

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. Taron 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 worldwide 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 JG 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^- and H_2O_2 (Gratão PL et al. 2005; Noctor and Foyer 1998; Sharma and Dubey 2007). This study aims to investigate in the rice seedlings (*Oryza sativa* L., cv. Taron 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. Taron 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$. 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 $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ 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):

$$\text{Zn Translocation Factor} = \frac{\text{Zn content of shoot}}{\text{Zn content of root}} \times 100$$

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 Na_2CO_3 (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 $\text{min}^{-1} \text{mg}^{-1}$ 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 H_2O_2 decomposition was measured at 240 nm (extinction coefficient of 0.036 $\text{mM}^{-1}\text{cm}^{-1}$) and enzyme specific activity was expressed as $\mu\text{mol H}_2\text{O}_2$ oxidized $\text{min}^{-1} \text{mg}^{-1}$ 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 ($\epsilon = 2.8 \text{ mM}^{-1} \text{ cm}^{-1}$). 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 ($\epsilon = 26.6 \text{ mM}^{-1} \text{ cm}^{-1}$). 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 3x3 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 (Fig. 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 .

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 (12.3 μg 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.

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

Antioxidative enzymes

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

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 . The minimum SOD activity was recorded (36 units mg⁻¹ protein) at the Zn level of 100 μM . As shown in Fig. 3b, the addition of Zn to nutrient solution was not significant on the CAT activity. The maximum CAT activity was recorded

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
5	9.88±1.21 ^b	28.46 ±3.47 ^a	288.0±36 ^b
50	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 \pm 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.

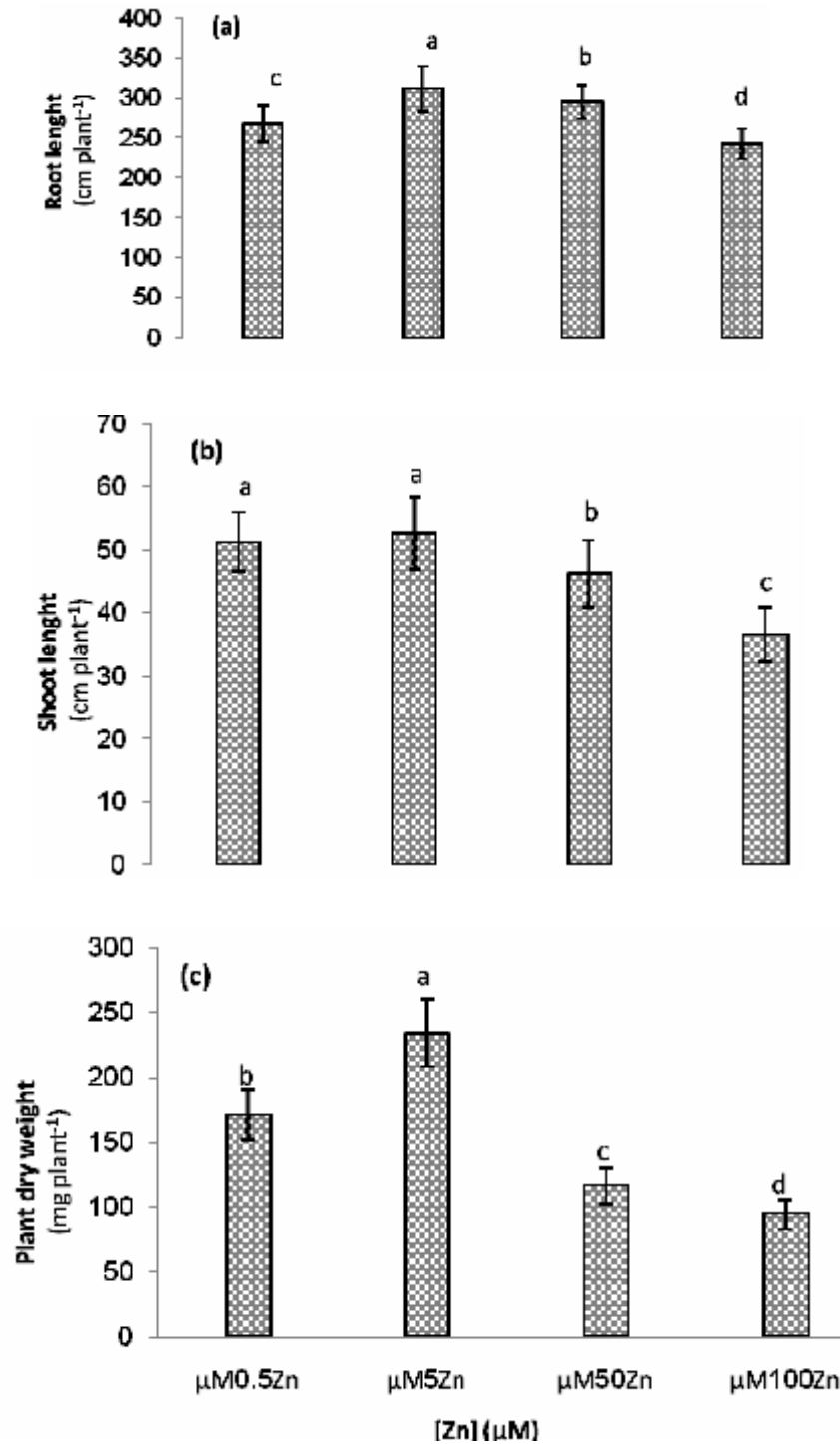


Fig. 1: Changes in growth responses; root length (a), shoot length (b) and plant dry weight (c) in rice subjected to different Zn levels in nutrient solution. All data are means \pm SE of three replications. Treatments with the same lower-case letters were not significantly different based on mean comparison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

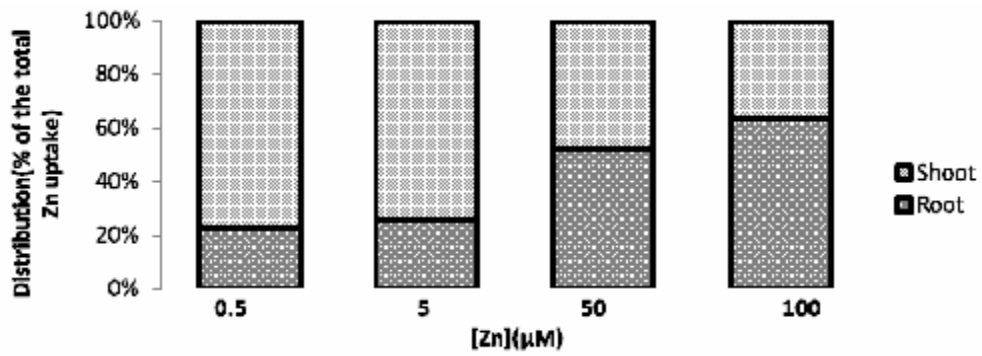
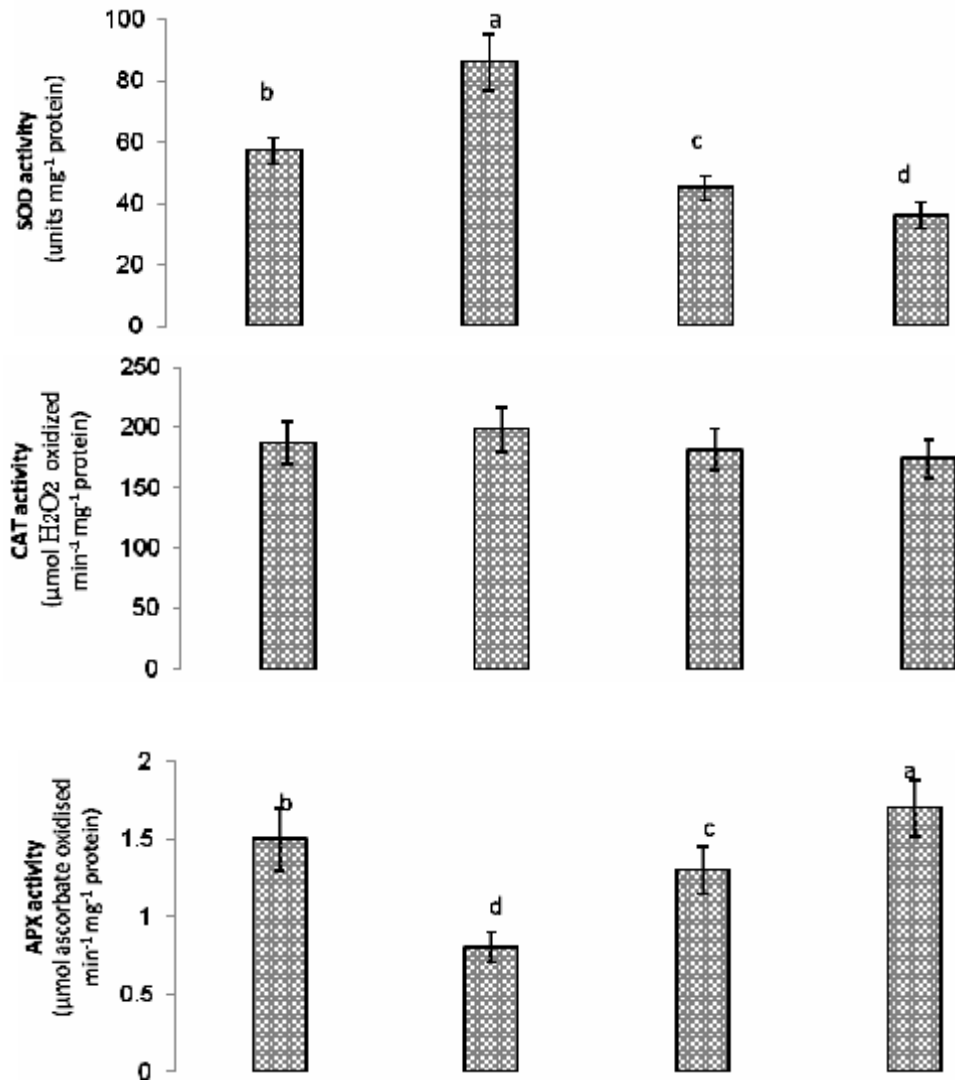


Fig. 2: The accumulation percent of Zn in different parts of rice (*Oryza sativa* L.) treated with different Zn levels (μM)



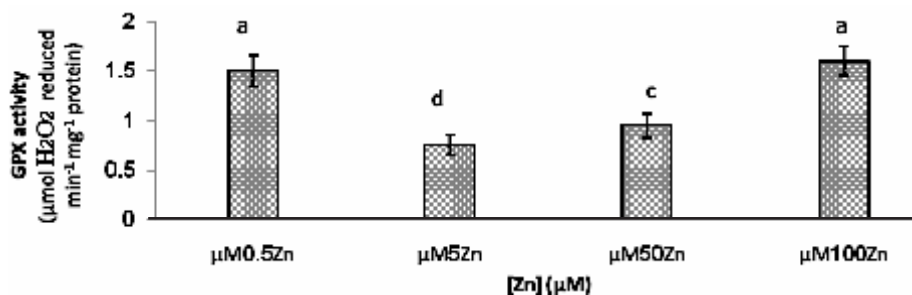


Fig. 3 Changes in antioxidant enzymes activities; SOD (a), CAT (b), APX (c) and GPX (d) in rice shoots subjected to different Zn levels in nutrient solution. All data are means \pm SE of three replications. Treatments with the same lower-case letters were not significantly different based on mean comparison by Duncan's test at $p < 0.05$

(198 $\mu\text{mol H}_2\text{O}_2$ oxidized $\text{min}^{-1} \text{mg}^{-1}$ protein) at the Zn level of 5 μM . The minimum CAT activity was recorded (174 $\mu\text{mol H}_2\text{O}_2$ oxidized $\text{min}^{-1} \text{mg}^{-1}$ protein) at the Zn level of 100 μM . Increasing Zn from 0.5 to 5 μM significantly decreased APX (Fig. 3c) and GPX (Fig. 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 $\text{min}^{-1} \text{mg}^{-1}$ protein and 1.6 $\mu\text{mol H}_2\text{O}_2$ reduced $\text{min}^{-1} \text{mg}^{-1}$ protein) at the Zn level of 100 μM . The minimum APX and GPX activities were recorded (0.8 μmol ascorbate oxidized $\text{min}^{-1} \text{mg}^{-1}$ protein and 0.75 $\mu\text{mol H}_2\text{O}_2$ reduced $\text{min}^{-1} \text{mg}^{-1}$ 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 (Fig. 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 (Fig. 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 H₂O₂ 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 RK *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; Cuyppers 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

1. Aebi H, Catalase in vitro. *Methods Enzymol* **52**: 121–126 (1984).
2. Ait Ali N, Bernal MP, Ater M, Tolerance and bioaccumulation of copper in *Zea mays* and *Phragmites australis*. *Plant and Soil* **239**: 103-111 (2002).
3. Alia KV, K.V.S.K. Prasad, and Pardha Saradhi P., Effect of zinc on free radicals and proline in *Brassica juncea* and *Cajanus cajan*. *Phytochemistry* **39**: 45-47 (1995).
4. Aravind P, Prasad MNV, Cadmium-Zinc interactions in a hydroponic system using *Ceratophyllum demersum* L: adaptive ecophysiology, biochemistry and molecular toxicology. *Braz. J. Plant Physiol.* **17**: 3-20 (2005).
5. Blokhina O, Virolainen E, Fagerstedt KV Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Annals of botany* **91**Spec No: 179-194 (2003).
6. Cakmak I, Öztürk L, Eker S, Torun B, Kalfa HI, A Y, Concentration of zinc and activity of copper/zinc superoxide dismutase in leaves of rye and wheat cultivars differing in sensitivity to zinc deficiency. *J Plant Physiol* **151**: 91-95 (1997).
7. Candan N, Tarhan L, 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 (2003)
8. Chaoui A, Mazhoudi S, Ghorbal MH, E EF Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.). *Plant science : an international journal of experimental plant biology* **127**: 139-147 (1997).
9. Cherif J, Mediouni C, Ammar WB, Jemal F Interactions of zinc and cadmium toxicity in their effects on growth and in antioxidative

- systems in tomato plants (*Solanum lycopersicum*). *Journal of Environmental Sciences* **23**: 837-844 (2011).
10. Cuypers A, Vangronsveld J, H C, Biphasic effect of copper on the ascorbate-glutathione pathway in primary leaves of *Phaseolus vulgaris* L. seedlings during the early stages of metal assimilation. *Physiol Plant* **110**: 512-517 (2000).
 11. Ebbs SD, Kochian ALV, Phytoextraction of zinc by oat (*Avena sativa*), barley (*Hordeum vulgare*), and Indian mustard (*Brassica juncea*). *Environmental Science and Technology* **32**: 802-806 (1998).
 12. Gao JG, Xiao Q, Ding LP, Chen MJ, Yin L, Li JZ, Zhou SY, GY H, Differential responses of lipid peroxidation and antioxidants in *Alternanthera philoxeroides* and *Oryza sativa* subjected to drought stress. *Plant Growth Regul* **56**: 89-95 (2008).
 13. Giannopolitis CN, Ries SK, Superoxide dismutases: I. Occurrence in higher plants. *Plant physiology* **59**: 309-314 (1977).
 14. Gratão PL, Polle A, Lea PJ, Azevedo R Making the life of heavy metal stressed plants a little easier *Funct Plant Biol* **132**: 481-494 (2005).
 15. Hajiboland R, Beiramzadeh N, Growth, gas exchange and function of antioxidant defense system in two contrasting rice genotypes under Zn and Fe deficiency and hypoxia. *Acta Biologica Szegediensis* **52**: 283-294 (2008).
 16. Hassan MJ, Zhang G, Wu F, Wei K, Chen Z Zinc alleviates growth inhibition and oxidative stress caused by cadmium in rice. *Journal of Plant Nutrition and Soil Science* **168**: 255-261 (2005).
 17. Jiménez A HJ, del Río LA, Sevilla F, Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. *Plant physiology* **114**: 275-284 (1997) .
 18. Noctor G, Foyer CH, Ascorbate and Glutathione: Keeping Active Oxygen Under Control. *Annual review of plant physiology and plant molecular biology* **49**: 249-279 (1998).
 19. Prasad AS., Zinc: an overview. *Nutrition* **11**: 93-99 (1995).
 20. Quessada MP MJ, Caractérisation d'une peroxidase impliquée spécifiquement dans la lignification en relation avec l'incompatibilité au greffage chez l'abricotier. *Physiologie Végétale* **22**: 533-540 (1984).
 21. Quijano-Guerta C, Kirk GJD, Portugal AM, Bartolome VI, McLaren GC, Tolerance of rice germplasm to zinc deficiency. *Field Crops Res* **76**: 123-130 (2002).
 22. Shah K, Kumar RG, Verma S, Dubey RS Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. *Plant Science* **161**: 1135-1144 (2001).
 23. Sharma P, Dubey RS, 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 (2007).
 24. Song A, Li P, Li Z, Fan F, Nikolic M, Liang Y The alleviation of zinc toxicity by silicon is related to zinc transport and antioxidative reactions in rice. *Plant and Soil* **344**: 319-333 (2011).
 25. Tavallali V, Rahemi M, Eshghi S, Kholdebarin B, and Ramezani A, Zinc alleviates salt stress and increases antioxidant enzyme activity in the leaves of pistachio (*Pistacia vera* L. 'Badami') seedlings. *Turk J Agric For* **34**: 349-359 (2010).
 26. Tennant D, A test of modified line intersect method of estimating root length. *J Ecol* **63**: 995-1001 (1975).
 27. Tewari RK, Kumar P, PN S, Oxidative stress and antioxidant responses in young leaves of mulberry plants under nitrogen, phosphorus or potassium deficiency. *J Integr Plant Biol* **49**: 313-322 (2007).
 28. Van Assche F, Clijsters H, Effects of metals on enzyme activity in plants. *Plant, Cell & Environment* **13**: 195-206 (1990).
 29. Yoshida S, Forno DA, Cook JH, Gomes KA 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 (1976).