Introduction

Predicting Cd concentrations in plants is essential for controlling Cd entry into the food chain. Cadmium uptake by plants is the result of root adsorption to cell walls and of absorption through root cell membranes. Concentration-dependent kinetics performed on seedlings of maize and alpine pennycress allowed short-term Cd uptake by both root uptake pathways to be parameterized (Redjala et al., n.d.). However, the uptake parameters were obtained on plants which were previously cultivated without exposure to Cd. The objective of the present work was to assess how chronic exposure of plants to different Cd levels would affect the root adsorption and absorption rates.

Indeed, pre-exposure to metals may substantially modify the kinetics of metal uptake. Stimulation of Cd uptake has been reported to occur in Fe deficient conditions (Cohen et al., 2004), showing some up-regulation of membrane proteins able to transport Cd. Moreover, Larsson et al. (2002) studied the effect of prior Cd^{2+} exposure on Cd uptake by roots of *Arabidopsis thaliana*, and found some up-regulation of total Cd uptake in the wild type, and down-regulation in the PC-deficient mutant. Furthermore, very few works have investigated the regulation of root cation exchange capacity by prior exposure to metal.

In the present study, we investigated the impact of plant Cd content on root Cd uptake characteristics, at both the cell wall and membrane levels. The experiment was performed on two species with contrasting demand for Cd: a hyperaccumulating ecotype of alpine pennycress (*Noccaea caerulescens* [J. Presl & C. Presl] F.K. Mey., also known as *Thlaspi caerulescens*), well-known for its ability to accumulate high concentrations of Zn and Cd in its shoots, and maize (*Zea mays*, INRA cv MB 862), which retains Cd in its roots.

Materials and Methods

Plant cultivation

After germination in wet cotton at 20 °C, seeds of maize and alpine pennycress were grown in hydroponics for twenty days and six weeks respectively. Control plants were cultivated without Cd. In order to obtain plants with significantly contrasting internal Cd contents, the nutrient solution was enriched with Cd (as $Cd(NO_3)_2$) during the last half of the growth period: the exposed maize and pennycress plants were grown in 0.1 µM and 10 µM Cd during the last ten days and the last three weeks, respectively.

Total Cd in plants

Half of the control and treated plants were sacrificed just after the growth period in order to be analyzed for quantification of the accumulated stable Cd. For that, shoots and root systems were separated, dried in an oven (three days at 72°C), ground in an agate mill (Retsch, Germany) and digested in a microwave oven (Mars 5, CEM Corporation, Matthews, Caroline du Nord, USA). Cadmium in the digested material was then quantified through electro thermal atomic absorption spectrometry (ETAAS, Spectra AA Zeeman 220, with Zeeman correction, Varian, Inc, Palo Alto, California, USA).

Characterization of root uptake

The remaining plants were exposed to a one-hour uptake in a radio-labelled solution in order to assess the absorption rate of Cd according to the level of Cd accumulated during the cultivation. Three Cd concentrations in the radio-labelled solution were used: 0.1 µM, 10 µM and 50 µM.
Before immersion of roots in the radio-labelled solution, they were rinsed and exposed to a

desorption treatment in order both to minimize contamination of the radio-labelled solution with Cd leakage from the cell walls, and to liberate all exchange sites able to adsorb Cd. For that, each root system was immersed for two hours in 80 ml of buffered solution (pH = 5.7) containing 5 mM of $Ca(NO_3)_2$ and 2 mM MES buffer, then for two further hours in 80 ml of buffered solution (pH = 5.7) containing 0.5 mM of $Ca(NO_3)_2$ and 2 mM MES buffer. After quick rinsing in distilled water, roots were immersed in 650 ml of ^{109}Cd radio-labelled solution containing 0.5 mM $CaCl_2$, 2 mM MES buffer (pH = 5.7) and $CdCl_2$ in the three different concentrations. Root exposure lasted one hour, without significant variation of Cd external concentration. Each root system was then separated from shoots before immersion in successive ice-cold MES-buffered baths (pH = 5.7) containing 2 mM $CdCl_2$ and 5 mM $CaCl_2$. This desorption procedure was interrupted by 2 minutes freezing in liquid nitrogen; roots were then thawed by agitation in a warm desorbing bath, and desorption kinetics went on in ice-cold desorbing solutions for the resulting disrupted root cells (Fig. 1). Cadmium collected in the desorbing solutions was quantified through 20 ml samples by gamma-counting (Wallac 1480 Wizard®3, 187 Perkin Elmer Life Sciences Wallac Oy, Turku, Finland). Cadmium bound to the apoplast at the end of desorption was also quantified through gamma-counting in dry matter. We previously showed (Redjala et al., n.d.) that the sudden unloading (40 minutes) of metal in the desorption solution caused by the freezing/thawing procedure represents symplastic Cd, while the gradual desorption before and afterwards, corresponds to leakage from the cell walls, regardless of the external concentration (Figure 1).

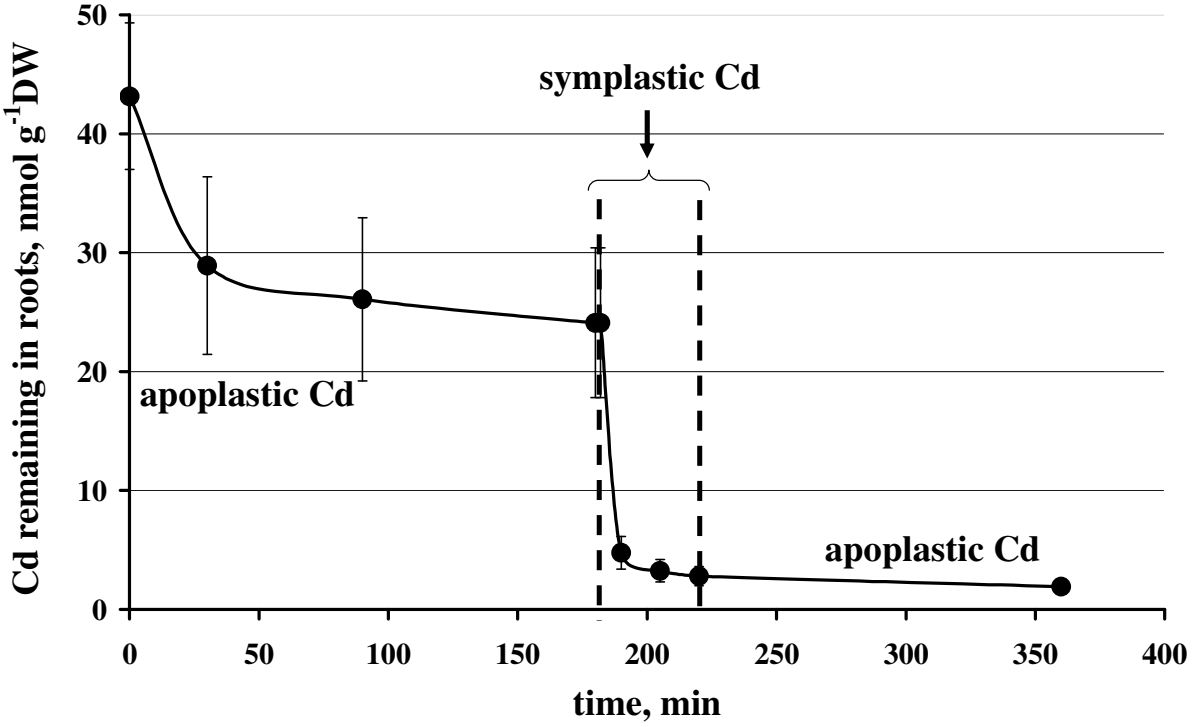

Figure 1: Time-desorption course of alpine pennycress root Cd after a one-hour uptake in 0.1 µM Cd; the hydroponic solution was contaminated with 0.1 µM Cd for the last six weeks of cultivation.

Statistical processing

For each short-term exposure concentration (0.1, 10 and 50 µM), a Student test was used to compare Cd uptaken by slightly-contaminated or highly-contaminated plants with Cd uptaken by unexposed (control) plants.

Results

After Cd accumulation during growth

Root dry mass was not affected by the contamination level during growth. On the other hand, some interveinal chlorosis was observed in the highly-contaminated alpine pennycress plants. Alpine pennycress accumulated more Cd in shoots than in roots, while it was the reverse for maize (data not shown). Moreover, Cd accumulated in roots and shoots was positively correlated with Cd contamination level, which showed that the contamination levels were efficient in generating contrasting internal Cd accumulations.

Short-term exposure after long-term treatment

Apoplastic and symplastic sorption increased with external concentration during the short-term exposure in both species, regardless of the amount of Cd internally accumulated during cultivation (Figures 2 and 3).

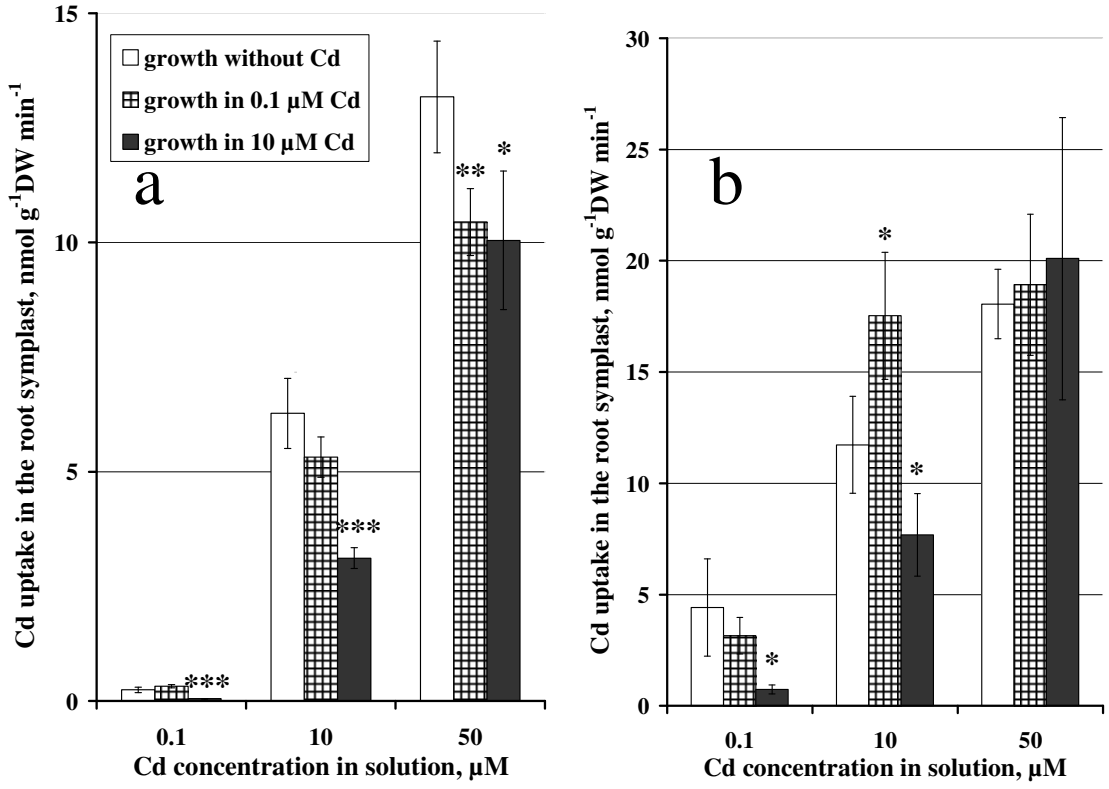

Figure 2. Symplastic Cd uptake during a one-hour exposure to 0.1, 10 or 50 µM $CdCl_2$, after growth in uncontaminated, slightly-contaminated (0.1 µM Cd) or highly-contaminated (10 µM Cd) hydroponic solutions. Bars represent the mean values of four and five replicates for maize (a) and alpine pennycress (b) respectively, and error bars represent a two-standard deviation segment.

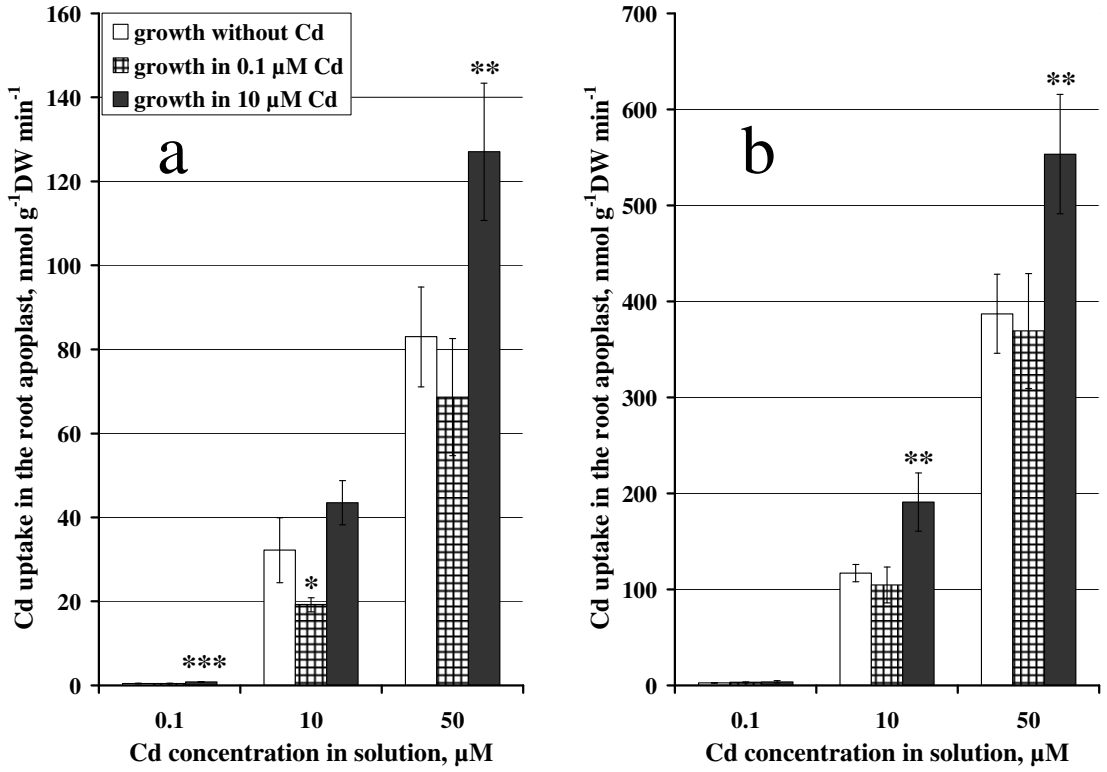

Figure 3. Apoplastic Cd uptake during a one-hour exposure to 0.1, 10 or 50 µM $CdCl_2$, after growth in uncontaminated, slightly-contaminated (0.1 µM Cd) or highly-contaminated (10 µM Cd) hydroponic solutions. Bars represent the mean values of four and five replicates for maize (a) and alpine pennycress (b) respectively, and error bars represent a two-standard deviation segment.

In both plant species, low internal accumulation of Cd (after cultivation in 0.1 µM Cd) did not affect the short-term Cd uptake in 0.1 µM Cd exposure solution, by either apoplastic and symplastic compartments. On the other hand, our contrasted plants showed different behaviours when exposed to 10 µM Cd or 50 µM Cd after cultivation in 0.1 µM. Indeed, roots of the hyper-accumulating plant showed a more or less significant increase in Cd membrane influx, but no impact on cell wall adsorption. On the contrary, maize roots showed a more or less significant decrease in both membrane influx and cell wall adsorption rate.

After high internal accumulation of Cd (after cultivation in 10 µM Cd), root symplastic absorption was generally reduced for both plants. This decrease in the absorption rate is much more significant in maize than in alpine pennycress. Nevertheless, when considering the lowest concentrations (0.1 µM Cd and 10 µM Cd) during short-term exposure, both plants showed a more reduced symplastic influx. As regards Cd binding to cell walls, cultivation in 10 µM Cd was responsible for a significant increase in the adsorption rate.

Discussion

In a previous study (Redjala et al., n.d.) we showed that Cd transport through root cell membranes was ensured by at least two different transport systems, one of them acting

predominantly at low external concentrations (high-affinity transport system, HATS) and the other one prevailing at higher concentrations (low-affinity transport system, LATS). For maize, the HATS prevailed at external concentrations below 1 µM, while it prevailed up to 30 µM for alpine pennycress. Thus, the short-term exposure to 0.1 µM will concern the HATS for both plants, while the highest exposure concentration will more concern the LATS.

According to our results, chronic exposure to Cd does not exert the same influence on the different components of root uptake and depends on the level of exposure.

Symplastic regulation

The HATS of maize and alpine pennycress is not affected at all by the low internal accumulation of Cd during growth. The withdrawal of free Cd ion from the cytosol by the complexation with phytochelatins (PCs) and eventual transport to the vacuole or to the shoots may depress any mechanism regulating the cytosolic Cd concentrations (Larsson, 2002). On the other hand, the plants do not show the same behaviour for the high short-term exposure concentrations: the 0.1 µM Cd contamination of the growth solution would down-regulate the LATS of maize but not that of alpine pennycress, for which some stimulating effect seems to happen.

After high Cd accumulation during growth, the Cd symplastic influx is significantly reduced for both plants. Such a diminution of the intracellular uptake of Cd had already been observed on wheat root protoplasts (Lindberg et al., 2007). It may come from down-regulation of the short-time Cd^{2+} uptake on both high- and low-affinity transport systems. In our study, this reduction of the Cd short-term uptake is higher for the lower exposure concentrations. This supposes that the down-regulation affects the HATS rather than the LATS. The decrease in Cd intracellular influx can also result from up-regulation of the Cd^{2+} extrusion from intracellular to extracellular space, through PC-Cd efflux (Jasinski et al., 2003), Cd^{2+}/H^{+} antiport (Salt and Wagner, 1993) or vesicle excretion (Seregin and Kozhevnikova, 2008). Another alternative explanation to the lower Cd uptake could be the changed appearance of the root system in the Cd-treated plants. Thus, although approximately the same root masses were achieved in all plants tested, the uptake surface might have been different, favoring Cd uptake in the control plants. Finally, some better sequestration of Cd by the cell walls might decrease the entry rate of Cd through cell membranes.

There may be some regulation controlled by a threshold value for Cd plant status, in both species. Long-term contamination with 0.1 µM would be below this threshold, thanks to the PC sequestration for example. On the other hand, long-term contamination with 10 µM would exceed this limit and result in down-regulation of the transport proteins of the HATS, possibly because of excess free Cd in the cytosol or because of some signal from the shoots.

Apoplastic regulation

For both plants, cell wall sorption efficiency appears to be improved by the high Cd concentration in the growth solution, whereas the low concentration had generally no significant impact. The increase in the cell wall binding efficiency after high internal Cd accumulation may be related to the down-regulation of intracellular uptake. However, the reduction in symplastic influx is very low and cannot account for the increase observed at the apoplastic level. On the contrary, the up-regulation of the adsorption rate may well account for the decrease in the symplastic uptake. The apparent up-regulation of Cd binding properties may be due to modifications of the root cell-wall adsorption characteristics, particularly the root CEC.

Cadmium stress is known to affect cell wall composition. First, Cd increases the proportion of acidic pectins (compared to neutral pectins). Secondly, the cell wall CEC may be increased through regulation of enzymes. For instance, pectinmethylesterase has been suggested to be stimulated in the outer cell wall domains of Cd-stressed plants, resulting in a strong decrease in the methylesterification of the acidic pectins. Thus, Cd strongly increases the acid pectins/esterified pectins ratio, hence the higher CEC, particularly in the middle lamellae (Douchiche et al., 2007). This low degree of esterification enhances the adsorption of all metallic trace elements (Kupchik et al., 2006), improving the plant tolerance of the metal.

As dry mass did not vary with the level of contamination, there may be no significant difference in the proportion of young roots and then no decrease in the root CEC due to the age of roots. Therefore, the insignificant effect of low Cd contamination on the apoplastic adsorption rate could be accounted for by the existence of some Cd-stress threshold below which there is no regulation mechanism.

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