Supplemental information

Zn distribution and speciation in *Arabidopsis halleri* × *Arabidopsis lyrata* progenies presenting various Zn accumulation capacities

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Materials and methods

Preparation of Zn model compounds

Aqueous Zn model compounds included aqueous Zn (1 M $Zn(NO_3)_2$, pH 4.0), Zn citrate (10 mM $Zn(NO_3)_2$ + 100 mM citrate, pH 4.5), Zn malate (10 mM $Zn(NO_3)_2$ + 100 mM malate, pH 5.5), Zn succinate (10 mM $Zn(NO_3)_2$ + 100 mM succinate, pH 5.5), Zn + 3 OAs (10 mM $Zn(NO_3)_2$ + 33 mM malate + 33 mM citrate + 33 mM succinate, pH 5.5), Zn nicotianamine (3 mM $Zn(NO_3)_2$ + 12 mM nicotianamine, pH could not be measured due to small volume), Zn oxalate (33 mM $Zn(NO_3)_2$ + 130 mM oxalate, pH 5.0), and Zn histidine (10 mM $Zn(NO_3)_2$+ 100 mM histidine, pH 5.5). Other aqueous Zn model compound spectra included Zn acetate with three ligand/Zn (L/Zn) molar ratios (L/Zn = 2, 3 and 5, pH 5.0), Zn aspartate (L/Zn = 4, pH 4.3), Zn citrate at L/Zn = 1, pH 5.0, Zn lactate (L/Zn = 5, pH 5.0), and Zn salicylate (L/Zn = 5, pH 5.0) (Fig. SI1A). All solutions were mixed with 30% (v/v) glycerol to minimize the formation of ice crystals during freezing. Solid state model compounds included various zinc minerals, Zn sorbed on various minerals at different surface loadings and pH conditions as inner and outer-sphere surface complexes, and Zn-OAs and Zn-amino acids complexes previously described (Sarret *et al.*, 2002). Zn-pectin complexes were prepared by introducing 166 mg of pectin extracted from apples esterified at 70 to 75% (Fluka) in 5.5 mL of 0.23 and 0.46 mM $Zn(NO_3)_2$ (7.6 and 15.3 µmol g^{-1} DW, respectively, *i.e.*, L/Zn = 767 and 383, with L = one galacturonic acid residue esterified at 70%), and stirring the gel for 3 h with the pH held at 5.0 by addition of 0.1 N NaOH or HNO_3. Since the Zn-pectin complexes could not be separated by centrifugation, the gel was directly freeze-dried at −50 °C. Zn-cell wall complexes were prepared using cell walls ghosts isolated from tobacco roots. Although the composition of the cell wall of Arabidopsis leaves and tobacco roots may differ, the sensitivity of EXAFS for this type of polymeric material is probably not sufficient to detect it. The same tobacco cell walls were used for the study of Cd speciation in *A. thaliana* roots and leaves (Isaure *et al.*, 2006). They were obtained by immersing fresh roots of 4-week old tobacco plants in a 1% v/v Triton X100 detergent solution with 1 mM $CaCl_2$ for 28 days to remove the cell content. The detergent was then removed by washing with a 1 mM $CaCl_2$ solution. The entire treatment was carried out at 4 °C. Then, 100 mg of cell walls were placed in 50 mL of 1.53×10^{-3}, 6.11×10^{-3}, 3.06×10^{-2} and 3.06×10^{-1} mM $Zn(NO_3)_2$ at pH 5.0, and the suspension was centrifuged after 3 days of contact (final pH= 5.4). The pellet was then freeze-dried. The final Zn concentrations in cell walls were 0.76, 1.4, 12.7 and 69.6 µmol g^{-1} DW, respectively. The preparation of Zn-sorbed hydroxylapatite at various Zn concentrations and pH was described previously (Panfili *et al.*, 2005). The sample ZnPhos contains 1% Zn, and was prepared at pH 5.0. Solid state model compounds were prepared as 5 mm pressed pellets for EXAFS analyses.

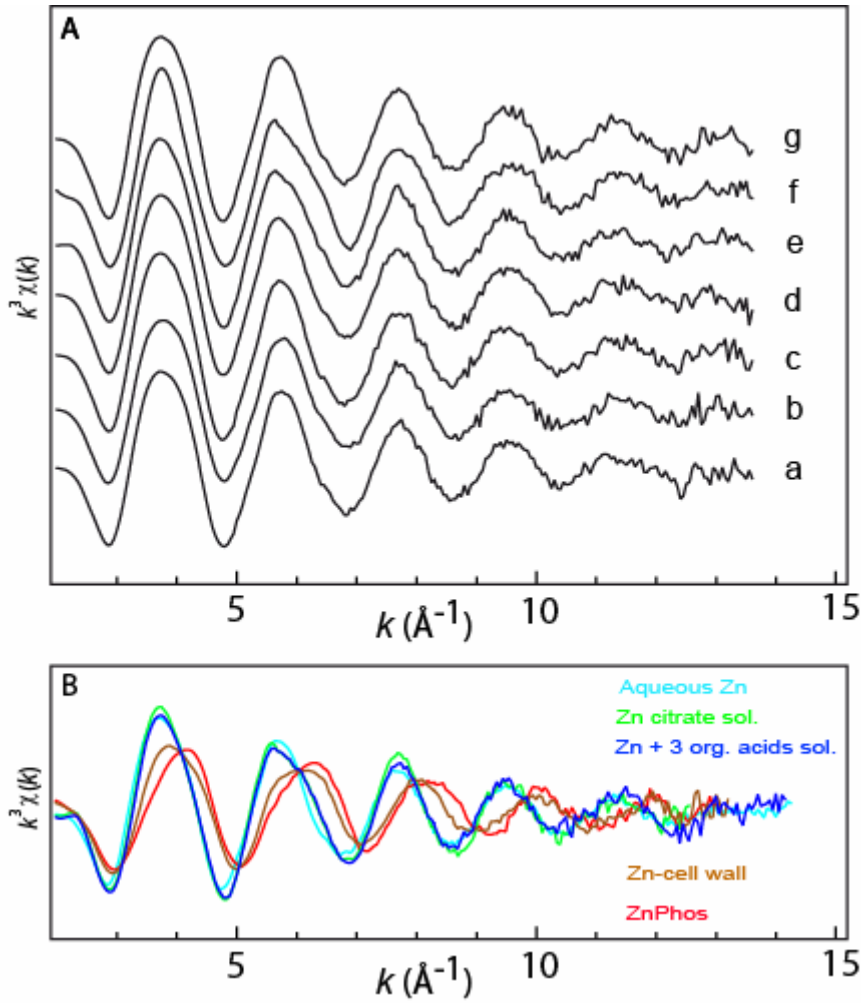

Figure SI1: A. Zn K-edge EXAFS spectra for aqueous Zn reference compounds: Zn acetate at pH 5.0 and ligand/Zn (L/Zn) molar ratio = 2 (a), 3 (b) and 5 (c); Zn aspartate (pH 4.3, L/Zn = 4, d); Zn citrate (pH 5.0, L/Zn = 1, e), Zn lactate (pH 5.0, L/Zn = 5, f), and Zn salicylate (pH 5.0, L/Zn = 5, g). B. Zn K-edge EXAFS spectra for aqueous Zn^{2+}, Zn citrate and Zn + 3 AOs in solution, Zn-cell wall and ZnPhos (spectra also shown in Fig. 2A)

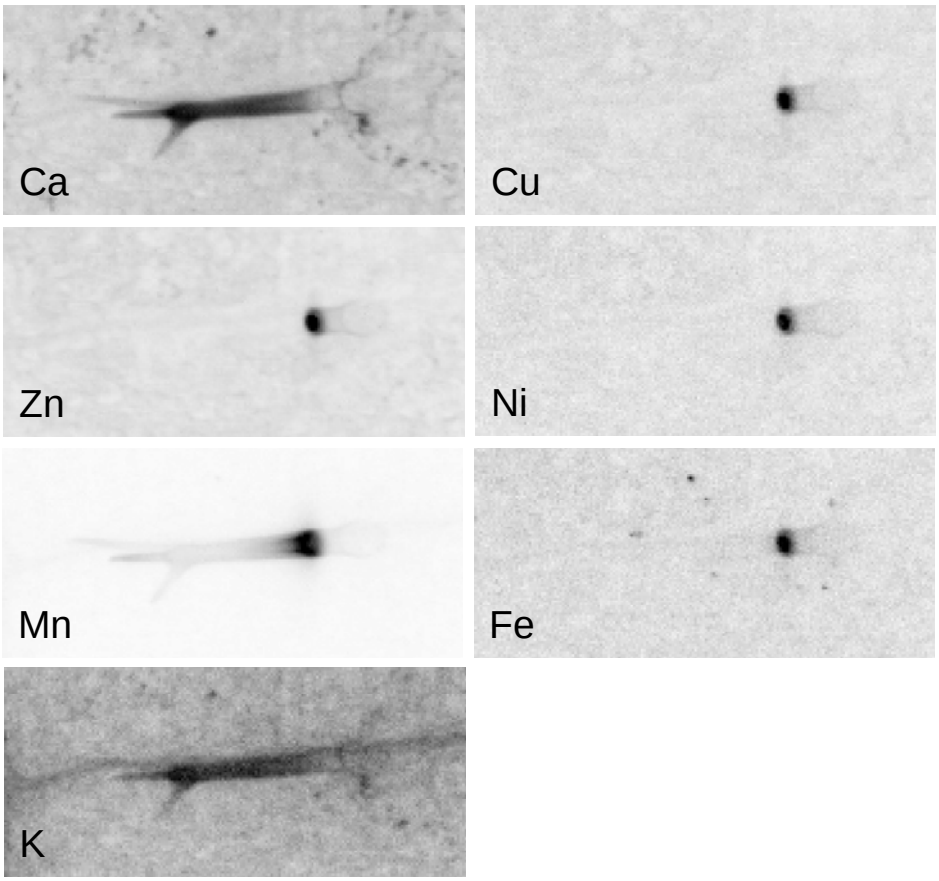

Figure SI2: µXRF maps of a trichome of *A. halleri* also presented in Figure 10, recorded at 10 keV with 4×4 µm^2 pixel size and a counting time of 100 ms pixel^{-1}. Darker regions correspond to higher concentrations. Bar: 100 µm.

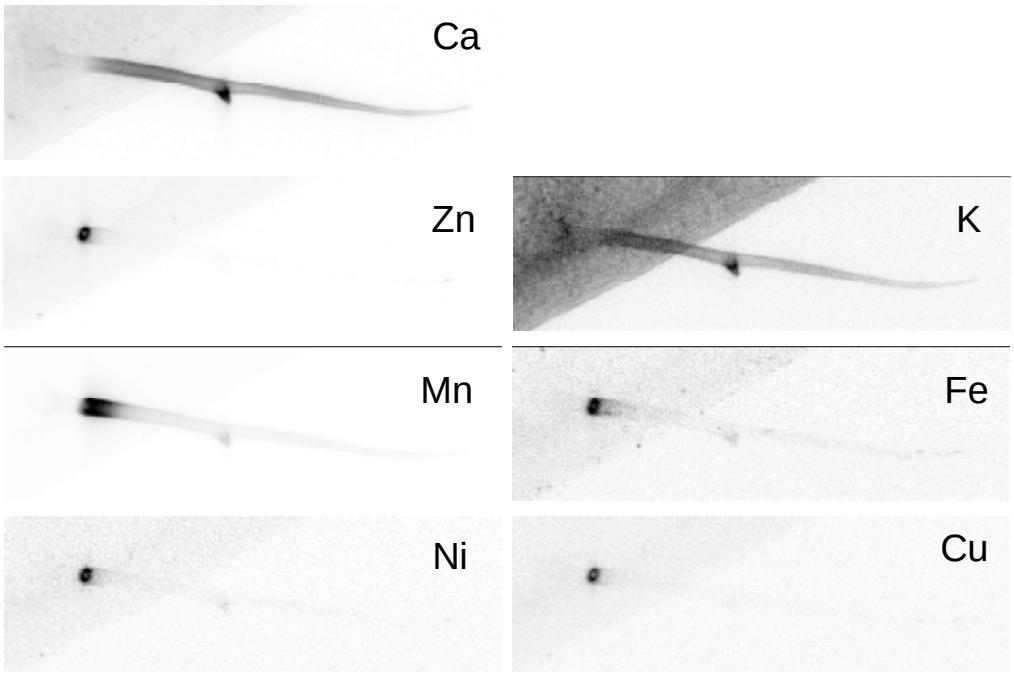

Figure SI3: µXRF maps of a trichome of *A. lyrata* also presented in Figure 10, recorded at 10 keV with 4 × 4 μm^2 pixel size and a counting time of 100 ms $pixel^{-1}$. Darker regions correspond to higher concentrations. Bar: 100 µm.

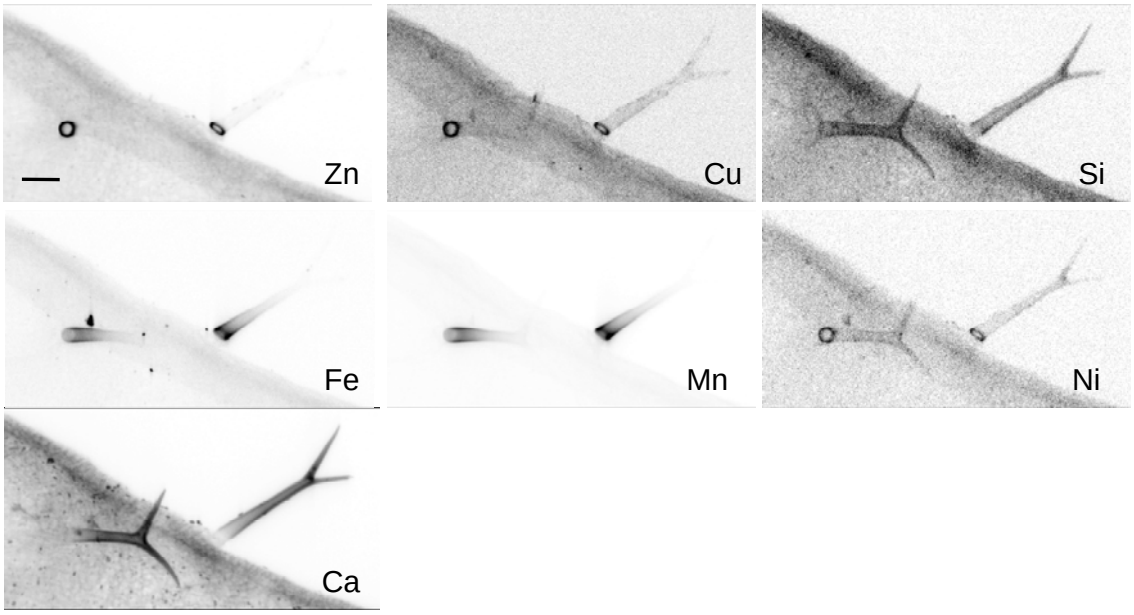

Figure SI4: µXRF maps of trichome of F2-3 also presented in Figure 10, recorded at 10 keV with 3×3 µm^2 pixel size and a counting time of 100 ms pixel^{-1}. Darker regions correspond to higher concentrations. Bar: 100 µm.