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Effects of Zn stress on antioxidant enzyme activity in *Lemna polyrrhiza L.*

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Abstract

The effect of deleterious concentration of zinc provided individually was investigated in order to assess the effect of metal interaction in *Lemna polyrrhiza L.* The present study also emphasizes on the response of catalase and guaiacol peroxidase enzymes under zinc stress. Both antioxidant enzymes exhibited an increasing trend under different treatment conditions but it was reverse at highly toxic metal concentration. The antioxidant activities of enzymes, i.e of catalase, ascorbate peroxidase, guaiacol peroxidase and their activity proportions were examined. Catalase activities were substantially increased in a stress environment as compared to guaiacol peroxidase. Further, catalase and showed increased activities in a combined stress environment. Physiological role of these enzymes in stress tolerance mechanism is discussed. The response of *Lemna polyrrhiza L* to toxic concentrations of Zn appears to induce oxidative damage as observed by the increase antioxidant metabolism.

Keywords- *Antioxidant, ascorbate peroxidase, catalase, enzyme activity, glutathione reductase, guaiacol peroxidase, Lemna polyrrhiza L. , stress*

Introduction

Environmental stress factors like drought, temperature, high salinity and heavy metals are the major constraints that limit plant growth and productivity, by disturbing the intracellular water balance. Usually, in fields or on agricultural land, unlike in a laboratory or even a greenhouse environment, plants are subjected to a manifold array of stress factor (Siddiqui *et al*. 2008 and Nawaz *et al*. 2010).However, most of the studies have been devoted to assess the physiological response of plants in a single stress environment like salinity (Sinha 1991 and Sharma and Gills 1994, Kumar and Kumar 1996), drought (Shinozaki and Yamaguchi 2000) and heavymetals (Hameed *et al*. 2000 and Jetley *et al*, 2004). Studies on the physiological responses of plants under a combination of such stresses are restricted to just a few reports (Wang et al. 2003 and Dudley and Shani 2003, Wang and Huang 2004 and Jakab *et al*. 2005) and even they are not directly related to the combination of stress factors like heavy metal.

Plants have utilized various mechanisms to combat with abiotic stresses. Among them, stress tolerance gene expression, compatible solutes, phenols and antioxidant enzyme production are some examples (Jakab *et al*. 2005, Siddiqui and Khan 2011). Antioxidant enzymes such as superoxide dismutase (SOD) ascorbate peroxidase (APX), glutathione reductase, guaiacol peroxidase and catalase are well-known defense systems providing protection against the hazards of reactive oxygen species (ROS) in different stressful conditions (Allen *et al*. 1997, Kwon *et al*. 2002). These defenses may also involve water-soluble antioxidants such as ascorbate, glutathione and phenolic compounds and lipid-soluble molecules such as carotenoids and tocopherols (Foyer *et al*,1994; Hodges *et al* . 1997 and Hodges and Forney 2000; Pastori *et al*, 2000). Oxidative stress is characterized by the synthesis of hydrogen peroxide, which is detoxified by CAT activity in the peroxisomes and by APX in the cytosol, mitochondria and chloroplasts (Foyer *et al.* 1994 and Asada, 1999). On the other hand, antioxidant molecules such as glutathione, ascorbate and soluble phenolic compounds are able to directly scavenge reactive oxygen radicals and, in the case of ascorbate and glutathione, they are also substrates for the antioxidant enzymes APX and GPX, respectively. Therefore, it seems likely that both ascorbate and glutathione might play a key role in buffering oxidative stress in most eukaryotic systems (Noctor and Foyer, 1998; Smirnoff, 2000 and Smirnoff *et al*. 2001).

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Activities of these antioxidant enzymes are frequently observed in a single stress environment but the response and the proportion of the relative activities of these enzymes in a combined heavy metal (lead) environment have seldom been reported. To make up for this lack, the present study examines the response of antioxidant enzymes and the proportions of their relative activities in heavy metal (zinc sulphate) stress environment.

Material and Methods

Plant Material:

The test plants *Lemna polyrrhiza L.* was collected from the pond at Harani, Vadodara (Fig 1 and 2). They were allowed to acclimatize for 15 days. Plants were washed thoroughly under a running tap water and were grown and propagated for 4 weeks in quarter strength Hoagland's solution (Hoagland and Arnon, 1950). Plants of same size were selected for the experiment. In the pilot scale experiment, the test plants were exposed to wide range of the metal ion concentrations i.e.10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ppm. It was noticed that the plants were unable to survive in the concentration range between 20-100 ppm Zn ions.

In the subsequent experiments it was revealed that the concentration mortality (LC50) of Zinc sulphate on exposed plants was 9 ppm during 240 hrs. Therefore, the trace element under study $ZnSO_4$ (Zn) were supplied at 1, 3, 5, 7 and 9 ppm for 3, 6 and 9 days. Nutrient solution devoid of trace element served as a control. Both the control and the treated solutions were maintained at pH 5.5 using dilute HCl or NaOH. Experimental plants (in triplicates) were placed in nutrient solution. Solutions were replenished every 3 days to prevent depletion of metals and nutrients. After each experimental period, harvested plants were washed in running tap water and rinsed with deionized water. Extraction and estimation of CAT and APX was followed the method of Thimmaiah, (1999).

Fig 1: Map of Collection site

Enzyme Extraction and Assay:

Catalase

Plants (100 mg) from each treatment were collected at 3, 6 and 9 days intervals and homogenized in 0.1 M sodium phosphate buffer (pH 7.0) and centrifuged at 1,000 g for 10 min at 4°C. One ml of the supernatant was added to a reaction mixture containing 1 ml of 0.1 M H_2O_2 and 3 ml of 0.1 M sodium phosphate buffer (pH 7.0). The reaction was stopped by adding 10 ml of 2% H_2SO_4 after 1 min incubation at 20°C. The acidified reaction mixture with or without supernatant was titrated against 0.01 M $KMnO_4$ to determine the quantity of H_2O_2 utilised by the enzyme. The catalase activity was expressed in enzyme units as µmol H_2O_2 destroyed mg protein^{-1} min^{-1}.

Fig 2: Lemna polyrhhiza

Guaiacol Peroxidase

Plants (100 mg) from each treatment were collected at 2 week intervals and homogenized with mortar and pestle in cold 0.1 M phosphate buffer (pH 6.1). The homogenate was filtered and centrifuged at 12000 g for 10 min at 4°C. The supernatant was used for the peroxidase assay. The assay mixture contained 0.1 M phosphate buffer (pH 6.1), 4 mM guaiacol, 3 mM H_2O_2 and 0.4 ml of crude enzyme extract. The total reaction volume was 1.2 ml. The rate of change in absorbance (OD) at 420 nm was measured using a UV-Spectrophotometer (Jasco, UVIDEC-650, Japan). The levels of enzyme activity were expressed as µmol H_2O_2 destroyed mg protein-1 min-1.

Results and Discussion

In the current research it was investigated that the activities of both catalase and peroxidase were significantly higher in tolerant plants in comparison with the uncontaminated ones (Fig. 3 and Fig 4.) Greater activities of catalase and Guaicol peroxidase indicated that the tolerant plant were under oxidative stress, a feature often associated with metal tolerance (Van and Clisters, 1990) Nashikkar and Chakrabarti, (1994) reported that both higher activity of catalase and peroxidase in crops grown on heavy metal polluted soil.

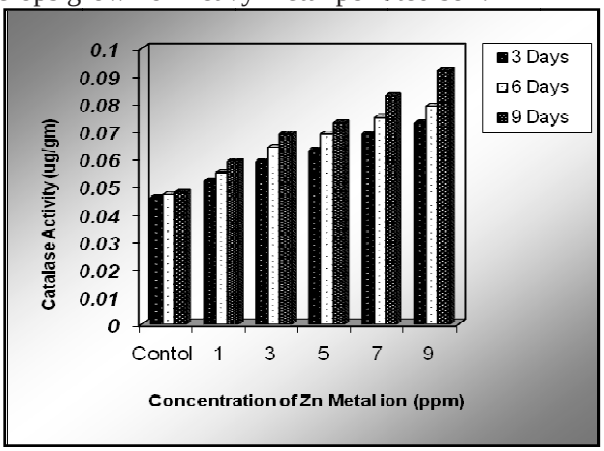

Fig.3:Catalase Activity

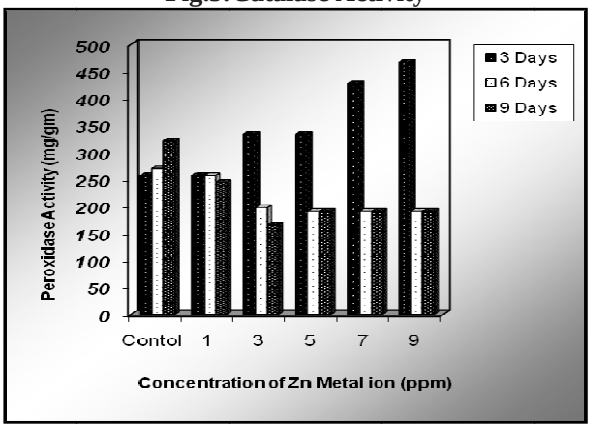

Fig: 4:Peroxidase activity

In the present investigation it was reported that activities of catalase enhanced linearly with increased metal ion concentration whereas the activity of guaicol peroxidase showed increase with metal ion concentration only at 3 day exposure period. Thereafter it decreased at 6 and 9 day exposure period. Heavy metals in soil, water and atmosphere, where plants are living are seen to demonstrate interactions between these heavy

metals and the plants. On the other hand, heavy metals show negative effects on plants by inhibiting growth, damaging the structure, affecting the physiological and biochemical activities and decreasing the functions of the plants. The effects and bioavailability of heavy metals depends on many factors including environmental conditions such as pH, species of heavy metals, and organic substances in the media as well as fertilization and the individual plant species. Plants have their own mechanisms of resistance against the negative effects of heavy metal by combining heavy metals with proteins and developing enzymes and nucleic acids to detoxify heavy metal pollution. Thus, the effects heavy metals on plants are revealed in several aspects and the plants show many kinds of resistance mechanisms. The changes in antioxidative enzyme activities in response to heavy metal stress are known to be dependent on heavy metal concentration (Shah *et al*.2001). Activities of these enzymes might increase in order to cope with the oxidative stress imposed by heavy metals on plants, as was repeatedly found in our experiments. Alternatively, they might be diminished if the toxic effects of higher concentration of heavy metals were greater than can be tolerated and combated by the antioxidant enzymes, as is the case in the present experiment, particularly catalase activity.

On the other hand, decreased activity of peroxidase in 6 and 9 days Zn treated plant indicated that the plants were under tremendous heavy metal stress. This might have resulted in the accumulation of ROS (reactive oxygen species). ROS products are reported to cause damage to the biomolecules by peroxidation, electrophilic substitution reaction, reduction of memberane lipids, proteins, chloroplast pigments, enzymes, nucleic acids etc. (Comba *et al*. 1998, Becana *et al*. 2000 and Majeed *et al*.2010). It is evident that each of Zn can separately cause significant reduction in most of the recorded structural parameters with detrimental physiological consequences. It might be concluded that exposure of *L.poyrhhiza L.* to toxic levels of Zn triggers a number of closely inter related s structural and functional events in the stressed plants.

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