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Dynamics of cadmium distribution in the intercellular space and inside cells in soybean roots, stems and leaves

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Abstract

Soybean (*Glycine max* L.) plants grown in nutrient solution were exposed to 1 mM $Cd(NO_3)_2$ for 24 h. Dynamics of distribution of cadmium among its different forms (water soluble, Ca-exchangeable and complexed) in the intercellular space and the ratio of the intercellular and intracellular cadmium in roots, stems and leaves were studied. In roots, in the beginning of treatment the largest portion of Cd was found in the intercellular space and 1 h later Cd content started to decrease, so that between 13- and 24-h treatment an equilibrium was reached in which about 70 % of Cd was found inside cells. In stems, already after 1-h treatment, the Cd concentrations in the cells and intercellular space were similar, the equilibrium being disturbed after 13 h, so that after 24-h treatment 80 % of Cd was found inside cells. In leaves, up to the 13 h Cd distribution showed fluctuation, after that equilibrium was reached, with 70 % of intracellular Cd. The highest contents of all Cd forms in the intercellular space was observed in roots.

Additional key words: apoplast, *Glycine max* L., water soluble, exchangeable and complexed cadmium.

Introduction

Cadmium is readily taken up by root and it is relatively mobile within the plant (Verkleij and Schat 1990). It is characterized by weak retranslocation so that its content in seeds and fruits is low, even with the plants grown on soils severely contaminated with Cd. The uptake mechanism of cadmium is still unclear. Both active and passive transport seem to be involved, the former prevailing at low concentrations, whereas the latter becomes more important at toxic levels (Godbold 1991). Related to these observations, the rate of Cd uptake and transport in plants shows a positive correlation with its concentration in the nutrient medium (Sanità di Toppi and Gabbrielli 1999). The Cd taken up by plant is transported by xylem in the form of Cd^{2+} (Leita *et al.* 1996).

The phytotoxicity of cadmium depends on the ability of the components of apoplast, symplast and xylem to immobilize it in the course of its uptake and transport (Sanità di Toppi and Gabbrielli 1999). Accumulation of heavy metals in particular organs, tissues, and cell compartments is different. Immobilization of heavy metals and plant tolerance to them may be either constitutional or induced. Most often, tolerance is based on different mechanisms such as chelate formation in the root and generation of a pH barrier, immobilization of heavy metals in the wall of root cells, complexing in the symplast with organic acids, proteins, or cystine-rich polypeptides (Vögeli-Lange and Wagner 1990, Nakazawa *et al.* 2001), as well as in vacuoles (Chardonnens *et al.* 1998).

Cadmium is to a greater extent accumulated in the plant root. Cadmium distribution in particular root parts depends primarily on the plant species. In *Pinus pinea* and *Pinus pinaster* significantly higher amounts of Cd were found in the cortex wall, cortex lumen or central cylinder wall (Arduini *et al.* 1994). Subcellular fractionation of Cd-containing root tissue of *Phaseolus vulgaris* showed that more than 70 % of Cd is localized in the cytoplasmatic fraction (Weigel and Jäger 1980).

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The apparent free space (AFS) of plant tissue represents the intercellular space freely accessible to ions. In AFS, cation exchange and complexation to cell wall take place. The amounts of water soluble (WS), exchangeable (EXCH) and complexed (COMPL) cadmium in the intercellular solutions from AFS washing were much greater in root tissue than in leaves (Leita *et al.* 1996).

Current literature concerning the response of the plant to cadmium exposure is mainly aimed at evaluating its effect on the development and plant growth, accumulation of Cd in roots and shoots (Leita *et al.* 1991, Costa and Morel 1993, Jalil *et al.* 1994, Fargašová 2001), biochemical and physiological aspects such as the neosynthesis of proteins and polypeptides (Robinson 1990), whereas little work is carried out on the cadmium chemical species involved in Cd-uptake and translocation within plant tissues, especially in first hours of plant treatment with this heavy metal.

The aim of this work was to study the changes in content of water soluble, exchangeable and complexed Cd in the intercellular space in young soybean plants during 24-h of treatment with Cd and its relationship to the intracellular Cd (INTRA Cd) content. Such investigations were at the same time aimed to point to the dynamics of induction of the apoplast mechanisms by which the plant can reduce phytotoxicity of cadmium.

Materials and methods

Experiments were carried out under semicontrolled conditions in a greenhouse on young plants of soybean (*Glycine max* L.). After germination of seeds in vermiculite at 25 °C the plants were grown in plastic pots with half-strength Hoagland's nutrient solution of the following composition: 2.5 mM $Ca(NO_3)_2$, 2.5 mM KNO_3, 1.0 mM KH_2PO_4, 1.0 mM $MgSO_4 . 7 H_2O$ and 23.1 µM H_3BO_3, 4.6 µM $MnCl_2 . 4 H_2O$, 0.38 µM $ZnSO_4$, 0.16 µM $CuSO_4$, 0.052 µM Na_2MoO_4 and 8.95 µM FeNaEDTA. In this nutrient solution plants were grown to the complete formation of physiologically active third leaf.

Treatment with Cd was carried out by transferring the plants into the nutrient solution to which 1 mM Cd was added in the form of $Cd(NO_3)_2$. Particular Cd fractions were separated after 0, 0.25, 0.50, 1, 2, 5, 8, 13 and 24 h. Plant roots were washed thoroughly with cool distilled water and dried between the sheets of filter paper. Intercellular Cd was extracted from roots, stems and leaves by the method of Leita *et al.* (1996). The intercellular solution was collected by infiltration-extraction procedure using successively distilled water, 5 mM $CaCl_2$, and 5 mM EDTA in order to collect separately the water soluble, exchangeable, and complexed Cd. Volume of the liquid obtained after each washing of the intercellular space was measured. To determine contents of intracellular Cd, the remaining plant material was dried in an oven at 70 °C to constant mass. Dry plant material was milled, weighed, ashed, and the obtained ash dissolved in 5 % HCl. Concentrations of Cd in the intercellular solution and in the solution after ash dissolution was determined by atomic absorption spectroscopy (*SpectraAA-10*, *Varian*, Mulgrave, Australia). Contents of particular forms of Cd in the intercellular space, as well as contents of intracellular Cd, were calculated on dry matter.

The data represent the average of three experiments. Variations between replicates were less than 5 %.

Results and discussion

Content of intracellular Cd as well as Cd content in particular fractions of intercellular spaces was different in roots, stems and leaves of young plants in the course of 24-h treatment with Cd (Figs. 1, 2). The highest content of all Cd forms was found in roots, whereby the content of intracellular Cd was extremely high. In stems, contents of all Cd forms were essentially lower (even ten times), and a significant increase in the intracellular Cd was especially observed between the 13th and 24th hour of treatment. The lowest contents of all Cd forms were observed in leaves, where a marked increase in the intracellular Cd was observed after 2-h treatment.

In the first 15 min of treatment, the main portion of Cd in roots was in ionic form (highest portion of Cd in WS). During the next 15 min Cd binding to cell walls took place (highest portion of Cd in COMPL) as well as its penetration to cells increase in the portion of intracellular Cd. In the further course of treatment (1 - 2 h), some structural and biochemical changes take probably place, yielding an increase in the portion of intracellular Cd, which reached its maximum after 24-h treatment. It can be supposed that a rapid transport of Cd to cells took place and its binding to numerous small organic molecules (citrate, malate and oxalate), and especially to peptides and phytochelatins, whose synthesis starts in this stage of plant development (Krotz *et al.* 1989, Chengbin and Oliver 1998). This confirms the point of view that root is the main obstacle to Cd distribution to other plant parts, especially to leaves (Cataldo *et al.* 1981, Guo and Marschner 1995, Salt *et al.* 1995).

In stems, 15 min after beginning of treatment a marked increase of Cd in the COMPL fraction was observed, which showed that Cd was primarily bound to the elements of cell walls. Later, an increase of the portion of intracellular Cd was observed, whereby the Cd portion in the COMPL fraction showed no significant decrease. These results indicated that xylem represented a second factor in the protection of the photosynthetic organs (leaves) from an excess of Cd. The capacity of Cd binding to xylem cell walls remained unchanged, as an approximately constant portion of Cd was in the COMPL fraction. When all binding sites were covered, some other protection mechanisms were activated, whereby Cd penetrated to the xylem cells and formed complexes of the type of metalothionein and/or phytochelatin (abrupt increase in the intracellular Cd was observed 13 h after the beginning of treatment). It is possible that this accumulation of Cd was a consequence of a slower flow of xylem sap during the night, which increased the possibility of Cd binding to the cell walls and its penetration to xylem cells.

In leaves, the transported Cd probably entered immediately the cell, as a significant increase in the portion of intracellular Cd was observed already in 15-min treatment, and the ratio of the intercellular and intracellular Cd reached its maximum after 2 h (Fig. 2). It is supposed that a saturation mechanism operated in leaves and probably in the Cd transport to leaves (Salt *et al.* 1995), since the increase in Cd content in leaves was not significant.

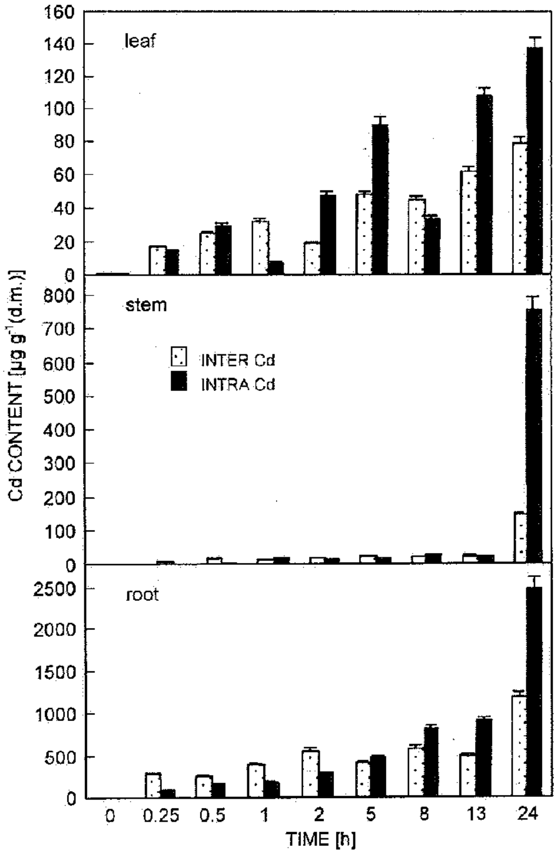

Fig. 1. Contents of water soluble, exchangeable and complexed Cd in intercellular space (INTER Cd), and contents of intracellular Cd (INTRA Cd) in roots, stems and leaves of young soybean plants in 24-h treatment with Cd.

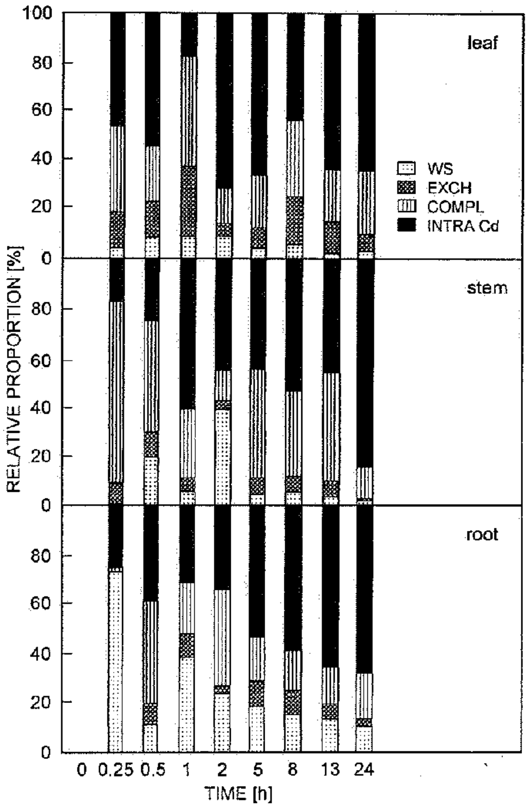

Fig. 2. Relative proportions of water soluble, exchangeable, and complexed Cd in intercellular space and intracellular Cd in roots, stems, and leaves of young soybean plants in 24-h treatment with Cd.

If we consider the Cd distribution in particular organs of young soybean plants to INTER Cd and INTRA Cd (expressed as the given Cd fraction in total Cd), a clear difference in Cd distribution in particular organs during treatment could be observed.

With roots, in the beginning of treatment, a highest portion of Cd was found in the intercellular space and already 1 h later began decrease, accompanied, normally, by an increase in the INTRA Cd. Between the 13th and 24th h equilibrium was reached, whereby about 70 % of Cd was inside cells. This indicated that the mechanisms by which the root protected other plant parts from the presence of Cd were activated. In stems, approximately equal Cd contents were observed in the intercellular space and inside the cells. This even distribution was disturbed after 13 h, so that after 24-h of treatment about 80 % of Cd was present as INTER Cd. In leaves, Cd distribution to the 13th h was characterized by fluctuations and the later equilibrium was attained in a similar way as in roots, with about 70 % of Cd being inside cells.

Previous studies suggested that in the majority of plant species, including soybean, Cd is taken up and transported in its ionic form, whereby with time it passes from the intercellular space to the cell. The results obtained in this and similar investigations, indicates that intercellular space of leaf, and especially of root, plays a significant role in Cd ion immobilization. Namely, study by Leita *et al.* (1996) showed that distilled water and 5 mM $CaCl_2$ solution extracted both from leaf and root only a small portion of Cd, whereas 5 mM EDTA showed a high capacity of Cd extraction from all binding sites of the plant cell walls. The role of root in protection of the fragile aboveground parts from phytotoxic action of Cd is especially evident with the genotypes tolerant to Cd. It was found (De Knecht *et al.* 1992) that Cd tolerant plants such as *Silene vulgaris* exhibited a higher Cd root to shoot ratio than sensitive plants. This difference may be due to a more efficient transport of Cd into the vacuoles of root cells in tolerant plants.

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