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Effect of citrate on the uptake of copper and cadmium by *Lupinus albus*, *Lupinus luteus* and *Lupinus angustifolius*

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Abstract

During P deficiency, several lupin species show an increased efflux of organic acid anions, which increases the solubility of P and metals such as Fe, Al, Zn, Cu and Cd. The effect of these organic acid anions on the uptake of Cu and Cd is not well known. To test their effect, we investigated the uptake of Cu and Cd by lupin species over 48 h from solutions in which the metals were present as free cations (1.8 µmol L^{-1} as nitrate) or as citrate complexes (1.8 µmol L^{-1} Cu or Cd nitrate plus 1 mmol citrate L^{-1}). Afterwards, the roots were transferred to a 2 mmol $Ca(NO_3)_2$ L^{-1} solution for 5 h to measure the quantities of exchangeable Cu and Cd. There was a strong reduction of the Cu concentration in the Cu nitrate treatment. Within 9 h the minimum concentration was reached by all the 3 plant species. But in contrast, the Cu uptake from the Cu nitrate + citrate solution was totally inhibited over the 48 h. In the case of Cd, the concentration of the Cd nitrate solution was gradually reduced and the minimum concentration was reached after 12 h (*L. albus*). Compared with this result, the Cd depletion of solution with Cd citrate complexes was significantly inhibited at least during the first hour. Later on, there were no significant differences between Cd uptake rate from Cd nitrate and Cd citrate solutions. Whereas only 5 to 12 % of the absorbed Cu (from Cu nitrate of the 3 lupin species) was exchangeable by Ca nitrate, the exchange of Cd reached 36 to 63 % regardless of the form of Cd supplied.

Introduction

Cultivars of white lupin (*Lupinus albus*), yellow lupin (*Lupinus luteus*) and blue lupin (*Lupinus angustifolius*) show an increased efflux of citrate, malate and other organic acid anions during phosphate deficiency (Neumann *et al.*, 1999; Egle *et al.*, 1999). The organic acid anions not only increase the solubility of phosphorus in soils but also that of metals such as Fe, Al, Zn, Cu and Cd (Gerke *et al.*, 1994; Keller, 2000).

The role of carboxylates on the uptake of metal cations by roots is not well known. Therefore we investigated the uptake by the root of several lupin species of Cu and Cd from solutions as free ions or complexed by citrate. Afterwards, the loosely bound apoplastic metal fraction was determined by exchanging the root-bound metals by incubating in Ca nitrate solution.

Materials and methods

PVC containers were filled with 14 L nutrient solution according to Neumann *et al.*, (1999). Sixteen plants of Lupinus albus (cv. Minori), Lupinus angustifolius (cv. Bordako) and Lupinus luteus (cv. Borsaja) per container were cultivated for 21 days in a climate chamber at a day / night regime of 14 h / 10 h at 22°C / 18°C, 70 % relative humidity, and a photosynthetic active radiation of 240 µE cm^{-2} s^{-1}.

Afterwards, two plants per species were placed in containers (3 L) with 1.8 µmol L^{-1} Cu or Cd as nitrate or nitrate + 1 mmol citrate. The plants remained in the metal-containing solutions for 48 hours at pH 5 to 6. Aliquots of the nutrient solutions were analysed by flame- or GF-AAS at intervals of 1-4 hours. Then the plants were transferred for 5 hours to a 2 mmol Ca nitrate solution (2 L) to remove sorbed or exchangeable Cu and Cd. The solutions were then analysed by flame- or GF-AAS to determine the quantities of desorbed Cu or Cd. Root length was determined by the method of Newman (1966).

Results and discussion

White and blue lupin readily absorbed Cu from Cu-nitrate solutions (Fig. 1). The lowest Cu concentration in solution (C_{min}) was reached after 6 to 9 h. When citrate was present at 1 mmol L^{-1} in the solution, Cu concentration fluctuated during the first 16 h. before stabilizing This fluctuation may be due to the equilibration of the concentration of the external solution with that of the solution of the apparent free space. After 48 h, there was no further depletion of Cu from the applied solution, thus no uptake of Cu. Similar results were found for yellow lupin (data not shown).

In the case of Cd, all three lupin species showed a strong depletion of the Cd nitrate solutions within about 12 h (shown for white lupin only, Fig. 2A). With citrate application, Cd depletion was restricted to the first hour. Afterwards, neither for depletion (Fig. 2A) nor for Cd uptake rate (Fig. 2B), was there any significant difference in the Cd solution with or without citrate.

At a concentration of 1 mmol citrate L^{-1} and a pH 5 to 6, it was calculated using the thermodynamic data of

Martell and Smith (1989) that more than 99 % of the Cu and 85 % of the Cd was complexed by citrate (Gerke, 1995)

Thus, the Cu complexes formed with citrate totally inhibited Cu uptake. The effect on Cd uptake, however, was much less because of the lower tendency of Cd to form complexes. Nevertheless these results support the hypothesis of Römer *et al.* (2000) that Cd uptake by lupin roots may be restricted by the formation of Cd citrate complexes.

The desorption data show that a high proportion of Cd is desorbable by Ca (36 – 63 %) indicating that this fraction is loosely bound in the apparent free space of roots (Table 1). In the case of Cu only 5 to 12 % was exchangeable. The consequences for the uptake of both elements into the root cells and their transport to the shoot are not quite clear because "this exchange adsorption in the apparent free space of the apoplast is not an essential step for ion uptake... into the cytoplasm" (Marschner, 1986).

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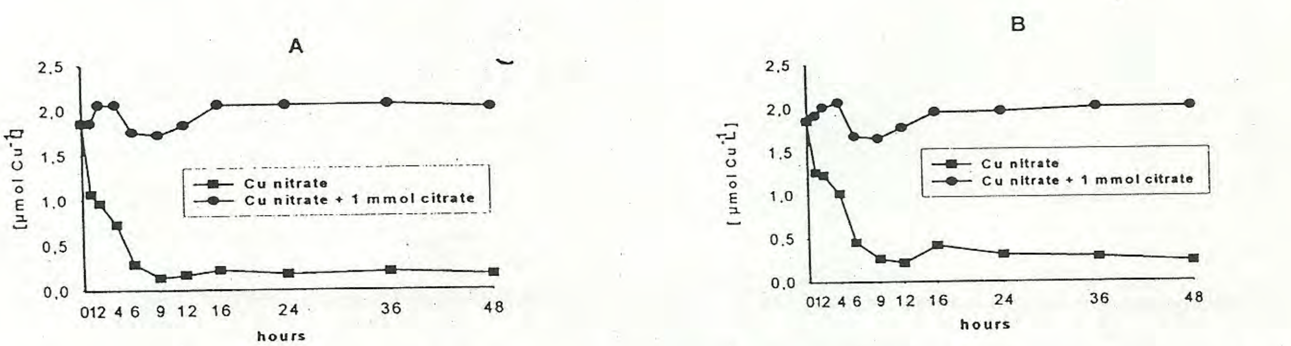

Figure 1. Changes of the Cu concentrations of the applied Cu solutions by *L. albus* (A) and *L. angustifolius* (B) within 48 h.

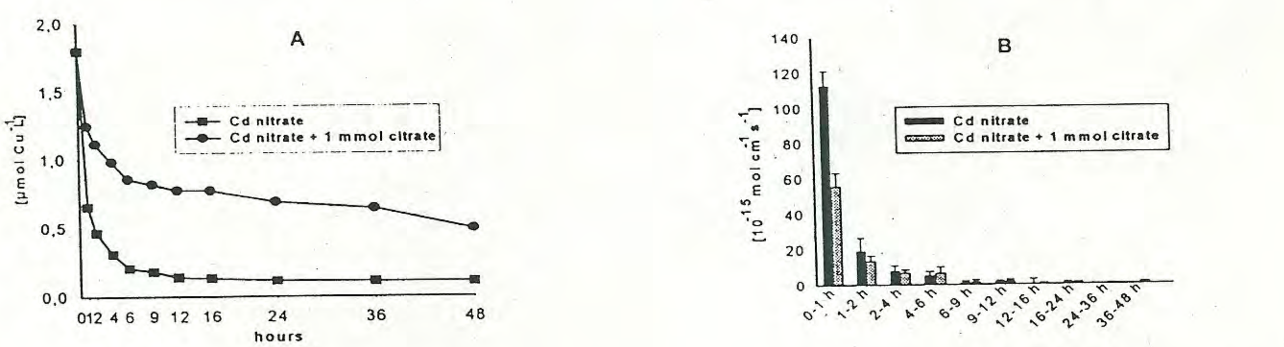

Figure 2. Changes of the Cd concentrations of the applied Cd solutions by *L. albus* (A) and the corresponding Cd net-uptake rates per unit root length (B) within 48 h.

Table 1. Proportions (% of total root content) of Cu and Cd which could be desorbed from the roots by incubation in a 2 mmol Ca nitrate solution for 5 hours after the 48 h uptake period of Cu and Cd in the presence or absence of citrate.

Plant species	$Cu(NO_3)_2$	$Cu(NO_3)_2$ + citrate	$Cd(NO_3)_2$	$Cd(NO_3)_2$ + citrate
Lolium albus	7	0	40	37
Lupinus angustifolius.	5	0	44	48
Lupinus luteus	12	0	36	63