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Effect of zirconium chelate on cadmium stressed wheat (*Triticum aestivum* L. MV.20) seedlings

Marietta Fodor*, Attila Hegedűs, Éva Stefanovits-Bányai

Deptartment of Applied Chemistry, Corvinus University of Budapest, Budapest, Hungary

ABSTRACT Wheat seedlings were grown hydroponically, for the heavy metal treatment 200 μM Cd acetate was applied. Nutrient solutions were also supplemented with a water-soluble zirconium ascorbate complex (Zr-ASC) at several concentrations. Our results indicated that wheat seedlings have accumulated great amounts of zirconium and cadmium in their roots and shoots. Zirconium uptake was increased under Cd stress; however, its translocation to the leaves was mitigated. Increased activities of guaiacol peroxidase (POD), ascorbic acid peroxidase (APX) and glutathione reductase (GR) reflected a Cd induced oxidative challenge in plant cells. Cadmium induced heavy metal stress symptoms could be alleviated by 10 and 33 μM Zr-ASC, which was also confirmed by the reduced activities of all the tested antioxidant enzymes.
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KEY WORDS

Zirconium
Cadmium
wheat
oxidative stress
peroxidases
glutathione reductase

Zirconium is the 20th most common element in the earth's crust, its total content in soils is deemed to be between 30 and 2000 mg kg^{-1} dry soil. Zr is present in soil in amounts higher than nickel, zinc, copper or lead. Due to the changing environmental conditions or sources of contamination, water soluble Zr may occur in ecosystems. Water solubility means that Zr uptake by the roots becomes possible and in this way it may enter the food chain. This may have unknown influences on the domestic animals or even on the human body.

Our knowledge on the effects of Zr on living organisms is especially restricted. However, in a previous study we could detect favourable physiological effects on wheat seedlings growing in 10-55 μM Zr-ASC (Fodor et al. 2005). We cannot rule out the possibility that this beneficial effect may even be more intensive in plants grown under severe abiotic stress conditions, as it was shown in case of titanium ascorbate, a very similar compound to Zr-ASC, in some crops (Alcaraz-Lopez et al. 2003).

Our experiments were carried out to survey that 10, 33 and 55 μM Zr-ASC treatment could have beneficial effects on Cd-treated plants or not.

Materials and Methods

For each treatment 3 g wheat caryopses (*Triticum aestivum* L. MV. 20) were imbibed for 24 hours and germinated in hydroponics of Knopp solution (Suba 1978), which was supplemented with 200 μM Cd acetate. K-ASC and different concentrations of Zr-ASC (10, 33 55 μM Zr) were used for Zr treatment. Zirconium was provided as Zr-ascorbate chelate. It was obtained from zirconyl chloride ($ZrOCl_2$ x 8 H_2O) and L-ascorbic acid (Fodor et al. 2003). Plants were kept in complete darkness during the first 5 days of their development, and then grown further at room temperature (25°C) under natural irradiation. Nine-day-old seedlings were rinsed with distilled water, dried, and prepared for analysis.

Cd content was measured by ICP-OES. Plant material was dehydrated at 105°C for 24 h and 0.2 g of powdered dry material was dissolved in 2 ml of HNO_3 : H_2O_2 mixture (1:1, v/v). Decomposition of plant tissues was completed by incubation at high pressure and temperature. Arsenazo III was used as a reagent to determine Zr content of the filtered solutions (Savvin 1961). The measurement was carried out by a PC-controlled *GBC 916 UV/VIS* spectrophotometer at λ = 665 nm (ε = 120 $mmol^{-1}$ cm^{-1}). Enzyme extraction and activity determination of guaiacol peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR) was carried out as described by Fodor et al. (2005).

Three independent experiments were conducted and plants growing in 200 μM cadmium acetate were taken as control. Statistical analyses were performed by Microsoft Excel software ($P \leq 0.05$).

Results

Zr content

Cadmium did not inhibit the Zr uptake of plants, in fact it was enhanced: 1200 μg/g dry matter in case of 55 μM Zr in contrast to the 430 μg/g dry matter without Zr.

Zr translocation from roots to leaves was also considerably affected. Leaves of control plants contained 120 μg Zr/g dry matter, while 55 μM Zr treatment caused a marked decrease as leaves contained only 30 μg Zr/g dry matter. Zirconium could not be detected in leaves of plants growing in Knopp solution.

*Corresponding author. E-mail: marietta.fodor@uni-corvinus.hu

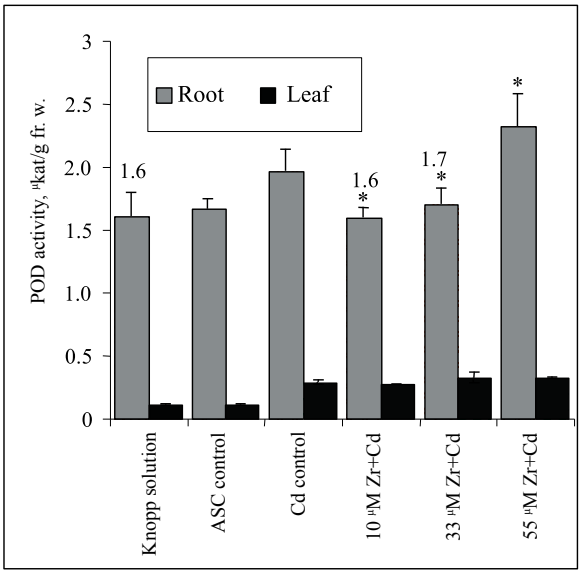

Figure 1. Zr-ASC induced changes in the POD activity in leaves and roots of Cd-stressed plants (* = P<0.05).

Determination of enzyme activities

Leaves and roots of control plants growing in Cd-containing nutrient solution performed increased POD activities compared to those growing in Cd-free solutions (Fig. 1). 10 and 33 μM Zr-ASC treatment significantly reduced the POD activity in roots of plants. Their values have practically reached the level of POD activity measured in plants growing in Cd-free nutrient solutions. In leaves no alterations attributable to the effect of zirconium could be observed.

Cd treatment has considerably increased the APX activity in roots and leaves, while the additional Zr-ASC treatment in 10 and 33 μM concentrations resulted in a significant reduction. 55 μM Zr-ASC, however, markedly increased the APX activity in roots.

Cd treatment also induced elevated GR activities. In roots, a slight but not significant decrease was measured in plants treated by Zr, however, in leaves, 10 and 33 μM Zr-ASC significantly decreased the GR activity to the level observed in plants grown in the K-ASC solution.

Discussion

Cadmium does not generate active oxygen species directly, but by modifying some of the enzymes' activity, it induces oxidative stress (Hegedűs et al. 2001). Cd treatment induced considerable increases in all enzyme activities (POD, APX, GR) both in roots and leaves, which indicates that plants suffered oxidative stress. In roots, 10 and 33 μM Zr-ASC significantly lowered the POD and APX activities, indicating that small amounts of Zr may alleviate Cd induced oxidative stress effects. Similar results were presented for titanium ascorbate by Hegedűs et al. (2002) in case of barley seedlings, and by Koczka et al. (2001) in *Ginkgo biloba* L.

There was no remarkable increase in POD activity, when plants were grown in 200 μM Cd-containing nutrient solution supplemented with 10-55 μM Zr-ASC. 33 and 55 μM Zr induced a slight but not significant elevation in POD activity, which can be attributed to a joint effect produced by the two different metals.

Activity increases of APX and GR were considerably restricted in leaves of plants treated by 10 and 33 μM Zr-ASC compared to that of the Cd-treated control plants. This may reflect that Cd induced oxidative stress some way could be moderated by Zr-ASC supplementation in a concentration range of 10 and 33 μM, and consequently, the activities of antioxidant enzymes were not as much elevated.

Enzyme activities in case of 55 μM Zr treatment were similar to those of the Cd-stressed control plants, which indicates a concentration threshold, over which no protective effects can be demonstrated.

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References

Alcaraz-Lopez C, Botia M, Alcaraz CF, Riquelme F (2003) Effects of foliar sprays containing calcium, magnesium and titanium on plum (*Prunus domestica* L.) fruit quality. J Plant Physiol 160:1441-1446.

Chance B, Maehly AC (1955) Assay of catalases and peroxidases. Methods Enzymol 2:764-817.

Fodor M, Hegóczki J, Vereczkey G (2003) The effects of zirconium, a less known microelement, on basic fermentation characteristics and protein composition of *Saccharomices cerevisiae*. Acta Aliment 32:353-362.

Fodor M, Hegedűs A, Stefanovits-Bányai É (2005) Zirconium induced physiological alterations in wheat seedlings. Biol Plantarum (in press).

Hegedűs A, Tóth E, Vajda K, Hauptmann G, Stefanovits-Bányai É, Erdei S (2002) Titán-aszkorbát kezelés hatása a növények antioxidáns védekező rendszerére. Proc. The 9th Symposium on Analytical and Environmental Problems, Szeged. 65-69.

Koczka N, Orlóci L, Dimény J, Stefanovits-Bányai É (2001) Effect of cadmium stress on peroxidase activity of *Ginkgo biloba* L. Proc. The 9th International Trace Element Symposium Budapest, pp.159-165.

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867-880.

Savvin SB (1961) Analytical use of Arsenazo III. Determination of thorium, zirconium, uranium and rare earth elements. Talanta 8:673-685.

Suba J (1978) Növényélettani gyakorlatok. Tankönyvkiadó, Budapest.