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Cadmium uptake, localization and detoxification in *Zea mays*

M. WÓJCIK and A. TUKIENDORF*

Department of Plant Physiology, Institute of Biology, Maria Curie-Skłodowska University, Akademicka 19, PL-20033 Lublin, Poland

Abstract

Cadmium uptake, translocation and localization in maize roots and shoots at the tissue and cellular level were investigated. Metal accumulation in plant organs as well as symptoms of Cd toxicity were closely correlated with an increase in Cd concentration applied (5 - 300 μM). Most of the metal taken up was retained in roots, mainly inside the cells of endodermis, pericycle and central cylinder parenchyma. Accumulation of phytochelatins and related peptides also depended on Cd concentration in the nutrient solution.

Additional key words: energy dispersive X-ray microanalysis, maize, phytochelatins.

Introduction

Elevated concentrations of both essential and nonessential heavy metals in the soil pose a serious environmental problem. Among them cadmium (Cd) appears to be one of the most dangerous element to all kinds of organisms (Sanitá di Toppi and Gabbrielli 1999). Although considered to be a nonessential element for metabolic processes, it is easily absorbed by plants and even in small amounts it causes toxicity symptoms. The toxic effect of Cd on plants is wide-spread and various and was extensively reviewed (Ernst 1980, Krupa and Baszyński 1995, Sanitá di Toppi and Gabbrielli 1999, Siedlecka *et al*. 2001). The most spectacular symptoms of Cd toxicity are: growth retardation, chlorosis and necrosis of leaves, red-brown coloration of leaf margins or veins. Cadmium changes root morphology, root and leaf anatomy, damages cell structures. It disturbs water balance, mineral nutrition, photosynthesis, respiration and plant development in general (Prasad 1995).

Plants have developed different mechanisms to deal with the excess of heavy metals in soils. They can prevent or restrict the uptake of metals through the root and into the protoplast (stress avoidance) or minimize the toxic effects of metal ions inside the protoplast (stress tolerance) (for review see Verkleij and Schat 1990, Ernst *et al.* 1992, Siedlecka *et al.* 2001). In the case of Cd, stress avoidance mechanisms are rather of minor relevance. They include metal retaining by mycorrhizal fungi on the root surface, its immobilization in cell wall, deposition in trichomes or removing it from plant organism with seasonally shedded leaves (Punz and Sieghardt 1993, Wagner 1993, Salt *et al*. 1995). Stress tolerance mechanisms are much more important and they rely on complexation of Cd ions in cytoplasm with organic acids, amino acids, cysteine rich peptides (PCs) or their compartmentation in the vacuole in the form of Cd-PC complexes, organic acids or phytate (Wagner 1993, Prasad 1995, Rauser 1999). In many plant species apart from PCs, of the general formula $(\gamma\text{-Glu-Cys})_n\text{-Gly}$, their structural variants called isophytochelatins are accumulated where the C-terminal Gly is replaced by β-alanine, serine, glutamate or glutamine (iso-PC-β-Ala, -Ser, -Glu, -Gln, respectively) or Gly is absent (desGly-PCs) (for references see Rauser 1995, Kubota *et al*. 2000). These commonalities in the structure between peptides lead to their collective designation as γ-Glu-Cys peptides. Phytochelatins are one of specific families of γ-Glu-Cys peptides but the trivial name "phytochelatins" for all five families of these peptides is used. Apophytochelatins (Apo-PCs) and their complexes with cadmium (PC-Cd complexes) synthesized in the cytosol in the form of LMW Cd-PC complexes, are transferred to the vacuole (by the transporter HMT1), where Cd

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Abbreviations: EDAX - energy dispersive X-ray microanalysis; HPLC - high performance liquid chromatography; PC - phytochelatins.
* Corresponding author; fax: (+48) 81 537 5901, e-mail address: antuken@biotop.umcs.lublin.pl

(transported by the Cd/H^+ antiporter in the tonoplast) and labile sulfide are added to form the HMW Cd-PC complexes – essential for maximal detoxification of Cd (Ortiz *et al*. 1995, Clemens and Simm 2003). Deposition of Cd in the vacuole further prevents its toxicity to plant metabolism and also its translocation within the plant.

The knowledge of the metal distribution in plant organs and cell compartments is important to better understand the tolerance mechanisms in plant species. Studies on the localization of heavy metals in plants, especially at the subcellular level, have recently attracted the attention of many scientists. New and more technically advanced methods of metal detection and sample preparation are still developed. Among them, energy dispersive X-ray microanalysis (EDAX) following glutaraldehyde fixation with sulphide supplement or freezing of the plant sample, is frequently used. It allows the precise localization of many elements in the cell compartments, although is not always useful at a very low metal concentrations.

Maize is a common and one of the most important agricultural crop and it is also used in many studies of elemental pollution and serves as a model system for studies on PCs. Maize is also a very interesting species due to its potential usefulness in phytoremediation of the areas contaminated with heavy metals, especially in one of the phytoremediation technologies – induced hyper-accumulation.

The aim of this study was to investigate cadmium uptake, translocation and localization in maize roots and shoots at the tissue and cellular level. Maize plants were grown in hydroponics and exposed to cadmium concentrations from values comparable to pore water concentrations on contaminated soils up to cadmium ranges not occurring on metal polluted soils but used in the studies on induced hyperaccumulation. We also examined the accumulation of phytochelatins and related peptides in Cd-treated plants and discussed their role in Cd detoxification.

Materials and methods

Plants: Seeds of maize (*Zea mays* L. cv. Hidosil) were germinated for 5 d in moisture filter paper and then seedlings were transferred into Hoagland solution (9 seedlings × 1.4 dm^3 solution in a pot). The plants were cultivated in a vegetation room at 23/17 °C (day/night) with 16-h photoperiod at photosynthetic photon flux density of 150 μmol m^{-2} s^{-1} and relative humidity of 70 %. After 2-d acclimation phase in Hoagland and Arnon nutrient solution Cd (5 - 300 μM) as $Cd(NO_3)_2 \cdot 4\ H_2O$ (*Sigma-Aldrich,* Steinheim, Germany) was added. The plants were analysed after 14 d since the metal addition. The medium was maintained at a constant volume by adding distilled water and continuously aerated during experiment.

Determination of cadmium content: Cadmium content in roots and shoots of seedlings was determined by atomic absorption spectrophotometry (*Perkin Elmer model 3300*, Norwalk, CT, USA) after wet-ashing of dry matter in a $HNO_3/HClO_4$ mixture (4:1, v/v).

Samples preparation for electron microscopy analysis and cadmium localization by EDAX: Plants grown in the presence of 300 μM Cd were used for electron microscopy analysis. Fragments of roots (apical segments approximately 5 mm in length) and leaves (with visible symptoms of cadmium toxicity) were fixed in 2 % glutaraldehyde in 50 μM buffer Na-PIPES, pH 7.5. To precipitate heavy metals in tissues and prevent their washing out or dislocation during further preparation procedure, the fixing mixture was added Na_2S (1 %, m/v). Fixation was conducted for 4 h at 4 °C. The samples were then rinsed in fresh buffer and dehydrated in ascending series of ethanol. After dehydratation the preparations were gradually saturated with LR-White resin (*Sigma-Aldrich*). Then the material was embedded in fresh activated LR-White resin and kept at 50 - 55 °C for 24 h. The embedded plant material was cut into thin sections (100 nm) with diamond knives and an ultramicrotome *Reichert Ultracut S* (Vienna, Austria). The specimens were used for X-ray microanalysis in a *JEOL JEM 1200EX* (Peabody, MA, USA) transmission electron microscope with a *LINK AN 10 000* (*Link Systems*, High Wycombe, Buckinghamshire, UK) energy dispersive X-ray microanalyser. The microanalysis was performed at an accelerating voltage of 80 kV with a take-off angle of 45°. Spectra from 0 to 10 keV with Cd Lα peaks were recorded after 200 s. Detection limit was 0.1 - 0.5 %.

Analysis of γ-Glu-Cys peptides by HPLC method: Roots and shoots were weighted and ground in a cooled mortar with a double volume of 0.1 M HCl. Homogenates were centrifuged at 14 000 *g*, and the obtained supernatants were used for chromatographic analyses.

A *Beckman* (Fullerton, USA) chromatograph (*model 126/166*) with a *Supelco* precolumn (4.6 × 10 mm) and column (4.6 × 250 mm), both filled with *Ultrasphere C-18*, were used. Samples were separated in acetonitrile (ACN, *Sigma-Aldrich*) linear gradient (0 - 20 %) in 0.05 % trifluoroacetic acid (TFA, *Sigma-Aldrich*) for 40 min at a flow rate of 1 cm^3 min^{-1}. Next, the column was washed with 50 % acetonitrile and equilibrated with 0.05 % TFA.

Separated peptide fractions flowing out of the column were directed into a mixer (0.001 cm^3) and mixed with 200 μM Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid, DTNB, *Sigma-Aldrich*) in 0.05 M potassium-phosphate buffer, pH 7.6 (0.5 cm^3 min^{-1}). The absorbance of the products of DTNB reactions with -SH groups was measured at 405 nm using a *Beckman* detector (*model 166*). The retention times and peak areas were determined with a computer program *Gold Nouveau* (*Beckman*). Peaks on chromatograms were identified as described previously by Wójcik and Tukiendorf (1999).

Statistical analysis: The data for the growth parameters and for Cd content were analysed from 4 replications, whilst X-ray microanalysis and analysis of thiol peptides were performed twice (always 9 plants were used per repetition). Means and standard errors (SE) were calculated using *SigmaStat 2.0* program with one-way *ANOVA* test for comparing the significance of the differences between means ($P < 0.05$).

Results

Cadmium accumulation in plants: Cadmium accumulation both in roots and in shoots of maize seedlings increased almost lineary with increasing Cd concentration in nutrient solution (Fig. 1). Its amount in roots increased over 3-fold and in shoots over 6-fold when comparing 5 and 300 μM Cd treatment. The roots accumulated higher amounts of Cd (73 - 85 %) than the above-ground parts (15 - 27 %), although at higher Cd concentration a tendency of sligthly enhanced Cd translocation to the shoots was observed.

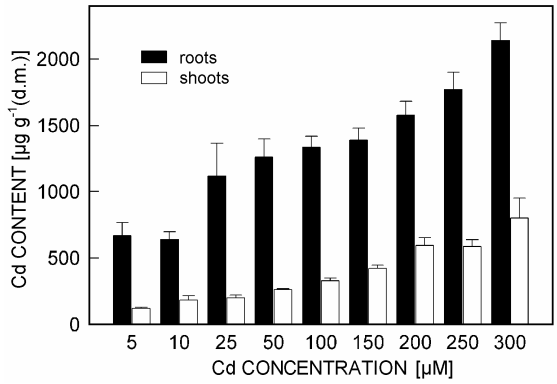

Fig. 1. Cadmium accumulation in maize plants. Means of four replicates (9 plants in each) ± SE.

Cadmium toxicity: Growth of maize seedlings depended on the Cd concentration used (Fig. 2). An increase in Cd concentration from 5 to 300 μM resulted in increased inhibition of root elongation (from 12.3 to 91 %), root fresh mass (from 12.7 to 98.8 %) and shoot fresh mass (from 11.5 to 78.2 %), compared with control plants. Also other morphological changes of plant appearance, especially at metal highest concentrations, were observed. They include: drying of older leaves, chlorosis and necrosis of young leaves, reduction in the number and length of lateral roots, their unnatural sidewards protrusion, browning of roots. Apart from these morphological symptoms of Cd toxicity on maize seedlings, some destructive changes in root and shoot ultrastructure were also shown. The highest Cd concentration (300 μM) induced serious damages to cells of root cortex parenchyma, endodermis, pericycle and, to a lesser extend, other stelar tissues. These damages were often visible in electron microscopy as protoplast complete destruction with fragments of destroyed membranes and nuclei relatively the best preserved (Fig. 3*A*,*B*,*C*), sometimes only as slight plasmolysis (Fig. 3*D*). The changes in ultrastructure of leaf cells were similar but much less pronounced (Fig. 5).

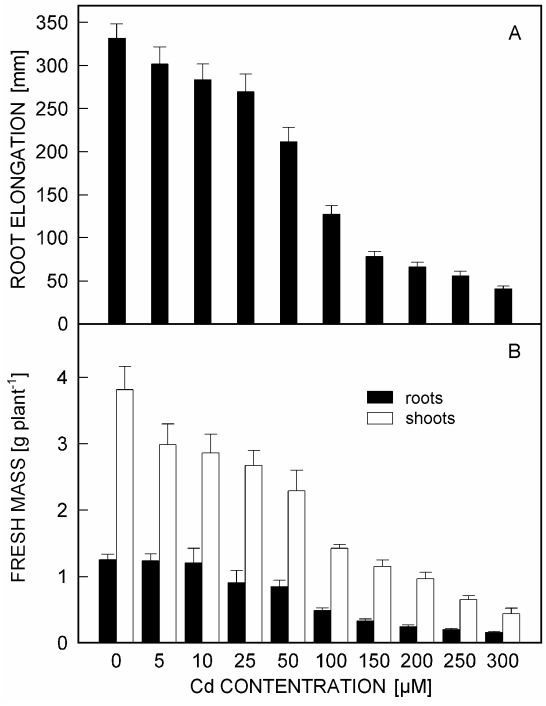

Fig. 2. Root elongation (*A*) and fresh mass (*B*) of control and Cd-treated maize plants. Means of four replicates (9 plants in each) ± SE.

Cadmium localization: EDAX analyses of root cross sections did not show Cd presence in epidermis, while in cortex parenchyma cells only its trace amounts were

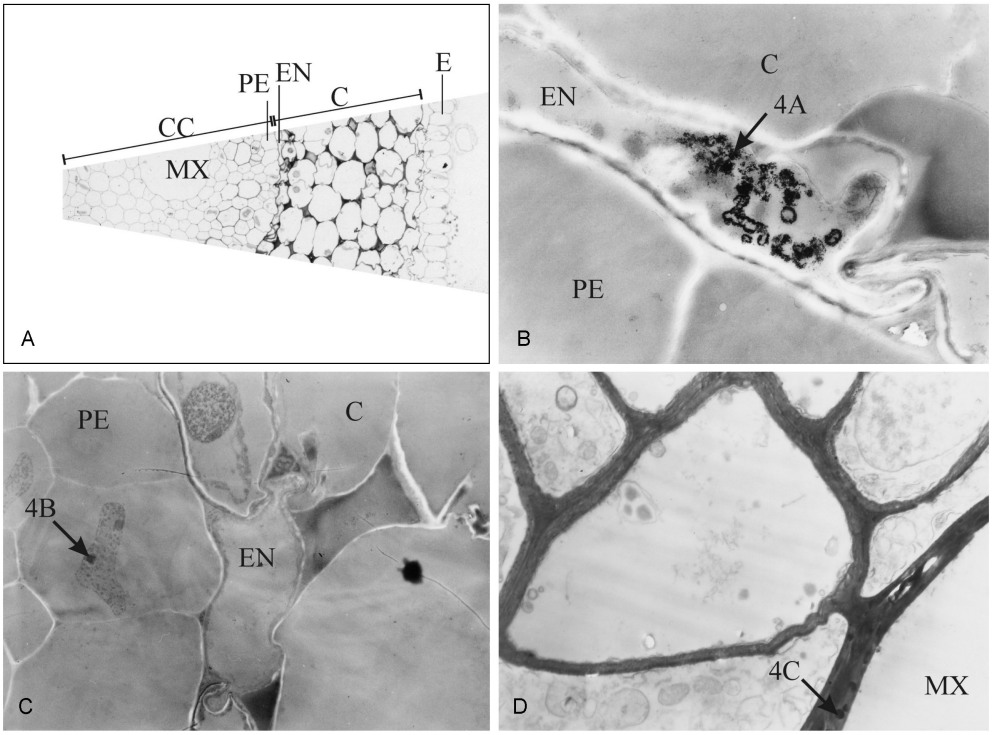

Fig. 3. Fragments of root cross-sections of maize exposed to 300 μM Cd. *A* - light microscope micrograph, *B*, *C*, *D* - transmission electron microscopy micrographs. E - epidermis, C - cortex, EN - endodermis, PE - pericycle, CC - central cylinder, MX - metaxylem. *Arrows* indicate localization of Cd-rich sites (of which EDAX spectra are presented in Fig. 4). *A* - 400×, *B* – 4000×, *C* - 1500×, *D* - 4000×.

detected. Relatively great amounts of Cd were found in endodermis - in dark deposits being the left-over of degenerated protoplasts and nuclei, as well as in deposits scattered along the cell walls (Figs. 3*B*, 4*A*). Large amounts of cadmium were also measured in cells of pericycle, mainly in their nuclei (Figs. 3*C*, 4*B*), in metaxylem cell walls (Figs. 3*D*, 4*C*) and inside some parenchyma cells adjacent to the xylem (not shown in the photography, Fig. 4D). In other cells of the central cylinder cadmium was not detected at all or only in trace amounts.

In the cross sections of maize leaves, relatively largest Cd amounts were detected inside the cells of lower (Figs. 5*A*, 6*A*) and upper epidermis (Figs. 5*B*, 6*B*). Cd was also found in electron-dense granules localized in vacuoles of mesophyll cells surrounding the vascular cylinder (Figs. 5*C*, 6*C*) and in spongy mesophyll cells (Figs. 5*D*, 6*D*). In leaves, the detected metal was always present in dark deposits within protoplasts (vacuoles) of the observed cells and it was not found in the cell walls or cuticle layers on the leaf surface.

Dark Cd-bearing deposits found in cells of maize roots and shoots contained also different concentrations of other elements, such as S, P, Cu, Ca, Fe (Figs. 4, 6).

Phytochelatin accumulation: In Cd treated maize plants 3 families of thiol peptides were determined: PCs - (γ- Glu-Cys)$_{2-4}$-Gly, iso-PCs(Glu) - (γ-Glu-Cys)$_{2-3}$-Glu and desGly-PCs - (γ-Glu-Cys)$_{2-3}$. In control plants these peptides were absent or only trace amounts of PCs (n = 2) appeared (data not shown).

Accumulation of thiol peptides in plants increased with increasing Cd concentration in the nutrient solution (Fig. 7). This was apparent for the total pool of these peptides as well as for their particular families of which desGly-PCs were the most abundant (40 - 53 % in roots and 44 - 63 % in shoots). The amounts of PCs and iso-PCs(Glu) were similar. PCs constituted 23 - 34 % in roots and 15 - 33 % in shoots, whereas iso-PCs(Glu) 15 - 32 % in roots and 14 - 37 % in shoots of the whole amount of the thiol peptides determined in maize plants. In the whole range of Cd concentrations, the predominating forms of PCs and desGly-PCs (both in roots and in shoots) were short-chain peptides (n = 2). However, the amount of longer-chain derivatives (n = 3 for desGly-PCs and n = 3 - 4 for PCs) continuously increased with increased Cd concentration applied. Iso-PCs(Glu) were accumulated mainly in the form n = 3. The accumulation of thiol peptides was higher in roots than in shoots with 52 - 74 % of all peptides determined in roots (Fig. 7).

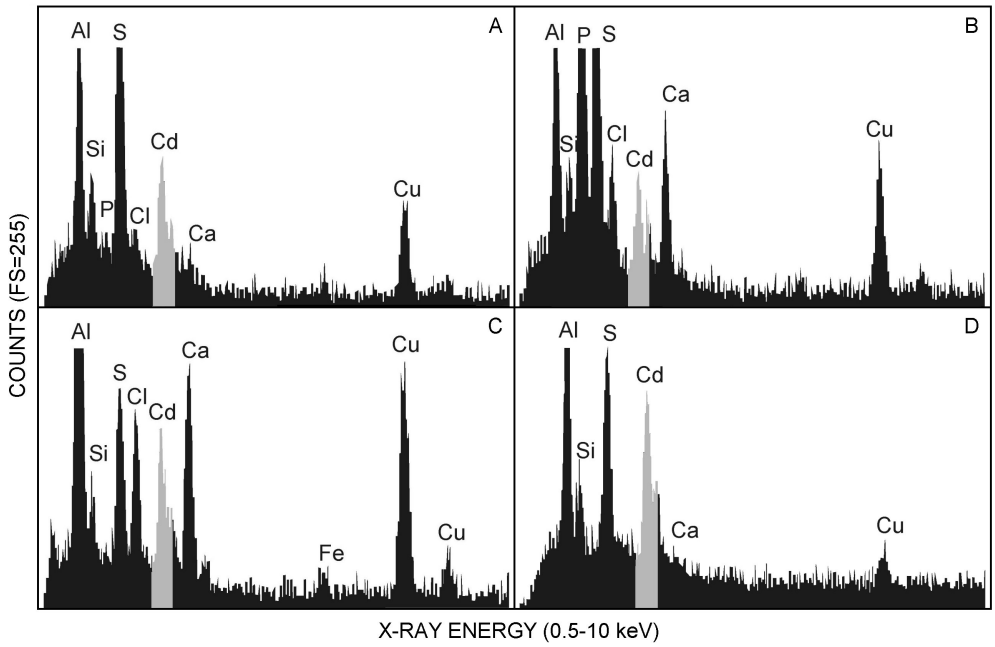

Fig. 4. EDAX spectra of maize root sections sites shown in the Fig. 3.

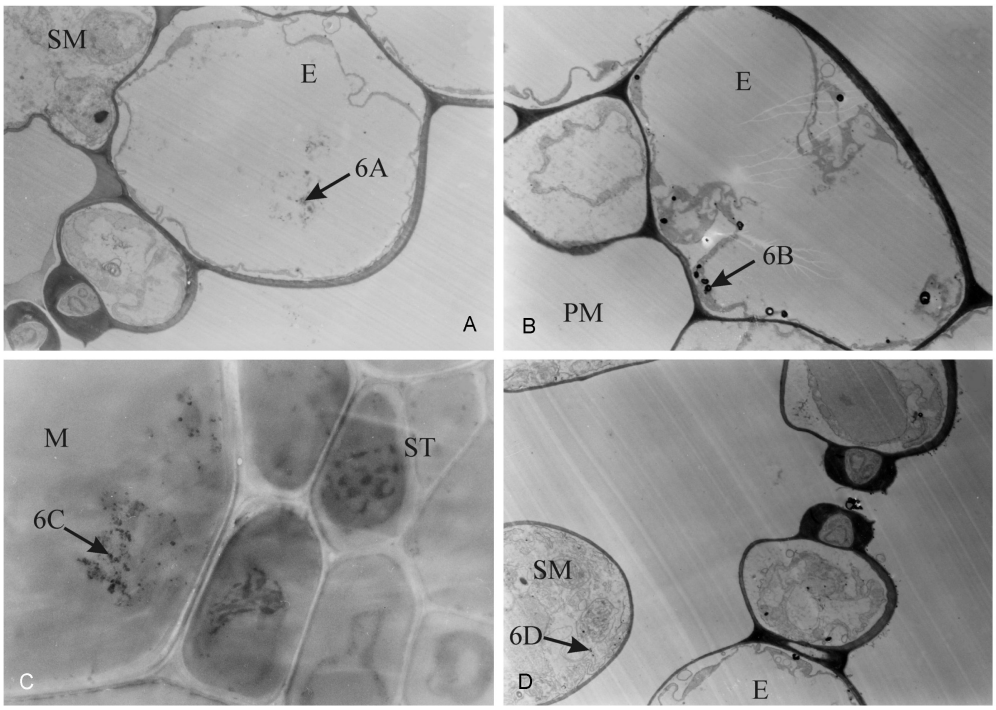

Fig. 5. Transmission electron microscopy micrographs of leaf cross-sections of maize exposed to 300 μM Cd. E - epidermis, SM - spongy mesophyll, PM - palisade mesophyll, M - mesophyll surrounding vascular cylinder, ST - sieve tube. *Arrows* indicate localization of Cd-rich sites (from which EDAX spectra are presented in Fig. 6). *A* - 2000×, *B* - 2000×, *C* - 6000×, *D* - 1500×.

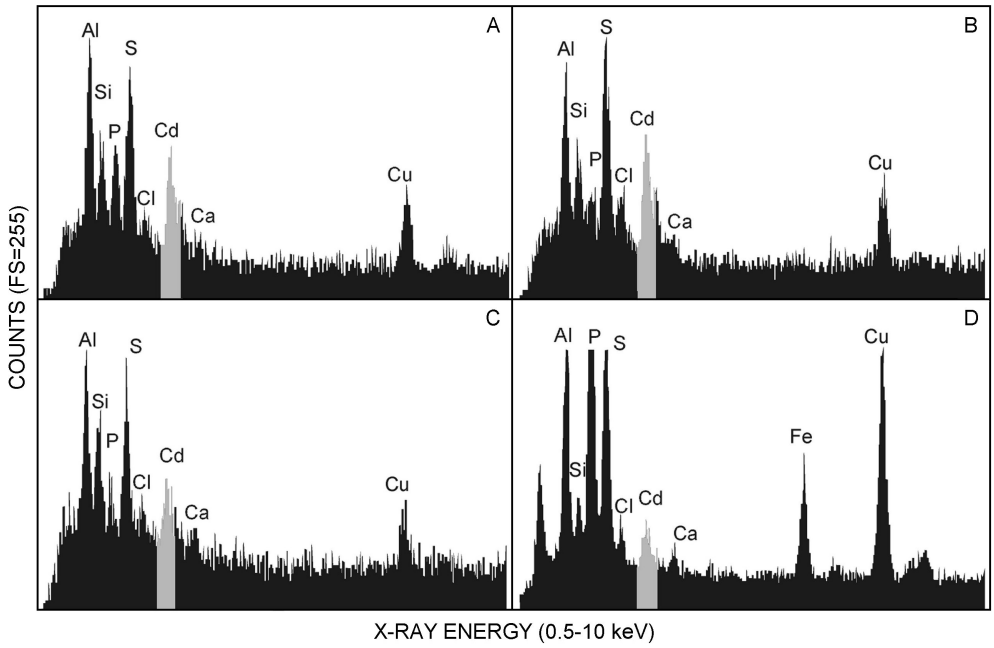

Fig. 6. EDAX spectra of maize leaf sections sites shown in the Fig. 5.

Discussion

Cd accumulation in maize plants as well as the external symptoms of metal toxicity were closely correlated with Cd concentration in the solution (Figs. 1, 2). Our results are in agreement with a number of reports which indicate that Cd accumulates more in roots than in maize shoots (Meuwly and Rauser 1992, Rauser and Meuwly 1995, Lozano-Rodríguez *et al*. 1997, Seregin and Ivanov 1997, Lagriffoul *et al*. 1998, Wójcik and Tukiendorf 1999). Studies of cadmium uptake and distribution in maize indicated the existence of two groups of inbred lines (Florijn and van Beusichem 1993). One of them are "shoot Cd excluders" in which the shoot/root metal ratio was approximately 0.02, and "non-shoot Cd excluders" exhibiting a much higher translocation of Cd to the shoot (shoot/root metal ratio of approximately 0.8). Seedlings of maize in our investigations seem to represent "shoot Cd excluder" strategy because the Cd shoot-to-root content ratio varied from 0.14 to no more than 0.35 (Fig. 1). However, despite a lower Cd content in shoots, these organs showed a greater growth reduction than roots. In our experiments plant growth was inhibited already at 5 μM Cd (Fig. 2). Some authors did not observe maize fresh mass reduction by Cd concentrations lower than 10 μM (Lagriffoul *et al.* 1998), whereas others could see it at 3 μM Cd (Meuwly and Rauser 1992).

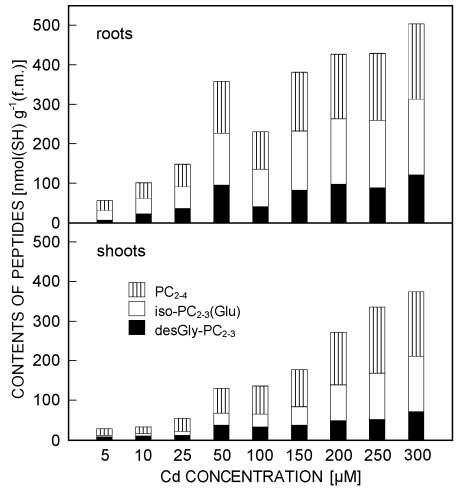

Fig. 7. Accumulation of γ-Glu-Cys peptides in maize plants. Bars represent mean of 2 replicates, SE = 10 - 30 % of the values.

Plants treated with 300 μM Cd were used by us to examine Cd localization in tissues and cells and its influence on root and leaf ultrastructure. We found damages of root ultrastructure - most cortical cells were destroyed as well as many cells of the endodermis and pericycle (Fig. 3). Electron-dense droplets were visible in the space surrounding the destroyed membranes and nuclei. Similar phenomenon was also observed by Seregin and Ivanov (1997) and Doncheva (1998) in Cd- and Cu-stressed maize, respectively. Cd-caused structural changes in root apices, rhizodermis and cortex were observed by Lunáčková *et al.* (2003/4) in cuttings of *Salix alba* and *Populus* × *euroamericana* grown in 10 μM $Cd(NO_3)_2$. The changes of leaf ultrastructure were much

less apparent probably due to much lower Cd content in these organs. Death of the particular cells may provide a mechanism by which roots can accumulate large amounts of heavy metals without causing a complete destruction or excessive damage to the tissue. However, in our studied plants not the most destroyed cortical cells accumulated relatively largest amounts of Cd.

EDAX method used by us enables only qualitative analysis of Cd localization in plant tissues or cell compartments. However, peak heights at EDAX spectra are strongly influenced by the thickness of the cuttings as well as data collection time and impulse number. Since we always used the same thickness of cuttings and other parameters of the analysis were constant (data collection time – 200 s, impulse number – FS = 255), the height of peaks give a comparative idea of "amount" and let us to compare Cd amounts reported by us at different sites.

Our EDAX analyses of maize root cross sections showed Cd presence mainly inside the cells of the endodermis, pericycle, central cylinder parenchyma and, to a lesser extend, inside cortex parenchyma cells. Its deposition in cell wall was found only in metaxylem cells. These results are not in agreement with the most of the reports concerning Cd localization in maize roots. The largest amounts of the metal were usually detected in apoplast – in cell walls of the cortex parenchyma, endodermis, pericycle and sieve tubes, and much smaller amounts were detected inside protoplasts (Khan *et al.* 1984, Lozano-Rodríguez *et al.* 1997, Seregin and Ivanov 1997). Also in other plant species apoplast and especially the cell wall appeared to be the main site of Cd accumulation in roots, *e.g*. in *Arabidopsis halleri* (Küpper *et al*. 2000) or in *Thlaspi caerulescens* (Vázquez *et al.* 1992a). However, Rauser and Ackerley (1987) could not find Cd in the cell walls of maize and *Agrostis* roots, instead they found Cd located in cytoplasm, vacuoles and nuclei, which is close to our results. Similarly, Vázquez *et al.* (1992b) showed the presence of Cd in vacuoles and nuclei but not in cell walls, cytoplasm and plastids of bean roots. Cadmium penetration from the outside environment through the successive tissues of the root and from the apoplast into the protoplast intensifies with metal concentration increase and extension of its action time (Seregin and Ivanov 1997). However, many of the results mentioned above are not consistent with this observation. The fact that we detected relatively largest amounts of Cd in inner tissues of the root (endodermis and stelar cells) as well as inside the protoplasts may have resulted from the metal concentration used and time of its action.

In maize leaves we found Cd in protoplasts of epidermis and mesophyll cells. Different results were obtained by Seregin and Ivanov (1997), who also found Cd in these tissues (in epidermis, outer layers of mesophyll and in xylem), but in their cell walls and not inside the cells. On the other hand, Lozano-Rodríguez *et al.* (1997) were able to show Cd presence in cytoplasm and vacuoles of maize leaf mesophyll, besides its presence in cell walls, which is more in agreement with our results. In leaves of other plant species Cd was detected in trichomes (*e.g*. in *Arabidopsis halleri* and *Brassica juncea* – Küpper *et al.* 2000, Salt *et al.* 1995, respectively), epidermis and mesophyll (Küpper *et al.* 2000).

In our experiments it was difficult to show the exact Cd localization in cell compartments of the maize root because of a high degree of protoplast damage caused by Cd toxicity. In leaves, vacuoles seemed to be the main cell compartment responsible for Cd accumulation. At the cellular level, Cd chelation and compartmentation within the vacuole protect the cell function under stress conditions. This mechanism is based mainly on the function of phytochelatins. Consistent with this, we found PCs accumulation in roots and shoots of maize seedlings exposed to Cd. Their accumulation increased with increasing metal concentration in the solution and in plant tissues and was higher in roots than in shoots, which was probably the consequence of retaining larger amounts of the metal in roots. However, the amount of synthesised PCs appeared to be not sufficient to detoxify Cd within the plants since symptoms of Cd toxicity occurred. With the increase in Cd concentration in the solution from 5 to 300 μM, the metal content in roots increased 3.2 times [from 669 to 2141.5 mg(Cd) kg^{-1}(d.m.)] (Fig. 1), γ-Glu-Cys peptides accumulation increased 10 times [from 47 to 474 nmol(SH) g^{-1}(f.m.)] (Fig. 7) and root growth was inhibited by about 82 % (root elongation by 78 % and fresh mass by 86 %) (Fig. 2). In shoots Cd content increased 6.6 times [from 121 to 803 mg(Cd) kg^{-1}(d.m.)] (Fig. 1), γ-Glu-Cys peptides accumulation increased 13 times [from 28 to 375 nmol(SH) g^{-1}(f.m.)] (Fig. 7) and fresh mass decreased by 66.5 % (Fig. 2). So, in spite of a higher ratio of γ-Glu-Cys peptides to Cd in plants treated with 300 μM Cd in comparison to those treated with 5 μM Cd, plants suffered much more from Cd toxicity. This can indicate that the amount of PCs is not *per se* the most important condition for efficient metal detoxification. According to Rauser`s (2003) experiments with maize seedlings exposed to 0.1 - 3 μM Cd, PCs do not bind all the Cd and the prominence of PC-based complexes depends on the time of treatment, the concentration of Cd used and the tissue analysed. At lower Cd concentrations probably significant levels of the metal entering the plant are retained in cell walls (Wagner 1993). It results not only in lower Cd toxicity for symplast but also in lower PCs synthesis, hence the ratio of accumulated PCs to detected Cd is lower. However, with increasing metal input to the plant, cell wall binding capacity is relatively diminished and considerable levels of Cd ions enter the protoplast causing serious damages, inducing PC synthase in the cytoplasm to PC production. Thus the higher PC accumulation could be rather the consequence

of PC synthase induction by Cd ions than an index of a more efficient system of Cd detoxification in the plant. Apart from this, the composition of accumulated PCs is also important for Cd binding capacity. Rauser and Meuwly (1995) found that among thiol peptides induced in maize roots by Cd action isoforms with longer chains chelated the metal more efficiently than those with shorter ones. In our studied plants, peptides with short chains (n = 2) (not so efficient in Cd detoxification) predominated.

Accumulation of PCs in Cd treated maize was recently quite often investigated, but the contribution of their particular isoforms in Cd detoxification is still not estimated. In our studies presented here as well as those of Rauser and Meuwly (1995) and Meuwly *et al.* (1995) desGly-PCs were present in the highest amounts. We found iso-PCs(Glu) as abundant as PCs, whereas other authors found their levels much lower. Our earlier studies (Tukiendorf and Rauser 1990, Wójcik and Tukiendorf 1999) showed that PCs glutathione derivatives, are first synthesised in maize response to Cd exposure. As it appears from all these results, the kind of synthesised peptide highly depends on the concentration and action time of Cd, suggesting that PCs play a dominating role in Cd detoxification at the beginning of Cd induced stress, and the contribution of the other families increases with extending time of Cd action and its concentration increase.

In summary we can conclude that the influence of Cd on maize plants is a complex phenomenon, and to understand it different aspects of Cd presence within the plant should be considered. Cd toxicity cannot be explained without the knowledge of the metal content in plants and also its distribution in plant organs, tissues and cell compartments. It is believed that only a part of metal, which interferes with cellular metabolism, is phytotoxic. Consequently, the metal bound to cell walls or accumulated in vacuoles has no physiological effect. Both these metal fractions can be detected and estimated using different methods of Cd localization in plants, including EDAX. Our results do not clearly indicate Cd distribution in roots but they confirm its deposition in vacuoles of leaf cells. Phytochelatins play an important role in exclusion of Cd toxicity out of sensitive cellular processes, either by metal chelation in cytoplasm or its deposition in the vacuole. We found PCs accumulation to depend on the metal concentration in plant tissues. However, the question arises whether their synthesis is only the result of the activation of enzyme of their synthesis by Cd ions, or it is really crucial for Cd detoxification and tolerance. To answer this question, further biochemical studies will be carried out.

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