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Influence of exposure solution composition and of plant cadmium content on root cadmium short-term uptake

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ABSTRACT

The aim was to evaluate the influence of (i) cations potentially present in soil solution and (ii) of plant Cd content on apoplastic and symplastic root uptake of Cd. Hydroponically grown maize (*Zea mays*) roots were exposed to two Cd concentrations together with a cation five times the strength of the Cd. The influence of three pH and four Ca levels was also assessed. In addition, maize and alpine penny-cress (*Noccaea caerulescens*) were grown so their tissues contained Cd at three levels when their roots were exposed for 1 h to three Cd concentrations. Maize Cd uptake in the 5 μM Cd solution was reduced by the presence of Pb^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+}, Mg^{2+} or Mn^{2+}. At a 1 μM Cd concentration, Cu^{2+}, as well as Ca^{2+} and H^+ reduced the Cd uptake. However, the Ca concentration had to be much higher than that of Cd to restrain its uptake. High plant Cd content was responsible for an increase in the apoplastic Cd uptake and a decrease in the symplastic absorption, for both species. Low plant Cd content neither affected the Cd apoplastic uptake whatever the exposure concentration, nor the symplastic uptake in 0.1 μM Cd exposure solution. Moreover, the hyperaccumulator symplastic uptake increased when exposed to 10 μM Cd, but not when exposed to 50 μM Cd. Maize roots showed a decrease in membrane net flux when exposed to 10 μM and 50 μM Cd. Finally, the results suggest that for plants exposed to usual soil solution concentrations, it is mainly calcium and protons which interfere with Cd internalization.

1. Introduction

When present in the soil even at relatively low concentrations, Cd tends to accumulate in plant organs. The presence of this metal in consumable parts of crop plants is a threat to human health. On the other hand, it suggests a way to decontaminate Cd polluted soil through phytoextraction, using low accumulating plants with high biomass production, such as maize (*Zea mays* L.), sunflower (*Helianthus annuus* L.) and willow (*Salix* spp.) or hyperaccumulating species like alpine pennycress (*Noccaea caerulescens* (J. Presl & C. Presl) F.K. Mey). From a practical point of view, Cd phytoaccumulation should be reduced in the first case and enhanced in the second. To reach both these goals there is a need to better understand the mechanisms of soil to plant Cd transfer, in which root uptake is a key process.

The Cd symplastic net flux (F_s, mol g^{-1} s^{-1} or mol m^{-2} s^{-1}) is generally modelled by the classical Michaelis–Menten equation

$$F_S = \frac{I_{max}([Cd] - [Cd]_{min})}{K_m + ([Cd] - [Cd]_{min})} - E,$$

where [Cd] represents the Cd concentration in the exposure solution (mol L^{-1}). I_{max} (mol g^{-1} s^{-1} or mol m^{-2} s^{-1}) and K_m (mol L^{-1}) are the maximum influx and the affinity coefficient, respectively. Considering that $[Cd]_{min}$, the concentration under which there is no possible uptake and the efflux E (mol g^{-1} s^{-1} or mol m^{-2} s^{-1}) are negligible, the equation becomes

$$F_S = \frac{I_{max}([Cd])}{K_m + ([Cd])}.$$

This supposes the existence of a transport system with a high affinity to Cd (HATS) in the plasma membrane (Cataldo et al., 1983; Hart et al., 1998; Lombi et al., 2001). It is not clear if this HATS is specific to Cd or if the metal enters the root cell using a Zn, Cu or Mn carrier, even a Ca or Mg cation channel (Welch and Norvell, 1999).

More recently, it has been shown that the uptake kinetics model could be completed with a linear component that might correspond to the flow controlled by a low affinity transport system (LATS) acting at higher concentrations (Redjala et al., 2009, 2010a):

$$F_S = \frac{F_{max}[Cd]}{K_m + [Cd]} + a[Cd],$$

where a is the slope of the linear component.

One can put forward the hypothesis that the Michaelis–Menten parameters (F_{max}, K_m and a) poorly reflect the root uptake in the

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field, at least for two reasons. The first one is that the plant used in the published works had not been exposed to Cd during cultivation before their roots were briefly exposed (generally for 20 min) to a Cd solution. In the field the Cd root uptake is that of a plant which contains Cd at varying concentrations. Knowing the numerous reactions that control the Cd homeostasis (Welch and Norvell, 1999; Clemens, 2001), it cannot be excluded that the Cd content in the plant tissue would have a feedback effect on the root uptake which is not taken into account in the classically measured Michaelis–Menten parameters.

The second reason is that the Cd uptake kinetics were carried out in quasi-standard solutions containing predominantly 0.2–0.5 mM Ca and 2–5 mM MES to buffer the pH around 6 (Cataldo et al., 1983; Hart et al., 1998, 2002, 2006; Lombi et al., 2001; Zhao et al., 2002, 2006). This matrix is different in composition from the soil solution, in which various cations are present, at relatively high levels such as Ca^{2+}, or at lower or trace levels, such as H^+, NH^{4+}, K^+, Zn^{2+} or Cu^{2+} (Wolt, 1994). The composition of the soil solution varies according to factors such as soil type, horizon, use and contamination. It can therefore be hypothesized that the Michaelis–Menten parameters measured *in vitro* do not take into account any possible competition between Cd and other cations for the sorption on the plasmalemma and also on the cell wall sites. As already mentioned by Hart et al. (2002), it has been demonstrated that Cd phytoaccumulation can be modulated by the presence of Zn. However, as most of the results are based on analysis of plants grown in soil or in nutrient solution, it is not possible to know to which of the soil chemistry, root uptake or translocation processes the interaction between Cd and Zn must be attributed. There are few studies of the interaction between Cd and other cations for the uptake at the root level *sensu stricto*. Competition between Cd and Zn for root uptake has been shown in bread and durum wheat (*Triticum aestivum* L. and *T. turgidum* L.) (Smeyers-Verbeke et al., 1978; Hart et al., 2002), a low Cd accumulating population (Prayon) of alpine pennycress (Lombi et al., 2001; Zhao et al., 2002) or *Arabidopsis halleri* (L) O'Kane & Al-Shehbaz. This Cd/Zn competition was not seen for the Ganges population of alpine pennycress which accumulates more Cd than the Prayon population (Lombi et al., 2001; Zhao et al., 2002). The Mn also depressed the Cd uptake of Cd by the Prayon population of alpine pennycress but not by the Ganges one (Zhao et al., 2002). Copper was found to interfere with Cd uptake by wheat roots (Smeyers-Verbeke et al., 1978), while Ca at high concentrations (5 mM) depressed the Cd uptake by the Prayon population of alpine pennycress but not the Ganges one (Zhao et al., 2002). In the latter work, Co, Fe(II) and Ni were shown not to interfere with Cd uptake by either the Prayon and Ganges populations. However, we found no results on the effect of other cations present in soil solutions, such as H^+, K^+, Na^+, NH^{4+} and Pb^{2+}.

It must added that in all the above-mentioned research, only symplastic uptake was considered, any apoplastic sorption being neglected. However, this component can represent 30–90% of the root Cd uptake after short term exposure (Redjala et al., 2009). To our knowledge, there is no information on the effect of both plant Cd content and ionic composition of the exposure solution on apoplastic uptake.

To summarize, it can be hypothesized that the plant regulates Cd internalization according to the metal concentration in its tissues and that cations in the soil solution compete with Cd for sorption both on the cell membrane transporters and on cell wall sites. The aim of the work presented here was therefore (i) to evaluate the interference of the various cations potentially present in soil solution (H^+, K^+, Na^+, NH_4^+, Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} or Zn^{2+}) on both the apoplastic and symplastic root uptake of Cd, and (ii) to test the hypothesis of a feedback effect of plant Cd content on the short term root uptake of the metal.

2. Materials and methods

2.1. Plant species

Maize (cv INRA MB 682) and alpine pennycress of the Viviez population from the south of France were chosen because of their contrasting capacities to tolerate and accumulate Cd. Maize plants accumulate the metal in their roots (Jarvis et al., 1976; Florijn and Van Beusichem, 1993). In contrast, alpine pennycress has been reported to accumulate more than 1400 mg kg^{-1} DW of Cd in its shoots (Reeves et al., 2001; Sirguey et al., 2006). Only maize was used for the study of the influence of the composition of the exposure solution on the Cd short term uptake, because the reactivity of its apoplastic adsorption and symplastic absorption sites could be regarded as similar to those of the hyperaccumulating plant (Redjala et al., 2009, 2010a).

2.2. Plant cultivation

Maize seeds were put on filter paper moistened with distilled water and placed in an incubator at 25 °C for 4 days. After germination, the seedlings were transferred into a growth chamber with a photon flux density of 300 μmol s^{-1} m^{-2}, a photoperiod of 16 h and day/night temperatures of 25/20 °C. They were placed in hydroponics, onto a sheet of polystyrene floating on 40 L of the following nutrient solution (in μM): 3000 $Ca(NO_3)_2$, 250 $Ca(H_2PO_4)_2$, 500 K_2SO_4, 1000 $MgSO_4$, 2000 NH_4NO_3, 46 H_3BO_3, 9 $MnSO_4$, 0.3 $CuSO_4$, 0.8 Na_2MoO_4, 0.8 $ZnSO_4$, 7.5 $FeSO_4$. CHESS software (van der Lee, 1998) was used to check the availability of each type of nutrient. The nutrient solution was continuously aerated thanks to air bubbling from capillary tubes. pH was adjusted to 5.7 through KOH or NaOH addition when the solution was renewed twice a week. Cultivation of the plants used for the solution composition study lasted 12 days. For the study of the influence Cd content, the maize plants were grown for 20 days and during the last 10 days, Cd was added to the nutrient solution to maintain three different exposure levels: 0 μM (control), 0.1 μM and 10 μM Cd.

Alpine pennycress seeds were sown onto a filter paper lying on cotton wool soaked with distilled water. Germination lasted 10 days in the dark, at 20 °C. Seedlings were then transferred into a growth chamber for cultivation in hydroponics, on floating polystyrene sheets. Plants were fed with an aerated solution containing (in μM): 3500 $Ca(NO_3)_2$, 1500 $MgSO_4$, 1200 KNO_3, 100 K_2HPO_4, 10 KCl, 10 H_3BO_3, 10 $MnCl_2$, 7.5 $FeSO_4$, 5 $ZnSO_4$, 0.7 $NiSO_4$, 0.2 $CuSO_4$, 0.2 Na_2MoO_4. The pH was adjusted to 5.7 by adding KOH or HNO_3. The growth chamber conditions were the same as for maize cultivation. The cultivation lasted 6 weeks, with weekly renewals of the nutrient solution. During the last 3 weeks, Cd was added to the nutrient solution to maintain an exposure concentration of 0 μM (control), 0.1 μM and 10 μM Cd. Half of the control and of the plants exposed to Cd during cultivations were harvested just after the growth period in order to be analyzed for quantification of the accumulated stable Cd.

2.3. Root exposure to Cd

Roots of the plants cultivated in Cd enriched nutrient solutions 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 2 h 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.