



SUPEROXIDE DISMUTASE ACTIVITY IN RESPONSE TO TIR TREATED TWO BLACK GRAM [*Vigna mungo* (L.) Hepper] VARIETIES

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Abstract:

Increase in the atmospheric mean temperature is mainly due to emission of green house gases (carbon dioxide (CO₂), methane (CH₄), ozone (O₃), nitrous oxide, chlorofluoro carbons (CFCs)) by anthropogenic (Eitzinger et al., 2010) and agricultural systems (Smith and Olesen 2010). Black gram [*Vigna mungo* (L.) Hepper] is a tropical leguminous plant, popularly known as Urd bean or Mash in India. Black gram is very nutritious as it contains high levels of protein. Plants improve protective mechanism of heat stress responses encompassing different pathways that provide tolerance during HT stress. Plants adapt tolerance to high temperature stress by inherent basal level as well as acquired (Hikosaka et al., 2006). Tolerance limits vary between genotypes, but are also subject to acclimation (Weis et al., 1988). TIR (Temperature Induction Response) is a potential and versatile one for identifying highly thermo-tolerant genotype from a large population (Srikantbabu et al., 2002). SODs play a significant role in protecting lysing cells against the toxicity and mutagenicity of active O₂ species by virtue of their capacity to scavenge the O₂⁻ (Sandalios, 2001). Enzymatic test was made to resistant (LBG-806) and susceptible variety (LBG-823) of control, lethal (non-induced) and induced treatments of at 0th, 1st, 2nd and 3rd day seedlings. There was no significant difference was found between control and non-induced treatments in any day. In contrast rise in content in induction treatment over control was significantly different at (P≤0.01) in all days.

Key Words: Black gram, TIR: Temperature Induction Response, SOD: Super Oxide Dismutase

I. Introduction:

In recent years, with the recognition of global climate change, studies on regional impacts in particular food crops come to forefront of scientific community. Minor deviations from the normal weather, efficiency of food production is seriously impaired. Inter-Governmental Panel on Climate Change assessed a range of scenarios of future greenhouse-gas emissions and concluded that these would lead to an increase in global mean temperatures between 1.6°C and 6.9°C by the end of the 21st century. The most worrying part of the prediction is the estimated increase in winter and summer temperatures by 3.2°C and 2.2°C respectively by 2050 (IPCC, 2007).

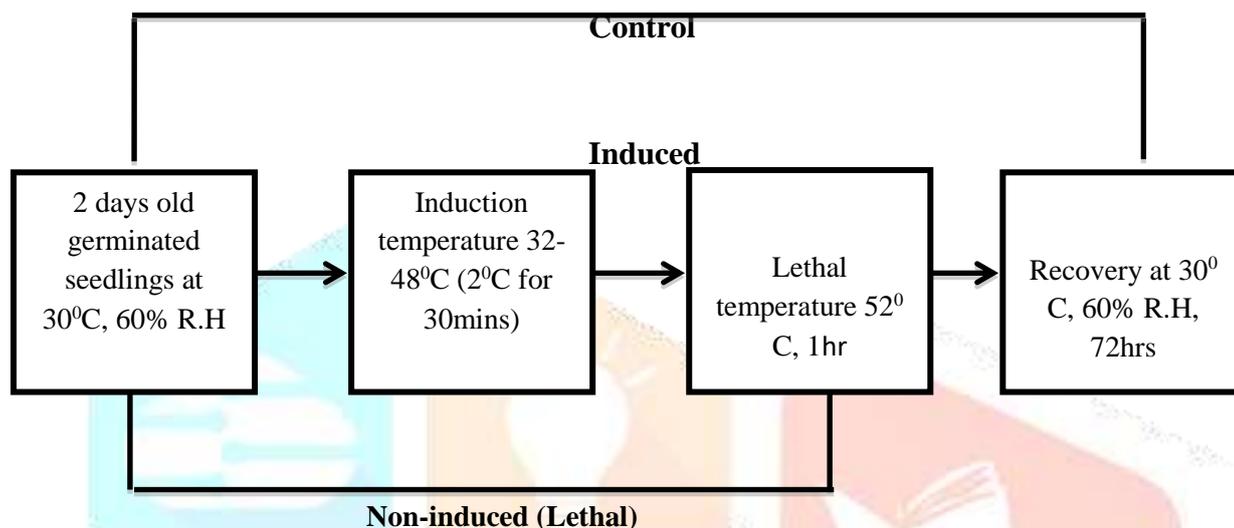
SODs play a significant role in protecting lysing cells against the toxicity and mutagenicity of active O₂ species by virtue of their capacity to scavenge the O₂⁻ (Sandalios, 2001). Zhao et al., (2006) expressed that increase in SOD activity and decrease in oxidative damage were closely related. Tolerance to temperature stress in plants showed positive correlation with increase in antioxidants (Hasanuzzaman et al., 2012; Almeselmani et al., 2006). The results indicated that xinmu No,1 was tolerant to salt or drought

stresses during germination as its activity of anti-oxidant enzymes (superoxide dismutase, peroxidase, ascorbate peroxidase and catalase). Combined increase of SOD and APX was observed in transgenic tobacco plants under high light intensity and low temperature stress (Gupta et al., 1993).

II. Materials and methods:

1) **PLANT SAMPLINGS:** Black gram temperature resistant variety LBG-806 and susceptible variety LBG-823 were selected based on the previous study through TIR technique (Sujatha B et al., 2018)

TIR technique involves series of experiments i.e., identification of lethal/challenging temperature, sub-lethal temperatures and finally screening of thermo-tolerant genotypes from large populations by subjecting aseptically germinated seedlings from each variety to three temperature treatments i.e. control, induced and non-induced



This method was developed by Srikanthbabu *et al.*, (2002), followed by Vijayalakshmi *et al.*, 2015 (rice), Babu *et al.*, 2013 (ragi), Chandola *et al.*, 2016 (tomato), Vidya *et al.*, 2017 (banana). The activity of the superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium adopting the method of Beauchamp and Fridovech (1971).

Enzymatic test was made to resistant (**LBG-806**) and susceptible variety (**LBG-823**) of control, lethal (non-induced) and induced treatments of at 0th, 1st, 2nd, and 3rd day seedlings.

Enzyme assay:

The 3 ml reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75µM nitro blue tetrazolium, 2µM riboflavin, 0.1 mM EDTA and 0.1 ml of enzyme extract. Riboflavin was added last and the test tubes were shaken and placed 30 cm below light blank consisting of two 15W florescent lamps. The reaction was started by switching on the light. The reaction was allowed to take place for 30 min and was stopped by switching off the light. The tubes were covered with a black cloth. The absorbance of the solution was measured at 560 nm in BIO-RAD Smart Spec plus UV visible spectrophotometer. The reaction mixture which was not exposed to the light did not develop colour and served as control.

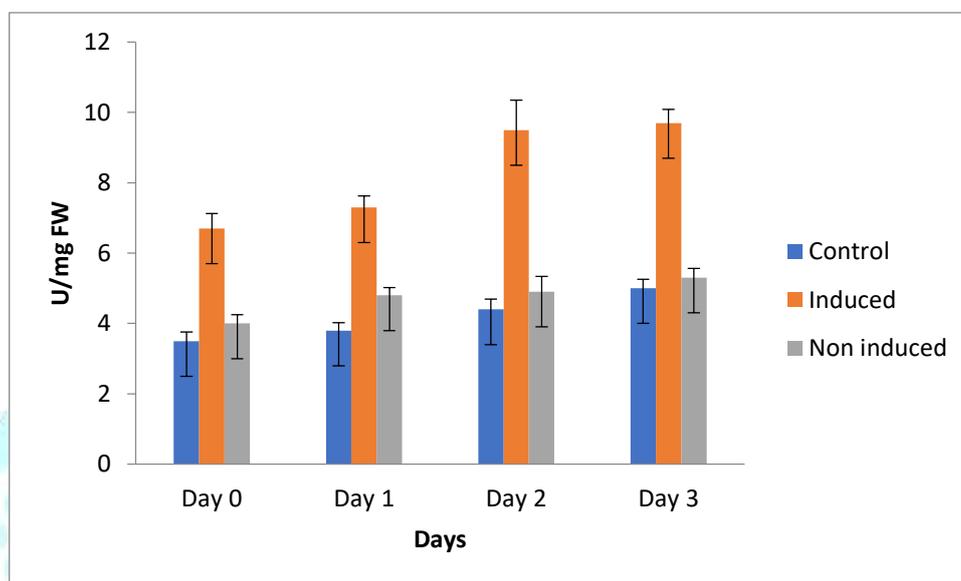
Log A560 was plotted as a function of the volume of enzyme extract used in the reaction mixture. From the resultant graph the volume of the enzyme extract corresponding to 50% inhibition of the reaction was read and was considered as one enzyme unit.

III. Results and Discussion

In resistant variety, at 0th day both induction and non-induction treatments showed increased content (91.42 %; 14.28 %) respectively over control was observed. In the subsequent recovery period i.e. from 0th to 3rd day rise in content was observed both in induction and non-induction treatments (44.77%; 32.5%) respectively. At the end of 3rd day, both induction and non-induction treatments showed increase in content (94%; 6%) over same day of control treatment (Fig. 1). There was no significant difference was

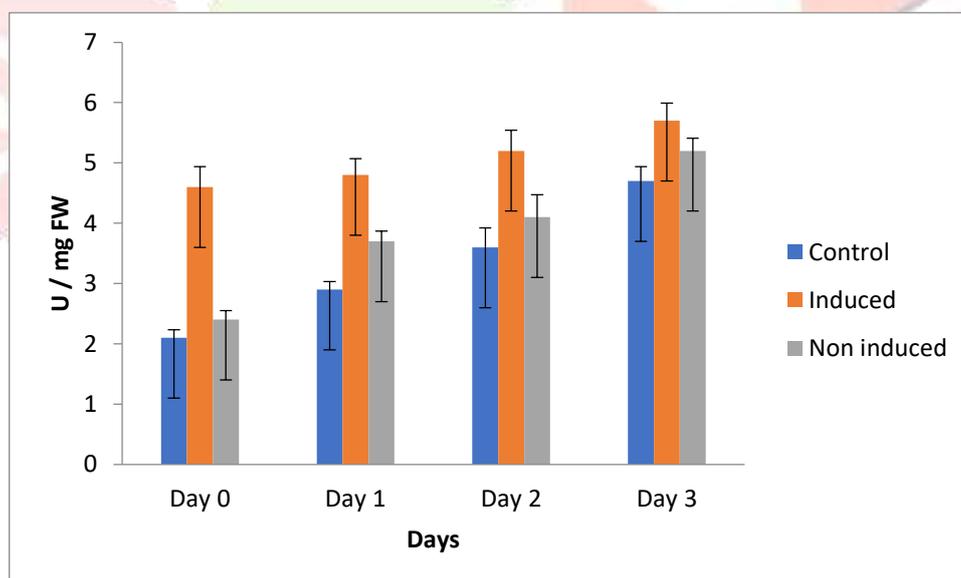
found between control and non-induced treatments in any day. In contrast rise in content in induction treatment over control was significantly different at ($P \leq 0.01$) in all days.

In susceptible variety, at 0th day both induction and non-induction treatments showed increased in content (119.04 %; 14.28 %) respectively over control was observed. Rise in content in induction treatment over control was significant at ($P \leq 0.01$). In recovery period from 0th to 3rd day both induction and non-induction treatments showed increase in content (23.91%; 116.66%) respectively. At the end of 3rd day, both induction and non-induction treatments showed increase in content (21.27%; 10.63%) over control of the same day (Fig. 2). Control treatment showed increase in content from 0th to 3rd day both in resistant and susceptible varieties (42.85%; 123.80%) respectively.



*Mean followed by the different letters on column were significantly different at $p \leq 0.01$

Fig. 1 Resistant variety (LBG-806)



*Mean followed by the different letters on column were significantly different at $p \leq 0.01$

Fig. 2 Susceptible variety (LBG-823)

In this research work significant increase in SOD content was observed in induction treatment of both resistant ((91.42 %) (Fig. 1) and susceptible (119.04 %) (Fig. 2) varieties, at 0th day over their respective control treatment. Results indicated that during induction treatment seedlings get acclimatized to face terminal lethal temperatures with the production of SOD content. SOD plays a potential role to scavenge ROS that are produced during induction period. In subsequent recovery period also, resistant variety showed increase in SOD activity over control treatment. In non-induction treatment, both varieties showed 14.28% increase in content at 0th day over control which is very less when compared with increase in induction treatment.

Heat acclimation during induction treatment led to a significant increase in SOD due to its strong ability to scavenge ROS in both resistant and susceptible varieties, as reported by Xu *et al.*, (2006), Wahid and Close (2007) and Yuan *et al.*, (2011). Rise in antioxidant enzyme activity may increase heat tolerance by detoxification of the heavy load of ROS. Stress induced signaling molecules may enhance the production of antioxidants in cells to provide thermo-tolerance (Sairam and Tyagi, 2004). Participation of the SODs in an intracellular signaling pathway in Arabidopsis was observed under severe salt stress (Alscher *et al.*, 2002).

References

1. Almeselmani, M., Deshmukh, P. S., Sairam, R. K., Kushwaha, S. R., & Singh, T. P. (2006). Protective role of antioxidant enzymes under high temperature stress. *Plant science*, 171(3), 382-388.
2. Alscher, R. G., Erturk, N., & Heath, L. S. (2002). Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *Journal of experimental botany*, 53(372), 1331-1341.
3. BABU, D. V., Sudhakar, P., & REDDY, Y. S. K. (2013). Screening of thermotolerant ragi genotypes at seedling stage using TIR technique. *Bioscan*, 8(4), 1493-1495.
4. Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical biochemistry*, 44(1), 276-287.
5. Chandola, A., Pandurangam, V., & Srivastava, J. P. (2016). Genotypic Variations in Tomato (*Lycopersicon Esculentum* Mill.) for Acquired Thermotolerance to Temperature Induction Response. *International Journal of Agriculture, Environment and Biotechnology*, 9(4), 649-657.
6. Eitzinger, J., Orlandini, S., Stefanski, R., & Naylor, R. E. L. (2010). Climate change and agriculture: introductory editorial. *The Journal of Agricultural Science*, 148(5), 499-500.
7. Gupta, A. S., Webb, R. P., Holaday, A. S., & Allen, R. D. (1993). Overexpression of superoxide dismutase protects plants from oxidative stress (induction of ascorbate peroxidase in superoxide dismutase-overexpressing plants). *Plant Physiology*, 103(4), 1067-1073.
8. Hasanuzzaman, M., Hossain, M. A., da Silva, J. A. T., & Fujita, M. (2012). Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In *Crop stress and its management: Perspectives and strategies* (pp. 261-315). Springer, Dordrecht.
9. Hikosaka, K., Ishikawa, K., Borjigidai, A., Muller, O., & Onoda, Y. (2006). Temperature acclimation of photosynthesis: mechanisms involved in the changes in temperature dependence of photosynthetic rate. *Journal of experimental botany*, 57(2), 291-302.
10. Sairam, R. K., & Tyagi, A. (2004). Physiology and molecular biology of salinity stress tolerance in plants. *Current science*, 407-421.
11. Sandalio, L. M., Dalurzo, H. C., Gomez, M., Romero-Puertas, M. C., & Del Rio, L. A. (2001). Cadmium-induced changes in the growth and oxidative metabolism of pea plants. *Journal of experimental botany*, 52(364), 2115-2126.
12. Smith, P., & Olesen, J. E. (2010). Synergies between the mitigation of, and adaptation to, climate change in agriculture. *The Journal of Agricultural Science*, 148(5), 543-552.
13. Srikanthbabu, V., Krishnaprasad, B. T., Gopalakrishna, R., Savitha, M., & Udayakumar, M. (2002). Identification of pea genotypes with enhanced thermotolerance using temperature induction response technique (TIR). *Journal of Plant Physiology*, 159(5), 535-545.
14. Sujatha, B., Sirisha, P., & Bharathi, Y. V. (2018). Screening of acquired thermotolerant ragi [*Eleusine coracana* (L.) Gaertn] genotypes using TIR technique. *International Journal of Plant Sciences (Muzaffarnagar)*, 13(1), 165-170.
15. Vidya, S. M., Laxman, R. H., Bhatt, R. M., Rekha, A., Sunoj, V. J., & Ravishankar, K. V. (2017). Temperature induction response technique: a screening tool for evaluation of banana cultivars for thermotolerance. *Indian journal of plant physiology*, 22(1), 79-84.
16. Vijayalakshmi, D., Srividhya, S., Vivitha, P., & Raveendran, M. (2015). Temperature induction response (TIR) as a rapid screening protocol to dissect the genetic variability in acquired thermotolerance in rice and to identify novel donors for high temperature stress tolerance. *Indian Journal of Plant Physiology*, 20(4), 368-374.
17. Wahid, A., & Close, T. J. (2007). Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves. *Biologia Plantarum*, 51(1), 104-109.
18. Weis, E. N. G. E. L. B. E. R. T., & Berry, J. A. (1988). Plants and high temperature stress. In *Symposia of the Society for Experimental Biology* (Vol. 42, pp. 329-346).

19. **Xu, S., Li, J., Zhang, X., Wei, H., & Cui, L. (2006).** Effects of heat acclimation pretreatment on changes of membrane lipid peroxidation, antioxidant metabolites, and ultrastructure of chloroplasts in two cool-season turfgrass species under heat stress. *Environmental and Experimental Botany*, 56(3), 274-285.
20. **Yuan, Y., Qian, H., Yu, Y., Lian, F., & Tang, D. (2011).** Thermotolerance and antioxidant response induced by heat acclimation in Freesia seedlings. *Acta physiologiae plantarum*, 33(3), 1001-1009.

