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THE EFFECT OF ZINC FROM THE SEED ON ANTIOXIDANT DEFENSE SYSTEM IN WINTER WHEAT (*Triticale aestivum* L.) SEEDLINGS

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ABSTRACT. Accumulation of zinc in seeds of winter wheat plants grown at four different levels of zinc (Zn) in soil ranging from 0 to 810 kg/ha of added Zn and remobilization of Zn from seeds to seedlings leaves were examined. Lipid peroxidation, soluble protein content and activity of enzymes of antioxidant defense were also studied in seedling leaves. The accumulation of Zn in seeds and its remobilization from seeds into primary leaves was proportional to Zn supply. Inducible effect of Zn was found on activity of most enzymes of antioxidative defense (superoxid dismutase, catalase and peroxidase). Activity of glutathione peroxidase was decreased. Although Zn is essential micronutrient for plants and could act as an antioxidant at lower doses, at high applied concentrations act as a prooxidant evoking oxidative stress.

Keywords: *antioxidative enzymes, lipid peroxidation, oxidative stress, Triticale aestivum L., winter wheat, zinc.*

INTRODUCTION

Zinc (Zn) is needed as micronutrient by all living organisms and is essential for normal growth and development of plants, as it is known to be required in several aspects of metabolism and reproductive development [1]. The critical soil Zn toxicity level for plants varies as a function of various soil and climatic factors as well as plant species and genotype [2]. Although Zn is an essential micronutrient, it become phytotoxic and inhibits cell growth in plants at excessive concentrations [3]. Leaf epinasty and chlorosis are easy visible symptoms of strong Zn phytotoxicity [4].

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Reactive oxygen species (ROS), which includes superoxide ($O_2^{\cdot -}$), hydroxyl radicals ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2), the ubiquitous products of single electron reductions of molecular oxygen are among the most reactive compounds known to be produced during and early metals stress. ROS are responsible for the majority of biological oxidative damage associated with oxidative stress, such as DNA, protein and the cell membrane damage [5]. The accumulation of ROS is limited by the action of various enzymatic and non-enzymatic mechanisms. These protective mechanisms include antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), various peroxidases and other enzymes [6].

RESULTS AND DISCUSSION

When applying increasing quantity of $ZnSO_4$ to the soil, the Zn contents of the seeds of winter wheat plants increased (Table 1). The remobilization of Zn from seed tissues during germination and early growth in seedlings was intensive. In response to an altered Zn content in the seeds, the Zn content of seedlings with highest amount of Zn in seeds reached a value almost double higher than the control. The accumulation of Zn in root and leaves of barley seedlings was proportional to metal concentration in culture medium [1, 7, 8]. Greater Zn concentration in nutrient solution significantly depressed the fresh and dry mass of leaves of different plant species [8, 9, 10]. Dry matter production of shoots was not altered by zinc content in shoot tissues. Only the highest Zn accumulation in shoots resulted in lower dry matter of shoots at early stage of growth. One of the first responses of plants to heavy metal stress is reduction of biomass, which can be considered as an index of plant tolerance [10].

Table 1. Dry matter content in shoots, and Zn concentrations in seeds and shoots of winter wheat plants at various Zn addition.

	Zn added (kg/ha)			
	0	90	270	810
Dry matter of shoots [mg/plant]	6.90 ± 0.63[a]	7.05 ± 0.66[a]	6.80 ± 0.61[a]	5.50 ± 0.50[b]
Zn content in seed [mg/kg]	12.56 ± 1.11[a]	49.00 ± 0.42[b]	56.28 ± 0.56[c]	65.37 ± 0.60[d]
Zn content in shoots [mg/kg dry matter]	43.58 ± 8.76[a]	71.87 ± 3.96[b]	73.45 ± 7.45[b]	81.90 ± 10.10[c]

[a, b, c, d] Values within columns without common superscript are significantly different ($P < 0.05$).
Values represent the mean ± SD

Zinc stimulates the production of ROS (Table 2). Lipid peroxidation, which is measured as MDA content, showed no significant alteration in the shoots of seedlings with lower content of Zn in tissues. However, the presence of Zn at higher amount in tissues of shoots expressed slightly enhanced MDA levels in comparison with that in control seedlings. As reported earlier, excess of Zn promoted MDA production in plants due to increased lipid peroxidation through excessive generation of free radicals [6, 7, 8]. Zn content in shoots did not affect level of soluble protein in tissues. Khudsar et al. [11] found that leaves of *Artemisia annua* had significantly lower protein content throughout the plant life in the Zn treated plants.

Table 2. Soluble protein content, lipid peroxidation, hydroxyl and superoxide radical accumulation, activities of enzymes of antioxidative response: superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and glutathione peroxidase (GSH-Px) in shoots of winter wheat at various Zn addition.

	Zn added (kg/ha)			
	0	90	270	810
Soluble protein content [g/kg]	25.99 ± 3.07^{a}	22.84 ± 2.13^{a}	23.24 ± 1.05^{a}	22.91 ± 3.32^{a}
Lipid peroxidation [nmol MDA/g]	60.41 ± 0.23^{a}	60.72 ± 3.73^{a}	66.21 ± 4.16^{b}	67.40 ± 2.84^{b}
Hydroxyl radical [nmol/g]	121.60 ± 11.61^{a}	122.21 ± 9.09^{a}	136.81 ± 6.24^{b}	158.71 ± 13.20^{c}
Superoxide radical [mmol/g]	4.25 ± 0.30^{a}	4.77 ± 0.41^{a}	51.52 ± 0.36^{b}	56.14 ± 0.51^{b}
SOD [U/g]	67.70 ± 6.04^{a}	82.32 ± 7.69^{b}	90.10 ± 8.52^{b}	121.37 ± 8.17^{c}
CAT [U/g]	145.03 ± 10.43^{a}	148.27 ± 9.95^{a}	164.63 ± 11.45^{b}	198.87 ± 13.77^{c}
POD [U/g]	9.77 ± 0.93^{a}	12.23 ± 1.49^{b}	12.41 ± 1.03^{b}	19.71 ± 2.13^{c}
GSH-Px [U/g]	116.03 ± 2.00^{a}	75.20 ± 11.91^{b}	60.52 ± 5.87^{c}	56.34 ± 10.22^{c}

$^{a, b, c}$Values within columns without common superscript are significantly different ($P < 0.05$).
Values represent the mean ± SD

The changes in the antioxidant enzyme activities, CAT, SOD, POD and GSH-Px, in shoots of winter wheat seedlings are shown in Table 2. Activities of CAT, SOD and POD displayed a progressive increase in response to Zn in dose-response manner, and the peak activity was found at highest Zn content. More than 2-fold increase in activity of these enzymes was

observed in seedlings with highest Zn content. Unlike others, the activity of GSH-Px declined as Zn content increase. To mitigate and repair damage initiated by ROS a significant enhancement in SOD activity was observed in seedlings with higher content of Zn. There are also reports of high SOD activity in different plant species [6, 8, 9].

The role of POD for scavenging different peroxides is generally recognized [12]. Significant increases in the activities of CAT and POD in the present investigation suggest their role in constant detoxification of H_2O_2 in seedlings under Zn toxicity [6, 7]. However, Hegedűs et al. [1] did not observe any change in CAT activity in Zn stressed barley seedlings. Cuypers et al. [13] and Hegedűs et al. [1] observed that a treatment with Zn resulted in an increase of the POD capacity.

CONCLUSIONS

Although Zn is not a redox active metal, it was shown that Zn toxicity could lead to oxidative damage as well as to induce antioxidative defense mechanisms against it.

In this study, Zn toxicity induces intracellular oxidizing conditions leading to the production of ROS, this was deducted from the stimulation of SOD, CAT and POD capacities in shoots of the seedlings.

EXPERIMENTAL SECTION

Plant material

A small-plot long-term field experiment was set up at the Nagyhörcsök Experimental Station of the Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, Budapest, to investigate the effect of high trace element rates. Winter wheat (*Triticale aestivum* L. cv. Kitarò) was sown on on the loamy textured calcareous soil formed on loess. Zn was applied at levels of 0, 90, 270 and 810 kg/ha. At harvest, seed were analysed for Zn content by inductive coupled plasma emission spectrometer (ICP) after digestion in ccHNO_3 and ccH_2O_2. Before sowing, seeds were surface sterilised by soaking in 70% (v/v) ethanol for 1 min, followed by 10% (v/v) sodium hypochlorite solution for 30 s, and rinsing in deionised water. Seed were placed in Petri dishes and germinated in a growth chamber. The seed were half submerged by periodically adding deionised water. Each treatment included five replicates with 35 seedlings per replicate. Samples were harvested on 23 day from the onset of imbibitions. Shoot

samples were analysed for Zn content by ICP. Leaf tissue homogenate were used for biochemical determinations and enzyme activity. All spectrophotometric analysis was conducted on a UV/visible light spectrophotometer (Jenway, 6505, UK).

Measurement of antioxidant activity

Lipid peroxidation was assayed by measuring malonyl dialdehide (MDA) production [14]. Superoxide radical production was determined by the adrenaline autooxidation [15] and hydroxyl radical production by the inhibition of deoxyribose degradation [16]. The total protein content in extracts was determined by a method described by Lowry et al. [17]. Total SOD activity was measured by the method of Mandal et al. (2008). CAT was assayed by monitoring the consumption of H_2O_2 [18]. Peroxidase (POD) was determined by measuring the oxidation of guaiacol [19]. Glutation peroxidase (GSH-Px) activity was assayed according to the method of Upadhyaya et al. [20].

Statistical analysis

Results are expressed as mean of determinations of 3 independent samples made in triplicates. Statistical significance was tested by analysis of variance followed by comparison by Duncan's multiple range test ($P < 0.05$) calculated using STATISTICA for Windows version 9.0 (StatSoft, Tulsa, OK, USA). Stepwise multiple regression analyses were used to determine correlation among variables.

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