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Research Article

NON-ENZYMATIC BIOCHEMICAL RESPONSE OF *OCIMUM SANCTUM* TO SODIUM CHLORIDE STRESS

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ABSTRACT

Objective: This study was undertaken to evaluate the non-enzymatic components of the antioxidant system in the leaves of *Ocimum sanctum* subjected to salt stress.

Methods: Leaves of *O. sanctum* exposed to different concentrations (0, 20, 40, 60, and 80 µM) of sodium chloride (NaCl) and were subjected to analysis of non-enzymatic biochemical parameters including ascorbic acid, proline, glutathione, and non-protein thiol (NPT) using the standard protocols.

Results: As the concentration of NaCl was increased, except NPT, all other three parameters displayed an overall decreasing trend as compared with control.

Conclusion: Our results clearly indicate that NPT is the key player among the non-enzymatic parameters which protect the *O. sanctum* against the deleterious effect of reactive oxygen species.

Keywords: *Ocimum sanctum,* Salt stress, Ascorbic acid, Glutathione, Proline, Non-protein thiol.

INTRODUCTION

Salinity is a serious environmental concern as it limits the plant development and productivity. As per Food and Agriculture Organization (FAO), it affects more than 6% of the world's land, and 23,222,000 ha land is salt stressed in India [1]. Salinity is caused by a number of natural and anthropogenic factors. Natural factors include evapotranspiration in arid and semi-arid regions, floods, intrusion of saline waters to rivers from coastal areas. Man increases the salinity content of the soil using poor quality of irrigation water, inappropriate practices of irrigation by untrained farmers, soil erosion by adopting unscientific methods of cultivation and deforestation [2]. Salt tolerance is a complex trait involving a number of plant adaptations such as cellular and metabolic adjustments, osmotic changes, and control of water through stomata [3].

Once plants are subjected to sodium chloride (NaCl) stress, they generate excessive reactive oxygen species (ROS) that are responsible for disturbing the cellular homeostasis of the plant cells thereby bringing the retardation in the growth and development of plants. Plants, however, have developed a number of ways to tolerate the oxidative damage caused due to overproduction of ROS like they generate osmoprotectants such as proline and glycine betaine, they trigger their enzymatic and non-enzymatic antioxidant defense system, they start synthesizing their compatible solutes such as polyamines, amides, amino acids, carbohydrates, and they also regulate ionic balance to maintain metabolism at the surface of roots and reduce the rate of photosynthesis to prevent water loss [4].

Medicinal plants are commercially cultivated for their bioactive component present in their plant parts, especially for the drugs [5]. A number of herbal drugs have their origin from medicinal plants such as *Azadirachta indica*, *Aloe barbadensis, Ocimum sanctum,* and *Mentha arvensis* [6]. Owing to their high medicinal value, they are considered suitable candidates for new reclaimed soils and semi-arid regions. The salt tolerant and high yielding varieties of these plants can be grown and utilized for new formulations. *O. sanctum* L. has been strongly recommended for the treatment of a number of ailments such as malaria, diarrhea, dysentery, arthritis, and bronchitis. The experimental plant has also been shown to exhibit antifertility, antidiabetic, anticancer, antimicrobial, cardioprotective, antispasmodic, and adaptogenic actions [7]. The present research work aims to explore the response of non-enzymatic components including ascorbic acid, proline, glutathione, and non-protein thiol (NPT), generated in the leaves of the experimental plant under different salt concentration.

METHODS

Plant materials and growth condition

O. sanctum seeds were procured from the Indian Agriculture Research Institute, Pusa, New Delhi. Seeds were sterilized with 1% sodium hypochlorite for 2 minutes, washed with distilled water several times and sowed during the month of July in pots containing a mixture of soil and manure in the ratio 3:1. The pots were placed in a net house located in Sharda University, Greater Noida. After four-leaf stage, the plants were treated with 0, 20, 40, 60, and 80 µM NaCl with Hoagland nutrient solution. The favorable conditions were maintained to avoid the fungal as well as insects infections. After the complete treatment, the leaves were harvested and immediately stored at −20°C.

Assay of non-enzymatic antioxidant molecules

Determination of ascorbic acid

Leaves weighing 0.1 g was homogenized in 10 ml of 0.4% oxalic acid and centrifuged at 8000 rpm at 4°C for 15 minutes. 500 µl of supernatant was taken in a tube, and 7 ml of dichlorophenol indophenol dye solution was added to the same tube. The absorbance was taken at 518 nm in a spectrophotometer [8].

Determination of proline

Proline concentration in leaves was determined spectrophotometrically by the method of Bates *et al*. [9]. Preserved leaves (0.1 g) were homogenized in 2 ml chilled sulfosalicylic acid and centrifuged at 10,000 rpm at 4°C for 35 minutes. The reaction mixture contained 1 ml of each supernatant, glacial acetic acid, and ninhydrin. The tubes were kept in a water bath for 1 hr and observed for change in color. After

cooling, 2 ml of toluene was added and shaken vigorously for 30 seconds, and absorbance was taken at 520 nm in the spectrophotometer.

Determination of glutathione

Total glutathione was determined by the glutathione recycling method of Anderson [10]. Leaves (0.1 g) were homogenized in 2 ml of 5% sulfosalicylic acid at 4°C. The homogenate was centrifuged at 10,000 rpm for 10 minutes. To a 0.5 ml of supernatant, 0.6 ml of reaction buffer (0.1 M Na-phosphate, pH 7, 1 mM ethylenediaminetetraacetic acid [EDTA]) and 40 µl of 0.15% 5,5-dithiobis-2-nitrobenzoic acid (DTNB) were added and absorbance was measured at 412 nm after 2 minutes. To the same, 40 µl of 0.4% nicotinamide adenine dinucleotide phosphate and 2 µl of glutathione reductase (GR; 0.5 enzyme unit) were added and the reaction was run for 30 minutes at 25°C. The samples were again read at 412 nm to determine total glutathione content.

Determination of NPT

NPTs were determined by homogenizing 0.1 g leaves in 5% (w/v) sulfosalicylic acid solution. The reaction mixture was centrifuged at 10,000 g at 4°C for 30 minutes. Supernatant was collected, and NPT was measured with Ellman's reagent. In short, 300 µl of the supernatant was added to 1.2 ml of 0.1 M potassium phosphate buffer (pH 7.6). After obtaining a stable absorbance at 412 nm, 25 µl of DTNB solution (6 mM DTNB dissolved in 5 mM EDTA and 0.1 M phosphate buffer solution pH 7.6) was added, and the increase in absorbance at 412 nm was read [11].

Statistical analysis

The experiments were conducted in triplicates. The obtained data were statistically analyzed using MS-OFFICE tools, and analysis of variance was performed using CROPSTAT software program for windows (7.2.2007.2 module), IRRI, Philippines.

RESULTS AND DISCUSSION

Effect of NaCl on non-enzymatic antioxidants defense mechanism

A number of non-enzymatic components of the antioxidant system were assayed and checked for their activity under NaCl stress conditions. Surprisingly, irregular trend in the measured values of non-enzymatic components of the antioxidant defense system was observed on application of NaCl stress. Ascorbic acid was measured highest in 20 µM concentration, and then, the value dipped in subsequent treatments with 80 µM concentration showing the lowest value of 25.23 (Fig. 1). Proline level was highest in control with a value of 18.61 and minimum in 80 µM concentration with a value of 4.82. The reduction in the proline amount was approximately 4-folds as compared to control (Fig. 2).

Comparable trends were also observed for glutathione as that of proline where the measured value showed a gradual decrease across the treatments. The glutathione control measured highest with 31.01 and 80 µM concentration giving the extreme low value of 8.7 nmoles/ml (Fig. 3). The variation in the measured value of NPTs was observed across the treatments. The value reached 456.82 in 20 µM concentration and then dipped in 40 µM concentration to 385.63 to increase further in 60 and 80 µM concentration to 623.31 and 731.40, respectively (Fig. 4).

Salinity poses a big challenge for agricultural production as it disturbs the ionic homeostasis of plants [12]. Land salinization is becoming a serious threat with the increasing scarcity of fresh water, increasing global warming, mal irrigation practices, and excessive use of fertilizers [13]. The need of the hour is to study how plants cope up with the stress and development of new breeds of salt-resistant plants.

Different crops exhibit a number of responses under salt stress conditions. Salinity affects plants at all stages of development adversely including germination, vegetative growth, and reproductive development. Salinity imposes nutrient deficiency, ion toxicity,

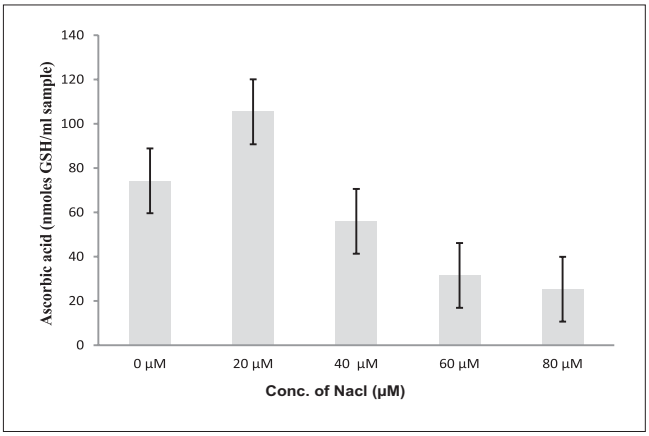

Fig. 1: Effects of different concentrations of sodium chloride on ascorbic acid content measured in leaves of *Ocimum sanctum* L. The data were analyzed for a significant difference (p≤0.05), and the error bars indicate standard error

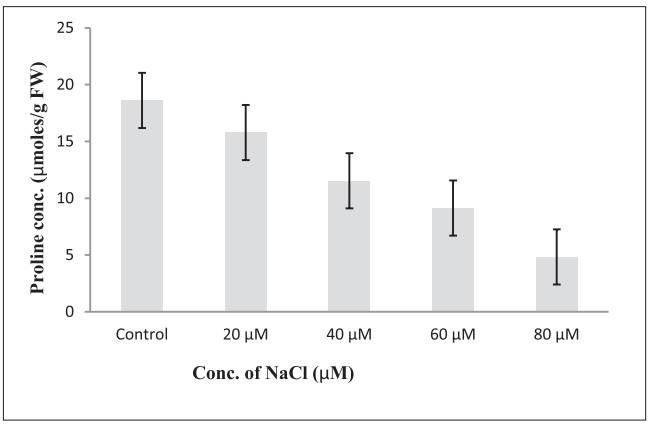

Fig. 2: Effects of different concentrations of sodium chloride on proline content measured in leaves of *Ocimum sanctum* L. The data were analyzed for a significant difference (p≤0.05), and the error bars indicate standard error

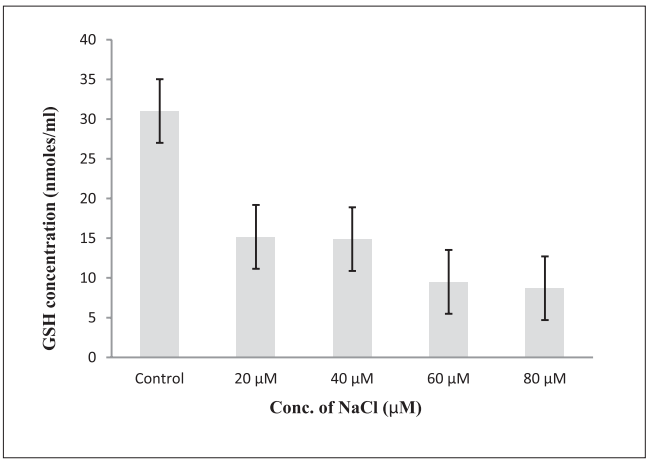

Fig. 3: Effects of different concentrations of sodium chloride on glutathione content measured in leaves of *Ocimum sanctum* L. The data were analyzed for a significant difference (p≤0.05), and the error bars indicate standard error

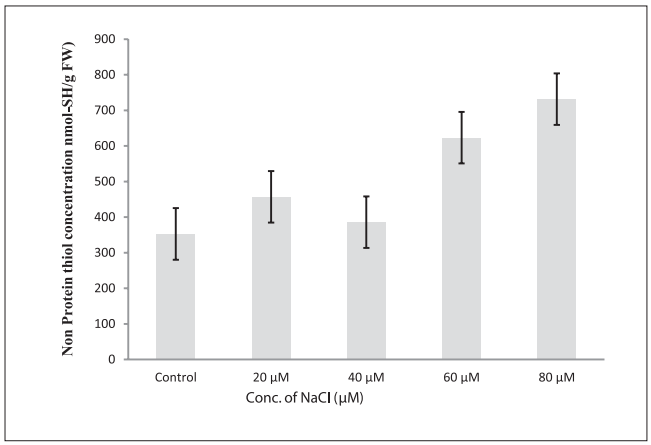

Fig. 4: Effects of different concentrations of sodium chloride on non-protein thiol content measured in leaves of *Ocimum sanctum* L. The data were analyzed for a significant difference (p≤0.05), and the error bars indicate standard error

osmotic, and oxidative stress [14]. Generation of ROSs causes oxidative stress [15-17].

To combat the ROS, ascorbic acid got triggered in 20 µM concentration, but its level came down in subsequent concentration. This may be attributed to the adaptive mechanism of the plant to stress.

Surprisingly, proline and glutathione, non-enzymatic component of the defense mechanism, do not indicate significant positive results. The concentration of NPT displayed a gradual increase but in 40 µM concentration the level decreased unexpectedly. In our previous work, we have investigated the enzymatic response of *O. sanctum* under NaCl stress [18]. From the results, authors can also suggest that exogenous application of ascorbic acid may be used to alleviate the oxidative stress in *O. sanctum* cultivated on mildly salt stressed soils [19].

CONCLUSION

O. sanctum is a well-known medicinal plant. It is the source of a number of commercially important formulations, which are known to cure many ailments. Authors, in past, have investigated the response of enzymatic components including catalase, ascorbate peroxidase, superoxide dismutase, and GR. The present investigation throws light on the behavior of non-enzymatic parameters of defense system under the same concentrations of salt stress. Results clearly indicate that the experimental plant protects itself from the adverse effects of salt mostly by the enzymatic defense, whereas among non-enzymatic parameters, ascorbic acid at low concentrations of salt, and NPT throughout the dosage played a significant role in defense mechanism against stress. Authors further suggest that exogenous application of ascorbic acid in mildly salt stressed soils may lead to alleviation in the oxidative stress in *O. sanctum*.

REFERENCES

1. FAO. The State of Food & Agriculture Biofuels: Prospects, Risks and Opportunities. Rome: Food & Agriculture Org.; 2008.
2. Yadav S, Irfan M, Ahmad A, Hayat S. Causes of salinity and plant manifestations to salt stress: A review. J Environ Biol 2011;32(2):667-85.
3. Abogadallah GM. Antioxidative defense under salt stress. Plant Signal Behav 2010;5(4):369-74.
4. Gupta B, Huang B. Mechanism of salinity tolerance in plants: Physiological, biochemical, and molecular characterization. Int J Genomics 2014;2014:701596.
5. Ahmad I, Khan MS, Singh Cameotra S. Quality assessment of herbal drugs and medicinal plant products. Encyclopedia of Analytical Chemistry, 2000.
6. Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TP. Indian herbs and herbal drugs used for the treatment of diabetes. J Clin Biochem Nutr 2007;40(3):163-73.
7. Pattanayak P, Behera P, Das D, Panda SK. *Ocimum sanctum* Linn. A reservoir plant for therapeutic applications: An overview. Pharmacogn Rev 2010;4(7):95-105.
8. Wegg SM. Ascorbic Acid Production in The Cultured Tissue of The Briar Rose, *Rosa rugosa*. PhD Dissertation, University of British Columbia: 1972.
9. Bates L, Waldren R, Teare I. Rapid determination of free proline for water-stress studies. Plant Soil 1973;39(1):205-7.
10. Anderson ME. Determination of glutathione and glutathione disulfide in biological samples. Methods Enzymol 1985;113:548-55.
11. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82(1):70-7.
12. Majumdar RS, Shukla M, Mishra PK. Cellular ion homeostasis mechanism in salt tolerant plants. Indian J Agric Biochem 2011;24(2):83-90.
13. Yu S, Wang W, Wang B. Recent progress of salinity tolerance research in plants. Russ J Genet 2012;48(5):497-505.
14. Shrivastava P, Kumar R. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J Biol Sci 2015;22(2):123-31.
15. Chaves MM, Oliveira MM. Mechanisms underlying plant resilience to water deficits: Prospects for water-saving agriculture. J Exp Bot 2004;55(407):2365-84.
16. Naureen G, Naqvi FN. Salt tolerance classification in wheat genotypes using reducing sugar accumulation and growth characteristics. Emirates J Food Agric 2010;22(4):308-17.
17. Gill M. Heavy metal stress in plants: A review. Int J Adv Res 2014;2(6):1043-55.
18. Bajpai SM, Gupta N, Majumdar SR. Biochemical adaptations of *Ocimum sanctum* to NaCl stress. Res J Chem Environ 2016;20(2).
19. Athar HR, Khan A, Ashraf M. Exogenously applied ascorbic acid alleviates salt induced oxidative stress in wheat. Environ Exp Bot 2008;63(1-3):224-31.