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Responses of wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) plants to cadmium toxicity in relation to magnesium nutrition

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Abstract – The influence of cadmium (Cd) on physiological processes in wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) plants exposed to either optimal mineral nutrition or the absence of magnesium (Mg) as well as the accumulation of cadmium and magnesium in plant organs were studied using the method of water culture in a greenhouse. Cd treatment reduced shoot fresh mass more strongly in Mg-supplied than in Mg-deficient plants. Negative effect of Cd on photosynthetic activity was more pronounced in *T. aestivum* than in *Z. mays* plants. Cd treatment decreased leaf chlorophyll and carotenoid concentration in both *Z. mays* and *T. aestivum*, irrespective of the Mg supply. Cd was preferentially accumulated in the roots of both species. Catalase activity in *T. aestivum* leaves and roots was unaffected by Cd and Mg supply. Cd treatment did not affect Fe accumulation in the leaves of either species, while in the roots a considerable increase occurred, irrespective of the Mg nutrition. Higher tolerance of *Z. mays* and *T. aestivum* plants to Cd toxicity exposed to Mg deficiency could partly be ascribed to the preservation of Fe nutrition.

Keywords: cadmium toxicity, growth, magnesium supply, photosynthetic activity, pigments, *Triticum aestivum*, tolerance, *Zea mays*

Introduction

Heavy metals are important and widespread pollutants in the environment. Due to their toxicity to most living organisms, control of the emission and accumulation of these contaminants into the biosphere has become a main task worldwide. Among other non-essential heavy metals, cadmium (Cd) has attracted considerable scientific attention due to its potential to endanger human health (Tran and Popova 2013). Soil contamination with Cd may come either from different anthropogenic sources including application of sewage sludge, wastewater, fertilizers, pesticides, as well as traffic and industrial activities, or from natural

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sources. Cd is readily taken up by roots and translocated to different plant organs (Amirjani 2012). Due to its high mobility in the phloem (Benavides et al. 2005), Cd may impede the acquisition of essential nutrients by various plant parts. Considering the role of nutrients in the creation and maintenance of the structure and function of plant cells (Wahid et al. 2007), optimal plant growth may be disrupted by the presence of Cd. This metal is generally considered a highly toxic element with negative effects on plant growth and development, photosynthesis, transpiration, stomatal regulation, enzymatic activities, water relation, mineral elements uptake, protein metabolism, and membrane functioning (Tran and Popova 2013). Possible mechanisms involved in the generation of the above disorders are induction of oxidative stress and replacement of essential elements such as Zn, Fe, and Mn, as they are cofactors of many enzymes (Seregin and Ivanov 2001, López-Millán et al. 2009).

The antioxidant compounds and antioxidative enzymes play important roles in the improvement of stress tolerance in plants (Noctor and Foyer 1998). Heavy metals are known to increase activity of antioxidant enzymes (Siddiqui 2013). Metalloenzymes (superoxide dismutase, catalase, peroxidase, ascorbate peroxidase) protect cellular components such as proteins, membrane lipids and nucleic acids, against oxidative injury caused by reactive oxygen species (Halliwell and Gutteridge 1989).

Magnesium (Mg) is an essential macroelement for plants as it is a constituent of the chlorophyll molecule (Bose et al. 2011). In addition, it plays an important role in the metabolism and translocation of carbohydrates, the production of oils and fats, activation of enzymes involved in the synthesis of nucleic acids, regulation of the other essential elements uptake, and transport of phosphate compounds throughout the plant (Tucker 1999). This implies that conservation of optimal Mg levels in organs of plants exposed to Cd stress postpones the toxicity effects of the heavy metal and improves plant performance. Earlier research has pointed to perturbation of both uptake and transport of different cations, including Mg, in various plant species exposed to Cd (Yang et al. 1996). Extensive studies on the level of Mg supply in alleviation of heavy metal toxicity have been undertaken recently. The results have shown that both Mg supply (10 mmol L^{-1}) and Mg deficiency may extenuate Cd toxicity in *Brassica rapa* L. var. *pervirdis* and *Arabidopsis thaliana*, respectively (Kashem and Kawai 2007, Hermans et al. 2011).

Negative effects of Cd on growth, physiological traits and biochemical processes in *Triticum aestivum* and *Zea mays* plants have been described previously (Florijn and Van Beusichem 1993, Lagriffoul et al. 1998, Ozturk et al. 2003, Lin et al. 2007, Ci et al. 2010). To our knowledge, these species have not been studied in relation to Cd stress under Mg deficiency. Therefore, the aim of the present study was to explore the role of this element in *T. aestivum* and *Z. mays* tolerance to Cd toxicity in relation to plant growth, rate of photosynthesis and transpiration, synthesis of chlorophylls and carotenoids, catalase activity, proline accumulation, and accumulation and translocation of Cd, Fe and Mg. The study was conducted on plants exposed to optimal and deficient Mg supply.

Materials and methods

Plant growth and treatments

Wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) seeds were surface sterilized with 3% H_2O_2 for 15 minutes and washed extensively with distilled water. The seeds were germinated in plastic boxes filled with sterilized sand, particle size between 0.01 and 0.075

mm. The seedlings were grown in a growth chamber at constant temperature of 25 °C and 16 h light/8 h dark photoperiod for twelve days. After that period, morphologically uniform plants were selected and transferred to 0.4 L pots containing Reid-York nutrient solution (Reid and York 1958), and cultivated in a greenhouse for 21 days. The f ollowing treatments were set: a) sufficient Mg supply without Cd (control), b) sufficient Mg supply with Cd (Cd), and c) Mg starvation with Cd (Cd-Mg). Nutrient solution with sufficient Mg supply contained the following macro- and micro-elements: 1.0 mmol L^{-1} KH_2PO_4, 5.0 mmol L^{-1} KCl, 4.0 mmol L^{-1} $CaCl_2 \times 2\ H_2O$, 2.0 mmol L^{-1} $MgSO_4 \times 7\ H_2O$, and 7.0 mmol L^{-1} NH_4NO_3 (Reid and York 1958). Deficiency of Mg was achieved by replacing $MgSO_4$ with 2.0 mmol L^{-1} K_2SO_4 in order to maintain the supply of sulfur. Cd stressed plants were supplied with 0.1 μmol L^{-1} of cadmium added as $CdCl_2$.

The nutrient solutions were continuously aerated and were replaced every 5 days. All parameters were measured after 21 days of the treatments. Rates of photosynthesis and transpiration were measured *in vivo*. The first three fully expanded leaves from the top of the plants were sampled and immediately used for analysis of photosynthetic pigments, catalase activity and proline accumulation.

Photosynthetic pigments, rates of photosynthesis and transpiration

The chlorophyll *a*, chlorophyll *b* and carotenoid contents were determined according to Wettstein (1957) after extraction in absolute acetone and expressed as mg g^{-1} DW. Rates of photosynthesis and transpiration were measured *in vivo* on intact leaves, using a LC Pro+ Portable Photosynthesis System (ADC BioScientific Ltd., UK). During the measurement, the saturated photosynthetically active radiation (PAR) with photon flux density of 1000 μmol m^{-2} s^{-1} was emitted, and the flow rate of ambient air to the leaf chamber was 100 μmol s^{-1}. All parameters were measured between 10:00 and 12:00 A.M., during a clear sunny day.

Biomass production and tolerance index

Plants were harvested after 21 days of cultivation, and fresh mass of shoots and roots was measured. *T. aestivum* and *Z. mays* susceptibility to Cd stress in Mg-supplied and Mg-starved plants in relation to control was evaluated using the tolerance index (TI). This parameter was calculated as follows: TI = fresh weight of treated plants × 100 / fresh weight of control plants (Wilkins 1978).

Determination of Mg, Cd and Fe

Following the biomass measurements, leaves and roots were oven-dried at 80 °C till the constant weight. Concentrations of Mg, Cd and Fe were determined by employing flame atomic absorption spectrophotometry (Varian, AAS240FS). The plant material was digested using closed vessel high pressure microwave digestion (Milestone D series Microwave Digestion System). For all treatments and both plant species, three independent replicates were used for analysis of metal accumulation in leaves and roots. The ability of plants to translocate Mg, Cd and Fe from the roots to the shoots in relation to Mg and Cd supply is indicated by the translocation factor (Tf). This factor was calculated according to Zacchini et al. (2009): Tf = concentration of the heavy metal in the shoot × 100 / concentration of the heavy metal in the root.

Determination of catalase activity and free proline accumulation in leaves

The leaf samples were homogenized using mortar and pestle in the presence of liquid nitrogen, and extracted in 5 mL of 50 mM phosphate buffer, pH 7.0, containing 1 mM ethylenediaminetetraacetic acid (EDTA) and 1% (w/v) polyvinylpyrrolidone (PVP). The extract was centrifuged at 4 °C at 15,000 rpm for 15 minutes. The supernatants were used for determination of catalase (CAT) activity according to the method of Claiborne (1984). CAT activity was determined by the rate of hydrogen peroxide disappearance measured spectrophotometrically at 240 nm. The total protein concentration in the supernatant was determined using the method of Bradford (1976) with bovine serum albumin as a standard.

Free proline content in leaves was measured according to Bates et al. (1973). Leaf samples were homogenized in 3% (w/v) sulfosalicylic acid and centrifuged for 10 minutes at 10,000 rpm. The reaction mixture consisted of 2 mL of supernatant, 2 mL of acid ninhydrin reagent (1.25 g ninhydrin in 30 mL of glacial acetic acid and 60 mL of 6 M phosphoric acid) and 2 mL of glacial acetic acid. Mixtures were boiled at 100 °C for 30 minutes and then they were transferred to an ice bath. Toluene (4 mL) was added to each sample to extract the proline-ninhydrin compound. The absorbance of the chromophore-containing toluene was measured spectrophotometrically (Spectrophotometer DU-65 BECKMAN) at 520 nm. The proline concentration was calculated from a standard curve and expressed on a fresh weight basis as μg proline g^{-1}.

Statistical analysis

The data were processed statistically by the analysis of variance. Differences between treatments were analyzed following Duncan's multiple range test at $P < 0.05$.

Results

Biomass production and plant tolerance to Cd

Cd treatment negatively affected shoot fresh mass in both species (Tab. 1). In *T. aestivum*, a significant reduction of 67.75% was recorded upon Cd treatment in comparison to the control. *Z. mays* shoots mass was lowered in treated plants, by 71.98 and 27.68% at Cd and Cd−Mg treatments, respectively. Shoot mass was less affected by Cd toxicity in both *Z. mays* and *T. aestivum* plants exposed to Mg starvation. In *T. aestivum*, root fresh mass was not affected by Cd, irrespective of Mg supply. A considerable decrease of 35.24% of the *Z. mays* root mass was observed at Cd treatment. The tolerance indices based on the biomass production were higher in plants exposed to Mg starvation (Tab. 1).

Photosynthetic pigments, rates of photosynthesis and transpiration

Chlorophyll *a* concentration was considerably lower in Cd stressed *T. aestivum* and *Z. mays* plants, irrespective of Mg supply (Tab. 2). Chlorophyll *b* was decreased in *T. aestivum* plants exposed to Mg starvation; however, Mg nutrition had no effect on the chlorophyll *b* level in *Z. mays* plants experiencing Cd stress. With respect to control, the chlorophyll *b* concentration was lower in both groups of *Z. mays* plants exposed to Cd stress. Concentration of carotenoids was diminished in both species exposed to Cd.

Tab. 1. Effect of Cd on plant growth and tolerance measured in Mg-supplied (Cd) and Mg-starved (Cd-Mg) *Z. mays* and *T. aestivum* plants. Values are means of five independent measurements ± SD. Data in parentheses present the percentage of control values. Statistically different values in each row are indicated by different letters according to Duncan's test ($P < 0.05$).

	Control	Cd	Cd-Mg
T. aestivum			
Shoot fresh mass (g)	1.12 ± 0.50 a	0.35 ± 0.06 b (31)	0.87 ± 0.06 a (78)
Root fresh mass (g)	0.30 ± 0.12 a	0.22 ± 0.08 a (73)	0.22 ± 0.06 a (73)
Tolerance index (%)	100.00 ± 0.00 a	41.99 ± 10.80 c	80.57 ± 18.27 b
Z. mays			
Shoot fresh mass (g)	5.96 ± 0.53 a	1.67 ± 0.14 c (28)	4.31 ± 0.21 b (72)
Root fresh mass (g)	2.27 ± 0.31 a	1.47 ± 0.26 b (65)	2.08 ± 0.29 a (92)
Tolerance index (%)	100 ± 0.00 a	38.09 ± 3.50 c	77.50 ± 3.98 b

Tab. 2. Effect of Cd on photosynthetic pigments concentration, rate of photosynthesis (A) and transpiration (E), leaf proline accumulation and CAT activity of leaves and roots in Mg-supplied (Cd) and Mg-starved (Cd-Mg) *Z. mays* and *T. aestivum* plants. Values are means of at least three independent measurements ± SD. Data in parentheses present the percentage of control values. Statistically different values in each row are indicated by different letters according to Duncan's test ($P < 0.05$).

	Control	Cd	Cd–Mg
T. aestivum			
Chlorophyll *a* (mg g^{-1} DW)	13.83 ± 0.77 a	7.76 ± 0.80 b (56)	7.32 ± 0.03 b (53)
Chlorophyll *b* (mg g^{-1} DW)	3.80 ± 0.17 a	2.87 ± 0.34 ab (76)	2.26 ± 0.08 b (59)
Carotenoids (mg g^{-1} DW)	3.82 ± 0.18 a	2.43 ± 0.26 b (64)	2.30 ± 0.02 b (60)
A (μmol of CO_2 m^{-2} s^{-1})	24.97 ± 0.29 a	8.92 ± 1.37 b (36)	10.05 ± 1.63 b (40)
E (mmol of H_2O m^{-2} s^{-1})	2.92 ± 0.26 a	2.54 ± 0.34 a (87)	2.51 ± 0.24 a (86)
Proline (μg g^{-1} FW)	4.51 ± 0.62 b	6.77 ± 0.05 b (150)	14.28 ± 1.83 a (317)
CAT activity (U mg^{-1} protein)			
of leaves	1.31 ± 0.29 a	0.96 ± 0.09 a (73)	1.04 ± 0.18 a (79)
of roots	0.14 ± 0.07 a	0.30 ± 0.08 a (214)	0.21 ± 0.07 a (150)
Z. mays			
Chlorophyll *a* (mg g^{-1} DW)	26.10 ± 1.66 a	13.22 ± 0.84 b (51)	14.49 ± 0.09 b (56)
Chlorophyll *b* (mg g^{-1} DW)	6.34 ± 0.34 a	3.89 ± 0.39 b (61)	3.76 ± 0.03 b (59)
Carotenoids (mg g^{-1} DW)	5.38 ± 1.63 a	2.85 ± 1.11 b (53)	4.77 ± 0.28 ab (89)
A (μmol of CO_2 m^{-2} s^{-1})	33.03 ± 1.59 a	27.10 ± 1.80 b (82)	22.92 ± 1.63 c (69)
E (mmol of H_2O m^{-2} s^{-1})	3.47 ± 0.03 a	3.21 ± 0.16 b (92)	3.17 ± 0.15 b (91)
Proline (μg g^{-1} FW)	7.31 ± 1.05 a	6.13 ± 0.51 a (84)	6.36 ± 1.34 a (87)
CAT activity (U mg^{-1} protein)			
of leaves	0.21 ± 0.07 a	0.20 ± 0.08 a (95)	0.30 ± 0.07 a (143)
of roots	0.35 ± 0.07 ab	0.41 ± 0.32 a (117)	0.28 ± 0.03 b (80)

Exposure of *T. aestivum* and *Z. mays* plants to Cd stress accompanied with different levels of Mg elicited a decrease of photosynthetic rate (Tab. 2). The negative effect of Cd on photosynthetic activity was more pronounced in *T. aestivum* (decrease by 64.28 and 59.75% at Cd and Cd–Mg treatments, respectively) than in *Z. mays* (decrease by 47.95 and 30.61% at Cd and Cd–Mg treatments, respectively). With respect to control, transpiration was not considerably changed in *T. aestivum*, while in *Z. mays* plants a decrease of about 8% occurred in both treatments.

Accumulation and translocation of Mg, Cd and Fe

Cd preferentially accumulated in the roots of *T. aestivum* and *Z. mays* plants (Tab. 3). Mg deficiency did not affect Cd accumulation in leaves. Cd concentration in roots was lower in *T. aestivum* plants exposed to Mg starvation in relation to Mg supplied plants, while the op-

Tab. 3. Effect of Cd on magnesium, iron and cadmium concentrations and translocation factors in Mg-supplied (Cd) and Mg-starved (Cd-Mg) *Z. mays* and *T. aestivum* plants. Values are means of three independent measurements ± SD. Statistically different values in each row are indicated by different letters according to Duncan's test ($P < 0.05$).

	Control	Cd	Cd-Mg
T. aestivum			
Magnesium			
Leaves (μg g^{-1})	3571.67 ± 64.44 b	4660.33 ± 49.74 a	708.33 ± 49.79 c
Roots (μg g^{-1})	1819.00 ± 15.00 b	2484.00 ± 6.41 a	962.00 ± 32.06 c
Translocation factor	196.37 ± 4.60 a	187.63 ± 2.46 b	73.64 ± 5.17 c
Iron			
Leaves (μg g^{-1})	396.67 ± 59.32 a	307.00 ± 8.39 a	320.33 ± 16.64 a
Roots (μg g^{-1})	1788.00 ± 17.41 c	3850.00 ± 45.51 b	5026.67 ± 168.39 a
Translocation factor	22.17 ± 3.26 a	7.97 ± 0.14 b	6.38 ± 0.48 b
Cadmium			
Leaves (μg g^{-1})	0.00 ± 0.00 b	818.6 ± 17.02 a	894.00 ± 37.85 a
Roots (μg g^{-1})	0.00 ± 0.00 c	1468.67 ± 34.13 a	1338.67 ± 34.64 b
Translocation factor	0.00 ± 0.00 c	55.73 ± 0.22 b	66.79 ± 2.16 a
Z. mays			
Magnesium			
Leaves (μg g^{-1})	2005.80 ± 127.13 b	3736.13 ± 84.05 a	1156.42 ± 17.75 c
Roots (μg g^{-1})	2460.25 ± 29.00 a	2537.50 ± 351.81 a	751.42 ± 18.38 b
Translocation factor	81.57 ± 6.08 b	149.40 ± 23.49 a	154.00 ± 6.08 a
Iron			
Leaves (μg g^{-1})	345.46 ± 98.08 a	288.58 ± 18.63 a	347.50 ± 57.72 a
Roots (μg g^{-1})	601.92 ± 7.62 c	2037.17 ± 10.85 a	1715.50 ± 181.01 b
Translocation factor	57.53 ± 16.92 a	14.16 ± 0.84 b	20.62 ± 5.52 b
Cadmium			
Leaves (μg g^{-1})	0.00 ± 0.00 b	399.42 ± 6.39 a	416.58 ± 76.94 a
Roots (μg g^{-1})	0.00 ± 0.00 c	2618.17 ± 21.81 b	3611.33 ± 309.87 a
Translocation factor	0.00 ± 0.00 b	15.26 ± 0.37 a	11.72 ± 3.19 a

posite trend occurred in *Z. mays* roots. Differences in root Cd accumulation probably results from variability in translocation of the metal between these species. The translocation factor was higher in Mg-starved *T. aestivum* plants.

In *T. aestivum*, Mg concentration in leaves was higher than in roots in control and Cd-treated plants (Tab. 3). A significant increase of Mg accumulation in *T. aestivum* in both shoots and roots was observed during Cd treatment with respect to control as well in *Z. mays* leaves (Tab. 3). In *T. aestivum* plants experiencing Mg starvation, higher concentration of Mg was measured in roots than in leaves, suggesting lower translocation of the element from roots to shoots (Tab. 3). Accumulation of Fe was stimulated in roots of *T. aestivum* and *Z. mays* plants under Cd stress, and translocation of this element was lower than in control plants.

Catalase activity in leaves and roots

Generally, higher CAT activity has been observed in leaves than in roots of *T. aestivum*, while the opposite trend occurred in Mg-supplied *Z. mays* plants (Tab. 2). CAT activity in *T. aestivum* leaves and roots was unaffected by Cd and Mg supply. However, decreased CAT activity was recorded in *Z. mays* roots in plants exposed to Mg starvation.

Discussion

Disorders in plant growth, photosynthetic activity, chlorophyll and carotenoid concentration, and mineral nutrition occurred in *T. aestivum* and *Z. mays* plants exposed to Cd. Responses of the studied plants to the toxic effect of Cd depended on the Mg supply.

Growth inhibition is the most general non-specific symptom of heavy metal stress (Pál et al. 2006). Heavy metals affect plant growth either through direct interaction with cell wall polysaccharides or indirectly, provoking perturbation of metabolic processes (Seregin and Ivanov 2001). In the present study, Cd affected shoot fresh mass of *T. aestivum* and Z. *mays* plants (Tab.1). Shoot biomass production was lower in Mg-supplied plants (Cd) than in those exposed to Mg starvation (Cd–Mg), suggesting the protective role of Mg deficiency. The results are in line with the findings of CHOU et al. (2011), who examined the effect of Mg starvation on Cd toxicity in rice seedlings, using higher concentration of Cd (5 μmol L^{-1} $CdCl_2$). The authors concluded that Mg starvation protected rice seedlings from the Cd toxicity, estimating the decrease of biomass production and chlorophyll content, and induction of oxidative stress. Root growth is more susceptible to heavy metals than shoot growth, which is correlated with the predominant accumulation of the metals in the roots of many plant species (reviewed in Seregin and Ivanov 2001). In the present work, Cd did not affect root growth in *T. aestivum* plants, irrespective of Mg supply. However, root growth in *Z. mays* was reduced by 35.24% at Cd treatment, with respect to control. Furthermore, the tolerance index, based on biomass production of treated and control plants, suggested higher susceptibility to Cd toxicity in both species cultivated under Mg sufficient conditions, than in plants exposed to Mg starvation. According to the sensitivity scale proposed by Lux et al. (2004), *Z. mays* and *T. aestivum* plants exposed to Cd under optimal Mg nutrition and Mg deficiency showed medium and high tolerance, respectively. The protective effect of Mg deficiency against Cd toxicity could be ascribed to the maintenance of Fe status, the increase in antioxidative capacity, detoxification and/or protection of the photosynthetic apparatus (Hermans et al. 2011).

Cd is a non-redox metal, unable to produce reactive oxygen species (ROS) such as superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\bullet}OH$) via Fenton and/or Haber Weiss reactions (Salin 1988). This metal was found to produce oxidative stress indirectly, by interfering with the antioxidant defense system (Benavides et al. 2005). Disorders in enzymatic and non enzymatic antioxidant mechanisms with concomitant increase in ROS accumulation were observed in *Pisum sativum* exposed to Cd (Rodríguez-Serrano et al. 2006). Cd reduced the glutathione and ascorbate contents, and activities of catalase, glutathione reductase and guaiacol peroxidase. Therefore, maintaining of the balance between ROS generation and scavenging under heavy metal stress contributes to plant tolerance. CAT activity was not considerably changed in *T. aestivum* and *Z. mays* leaves exposed to Cd (Tab. 2). These results are not in line with the findings of Chou et al. (2011), who demonstrated activities of antioxidative enzymes, including CAT, that were higher in Mg-deficient than in Mg-sufficient rice leaves. However, Szalai and co-workers (2005) observed low and unchanged CAT activity in *Z. mays* inbred lines exposed to Cd.

In the present work, a negative effect of Cd and Mg starvation upon CAT activity was evident in *Z. mays* roots (Tab. 2), and the result may be related to decrease of Fe concentration. CAT is a metalloenzyme, containing the porphyrin prosthetic group, and Fe plays an important role in its activity. The strong correlation between the presence of Fe and the activity of the metalloenzymes, CAT and ascorbate peroxidase, has been previously observed in *Borage officinalis* (Mohamed and Aly 2004) and in hydroponically cultivated rice (Chalmardi and Zadeh 2013). The CAT activity was reduced in *Salvinia natans* exposed to Cd (Mohan and Hosetti 2006). Proteins form complexes with Cd, with concomitant changes of the protein conformation and solubility. The authors concluded that the decline in CAT activity in *Salvinia natans* was the consequence of the interaction between the protein and Cd. Considering the role of CAT in the decomposition of toxic H_2O_2 to water and oxygen, decrease of its activity may result in the H_2O_2 increase in *Z. mays* root cells, since Cd stimulates H_2O_2 accumulation (Semane et al. 2010).

Cd is known to affect photosynthetic pigments concentration in numerous plant species (Amirjani 2012). In the present study, cadmium decreased the concentration of leaf chlorophylls and carotenoids in both *Z. mays* and *T. aestivum*, and differences between Cd and Cd–Mg treatments were not statistically significant (Tab. 2). These results suggest that effect of Cd was more noticeable on the chlorophyll and carotenoid concentrations than that of the lack of Mg, although it is the central atom of the chlorophyll molecule. In their work with water plants, Kupper et al. (1998) reported that Cd replaced the central Mg ion in the chlorophyll structure. In the experiment with young *Z. mays* plants, Lagriffoul et al. (1998) concluded that the inhibition of electron transport at the level of the water-splitting complex and simultaneous decrease in chlorophyll pigment contents may have resulted from the interaction between Cd and Mn. Concentration of carotenoids was higher in *Z. mays* leaves during Cd-Mg treatment in comparison to Cd treatment, but these differences were not statistically significant (Tab. 2). Carotenoids are important light-harvesting pigments, able to quench triplet chlorophyll and remove reactive oxygen species, protecting chlorophyll and membranes from destruction (Amirjani 2012). According to the present results, increased carotenoid concentration could have contributed to the higher tolerance of *Z. mays* to Cd toxicity in Mg-starved plants. Furthermore, it has been reported that Cd may disturb Fe metabolism in plants, resulting in induced Fe deficiency despite optimal availability of Fe in the growth substrate (Sidlecka and Krupa 1999). Fe is important in the formation of precur-

sors of the chlorophyll molecule, i.e. δ-aminolevulinic acid and protochlorophyllide (Marschner 1986). In the present study, decreased chlorophylls content in *Z. mays* and *T. aestivum* leaves exposed to Cd was not attributable to Fe deficiency, since the concentration of Fe in leaves of both species was unaffected by the treatments. Previous studies showed that Cd regulates chlorophyll synthesis due to interaction with chlorophyll-synthesizing enzymes. Experiments with *Hordeum vulgare* showed that interaction of Cd with SH-groups may inhibit activity of protochlorophyllide reductase, with concomitant decrease of chlorophyll concentration (Stobart et al. 1985). In the experiments with *Phaseolus vulgaris* L. seedlings treated with different concentrations of cadmium acetate under both light and dark growth conditions, Padmaja et al. (1990) showed that Cd inhibited 5-aminolevulinic acid synthesis and ALA-dehydratase activity.

Cd decreased photosynthetic rate more markedly in *T. aestivum* than in *Z. mays*, irrespective of the Mg supply (Tab. 2). The decline of the photosynthetic rate under Cd stress can result from various factors: the disturbance of chloroplast ultrastructure, synthesis of chlorophyll, plastoquinone, and carotenoids, electron transport, activity of the Calvin cycle enzymes, CO_2 fixation, and stomatal conductance (Ernst 1980, Seregin and Ivanov 2001, Xue et al. 2013). In the present work, a decrease in the photosynthetic activity may be partly due to the reduced chlorophyll content in both species. Previous investigations depicted strong reduction of the stomatal conductance in Cd treated plants (Baryla et al. 2001). The depression of photosynthesis in *Z. mays* plants may be attributed, at least partly, to stomatal closure, considering the decreased transpiration rate in plants exposed to Cd. These results are in line with the report of Bazzaz et al. (1974), who found a direct correlation between the Cd concentration and the inhibition of net photosynthesis and transpiration in *Z. mays*, indicating the impact of Cd on stomatal resistance. In present work, the reduction of growth in *Z. mays* and *T. aestivum* plants exposed to Cd might be the consequence of inhibition of carbon fixation due to a decrease of photosynthetic rate and chlorophyll content, as reported also by Hassan et al. (2005). However, it seems that reduction of the photosynthetic rate in the Cd-exposed *T. aestivum* plants results from non-stomatal limitations, which is in accordance with the observations of Xue et al. (2013) in *Glycine max*.

Cd affects the uptake, translocation, and subsequent distribution of nutrient elements in plants (Tran and Popova 2013). Disorders of mineral nutrition evoked by high levels of Cd lead to nutrient deficiencies, oxidative burst, and consequent depression of plant growth and development (Nazar et al. 2012). Decreased photosynthesis and plant growth strongly correlated with nutrient disorders under Cd exposure (Sun and Shen 2007). It is considered that disbalance in acquisition of nutrients results from the competition of Cd with other cations in their transport across membranes (Llamas et al. 2000). Cd uses the same cation transport systems as the essential elements, because of the lack of specificity of these proteins (López-Millán et al. 2009), such as members of ZIP and NRAMP families (Perfus-Barbeoch et al. 2002). The uptake of Cd seems to occur mainly via Ca^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{2+} transporters (Clemens 2006). However, Cd-induced inhibition of the uptake of macro- and micronutrients relies on the size of metal ion radii and disorders in the cell metabolism with concomitant changes in membrane enzyme activities and membrane structure (Seregin and Ivanov 2001).

In the present study, the Cd concentration was higher in roots of both *Z. mays* and *T. aestivum* than in their leaves (Tab. 3). Nontolerant plants and tolerant excluders mainly accumulate Cd in roots (Verbruggen et al. 2009). Generally, the most of the Cd accumulated

in the root remains in this organ, and only 2% is transported to leaves in higher plants (Xue et al. 2013). There were no considerable differences in Cd concentration of leaves between Mg-supplied and Mg-starved *T. aestivum* and *Z. mays* plants. These results suggest that Mg supply did not considerably affect Cd accumulation in the plant leaves and that the higher tolerance of Mg-starved plants was not related to decreased accumulation of Cd. The obtained results disagree with the findings of Chou et al. (2011), who reported a shoot Cd concentration of Mg–deficient seedlings higher than that of Mg-sufficient rice seedlings. However, additional Mg in the nutrient solution (10 mmol L^{-1}) may enhance the growth of plants suffering from Cd toxicity, due to reduction in Cd concentration in the plant (Kashem and Kawai 2007).

Higher concentration of Cd in roots of Mg-supplied *T. aestivum* plants than in plants exposed to Mg starvation probably results from lower translocation of the heavy metal. In *Z. mays*, the translocation factors have not differed in relation to Mg supply. Higher Cd concentration occurred in plants exposed to Mg deficiency. These results suggest a species-specific response of *Z. mays* and *T. aestivum* plants experiencing Cd toxicity with respect to Mg nutrition. However, differences in Cd distribution within plants occur also between genotypes. In the experiment with *Z. mays* inbred lines Florijn and van Beusichem (1993) distinguished genotypes with higher Cd concentration in the roots than in shoots from genotypes with similar Cd concentration in the shoots and roots. Furthermore, a considerable difference in Cd concentration among plant parts and genotypes, with the highest concentration in roots, was described in *T. aestivum* genotypes in response to Cd addition (Zhang et al. 2002).

In neither species did Cd affect Fe accumulation in the leaves, while a considerable increase occurred in the roots, irrespective of the Mg supply (Tab. 3). A contradiction exists between these results and the findings of other authors. The increase in the Fe concentration of leaves in Cd-treated plants exposed to Mg deficiency was observed by Hermans et al. (2011), while Fodor et al. (2005) reported decreased Fe concentration in shoots of Cd treated *Populus alba*. Enhanced accumulation of Fe in *Z. mays* and *T. aestivum* roots observed in the present work could be the consequence of lower translocation of the element from roots to aerial plant parts. Depression of Fe translocation followed by increased root Fe concentrations has been found in *Cucumis sativus* exposed to Cd (Fodor et al. 1996). Fe limits Cd uptake and translocation, and prevents disturbances in plant growth, photosynthetic activity and photosynthetic pigments accumulation, and alleviates Cd toxicity (Nazar et al. 2012). However, increased Fe concentration in roots of *T. aestivum* and *Z. mays* plants suggests that Cd treatments impeded assimilation of Fe in the roots, considering the same translocation factors for both treatments. Similar observations were reported for *Nicotiana tabacum* plants exposed to Cd (Yoshihara et al. 2006).

In the present work, significant increase of Mg concentrations in leaves occurred in Cd-treated plants, with respect to the control (Tab. 3). However, in *T. aestivum* and *Z. mays* roots, the values were considerably and negligibly increased, respectively, during the same treatment. The literature data are inconsistent regarding the Mg accumulation in certain plant organs. In *Brassica juncea*, Mg concentration of the roots was unaffected, while the concentration of the shoots increased slightly by Cd exposure (Jiang et al. 2004). In a pot experiment with different rice genotypes exposed to Cd stress, Liu et al. (2003) investigated the absorption and accumulation of Cd, Fe, Zn, Mn, Cu and Mg in roots and shoots, at both heading and ripening periods. No significant correlation between Cd and Mg was found in

roots at the two periods. However, while in the leaves Cd showed no significant correlation with Mg at heading, a significant positive correlation appeared at the ripening stage. Soil contamination with cadmium increased the content of Mg and Ca in aboveground parts and roots of *Lupinus arboreus* (Wyszkowski 2002). No significant change in Mg content in *Z. mays* leaves or roots in response to the Cd accumulation was observed (Lagriffoul et al. 1998). The incongruity of the literature data related to disorders in the mineral elements uptake and distribution is probably the result of several factors: specific response of certain species and genotypes exposed to Cd stress, Cd concentration applied, and experimental conditions. Optimal plant nutrition is a good strategy in alleviation of the deleterious effects of Cd on plants and can contribute to the increase of plant tolerance to Cd (Sarwar et al. 2010). Maintenance of uptake and transport of Mg to shoots may contribute to the chlorophyll biosynthesis and protect photosynthetic system under Cd stress (Gomes et al. 2013).

Heavy metals inhibit the formation of root hairs, causing disbalance in plant water relationships (Pál et al 2006). The stimulation of proline accumulation in plants exposed to excess heavy metals has been reported in other species and might be related to Cd induced changes of water regime. Osmolytes, including proline, can improve leaf relative water content, scavenge reactive oxygen species and protect the integrity of membranes (Changhai et al. 2010). In the present study, the accumulation of proline was stimulated by Cd only in *T. aestivum*, irrespective of the Mg supply (Tab. 3). The osmotic adjustment under water-deficient conditions plays a crucial role in maintaining high transpiration rates (Damatta et al. 2003), and this is in agreement with the results obtained in the present work.

In conclusion, Mg starvation contributed to higher tolerance of *Z. mays* and *T. aestivum* plants to Cd toxicity. The results obtained might partly be ascribed to the preservation of Fe nutrition, as reported for other plant species. Further investigations are needed to elucidate other mechanisms involved in alleviation of Cd toxicity in *Z. mays* and *T. aestivum* plants.

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