J. Phytol. Res. 16(1) : 85-88, 2003

INDUCTION OF OXIDATIVE STRESS IN THE ROOTS OF *LEMNA MINOR* L.: A RESPONSE TO NaCl-SALT STRESS

R.K. UPADHYAY and S.K. PANDA
Plant Biochemistry Laboratory, School of Life Sciences, Assam (Central) University, Silchar-788011, India.

Implication of NaCl-salt stress induced oxidative reactions in the roots of *Lemna minor* L. and its response to salt tolerance was experimentally investigated. *Lemna* roots were acclimatized under control (0) and 200 mM NaCl solution. An increase in peroxide (H_2O_2) contents, superoxide dismutase (SOD), guaiacol peroxidase (GPx) and glutathione reductase (GR) activities were marked with an uniform decrease in catalase (CAT) activity. At higher salt concentration, Na^+ was found to accumulate more with a decrease in K^+content.

Keywords : *Lemna minor* L; NaCl salinity; Oxidative stress.

Introduction

Plants experience a great number of stress conditions, of which salt stress is known to be one of the great environmental stress affecting tremendously the physiology of plants growing in both soil and aquatic environment. *Lemna minor* L., a fast growing aquatic duckweed is one of the components of the aquatic ecosystem which is badly affected by various ionic pollutants[1]. Salt stress not only alters the growth and metabolic status, like accumulation of proline, inhibition of protein and nucleic acid hydrolysis etc. but also induces reactive oxygen species (ROS) like superoxide radicals (O_2), alkoxyl radicals (RO), hydroxyl radicals (OH), prehydroxyl radicals (HO_2), etc. in various plant tissues which changes the redox homeostasis of the cell causing oxidative stress and above all these reactive oxygen species (ROS) can damage almost every macromolecule[2-6]. Plants have both enzymic (CAT, APX, GPx, SOD, GR) and non enzymic (ascorbate, glutathione, α-tocopherol, carotenoid) antioxidant defence system to succeed in controlling oxidative stress[4,7,8]. Salt stress has a great extent of physiological and biochemical responses including oxidative stress[6,9-11]. To test the hypothesis, that imposition of salt stress induces oxidative reactions in the root cells of *Lemna minor* L., the present investigation was undertaken.

Materials and Method

Floating aquatic microphyte, *Lemna minor* L. was collected from the uncontaminated pond nearby University (90°40/E longitude and 20°04/N latitude) and grown under laboratory conditions under continuous light. Light was provided with white fluorescent tube lights (Philips 36 W TLD) giving a photon flux density (PFD) of 52μ $Em^{-2}S^{-1}$ (PAR). For salt treatment plants were washed with double distilled water several times and soaked dry and fifty plants were transfered to petriplates with control (0) and 200 mM NaCl solution with three replicates each. The petriplates were incubated under light at 29° C for 24h, 48h and 72h. After the treatment the minute roots were excised out, soaked dry and sampled for various biochemical and enzymic estimations.

The estimations of NA^+ and K^+ ions were done using the method of Humpries[12]. The tissue of *Lemna minor* L. was weighed and oven dried and acid digested in standard volume of HNO_3 and the Na^+ and K^+ ions were estimated with the help of Flame photometer (Systronics, India). 0.2g of root tissue was taken and homogenized with 5% Trichloroacetic acid (TCA) and the homogenate was used for the extraction and estimation of total peroxide content[13]. Lipid perioxidation was measured as the amount of thiobarbituric acid reactive substance (TBARS) determined by the thiobarbituric acid (TBA) reaction as described by Heath and Packer[14]. 0.2g of root tissue was homogenized in 2.0ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 12000g. for 20 min. To 1ml of resulting supernatant, 1ml of TCA

(20%) containing (0.5% w/v) of TBA and 10µ of BHT in (40% ethanol) were added. The mixture was heated at 95°C for 30 min. and then cooled in ice. The contents were centrifuged at 12000g. for 15 min. and absorbancy was measured at 532 nm and corrected for 600 nm. The concentrations of TBARS was calculated using an extinction coefficient of 155 mM cm^{-1}.

The root tissue (0.2g) was homogenized with phosphate buffer, pH6.8 (0.1M) in prechilled motor and pestle. The extract was centrifuged at 4°C for 15 min. at 14000g. in a cooling centrifuge. The supernatant was used for the assay of catalase (CAT), guaiacol peroxidase (GPx), superoxide dismutase (SOD) and glutathione reductase (GR). The CAT and GPx activities were assayed as per the method of Chance and Maehyl[15]. The 5.0 ml mixture comprise of 3.0 ml phosphate buffer (pH 6.8), 1 ml (30 mM) H_2O_2 and 1 ml enzyme extract. The reaction was stopped by adding 10ml 2% H_2SO_4 after 1 min. incubation at 20°C. The acidified reaction mixture was titrated against 0.01N $KMNO_4$ to determine the quantity of H_2O_2 utilized by the enzyme. The CAT activity was expressed as µ mole H_2O_2 determine min^{-1} g. fr. wt^{-1}. The 3.0 ml reaction comprise of 0.1 M phosphate buffer (pH 6.8), guaiacol (30mM), H_2O_2 (30mM) and 0.3ml enzyme extract. The rate of change in absorbancy at 420 nm was measured using UV-visible spectrophotometer (Systronic, India). The levels of enzyme activity was expressed as µ moleH_2O_2 destroyed min^{-1} g.fr. $wt.^{-1}$. The assay of SOD was done as per the method of Giannopolitis and Reis[16]. 3ml assay mixture for SOD contains 79.2 mM Tris-HC1 buffer (pH 6.8), containing 0.12 mM EDTA and 10.8 mM Tetraethylene diamine, Bovine serum albumin ($3.3 \times 10^{-3\%}$), 6mM nitroblue tetrazolium (NBT), 600 µM riboflavin in 5mM KOH and 0.2ml enzyme extract. Reaction mixture was initiated by placing the glass test tubes i n between two fluorescent tubes (Philips 20 W). Switching the light on and off, the reaction mixture was initiated and terminated. The increase in absorbancy due to formazan formation was read at 560 nm. Under the above condition, the increase in absorbancy in the absence of enzyme was 100% and 50% initial was taken an equivalent to 1 unit of SOD activity. Glutathione reductase (GR) was assayed by the method of Smith *et al.*[17]. The reaction mixture contained 0.2M potassium phosphate buffer (pH 7.5) containing 1mM EDTA, 3mM DTNB (5,5′-ditiobis-2 nitrobenzoic acid) in 0.01M potassium phosphate buffer (pH 7.5), 2mM NADPH 1ml enzyme extract and distilled water to make up a volume of 2.9ml. Reaction was initiated by adding 2mM GSSG (oxidised Glutathione or Glutathione disulphide). The increase in absorbancy at 412 nm was recorded at 25°C over a period of 5 min. spectrophotometrically. The activity is expressed as absorbancy change (ΔA_{412}) g. fr. wt^{-1} sec^{-1}. All the observations were done in triplicates and repeated thrice and the data represents mean±standard error of mean (S.E.M.).

Results and Discussion

With the increase in day of exposure to 200mM NaCl concentration, a uniform increase in lipid peroxidation detected as TBARS (74.3%) was marked as compared to control. An increase in superoxide dismutase (SOD) activity in root cells from control (149.5%) under salt stress must have dismutated superoxide radicals forms to hydrogen peroxide which is also substantiated by an accumulation of cytotoxic (H_2O_2) (1300%) in root cells. The peroxide scavenging enzyme, catalase (CAT), guaiacol peroxidase (GPx) and glutathione reductase (GR) showed a decrease in catalase (CAT) activity (41.03%) and an increase in guaiacol (GPx) peroxidase (454.4%) and glutathione (GR) reductase (468.9%) activities under salt stress which indicated a situation of salt stress imposed oxidative stress in root cells and the loss of capacity of cellular protection

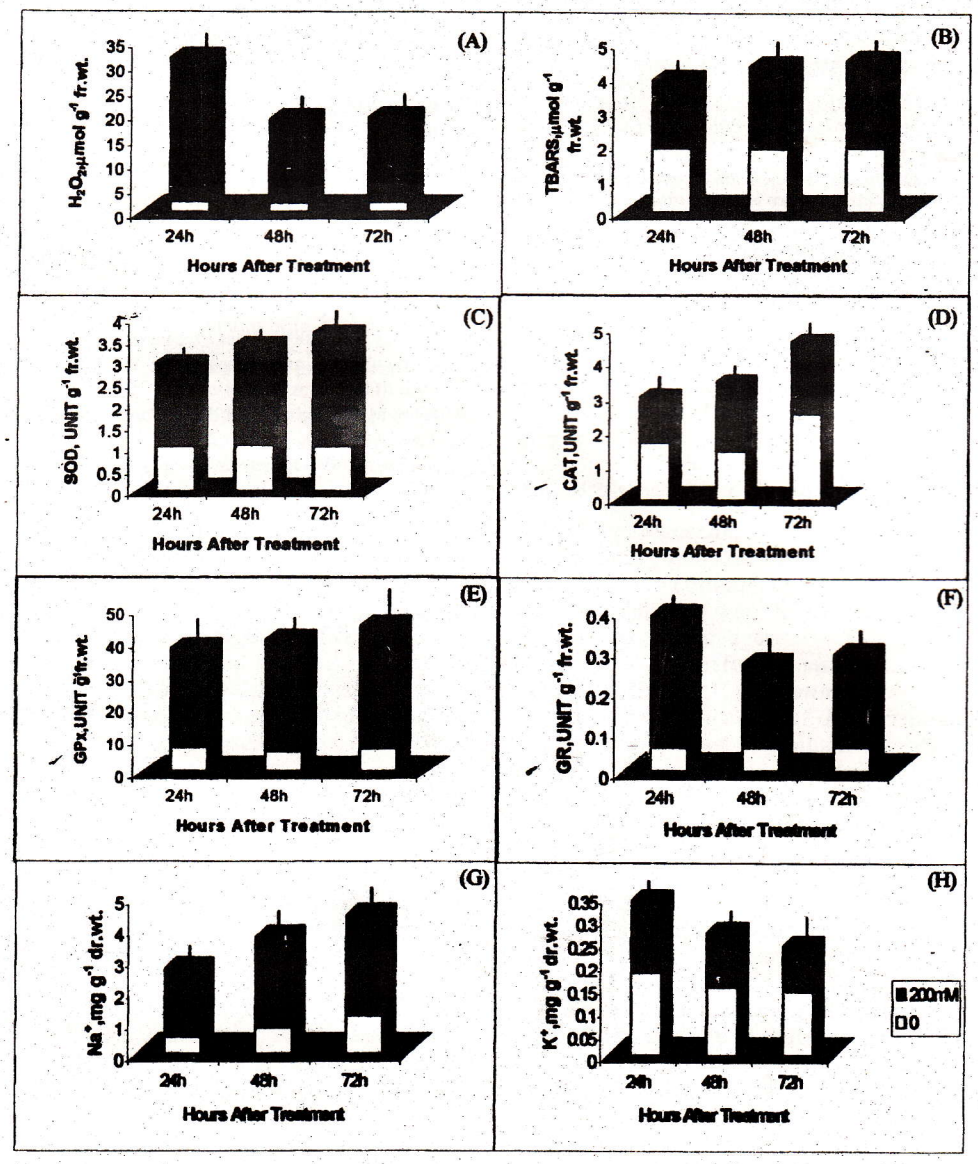

Fig. 1. Changes in H_2O_2 (A), TBARS (B), and activities of SOD (C), CAT (D), GPx (E), GR (F), Na^+ (G) and K^+ ion (H) contents in the roots of *Lemna minor* L. subjected to NaCl-salinity stress. Data presented are means of three separate experiments± Standard errors.

in roots[18-20]. The increase in sodium ion (NA^+) content (262.5%) with a decrease in potassium ion (K^+) content (28.5%) showed sodium ion toxicity and salt sensitivity of *Lemna* as also correlated with the induction of oxidative stress[21].

References

1. Arber A 1963, *Harper Publishing Co* New York Pp. 73.83.
2. Scandalios JG 1993, *Plant Physiol.* **101** 7
3. Hendry G A F 1993, *Seed Sci. Res.* **3** 141
4. Alscher R G, Donahue JL and Crammer C L 1997, *Physiol. Plant.* **100** 224
5. Hernandez J A, Jimenez A, Mullineaux P and Sevilla F 2000, *Plant Cell Environ.* **23** 853
6. Khan MH and Panda S K 2002, *Biol. Plant.* **45** 625
7. Hendry G A F and Crawford RMM 1994, *Proc. Ro. Soc. Edinburgh* **102B** 1
8. Panda S K 2002, *Advances in stress physiology of plants,* Scientific Publishers Pp. 1
9. Bowler C, Montagu M V and Inze D 1992, *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **43** 83
10. Shalata A and Tal M 1998, *Physiol. Plant.* **104** 169.
11. Lee D H Kim YS and Lee CB 2001, *J Plant Physiol.* **158** 737
12. Humpries E C 1956, In : *Modern Methods of Plant Analysis* (ed.) Peach K and Tracey N.V. Pp. 468
13. Sagisaka S 1976, *Plant Physiol.* **57** 308
14. Heath RL and Packer L 1968, *Arch. Biochem. Biophys.* **125** 189
15. Chance B and Maehly AC 1955, *Methods Enzymol.* **2** 764
16. Giannapolitis CN and Ries SK 1977, *Plant Physiol.* **59** 309
17. Smith I K, Vierheller T L and Thorne CA 1988, *Anal. Biochem.* **175** 408
18. Hernandez JA, Ferrer MA, Jimenez A, Barcelo AR and Sevilla F 2001, *Plant Physiol.* **127** 817.
19. Hernandez J A and Almansa M S 2002, *Physiol. Plant.* **115** 251
20. Khan MH, Singha Ksh LB and Panda S K 2002, *Acta Physiol. Plant.* **24** 145
21. Subbarao GV, Johanson C, Jana MK and Rao JVDK 1990, *J. Plant Physiol.* **137** 64