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Cadmium uptake by roots: Contribution of apoplast and of high- and low-affinity membrane transport systems

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ABSTRACT

The aim was to measure the respective contributions of apoplast and symplast to the Cd root uptake and to explain the linear component of the symplastic absorption. Two plants were used, maize (*Zea mays* L.) and two ecotypes of alpine pennycress (*Noccaea caerulescens* (J. Presl & C. Presl) F.K. Mey.), with contrasted abilities to accumulate Cd. Their roots were exposed to labelled Cd solutions of increasing concentrations. Root Cd was physico-chemically fractioned to obtain the exchanged apoplastic, non-exchanged apoplastic and symplastic pools. For both species, the proportion of Cd retained by the cell walls increased with Cd concentration in the exposure solution (ranging from 0.05 to 50 μmol L^{-1}), from approximately 30% to 90% of the total root Cd. This was modeled using Freundlich isotherms. The non-exchanged apoplastic Cd was negligible at the highest exposure concentrations, but reached almost 30% of the total root uptake at the lowest ones. The symplastic influx in roots of both species fitted a Michaelis-Menten function associated with a linear one. The linear component of the symplastic influx could reflect absorption through a low-affinity transport system (LATS). The strong adsorption of Cd on root apoplast might act as a driving force to extract the metal from the soil, compete with the symplastic absorption and contribute to the amount of element taken up by the plant, at least in its roots.

1. Introduction

Over the last century, there has been significant accumulation of Cd in soils, because of industrial and urban activities, transport and agricultural practices. Despite its toxicity to almost all biota, and because of its non-degradable nature and its chemical mobility, the metal can pass through food chains and cause various diseases to living organisms, including human beings. That is why Cd contamination is a serious threat to ecosystems, agricultural land, ground water, human and animal health. Although Cd is neither an essential nor beneficial element for plants (except presumably for hyperaccumulating species), plant organs generally exhibit measurable Cd concentrations, as a result of their root uptake and translocation. Soil-to-plant transfer of Cd is an important research topic as vegetal foods constitute a major source of human exposure to Cd (Leblanc et al., 2004) and also because plants are, through phytoextraction, a potentially efficient means of remediating soils contaminated with trace metals.

Understanding the mechanisms involved in the soil-to-plant transfer of Cd is necessary either to reduce these transfers towards edible plant parts or to increase them towards the organs harvested in phytoextraction. Cadmium accumulation in plants is regulated by several physiological processes, including Cd uptake from soil (*via* roots) and atmosphere (*via* shoots), xylem translocation from root to shoot and phloem movement into grain during maturation (Hart et al., 1998). Root uptake of the metal from contaminated soil is believed to be the major source of Cd in crops in rural areas (Smolders, 2001) and is the pathway favored in phytoextraction. Thus, comprehension of Cd phytoaccumulation starts with describing the mechanisms by which Cd is absorbed by root cells.

The influx of Cd across the plasmalemma of root cells has been shown to occur *via* a concentration-dependent process exhibiting saturable kinetics in soybean (*Glycine max*) (Cataldo et al., 1983), lupine (*Lupinus albus*) (Costa and Morel, 1993), lettuce (*Lactuca sativa*) (Costa and Morel, 1994), maize (*Zea mays*) (Mullins and Sommers, 1986), durum wheat (*Triticum turgidum*) (Hart et al., 1998), and oats (*Avena sativa*) (Salt and Wagner, 1993). The saturable nature of Cd uptake in these studies indicates a transporter-limited process that exhibits Michaelis-Menten enzyme kinetics, and suggests that Cd uptake by roots is controlled by a transport protein in the plasmalemma (Kochian, 1991). Until now, no specific membrane carrier has been identified for Cd uptake. Several results indicate that Cd gets through the plasmalemma of root cells in an opportunistic way, *via* other divalent cation carriers or channels, such as those for Zn^{2+}, Cu^{2+}, Fe^{2+} or Ca^{2+} (Cataldo et al., 1983; Costa and Morel, 1993; Welch and Norvell, 1999; Zhao et al., 2002; Lindberg et al., 2004; Han et al., 2006). At higher Cd^{2+} concentrations, the saturable kinetics are associated with a linear

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component in Indian mustard (*Brassica juncea*) (Salt et al., 1995), wheat (*Triticum aestivum*) (Hart et al., 1998, 2002; Harris and Taylor, 2004), *Noccaea caerulescens* (Lombi et al., 2001, 2002; Zhao et al., 2002), maize (Han et al., 2006), pea (*Pisum sativum*) (Cohen et al., 1998) and *Arabidopsis halleri* (Zhao et al., 2006). Their linear kinetics are attributed to apoplastic sorption. However, it may represent some low-affinity transport system through carriers or channels as demonstrated in animal cells (Lecoeur et al., 2002) and plant organelles (Hinkle et al., 1987). This uncertainty lays in the method used to determine the symplastic influx, which does not allow a perfect discrimination of the metal bound onto the cell walls from the metal passed through the plasmalemma (Reid and Liu, 2004).

On the other hand, interactions of Cd with the apoplast could contribute to the root sink for this metal. To forecast the soil-to-plant transfer by means of mechanistic modeling (Sterckeman et al., 2004), this sink should be completely and precisely assessed. In the various works cited above, the uptake by root apoplast was more or less eliminated, through a desorption step after root exposure to Cd. Therefore, the apoplastic uptake function that could be useful for modeling the Cd transport in the rhizosphere is not available from the literature.

The aim of the study presented here was to clarify the function of Cd root influx. We developed an original method to fractionate Cd in roots, in order to discriminate between the contributions of apoplast and of symplast to the influx. This fractionation also aimed to verify the existence of a linear component in the symplastic influx, which would give evidence for some low-affinity transport system.

2. Materials and methods

2.1. Plants and cultivation

Maize (*Zea mays* L. cv INRA MB 682) and alpine pennycress (*N. caerulescens* (J. Presl & C. Presl) F.K. Mey., also designated as *Thlaspi caerulescens* J. Presl & C. Presl) were chosen because of their contrasting capacities to tolerate and accumulate Cd. Maize is known to accumulate the metal in their roots (Wójcik and Tukiendorf, 2005), and to privilege Cd binding to the cell walls (Lozano-Rodriguez et al., 1997). Two ecotypes of alpine pennycress were cultivated: Nc-H, from the South of France, is known to hyperaccumulate Cd in its leaves and Nc-L, from the Vosges mountains in France, is known to hyperaccumulate Ni.

Maize caryopses were put on filter paper moistened with distilled water and placed for five days in an incubator at 25 °C. 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 $FeSO_4$. The pH of the nutrient solution was adjusted to 5.7, through KOH addition. CHESS software (van der Lee, 1998) was used to check the availability of all the nutrients. The cultivation lasted twelve days. The nutrient solution was aerated continuously by bundles of capillary tubes and renewed twice a week.

Seeds of alpine pennycress were sown on filter paper lying on cotton soaked with distilled water. Germination lasted ten days in the dark, inside an incubator, at 20 °C. Seedlings were then transferred to a growth chamber for cultivation in hydroponics, onto floating polystyrene sheets. Both ecotypes were fed with the same 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 NaOH or HNO_3. Day/night temperatures were 20 °C and 15 °C, with a photoperiod of 16 h, a relative humidity of 80%, and a light intensity of 300 μmol s^{-1} m^{-2}. The cultivation lasted three months, with solution renewals once a week.

2.2. Concentration-dependent kinetics with cadmium fractionation

The seedling roots were first rinsed in tap water, then in distilled water. Roots were then immersed in 650 mL of a solution containing 0.5 mM $CaCl_2$, 2 mM MES buffer (pH 5.7) and $CdCl_2$ in different concentrations ranging from 0.05 to 50 μmol L^{-1}. Cadmium was labelled with ^{109}Cd (GE Healthcare, Chalfont St. Giles, United Kingdom) dissolved in diluted HCl; the absorption solutions for alpine pennycress contained 2 kBq L^{-1}. For maize, 5 kBq L^{-1} were added to the low-concentration solutions while the high-concentration solutions received 54 kBq L^{-1}. Root exposure to Cd lasted one hour, at external concentrations that can be considered as constant, as their decrease due to root uptake was always lower than 10% of the initial concentration. Each bulk solution was aerated and agitated during the root exposure, by air bubbling from a plastic capillary tube. Water loss by evapo-transpiration was negligible. After exposure, roots were rinsed in distilled water for about ten seconds, and gently blotted using paper towels. They were then immersed in three successive ice-cold desorption baths of 10 min each (Hart et al., 1998), containing 5 mmol L^{-1} $CaCl_2$, 2 mmol $CdCl_2$ and 2 mmol L^{-1} MES.

Extraction of the intracellular content (symplastic content) was carried out using a chemical method, which disrupts the cell membranes by causing their permeabilization. This was achieved through a mixture of methanol and chloroform (2/1, v/v) that acts by creation of canals through the cell membrane. This treatment was demonstrated to produce a morphologically intact root cell wall preparation essentially devoid of membrane lipids (DiTomaso et al., 1992; Liu et al., 2007). After separation from shoots, roots were immersed for three days in pots containing the methanol–chloroform mixture (M–C). Each root system was then immersed into two successive one-day baths of distilled water. Vigorous manual shaking was performed twice a day, to make intracellular compounds come out of the roots. Root systems were finally immersed again in pots of desorption solution (the same as described above), for half an hour, in order to desorb some cytoplastic Cd which may have adsorbed onto the cell walls after cell lysis. Root apoplasts and shoots were then oven-dried at 72 °C for three days, and weighed.

2.3. Cadmium determination

All solutions (rinsing, desorption, M–C) were weighed for determination of their volumes. Their ^{109}Cd content was quantified using a gamma spectrophotometer (Wallac 1480 Wizard®3 from Perkin Elmer Life Sciences Wallac Oy, Turku, Finland), on the basis of liquid aliquots or total dry matter of each root system. To relate Cd content found in the root dry matter to that in solution, we used close linear correlations (DR = 0.7935 UR, R^2 = 0.99) found between gamma-counting in un-ground (UR, cpm) and ground and digested root material (DR, cpm).

2.4. Data analysis

The Cd content in the shoots being negligible, the uptake isotherms were plotted using the data produced by the root Cd fractionation by the successive baths. Values presented in the figures and tables are means of four replicates for maize, and of three to six replicates for alpine pennycress.