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COMPARATIVE MORPHOLOGICAL AND HISTOLOGICAL STUDY ON ZINC- AND CADMIUM-TREATED DURUM WHEAT PLANTS WITH SIMILAR GROWTH INHIBITION

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Summary. A comparative study of the effects of excess Zn^{2+} and high Cd^{2+} concentrations on some morphoplogical and histological characterstics of durum wheat plants was conducted in hydroponic experiments. Treatment with the tested heavy metals was applied when plants were 8-day-old and carried on for the next 10 days. Cd^{2+} and Zn^{2+} were added to the nutrient solution at concentrations 50 and 600 µM, respectively. These concentrations were chosen as a result of a preliminary study, where they have been found to produce similar relative growth rate (RGR) inhibition of about 50%. The results revealed that Zn^{2+} and Cd^{2+} toxic effects on wheat plants were clearly distinguished at both morphological and histological levels. Cd^{2+} inflicted more substantial changes in leaf structure, significantly increasing the number of the epidermal cells and stomata density on mm^2, as well as reducing the mesophyll thickness. Wheat plants' response to all these xeromorphic changes in leaf structure could be presumed to be the result of cadmium negative impact on plant water relations. On the other hand, Zn^{2+} reduced rather insignificantly the number of the epidermal cells and stomata frequency on mm^2 on both leaf surfaces, and it also reduced the mesophyll thickness by only 12%. Both metals reduced significantly the size of the root cortex. Neither Cd^{2+} nor Zn^{2+} applied in such concentrations caused abnormalities in leaf and root cells.

Key words: anatomy, cadmium, durum wheat, zinc, growth.

Abbreviations: ICP – AES (atomic emission spectrophotometer with inductively coupled plasma); FAA – formaldehyde - alcohol solution; FA – formaldehyde solution; RGR – relative growth rate.

INTRODUCTION

Cadmium and zinc are well-known soil pollutants in many industrial sites. In addition to the risk created by these metals for food chain safety, they often become toxic to plants themselves. There is a general assumption that excess Zn^{2+}

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and high Cd^{2+} concentrations have similar phytototoxic mode of action (Breckle, 1991). In fact, some of the visible toxicity symptoms and functional disorders in Cd^{2+}- and Zn^{2+}-treated plants are similar. However, as the role of these metals in plant metabolism is completely different, their impact on plant performance can also be expected to differ. Cadmium has no biological function, while Zn^{2+} is an essential micronutrient for plants, which makes it phytotoxic at supra optimal concentrations (Demirevska-Kepova et al., 2006; Cuypers et al., 1999). Unfortunately, some of the existing information about the impact of these heavy metals on plants should be treated with caution due to significant variation of the used experimental designs, such as the effects of Zn^{2+} and Cd^{2+} when the two metals are compared at equal external concentrations or when the chosen concentrations lack satisfactory rationale. The results presented here were obtained in a comparative study using Zn^{2+} and Cd^{2+} concentrations, which produced about 50% inhibition of RGR in young durum wheat plants.

MATERIALS AND METHODS

Seeds of *Triticum durum* Desf. (cv. Beloslava) were germinated on wet filter paper for 3 days and then transferred to plastic pots with modified ½ strength Hoagland nutrient solution. The solution was renewed every other day and aerated regularly. Seedlings were cultivated at controlled conditions: 26/22ºC day/night temperature, 16/8 h photoperiod and 250 $\mu mol\ m^{-2}\ s^{-1}$ light intensity for 18 days. An experimental design was set up including: (1) untreated plants (control), (2) Cd^{2+}- and (3) Zn^{2+}-treated plants. The metal ions were added when plants were 8-day-old to the nutrient solution in a sulfate form at concentrations of 50 µM and 600 µM for Cd^{2+} and Zn^{2+}, respectively. After the treatment, the plants were left to grow for 10 more days and were then used for chemical, biometrical and anatomical analyses. The total content of Zn and Cd in the roots and leaves was determined by ICP – AES after dry mineralization at 500°C precided by HNO_3 treatment. Samples from the roots and second leaf were fixed in FAA solution for 24 h and were then preserved in FA for further anatomical study. Permanent glycerin slides were prepared and examined with a light microscope at x100 magnification. The structural changes in the leaf mesophyll thickness, number of epidermal cells, stomata frequency on mm^2, and size of the root cortex and stele were measured. Statistical analysis was performed using a one-way ANOVA (for $P < 0.05$). Based on ANOVA results, a Tukey's test for main comparison at a 95% confidential level was applied.

RESULTS AND DISCUSSION

The results presented in Table 1 showed that both Cd^{2+} and Zn^{2+} inhibited RGR of durum wheat plants by about 50%. The plants treated with Cd^{2+}- and Zn^{2+}- manifested well-expressed toxicity symptoms, such as the appearance of chlorotic and necrotic spots in the leaves, weaker development of root branches and root browning (Das et al., 1997; Doncheva at al., 1999; Vassilev et al., 2007). We observed strongly expressed toxicity symptoms in the roots of plants, which were known to have metals accumulation and distribution. Total Cd^{2+} and Zn^{2+}

content in the roots of treated plants were several-fold higher than that in the leaves. The respective data in both roots and leaves were as follows: Zn^{2+} – 3029 and 880 mg.kg^{-1} dry weight; Cd^{2+} – 936 and 150 mg.kg^{-1} dry weight. The visible toxicity symptoms in the leaves of Zn^{2+}- and Cd^{2+}-treated plants were different. While Zn^{2+} caused necrotic spots, Cd^{2+} induced chlorosis. Treatment with Cd^{2+}- resulted in browning and a stronger inhibition of root length growth and branching, whereas Zn^{2+} made roots lighter in color and thinner in diameter.

Table 1. Effects of different Cd^{2+} and Zn^{2+} concentrations on some growth characteristics in durum wheat plants. L – root length; FW – fresh weight; DW – dry weight; LA – leaf area; RGR – relative growth rate.

Treatments	Parameters								
	Roots			Leaves				Whole plant	
	L [cm]	FW [g]	DW [g]	L [cm]	FW [g]	DW [g]	LA [cm^2]	RGR [mg.g^{-1}.day^{-1}]	Inhibition %
Untreated (control)	22.5^a	1.15^a	0.08^a	21.7^a	3.36^a	0.40^a	61.0^a	140.6^a	0
Cd 50 µM	9.5^b	0.50^b	0.04^b	14.4^b	1.53^b	0.23^b	21.2^b	68.8^b	51
Zn 600 µM	11.8^b	0.53^b	0.04^b	15.1^b	1.26^b	0.21^b	16.2^b	64.1^b	53

*Values followed by the same letter (a, b or c) within a column are not significantly different at $P < 0.05$.

Table 2. Anatomical parameters in leaf and root cells of Cd^{2+}- and Zn^{2+}-treated durum wheat plants with a similar degree of growth inhibition.

Treatments	Plant organ						
	Leaf					Root	
	Epidermis				Mesophyll	Cortex	Stele
	Upper		Lower				
	Stomata frequency/mm^2	Epidermal cells/mm^2	Stomata frequency/mm^2	Epidermal cells/mm^2	Thickness (µm)	Thickness (µm)	Diameter (µm)
Untreated (control)	199^a	628^a	135^a	542^a	110.8^a	22.1^a	30.8^a
Cd 50 µM	266^b	1076^b	193^b	627^b	77.4^b	13.2^b	20.1^b
Zn 600 µM	154^c	646^a	121^a	432^c	97.2^c	11.9^c	20.5^b

*Values followed by the same letter (a, b or c) within a column are not significantly different at $P < 0.05$.

The anatomical studies revealed that Zn^{2+} and Cd^{2+} toxic effects on the wheat plants were clearly distinguished at a histological level. In addition, plants responded to excess Cd^{2+} and Zn^{2+} with very different structural changes (Table 2). Cd^{2+} caused more substantial changes in leaf structure, significantly increasing (by 71%) the number of the epidermal cells in the upper epidermis as well as stomata density (by about 38%) on both leaf surfaces. Cd^{2+} also reduced the mesophyll thickness by 30%. All these xeromorphic changes could be considered to be the result of cadmium's negative impact on plant water relations. Zn^{2+} reduced rather insignifically the number of the epidermal cells and stomata frequency on mm^2 on both leaf surfaces, and it also reduced the mesophyll thickness by 12% only. Both metals caused significant changes in the root cortex, reducing the size of the cortex to a similar extent (46% and 40% in the presence of Zn^{2+} and Cd^{2+}, respectively). No significant changes were observed in the size of the root stele.

In conclusion, when Zn^{2+} and Cd^{2+} were applied at concentrations producing 50% inhibition of RGR in young durum wheat plants, the changes at both morphological and histological levels were expressed with different stress symptoms. Briefly, Cd^{2+} induced classic xeromorphic changes in leaf structure, which were not observed in Zn^{2+}-treated plants.

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