g of callus		Catalase specific activity		SOD specific activity		Peroxidase specific activity	
			(S.D)		(S.D)		(S.D)		(S.D)
D	20	0.139	0.342	0.010	0.000879	0.082	0.0101	0.007	0.0103
	50	0.131	0.302	0.008	0.000854	0.088	0.0106	0.009	.0113
	100	0.131	0.300	0.008	0.000834	0.091	0.0117	0.012	0.0117
E	20	0.141	0.304	0.008	0.00822	0.073	0.0109	0.091	0.0111
	50	0.131	1.063	0.090	0.0038	0.078	0.0112	0.098	0.0100
	100	0.121	0.698	0.090	0.0022	0.081	0.0106	0.100	.0116
F	20	0.145	0.542	0.089	0.0017	0.088	0.0058	0.082	0.0051
	50	0.142	0.312	0.099	0.0100	0.089	0.0062	0.087	0.0068
	100	0.141	0.310	0.093	0.0100	0.091	0.0084	0.090	0.0076
controlled		0.152	0.306	0.011	0.00078	.0842	0.0095	0.006	0.0061

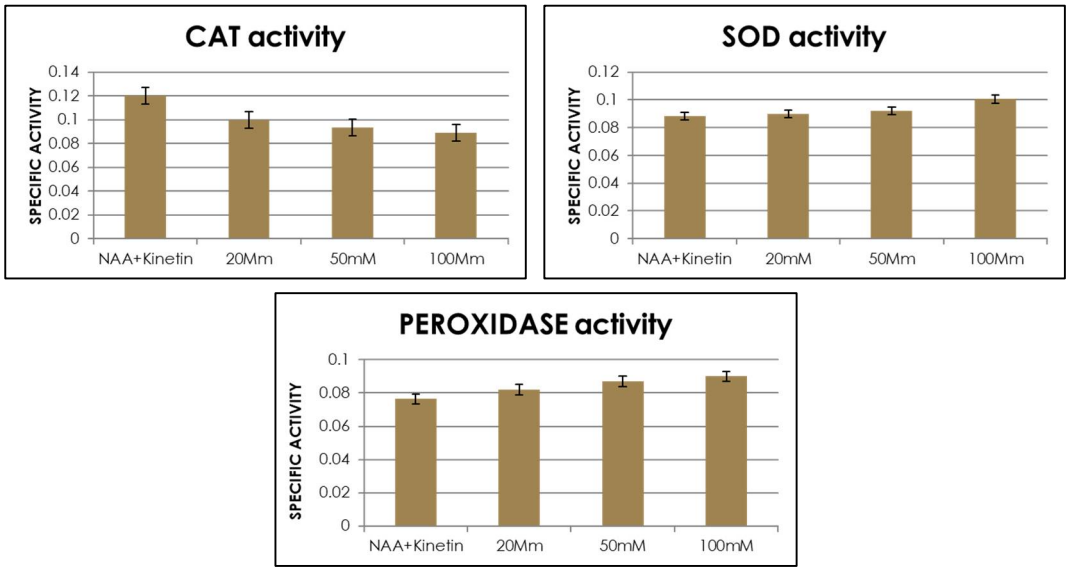

Figure 2 Graphical Presentation of Catalase, SOD, Peroxidase activity

The calluses were subsequently cultured in the same medium, which contained specific concentrations of plant growth regulators (PGRs), namely NAA and Kinetin. After a period of 20 days, these calluses were subjected to salt stress treatment using varying concentrations of sodium chloride (NaCl) at 20 mM, 50 mM, and 100 mM, alongside a control group that received only the medium and PGRs without salt. Following the inoculation of 0.2 g of callus into the salt-containing media, an enzyme assay was conducted 20 days later to evaluate enzyme activity, yielding satisfactory results. The assay focused on the enzymes catalase, superoxide dismutase (SOD), and peroxidase, measuring their activity relative to a specific protein concentration [3, 7, 8].

Catalase activity was observed to decline under stress conditions, attributed to a reduction in its turnover, which reflects the disruption of metabolic processes during stress. In contrast, SOD exhibited increased turnover and activity in response to stress, with higher levels of SOD activity noted at elevated salt concentrations. Under controlled conditions (media with PGRs), both SOD turnover and activity were lower; however, as salt concentration increased, SOD activity correspondingly rose. A similar trend was noted for peroxidase, where enzyme activity and turnover increased with rising salinity levels compared to the control condition [9, 10].

These findings indicate that as the stress level (NaCl concentration) escalates, there is a corresponding increase in reactive oxygen species (ROS) generation. Consequently, this leads to a heightened turnover of ROS scavenging enzymes, resulting in increased enzyme activity under higher stress conditions. In contrast, the control condition exhibited lower enzyme concentrations and activity, suggesting reduced ROS generation and, therefore, a lower production of ROS scavenging enzymes.

REFERENCES

1. Azeem, M., Pirjan, K., Qasim, M., Mahmood, A., Javed, T., Muhammad, H., Yang, S., Dong, R., Ali, B., & Rahimi, M. (2023). Salinity stress improves antioxidant potential by modulating physio-biochemical responses in Moringa oleifera Lam. *Dental Science Reports*, *13*(1). https://doi.org/10.1038/s41598-023-29954-6
2. Bharti, N., & Barnawal, D. (2019). *Amelioration of Salinity Stress by PGPR: ACC Deaminase and ROS Scavenging Enzymes Activity*. 85–106. https://doi.org/10.1016/B978-0-12-815879-1.00005-7
3. Grinn-Gofroń, A., Bosiacka, B., Bednarz, A., & Wolski, T. (2018). A comparative study of hourly and daily relationships between selected meteorological parameters and airborne fungal spore composition. *Aerobiologia*, *34*, 45–54.
4. Jia, P., Melnyk, A. V, Li, L., Kong, X., Dai, H., & Zhang, Z. (2022). Differential adaptation of roots and shoots to salt stress correlates with the antioxidant capacity in mustard (Brassica juncea L.). *Pakistan Journal of Botany*, *54*(6). https://doi.org/10.30848/pjb2022-6(32)
5. Kesawat, M. S., Satheesh, N., Kherawat, B. S., Kumar, A., Kim, H. U., Chung, S. M., & Kumar, M. (2023). Regulation of Reactive Oxygen Species during Salt Stress in Plants and Their Crosstalk with Other Signaling Molecules—Current Perspectives and Future Directions. In *Plants* (Vol. 12, Issue 4). https://doi.org/10.3390/plants12040864
6. Mondal, S., Burgert, S., Asch, J., Rahaman, E. H. M. S., & Asch, F. (2023). Salinity effects on the activities of ROS scavenging enzymes in leaves of two sweet potato clones. *Journal of Agronomy and Crop Science*, *209*(6). https://doi.org/10.1111/jac.12657

7. Pilon-Smits, E., Terry, N., Sears, T., Kim, H.-K., Zayed, A., Hwang, S., Dun, K. V, Voogd, E., Verwoerd, T., Krutwagen, R. W. H. H., & Goddijn, O. (1998). Trehalose-producing transgenic tobacco plants show improved growth performance under drought stress. *Journal of Plant Physiology*, *152*, 525–532. https://doi.org/10.1016/S0176-1617(98)80273-3
8. Romero, C., Bellés, J., Vayá, J., Serrano, R., & Culiáñez-macià, F. A. (1997). Expression of the yeast trehalose-6-phosphate synthase gene in transgenic tobacco plants: pleiotropic phenotypes include drought tolerance. *Planta*, *201*, 293–297. https://doi.org/10.1007/s004250050069
9. Sultana, Most. S., Yamamoto, S., Biswas, Md. S., Sakurai, C., Isoai, H., & Mano, J. (2022). Histidine-Containing Dipeptides Mitigate Salt Stress in Plants by Scavenging Reactive Carbonyl Species. *Journal of Agricultural and Food Chemistry*, *70*(36), 11169–11178. https://doi.org/10.1021/acs.jafc.2c03800
10. Talaat, N. B. (2015). Effective Microorganisms Improve Growth Performance and Modulate the ROS-Scavenging System in Common Bean (Phaseolus vulgaris L.) Plants Exposed to Salinity Stress. *Journal of Plant Growth Regulation*, *34*(1), 35–46. https://doi.org/10.1007/S00344-014-9440-2
11. Ying, C., Binling, Y., Zihan, W., Chen, X., Yanqiong, C., & Qiu, N. (2018). The ion homeostasis and ROS scavenging responses in 'NL895' poplar plantlet organs under in vitro salinity stress. *In Vitro Cellular & Developmental Biology – Plant*, *54*(3), 318–331. https://doi.org/10.1007/S11627-018-9896-Z
12. Zeid, I. M. (2009). Trehalose as osmoprotectant for maize under salinity-induced stress. *Research Journal of Agriculture and Biological Sciences*, *5*, 613–622.
13. Aebi, H. (1974) Catalase. In: Bergmeyer, H.U., Ed., Methods of Enzymatic Analysis, Verlag Chemie/Academic Press Inc., Weinheim/NewYork, 673-680.
14. Bradford, Marion (1976). "A Rapid and Sensitive Method for the Quantification of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding" (PDF). *Analytical Biochemistry*. 72 (1–2): 248–254.