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Silicon Influence on Maize, *Zea mays* L., Hybrids Exposed to Cadmium Treatment

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Abstract Thirty *Zea mays* L. hybrids were screened using hydroponically—grown seedlings treated in the medium with high cadmium content (100 μM $Cd(NO_3)_2 \cdot 4H_2O$). Measurements showed conspicuous differences between the hybrids in the growth parameters in Cd treated plants. Hybrids differed greatly in Cd accumulation and translocation. Root/shoot ratio in Cd concentration ranged from 2.78 to 12.83. The majority of the heavy metal was localized in the root system. Five hybrids were chosen and the effect of silicon (5 mM) effect on high-level cadmium toxicity symptoms was investigated. Silicon decreased Cd accumulation in roots and its translocation into the shoots.

Keywords Cadmium · Silicon · Maize · Hydroponics

There are ~90 elements present in the earth's crust, of which about 80% are metals and 60% are heavy metals. Heavy metal contamination of soils has become one of the most serious environmental problems nowadays.

Cadmium is a non-essential element that negatively affects plant growth and development and is recognized as an extremely significant pollutant due to its high toxicity and high solubility in water (Pinto et al. 2004). Like many other heavy metals, Cd is not very abundant, but its concentration in agricultural soils has gradually increased over the years due to anthropogenic activities (Clemens 2006).

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The concentration of Cd in soils varies. The soil monitoring system in Slovakia has been developed at the Soil Fertility Research Institute in Bratislava since 1986. The values of trace elements in Slovakia are not particularly high. The average range of Cd is 0.1–0.5 mg kg^{-1} (limit 0.8 mg kg^{-1}) (Kobza 1995), however, in some areas the limit values are exceeded.

Silicon is the second most abundant element in the earth's crust, second only to oxygen and together they make up more than 75% of the earth's crust. Silicon is present in the soils in the form of silicic acid, generally found at concentrations ranging from 0.1 to 0.6 mM. Its concentrations vary greatly in plant aboveground parts, ranging from 0.1 to 10% of the dry weight (Epstein 1994).

Silicon induced alleviating effect of Cd toxicity in some plants has been well documented in some species. The amelioration effect of Si may be correlated with a reduction in the Cd translocation into the shoots. Shi et al. (2005) reported an increasing Cd accumulation in roots of the rice seedlings, due to the Si treatment, and restricted transport of Cd from roots to shoots. Liang et al. (2005) studied a Si—alleviative effect on maize grown in cadmium contaminated soil. Their results suggest that Si can effectively alleviate the Cd toxicity in maize and the effect of Si on Cd toxicity can be attributed not only to Cd immobilization and its low phytoavailability arising from pH—rise in the Si amended soil, but also to the Si—mediated Cd detoxification in plants. Cd was found to be in the form of specific adsorbed or Fe–Mn oxides-bound fraction. Shoot Cd concentration in the Si-amended Cd treatments significantly decreased compared to the non-Si-amended Cd treatments. Total Cd in the shoots and roots was considerably and significantly higher in the Si-amended Cd treatments than in the non-Si-amended Cd treatments because of significantly higher biomass in the Si amended

Cd treatments. Da Cunha and do Nascimento (2009) studied the relationship between the accumulation of Si, Cd, and Zn and the tolerance and structural alterations displayed by maize plants under the influence of these elements. Their results showed that the maize plants treated with Si exhibited not only biomass increase but also higher metal accumulation. Authors also suggest that the deposition of silica in the endodermis and pericycle of roots plays an important role on the maize tolerance to Cd and Zn stress. Vaculík et al. (2009) studied the effect of Si on Cd—treated young maize plants (hybrid Jozefina). Si improved all growth parameters compared with the control and also plants treated in Si + Cd solution exhibited better growth compared to the Cd treatment. However, concentration of Cd in roots and shoots of this maize hybrid and the total amount of Cd per plant were significantly higher in the Si + Cd treated plants than in the Cd treatment. In some species Si doesn't affect the uptake, but can alleviate the toxicity by co-precipitation of Si and metals (Neumann and Zur Neiden 2001).

Maize is one of the world's most important crops, ranking third after wheat and rice, so the changes induced by Cd, one of the most toxic heavy metals, is a cause of some concern. Considering maize as a crop which may be cultivated on contaminated soils, it is very important to minimize the cadmium transport from roots to the shoots and reproductive organs. Another important factor is selection of hybrids and cultivars with low root to shoot translocation.

The first aim of this work was to screen 30 *Zea mays* (L.) hybrids cultivated in Central Europe to investigate the influence of high Cd concentration (100 μM) on seedlings. Differences in the sensitivity, accumulation capability and translocation of cadmium were investigated. The second aim was to examine the effect of silicon (5 mM) on five Cd treated maize hybrids differing in the Cd uptake and translocation capability.

Materials and Methods

Seeds of 30 selected maize (*Zea mays* L.) hybrids providing from Sempol spol. s.r.o. were germinated in filter—paper at 26°C. The following hybrids were used for the screening of their reaction to Cd treatment:

1. Acka
2. Karolína
3. Zuzana
4. Tereza
5. Opoka
6. Maikada
7. Torena
8. Hital
9. Ankora
10. Amanita
11. Novania
12. Szegedi
13. Helena
14. Quintal
15. Mária
16. Erikana
17. Tiara 235
18. Markíza
19. Valentína
20. Bosman
21. Jozefína
22. Omeral
23. Anna
24. Almansa
25. Luciana
26. Reduta
27. Tequila
28. Nimba
29. Leona
30. Nova

After 5 days, the seedlings were transplanted into 8-L plastic pots filled with 0.5 Knop nutrient solution (pH 5.8) as a control and in medium supplied with 100 μM $Cd(NO_3)_2 \cdot 4H_2O$ (Cd) and cultivated for 7 days. All nutrient solutions were replaced after 3 days. To evaluate the effect of silicon on the Cd stress in maize, 5 selected hybrids (Novania, Valentina, Reduta, Almansa and Szegedi) were grown in hydroponics and treated as in the first experiment, as a control, with addition of 100 μM $Cd(NO_3)_2 \cdot 4H_2O$ (Cd), with addition of 5 mM Si (in the form of sodium silicate solution) (Si) and in medium supplied with the combination of Cd and Si (100 μM and 5 mM, respectively) (Si + Cd). Plants were harvested after 7 days of cultivation with 20–25 plants per treatment. The roots and shoots were detached and all roots were thoroughly washed in the distilled water three times. The growth parameters were evaluated. The growth tolerance index (GTI) was calculated as a fresh weight of Cd treated plants to fresh weight of control plants ratio × 100. The samples were dried at 70°C and the percentage of the dry weight was established. Experiment has been replicated for three times.

The concentrations of Cd and Si in shoots and roots were determined by atomic absorption spectrometry (AAS). For Cd dry plant samples were ground in a mortar and dissolved in concentrated HNO_3. After heating at 160°C for 3 h, concentrated HF was added. Thereafter the samples were dried and a mixture of concentrated HNO_3 and H_3BO_3 was added. For Si, dry plant samples were dissolved in concentrated HNO_3 and heated at 150°C for 2 h. After that, H_2O_2 and HF were added and after heating at 150°C for 2 h, H_3BO_3 was added. The Cd and Si concentrations in the roots and shoots were determined by flame atomic absorption spectroscopy (AAS) (PerkinElmer, #1100).

ANOVA was performed by using STATGRAPHICS Centurion XV.I software for statistical significance at $p < 0.05$. Means were separated using LSD (least significant difference) test at 5% level of significance.

Results and Discussion

Thirty *Zea mays* (L.) hybrids were screened to answer the question, if there are any considerable differences in Cd uptake and accumulation or in translocation capability. High concentration of Cd in the nutrient solution and the use of hydroponics do not allow relating the results directly with conditions of field cultivation on polluted soils. However, it allows identifying potentially interesting hybrids for further field experiments. The differences between the hybrids were found in shoots as well as in roots between variants. In the cadmium treated plants, the main noticeable changes were the retarded root growth and decreased leaf areas. The negative effect on the root growth was significant among the hybrids with exception of hybrid Zuzana, Tereza and Jozefína. The primary seminal root growth was reduced from 1.03 (Tereza) to 61.8% (Quintal) (Fig. 1), roots were not only shorter, but also roughen, often with light brown pigmentation. Shoot growth was due to Cd treatment inhibited in all investigated hybrids. At this stage, the plants had two fully developed leaves; the third leaf was variably developed, depending on the variant and hybrid. Leaf blades were often chlorotic or necrotic and the leaf area was reduced by Cd treatment (Fig. 2). The growth reduction in the Cd treated plants was more visible in the case of the second than in the first leaf. The roots in Cd treatment had in all cases higher percentage of dry mass than in control, 10 of hybrids treated by Cd solution had higher d. w. percentage in roots than in the shoots part (Fig. 3). Figure 4 shows the fresh weights of shoots and roots of maize. This parameter was significantly negatively affected by Cd in all hybrids, weight of the roots decreased from 23 (Acka) to 72% (Nova). The fresh weight of shoots decreased from 24 (Jozefína) to 67% (Almansa). The negative effect of Cd treatment was more prominent in roots (the decrease was by 55 and 51% at an average, roots and shoots, respectively). An average growth tolerance index was established for roots (89%) and shoots (97%) (data not shown). Cadmium concentration in the roots and shoots is shown in Fig. 5. The results demonstrated great differences in Cd accumulation among hybrids. The hybrids Maikada and Reduta accumulated the highest amounts of Cd in their roots ($\sim$5,800 mg kg^{-1} d. w.) and hybrid Leona and Tereza translocated the highest amounts of Cd to their shoots ($\sim$700 mg kg^{-1} d.w.). Even though the R/S ratio in Cd concentration ranged from 2.78 (Nova) to 12.83 (Reduta) (Table 1), the majority of the studied heavy metal was localized in the root system and only a small amount was translocated into the shoot. Such screenings have been done previously by several authors using different plant species. Nehnevajova et al. (2005) screen 15 commercial cultivars of sunflower, *Helianthus annuus* L., grown on metal-contaminated soil, to find out the variety with the highest metal extraction. Highly significant differences were observed within tested varieties for metal accumulation and extraction efficiency. Greger

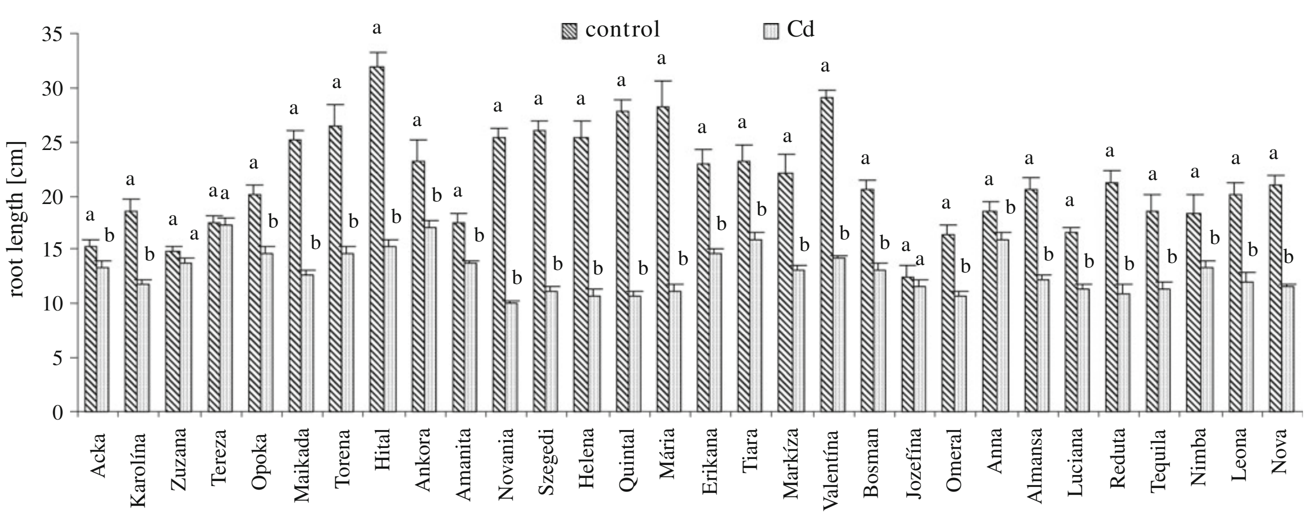

Fig. 1 Length of the primary seminal root in the control and Cd treatment (100 μM) of 30 maize hybrids after 7 days of cultivation in hydroponics. Values are mean ± SE of 15 plants. *Different letters* indicate significant differences between the treatments at 0.05% level

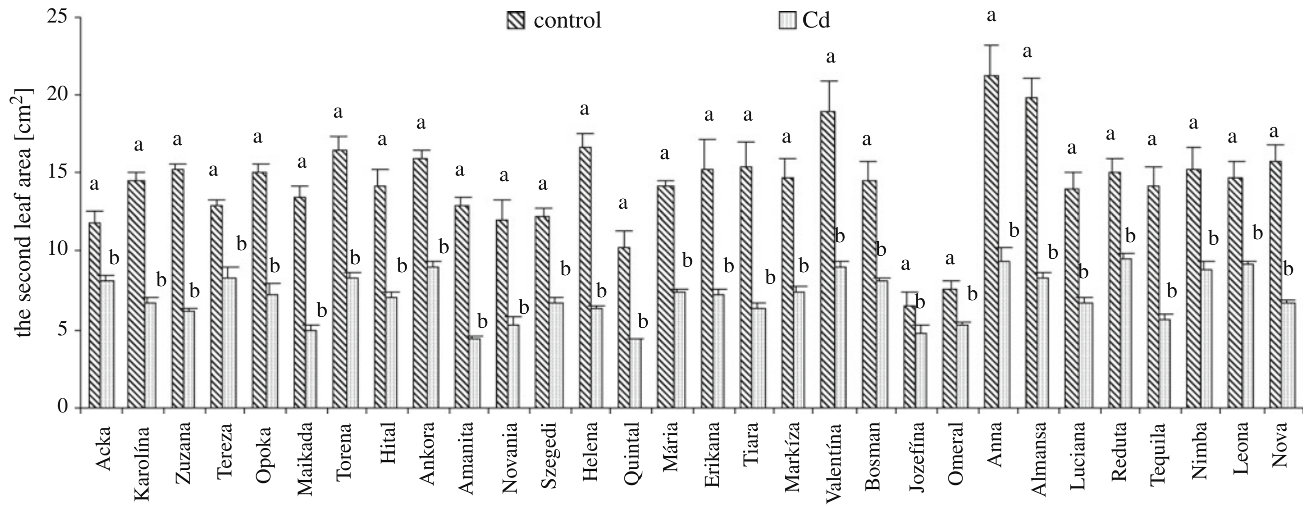

Fig. 2 The second leaf area in the control and Cd (100 µM) treatment of 30 maize hybrids in hydroponics after 7 days of cultivation. Values are mean ± SE of 25 plants. *Different letters* indicate significant differences between the treatments at 0.05% level

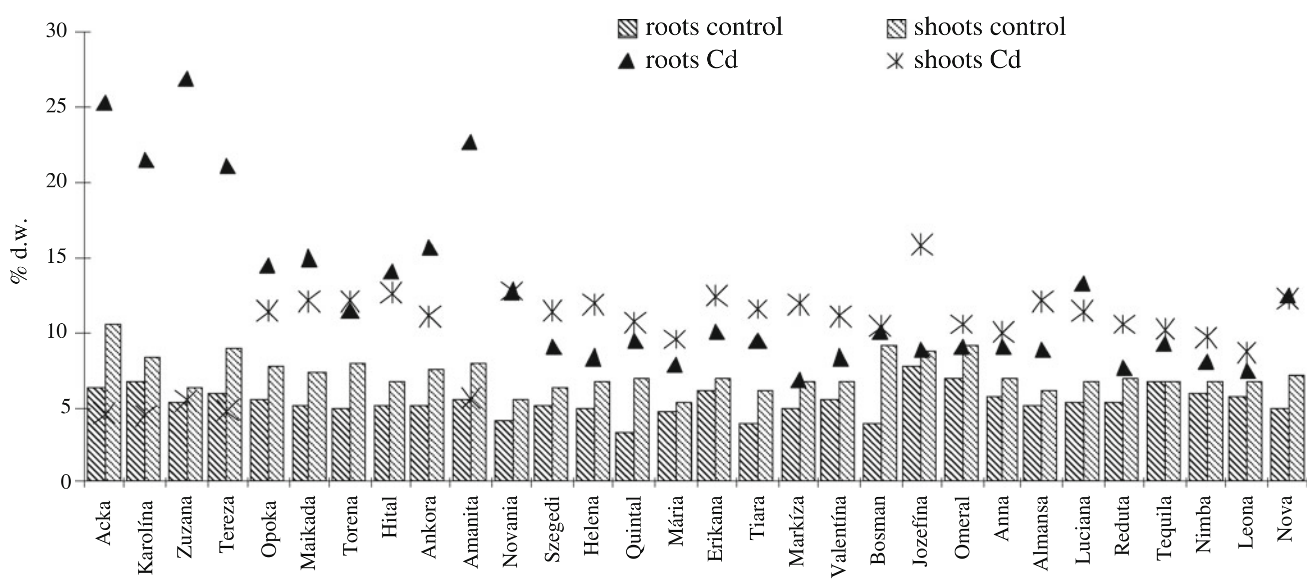

Fig. 3 The percentage of the dry weight of 30 maize hybrids in control and plants treated with Cd (100 µM) after 7 days of cultivation in hydroponics

and Löfstedt (2004) compared uptake and distribution of cadmium in different cultivars of bread and durum wheat and conclude that the variation between the cultivars in each wheat type was greater than the differences between the wheat types. Genotypic variation in uptake and distribution of cadmium in maize inbred lines were studied by Florijn and van Beusichem (1993). The Cd concentration in the shoots showed large genotypic variation and two main groups of imbreds were distinguished: a group with low shoot, but high root Cd concentrations ('shoot Cd excluder') and a group with similar shoot and root Cd concentration ('non-shoot Cd excluder'). In the present study hybrids Opoka, Tequila, Karolina, Luciana, Szegedi and Reduta with highest Cd accumulation in roots, low accumulation in shoots and highest R/S ratio including, can be considered as a 'shoot Cd excluders' (Fig. 5, Table 1). The role of roots in differences in seed cadmium concentration among soybean cultivars was investigated by Sugiyama et al. (2007). Authors concluded that there were differences among cultivars in translocation of Cd to the seed and in Cd accumulation capacity of the roots. The amount of Cd accumulated in shoots was determined by the Cd accumulation capacity of roots: cultivars with small capacity to accumulate Cd in roots translocated more Cd and accumulate it in shoots. Greger and Löfstedt (2004) considered, that the differences that were found between Cd

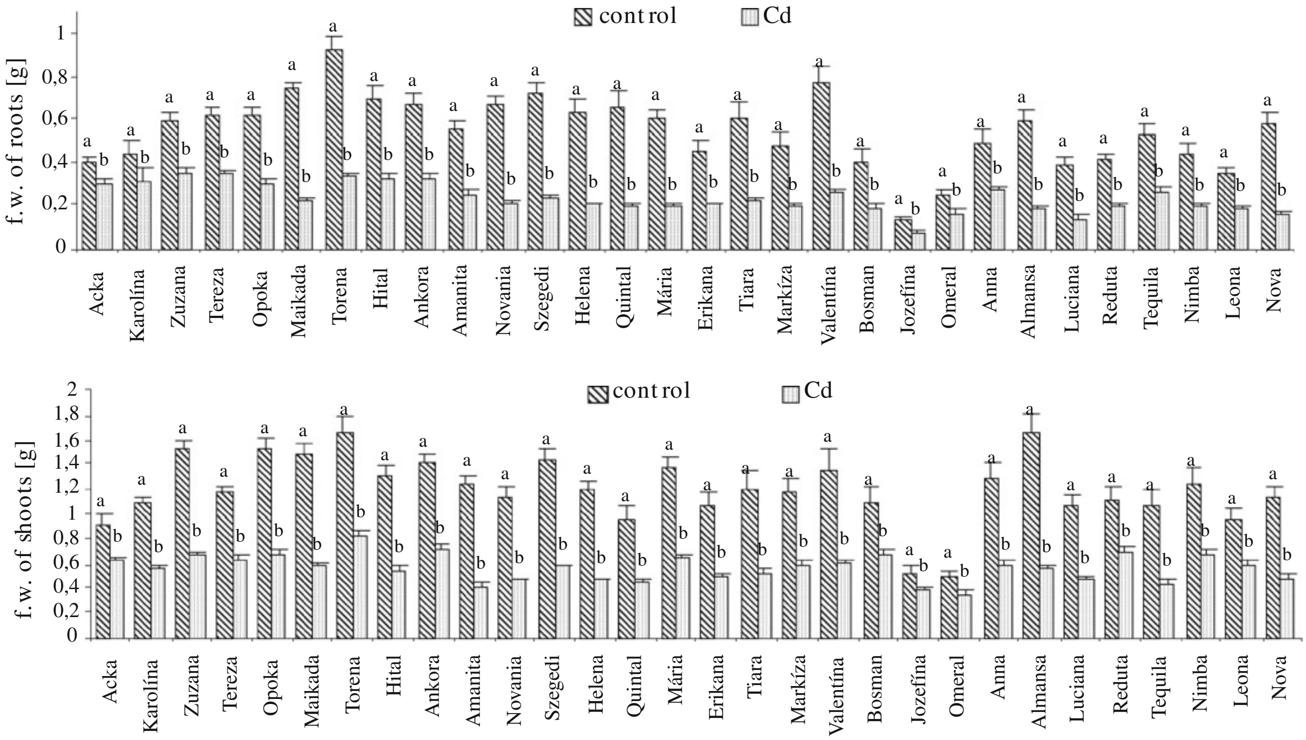

Fig. 4 The fresh weight of roots and shoots of 30 maize hybrids treated as a control and in Cd (100 μM) solution in hydroponics for 7 days. Values are mean ± SE of 25 plants. *Different letters* indicate significant differences between the treatments at 0.05% level

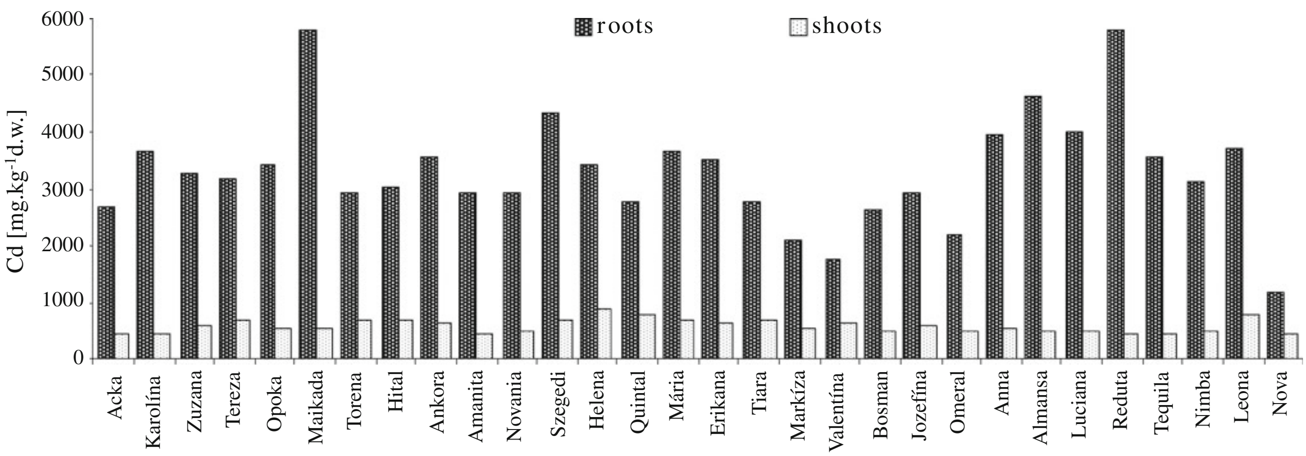

Fig. 5 The Cd content in plants of 30 maize hybrids treated with 100 μM Cd solution in hydroponics for 7 days

Table 1 The root/shoot ratio in Cd concentration of 30 maize hybrids treated with 100 μM Cd solution in hydroponics for 7 days

Acka	6.34	Torena	4.54	Helena	3.92	Valentína	2.84	Luciana	7.98
Karolína	8.04	Hital	4.71	Quintal	3.59	Bosman	5.21	**Reduta**	**12.83**
Zuzana	5.58	Ankora	5.66	Mária	5.64	Jozefína	5.27	Tequila	7.62
Tereza	4.38	Amanita	6.55	Erikana	5.79	Omeral	4.3	Nimba	6.4
Opoka	6.25	Novania	5.84	Tiara	4.11	Anna	7.52	Leona	4.81
Maikada	10.91	Szegedi	6.65	Markíza	3.93	Almansa	9.08	**Nova**	**2.78**

The two hybrids in bold represent the most extreme values accumulation in grains of bread and durum wheat were due to variation in the translocation of Cd into the shoots, rather than to Cd uptake in the roots. The Cd tolerance of shoots in maize hybrids investigated by us differed among cultivars (Figs. 2, 3, 4). Sugiyama et al. (2007) found a relationship between Cd accumulation capacity of roots and Cd tolerance of shoots of soybean cultivars and concluded, that cultivar with high Cd accumulation capacity of roots had a low Cd tolerance of shoots. Using the correlation analysis, no correlation between the Cd accumulation and growth of plant parts was found in the present study, thus no relation between the sensitivity and Cd accumulation and translocation capability was recognized in maize.

Silicon is beneficial element for many plants. It is known, that silicon deposits in inner tangential walls of root endodermal cells in some species (Lux et al. 2003) where it can act as a physical barrier, but it's also a modulator of abiotic stress and host resistance. Liang et al. (2005) found out, that after addition of Si into the contaminated soil, Cd was presented in the form of specific adsorbed or Fe–Mn oxides bound fraction. An alleviating effect of silicon on the heavy metal toxicity has been reported; however, the mechanism is still poorly understood. Focusing on the root Cd content and R/S ratio, five contrasting hybrids were chosen in the present study (Novania, Valentina, Reduta, Almansa, Szegedi). Valentina had the lowest R/S ratio (2.84) followed by Novania, Szegedi, Almansa and Reduta with the highest R/S ratio (12.83) (Table 1). Additionally, Valentina and Novania belong to the hybrids accumulating the lowest amounts of Cd in their roots, while hybrid Szegedi, Almansa and Reduta to hybrids with highest root Cd accumulation capacity (Fig. 5). The GTI of roots of Cd treated plants was 34% in an average while the GTI of Si + Cd treated plants was 29% in an average. Only hybrids Novania and Valentina exhibited the slight increase in the GTI of Si + Cd treated plants. The increase of GTI was notable in the shoots (43 and 47%, Cd and Si + Cd treated plants, respectively), while the most prominent increase of this parameter occurred in hybrid Almansa (data not shown). The growth of the primary seminal root in the Si + Cd variant comparing to the Cd variant was not affected positively (hybrid Novania, Valentína, Almansa and Szegedi) (Fig. 6). The root growth promoting effect of Si compared to Cd treatment was achieved in the Si + Cd treatment only in the hybrid Reduta (the increase was in 16%). The root system of Cd treated plants was shorter and contained less lateral roots than those treated in the control medium and with silicon supplementation. As already mentioned, the primary seminal root was due to Cd treatment roughen and had often altered pigmentation (yellow brown), however, in the Si + Cd treatment, less pigmentation of roots was observed comparing with the control. All hybrids

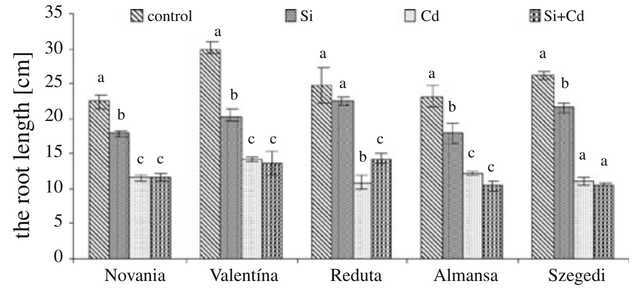

Fig. 6 The primary seminal root length of five maize hybrids treated with Cd (100 μM), Si (5 mM) and Si + Cd solution. Values are mean ± SE of 20 plants per replicate. Three replicates have been done. *Different letters* indicate significant differences between the treatments at 0.05% level

achieved higher percentage of the dry weight in Si, Cd or Si + Cd variant than in the control (Fig. 7). The increase of the percentage of the d.w. was more notable in the Cd or Si + Cd treated plants compared to Si treatment. The highest value of the percentage of the d.w. in the Si + Cd treated plants had hybrids Valentina and Reduta (in roots) and Reduta and Almansa (in shoots). According to Sridhar et al. (2007) in Cd treated barley plants, statistically significant negative correlation was found only between Cd accumulation in shoots and roots to water content. More visible leaf area reduction occurred by Cd treatment on the

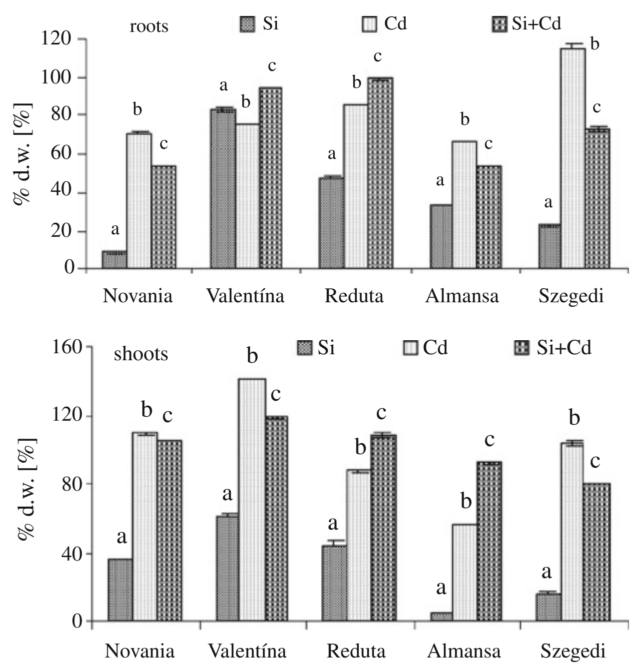

Fig. 7 The percentage of the dry weight of the roots and shoots of five maize hybrids treated with the Si (5 mM), Cd (100 μM) or Si + Cd for 7 days in hydroponics. The values express the increase of the parameter of plants with altered cultivation compared to the control plants calculated as the 100% + the increase of the percentage of the d.w. *Different letters* indicate significant differences between the treatments at 0.05% level

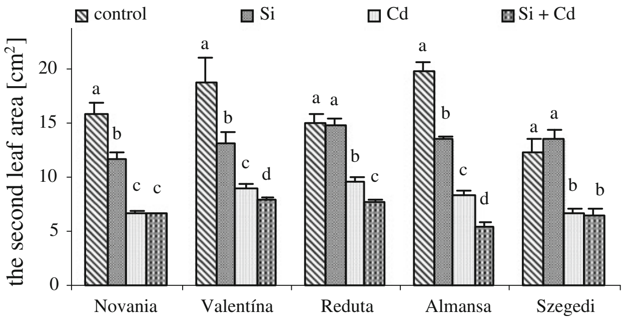

Fig. 8 The area of the second leaf of five maize hybrids treated with Si (5 mM), Cd (100 μM) or Si + Cd in hydroponics for 7 days. Values are mean ± SE of 20 plants per replicate. Three replicates have been done. *Different letters* indicate significant differences between the treatments at 0.05% level

second leaf (Fig. 8). Relatively small amount of Cd was translocated from roots to the shoot. While root Cd content varied from 1,861 ± 537 (Novania) to 3,369 ± 350 (Szegedi) mg kg^{-1} d.w, plants translocated only from 317 ± 21 (Szegedi) to 421 ± 69 mg kg^{-1} (Reduta) Cd to their shoots. Hybrids accumulated from 17.3 ± 2 (Reduta) to 23 ± 8% (Almansa) of Si in the roots and 3.6 ± 0.7 (Szegedi) to 5.3 ± 1.1% (Reduta) in the shoots (Fig. 9). Addition of silicon to the medium containing Cd reduced Cd content from 12% (Almansa) to 43% (Reduta) (in roots) and from 6 (Valentina) to 35% (Reduta) (in shoots). Silicon content in Si + Cd treatment was also decreased in the roots and shoots in comparison with Si treated plants; the decrease was more intensive in the shoots than in the roots. The silicon content was reduced from 15 (Valentina) to 59% (Novania, Szegedi) (in roots) and from 42 (Valentina) to 75% (Almansa) (in shoots) in the Si + Cd treatment compared to Si treatment (Fig. 10). Concerning the alleviating effect of silicon on abiotic stress caused by heavy metals many authors confirmed the decreasing Cd accumulation in roots and shoots in the medium enriched with silicon. Silicon induced inhibition of Cd uptake in rice (Nwugo and Huerta 2008). Shi et al. (2005) reported amelioration of negative effect of Cd by addition of Si in rice plants, which was correlated with a reduction in Cd uptake. However, Cd concentration in below and above-ground plant parts and the total amount of Cd per plant in a specific maize hybrid Jozefina, were significantly higher in the Si + Cd plants than in the Cd treatment in the study of Vaculík et al. (2009). In the present study, only in one hybrid—Almansa—such increase of the Cd content per the whole plant was confirmed (Fig. 11). This phenomenon can be explained as a hybrid or varietal specific reaction (Table 2).

Metal pollution is a global problem. To prevent the unwanted effects of heavy metals and to ensure the foot safety it is necessary to understand their physical and chemical behavior in the soil and the accumulation capability of the metals which varies between the plants species. In the present study it was found that maize hybrids

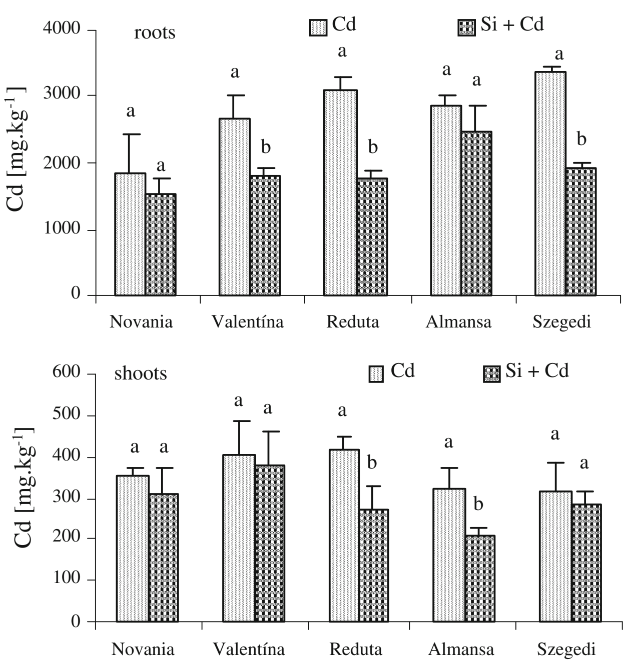

Fig. 9 The cadmium content in roots and shoots of five maize hybrids cultivated in the 100 μM Cd and 5 mM Si + 100 μM Cd solution for 7 days. Values are mean ± SE of three replicates with 20 plants per replicate. *Different letters* indicate significant differences between the treatments at 0.05% level

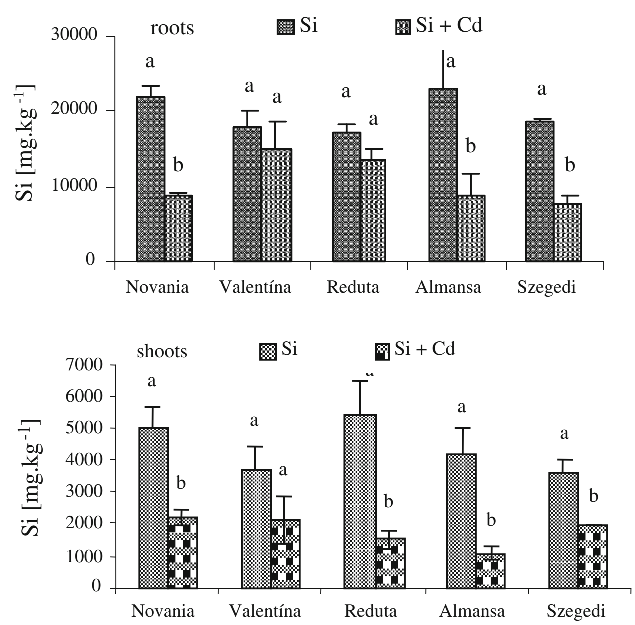

Fig. 10 The silicon content in roots and shoots of five maize hybrids cultivated in the 100 μM Cd and 5 mM Si + 100 μM Cd solution for 7 days. Values are mean ± SE of three replicates with 20 plants per replicate. *Different letters* indicate significant differences between the treatments at 0.05% level

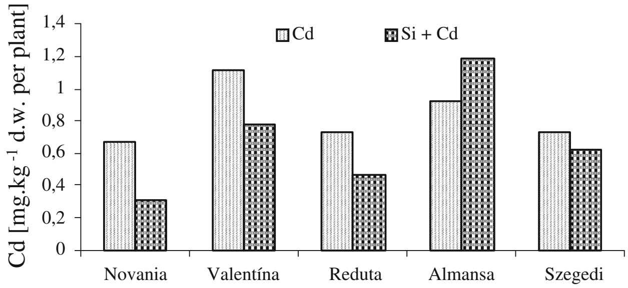

Fig. 11 The cadmium content calculated per the whole plant of five maize hybrids cultivated in the 100 μM Cd and 5 mM Si + 100 μM Cd solution for 7 days

Table 2 The influence on root/shoot ratio comparing Cd (100 μM) and Si + Cd treatment (5 mM + 100 μM) of five maize hybrids cultivated in hydroponics for 7 days

	R/S ratio in Cd variant	R/S ratio in Si + Cd variant
Novania	5.2	4.9
Valentina	6.6	4.8
Reduta	7.4	5.4
Almansa	8.7	11.5
Szegedi	7.3	10.1

differ considerably in their characteristics of Cd accumulation in roots and translocation of Cd to the shoots. In was found also, that a great variability exists in response of maize plants to alleviating effect of Si on Cd treated plants. Variability in responses of maize to both Cd and Si + Cd treatment underlay the necessity of care to be taken in generalization of the effect of these ions on plant behavior. Great differences in reaction to Cd, and Si + Cd exist not only between the species but also intraspecifically; this can be used in agronomical praxis.

It can be concluded, that there is great variability in the reactions of maize hybrids to Cd toxicity. From the results obtained in this study it emerges also, that Cd interferes with the plant metabolism resulting in reduced Si uptake by plant roots; an active process via transporters characterized by Mitani et al. (2009).

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