Influence of complexing ligands on Zn uptake and translocation in tobacco and celery plants

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Summary

Substances releasing metals sorbed onto soil particles, or substances changing ionic character influence bioavailability of metals and ability for root uptake as well. In our paper in short-term laboratory experiments, the influence of complexing ligands on the uptake and translocation of $^{65}ZnCl_2$ in hydroponically grown tobacco (*Nicotiana tabacum* L.) and celery (*Apium graveolens* L.) were studied by gamma-spectrometry. EDTA added in equimolar ratio to $ZnCl_2$ (5 μmol dm^{-3}) caused four-times decrease of Zn uptake within 8 d cultivation in 25% diluted Hoagland medium compared with control experiments. Similar effect of NTA and citrate under the same conditions was not observed. On the other hand NTA, but not EDTA and citrate, stimulated Zn transport from roots to shoots in both tobacco and celery plants. Observed data are discussed in connection with the role of free metal ions and metal-ligand complexes in transport processes.
Keywords: ^{65}Zn, uptake, translocation, tobacco, celery, complexing ligands.

Introduction

Zinc is an element of special interest with regard to soil fertility. All plants need it as a micronutrient. Zinc deficiency concerns farmers in many regions around the world. Zinc is a trace element essential for cell proliferation and differentiation. It is a structural constituent of many enzymes and proteins, including metabolic enzymes, transcription factors, and cellular signaling proteins. The problems related to micronutrients are not dependent only on their total content in the soil, but rather on their form of binding and therefore their bioavailability. Therefore, several studies have been conducted using seedlings or adult plant, which have been cultivated in hydroponic conditions (see e.g. *Page* and *Feller* 2005, *Wenger et al.* 2003, *Sarret et al.* 2001). The chemical speciation is a major factor determining the impact of Zn content on soil fertility.

For more than 40 years, synthetic chelates have been used to supply plants with micronutrients in both soil and hydroponics. Ethylenediamine tetraacetate (EDTA) and nitrilotriacetate (NTA) are the common complexing ligands found in synthetic fertilizers. The formation of chelates prevents precipitation and sorption of the metals thereby maintaining their availability for plant uptake. Yet the mechanisms by which chelates enhances metal accumulation are still not well characterized (*Bell et al.* 2003, *Vassil et al.* 1998). Within this context, the objective of our project was firstly to study the changes in chemical speciation of micronutrients, especially zinc in mineral medium supplemented with complexing ligands. The second objective was to study the influence of complexing ligands on the uptake and translocation of Zn in hydroponically grown tobacco (*Nicotiana tabacum* L.) and celery (*Apium graveolens* L.) in short-term laboratory experiments.

Material and methods

Seeds of tobacco (*N. tabacum* L.) and celery (*A. graveolens* L.) were germinated and grown at 22 °C in pots filled with granulated perlite watered with diluted Hoagland medium (*Hoagland* 1920), pH 5.5 at light/dark period 12/12 h and illumination with 2 tubes (2000 lx, Brilliant daylight and Tropic sun, SERA, D). After 8 weeks (tobacco) or 5 weeks (celery), seedlings were transferred into Erlenmeyer flask with 25% Hoagland medium (HM) spiked with $^{65}ZnCl_2$. In time intervals aliquot samples of cultivation media were taken and ^{65}Zn radioactivity was measured by the well type NaI(Tl) scintillation gamma-spectrometer (Scionix, NL) with data processing software Scintivision32 (Ortec, USA). At the end of experiments plants were harvested and roots carefully rinsed in distilled water. In roots, stalks and leaves incorporated radioactivity was measured by gamma-spectrometry. Standardized $^{65}ZnCl_2$ solution (0.88 MBq cm^{-3}, 50 mg dm^{-3} $ZnCl_2$, 3 g dm^{-3} HCl) was obtained from CMI (CZ). Prediction of the Zn speciation in the nutrient solutions as a function of the total salt concentrations, solution pH and temperature was performed using the software Visual MINTEQ ver. 2.52. This speciation model allows the calculation of the composition of solution of specified conditions.

Results and discussion

Numerous Zn-ligand complexes can exist in solution which can be difficult to measure directly, and speciation models, based on total dissolved concentrations of elements and ligands and their stability constants are often used to infer Zn^{2+} concentration in soil solution (*Zhang* and *Young* 2006). As can be calculated by Visual MINTEQ speciation program, zinc in HM nutrient medium at pH 5.5 occurs practically as free cation (> 95% Zn^{2+}). According to the speciation calculations, the addition of 10 µM of NTA or EDTA led to an almost 90% and 96% Zn complexation, respectively. The addition of 5 µM of NTA or EDTA led to 60–68% Zn complexation, the addition of 5 or 10 µM of citric acid caused only negligible Zn complexation (*Table 1.*). It corresponds well with known stability constants of Zn-ligand complexes expressed as log β = 16.5 (for EDTA-Zn), 10.7 (for NTA-Zn) and 4.9 (for Citrate-Zn) at µ = 0.1 and 20 °C (*Sillén* and *Martell* 1964).

Table 1. Content of Zn^{2+} ions and corresponding Zn-ligand complexes (%) at equilibrium in 25% HM* at pH 5.5 and 22 °C in the presence of EDTA, NTA and CA. Calculated by program Visual MINTEQ ver. 2.52.

Metal	Ligand	Zn speciation	
5 µM $ZnCl_2$	–	95% Zn^{2+}	
5 µM $ZnCl_2$	5 µM Citric acid	92.0% Zn^{2+};	3.3% $ZnCitrate^{(-)}$
	10 µM Citric acid	88.0% Zn^{2+};	6.5% $ZnCitrate^{(-)}$
5 µM $ZnCl_2$	5 µM EDTA	30.0% Zn^{2+};	68.0% $ZnEDTA^{(2-)}$
	10 µM EDTA	3.2% Zn^{2+};	96.0% $ZnEDTA^{(2-)}$
5 µM $ZnCl_2$	5 µM NTA	38.0% Zn^{2+};	60.0% $ZnNTA^{(-)}$
	10 µM NTA	11.0% Zn^{2+};	89.0% $ZnNTA^{(-)}$

* Initial concentration of salts in medium (mg dm^{-3}): $MgSO_4 \cdot 7H_2O$ – 92.4; KNO_3 – 101.1; $CaCl_2$ – 111; $NaH_2PO_4 \cdot 2H_2O$ – 72.9; $Na_2HPO_4 \cdot 12H_2O$ – 11.6; $FeSO_4 \cdot 7H_2O$ – 4.5; $NaNO_3$ – 84.9; NH_4Cl – 53.5; NH_4NO_3 – 40.0; H_3BO_3 – 2.1; $Na_2MoO_4 \cdot 2H_2O$ – 0.015; $MnSO_4 \cdot 5H_2O$ – 1.25; $ZnSO_4 \cdot 7H_2O$ – 0.16; $CuSO_4 \cdot 5H_2O$ – 0.2, pH 5.5

EDTA added in equimolar ratio to $ZnCl_2$ (5 µmol dm^{-3}) caused four-times decrease of Zn uptake by celery within 8 d cultivation in 25% diluted HM, compared with control experiments. Similar effect of NTA and citrate under the same conditions was not observed (*Figure 1.*). The same effect was also observed in the case of tobacco plants (data not shown). Speciation analysis showed that Zn root uptake data correspond well with the concentration of zinc in free Zn^{2+} ionic form. The uptake of another bivalent metals in the presence of EDTA will decrease with increasing metal-ligand stability constants in the order $Co^{2+} \geq Zn^{2+} \gg Cu^{2+}$. *Wenger et al.* (2003) remarked that the formation of negatively charged Cu-NTA complexes prevented the Cu from binding to the cation exchange sites in the cell walls of the tobacco roots. The Cu-NTA complex might be taken up and translocated within the plant as an anion and thus overcome the control mechanism of the plants. We suppose that the same mechanism is involved also in the case of Zn-NTA complex uptake by tobacco and celery plants. Zinc is in prevailing part trapped in the tobacco and celery roots and only partially transported to shoots. The presence of EDTA in cultivation media caused decrease of Zn transport from roots to shoot, what can be seen from the increase of $[Zn]_{root}$/ $[Zn]_{shoot}$ concentration ratio comparing with control experiments (*Table 2.*). On the contrary NTA slightly supports Zn transport from roots to shoots, effect of citrate is not significant under given experimental conditions. Zn found in the roots and shoots in per cent of the total amount of accumulated zinc are shown in *Figure 2A, 2B*. *Bell et al.* (2003) found, that citrate and histidine are accumulated average 20-fold greater than EDTA in plant tissues of swiss chard. That may explain the fact that the Zn in the presence of EDTA was preferentially accumulated in roots of tobacco and celery plants.

Figure 1. Influence of complexing ligands on Zn uptake by celery (*A. graveolens* L.) cultivated in 25% HM containing 5 μmol dm^{-3} $ZnCl_2$ and equimolar concentration of NTA, EDTA or CA, pH 5.5 and 22 ^{o}C. Total Zn uptake after 8 d cultivation (%): No ligands – 40.2; NTA – 52.0; EDTA – 19.9; and CA – 41.5

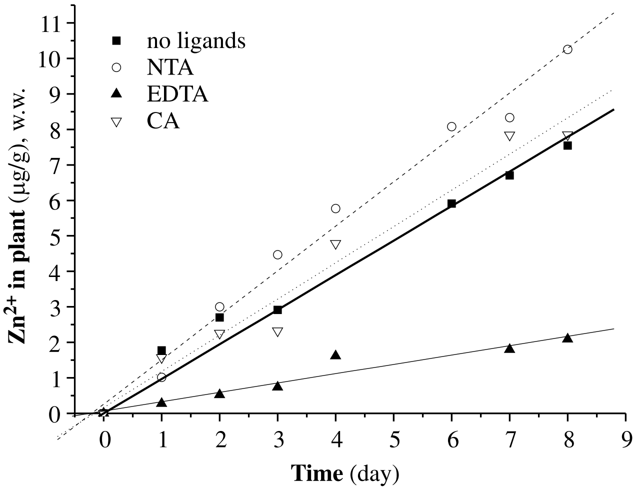

Figure 2. Influence of complexing ligands on distribution of Zn in roots and shoots of tobacco (*N. tabacum* L.) (A) and celery (*A. graveolens* L.) (B) after 8 day cultivation in 25% HM containing 5 μmol dm^{-3} $ZnCl_2$. Composition of nutrient media and Zn speciation see in *Table 1.*

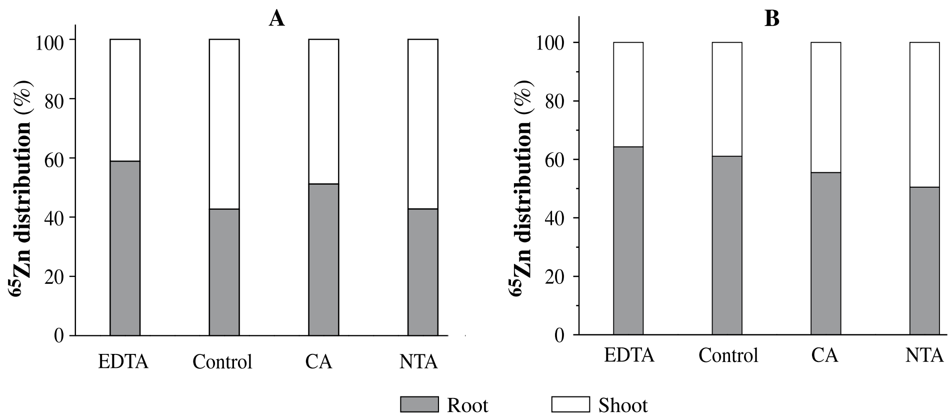

Table 2. Influence of complexing ligands on $[Zn]_{root}$ / $[Zn]_{shoot}$ concentration ratio [μg g^{-1}]/[μg g^{-1}] (dry weight basis) in tobacco (*N. tabacum* L.) and celery (*A. graveolens* L.) plants after 8 d cultivation in 25% HM containing 5 μmol dm^{-3} $ZnCl_2$

Plant	Complexing ligand	$[Zn]_{root}$ / $[Zn]_{shoot}$
Tobacco	–	10:1
	10 μmol dm^{-3} CA	11:1
	5 μmol dm^{-3} NTA	8:1
	10 μmol dm^{-3} EDTA	17:1
Celery	–	8:1
	5 μmol dm^{-3} CA	9:1
	5 μmol dm^{-3} NTA	6:1
	5 μmol dm^{-3} EDTA	15:1

Conclusions

EDTA in mineral nutrient hydroponic media diminishes both Zn root uptake and Zn transport from roots to shoots of tobacco and celery plants. NTA slightly accelerate Zn root uptake and Zn transport from roots to shoots. The effect of citrate on both processes was not significant. These facts are necessary to take in consideration at development of new formula of mineral nutrients, supplemented with comlexing ligands.

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A komplexképző ligandumok hatása a Zn felvételre és a Zn felhalmozódás a dohány és zeller növényekben

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Összefoglalás

Olyan anyagok, amelyek a talajok összetevőin szorbeált fémek kioldását teszik lehetővé, illetve olyanok, amelyek változtatják azoknak az ion-jellemzőit, jelentősen befolyásolják a fémek biológiai felhasználhatóságát és a gyökerek általi felvételt. Értekezésünkben, gamma-spektrometria segítségével, rövid lejáratú laboratóriumi kísérleteket alkalmazva, komplexképző ligandumok befolyását tanulmányoztuk, ^{65}Zn-kel jelölt cink-klorid felvételét és transzlokációját illetően, hidroponikusan termesztett dohányra (*Nicotiana tabacum* L.) és zellerre (*Apium graveolens* L.). $ZnCl_2$-hoz (5 µmol dm^{-3}) ekvimoláris arányban adagolt EDTA négyszeres Zn felvételcsökkenéshez vezetett 8 napi kultiválás és 25%-os Hoagland-médium alkalmazásánál. Egyforma feltételek mellett nem észleltünk hasonló befolyást sem NTA, sem pedig citrát alkalmazásánál. Másrészt, az NTA jelentősen elősegítette a cink, a gyökerekből a sarjakba való transzlokációját, mind dohánynál, mind pedig zellernél. EDTA és citrát alkalmazásánál ezt az effektust nem észleltük. A kapott adatokat a szabad fém ionok és fém-komplexek szerepét illetően tárgyaljuk.

Kulcsszavak: ^{65}Zn, felvétel, transzlokáció, dohány, zeller, komplexképző ligandumok.

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