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Research Article

EFFECT OF ZINC (ZN) ON SOME PHYSIOLOGICAL CHARACTERISTICS OF RICE SEEDLING

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ABSTRACT
Hydroponically studies were conducted in rice seedlings (*Oryza sativa* L., cv. Tarom Hashemi) under different Zn levels. The nutrient solution treatments were prepared of three 4 zinc levels (0.5, 5,50 and 100 µM $ZnSO_4$). Zn decreased Zn-TF, increased Zn accumulation in roots. Addition of Zn from 0.5 to 5 µM in nutrient solution increased shoot length, plant dry weight, superoxide dismutase (SOD) and decreased ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) while addition of Zn from 5 to 100 µM had the reverse effects.

Key Words: *Zinc, Accumulation, Growth, Oryza, Sativa, Antioxidant, Enzymes*

INTRODUCTION
Zinc (Zn) is essential for normal plant growth and development since it is constituents of many enzymes and other proteins. Zinc deficient soils are common in various climatic regions world-wide and Zn deficiency is one of the most important nutritional problems after macronutrients deficiency in crop production. Zn deficiency is now considered the most widespread nutrient disorder in lowland rice (Quijano-Guerta *et al.,* 2002). On the other hand, elevated concentrations of Zn can lead to toxicity symptoms and growth inhibition in rice (Song *et al.,* 2011). Essential and nonessential heavy metals stresses disrupt the redox homeostasis of cells and cause rapid formation of reactive oxygen species (ROS). The ROS cause oxidative damage to vital biomolecules such as lipids, proteins and nucleic acids, which is detrimental to plant growth and development (Blokhina *et al.,* 2003; Gao *et al.,* 2008; Shah *et al.,* 2001; Sharma and Dubey 2007). To scavenge ROS and to avoid oxidative damage, antioxidant plant enzymes such as superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GPX) control the cellular concentrations of $O_2^{\cdot-}$ and H_2O_2 (Gratão *et al.,* 2005; Noctor and Foyer 1998; Sharma and Dubey 2007). This study aims to investigate in the rice seedlings (*Oryza sativa* L., cv. Tarom Hashemi) grown hydroponically under different Zn levels: 1) growth responses; 2) changes of Zn accumulation in different parts of the rice; 3) uptake and distribution patterns of zinc in different parts of the rice; 4) induction of antioxidant enzymes activity.

MATERIALS AND METHODS
Plants Culture and Treatments
Seeds of rice (*Oryza sativa* L. cv. Tarom Hashemi) were provided by Iranian Rice Research Institute, Guilan Province, Iran. Seeds were germinated in darkness on filter paper. Germinated seeds were then transferred onto nylon net and pre-cultured for 2 days on 0.02 mM $CaSO_4$, 3 days on 25% and 3 days on 50% nutrient solution (Yoshida *et al.,* 1976). 13-day-old seedlings with similar sizes were selected and transferred to 2.5 L plastic pots with 5 bundles consisting of 3 plants per bundle. The plants were cultivated in a growth chamber. The temperature regime was 30-25°C day-night, with 14-10 hours light-dark period, and relative humidity of 80-90%, under photon flux density of 350 µmol m^{-2} s^{-1}. The nutrient solutions were not aerated and replaced every 5 days.The nutrient solution had the following composition

(mM): NH_4NO_3 1.5, $CaCl_2$ 1.00, $MgSO_4$ 1.60, K_2SO_4 1.00, KH_2PO_4 0.30 and (µM):H_3BO_3 2.0, $MnSO_4$ 5.0, $ZnSO_4$ 0.5, $CuSO_4$ 0.2 and $(NH_4)6Mo_7O_{24}$ 0.05. Iron was supplied as FeEDTA at 0.1 Mm. The concentration of Zn at 5, 50 and 100 µM was supplied by addition of $ZnSO_4$ at the defined concentrations to the nutrient solution. The pH of nutrient solution was 6.0 and was adjusted every day.

Study of Growth Parameters

After two weeks of treatment, the seedlings were harvested. The seedlings roots were washed in deionized water, and blotted using rough filter paper, then the seedlings were divided into shoots and roots whose fresh weights were determined immediately. Plant shoots and roots were, then, in the oven dried at 70°C for 48 h, and their dry weights were determined. Another group of plants was used for determination of root length(Tennant 1975).

Estimation of Zn Accumulation in Roots and Shoots

For determining Zn, the dried materials were ashed at 550°C for 20 h. The ash residue was incubated with 31% HNO_3 and 17.5 % (v/v) H_2O_2 at 72°C for 2 h, and dissolved in 0.1 N HCl. Cd was then quantified using an atomic absorption spectrophotometer (Model AA-6800; Shimadzu, Kyoto, Japan). The amount of Zn was expressed on the basis of dry weight (DW). Zn Translocation Factor was calculated with the following formula (Ait Ali *et al.*, 2002):

Zn Translocation Factor = (Zn content of shoot / Zn content of root) *100

Extraction and Assay of Antioxidative Enzymes

Fresh weight of root or shoot (0.2 g) was homogenized at 4°C in 5 ml extraction buffer (0.1 M phosphate buffer pH 6.8) with mortar and pestle. The homogenate was then centrifuged at 15,000g for 20 min and the homogenate was used as the crude extract for the superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) assay.SOD (EC 1.15.1.1) activity was assayed according to the method of Giannopolitis and Ries (1977). The reaction mixture contained 100 µL 1 µM riboflavin, 100 µL 12 mM L-methionine, 100 µL 0.1 mM EDTA (pH 7.8), 100 µL 50 mM Na2CO3 (pH 10.2), and 100 µL 75 µM nitroblue tetrazolium (NBT) in 2,300 µL 25 mM sodium phosphate buffer (pH 6.8), with 200 µL crude enzyme extract in a final volume of 3 mL. SOD activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of NBT. Glass test tubes containing the mixture were illuminated with a fluorescent lamp (120 W); identical tubes that were not illuminated served as blanks. After illumination for 15 min, the absorbance was measured at 560 nm. One unit of SOD was defined as the amount of enzyme activity that was able to inhibit by 50% the photoreduction of NBT to blue formazan. The SOD activity of the extract was expressed as SOD unit min^{-1} mg^{-1} protein.CAT (EC 1.16.1.6) was extracted similarly to SOD except that 50 mM Tris-NaOH buffer was used and was assayed according to the method of Aebi (1984). The rate of H2O2 decomposition was measured at 240 nm (extinction coefficient of 0.036 $mM^{-1}cm^{-1}$) and enzyme specific activity was expressed as µmol H2O2 oxidized min^{-1} mg^{-1} protein.

APX (EC 1.11.1.11) activity was assayed as described by Jimenez *et al.*, (1997) by monitoring the oxidation of ascorbate at 240 nm for 2 min at 25°C (ε = 2.8 mM^{-1} cm^{-1}). The reaction mixture (1 ml) consisted of 50 mM HEPES-NaOH buffer (pH 7.6) containing 0.2 mM ascorbate, 0.3 mM H_2O_2, and 100 µl of supernatant. The reaction was started by the addition of H_2O_2. Corrections were made for the nonenzymatic oxidation of ascorbate by H_2O_2 and for the oxidation of ascorbate in the absence of H_2O_2. APX was measured in the presence and absence of the specific inhibitor 4-chloromercuribenzoic acid (0.5 mM). One unit of APX was defined as the amount of enzyme required to oxidize 1 nmol (ascorbate) min^{-1}. GPX (EC 1.11.1.7) activity was determined according to Quessada and Macheix (1984)by monitoring the increase in absorbance at 470 nm and 25°C for 2 min (ε = 26.6 mM^{-1} cm^{-1}). The reaction mixture (1 ml) consisted of 50 mM potassium phosphate buffer (pH 6.1), 6.25 mM guaiacol, and 25 µM H2O2. The reaction was started by the addition of 10 µl of the enzyme extract. One unit of GPX was defined as the amount of enzyme required to decompose 1 µmol (H2O2) min^{-1}.

Statistical Analysis

Experiments were carried out under a completely randomized design in a 3×3 factorial array with three replications. The factors were 4 levels of Zn. The analysis of variance (ANOVA) was performed using the SPSS 16.0 software, and the means were compared by Duncan's test.

RESULTS

Plant Growth

Plant growth response to Zn Levels was obviously different (Figure 1). The highest root length (312 cm), shoot length (52.7 cm) and plant dry weight (234.3 mg plant^{-1}) were observed at the Zn level of 100 µM. The lowest root length (243 cm), shoot length (36.7 cm) and plant dry weight (94.5 mg plant^{-1}) were observed at the Zn level of 100 µM.

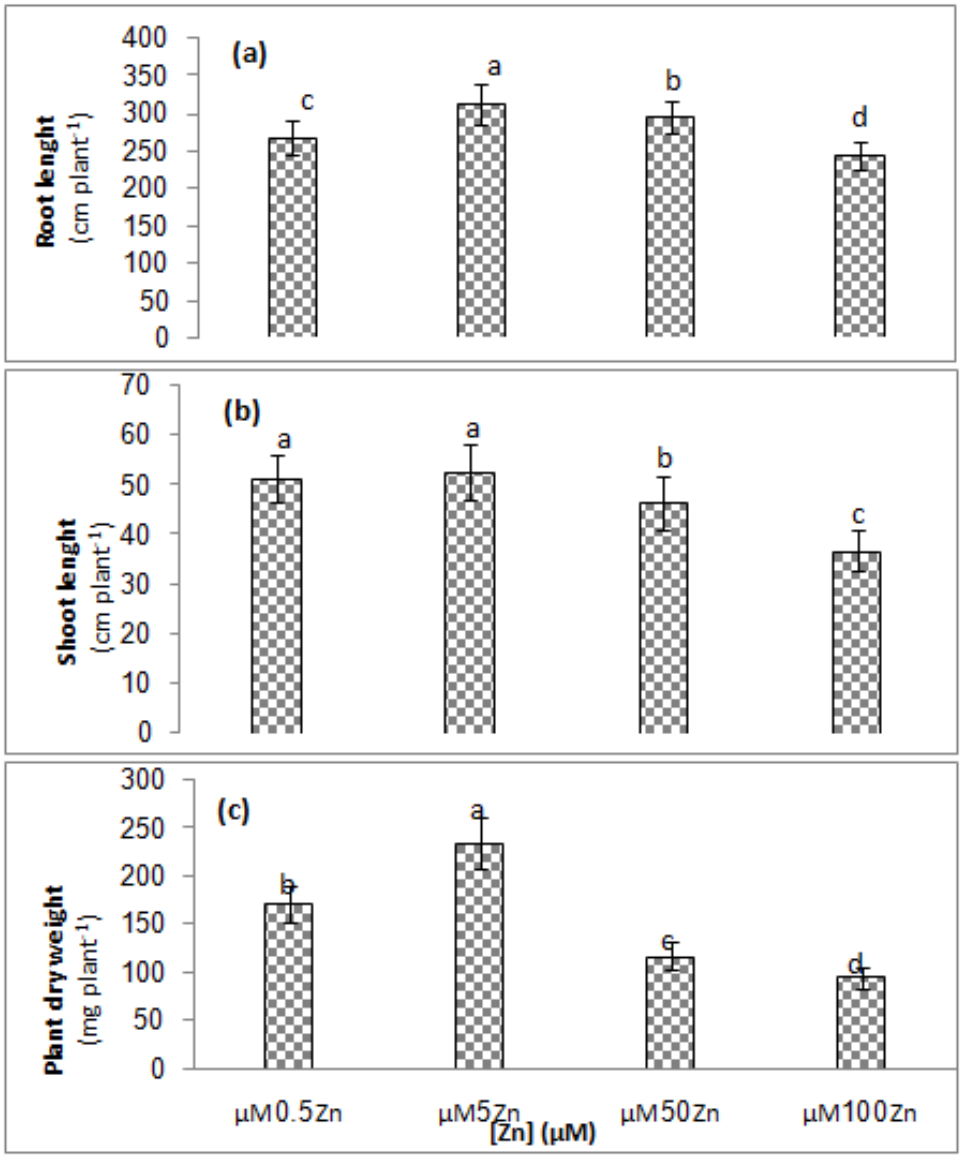

Figure 1: Changes in growth responses; root length (a) shoot length **(b)** plant dry weight **(c)** in rice subjected to different Zn levels in nutrient solution. All data are means ± SE of three replications. Treatments with the same lower-case letters were not significantly different based on mean comparison by Duncan's test at $p< 0.05$

Zn Accumulation in Roots and Shoots

Table 1 shows the effects Zn on the Zn accumulation in the roots and shoots and Zn Translocation Factor. The Zn accumulation in roots significantly increased with increasing Zn supply levels. This increase was 117%. Increasing Zn from 0.5 to 5 µM significantly increased Zn accumulation in shoots while that increasing Zn from 5 to 100 µM decreased Zn accumulation. The maximum Zn accumulation was recorded in roots (12.3µg Plant^{-1}) at the Zn level of 100 µM. The minimum Zn accumulation was recorded in roots (5.67 µg Plant^{-1}) at the Zn level of 0.5 µM. The highest Zn accumulation was observed in shoots (28.46 µg Plant^{-1}) at the Zn complex level of 5 µM. The lowest Zn accumulation was observed in shoots (7.1 µg Plant^{-1}) at the Zn complex level of 100 µM.. Zn Translocation Factor significantly decreased with increasing Zn supply levels.

Table 1: Effects of Zn on the Zn accumulation (µg Plant^{-1}) in the roots and shoots and Zn Translocation Factor in rice (*Oryza sativa* L.).

Zn levels (µM)	Root Zn Accumulation	Shoot Zn Accumulation	Zn Translocation Factor
0.5	5.67±0.92^{c}	19.10± 2.75^{b}	336.9±24^{a}
005	9.88±1.21^{b}	28.46 ±3.47^{a}	288.0±36^{b}
050	9.64±1.43^{b}	8.95±1.02^{c}	92.9±12^{c}
100	12.3±1.21^{a}	7.10±2.40^{d}	58.0±16^{d}

The data are expressed as means ± SE (n = 3). The means marked with the different letter in the same column are significantly (P < 0.05) different. ns: non- significant; ∗∗significant at p < 0.01; ∗significant at p < 0.05.

The accumulation percent of Zn in root and shoot component of plants is shown in Figure 2. The accumulation percent of Zn was highest in root followed by shoot at 50 and 100 µM Zn supply level.

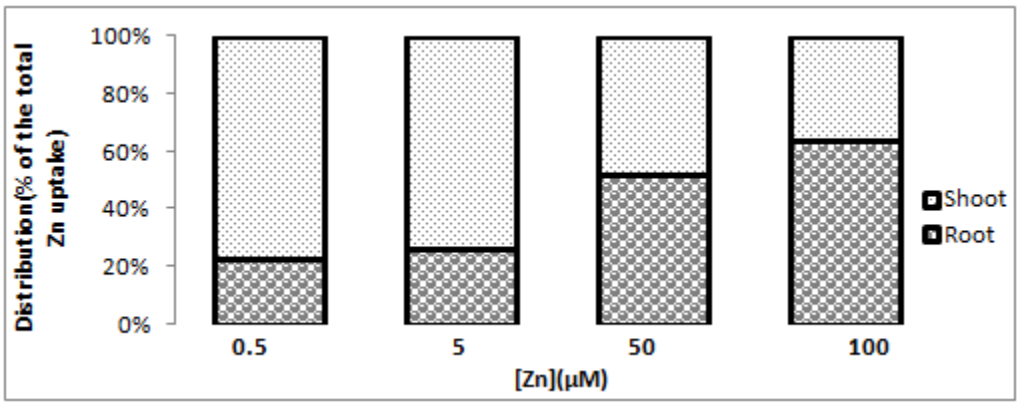

Figure 2: The accumulation percent of Zn in different parts of rice (*Oryza sativa* L.) treated with different Zn levels (µM).

Antioxidative enzymes

Figure 3 shows the effects of Zn on the antioxidant enzymes activities in shoots.

Treatments with the same lower-case letters were not significantly different based on mean comparison by Duncan's test at p<0.05. Increasing Zn from 0.5 to 5 µM significantly increased SOD activity while that increasing Zn from 5 to 100 µM decreased SOD activity. The maximum SOD activity was recorded (86 units mg^{-1} protein) at the Zn level of 5 µM.

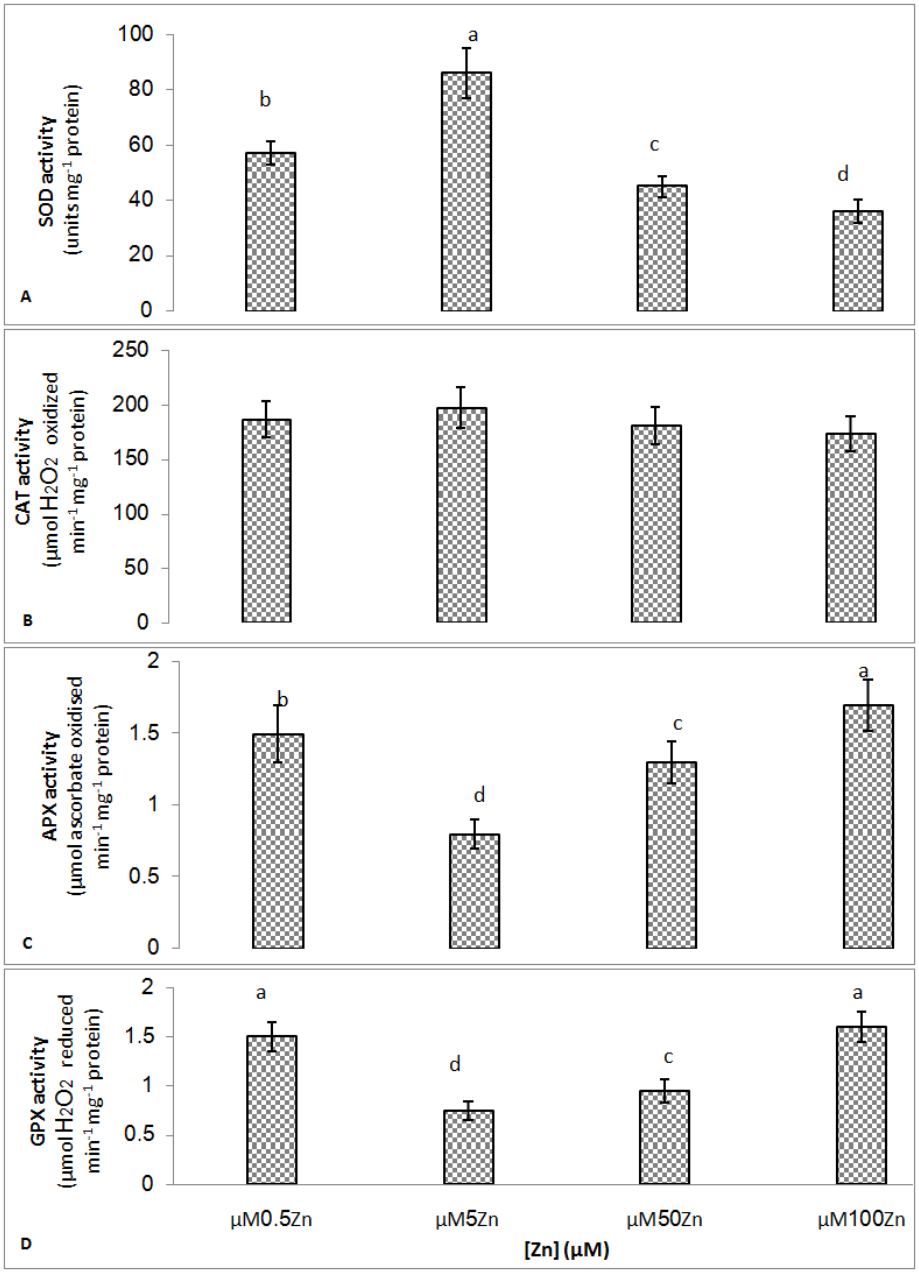

Figure 3: Changes in antioxidant enzymes activities; (A) SOD **(B)** CAT **(C)** APX and **(D)** GPX in rice shoots subjected to different Zn levels in nutrient solution. All data are means ± SE of three replications. The minimum SOD activity was recorded (36 units mg^{-1} protein) at the Zn level of 100 µM.As shown in Figure 3B, the addition of Zn to nutrient solution was not significant on the CAT activity. The maximum CAT activity was recorded (198 µmol H_2O_2 oxidized min^{-1} mg^{-1} protein) at the Zn level of 5 µM. The minimum CAT activity was recorded (174 µmol H_2O_2 oxidized min^{-1} mg^{-1} protein) at the Zn level of 100 µM. Increasing Zn from 0.5 to 5 µM significantly decreased APX (Figure 3C) and GPX (Figure 3D) activities while that increasing Zn from 5 to 100 µM increased APX and GPX activities. The maximum APX and GPX activities were recorded (1.7 µmol ascorbate oxidized min^{-1} mg^{-1} protein and 1.6 µmol H_2O_2 reduced min^{-1} mg^{-1} protein) at the Zn level of 100 µM. The minimum APX and GPX activities were recorded (0.8 µmol ascorbate oxidized min^{-1} mg^{-1} protein and 0.75 µmol H_2O_2 reduced min^{-1} mg^{-1} protein) at the Zn level of 5 µM.

DISCUSSION

Addition of Zn from 0.5 to 5 µM in nutrient solution increased shoot length and plant dry weight while that increasing Zn from 5 to 100 µM decreased them (Figure 1). The element Zn is essential for plant growth (Prasad 1995). High rates level of Zn showed severe phytotoxic effects on rice and significantly inhibited its growth. High level of Zn is likely to destroy the metabolic balance in plants to result in disorder of other mineral nutrients states. Zn at higher concentrations has retarded the growth and development of plants by interfering with certain important metabolic processes (Alia 1995; Cherif *et al.*, 2011; Ebbs and Kochian 1998). These results suggest that 0.5, 5 and ≥50 µM Zn supply levels are deficiency, adequate and toxic levels for this cultivar, respectively. Addition of Zn to nutrient solution increased Zn accumulations in roots and shoot. Increasing Zn from 0.5 to 5 µM increased Zn accumulation and then its increase from 5 to 100 µM significantly decreased Zn accumulations in shoots. The decrease of shoots Zn accumulation in high level of Zn is due to severe phytotoxic effects on rice. The high level of Zn is likely to destroy the metabolic balance in plants, to result in disorder of other mineral nutrients states(Table 1).With increasing Zn levels decreased Zn Translocation Factor (Table 1). These results indicate that the rice can considerably increase Zn accumulation in roots with addition of Zn to nutrient solution and inhibited of their transport to shoots.However, the highest amount of the Zn was accumulated in the shoots of rice, but, low Zn Translocation Factor in 50 and 100 µM of Zn levels which also had toxic effects on growth parameters, show that one of the defense mechanism of rice against heavy metal stress can be their accumulation in the roots.However, addition of Zn to nutrient solution from 0.5 to 5 µM significantly increased SOD activity and significantly decreased APX, GPX activities in shoots, but increasing Zn from 5 to 100 µM significantly decreased SOD activity and significantly increased APX, GPX activities in shoots (Figure 3). Addition of Zn to nutrient solution was not significant on the CAT activity in shoots. Zn is able to contribute in the structure of Cu/Zn SOD isozyme, therefore its addition to nutrient solution up to adequate level (5 µM Zn) increased SOD activity. Similar to our observations was reported before (Aravind and Prasad 2005; Cherif *et al.*, 2011; Hajiboland and Beiramzadeh 2008; Tavallali *et al.*, 2010). The excess of Zn decreased SOD activity that may be due to the Zn toxicity in high level (Candan and Tarhan 2003; Song *et al.*, 2011). Activity of SOD was suggested to be an indicator of Zn nutritional status of plants (Cakmak I *et al.*, 1997). Activity of two H_2O_2 scavenging enzymes, APX and GPX was induced by low Zn supply. Induction of APX and particularly GPX under the effect of deficiency of macro (Tewari *et al.*, 2007) or micronutrients (Candan and Tarhan 2003; Hajiboland and Beiramzadeh 2008; Hassan *et al.*, 2005) was frequently reported. The least level of APX and GPX activity in the 5 µM Zn may be due to optimal growth conditions. There is rather a negative relationship between APX and GPX activity and plants growth, e.g. higher growth was accompanied by lower activity of APX and GPX. Similar to our results about CAT activity was also reported in rice (Hajiboland and Beiramzadeh 2008).The present results indicate that Zn significantly decreased APX and GPX activities in shoots. It implies that, APX and GPX activities only monitored stress conditions without any protecting role. On the other hand, the functional significance of APX and GPX in the protection of plants against oxidative stresses has been questioned by many authors(Chaoui A *et al.*, 1997; Cuypers A *et al.*, 2000; Hajiboland and Beiramzadeh 2008; Van Assche and Clijsters 1990).

CONCLUSION

The results showed that optimal growth was found when the plant grew at the Zn level of 5 µM. The distributions of Zn in different plant parts decreased in the order: shoot> root for Zn. Results suggest that increasing Zn to nutrient solution up to adequate level (5 µM Zn) by improving growth increased SOD activity, and within this condition decreased APX and GPX activities in shoots.

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REFERENCES

Aebi H (1984). Catalase in vitro. *Methods Enzymol* **52** 121-126.
Ait Ali N, Bernal MP and Ater M (2002). Tolerance and bioaccumulation of copper in andlt; iandgt; Phragmites australis and andlt; iandgt; Zea maysandlt; /iandgt. *Plant and Soil* **239** 103-111.
Alia KV, Prasad SK and Pardha Saradhi P (1995). Effect of zinc on free radicals and proline in Brassica juncea and Cajanus cajans. *Phytochemstry* 39: 45-47.
Aravind P and Prasad MNV (2005). Cadmium-Zinc interactions in a hydroponic system using ceratophyllum demersum L: adaptive ecophysiology, biochemistry and molecular toxicology. *Brazilian Journal of Plant Physiology* **17** 3-20.
Blokhina O, Virolainen E and Fagerstedt KV (2003). Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Annals of Botany* **91** 179-194.
Cakmak I, Öztürk L, Eker S, Torun B and Kalfa HI AY (1997). Concentration of zinc and activity of copper/zinc superoxide dismutase in leaves of rye and wheat cultivars differing in sensitivity to zinc deficiency. *Journal of Plant Physiology* **151** 91-95.
Candan N and Tarhan L (2003). The correlation between antioxidant enzyme activities and lipid peroxidation levels in Mentha pulegium organs grown in Ca2+, Mg2+, Cu2+, Zn2+ and Mn2+ stress conditions. *Plant Science : An International Journal of Experimental Plant Biology* **165** 769-776.
Chaoui A, Mazhoudi S and Ghorbal MH (1997). Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (Phaseoulus vulgaris L). *Plant Science: An International Journal of Experimental Plant Biology* **127** 139-147.
Cherif J, Mediouni C, Ammar WB and Jemal F (2011). Interactions of zinc and cadmium toxicity in their effects on growth and in antioxidative systems in tomato plants (Solarium lycopersicum). *Journal of Environmental Sciences* **23** 837-844.
Cuypers A and Vangronsveld JHC (2000). Biphasic effect of copper on the ascorbate-glutathione pathway in primary leaves of Phaseolus vulgaris L. seedlings during the early stages of metal assimilation. *Physiology Plant* **110** 512-517.
Ebbs SD and Kochian ALV (1998). Phytoextraction of zinc by oat (Avena sativa), barley (Hordeum vulgare), and Indian mustard (Brassica juncea). *Environmental Science and Technology* **32** 802-806.
Gao JG, Xiao Q, Ding LP, Chen MJ, Yin L, Li JZ, Zhou SY and GY H (2008). Differential responses of lipid peroxidation and antioxidants in Alternanthera philoxeroids and *Oryza sativa* subjected to drought stress. *Plant Growth Regulation* **56** 89-95.
Giannopolitis CN and Ries SK (1977). Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiology* **59** 309-314.
Gratão PL, Polle A, Lea PJ and Azevedo R (2005). Making the life of heavy metal stressed plants a little easier. *Functional Plant Biology* **132** 481-494.
Hajiboland R and Beiramzadeh N (2008). Growth, gas exchange and function of antioxidant defense system in two contrasting rice genotypes under Zn and Fe deficiency and hypoxia. *Acta Biological Szegediensis* **52** 283-294.
Hassan MJ, Zhang G, Wu F, Wei K and Chen Z (2005). Zinc alleviates growth inhibition and oxidative stress caused by cadmium in rice. *Journal of Plant Nutrition and Soil Science* **168** 255-261.
Jiménez A HJ, del Río LA and Sevilla F (1997). Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. *Plant Physiology* **114** 275-284.
Noctor G and Foyer CH (1998). ASCORBATE AND GLUTATHIONE: Keeping Active Oxygen Under Control. *Annual Review of Plant Physiology and Plant Molecular Biology* **49** 249-279.
Prasad AS (1995). Zinc: an overview. *Nutrition* **11** 93-99.
Quessada MP (1984). Caractérisation d'une peroxidase impliquée spécifiquement dans la lignification en relation avec l'incompatibilité au greffage chez l'abricotier. *Physiologie Végétale* **22** 533-540.

Quijano-Guerta C, Kirk GJD, Portugal AM, Bartolome VI and McLaren GC (2002). Tolerance of rice germplasm to zinc deficiency. *Field Crops Research* **76** 123-130.

Shah K, Kumar RG, Verma S and Dubey RS (2001). Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. *Plant Science* **161** 1135-1144.

Sharma P and Dubey RS (2007). Involvement of oxidative stress and role of antioxidative defense system in growing rice seedlings exposed to toxic concentrations of aluminum. *Plant Cell Reports* **26** 2027-2038.

Song A, Li P, Li Z, Fan F, Nikolic M and Liang Y (2011). The alleviation of zinc toxicity by silicon is related to zinc transport and antioxidative reactions in rice. *Plant and Soil* **344** 319-333.

Tavallali V, Rahemi M, Eshghi S, Kholdebarin B and Ramezanian A (2010). Zinc alleviates salt stress and increases antioxidant enzyme activity in the leaves of pistachio (Pistacia vera L 'Badami') seedlings. *Turkish Journal of Agriculture and Forestry* **34** 349-359.

Tennant D (1975). A test of modified line intersect method of estimating root length. *Journal of Ecologycal* **63** 995-1001

Tewari RK, Kumar P and PN S (2007). Oxidative stress and antioxidant responses in young leaves of mulberry plants under nitrogen, phosphorus or potassium deficiency. *Journal of Integral Plant Biology* **49** 313-322.

Van Assche F and Clijsters H (1990). Effects of metals on enzyme activity in plants. *Plant, Cell and Environment* **13** 195-206.

Yoshida S, Forno DA, Cook JH and Gomes KA (1976). Routine procedure for growing rice plants in culture solution. In: *Laboratory Manual for Physiological Studies of Rice*. The International Rice Research Institute: Los Banos, Laguna, Philippines.