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Study of the Alleviation of Salinity Effect Due to Enzymatic and Non-Enzymatic Antioxidants in *Glycine Max*

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ABSTRACT

Salinity has an adverse effect on plants particularly legumes. The aim of the present study was to investigate the effect of saline stress on the grain legume soybean. Therefore many enzymatic, non-enzymatic scavengers and physicochemical parameters were estimated. Hydroponic culture of 14 day old seedlings was carried out experimentally. The seedlings were further subjected to four levels of salt stress treatments of 50, 75, 100, 200 mM concentrations. Enzymatic scavengers such as Superoxide Dismutase, Peroxidase resulted in the significant increase at 100 mM concentration whereas Catalase enzyme showed the significant decrease at the same level. Ascorbic Acid, Carotenoid content, Lipid Peroxidation level was remarkably increased. Physicochemical parameters such as Proline, Protein, and Chlorophyll significantly decreased at the highest salt level unlike Phenol which increased at 200 mM concentration. These results concluded that soybean is a salt tolerant plant which has an efficient scavenging system to work against the reactive oxygen species produced due to salinity.

Keywords: Superoxide Dismutase, Catalase, Peroxidase, Non Protein Thiol, antioxidants, Lipid Peroxidation

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INTRODUCTION

Salinity is a major environmental problem caused mainly by the use of different chemical fertilizers and the agricultural practices performed on the fields. Most of the salt stresses in nature are due to Na^+ salts particularly NaCl [1].High salinity lowers water potential and induces ionic stresses and results in generation of reactive oxygen species. These species drastically limits growth and development of plants by affecting different metabolic processes such as CO_2 assimilation, oil and protein synthesis [2]. These molecules are highly damaging to lipids, nucleic acids, and proteins [3]. Antioxidants like superoxide dismutase, Guaiacol peroxidase, catalase, ascorbic acid, carotenoids, lipid peroxidase, phenolic compounds provide defense mechanism against the oxidative damage produced by ROS. Soybean plant is categorized as moderately salt tolerant [4] and therefore its antioxidant activities due to salt stress had been experimentally estimated.

MATERIALS AND METHODS

Plant material and Treatments

The *Glycine max* (L.) Merr.seeds used in the experiment were obtained from Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, India. Seeds obtained were surface sterilized with 1% sodium hypochlorite for 2 min and washed thoroughly with tap water and then rinsed with distilled water. The seeds were then germinated for 4 days in the dark in petridishes. Four day old seedlings were then cultivated hydroponically and transferred to ¼ strength modified Hoagland nutrient solution [5] in the growth chamber at the light intensity of 4500 lux (16 hr light/8 hr dark) at 25ºC. After 10 days period of growth, the solutions were treated with 0(control) 50, 75, 100, 200 mMNaCl concentrations and the solution was renewed on alternate days. The root and shoot of the plants were taken for the antioxidants study.

Assay of Enzymatic parameters

Superoxide dismutase (SOD, EC 1.15.1.1) was assayed on the basis of its ability to reduce the photoreduction of nitrobluetetrazolium to blue formazone by 50% [6]. 3 mL of the reaction mixture contained 50 mM sodium phosphate buffer (pH 7.0), 10 mM methionine, 1.17 mM riboflavin, 56 mM NBT and 100 µL enzyme extract and the absorbance of the solution was taken at 560 nm with the help of Perkin Elmer Lambda 900 UV/Vis spectrophotometer.. SOD activity was expressed as Units/mg protein/hr.

Catalase activity (CAT, EC 1.11.1.6) was determined by the decrease in the absorbance of the reaction mixture at 240 nm. 3 mL of the reaction mixture contains 50 mM (pH 7.0) of potassium phosphate buffer, 7.5mM of H_2O_2 and 50 µL of the crude extract. CAT activity was expressed as µmoL H_2O_2 decomposed/min/mg protein [7].

3 mL of the reaction mixture for peroxidase (POD, E.C. 1.11.1.7) contained 2.8 mL of guaiacol (3%), 0.1 mL H_2O_2 (2%), and 0.1 mL enzyme extract. POD activity was measured by the increase in the absorbance at 470 nm with the help of Perkin Elmer Lambda 900 UV/Vis spectrophotometer. Its activity was expressed as nmoLguaiacol oxidized/min/mg protein [8].

Assay of Physicochemical parameters

Phenol estimation method was described by Malick and Singh, 1980 [9].1 g of the root tissue was grounded with 10 mL of 80% ethanol and the homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was evaporated to dryness and the residue was dissolved with 5 mL of distilled water. This was then used as an extract. To 2 mL of the extract, 0.5 mL of Folin's reagent was added. After 3 min, 2 mL of 20% Na_2CO_3 solution was mixed properly. The mixture was kept in boiling water for 1 min and then the absorbance was read at 650 nm with the help of Perkin Elmer Lambda 900 UV/Vis spectrophotometer

Total soluble protein content in root tissues was quantified by the method of Lowry *et al*., (1951) [10] using bovine serum albumin as standard.

Proline content in the root tissues of the plants were extracted with 3% aqueous 5-sulphosalicylic acid, centrifuged at 5000 rpm. The supernatant used for proline assay and its absorbance was measured at 520 nm. The proline content was expressed as µmol / g fresh weight. This method was described by Bates *et al*., 1973 [11].

Chlorophyll estimation was assayed by the method of Arnon and Stout (1939) [12] in the leaves samples with 80% acetone.

Assay of non enzymatic parameters

Lipid peroxidation was measured by the level of malondialdehyde which is the product of lipid peroxidation content determined by the thiobarbituric acid (TBA) according to the method of Heath and Packer (1968) [13].
Ascorbic acid content was assayed by the method of Omaye*et al*., (1979) [14]. 1 g of root tissue was grinded with 5 ml of 10% TCA, centrifuged at 3500 rpm for 20 min and the supernatant obtained was used for the assay. 1 ml of DTC reagent (2, 4- dinitrophenyl hydrazine-thiourea) was added to the 0.5 ml of the extract and incubated at 37ºC for 3 hour. To this extract 0.75 ml of ice-cold 65% H_2SO_4 was allowed to stand at 30ºC for 30 min and the resulting color was read at 520 nm by spectrophotometer.

Statistical analysis

The values shown in the tables and figures are the mean of five replicated treatments. The results were statistically evaluated using two way analysis of variance where significant differences ($p \leq 0.05$) were obtained using Fisher (1936) [15] technique.

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RESULTS AND DISCUSSION

The present study brings forth the effect of NaCl concentration on the enzymatic and non-enzymatic scavengers of the plant. SOD is the most significant enzymatic antioxidant found in all aerobic organisms. It has been well established that SOD is the first line of defense against oxidative stress. SOD activity was observed to be at the highest levels at 200mM concentration of NaCl. The values at 50, 75, 100, 200 mM concentration was found to be significantly increased at 7.48%, 24.02%, 41.01%, and 47.58% respectively as compared to control (Fig. 1). The increase in SOD activity in *Anabaena doliolum* under NaCl stress has been reported [16]. Significant increase in SOD activity under salt stress has been observed in plants like mulberry [17], *C. arietinum*[18] and *Lycopersiconesculentum*[19].

The Peroxidases make use of guaiacol as electron donor and use H_2O_2 in the oxidation of various organic and inorganic substrates [20]. The GPOX content was markedly increased with 71.94% at the highest level of concentration (200 mM). The significant increase of 39%, 59%, 70.98% was observed at 50, 75, 100 mM concentration respectively as compared to control (Fig. 2). A concomitant increase in GPOX activity in both the leaf and root tissues of *Vignaradiata* [21], *O. sativa* [22] had been reported under salinity stress.

Catalase activity showed the significant decrease at the highest NaCl concentration (200 mM). The significant decrease in CAT activity was observed at 50 mM (15.59%) and 75 mM (40.21%) concentration respectively as compared to control. However no significant difference was observed at 100mM and 200 mM concentration (Fig. 3). CAT enzyme is responsible for the dissociation of H_2O_2 into H_2O and O_2 and is important for ROS detoxification during stress condition. The response of CAT enzyme in different plant varies. Decreased activity is observed in *Glycine max* [23], *Phragmitesaustralis* [24], *Capsicum annuum* [25] and *Arabidopsis thaliana* [26], whereas its activity is increased in *O.sativa* [27], *B. juncea*[28], *T. aestivum* [29], *C.arietinum* [30] and *Vignamungo* roots [31] under Cd stress. Furthermore, the response of CAT activity under osmotic stress has been frequently contradictory. Accordingly some workers have shown enhanced CAT activity [32], [33] whereas others have reported a salt induced down regulation [34], [35].

For the further assessment of this study, non-enzymatic scavengers such as LP, AsA, Car, NPT was also estimated. Changes in LP activity in the roots under salt stress was increased by about 9.15%, 10.44%, 18.76% and 46.01% at 50, 75, 100, 200 mM concentration with respect to control (Fig. 4). During severe stress conditions, peroxidation of membrane lipids occurs in every living organism. During LP process, compound such as malondialdehyde reacts with Thiobarbituric acid to form coloured products called Thiobarbituric Acid Reactive substances (TBARS) [13]. Further studies by Kukreja*et al*., (2005) [18] revealed marked increase in lipid peroxidation in *Cicerarietinum* roots under salinity stress.

Car protects photosynthetic apparatus of the plants against reactive oxygen species produced under stress conditions. Car content at 50, 75, 100, 200 mM concentration was estimated to increase significantly by 4.25%, 10.68%, 13.37% and 17.49% as compared to

control (Fig. 5). In plants like *Phyllanthusamarus* and *V. mungo* there is a reported decrease in carotenoid content [36] whereas the increase in carotenoid content was reported in Cd stress.

Ascorbic acid is one of the most effective antioxidants in alleviating the damage caused by reactive oxygen species in plants [37, 38]. It has been reported by earlier that ascorbic acid plays an important role in the removal of H_2O_2. AsA content in the roots of the soybean plant increased progressively as the salt concentration increases. The significant increase of 44.44%, 68.01%, 78.78%, and 83.27% was observed at 50, 75, 100, 200 mM concentration as compared to control (Fig. 6).

Salt stress on the physicochemical parameters was further studied. Chlorophyll content of the leaves decreased significantly as the salt concentration increases. The content in leaves at NaCl concentrations of 50, 75, 100, 200 mM was increased by 8.91%, 16.70%, 36.23%, 41.27% respectively as compared to control (Fig. 7). Decrease in chlorophyll content of soybean leaves with increasing salinity could be related to increasing the activity of chlorophyll degrading enzyme, chlorophyllase [39], and the destruction of the chloroplast structure and the instability of pigment protein complexes [40]. Similar results were reported for pea [41], sunflower [42], sorghum [43], and wheat [44].

Phenols play an important role in the detoxification of ROS [45]. Phenol content was increased by about 24.61%, 35.11%, 42.89% and 50.89% at NaCl concentrations of 50, 75, 100, 200 mM respectively with respect to control (Fig. 8). Synthesis and accumulation of phenolic compounds in plants can be attributed to the stress response [46].

The promotion of protein content loss in the roots of the soybean plants was observed at 50, 75, 100, 200 mM concentration with 17.06%, 23.09%, 42.91%, and 44.51% as compared to control (Fig. 9). It has been reported earlier by Kastori*et al*., 1992 [47] that in *Helianthus annus* protein content is decreased at high concentration of heavy metals. This decrease might be attributed to the disturbance in nitrogen balance under stress conditions [48].

Proline accumulation can be considered as indicator for the heavy metal stress. The significant increase in proline content was observed with 11.71%, 30.61%, 40.51%, 45.70% at 50, 75, 100, 200 mM concentration as compared to control (Fig. 10). Much literature has been provided regarding proline acting as an osmoprotectant and is the efficient quencher of ROS under salt, metal, drought stress. Proline is described and evident in many research papers as an osmoprotectant, inhibitor of LP, OH and $^1O_2$ scavenger [49, 50].

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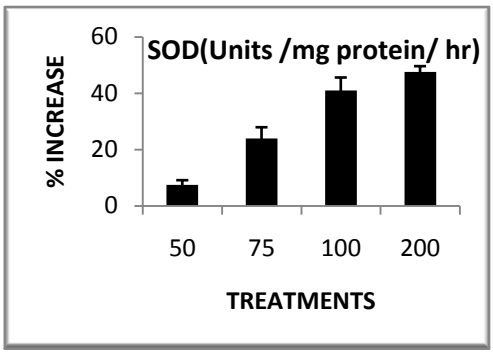
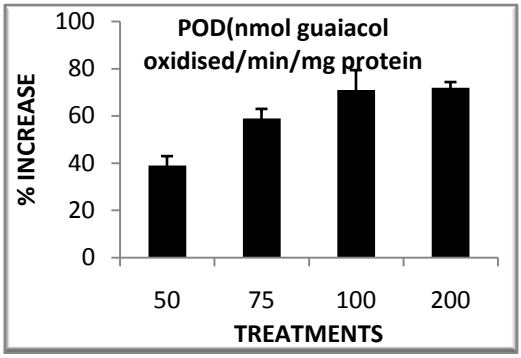


Fig 1



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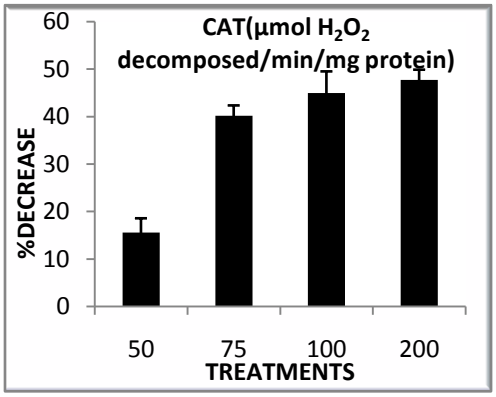

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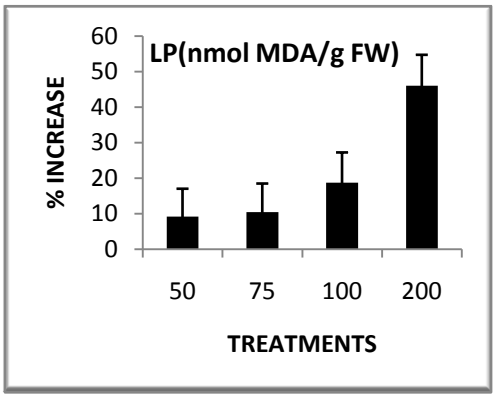
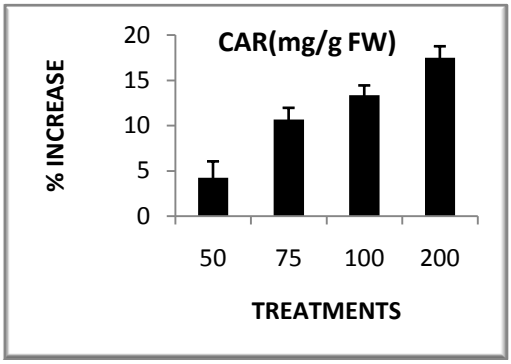


Fig3



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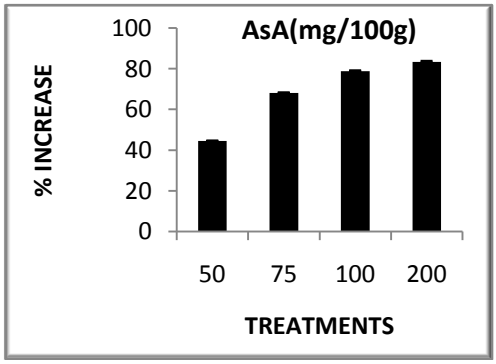

Fig 6

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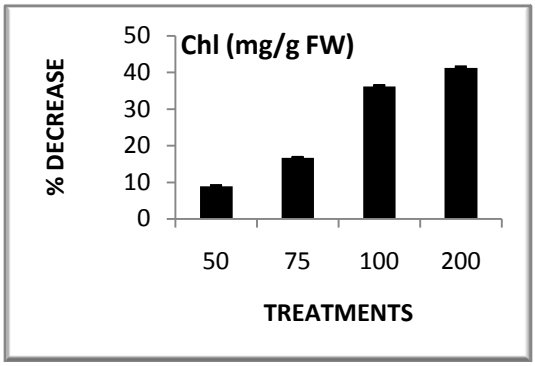

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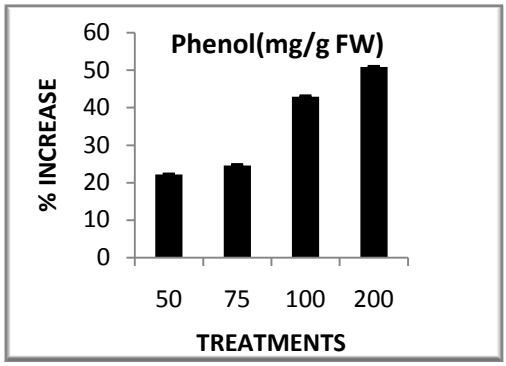

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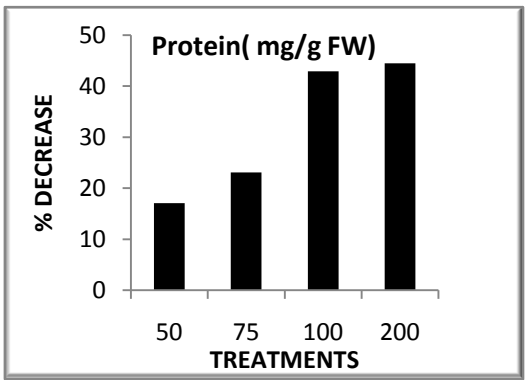

Fig 9

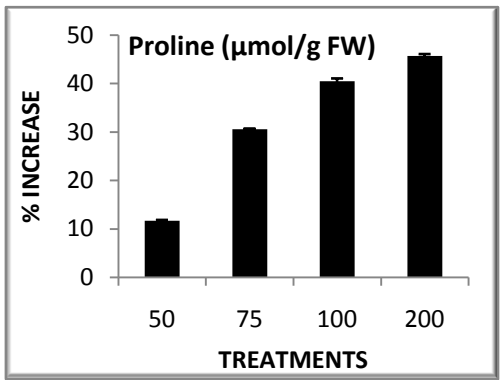

Fig 10

CONCLUSION

The present study revealed the effect of salt stress on the antioxidants in soybean plant. Antioxidants level in the soybean plants had been increased as shown in the results of the experimental study making it the salt-tolerant crop. The study throws the light on the defense mechanisms of the plant to alleviate the level of salt stress.

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