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ORIGINAL ARTICLE

Zinc toxicity on antioxidative response in (Zea mays L.) at two different pH

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Zn is the second most abundant transition metal after iron (Fe). Excess Zn can have negative effects on plants. The effect of Zn at two different pH on lipid peroxidation (MDA), membrane permeability (EC), hydrogen peroxide (H_2O_2), non-protein thiols (NPT) and the activities of major antioxidant enzymes *Zea mays* were investigated under controlled growth conditions. Zn-excess conditions increased the EC, MDA, H_2O_2 content and non-protein thiols and also activities of antioxidant enzymes were increased. Also zinc toxicity was higher in 4.5 pH than 7.5 pH.

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Soil pollution by toxic metals is a serious problem for the environment and is also one of the environmental stresses for higher plants. Evidence has suggested that heavy metal toxicity is manifested as oxidative stress, which is caused by the stimulation of free oxygen radical production (Gao *et al*., 2011). Zinc is a heavy metal. It acts as a plant nutrient and plays an important role in many metabolic reactions but it is toxic at higher concentrations and causes growth inhibition (Vaillant *et al*., 2005) and induction of oxidative damage in various plant species (Panda *et al*., 2003). At the cellular level, excess Zn can significantly alter mitotic activity (Rout and Das, 2003), affect membrane integrity and permeability (Stoyanova and Doncheva, 2002), and even kill cells (Chang *et al*., 2005). Various studies indicated that the availability of elements for leaching and plant uptake depend on their solubility in the soil solution (Gao *et al*., 2010). As a result of the strong influence of pH on metal solubility (McBride *et al*., 1997), anthropogenic processes which result in the lowering of substrate pH can cause metal toxicities, even if no extra metal has been added to the system (Robinson *et al*., 1995). The goals of the recent study were to investigate the effect of zinc toxicity on antioxidative response and role of pH in toxicity on *Zea mays* plants.

MATERIALS AND METHODS

Seeds were surface sterilized in 5% sodium hypochlorite solution for 10 minutes before use, to avoid fungal contamination. Seeds were then washed thoroughly with deionized, imbibed overnight in distilled water and germinated on moistened filter paper in trays for 5 days in darkness at 25°C. After 5 days, uniformly germinated seedlings were transferred to plastic cups. The plants were irrigated over a period 10 days with nutrient solution containing zinc between 0, 400 and 600 µM as sulfate ($ZnSO_4$) and two pH 4.5 and 7.5. After 12 days of Zn treatment, 9 plants from each group (control, Zn 400-pH4.5, Zn 400-pH7.5, Zn 600-pH4.5, Zn 600-pH7.5) were taken and divided into shoot and root fractions and analyzed.

Concentration of H_2O_2 was determined following Jana and Choudhuri (1981). Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) content according Heath and Packer (1968). Ascorbate peroxidase activity was measured according to Nakano and Asada (1981) by monitoring the rate of ascorbate oxidation at 290 nm. Catalase activity was assayed according to Aebi (1983). Guaiacol peroxidase activity was measured according to Upadhyaya *et al* (1985). GR Activity was assayed according to Foyer (1976). Percentage electrolyte leakage was assayed according to Lutts *et al* (1996). Non-protein thiols were measured with Edreva et al (1984).

Statistical analysis

Results were based on three replicates and statistical analysis was performed using ANOVA (for $P < 0.05$). Based on the ANOVA results, a Tukey test for mean comparison was performed, for a 95% confidential level, to test for significant differences among treatments.

RESULTS

In the *Zea mays*, lipid peroxidation, which is measured as Malondialdehyde (MDA) content, increased at all Zn treatments than control and maximum increase was observed at 600 µM (pH 4.5) (Fig. 1, A and B). H_2O_2 content increased at all Zn treatments than those in the control while the maximum increase was observed at 600 µM (pH 7.5). But no significant difference was observed between treatments and control (Fig. 1, C and D). Effect of zinc on APX and GR activities is shown in Fig. 2. APX and GR activity increased in all Zn treatments in both shoot and root compared with control. The highest value was occurred in the 600 µM (pH 4.5) Zn treatment. APX and GR activity were higher in 600 µM than 400 µM concentration of zinc. But no significant difference was abserved between pH 7.5 and pH 4.5 in APX activity while GR activity significantly was higher at pH 4.5 than pH 7.5 in both 400 µM and 600 µM concentration. CAT and GPX activity increased in all Zn treatments in both shoot and root compared with control (Fig. 3) and maximum increase was observed at 600 µM (pH 4.5) treatment of zinc. CAT activity significantly was higher at pH 4.5 than pH 7.5 in shoot and there was significant difference between pH 7.5 and pH 4.5 of 600 µM concentration in root. GPX activity significantly was higher at pH 4.5 than pH7.5 in 600 µM concentration in root. But no significant difference was abserved between pH 7.5 and pH 4.5 in shoot.

Non-protein thiol compounds are involved in heavy metal detoxification. Non-protein thiols increased in all Zn treatments than those in the control and this increase was higher in 600 µM than 400 µM of zinc (Fig. 4). Also Non-protein thiol compounds were significantly higher in pH 4.5 than pH 7.5 in shoot. Electrolyte leakage (Fig. 4, C)

reflected the plasmalemma damage caused by the Zn. Electrolyte leakage percentage in all Zn treatments increased as compared to the control. But no significant difference was observed between Zn 400 µM and Zn 600 µM also between pH 7.5 and pH 4.5.

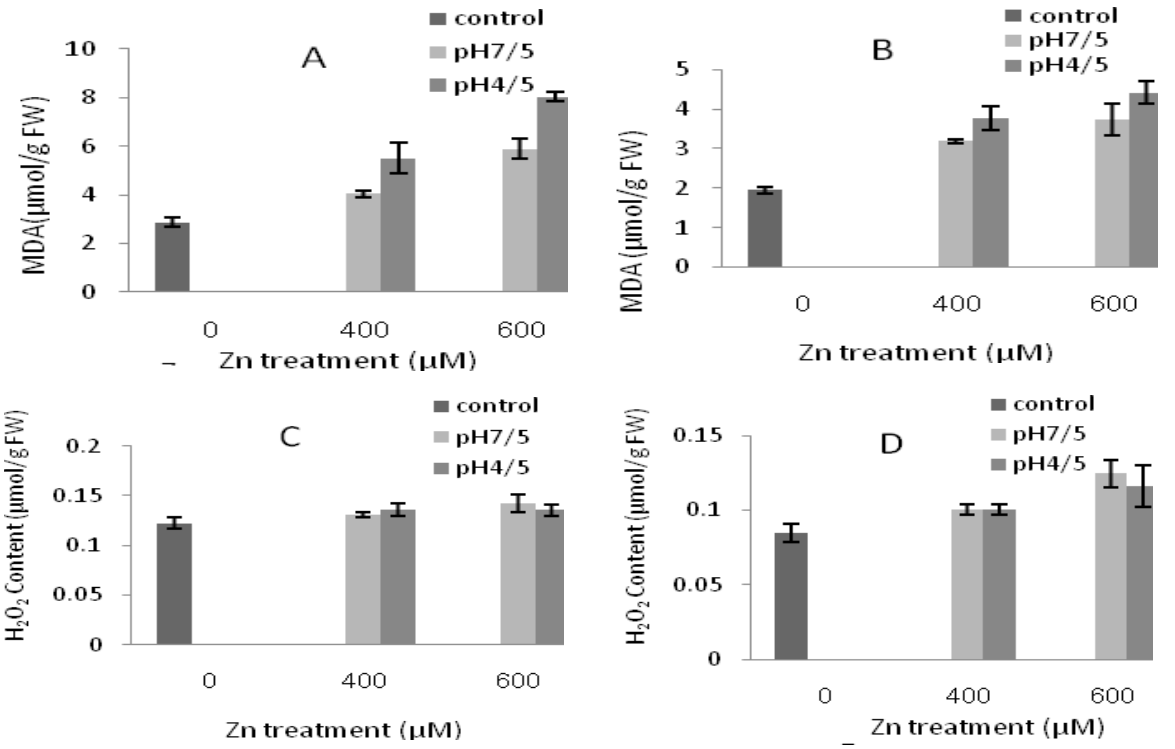

Figure 1. The effects of Zinc on Malondialdehyde content in shoot (A) and root (B) and H_2O_2 content in shoot (C) and root (D) of *Zea mays.* Values are means of three replicates ± S.E.

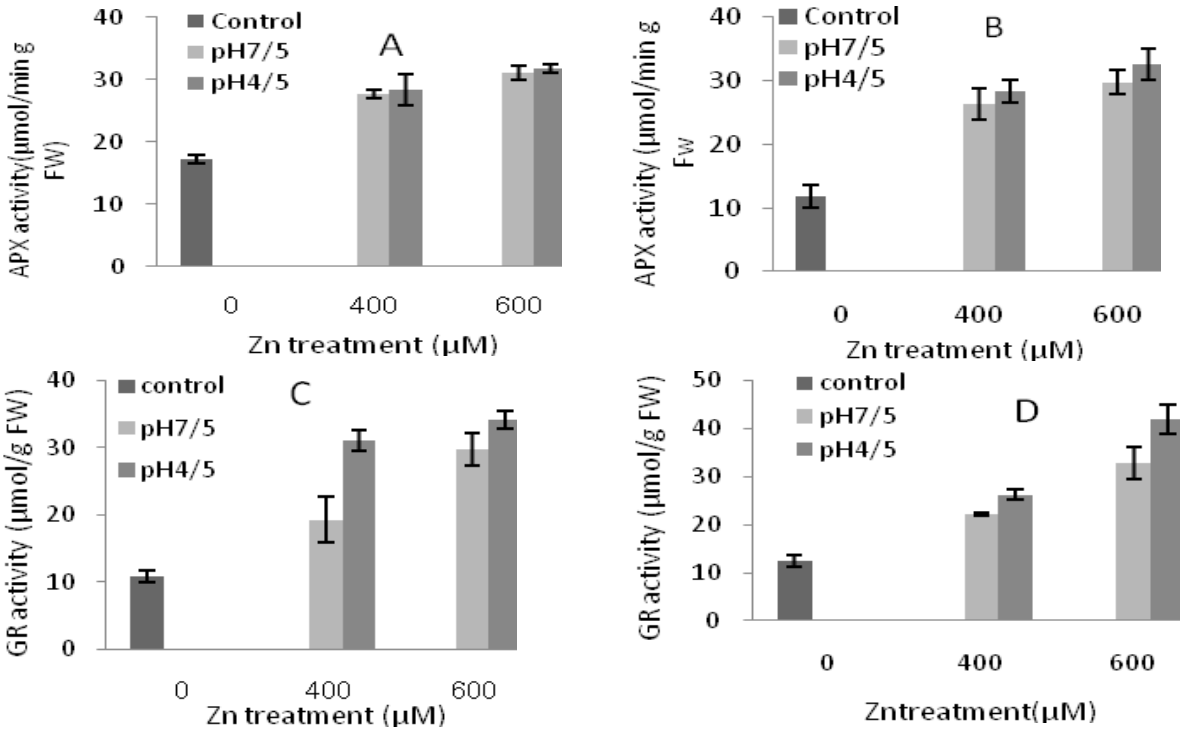

Figure 2. The effects of Zinc on APX activities in shoot (A) and root (B) and GR activities in shoot (C) and root (D) of *Zea mays.* Values are means of three replicates ± S.E.

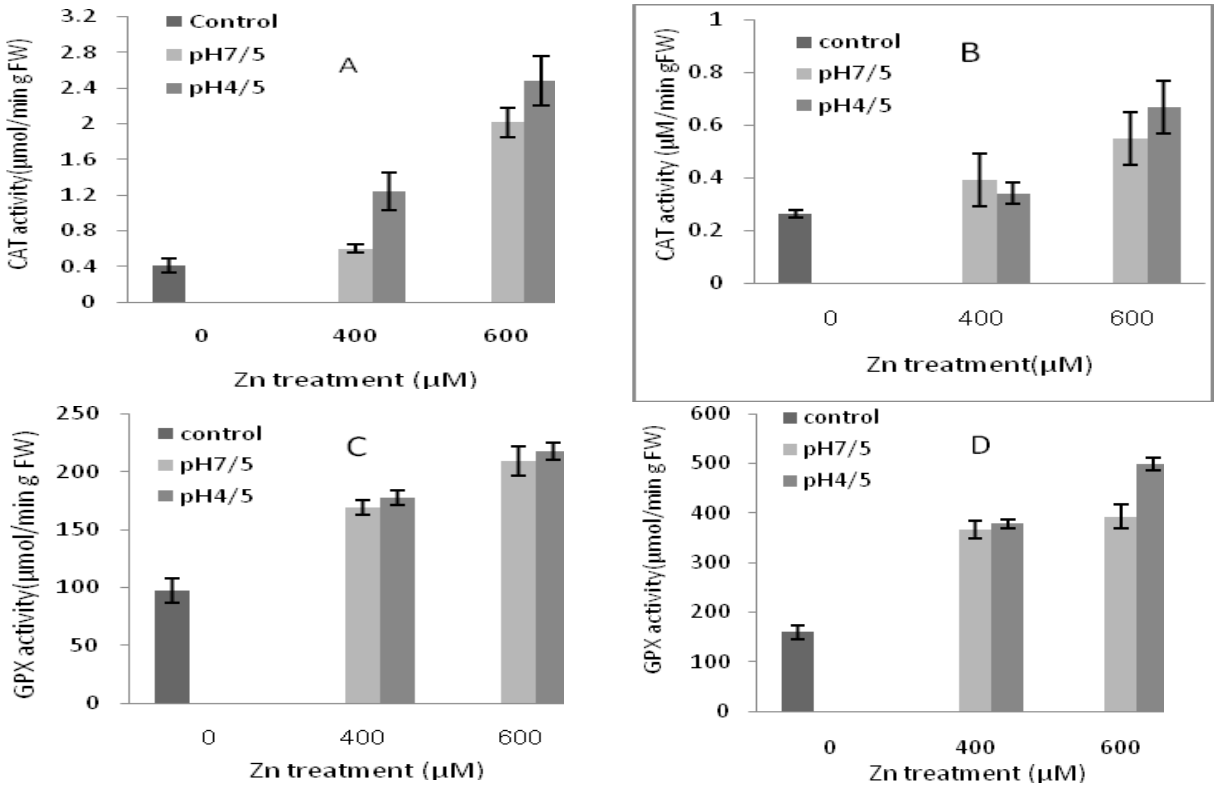

Figure 3. The effects of Zinc on CAT activities in shoot (A) and root (B) and GPX activities in shoot (C) and root (D) of *Zea mays.* Values are means of three replicates ± S.E.

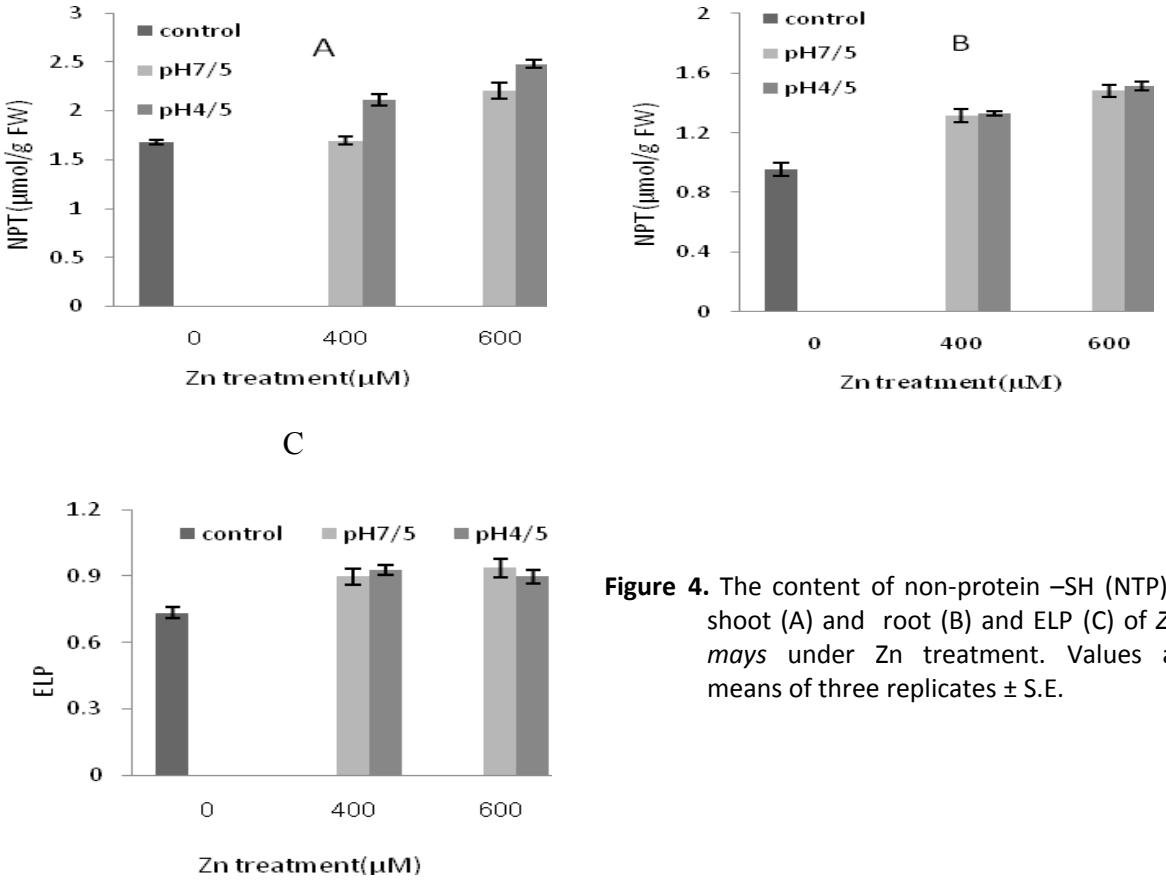

Figure 4. The content of non-protein –SH (NTP) in shoot (A) and root (B) and ELP (C) of *Zea mays* under Zn treatment. Values are means of three replicates ± S.E.

DISCUSSION

Heavy metal accumulation in plant lead to some physiological and biochemical changes. Production of free radicals in plants under heavy metal stress has been documented (McGeer *et al*., 2000). Like other metals zinc also induces free radical generation in plants (Rout and Das, 2003). During present study zinc stimulated MDA production in *Zea mays*. As a product of membrane lipid peroxidation, MDA is used to assess the extent of oxidative stress in plants, and its level is increased under stress conditions (Liu *et al*., 2009). In plants, heavy metals induce oxidative stress by generation of superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^\bullet$), and singlet oxygen ($^1O2$), collectively termed ROS (Jain *et al*., 2010). These ROS are highly reactive and cause the death of plants by damaging membrane lipids, proteins, pigments and nucleic acids. To cope up with the damages caused by the ROS, cells possess their own comprehensive and integrated endogenous antioxidant defense system composed of both enzymatic as well as non-enzymatic components (Miller et al., 2008). Similar to our results an increase in MDA and H_2O_2 content under excess zinc has been reported in sugarcane (Jain et al., 2010). In the present study increased electrolyte leakage under zinc stress. Generation of free radicals and increased lipid peroxidation under zinc stress may have resulted in an increase in membrane permeability and loss of membrane integrity (Rout and Das, 2003). Jain *et al*. (2010) reported that elevated levels of H_2O_2 and $O2^{\bullet-}$ caused by stress facilitate the formation of highly active hydroxyl radicals ($OH^\bullet$). These hydroxyl radicals are generally considered to be the most likely ROS to initiate the peroxidation destruction of lipids that lead to membrane damage. Non-protein thiols, including glutathione, thiol-rich peptides and other SH compounds, fulfill an important role in the detoxification of heavy metals in plants (Tiryakioglu *et al*., 2006). In our study, an increase in the level of non-protein thiols observed with the increase Zn. Similarly, a marked increase was seen in non-protein thiols as a response to different metal stress in a number of plants (Tiryakioglu *et al.*, 2006). The induction of certain enzymes that detoxify ROS is considered to play an important role in the defense against oxidative stress caused by toxic metal concentrations (Miller *et al*., 2008). In plants, a number of enzymes regulate H_2O_2 intracellular levels, but CAT, APX and GPX are considered the most important (Liu *et al*., 2009). These enzymes work independently in different parts of the plants to break up H_2O_2. The results of the present study show that the plants treated with Zn had high GPX (Fig. 3), CAT (Fig. 3), APX (Fig. 2) and GR activity (Fig. 2), indicating that there was efficient ROS scavenging activity in the system. Previous studies have also shown that induction of GPX activity has been reported in many plant species, when exposed to zinc stress (McGeer *et al*., 2000).

The results also showed that at the low pH zinc toxicity higher than that at high pH in *Zea mays* because MDA content, electrolyte leakage, non-protein thiol and antioxidant enzymes activity was higher at pH 4.5 in comparison with that at pH 7.5. High toxicity in low pH might be the resulting in enhance zinc bioavailability in *Zea mays*. Lowering pH increased easily available Zn concentrations and enhanced metal uptake. Zn deficiency is a common problem in plants grown in high pH, calcareous soils (Cakmak *et al*. 1996), whereas in low pH soils Zn availability is generally high (Chaney, 1993). Autumn *et al*. (2006), reported that lowering pH is

an effective method to enhance metal bioavailability and *Thlaspi caerulescens* uptake for both Cd and Zn. The most obvious method for reducing plant Zn toxicity is to raise soil pH. Havlin and co-authors (2005) reported that available Zn and Mn concentrations decreased with raising the soil pH.

In conclusion, our results have demonstrated Zn phytotoxicity in *Zea mays*. Higher H_2O_2, MDA contents and increased activity of antioxidant enzymes in *Zea mays* indicating oxidative damage by zinc toxicity. Also at the low pH zinc toxicity was higher in *Zea mays.*

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