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Review

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Zinc in plants

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Contents

Summary

Key words: Brassicaceae, cadmium (Cd), Genechip, genetics, hyperaccumulation, ion transport, transcriptomics, uptake kinetics.

Zinc (Zn) is an essential component of thousands of proteins in plants, although it is toxic in excess. In this review, the dominant fluxes of Zn in the soil–root–shoot continuum are described, including Zn inputs to soils, the plant availability of soluble Zn^{2+} at the root surface, and plant uptake and accumulation of Zn. Knowledge of these fluxes can inform agronomic and genetic strategies to address the widespread problem of Zn-limited crop growth. Substantial within-species genetic variation in Zn composition is being used to alleviate human dietary Zn deficiencies through biofortification. Intriguingly, a meta-analysis of data from an extensive literature survey indicates that a small proportion of the genetic variation in shoot Zn concentration can be attributed to evolutionary processes whose effects manifest above the family level. Remarkable insights into the evolutionary potential of plants to respond to elevated soil Zn have recently been made through detailed anatomical, physiological, chemical, genetic and molecular characterizations of the brassicaceous Zn hyperaccumulators *Thlaspi caerulescens* and *Arabidopsis halleri*.

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I. Physical and chemical properties of zinc

Zinc is a transition metal of atomic number 30 and is the 23rd most abundant element on earth. Zinc has five stable isotopes: ^{64}Zn (48.63%), ^{66}Zn (27.90%), ^{67}Zn (4.90%), ^{68}Zn (18.75%) and ^{70}Zn (0.62%). Heavy and light isotopic enrichments of root and shoot Zn fractions, respectively, have been reported in plants (Weiss *et al.*, 2005). Approximately 30 short-lived Zn radioisotopes occur in the atomic mass range 54–83, and the longest-lived (^{65}Zn, $t_{1/2}$ = 244.26 d) is frequently used as a Zn tracer in plants. In solution, Zn exists in the +2 oxidation state and, unlike Fe^{2+} and Cu^{2+}, is redox-stable under physiological conditions as a result of a complete *d*-shell of electrons (Barak & Helmke, 1993; Auld, 2001). Additionally, Zn^{2+} has pronounced Lewis acid characteristics because of its small radius to charge ratio (i.e. 0.83 Å, coordination number, CN = 6) compared, for example, with Ca^{2+} (1.08 Å, CN = 6), and thus forms strong covalent bonds with S, N and O donors. This electron configuration of aqueous Zn^{2+} complexes favours octahedral coordination geometries (CN = 6), although CN = 4 and CN = 5 geometries also occur (Barak & Helmke, 1993). Zinc forms numerous soluble salts, including halides, sulphates, nitrates, formates, acetates, thiocyanates, perchlorates, fluosilicates, cyanides, alkali metal zincates and Zn-ammonia salts; sparingly soluble compounds, including Zn-ammonium phosphate, Zn hydroxide and Zn carbonate; and a range of soluble and insoluble organic complexes (Lindsay, 1979; Barak & Helmke, 1993).

II. Biochemical properties of zinc

Zinc is typically the second most abundant transition metal in organisms after iron (Fe), and the only metal represented in all six enzyme classes (Enzyme Commission number, EC 1–6; (oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases; http://www.chem.qmul.ac.uk/iubmb/enzyme/; Webb, 1992). Enzymatic function and reactivity are determined by the geometric and binding characteristics of Zn^{2+}-ligand complexes and three primary Zn^{2+}-ligand binding sites are recognized: structural, catalytic and cocatalytic (Auld, 2001; Maret, 2005). Structural Zn sites, in which Zn ensures appropriate protein folding (e.g. alcohol dehydrogenases, protein kinases), comprise four ligands, frequently cysteine (Cys), and no bound water molecule. In catalytic sites, Zn is directly involved in the catalytic function of the enzyme (e.g. carbonic anhydrases); histidine (His) is the primary amino acid and Zn^{2+} is complexed with water and any three S, N or O donors. In cocatalytic sites, Zn^{2+} can be used for catalytic, regulatory and structural functions (e.g. superoxide dismutases, purple acid phosphatases, metallo-β-lactamases). At such sites, two or three Zn^{2+} occur in close proximity and are bridged by amino acid residues, principally aspartic acid (Asp) or glutamic acid (Glu), but also His and potentially a water molecule, but not Cys. A fourth type of Zn^{2+}-ligand binding or protein interface site can behave as a catalytic or structural site. These occur when ligands from the surface of two protein molecules bind to a single Zn atom (e.g. nitric oxide synthases). Zinc binding sites also occur in a wide range of other proteins, membrane lipids and DNA/RNA molecules. The largest class of Zn-binding proteins in organisms is the zinc finger domain containing proteins, which can regulate transcription directly through effects on DNA/RNA binding, and also through site-specific modifications, regulation of chromatin structure, RNA metabolism and protein–protein interactions (Klug, 1999; Englbrecht *et al.*, 2004).

III. Proteins interacting with zinc

In *Escherichia coli*, femtomolar (1×10^{-15} M) cytosolic concentrations of free Zn^{2+} induce the activity of Zn influx (Zur) and efflux (ZntR) proteins (Outten & O'Halloran, 2001). Since these concentrations are 10^6 times lower than one Zn^{2+} ion per cell, contrasting with a minimal Zn content of an *E. coli* cell in the millimolar range (*c.* 200 000 atoms per cell), cytosolic free Zn^{2+} pools are not thought to persist. Ionic Zn is likely to be excluded from the cytosol via direct transfer between proteins, with > 10% of cellular Zn thought to be tightly bound to just six proteins, including an RNA polymerase expressed at 5000 copies per cell with two Zn atoms bound per copy, and five tRNA synthetases expressed at 2000–3000 copies per cell with a single Zn atom bound per copy (Outten & O'Halloran, 2001). Since there are > 30 further proteins of unknown copy number which require tightly bound Zn (Katayama *et al.*, 2002), and many other proteins, amino acids and nucleotides with lower affinities for Zn, a large Zn-binding overcapacity in the cytosol of *E. coli* has been predicted (Outten & O'Halloran, 2001). In plant cells, high Zn-status leaf epidermal cell vacuoles, cell walls and cytoplasm (i.e. cytosol and organelles excluding the vacuole) can contain, respectively, 74 305, 11 577 and 3205 μg Zn g^{-1} DW (dry weight); lower Zn-status leaf mesophyll cell vacuoles, cell walls and cytoplasm contain, respectively, 327, 9353 and ≤ 262 μg Zn g^{-1} DW; and root cortical vacuoles, cell walls and cytoplasm contain, respectively, ≤ 262, 589 and ≤ 262 μg Zn g^{-1} DW (Frey *et al.*, 2000). Thus, 9.6×10^{10}, 2.7×10^9 and 1.8×10^9 atoms of Zn can occur in leaf epidermal cell vacuoles, cell walls and cytoplasm, respectively. Leaf mesophyll cell vacuoles, cell walls and cytoplasm contain 4.2×10^8, 2.2×10^9 and $\leq 1.5 \times 10^8$ atoms of Zn respectively, and root cortical cell vacuoles, cell walls and cytoplasm contain $\leq 3.4 \times 10^8$, 1.4×10^8 and $\leq 1.5 \times 10^8$ atoms of Zn, respectively. These calculations assume that a plant cell comprises 1×10^{-15} m^3 with a mass of 1×10^{-9} g FW (fresh weight); the vacuole, cell wall and cytoplasm occupy 70, 5 and 25%, respectively, of the cell volume; and FW : DW ratios are five, two and four for vacuoles, cell wall and cytoplasm, respectively (Flowers & Yeo, 1992; Frey *et al.*, 2000). It is not yet known what proportion of plant cytoplasmic Zn is present as free Zn^{2+} or as Zn bound to protein, amino acid, nucleotide or lipid ligands at lower affinities, or compartmentalized into organelles. However, by

analogy with *E. coli*, it seems likely that (i) cytosolic Zn^{2+} concentration ($[Zn^{2+}]_{cyt}$) will be vanishingly low to prevent interference with metalloregulatory and other signalling proteins; and (ii) low $[Zn^{2+}]_{cyt}$ will be maintained through high-affinity binding of Zn in the cytosol and through compartmentalization of Zn into cytoplasmic organelles. In mammals, cytoplasmic Zn can be sequestered into vesicles ('zincosomes'; Beyersmann & Haase, 2001), and this process might also occur in plants.

In the human genome, annotation- and Zn-binding domain-based searches reveal that *c.* 10% of proteins (i.e. 2800) potentially bind Zn (Andreini *et al.*, 2006), with hundreds more involved in Zn transport and trafficking (Beyersmann & Haase, 2001). A similar *in silico* study of the complement of Zn-binding proteins in *Arabidopsis thaliana* (L.) Heynh. was undertaken here. First, protein domains with observed or predicted capabilities for binding Zn were identified from the Pfam database (http://www.sanger.ac.uk/Software/Pfam/; 25 July 2006). Approximately 120 putative Zn-binding protein domains were identified, with 2042 *A. thaliana* proteins (TAIR6) containing one or more of these domains (Supplementary material, Table S1). Secondly, annotation searches using the words 'zinc' or 'Zn', corrected for false positives, revealed 1245 genes (Table S2), of which 1096 were common to the domain-based search. Finally, proteins implicated in Zn homeostasis were hand-compiled (Table S3). These included the remaining carbonic anhydrases, alcohol dehydrogenases and proteins with putative Zn transport functions, including P_{1B}-type ATPases, divalent cation transporters and non-specific cation channels. This third list contained 1635 proteins, 176 of which were unique. In total, 2367 proteins (Table S4) in 181 gene families (Table S5) were identified as Zn-related. Briefly, one or more Gene Ontology molecular function (GO:3674) subcategories were assigned to each gene using GeneSpring GX (Agilent Technologies Inc., Palo Alto, CA, USA) (Table 1). The largest group of Zn-binding proteins in *A. thaliana* are Zn finger domains, assigned to transcription regulator activity (GO:30528) and binding (GO:5488) functional subcategories. The catalytic activity (GO:3824) subcategory comprises numerous proteins, including those with hydrolase activity (GO:16787, e.g. P_{1B}-ATPases) and transferase activity (GO:16740, e.g. mitogen-activated protein kinases (MAPKs)). The transporter activity (GO:5215) subcategory includes ABC transporters, P_{1B}-ATPases, various divalent cation transporters (for example, the cation diffusion facilitator family (CDFs)), Zn-Fe permeases (ZIPs) and non-specific cation channels.

IV. Zinc fluxes in the soil–root–shoot continuum

1. Zinc inputs to soils

The primary input of Zn to soils is from the chemical and physical weathering of parent rocks. The lithosphere typically comprises 70–80 μg Zn g^{-1}, whilst sedimentary rocks contain 10–120 μg Zn g^{-1} (Friedland, 1990; Barak & Helmke, 1993; Alloway, 1995). Mean soil Zn concentrations ($[Zn]_{soil}$) of 50 and 66 μg total Zn g^{-1} soil are typical for mineral and organic soils, respectively, with most agricultural soils containing 10–300 μg Zn g^{-1} (Alloway, 1995; Barber, 1995). Zinc occurs in rock-forming minerals as a result of the nonspecific replacement of Mg and Fe with Zn (Barak & Helmke, 1993). Rocks containing weathered Zn minerals, including Zn sulphide (sphalerite, wurtzite), sulphate (zincosite, goslarite), oxide (zincinte, franklinite, gahnite), carbonate (smithsonite), phosphate (hopeite) and silicate (hemimorphite, willemite) minerals, can form 'calamine' soils containing extremely high concentrations of Zn and other metals (Barak & Helmke, 1993). For example, in Plombières in Belgium, $[Zn]_{soil}$ exceeds 100 000 μg Zn g^{-1} (Cappuyns *et al.*, 2006). Such sites are usually localized to a few hectares, although adjacent soils can also have high $[Zn]_{soil}$ through water seepage from ore bodies (Chaney, 1993). Secondary natural inputs of Zn to soils arise because of atmospheric (e.g. volcanoes, forest fires, and surface dusts) and biotic (e.g. decomposition, leaching/washoff from leaf surfaces) processes (Friedland, 1990).

Humans have long influenced Zn inputs to soils. Two thousand years ago, approx. 10 000 tonnes Zn yr^{-1} were emitted as a result of mining and smelting activities (Nriagu, 1996). Since 1850, emissions have increased 10-fold, peaking at 3.4 Mt Zn yr^{-1} in the early 1980s, and then declining to 2.7 Mt Zn yr^{-1} by the early 1990s (Nriagu, 1996). Arctic troposphere Zn concentrations (*c.* 2 ng Zn m^{-3} in winter months) are yet to reflect this decline (Gong & Barrie, 2005). The ratio of Zn emissions arising from anthropogenic and natural inputs is estimated to be > 20 : 1 (Friedland, 1990). Other anthropogenic inputs of Zn to soils include fossil fuel combustion, mine waste, phosphatic fertilizers (typically 50–1450 μg Zn g^{-1}), limestone (10–450 μg Zn g^{-1}), manure (15–250 μg Zn g^{-1}), sewage sludge (91–49 000 μg Zn g^{-1}), other agrochemicals, particles from galvanized (Zn-plated) surfaces and rubber mulches (Chaney, 1993; Alloway, 1995). Crop Zn toxicity can occur in Zn-contaminated soils (discussed in Section VI.1).

2. Zinc behaviour in soils

Soil Zn occurs in three primary fractions: (i) water-soluble Zn (including Zn^{2+} and soluble organic fractions); (ii) adsorbed and exchangeable Zn in the colloidal fraction (associated with clay particles, humic compounds and Al and Fe hydroxides); and (iii) insoluble Zn complexes and minerals (reviewed by Lindsay, 1979; Barrow, 1993; Alloway, 1995; Barber, 1995). The distribution of Zn between soil fractions is determined by soil-specific precipitation, complexation and adsorption reactions. The dominant factor determining soil Zn distribution is pH; Zn is more readily adsorbed on cation exchange sites at higher pH and adsorbed Zn is more readily displaced by $CaCl_2$ at lower pH. Thus, soluble Zn and the ratio of Zn^{2+} to

Table 1 Gene Ontology molecular function (GO:3674) assigned to 2367 Zn-related genes in *Arabidopsis thaliana*, identified through annotation-, domain- and literature-based searches using GeneSpring GX (Agilent Technologies, Inc., Palo Alto, CA, USA) (primary data in Supplementary material, Table S4)

Gene Ontology: molecular function (GO:3674) subcategories	Example gene families	Number of genes with potential role in Zn homeostasis	Selected references
Binding (GO:5488)	Zinc finger proteins	1503	Berg & Shi (1996), Klug (1999), Auld (2001)
	Squamosa promoter binding proteins		Yamasaki *et al*. (2004)
	Metallothioneins		Blindauer & Sadler (2005), Roosens *et al*. (2005), Zimeri *et al*. (2005)
	WRKY family transcription factors		Eulgem *et al*. (2000)
Catalytic activity (GO:3824)	Alcohol dehydrogenases	634	Chase (1999), Kim *et al*. (2004)
	Carbonic anhydrases		Moroney *et al*. (2001), Tiwari *et al*. (2005)
	Superoxide dismutases		Mittler *et al*. (2004)
	Glutathione transferases		Edwards & Dixon (2005)
	Metallo β-lactamases		Auld (2001)
	Purple acid phosphatases		Olczak *et al*. (2003)
	Mitogen-activated protein kinases (MAPK)		Morris (2001)
	SET-domain transcriptional regulators		Cheng *et al*. (2005)
	P_{IB}-ATPases		Williams & Mills (2005)
Transcription regulator activity (GO:30528)	Zinc finger proteins (e.g. CCHC, CONSTANS B-Box, Cys_2/His_2, Dof, GATA)	379	Clay & Nelson (2005), Robson *et al*. (2001), Reyes *et al*. (2004), Sakamoto *et al*. (2004), Yanagisawa (2004)
	Squamosa promoter binding proteins		Yamasaki *et al*. (2004)
	WRKY family transcription factors		Ülker & Somssich (2004), Zhang & Wang (2005)
Transporter activity (GO:5215)	ABC transporters	254	Hall & Williams (2003), Hantke (2005)
	P_{IB}-ATPases		Williams & Mills (2005)
	Divalent cation transporters from several families (e.g. cation diffusion facilitators, CDFs, Zn-Fe permeases, ZIPs)		Colangelo & Guerinot (2006), Mäser *et al*. (2001)
	Nonspecific cation channels		White *et al*. (2002a)
Molecular function unknown (GO:5554)		241	
Signal transducer activity (GO:4871)	Mitogen-activated protein kinases (MAPK)	26	Morris (2001)
Structural molecule activity (GO:5198)	40S ribosomal protein S27	12	McIntosh & Bonham-Smith (2006)
Translation regulator activity (GO:45182)	Translation initiation factors	10	Browning (2004)
Enzyme regulator activity (GO:30234)	GTPase activator proteins of Rab-related small GTPases-like protein	7	Saito *et al*. (2002)

organic Zn-ligand complexes increase at low pH, especially in soils of low soluble organic matter content. Soil type, soil moisture, mineral and clay types and contents, diffusion and mass flow rates, weathering rates, soil organic matter, soil biota and plant uptake will also affect Zn distribution. Insoluble Zn comprises > 90% of soil Zn and is unavailable for plant uptake. Exchangeable Zn typically ranges from 0.1 to 2 μg Zn g^{-1}. Concentrations of water-soluble Zn in the bulk soil solution ($[Zn]_{bss}$) are low, typically between 4×10^{-10} and 4×10^{-6} M (Barber, 1995), even in Zn-contaminated soils (Knight *et al.*, 1997). 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, their stability constants and mineral equilibria reactions, are often used to infer $[Zn^{2+}]_{bss}$ (Barak & Helmke, 1993; Zhang & Young, 2006). Zn^{2+} typically accounts for up to 50% of the soluble Zn fraction and is the dominant plant-available Zn fraction. However, in calcareous soils, Zn^{2+} may be as low as 10^{-11}–10^{-9} M and can limit crop growth (Hacisalihoglu & Kochian, 2003; discussed in Section V.1).

Soil Zn fractions in the solid phase can be quantified using sequential extractions or isotopic dilution techniques (Young *et al.*, 2006). For example, Zn extracted by H_2O ('water-soluble'), KNO_3 ('exchangeable'), $Na_4P_2O_7$ ('organically bound'), EDTA ('carbonate/noncrystalline iron occluded'), NH_2OH ('manganese oxide occluded'), $Na_2S_2O_4$ ('crystalline iron oxide occluded'), HNO_3 ('sulphides'), $HNO_3 + H_2O_2$ ('residual') represented 0.2, 10.0, 32.5, 7.9, 7.2, 7.5, 3.3 and 32.3% of total soil Zn, respectively, in a mineral soil from Indiana, in the USA (Miller & McFee, 1983). Despite recent methodological advances in measuring metal species in the soil solution (Zhang & Young, 2006), Zn availability at the soil–root interface ($[Zn]_{ext}$) can still be difficult to determine satisfactorily, especially if Zn uptake by roots is high and Zn-depletion zones develop around the root. Thus, modelling approaches to determine soil and plant effects on Zn dynamics have been developed (Barber & Claassen, 1977; Bar-Yosef *et al.*, 1980; Barber, 1995; Whiting *et al.*, 2003; Qian *et al.*, 2005; Lehto *et al.*, 2006). For example, Whiting *et al.* (2003) adapted a transport model of Baldwin *et al.* (1973) to calculate $[Zn]_{ext}$, using empirically derived soil parameters (Barber, 1995) and empirically derived or inferred plant parameters. The parameters were $[Zn]_{bss}$ (concentration of Zn in the bulk soil solution), D (the effective diffusion coefficient of Zn in the soil solution), b (the Zn-buffering power of the soil), x (the radius of the soil cylinder that can be exploited by the root), α (the root absorption power of plant for Zn), $\bar{o}$ (the water flux from the soil to the root surface) and a (the root radius). Values for $[Zn]_{bss}$ vary widely, as described previously, typically in the range 1×10^{-8} to 1×10^{-6} M. Values of D also vary widely, increasing at high $[Zn]_{soil}$, high soil bulk density (air-filled pores increase diffusion resistance), high soil water content and low pH, typically within the range 10^{-10}–10^{-8} cm^2 s^{-1} for Zn (Barber, 1995). Values of b represent the distribution of Zn between the solution and the solid phases and are estimated from empirically derived Zn adsorption isotherms (Barber, 1995). Values of b are highest at low $[Zn]_{bss}$, and thus at high cation exchange capacity (CEC) and pH. Values of b typically range from 2.4 to 571 (Barber, 1995), and for vertisols from 217 to 790 (Dang *et al.*, 1994). Paramaters $\bar{o}$ and α represent plant-specific transpiration and Zn uptake rates and root morphology. For plants of the same size and with the same root Zn and water-absorbing power, the primary drivers of $[Zn]_{ext}$ are $[Zn]_{bss}$ and b (Whiting *et al.*, 2003). At high b (> 200), $[Zn]_{ext}$ is proportional to, or approximates, $[Zn]_{bss}$, that is, plant-available Zn is determined by the capacity of the soil to replenish the soluble Zn fraction as it is removed by the plant. However, when $b < 200$, $[Zn]_{ext}$ is << $[Zn]_{bss}$, and especially so when $b < 50$.

Modelling $[Zn]_{ext}$ requires assumptions to minimize complexity and to compensate for a lack of adequate input data. For example, Whiting *et al.* (2003) assumed that: (i) $[Zn]_{ext}$ was in steady state with roots nonrandomly dispersed at constant density in a finite volume of soil; (ii) only radial transport of Zn occurred; (iii) all Zn in the soil solution was available for uptake; and (iv) no compounds mobilizing Zn from the solid phase were secreted from roots, mycorrhiza or other soil-dwelling organisms. However, models to predict $[Zn]_{ext}$ are continually being improved for both soil (Lehto *et al.*, 2006) and plant (Qian *et al.*, 2005) parameters, and are likely to become invaluable in improving our understanding of whole-plant physiological processes, optimizing crop Zn nutrition through the application of fertilizers and soil amendments, and improving risk assessments for metal-contaminated environments.

3. Zinc fluxes into plants

Zinc is acquired from the soil solution primarily as Zn^{2+}, but also potentially complexed with organic ligands, by roots which feed the shoots via the xylem. The relationship between Zn influx (and uptake or accumulation) to excised roots and intact plants, V, and $[Zn]_{ext}$ is often characterized by the sum of one or more Michaelis–Menten functions, each defined by a V_{max} (the rate at $[Zn]_{ext} = \infty$) and an affinity constant, K_m ($[Zn]_{ext}$ when $V = 0.5\,V_{max}$), plus a linear term, k ($V/[Zn]_{ext}$). Most detailed kinetic studies report a Michaelis–Menten function with a K_m of 1.5–50 μm (with V_{max} values of up to 5.74 μmol g^{-1} FW h^{-1}), and, occasionally, additional Michaelis–Menten functions with higher K_m values (Table 2). These K_m values are generally higher than $[Zn^{2+}]_{bss}$ (10^{-11}–10^{-6} M; see Section IV.2). Two studies have reported Michaelis–Menten functions for wheat plants with K_m values of 0.6–2.3 nM for Zn^{2+} uptake (Wheal & Rengel, 1997; Hacisalihoglu *et al.*, 2001). Whilst K_m and V_{max} do not differ greatly between Zn-efficient and Zn-inefficient wheat varieties, suggesting that the kinetics of Zn influx *per se* do not play a significant

Table 2 Published Michaelis–Menten functions, describing the relationship between zinc influx (and uptake or accumulation) and excised roots and intact plants, V, according to the terms V_{max} (V, when $[Zn]_{ext} = \infty$), K_m ($[Zn]_{ext}$, when $V = 0.5V_{max}$), and a linear term k ($V/[Zn]_{ext}$)

		Function 1		Function 2		Function 3			
Reference	$[Zn]_{ext}$ (μM)	K_m (μM)	V_{max}[a] (nmol g^{-1} h^{-1})	K_m (μM)	V_{max}[a] (nmol g^{-1} h^{-1})	K_m (μM)	V_{max}[a] (nmol g^{-1} h^{-1})	k	Notes[b]
Schmid *et al*. (1965)	0.5–10	21.6	714 FW	No	No	No	No	No	Excised barley roots, [A], [C], [F], inhibition by 10 μM Cu
Chaudhry & Loneragan (1972a)	0.5–10	3.1	275 FW	No	No	No	No	No	Wheat seedlings, [A], [D], [F], noncompetitive inhibition by 250 μM Mg, Ba, Sr, Ca
Chaudhry & Loneragan (1972b)	0.05–5	1.0	6.31 FW	No	No	No	No	No	Wheat seedlings, [A], [D], [F]
Chaudhry & Loneragan (1972c)	0.5–10	36	50.8 FW	No	No	No	No	No	Wheat seedlings, [A], pH 4, [D], [F], competitive inhibition by 1 μM Cu
Bowen (1973)	8–500	a) 18 b) 103	4000 DW 18 300 DW	No	No	No	No	No	Excised sugarcane roots of (i) Zn-efficient [H53-263] and (ii) Zn-inefficient [H57-5174] clones, [A], [D], [F]
Brar & Sekhon (1976)	0.5–2	5.3	4154 DW	No	No	No	No	No	Wheat plants, [A], [D], [F], competitive inhibition by 5 μM Cu
Hassan & van Hai (1976)	0.01–500	(a) 1.6 ± 1.0 (b) 3.9 ± 3.2	(a) 210 DW (b) 100 DW	(a) 83 ± 24 (b) 53 ± 18	(a) 3880 DW (b) 1790 DW	(a) 120 ± 30 (b) 150 ± 60	(a) 7810 DW (b) 7220 DW	No	(a) Excised roots and (b) intact plants of sour orange, [B], [C], [G]
Veltrup (1978)	1.5–1380	3.18 ± 1.8	530 DW	151 ± 43.8	10.6 DW	490 ± 200	18.2 DW	No	Barley plants, [B], [D], rinse, [G], unaffected by 16 μM Cu
Bowen (1981)	20–250	16	5710 DW	42	20 400 DW	No	No	No	Excised barley roots, [A], [C], [F], [G]
Homma & Hirata (1984)	0.089–8.9	0.37	28.7 FW	5.4	194.1 FW	No	No	No	Rice seedlings, [A], [D], [F]
Bowen (1986)	10–500	(a) 6 (b) 13	(a) 2900 FW (b) 5740 FW	No	No	No	No	No	Excised root apices of (a) Zn-efficient [M101] and (b) Zn-inefficient [IR26] rice varieties, [A], [D], [F]
Mullins & Sommers (1986)	0–10	1.5–2.2	2.9–4.0 FW[c]	No	No	No	No	No	Maize plants, [B], [E]
Bowen (1987)	10–500	(a) 50 (b) 57	(a) 7220 (b) 2340	No	No	No	No	No	Excised root apices of (a) Zn-efficient [Kewalo] and (b) Zn-inefficient [Sel 7625-2] tomato, [A], [C], [F], competitive inhibition by Cu
Lasat *et al*. (1996)	0.5–100	8	270 FW	No	No	No	No	[H]	*Thlaspi caerulescens* seedlings, [A], [C], [F]
Lasat *et al*. (1996)	0.5–100	6	60 FW	No	No	No	No	[H]	*Thlaspi arvense* seedlings, [A], [C], [F]
Rengel & Wheal (1997)	0.03–3	(a) 0.86 ± 0.09 (b) 0.76 ± 0.06	(a) 5.5 DW (b) 3.5 DW	No	No	No	No	No	Wheat seedlings of (a,b) Zn-efficient [Excalibur] and (c,d)

Table 2 *continued*

Reference	$[Zn]_{ext}$ (μM)	Function 1 K_m (μM)	Function 1 V_{max}[a] (nmol g^{-1} h^{-1})	Function 2 K_m (μM)	Function 2 V_{max}[a] (nmol g^{-1} h^{-1})	Function 3 K_m (μM)	Function 3 V_{max}[a] (nmol g^{-1} h^{-1})	k	Notes[b]
		(c) 0.92 ± 0.14 (d) 0.88 ± 0.07	(c) 3.7 DW (d) 2.3 DW						Zn-inefficient [Gatcher] varieties, grown in (a,c) Zn-deficient or (b,d) Zn-replete solutions, [B], [E]
Rengel & Wheal (1997)	0.03–3	(a) 0.95 ± 0.10 (b) 0.93 ± 0.09	(a) 2.8 DW (b) 2.5 DW	No	No	No	No	No	*Triticum turgidum* seedlings grown in (a) Zn-deficient and (b) Zn-replete solutions, [B], [E]
Wheal & Rengel (1997)	0.0008–0.0123	0.0025	39 FW	No	No	No	No	No	Wheat plants, [B], [D], [E].
Hart *et al*. (1998)	0–80	2.3 ± 0.4	162 FW	No	No	No	No	[H]	Wheat seedlings, [A], [C], [F].
Hart *et al*. (1998)	0–80	3.9 ± 0.5	175 FW	No	No	No	No	[H]	*Triticum turgidum*, [A], [C], [F].
Santa-Maria & Cogliatti (1998)	0.2–10	22.3 ± 4.1	11 200 DW	No	No	No	No	No	Wheat plants, [B], [C], [F].
Hacisalihoglu *et al*. (2001)	0.0001–80	(a) 0.0006 (b) 0.0012 (c) 0.0023 (d) 0.0007	(a) 10.9 FW (b) 9.0 FW (c) 30.9 FW (d) 9.7 FW	(a) 1.9 0.3 (b) 4.9 ± 1.7 (c) 4.1 ± 1.5 (d) 3.4 ± 1.1	(a) 446 FW (b) 143 FW (c) 521 FW (d) 294 FW	No	No	[H]	Wheat seedlings of (a,b) Zn-efficient [Dagdas] and (c,d) Zn-inefficient [BDME-10] varieties, grown in (a,c) Zn-deficient or (b,d) Zn-replete solutions, [A], [C], [F]
Hart *et al*. (2002)	0.050–50	2.3 ± 0.3	171 FW	No	No	No	No	[H]	Wheat seedlings [A], [C], [F], competitive inhibition by 10 μM Cd
Hart *et al*. (2002)	0.050–50	3.3 ± 0.4	166 FW	No	No	No	No	[H]	*Triticum turgidum*, [A], [C], [F], competitive inhibition by 10 μM Cd

[a]V_{max} data expressed on a root fresh weight (FW) or root dry weight (DW) basis.
[b]Notes key: [A], uptake from 'single salt' solution containing both Zn^{2+} and Ca^{2+} [B], uptake from a complete nutrient solution; [C] influx measurement (≤ 30 min); [D], uptake measurement (60–120 min); [E], calculated from Zn^{2+} depletion in uptake solution; [F], desorbed in cationic solution following uptake; [G], abrupt transitions between 'phases' described by single Michaelis–Menten functions; [H], a linear component was assumed to be cell wall binding.
[c]V_{max} data recalculated assuming a root area/FW quotient of 20 cm^2 g^{-1}.

role in Zn efficiency in wheat (Hart *et al.*, 1998, 2002; Hacisalihoglu *et al.*, 2001; Hacisalihoglu & Kochian, 2003), differences in these parameters between Zn-efficient and Zn-inefficient genotypes of sugarcane, rice and tomato have been reported (Bowen, 1973, 1986, 1987). In general, Zn-deficient plants have higher V_{max} and comparable K_m values to Zn-replete plants (Rengel & Wheal, 1997; Hacisalihoglu *et al.*, 2001). Several authors have attributed a linear term, which is not removed by washing the roots in solutions containing excess cations, to the accumulation of Zn bound strongly to cell walls (Lasat *et al.*, 1996; Hart *et al.*, 1998, 2002; Hacisalihoglu *et al.*, 2001). There has been some discourse about the consequences of Zn binding to cell walls in experiments to determine kinetic parameters for Zn influx to roots (Reid *et al.*, 1996). In the giant alga, *Chara*, cell walls can be removed and apoplastic effects on Zn influx can be determined directly (Reid *et al.*, 1996). Using this technique, Zn influx to the cytoplasm was described by the sum of a Michaelis–Menten function with a low K_m (< 0.1 μM) plus a linear term for $[Zn]_{ext}$ up to 50 μM. These data suggest that most experiments to determine the kinetic parameters of Zn influx to roots may have overestimated K_m values and distorted the linear term.

Transport from epidermal and cortical cells to the root xylem can occur via the cytoplasmic continuum of cells, linked by plasmodesmata, from which Zn is pumped into the stelar apoplast (Lasat & Kochian, 2000). This 'symplastic' pathway is catalysed by plasma membrane and tonoplast transport activity. Zinc can also be delivered extracellularly to the stelar apoplast in regions where the Casparian band is not fully formed (White, 2001; White *et al.*, 2002b). Apoplastic mineral fluxes are dominated by the cell wall cation exchange capacity (CEC), Casparian band formation and water flows (Sattelmacher, 2001). Both symplastic and apoplastic fluxes may contribute to net Zn fluxes to the shoot. If symplastic fluxes dominate, Zn accumulation in the shoot approximates the sum of unidirectional influx (ϕ_{oc}) of Zn to root cells, minus efflux (ϕ_{co}) and net vacuolar Zn sequesteration (i.e. unidirectional vacuolar influx, ϕ_{cv}, minus unidirectional vacuolar efflux, ϕ_{vc}) (Lasat & Kochian, 2000; White *et al.*, 2002b). Thus, the maximal $[Zn]_{shoot}$ supported by symplastic root Zn fluxes can be simulated for different shoot : root FW ratios, relative growth rates (RGRs) and ϕ_{oc} values if one assumes steady-state conditions (White *et al.*, 2002b). In *Thlaspi caerulescens* J. & C. Presl., which can accumulate > 30 000 μg Zn g^{-1} DW (see Section VI.3), the delivery of Zn to the root xylem exclusively via the symplast is kinetically challenging at high $[Zn]_{ext}$ (White *et al.*, 2002b). Substantial symplastic Zn fluxes may occur at high $[Zn]_{ext}$ (White *et al.*, 2002b). However, it is noteworthy that the Ca^{2+}-transporting ATPase activity required to catalyze the export of Ca^{2+} *alone* from the symplast into the root stelar apoplast may exceed total membrane protein quotas in a typical dicot (White, 1998; White *et al.*, 2002b). *Thlaspi caerulescens* has substantial shoot Ca, Mg *and* Zn concentrations (> 60,000 μg g^{-1} DW; Meerts *et al.*, 2003; Dechamps *et al.*, 2005). *Thlaspi caerulescens* also has altered Casparian band formation (Section VI.iii), and Cd^{2+} fluxes are substantially greater at the root tips in wheat, *T. caerulescens* and *T. arvense* L. (i.e. in regions where the Casparian band is not fully formed; Piñeros *et al.*, 1998). Integrating Zn flux analyses with models to predict $[Zn]_{ext}$ may ultimately enable quantification of the relative symplastic and apoplastic root fluxes, provided fluxes and intracellular Zn distibutions of intact root cells can be determined accurately.

V. Zinc in plants

1. Zinc is an essential plant nutrient

The essentiality of Zn in plants was first shown in maize (Mazé, 1915), and subsequently in barley and dwarf sunflower (Sommer & Lipman, 1926). Early reports of severe Zn deficiency symptoms included impaired stem elongation in tomato (Skoog, 1940). Incipient Zn deficiency symptoms in tomato, remedied by resupply of Zn, included reduced protein and starch synthesis whilst sugar content was unaffected (Hoagland, 1948). Severe Zn deficiency symptoms have since been catalogued for many crops (Scaife & Turner, 1983; Marschner, 1995; Sharma, 2006; Fig. 1). Severe Zn deficiency is characterized by root apex necrosis ('dieback'), whilst sublethal Zn deficiency induces spatially heterogeneous or interveinal chlorosis ('mottle leaf'), the development of reddish-brown or bronze tints ('bronzing'), and a range of auxin deficiency-like responses such as internode shortening ('rosetting'), epinasty, inward curling of leaf lamina ('goblet' leaves) and reductions in leaf size ('little leaf'). In most crops, the typical leaf Zn concentration ($[Zn]_{leaf}$) required for adequate growth approximates 15–20 mg Zn kg^{-1} DW (Marschner, 1995).

Zinc is the most common crop micronutrient deficiency, particularly in high-pH soils with low $[Zn]_{bss}$ (Graham *et al.*, 1992; White & Zasoski, 1999; Cakmak, 2002, 2004; Alloway, 2004). Notably, 50% of cultivated soils in India and Turkey, a third of cultivated soils in China, and most soils in Western Australia are classed as Zn-deficient. Zinc deficiency in crop production can be ameliorated through agronomy or genetic improvement. Early agronomic successes included the treatment of little leaf in peach orchards, using soil-applied $FeSO_4$ fertilizers containing Zn impurities, and subsequently the treatment of mottle leaf in citrus orchards, rosetting in pecan and stunted pineapple growth using foliar sprays containing Zn (Hoagland, 1948). More recently, substantial arable crop responses to Zn fertilization have been reported in Australia, India, and Central Anatolia in Turkey, where wheat grain yields have increased by over 600% since the mid-1990s, with a concomitant annual economic benefit of US$100 million (Cakmak, 2004). It may also be possible to improve crop yields on Zn-deficient soils by exploiting genotypic differences

Fig. 1 Zinc deficiency induced by prolonged flooding in rice. Typical symptoms include high plant mortality, stuntedness, leaf bronzing and a delay in flowering. (Photograph courtesy of Dr Matthias Wissuwa, Japan International Research Center for Agricultural Sciences, Tsukuba, Japan.)

in Zn uptake and tissue-use efficiency that exist within crop species (Rengel, 2001; Cakmak, 2002; Hacisalihoglu & Kochian, 2003; Alloway, 2004). For example, in rice, Zn uptake efficiency correlates with exudation rates of low-molecular-weight organic anions and a substantial proportion of the phenotypic variation in Zn uptake efficiency is under genetic control (Hoffland *et al.*, 2006; Wissuwa *et al.*, 2006).

2. Evolutionary aspects of shoot Zn concentration among angiosperms

Previously, a meta-analysis of 70 species from 35 studies in the literature implied that ancient evolutionary processes might impact on $[Zn]_{shoot}$ in angiosperms (Broadley *et al.*, 2001). A much larger survey was undertaken here. Primary $[Zn]_{shoot}$ data, that is, Zn concentrations reported in leaf or nonwoody shoot tissues, were obtained from 1108 studies, contained in 204 published papers and three unpublished datasets (Tables S6–S9). These primary data define the largest set of interlinked studies, in which $[Zn]_{shoot}$ values are reported for more than two species in controlled experiments where each study contains more than one species common to another study. Thus, 365 species from 48 families and 12 key clades were sampled. Primary $[Zn]_{shoot}$ data were ln-transformed and a variance-components model was fitted to the data using residual maximum likelihood (REML) procedures (Broadley *et al.*, 2001, 2003). Species, family and key clade variance components were estimated using a random model factor of (study + (key clade/family/species)) with no fixed model factor. Mean data for phylogenetic groups and significance tests (Wald tests) were conducted using (key clade/family/species) as a fixed factor, retaining (study + (key clade/family/species)) as a random factor. All analyses were performed using GenStat (Release 9.1.0.147; VSN International, Oxford, UK).

Study effects dominated the variance components because of the vast range of $[Zn]_{ext}$ across all studies and because of differences in the relationship between $[Zn]_{ext}$ and $[Zn]_{shoot}$ between species (Table S9). After removing study effects, key clade (Wald statistic = 592.15, d.f. = 10, $P < 0.001$) and family within key clade (Wald statistic = 298.71, d.f. = 36, $P < 0.001$), variance components accounted for 22.1% of the variation in $[Zn]_{shoot}$ among angiosperm species (Table S9). The analysis was repeated with *Thlaspi* and *Arabidopsis* genera excluded to remove the influence of Zn 'hyperaccumulation' (defined in Section VI.3) from the analysis. Adjusted key clade and family variance components accounted for 26.5% of the variation in $[Zn]_{shoot}$ (Table S9). After removing the effect of study, and with *Thlaspi* and *Arabidopsis* genera excluded, mean relative $[Zn]_{shoot}$ among key clades ranged from < 40 (Ericales) to > 100 (Caryophyllales and noncommelinoid monocotyledons; NB: units approximate μg Zn g^{-1} DW) (Table S9). Among well-replicated families, lower $[Zn]_{shoot}$ occurred in Linaceae (52, $n = 29$), Poaceae (59, $n = 1527$) and Solanaceae (66, $n = 181$), and higher $[Zn]_{shoot}$ occurred in Amaranthaceae (108, $n = 214$) and Salicaceae (195, $n = 45$) (Table S9). Compared with other essential elements, variation in $[Zn]_{shoot}$ manifesting above the family level is less than for Ca, K, Mg and Si (Broadley *et al.*, 2004; Hodson *et al.*, 2005), but greater than for N and P (Broadley *et al.*, 2004). Furthermore, variation in $[Zn]_{shoot}$ manifesting above the family level is

less than previously reported for $[Zn]_{shoot}$ among a limited dataset of 70 species (Broadley *et al.*, 2001). However, despite the large study effects within this dataset, evidence of ancient evolutionary processes influencing $[Zn]_{shoot}$ can still be observed using this approach. A considerable additional sampling effort is required to explore this phenomenon further.

After removing study effects, > 70% of the remaining variation in $[Zn]_{shoot}$ occurs within-family. Thus, substantial differences in $[Zn]_{shoot}$ exist between and within genera and species. Several billion people worldwide have Zn-deficient diets, and this within-species genetic variation is providing a valuable genetic resource to select or breed crops with increased Zn concentrations in their edible portions, notably in several cereal, legume, root and leafy vegetable crops (Graham *et al.*, 2001; Welch & Graham, 2004; Grusak & Cakmak, 2005; Vreugdenhil *et al.*, 2005; White & Broadley, 2005; Ghandilyan *et al.*, 2006). Accelerated breeding to increase the delivery of dietary Zn in crops may be possible following identification of intraspecific genetic variation in Zn composition. In common bean (*Phaseolus vulgaris* L.), seed Zn concentration ($[Zn]_{seed}$) is a quantitative trait which can be mapped to genetic loci using quantitative trait loci (QTL) analyses (Ghandilyan *et al.*, 2006). QTLs for $[Zn]_{seed}$ have also been mapped in *A. thaliana* (Vreugdenhil *et al.*, 2004). Following the identification of QTL, candidate genes or loci can be resolved through fine mapping and map-based cloning, and this information could be used for gene-based selection or marker-assisted breeding strategies. An advantage of this strategy is that knowledge of the genes and/or chromosomal loci controlling $[Zn]_{seed}$ or $[Zn]_{shoot}$ in one plant species could be used in a different target crop species by exploiting gene homology and/or genome collinearity (Ghandilyan *et al.*, 2006).

VI. Plant responses to elevated soil Zn

The response of plants to elevated $[Zn]_{soil}$ has generated a substantial literature, driven by the primary questions, 'Can crops be grown safely and productively at elevated $[Zn]_{soil}$?', and, 'How and why do certain taxa thrive at elevated $[Zn]_{soil}$?

1. Zn toxicity in crops

Zinc toxicity in crops is far less widespread than Zn deficiency. However, Zn toxicity occurs in soils contaminated by mining and smelting activities, in agricultural soils treated with sewage sludge, and in urban and peri-urban soils enriched by anthropogenic inputs of Zn, especially in low-pH soils (Chaney, 1993). Toxicity symptoms usually become visible at $[Zn]_{leaf}$ > 300 mg Zn kg^{-1} leaf DW, although some crops show toxicity symptoms at $[Zn]_{leaf}$ < 100 mg Zn kg^{-1} DW (Chaney, 1993; Marschner, 1995), and toxicity thresholds can be highly variable even within the same species. For example, $[Zn]_{leaf}$ associated with a 50% yield reduction in radish ranged from 36 to 1013 mg kg^{-1} DW (Davies, 1993). Zn toxicity symptoms include reduced yields and stunted growth, Fe-deficiency-induced chlorosis through reductions in chlorophyll synthesis and chloroplast degradation, and interference with P (and Mg and Mn) uptake (Carroll & Loneragan, 1968; Boawn & Rasmussen, 1971; Foy *et al.*, 1978; Chaney, 1993). Crops differ markedly in their susceptibility to Zn toxicity. In acid soils, graminaceous species are generally less sensitive to Zn toxicity than most dicots, although this is reversed in alkaline soils (Chaney, 1993). Among dicots, leafy vegetable crops are sensitive to Zn toxicity, especially spinach and beet, because of their inherent high Zn uptake capacity (Boawn & Rasmussen, 1971; Chaney, 1993). There is also genetic variation in sensitivity to Zn toxicity within species, including soybean (*Glycine max* L.; Earley, 1943; White *et al.*, 1979a,b,c) and rice (*Oryza sativa* L.), in which QTLs impacting on sensitivity to Zn toxicity have recently been mapped (Dong *et al.*, 2006).

2. Plant tolerance to elevated soil Zn (hypertolerance)

Numerous species of metallophyte thrive at a $[Zn]_{soil}$ that is toxic to most crop plants. Early studies on Zn hypertolerance focused on elevated $[Zn]_{soil}$ at mine sites and/or near corroded galvanized materials, such as electricity pylons (reviewed by Antonovics *et al.*, 1971; Baker, 1987; Ernst *et al.*, 1990, 1992; Macnair, 1993). Initially, Zn hypertolerance was thought to occur most frequently in species of Poaceae, Caryophyllaceae and Lamiaceae, although subsequent studies have shown no phylogenetic predisposition to the evolution of Zn hypertolerance (Baker, 1987). However, this hypothesis is difficult to test directly. At a species level, elegant theoretical and experimental frameworks have been developed to characterize the genetics of Zn hypertolerance (Macnair, 1990, 1993). Briefly, Zn hypertolerance tends to manifest as a dominant phenotype and spread rapidly in a population. However, dominance is not a fixed property of a gene but a measure of the phenotypic deviation of the heterozygote from the mean of the two homozygotes. Thus, G × E interactions will affect whether a gene confers a dominant or recessive phenotype under any particular condition, rendering the study of Zn tolerance nontrivial (Macnair, 1990, 1993). Nevertheless, Zn hypertolerance is likely to be under the control of a small number of major genes, as in *Silene vulgaris* (Moench) Garcke (Schat *et al.*, 1996) and *Arabidopsis halleri* (L.) O'Kane & Al-Shehbaz (Macnair *et al.*, 1999). In addition to dominance (i.e. interallelic/intragenic) effects, epistatic (i.e. intergenic) interactions have an impact on Zn hypertolerance, although these effects are more difficult to quantify (Macnair, 1993). Zinc-hypertolerant plants show fitness costs at low $[Zn]_{soil}$, for example, suboptimal carbonic anyhdrase and nitrate reductase activity occurs in *S. vulgaris* (Ernst *et al.*, 1992). In *Silene dioica* (L.) Clairv., pollen selection may accelerate reproductive isolation between adjacent populations which differ in Zn tolerance (Searcy & Mulcahy, 1985a,b,c).

At a whole-plant scale, natural Zn hypertolerance is thought to be conferred by Zn exclusion or by compartmentalization, for example, through mycorrhizal symbioses, altered root-to-shoot translocation or accumulation in older leaves (Baker, 1981; Ernst *et al.*, 1992; Hall, 2002). Key subcellular processes enabling Zn hypertolerance are likely to be increased organic acid production and vacuolar compartmentalization, including increased Zn efflux across the plasma membrane (Ernst *et al.*, 1992; Verkleij *et al.*, 1998; Chardonnens *et al.*, 1999; Clemens, 2001; Hall, 2002). Early hypotheses that cell-wall Zn binding, reduced membrane leakage or increased phytochelatin production increased Zn tolerance have not been supported by subsequent studies (Ernst *et al.*, 1992; Harmens *et al.*, 1993; Clemens, 2001; Schat *et al.*, 2002).

In general, Zn hypertolerance does not segregate with other metal tolerance phenotypes, although Cd, Co, Cu, Cd, Ni and/or Pb cotolerance can occur (Gregory & Bradshaw, 1965; Wu & Antonovics, 1975; Cox & Hutchinson, 1980; Symeonidis *et al.*, 1985; Macnair, 1990, 1993; Brown & Brinkmann, 1992; Schat & Vooijs, 1997). Cotolerance can be either pleiotropic (i.e. strict cotolerance) or the result of multiple hypertolerance mechanisms. Multiple hypertolerances arise through non-random association of favourable alleles at two or more loci (i.e. linkage disequilibrium), potentially in response to the co-occurrence of several metals in soils, gene flow from adjacent sites or seed transport between different metalliferous sites (Macnair, 1993; Schat & Vooijs, 1997). For example, Zn/Co/Ni cotolerance phenotypes cosegregated in *Silene vulgaris*, implying a pleiotropic effect, whilst Zn/Cd/Cu cotolerance phenotypes segregated independently, implying multiple mechanisms (Schat & Vooijs, 1997). Zinc/Cu cotolerance phenotypes also segregated independently in populations of *Agrostis stolonifera* L. (Wu & Antonovics, 1975) and *Mimulus guttatus* DC. (Macnair, 1990). Zinc-hypertolerant cultivars of grass species have been successfully bred and used to revegetate soils contaminated with Zn, Pb and other heavy metals, following mining activities, including *Festuca rubra* L. cv. Merlin and *Agrostis capillaris* L. cv. Groginan for calcareous and acid wastes, respectively (Smith & Bradshaw, 1979; Whiting *et al.*, 2005).

3. Zinc hyperaccumulation

The ability of some Zn-hypertolerant metallophytes to accumulate exceptional concentrations of Zn in their aerial parts was probably first reported among 'galmei' (calamine) flora of the Aachen region on the border of Belgium and Germany, where the presence of > 1% Zn in the plant ash of *Viola calaminaria* (Ging.) Lej. was recorded in 1855 (Reeves & Baker, 2000). A $[Zn]_{shoot}$ > 1% DW in *Thlaspi alpestre* L. (= *T. caerulescens*) was reported shortly thereafter in 1865 (Reeves & Baker, 2000). Zinc hyperaccumulation has since been defined as the occurance of > 10 000 μg Zn g^{-1} DW in the aerial parts of a plant species when growing in its natural environment (Baker & Brooks, 1989). These species (Table 3) have come to dominate the literature, in part because of the desire to transfer the character into crop species for use in phytoremediation, phytomining and biofortification (Chaney, 1983; Baker & Brooks, 1989; Brooks, 1998; Salt *et al.*, 1998; Guerinot & Salt, 2001; Macnair, 2003; Krämer, 2005a). Typically, 10–20 species are reported to be Zn hyperaccumulators, with a smaller number of these able to accumulate Cd to very high concentrations as well. This raises the intriguing question: how often has the Zn hyperaccumulation character evolved? The answer is uncertain because: (i) 3000 μg Zn g^{-1} DW might be a more suitable evolutionary definition of extreme $[Zn]_{shoot}$ based on the frequency distribution of values observed within the genus *Thlaspi* s.l., which contains most Zn hyperaccumulators (Reeves & Brooks, 1983; Reeves & Baker, 2000); (ii) records for several nonbrassicaceous Zn hyperaccumulators are uncertain (Macnair, 2003; R. D. Reeves, pers. comm.) (NB: plant samples collected from metal-rich substrates can easily become contaminated by soil particles); and (iii) taxonomic uncertainties and the use of synonyms can affect the identification of field and herbarium samples.

The genus *Thlaspi* L. s.l. is likely to be polyphyletic and its taxonomy is controversial. Seed-coat anatomical and sequence data have thus been used to split the genus into several alternative genera, including *Thlaspi* s.s., *Vania* and a clade containing *Thlaspiceras*, *Noccaea*, *Raparia*, *Microthlaspi* and *Neurotropis* (Meyer, 1973, 1979; Mummenhoff & Koch, 1994; Mummenhoff *et al.*, 1997; Koch & Mummenhoff, 2001; Koch & Al-Shehbaz, 2004). High $[Zn]_{shoot}$ is probably a general feature of *Noccaea* and its sister clade *Raparia* (Macnair, 2003; Taylor, 2004), but not of *Thlaspiceras*, which nevertheless contains Zn-hypertolerant species (e.g. *Thlaspiceras oxyceras* (Boiss.) F. K. Mey; Peer *et al.*, 2003), nor of the more distantly related nonZn-hypertolerant *Microthlaspi* and *Neurotropis* clades. Thus, the high $[Zn]_{shoot}$ character most likely evolved at the base of the *Noccaea*/*Raparia* clade, or less likely at the base of the *Noccaea*/*Raparia*/*Thlaspiceras* clade with a subsequent reversion to the low $[Zn]_{shoot}$ character in *Thlaspiceras* (Macnair, 2003; Taylor, 2004). Intriguingly, since Ni hyperaccumulation also evolved at the base of the *Noccaea*/*Raparia*/*Thlaspiceras* clade, high $[Zn]_{shoot}$ may be a modification of the Ni hyperaccumulation character (Taylor, 2004). Nickel hyperaccumulation is more common than Zn hyperaccumulation among angiosperms (> 300 species, in *c.* 34 families), although > 80% of temperate Ni hyperaccumulators are Brassicaceae species (Reeves & Baker, 2000; Borhidi, 2001). The only other Brassicaceae species with an unequivocally high $[Zn]_{shoot}$ character is *Arabidopsis halleri*. Thus, Zn hyperaccumulation has probably only arisen during two relatively recent evolutionary events within the Brassicaceae, and possibly on very few isolated occasions elsewhere in the angiosperms. The following sections focus primarily on *Thlaspi caerulescens*.

Genetics of Zn hyperaccumulation *Thlaspi caerulescens* is a short-lived, self-compatible biennial/perennial species which

Table 3 Plant species whose $[Zn]_{shoot}$ has been observed to exceeds 0.3% DW (unless stated) when grown under natural conditions

Species[a]	Potential synonyms[a]	Family (Order)	Locality	Maximum $[Zn]_{shoot}$ (% DW) observed).	References and Comments
Acer pseudoplatanus L.	–	Sapindaceae (Sapindales)	UK	0.35	Johnston & Proctor (1977)
Arenaria patula Michx.	*Minuartia patula* (Michx.) Mattf.	Caryophyllaceae (Caryophyllales)	USA	1.31	Brooks (1998). Uncertain record (Macnair, 2003; R. D. Reeves, pers. comm.)
Arabidopsis arenosa (L.) Lawalrée	*Cardaminopsis arenosa* (L.) Hayek	Brassicaceae (Brassicales)	France	0.52	Reeves *et al.* (2001)
Arabidopsis halleri (L.) O'Kane & Al-Shehbaz	*Arabis gemmifera* Makino, *Cardaminopsis halleri* (L.) Hayek, *C. ovirensis* (Wulf.) O. Schwarz	Brassicaceae	France	2.07	R. D. Reeves, pers. comm.
Arabidopsis thaliana (L.) Heynh.	*Arabis thaliana* L., *Sisymbrium thalianum* (L.) J. Gay & Monnard, *Stenophragma thalianum* (L.) Čelak.	Brassicaceae	USA	2.67	Reeves (1988)
Biscutella laevigata L.	*Biscutella alsatica* Jord., *B. austriaca* Jord., *B. longifolia* Vill., *B. lucida* Balb. ex DC., *B. sempervirens* L., *B. varia* Dumort., *B. variegata* Boiss. & Reut., *B. vincentina* (Samp.) Rothm. ex Guinea	Brassicaceae	France	0.41	R. D. Reeves, pers. comm.
Cochlearia pyrenaica DC.	*Cochlearia officinalis* L. subsp. *pyrenaica* (DC.) Rouy & Foucaud	Brassicaceae	UK	0.53	Reeves (1988)
Dianthus sp.	–	Caryophyllaceae	France	0.49	R. D. Reeves, pers. comm.
Dichapetalum gelonioides (Roxb.) Engl.	*Chailletia gelonioides* (Roxb.) J. D. Hook., *Dichapetalum howii* Merrill & Chun., *Moacurra gelonioides* Roxb.	Dichapetalaceae (Malpighiales)	Indonesia, Malaysia, Philippines	3.00	Reeves & Baker (2000)
Galium mollugo L.	*Galium album* Mill., *G. cinereum* All., *G. corrudifolium* Vill., *G. elatum* Thuill., *G. insubricum* Gaudin, *G. kerneranum* Klokov, *G. lucidum* All., *G. neglectum* Le Gall ex Gren., *G. tyrolense* Willd.	Rubiaceae (Gentianales)	France	0.30	Reeves *et al.* (2001)
Gomphrena canescens R. Br.	–	Amaranthaceae (Caryophyllales)	Australia	0.90	Nicolls *et al.* (1965)
Haumaniastrum katangense (S. Moore) Duvign. & Plancke.	–	Lamiaceae (Lamiales)	D. R. of the Congo	1.98	Brooks (1998). Uncertain record (Paton & Brooks, 1996; Macnair, 2003)
Minuartia verna (L.) Hiern	*Alsine verna* (L.) Wahlenb., *Arenaria verna* L., *Minuartia caespitosa* (Ehrh.) Degen	Caryophyllaceae	Yugoslavia	1.14	Various studies cited in Reeves & Baker (2000)
Noccaea boeotica F. K. Mey.	–	Brassicaceae	Greece	0.31	R. D. Reeves, pers. comm.
Noccaea eburneosa F. K. Mey.	*Noccaea salisii* (Brugger) F. K. Mey, *Thlaspi salisii* Brugger	Brassicaceae	Switzerland	1.05	Reeves & Brooks (1983), taxonomic status uncertain (R. D. Reeves, pers. comm.)
Polycarpaea synandra F. Muell.	–	Caryophyllaceae	Australia	0.70	Reeves & Baker (2000)
Rumex acetosa L.	–	Polygonaceae (Caryophyllales)	UK	1.10	Johnston & Proctor (1977)
Rumex acetosella L.	*Acetosella vulgaris* Fourr., *Rumex acetoselloides* Balansa, *R. angiocarpus* Murb., *R. fascilobus* Klokov, *R. multifidus* L., *R. pyrenaicus* Pourr. ex Lapeyr., *R. salicifolius* auct., *R. tenuifolius* (Wallr.) Á. Löve	Polygonaceae	France	0.31	Reeves *et al.* (2001)

Table 3 *continued*

Species[a]	Potential synonyms[a]	Family (Order)	Locality	Maximum $[Zn]_{shoot}$ (% DW) observed).	References and Comments
Sedum alfredii Hance	–	Crassulaceae (Saxifragales)	China	0.50	Yang *et al.* (2002)
Silene vulgaris (Moench) Garcke	*Silene angustifolia* Mill., *S. campanulata* Saut., *S. cucubalus* Wibel, *S. inflata* Sm., *S. latifolia* (Mill.) Britten & Rendle, non Poir., *S. tenoreana* Colla, *S. venosa* Asch., *S. vulgaris* (Moench) Garck	Caryophyllaceae	USA	0.47	Brooks (1998)
Thlaspi apterum Velen.	*Noccaea aptera* (Velen.) F. K. Mey.	Brassicaceae	Bulgaria	0.31	Reeves & Brooks (1983). Uncertain record (R. D. Reeves, pers. comm.)
Thlaspi alpinum Crantz	*Noccaea alpestris* (Jacq.) Kerguélen, *N. sylvia* (Gaudin) F. K. Mey., *T. sylvium* Gaudin	Brassicaceae	France	0.54	Reeves *et al.* (2001)
Thlaspi brachypetalum Jord.	*Noccaea brachypetala* (Jord.) F. K. Mey.	Brassicaceae	France	1.53	Reeves & Brooks (1983)
Thlaspi brevistylum (DC.) Mutel.	*Noccaea brevistyla* Steud.	Brassicaceae	Corsica	0.31	Taylor (2004)
Thlaspi bulbosum Spruner ex Boiss.	*Raparia bulbosa* (Boiss.) F. K. Mey.	Brassicaceae	Greece	1.05	Brooks (1998)
Thlaspi caerulescens J. & C. Presl.	*Noccaea arenaria* (J. E. Duby) F. K. Mey., *N. caerulescens* (J. & C. Presl) F. K. Mey., *N. occitanica* (Jord.) F. K. Mey., *Thlaspi alpestre* L., *T. arenarium* Jord., *T. caerulescens* subsp. *caerulescens*, *T. caerulescens* subsp. *calaminare* (Lej.) Lej. & Court, *T. caerulescens* subsp. *occitanicum* (Jord.) M. Laínz, *T. caerulescens* subsp. *tatrense* (Zapał.) Dvořáková, *T. gaudinianum* Jord., *T. huteri* A. Kern., *T. mureti* Gremli, *T. occitanicum* Jord., *T. pratulorum* Gand., *T. rhaeticum* Jord., *T. salisii* Brugger, *T. suecicum* Jord., *T. sylvestre* Jord., *T. tatrense* Zapał., *T. virgatum* Gren. & Godr., *T. villarsianum* Jord., *T. vogesiacum* Jord., *T. vulcanorum* Lamotte	Brassicaceae	W. and C. Europe	4.37	Various studies (Reeves & Baker, 2000). Also a Cd hyperaccumulator
Thlaspi cepaeifolium (Wulfen) W. D. J. Koch	*Noccaea cepaeifolia* (Wulfen) Rchb., *N. limosellifolia* (Burnat) F. K. Mey., *N. rotundifolia* (L.) Moench, *Thlaspi cepaeifolium* subsp. *rotundifolium* (L.) Greuter & Burdet, *T. limosellifolium* Reut. ex Rouy & Fouc., *T. rotundifolium* (L.) Gaudin subsp. *cepaeifolium* (Wulfen) Rouy & Fouc.	Brassicaceae	Italy	2.10	Various studies (Reeves & Baker, 2000)
Thlaspi epirotum Hal.	*Noccaea epirota* (Hal.) F. K. Mey.	Brassicaceae	Greece	<0.30	Reeves & Brooks (1983)
Thlaspi goesingense Hal.	*Noccaea goesingensis* (Hal.) F. K. Mey., *Thlaspi tymphaeum* Hausskn., *T. umbrosum* Waisb.	Brassicaceae	Austria	0.38	Reeves & Baker (1984)
Thlaspi graecum Jord.	*Noccaea graeca* (Jord.) F. K. Mey., *Thlaspi taygeteum* Boiss.	Brassicaceae	Greece	<0.30	Reeves & Brooks (1983)
Thlaspi kovatsii Heuff.	*Noccaea kovatsii* (Heuffel) F. K. Mey., *Thlaspi affine* Schott & Kotschy, *T. avalanum* Panč., *T. jankae* Kern., *T. trojagense* Zapał.	Brassicaceae	Bulgaria	0.49	Reeves & Brooks (1983)
Thlaspi magellanicum Pers.	*Noccaea magellanica* (Pers.) J. Holub	Brassicaceae	Argentina	0.39	Reeves (1988)
Thlaspi montanum L.	*Noccaea alpestris* (Jacq.) Kerguélen subsp. *sylvium* (Gaudin), *N. montana* (L.) F. K. Mey., *Thlaspi lotharingum* Jord.	Brassicaceae	USA	0.43	Hobbs & Streit (1986)

Table 3 *continued*

Species[a]	Potential synonyms[a]	Family (Order)	Locality	Maximum $[Zn]_{shoot}$ (% DW) observed).	References and Comments
Thlaspi ochroleucum Boiss. & Heldr.	*Noccaea lutescens* (Velen.) F. K. Mey., *N. ochroleuca* (Boiss. & Heldr.) F. K. Mey., *N. phrygia* (Bornm.) F. K. Mey., *N. rhodopensis* F. K. Mey., *N. versicolor* (Stoj. & Kitanov) F. K. Mey., *Thlaspi balcanicum* Janka, *T. heterochroum* Boiss., *T. lutescens* Velen., *T. phrygium* Bornm.	Brassicaceae	Turkey	0.63	Reeves & Brooks (1983), Reeves (1988)
Thlaspi parviflorum A. Nels.	*Noccaea parviflora* (A. Nels.) Holub	Brassicaceae	USA	0.31	Reeves *et al.* (1983)
Thlaspi pindicum Hausskn.	*Noccaea pindica* (Hausskn.) J. Holub, *N. tymphaea* (Hausskn.) F. K. Mey., *Thlaspi tymphaeum* Hausskn.	Brassicaceae	Greece	<0.10	Taylor & Macnair, 2006. Note, plants collected from serpentine soils with low $[Zn]_{soil}$. $[Zn]_{shoot}$ >1.00% DW observed under laboratory conditions.
Thlaspi praecox Wulf.	*Noccaea praecox* (Wulf.) F. K. Mey, *Thlaspi affine* Schott & Kotschy ex Bioss.	Brassicaceae	Bulgaria	2.10	Brooks (1998). Cd hyperaccumulator (Vogel-Mikuš *et al.*, 2005)
Thlaspi stenopterum Boiss. & Reut.	*Noccaea stenoptera* (Boiss. & Reut.) F. K. Mey.	Brassicaceae	Spain	1.60	Brooks (1998)
Thlaspi viridisepalum (Podp.) Greuter & Burdet	*Noccaea viridisepala* (Podp.) F. K. Mey.	Brassicaceae	Bulgaria	0.63	Reeves & Brooks (1983)
Viola calaminaria (Gingins) Lej.	*Viola tricolor* L.	Violaceae (Malpighiales)	Germany	1.00	Brooks (1998). Note, uncertain record (Macnair, 2003; R. D. Reeves, pers. comm.)

[a]Nomenclature and potential synonyms compiled from (i) original data sources, (ii) Flora Euopaea (digital online edition; http://rbg-web2.rbge.org.uk/FE/fe.html), (iii) http://www.diversityoflife.org/, (iv) USDA, ARS, National Genetic Resources Program.*Germplasm Resources Information Network - (GRIN)* [Online Database]. National Germplasm Resources Laboratory, Beltsville, Maryland, http://www.ars-grin.gov/cgi-bin/npgs/html/taxgenform.pl, (v) Muséum national d'Histoire naturelle [Ed]. 2003-2006 . Inventaire national du Patrimoine naturel, http://inpn.mnhn.fr., (vi) CWRIS PGR Forum Crop Wild Relative Information System, http://www.pgrforum.org/cwris/cwris.asp?fact=426656.

hyperaccumulates Zn/Cd/Ni. It occurs on calamine, serpentine (derived from ultramafic Mg and Fe-rich rocks, also enriched with Co, Cr and Ni), and nonmineral soils, with a wide distribution in central, northern and western Europe (Reeves & Brooks, 1983; Baker & Brooks, 1989; Reeves *et al.*, 2001). It is functionally nonmycorrhizal (Regvar *et al.*, 2003). In *T. caerulescens*, Zn hyperaccumulation and hypertolerance are constitutive species-level traits although inter- and intrapopulation variations in $[Zn]_{shoot}$ and/or Zn tolerance occur (Mathys, 1977; Ingrouille & Smirnoff, 1986; Baker *et al.*, 1994; Lloyd-Thomas, 1995; Pollard & Baker, 1996; Meerts & Van Isacker, 1997; Escarré *et al.*, 2000; Reeves *et al.*, 2001; Pollard *et al.*, 2002; Assunção *et al.*, 2003a,b, 2006; Frérot *et al.*, 2003, 2005; Macnair, 2003; Roosens *et al.*, 2003; Zha *et al.*, 2004; Molitor *et al.*, 2005). Populations from soils with high-$[Zn]_{soil}$ are frequently described as 'metallicolous' with an explicit adaptive connotation. Notably, outcrossing rates of *c.* 5.25% have been reported (Riley, 1956), although rates are population-specific and allozyme variation and pollen : ovule ratios indicate that low-$[Zn]_{soil}$ populations are more self-fertile than high-$[Zn]_{soil}$ populations (Koch *et al.*, 1998; Dubois *et al.*, 2003). Conversley, in nonhyperaccumulator species, metal-hypertolerant populations are more self-fertile than nontolerant populations (Antonovics, 1968, 1972; Ducousso *et al.*, 1990).

When *T. caerulescens* are grown under identical experimental conditions, Zn tolerance is generally greater, and $[Zn]_{shoot}$ is lower in high-$[Zn]_{soil}$ populations than in low-$[Zn]_{soil}$ populations. For example, $[Zn]_{shoot}$ was higher in field-sampled high-$[Zn]_{soil}$ populations than in low-$[Zn]_{soil}$ populations from the UK, but half-sib families of high-$[Zn]_{soil}$ populations had lower $[Zn]_{shoot}$ than low-$[Zn]_{soil}$ populations when grown subsequently in hydroponics (Pollard & Baker, 1996). Among a wider sample of European populations, $[Zn]_{shoot}$, but not $[Zn]_{xylem}$ (ranges 63–93 and 610–700 μM at 10 and 100 μM $[Zn]_{ext}$, respectively), varied similarly (Roosens *et al.*, 2003). In soil-based experimental studies lasting up to 1 yr, Zn tolerance was lower and $[Zn]_{shoot}$ higher in low-$[Zn]_{soil}$ populations than in high-$[Zn]_{soil}$ populations (Meerts & Van Isacker, 1997; Escarré *et al.*, 2000). Variation in $[Zn]_{shoot}$ between and within half-sib families was significant, but less than variation between populations, consistent with the self-fertilizing character of *T. caerulescens*. Notably, genetic variation in $[Zn]_{shoot}$ can occur within a few kilometres (Molitor *et al.*, 2005). In *A. halleri*, Zn hyperaccumulation and hypertolerance are also constitutive traits at the species level, although interspecific crosses between *A. halleri* and *A. lyrata* ssp. *petraea* (L.) O'Kane & Al-Shehbaz reveal that Zn hyperaccumulation and hypertolerance are genetically independent (Macnair *et al.*, 1999). Again, low-$[Zn]_{soil}$ populations accumulate more Zn than high-$[Zn]_{soil}$ populations of *A. halleri* under identical experimental conditions (Bert *et al.*, 2000, 2002).

There is a substantial G × E interaction impacting on $[Zn]_{shoot}$. For example, Assunção *et al.* (2003a,b, 2006) studied low-$[Zn]_{soil}$ populations of *T. caerulescens* from a nonmetalliferous (Lellingen, Luxembourg (LE)) site and a serpentine (Monte Prinzera, Italy (MP)) site, and high-$[Zn]_{soil}$ populations from two calamine sites (La Calamine, Belgium (LC) and Ganges, France (GA)). In field samples, $[Zn]_{shoot}$ decreased GA ≥ LC > LE > MP. In hydroponics, $[Zn]_{shoot}$ decreased MP > GA > LE > LC and Zn tolerance decreased GA = LC > MP > LE. A controlled intraspecifc cross was made from LE and LC; Zn accumulation segregated in F_3s with a continuous phenotypic distribution indicative of a polygenic trait. However, the genetic independence of Zn tolerance and accumulation in *T. caerulescens* could not be confirmed because of the negative correlation between the two traits. A genetic linkage map was subsequently constructed for this population and two QTLs were mapped for $[Zn]_{root}$ (on chromosomes 3 and 5), although no QTLs were identified for $[Zn]_{shoot}$ (Assunção *et al.*, 2006). However, in an F_2 population obtained from an LC × GA cross, three QTLs explained 44.5% of the phenotypic variance in $[Zn]_{shoot}$, with positive effects on $[Zn]_{shoot}$ arising as a result of both LC and GA alleles (Deniau *et al.*, 2006). Similarly, in controlled crosses from high- and low-$[Zn]_{soil}$ populations of *T. caerulescens* (Frérot *et al.*, 2003, 2005), F_1 progeny with at least one parent from a high-$[Zn]_{soil}$ population were more sensitive to Zn deficiency, but F_1 and F_2 progenies were more tolerant to high $[Zn]_{ext}$ and had lower $[Zn]_{shoot}$ than progenies from exclusively low-$[Zn]_{soil}$ population crosses. Zha *et al.* (2004) also studied $[Zn]_{shoot}$ in F_2 progenies of a controlled cross between a high (Ganges, southern France) and a low (Prayon, Belgium) Cd accumulator. When plants were grown in a Zn-supplemented compost, Ganges accumulated *c.* 50% higher $[Zn]_{shoot}$ than Prayon and parental frequency distributions overlapped. In the F_2s, $[Zn]_{shoot}$ had a continuous distribution. There was significant transgression below the distribution of Prayon parents, but not above Ganges parents. Conversely, in hydroponics in the presence of higher $[Cd]_{ext}$, $[Zn]_{shoot}$ was lower in Ganges than in Prayon, perhaps because of a toxic effect of Cd on the growth of Prayon. Again, the F_2s showed a continuous distribution for $[Zn]_{shoot}$ and significant transgression below the lower limit of Ganges parental distributions, but not above Prayon (Zha *et al.*, 2004). Intriguingly, other studies have also shown that high $[Cd]_{ext}$ inhibits Zn accumulation in Ganges, but not in Prayon, whilst high $[Zn]_{ext}$ inhibits Cd accumulation in Prayon, but not in Ganges populations (Lombi *et al.*, 2001; Zhao *et al.*, 2002; Roosens *et al.*, 2003). In two species endemic to serpentine soils (*Thlaspi pindicum* Hausskn. and *T. alpinum* Crantz var. *sylvium* Gaudin), $[Zn]_{shoot}$ was higher when Ni was also present in the hydroponic solution (Taylor & Macnair, 2006).

Compartmentalization of Zn in hyperaccumulators Generally, in Zn hyperaccumulators, $[Zn]_{shoot} > [Zn]_{root}$ by up to 10-fold, although this depends on $[Zn]_{ext}$ (Shen *et al.*, 1997; Zhao *et al.*, 2000; Roosens *et al.*, 2003). Vázquez *et al.* (1992,

1994) used scanning transmission (STEM) electron microscopy/energy-dispersive X-ray microanalysis (EDXMA) on freeze-substituted tissues to study cellular Zn distribution in *T. caerulescens*. Vacuoles of leaf epidermal and mesophyll cells, and leaf cell walls contained 296, 80 and *c.* 110 μg Zn g^{-1} DW, respectively, in plants grown at low $[Zn]_{ext}$. At high $[Zn]_{ext}$, vacuoles of leaf epidermal cells and their cell walls contained up to 13 600 and 929 μg Zn g^{-1} DW, respectively. Vacuoles of leaf mesophyll cells and their cell walls contained up to 4610 and 333 μg Zn g^{-1} DW, respectively. Zinc also accumulated in leaf subepidermal cells. In root epidermal and subepidermal cells, the Zn concentration ratio was approx. 1 (56 μg Zn g^{-1} DW) : 1.7 : 3 : 3.5 (vacuole centre : vacuole periphery : cell wall : intercellular space) at low $[Zn]_{ext}$. At high $[Zn]_{ext}$, background vacuolar Zn concentration was 175 μg Zn g^{-1} DW; scattered, electron-dense deposits of up to 18 300 μg Zn g^{-1} DW (likely to be artefacts of sample preparation; Küpper *et al.*, 1999; Frey *et al.*, 2000; Ma *et al.*, 2005) were also reported. The Zn concentration ratio was *c.* 1 : 0.5 : 1 (background vacuole : cell wall : intercellular space). Thus, root cell wall Zn concentrations were, in general, similar at different values of $[Zn]_{ext}$ but vacuolar Zn concentrations differed. Küpper *et al.* (1999) used direct microcapillary sampling and scanning electron microscopy (SEM)-EDXMA on freeze-fractured *T. caerulescens* leaves. Epidermal and mesophyll cell saps contained 385 and 60 mM, respectively, but the latter represented *c.* 60% of the leaf Zn content. Zinc did not accumulate in subepidermal cells. Vacuolation promoted Zn accumulation, that is, Zn concentrations were lower in younger leaves and correlated with epidermal cell length (Küpper *et al.*, 1999). *Arabidopsis halleri* has thinner leaves and smaller epidermal cells (length 8–30 μm) than *T. caerulescens* (10–100 μm) and also has trichomes (Küpper *et al.*, 2000; Zhao *et al.*, 2000). In contrast to *T. caerulescens*, SEM-EDXMA on freeze-fractured *A. halleri* tissues revealed Zn to be more uniformly distributed across the leaf, although trichome bases were substantially enriched with Zn (up to 1 M; Küpper *et al.*, 2000; Zhao *et al.*, 2000). Mesophyll cells had two- to threefold higher Zn concentrations than smaller-vacuoled epidermal cells and, at high $[Zn]_{ext}$, relative Zn enrichment of the mesophyll cells was greater than in the trichomes. Consistent with EDXMA studies, not all leaf protoplasts of *A. halleri* accumulate Zn, although all leaf protoplasts of *A. halleri* (and *T. caerulescens*) are Zn-hypertolerant (Marquès *et al.*, 2004). In *A. halleri* roots, Zn-phosphate precipitation in the rhizodermal outer wall prevented substantial Zn accumulation in other root compartments (Küpper *et al.*, 2000).

Frey *et al.* (2000) quantified subcellular Zn distribution in ultrathin (100 nm) cryosections of *T. caerulescens* using STEM-EDXMA. Zinc localized to upper and lower leaf epidermal cells. Leaf epidermal cells contained 74 305, 11 577 and 3205 μg Zn g^{-1} DW in their vacuoles, cell walls/apoplast and cytoplasm, respectively. In contrast, leaf mesophyll (327, 9353, 262), guard (1439, 8438, 589) and subsidiary (3009, 9615, 2420) cells and root cortical cells (262, 589, 262) contained substantially less Zn (values given in parentheses represent vacuole, cell wall/apoplast and cytoplasm compartments, respectively, in μg Zn g^{-1} DW). Thus, a substantial fraction of total shoot Zn content was localized to the cell wall/apoplast, for example, 79% of the mesophyll cell Zn quota. Studies using the Zn-specific dye Newport green diacetate confirm that extremely low cytoplasmic Zn concentrations are maintained in Zn hyperaccumulators (Marquès *et al.*, 2004). In direct analyses of mesophyll and epidermal protoplasts, mesophyll vacuoles and peeled leaf fractions, epidermal : mesophyll Zn ratios were reportedly approx. 2.5 : 1, with the mesophyll accounting for 65% of the leaf Zn quota (Ma *et al.*, 2005). However, only 23% of the leaf mesophyll cell Zn content was localized to the cell wall/apoplast. Similarly, *c.* 9–35% of mesophyll cell Cd quota has been attributed to apoplastic compartments (Cosio *et al.*, 2005; Ma *et al.*, 2005). Although different quantification methods will lead to discrepancies, compartmentalization of Zn in leaf cell vacuoles and cell walls is clearly an important facet of Zn hyperaccumulation.

In what form is Zn stored in hyperaccumulators? In *T. caerulescens* and *A. halleri*, up to 80% of shoot Zn is soluble in water or weak acids (Tolrà *et al.*, 1996; Zhao *et al.*, 1998, 2000; Ma *et al.*, 2005). In contrast to many crop species, insoluble Zn : P complexes such as $Zn_3(PO4)_2$ and Zn-phytates are not present in significant quantities (Zhao *et al.*, 1998, 2000; Sarret *et al.*, 2002). In roots, insoluble fractions make up a much larger proportion of the Zn content. For example, 25–57% of root Zn content is soluble but this declines at high $[Zn]_{ext}$, whilst insoluble Zn correlates with insoluble P in the stoichiometric ratio expected of $Zn_3(PO_4)_2$, that is, 0.27 P : Zn (Zhao *et al.*, 1998, 2000). In *T. caerulescens* leaf cells, millimole equivalents (meq) of the major cations (Ca + K + Mg + Zn) ranged from 167 to 562 meq except in the vacuoles of mesophyll (756 meq) and epidermal (1100 meq) cells (Frey *et al.*, 2000) and soluble vacuolar Zn concentrations did not associate with P, S or Cl (Küpper *et al.*, 1999; Frey *et al.*, 2000). Phytochelatins, small cystein-rich peptides, do not have an important role in binding Zn in hyperaccumulators, or in conferring Zn hypertolerance in general (Ernst *et al.*, 1992; Harmens *et al.*, 1993; Küpper *et al.*, 2000, 2004; Clemens, 2001; Schat *et al.*, 2002; Callahan *et al.*, 2006; Wójcik *et al.*, 2006). Further, the role of many other organic/amino acids, peptides and proteins (e.g. metallothioneins and the phytosiderophore precursor nicotianamine), which can also bind Zn, in Zn hyperaccumulation is not yet known (Callahan *et al.*, 2006). However, since inorganic cation and organic anion equivalents correlate significantly in *T. caerulescens* shoots (Tolrà *et al.*, 1996), and since carboxylic acids, primarily malate, citrate and oxalate, and amino acids are abundant in plant materials, their role in Zn hyperaccumulation has been studied widely (Tolrà *et al.*, 1996; Shen *et al.*, 1997; Zhao *et al.*, 1998, 2000; Salt *et al.*, 1999; Küpper *et al.*, 2000, 2004;

Sarret *et al.*, 2002; Ma *et al.*, 2005; Callahan *et al.*, 2006; Wójcik *et al.*, 2006).

In *T. caerulescens* shoots, concentrations of malate (164–248 μmol g^{-1} FW) > citrate (50–87 μmol g^{-1} FW) > succinate (23–38 μmol g^{-1} FW) > oxalate (1.9–4.9 μmol g^{-1} FW) > fumarate (0.31–1.25 μmol g^{-1} FW) > *cis*-aconitate (0.17–0.24 μmol g^{-1} FW), *trans*-aconitate (< 0.06 μmol g^{-1} FW); formate and acetate are detectable only at high $[Zn]_{ext}$ (Tolrà *et al.*, 1996). Soluble Zn, malate and oxalate are correlated in *T. caerulescens* shoots, although organic acid concentrations are constitutively high (Tolrà *et al.*, 1996; Shen *et al.*, 1997; Wójcik *et al.*, 2006), as they are in *A. halleri* (Zhao *et al.*, 2000). The molar ratios of malate : Zn in *T. caerulescens* (4.8–72, (Tolrà *et al.*, 1996), 9.7–23.6 (Wójcik *et al.*, 2006)) are sufficient to support a Zn-malate shuttle hypothesis (reviewed by Ernst *et al.*, 1992), that is, the transport of Zn-malate across the tonoplast and dissociation and subsequent Zn^{2+} binding to stronger chelators such as citrate or oxalate, and export of malate back across the tonoplast to the cytosol. However, malate does not have a strong affinity for Zn (Tolrà *et al.*, 1996). Whilst oxalate : Zn molar ratios are low in *T. caserulescens* (0.1–0.68; Tolrà *et al.*, 1996), molar ratios of citrate : Zn are 2.1–5.9 in *T. caerulescens* (Wójcik *et al.*, 2006) and 0.37–49.0 in *A. halleri* (Zhao *et al.*, 2000). In *T. caerulescens* roots, organic acid concentrations are substantially lower than in shoots; malate (2.1–10 μmol g^{-1} FW) = citrate (3.2–16.5 μmol g^{-1} FW) = succinate (1.8–10.6 μmol g^{-1} FW) > fumarate (0.027–0.18 μmol g^{-1} FW) > *cis*-aconitate (< 0.07 μmol g^{-1} FW) = *trans*-aconitate (< 0.07 μmol g^{-1} FW). Acetate and oxalate (1.4 μmol g^{-1} FW) were only detectable at high $[Zn]_{ext}$ (Tolrà *et al.*, 1996). Whilst some studies report little correlation between Zn accumulation and altered root organic acid status (Tolrà *et al.*, 1996), Zn might stimulate citrate production in *T. caerulescens* roots (Shen *et al.*, 1997).

Zinc coordination to O, N, S and His ligands can be predicted using noninvasive X-ray absorption spectroscopy (XAS) and extended X-ray absorption fine structure (EXAFS) analysis (Salt *et al.*, 1999; Sarret *et al.*, 2002; Küpper *et al.*, 2004; Callahan *et al.*, 2006). In *T. caerulescens*, up to 70% of root Zn may be associated with His, the remaining 30% with the cell wall, although it is difficult to discriminate between $Zn(His)_2$ and Zn^{2+} bound to O ligands, potentially representing hydroxyl groups of water, using these techniques (Salt *et al.*, 1999; Callahan *et al.*, 2006). In xylem saps, 21% of Zn was found to be associated with Zn citrate, the reminder probably being free Zn^{2+}. In shoots, 38, 9, 16 and 12% of total Zn was associated with citrate, oxalate, His and the cell wall, respectively, with 26% as free Zn^{2+} (Salt *et al.*, 1999). No evidence of Zn-malate coordination was found. In other studies, Zn was predominantly coordinated with O ligands, potentially representing hydroxyl groups of water, or potentially carboxyl groups of malate, citrate or other organic acids (Küpper *et al.*, 2004). Stronger Zn binding to His occurred in young leaves, potentially to avoid toxicity, and in senescing leaf tissues, which could be as a result of the breakdown in cell compartmentation. No associations between Zn and S ligands such as phytochelatins, metallothioneins or other Cys-rich peptides were observed (Küpper *et al.*, 2004). In *A. halleri* shoots, Zn was primarily (octahedrally) coordinated with malate. In the trichomes, a secondary unidentified organic Zn compound was present, tetrahedrally coordinated and complexed to carboxyl and/or hydroxyl functional groups (Sarret *et al.*, 2002). In *A. lyrata* ssp. *petraea*, shoot Zn was coordinated primarily with phosphate.

Two unusual root properties of Zn hyperaccumulators Two remarkable features of *T. caerulescens* roots may be linked to its ability to hyperaccumulate Zn. First, a zincophilic root foraging response to heterogeneous $[Zn]_{soil}$ has been shown, analogous to the exploitation of spatially heterogeneous soil macronutrients (Schwartz *et al.*, 1999; Whiting *et al.*, 2000; Haines, 2002). Increases in root biomass (including root length and root hair production) occur in high-Zn-containing patches compared with adjacent Zn-deficient patches. These responses are not thought to be constitutive at the species level (Whiting *et al.*, 2000; Haines, 2002) and warrant further genetic and molecular investigations. Second, a 'peri-endodermal' layer of cells with irregularly thickened inner tangential walls extending to < 1 mm from the root tip has been reported recently in *T. caerulescens* (Zelko *et al.*, in press; Figs 2, 3). This layer is composed of secondary cell walls impregnated by suberin/lignin, forming a compact cylinder surrounding the endodermis from the outer side. This layer is not seen in *T. arvense* (Zelko *et al.*, in press) or *A. thaliana* (van de Mortel *et al.*, 2006) when compared with *T. caerulescens*. The development of the endodermis also differs between *T. caerulescens* and *T. arvense* (Zelko *et al.*, in press). In *T. caerulescens*, Casparian bands (the first stage of endodermal development) form < 1 mm from the root tip, and suberin lamellae (the second stage of endodermal development) are formed in all endodermal cells *c.* 5–6 mm from the apex. In *T. arvense*, Casparian bands develop *c.* 2 mm from the root tip and suberin lamellae are formed in all endodermal cells > 10 mm from the root tip. Although a similar feature to this peri-endodermal layer was observed by early anatomists in some Brassicaceae, being described as 'réseau sus-endodermique' in 1887 (van Tieghem, 1887; Zelko *et al.*, in press), the precise function of this layer of cells and its impact on apoplastic and symplastic fluxes of Zn into the root stele (and its effects on Zn efflux from the stele) remain unclear, as does the distribution of this character among closely related taxa. Further anatomical studies in the *Thlaspiceras|Noccaea|Raparia|Microthlaspi|Neurotropis* clade are likely to be revealing in this respect. In a closely related Brassicaceae species, *Thellungiella halophila* O. E. Schulz, a second layer of endodermis is developed and it is probably related to the salt tolerance of this halophyte (Inan *et al.*, 2004). Thus, the structural/functional adaptations of roots associated with metallophily are highly variable in this interesting group of plants.

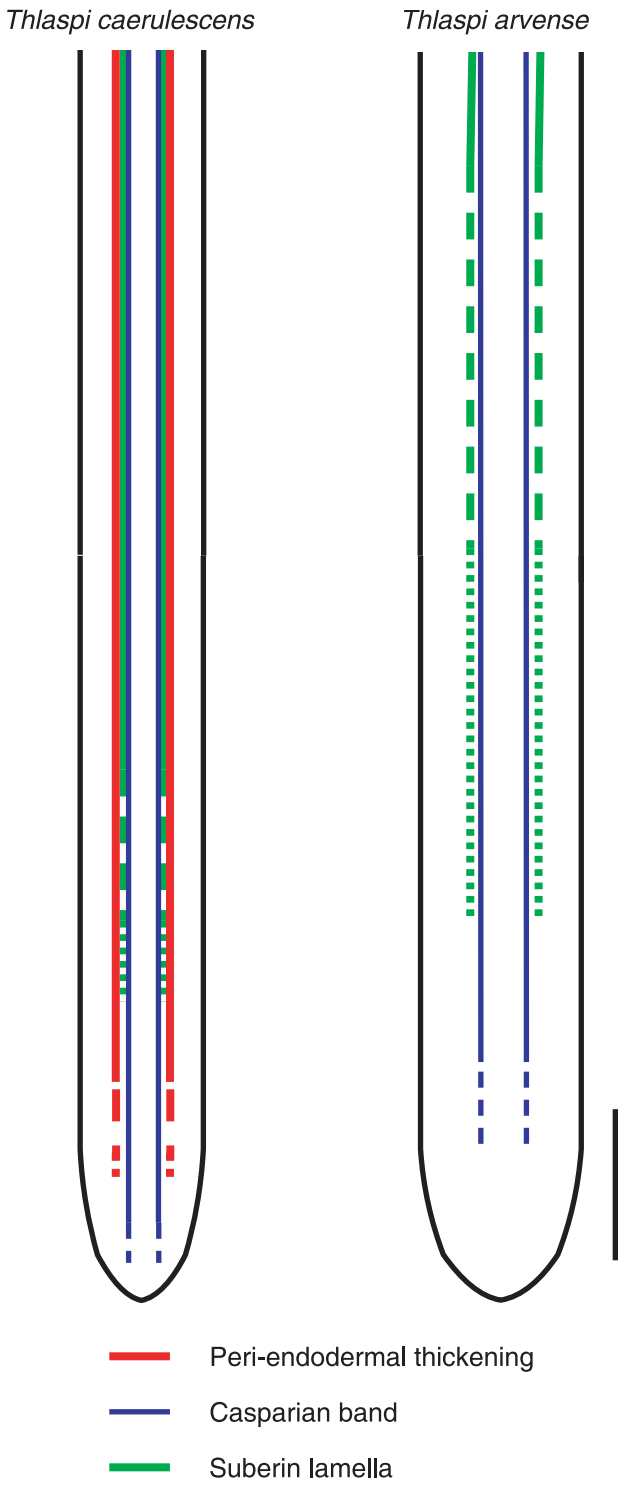

Fig. 2 Development scheme of apoplastic barriers along the root axis of *Thlaspi caerulescens* (hypertolerant Zn-accumulator) and *T. arvense* (nontolerant Zn nonaccumulator). In the first stage of endodermal ontogenesis, Casparian bands, and in the second stage, suberin lamellae deposition, develop closer to the root tip in *T. caerulescens* than in *T. arvense*. Note the early formation of a peri-endodermal layer close to the apex in *T. caerulescens* but not in *T. arvense*. Bar, 2 mm in the longitudinal axis. Transverse axes are not represented to scale.

Molecular insights into Zn hyperaccumulation Gene and protein expression profiling (Assunção *et al.*, 2001; Becher *et al.*, 2004; Weber *et al.*, 2004; Craciun *et al.*, 2006; Filatov *et al.*, 2006; Hammond *et al.*, 2006; Rigola *et al.*, 2006; Talke *et al.*, 2006; Tuomainen *et al.*, 2006; van de Mortel *et al.*, 2006; Weber *et al.*, 2006), and functional analyses of proteins in heterologous plant, yeast and *Xenopus laevis* oocyte systems are providing remarkable and rapidly advancing insights into Zn hyperaccumulation – and Zn homeostasis in general – at the molecular level. Fortunately, *T. caerulescens* and *A. thaliana* share > 87% sequence identity (Peer *et al.*, 2003; Rigola *et al.*, 2006), enabling *A. thaliana* sequence data to be easily exploited. To illustrate, global shoot transcriptome data published using the full-genome Affymetrix *A. thaliana* ATH1-121501 (ATH1) GeneChip array (Affymetrix Inc. Palo Alto, CA, USA), for *T. caerulescens* vs *T. arvense* (Hammond *et al.*, 2006), and *A. halleri* vs *A. lyrata* ssp. *petraea* (Filatov *et al.*, 2006) and *A. thaliana* (Talke *et al.*, 2006) comparisons, are combined and re-analysed here. Briefly, raw expression data files (CEL files) of shoot transcriptome data from either the hyperaccumulator (five *T. caerulescens*, eight *A. halleri*) or nonhyperaccumulator (five *T. arvense*, six *A. lyrata* ssp. *petraea*, two *A. thaliana*) condition, were analysed using a global Robust Multichip Average (RMA) algorithm (Irizarry *et al.*, 2003), with a custom GeneChip definition file (CDF) in GeneSpring GX (Agilent Technologies Inc.). This CDF was designed to exclude ATH1 GeneChip array oligonucleotide probe pairs that hybridized to both *T. caerulescens* and *T. arvense* genomic DNA (gDNA) below an intensity threshold of 300 (*sensu* Hammond *et al.*, 2005, 2006). Thus, 22 131 probe sets (out of 22 746), most likely to be common to *Thlaspi* s.l and *Arabidopsis*, were used for transcriptome comparisons. Data were normalized to the median expression value of either the hyperaccumulator or the non-hyperaccumulator condition. Putative genes with different amounts of expression between the two conditions were identified using a Welch's *t*-test with a Benjamini-Hochberg False Discovery Rate (FDR) correction of 0.05 (Table S10).

In total, homologues of 60 *A. thaliana* genes are significantly differentially expressed between hyperaccumulators and nonhyperaccumulators and may have conserved roles in brassicaceous Zn hyperaccumulation (Table S10). Six of these genes encode proteins with putative roles in Zn transport: three cation diffusion facilitator (CDF) family members (At2g39450 (TAIR6: *AtMTP11*), At2g46800 (*AtZAT1/AtMTP1*) and At3g58060 (*AtMTPc3*)); a member of the Zn-Fe permease (*ZIP*) family (At1g60960 (*AtIRT3/TcZNT2*)); and a P_{IB}-type heavy-metal-associated domain-containing ATPase (At4g30120 (*AtHMA3*)). CDFs appear to mediate vacuolar sequestration of Zn through Zn efflux from the cytoplasm (van der Zaal *et al.*, 1999; Persans *et al.*, 2001; Blaudez *et al.*, 2003; Delhaize *et al.*, 2003; Hall & Williams, 2003; Dräger *et al.*, 2004; Kim *et al.*, 2004; Kobae *et al.*, 2004; Desbrosses-Fonrouge *et al.*, 2005; Krämer, 2005b; Arrivault

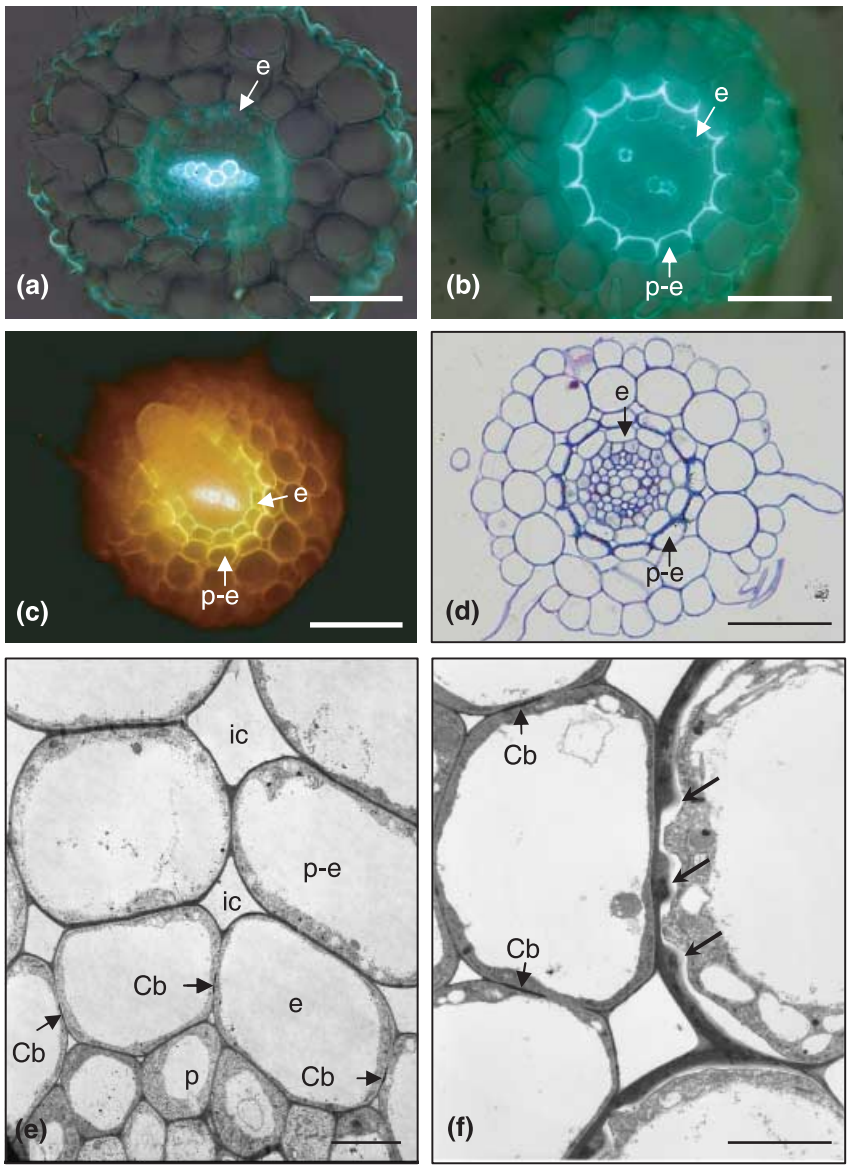

Fig. 3 Structure of primary roots in cross-sections of *Thlaspi arvense* (a) and *T. caerulescens* (b–f). (a) Autofluorescence of *T. arvense* root section showing typical dicotyledonous root structure with three cortical layers; the innermost is the endodermis exhibiting fluorescence of Casparian bands. (b) Autofluorescence of *T. caerulescens* root section showing the intensive fluorescence of inner tangential and radial walls of the peri-endodermal layer and light fluorescence of thin endodermal cell walls. (c) *T. caerulescens* primary root with emerging lateral root primordium in fluorescence microscope. After Fluorol yellow-toluidine blue staining, endodermal cell walls exhibit intensive bright yellow fluorescence as a result of suberin lamellae deposition (second stage of endodermal development). (d) Semithin section of Spurr embedded *T. caerulescens* root showing prominent wall ingrowths of the peri-endodermal layer (toluidine blue-basic fuchsin staining). (e) Transmission electron microscopy (TEM) of the young root part of *T. caerulescens* (< 1 mm from the apex) stained by $KMnO_4$ to visualize Casparian bands, which are present in radial walls in endodermis, but absent in the peri-endodermal layer. (f) TEM of the older root part of *T. caerulescens* (*c.* 2.5 mm from the apex) with irregular wall thickening in the peri-endodermal layer. Cb, Casparian band; e, endodermis; ic, intercellular space; p, pericycle; p-e, peri-endodermal layer; arrow, wall ingrowths in peri-endodermal cells. Bars, 50 μm (a–d); 4 μm (e, f).

et al., 2006; Colangelo & Guerinot, 2006), ZIPs are likely to mediate cellular Zn uptake (Pence *et al.*, 2000; Assunção *et al.*, 2001; López-Millán *et al.*, 2004; Colangelo & Guerinot, 2006), and HMAs have Zn-transport functions throughout the cell (Williams *et al.*, 2000; Mills *et al.*, 2003; Bernard *et al.*, 2004; Eren & Argüello, 2004; Gravot *et al.*, 2004; Hussain *et al.*, 2004; Papoyan & Kochian, 2004; Verret *et al.*, 2004, 2005; Williams & Mills, 2005; Colangelo & Guerinot, 2006). However, the function of other nutritionally important transporter genes, for example, a phosphate-starvation-inducible high-affinity phosphate transporter *AtPT2/AtPHT4/AtPHT1;4* (Misson *et al.*, 2004), which is highly expressed in Zn hyperaccumulators (Hammond *et al.*, 2006; Talke *et al.*, 2006), remains unknown. Other genes expressed highly in Zn hyperaccumulators include plant defensins (PDFs), which confer Zn tolerance and accumulation in heterologous systems, and may act as blockers of Zn-permeable channels (Mirouze *et al.*, 2006). Whilst it remains extremely challenging to elucidate how the function of single proteins in heterologous systems relates to the behaviour at whole-plant and crop scales, the use of homologous transformation (Peer *et al.*, 2003) and the increasing availability of species-specific sequence information (Rigola *et al.*, 2006) will undoubtedly hasten this process.

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References

Alloway BJ. 1995. *Heavy metals in soils, 2nd edn.* London, UK: Blackie Academic & Professional.

Alloway BJ. 2004. *Zinc in soils and crop nutrition.* Brussels, Belgium: International Zinc Association.

Andreini C, Banci L, Bertini I, Rosato A. 2006. Counting the zinc-proteins encoded in the human genome. *Journal of Proteome Research* **5**: 196–201.

Antonovics J. 1968. Evolution in closely adjacent plant populations. V. Evolution of self-fertility. *Heredity* **23**: 219–238.

Antonovics J. 1972. Population dynamics of the grass *Anthoxanthum odoratum* on a zinc mine. *Journal of Ecology* **60**: 351–365.

Antonovics J, Bradshaw AD, Turner RG. 1971. Heavy metal tolerance in plants. *Advances in Ecological Research* **7**: 1–85.

Arrivault S, Senger T, Krämer U. 2006. The *Arabidopsis* metal tolerance protein AtMTP3 maintains metal homeostasis by mediating Zn exclusion from the shoot under Fe deficiency and Zn oversupply. *Plant Journal* **46**: 861–879.

Assunção AGL, Bookum WM, Nelissen HJM, Vooijs R, Schat H, Ernst WHO. 2003a. Differential metal-specific tolerance and accumulation patterns among *Thlaspi caerulescens* populations originating from different soil types. *New Phytologist* **159**: 411–419.

Assunção AGL, Da Costa Martins P, De Folter S, Vooijs R, Schat H, Aarts MGM. 2001. Elevated expression of metal transporter genes in three accessions of the metal hyperaccumulator *Thlaspi caerulescens*. *Plant, Cell & Environment* **24**: 217–226.

Assunção AGL, Pieper B, Vromans J, Lindhout P, Aarts MGM, Schat H. 2006. Construction of a genetic linkage map of *Thlaspi caerulescens* and quantitative trait loci analysis of zinc accumulation. *New Phytologist* **170**: 21–32.

Assunção AGL, Ten Bookum WM, Nelissen HJM, Vooijs R, Schat H, Ernst WHO. 2003b. A cosegregation analysis of zinc (Zn) accumulation and Zn tolerance in the Zn hyperaccumulator *Thlaspi caerulescens*. *New Phytologist* **159**: 383–390.

Auld DS. 2001. Zinc coordination sphere in biochemical zinc sites. *Biometals* **14**: 271–313.

Baker AJM. 1981. Accumulators and excluders – strategies in the response of plants to heavy metals. *Journal of Plant Nutrition* **3**: 643–654.

Baker AJM. 1987. Metal tolerance. *New Phytologist* **106**: 93–111.

Baker AJM, Brooks RR. 1989. Terrestrial higher plants which hyperaccumulate metallic elements – a review of their distribution, ecology and phytochemistry. *Biorecovery* **1**: 81–126.

Baker AJM, Reeves RD, Hajar ASM. 1994. Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens*. J. & C. Presl (Brassicaceae). *New Phytologist* **127**: 61–68.

Baldwin JP, Nye PH, Tinker PB. 1973. Uptake of solutes by multiple root systems from soil III: A model for calculating the solute uptake by a randomly dispersed root system developing in a finite volume of soil. *Plant and Soil* **38**: 621–635.

Barak P, Helmke PA. 1993. The chemistry of zinc. In: Robson AD, ed. *Zinc in soil and plants.* Dordrecht, the Netherlands: Kluwer Academic Publishers, 1–13.

Barber SA. 1995. *Soil nutrient bioavailability, 2nd edn.* New York, NY, USA: John Wiley & Sons, Inc.

Barber SA, Claassen N. 1977. A mathematical model to simulate metal uptake by plants growing in soil. In: Drucker H, Wildung RE, eds. *Biological implications of metals in the environment. Proceedings of the Fifteenth Hanford Life Sciences Symposium.* Oak Ridge, TN, USA: Technical Information Center, Energy Research and Development Administration, 358–364.

Barrow NJ. 1993. Mechanisms of reaction of zinc with soil and soil components. In: Robson AD, ed. *Zinc in soil and plants.* Dordrecht, the Netherlands: Kluwer Academic Publishers, 15–31.

Bar-Yosef B, Fishman S, Talpaz H. 1980. A model of zinc movement to single roots in soils. *Soil Science Society of America Journal* **44**: 1272–1279.

Becher M, Talke IN, Krall L, Krämer U. 2004. Cross-species microarray transcript profiling reveals high constitutive expression of metal homeostasis genes in shoots of the zinc hyperaccumulator *Arabidopsis halleri*. *Plant Journal* **37**: 251–268.

Berg JM, Shi Y. 1996. The galvanization of biology: a growing appreciation for the roles of zinc. *Science* **271**: 1081–1085.

Bernard C, Roosens N, Czernic P, Lebrun M, Verbruggen N. 2004. A novel CPx-ATPase from the cadmium hyperaccumulator *Thlaspi caerulescens*. *FEBS Letters* **569**: 140–148.

Bert V, Bonnin I, Saumitou-Laprade P, de Laguérie P, Petit D. 2002. Do *Arabidopsis halleri* from nonmetallicolous populations accumulate zinc and cadmium more effectively than those from metallicolous populations? *New Phytologist* **155**: 47–57.

Bert V, Macnair MR, de Laguerie P, Saumitou-Laprade P, Petit D. 2000. Zinc tolerance and accumulation in metallicolous and nonmetallicolous populations of *Arabidopsis halleri* (Brassicaceae). *New Phytologist* **146**: 225–233.

Beyersmann D, Haase H. 2001. Functions of zinc in signaling, proliferation and differentiation of mammalian cells. *Biometals* **14**: 331–341.

Blaudez D, Kohler A, Martin F, Sanders D, Chalot M. 2003. Poplar Metal Tolerance Protein 1 confers zinc tolerance and is an oligomeric vacuolar zinc transporter with an essential leucine zipper motif. *Plant Cell* **15**: 2911–2928.

Blindauer CA, Sadler PJ. 2005. How to hide zinc in a small protein. *Accounts of Chemical Research* **38**: 62–69.

Boawn LC, Rasmussen PE. 1971. Crop response to excessive zinc fertilization of alkaline soil. *Agronomy Journal* **63**: 874–876.

Borhidi A. 2001. Phylogenetic trends in Ni-accumulating plants. *South African Journal of Science* **97**: 544–547.

Bowen JE. 1973. Kinetics of zinc absorption by excised roots of two sugarcane clones. *Plant and Soil* **39**: 125–129.

Bowen JE. 1981. Kinetics of active uptake of boron, zinc, copper and manganese in barley and sugarcane. *Journal of Plant Nutrition* **3**: 215–223.

Bowen JE. 1986. Kinetics of zinc uptake by two rice cultivars. *Plant and Soil* **94**: 99–107.

Bowen JE. 1987. Physiology of genotypic differences in zinc and copper uptake in rice and tomato. *Plant and Soil* **99**: 115–125.

Brar MS, Sekhon GS. 1976. Interaction of zinc with other micronutrient cations. I. Effect of copper on zinc^{65} absorption by wheat seedlings and its translocation within the plants. *Plant and Soil* **45**: 137–143.

Broadley MR, Bowen HC, Cotterill HL, Hammond JP, Meacham MC, Mead A, White PJ. 2003. Variation in the shoot calcium content of angiosperms. *Journal of Experimental Botany* **54**: 1431–1446.

Broadley MR, Bowen HC, Cotterill HL, Hammond JP, Meacham MC, Mead A, White PJ. 2004. Phylogenetic variation in the shoot mineral concentration of angiosperms. *Journal of Experimental Botany* **55**: 321–336.

Broadley MR, Willey NJ, Wilkins JC, Baker AJM, Mead A, White PJ. 2001. Phylogenetic variation in heavy metal accumulation in angiosperms. *New Phytologist* **152**: 9–27.

Brooks RR. 1998. *Plants that hyperaccumulate heavy metals*. Wallingford, UK: CAB International.

Brown G, Brinkmann K. 1992. Heavy metal tolerance in *Festuca ovina* L. from contaminated sites in the Eifel mountains, Germany. *Plant and Soil* **143**: 239–247.

Browning KS. 2004. Plant translation initiation factors: it is not easy to be green. *Biochemical Society Transactions* **32**: 589–591.

Cakmak I. 2002. Plant nutrition research: priorities to meet human needs for food in sustainable ways. *Plant and Soil* **247**: 3–24.

Cakmak I. 2004. Identification and correction of widespread zinc deficiency in Turkey – a success story (a NATO-Science for Stability Project). *Proceedings of the International Fertiliser Society* **552**: 1–26.

Callahan DL, Baker AJM, Kolev SP, Wedd AG. 2006. Metal ion ligands in hyperaccumulating plants. *Journal of Biological Inorganic Chemistry* **11**: 2–12.

Cappuyns V, Swennen R, Vandamme A, Niclaes M. 2006. Environmental impact of the former Pb-Zn mining and smelting in East Belgium. *Journal of Geochemical Exploration* **88**: 6–9.

Carroll MD, Loneragan JF. 1968. Response of plant species to concentrations of zinc in solution. I. Growth and zinc content of plants. *Australian Journal of Agricultural Research* **19**: 859–868.

Chaney RL. 1983. Plant uptake of inorganic waste constituents. In: Parr JF, Marsh PB, Kla JM, eds. *Land treatment of hazardous wastes.* Park Ridge, NJ, USA: Noyes Data Corporation, 50–76.

Chaney RL. 1993. Zinc phytotoxicity. In: Robson AD, ed. *Zinc in soil and plants.* Dordrecht, the Netherlands: Kluwer Academic Publishers, 135–150.

Chardonnens AN, Koevoets PLM, van Zanten A, Schat H, Verkleij JAC. 1999. Properties of enhanced tonoplast zinc transport in naturally selected zinc-tolerant *Silene vulgaris*. *Plant Physiology* **120**: 779–785.

Chase T Jr. 1999. Alcohol dehydrogenases: identification and names for gene families. *Plant Molecular Biology Reporter* **17**: 333–350.

Chaudhry FM, Loneragan JF. 1972a. Zinc absorption by wheat seedlings and the nature of its inhibition by alkaline earth cations. *Journal of Experimental Botany* **23**: 552–560.

Chaudhry FM, Loneragan JF. 1972b. Zinc absorption by wheat seedlings. I. Inhibition by macronutrient ions in short-term experiments and its relevance to long-term zinc nutrition. *Soil Science Society of America Proceedings* **36**: 323–327.

Chaudhry FM, Loneragan JF. 1972c. Zinc absorption by wheat seedlings. II. Inhibition by hydrogen ions and by micronutrient cations. *Soil Science Society of America Proceedings* **36**: 327–331.

Cheng X, Collins RE, Zhang X. 2005. Structural and sequence motifs of protein (histone) methylation enzymes. *Annual Review of Biophysics and Biomolecular Structure* **34**: 267–294.

Clay NK, Nelson T. 2005. The recessive epigenetic swellmap mutation affects the expression of two step II splicing factors required for the transcription of the cell proliferation gene STRUWWELPETER and for the timing of cell cycle arrest in the *Arabidopsis* leaf. *Plant Cell* **17**: 1994–2008.

Clemens S. 2001. Molecular mechanisms of plant metal tolerance and homeostasis. *Planta* **212**: 475–486.

Colangelo EP, Guerinot ML. 2006. Put the metal to the petal: metal uptake and transport throughout plants. *Current Opinion in Plant Biology* **9**: 322–330.

Cosio C, DeSantis L, Frey B, Diallo S, Keller C. 2005. Distribution of cadmium in leaves of *Thlaspi caerulescens*. *Journal of Experimental Botany* **56**: 765–775.

Cox RM, Hutchinson TC. 1980. Multiple metal tolerances in the grass *Deschampsia cespitosa* (L.) Beauv. from the Sudbury smelting area. *New Phytologist* **84**: 631–647.

Craciun AR, Courbot M, Bourgis F, Salis P, Saumitou-Laprade P, Verbruggen N. 2006. Comparative cDNA-AFLP analysis of Cd-tolerant and -sensitive genotypes derived from crosses between the Cd hyperaccumulator *Arabidopsis halleri* and *Arabidopsis lyrata* ssp. *petraea*. *Journal of Experimental Botany* **57**: 2967–2983.

Dang YP, Dalal RC, Edwards DG, Tiller K. 1994. Zinc buffer capacity of vertisols. *Australian Journal of Soil Research* **32**: 1231–1242.

Davies BE. 1993. Radish as an indicator plant for derelict land – uptake of zinc at toxic concentrations. *Communications in Soil Science and Plant Analysis* **24**: 1883–1895.

Dechamps C, Roosens NH, Hotte C, Meerts P. 2005. Growth and mineral element composition in two ecotypes of *Thlaspi caerulescens* on Cd contaminated soil. *Plant and Soil* **273**: 327–335.

Delhaize E, Kataoka E, Hebb DM, White RG, Ryan PR. 2003. Genes encoding proteins of the cation diffusion facilitator family that confer manganese tolerance. *Plant Cell* **15**: 1131–1142.

Deniau AX, Pieper B, Ten Bookum WM, Lindhout P, Aarts MGM, Schat H. 2006. QTL analysis of cadmium and zinc accumulation in the heavy metal hyperaccumulator *Thlaspi caerulescens*. *Theoretical and Applied Genetics* **113**: 907–920.

Desbrosses-Fonrouge A-G, Voigt K, Schröder A, Arrivault S, Thomine S, Krämer U. 2005. *Arabidopsis thaliana* MTP1 is a Zn transporter in the vacuolar membrane which mediates Zn detoxification and drives leaf Zn accumulation. *FEBS Letters* **579**: 4165–4174.

Dong Y, Ogawa T, Lin D, Koh H-J, Kamiunten H, Matsuo M, Cheng S. 2006. Molecular mapping of quantitative trait loci for zinc toxicity in rice seedling (*Oryza sativa* L.). *Field Crops Research* **95**: 420–425.

Dräger DB, Desbrosses-Fonrouge A-G, Krach C, Chardonnens AN, Meyer RC, Saumitou-Laprade P, Krämer U. 2004. Two genes encoding *Arabidopsis halleri* MTP1 metal transport proteins co-segregate with zinc tolerance and account for high MTP1 transcript levels. *Plant Journal* **39**: 425–439.

Dubois S, Cheptou P-O, Petit C, Meerts P, Poncelet M, Vekemans X, Lefèbvre C, Escarré J. 2003. Genetic structure and mating systems of metallicolous and nonmetallicolous populations of *Thlaspi caerulescens*. *New Phytologist* **157**: 633–641.

Ducousso A, Petit D, Valero M, Vernet P. 1990. Genetic variation between and within populations of a perennial grass – *Arrhenatherum elatius*. *Heredity* **65**: 179–188.

Earley EB. 1943. Minor element studies with soybeans. I. Varietal reaction to concentrations of zinc in excess of the nutritional requirement. *Journal of the American Society of Agronomy* **35**: 1012–1023.

Edwards R, Dixon DP. 2005. Plant glutathione transferases. *Methods in Enzymology* **401**: 169–186.

Englbrecht CC, Schoof H, Böhm S. 2004. Conservation, diversification and expansion of C_2H_2 zinc finger proteins in the *Arabidopsis thaliana* genome. *BMC Genomics* **5**: 39.

Eren E, Argüello JM. 2004. Arabidopsis HMA2, a divalent heavy metal-transportnig P_{IB}-type ATPase, is involved in cytoplasmic Zn^{2+} homeostasis. *Plant Physiology* **136**: 3712–3723.

Ernst WHO, Schat H, Verkleij JAC. 1990. Evolutionary biology of metal resistance in *Silene vulgaris*. *Evolutionary Trends in Plants* **4**: 45–51.

Ernst WHO, Verkleij JAC, Schat H. 1992. Metal tolerance in plants. *Acta Botanica Neerlandica* **41**: 229–248.

Escarré J, Lefèbvre C, Gruber W, Leblanc M, Lepart J, Rivière Y, Delay B. 2000. Zinc and cadmium hyperaccumulation by *Thlaspi caerulescens* from metalliferous and nonmetalliferous sites in the Mediterranean area: implications for phytoremediation. *New Phytologist* **145**: 429–437.

Eulgem T, Rushton PJ, Robatzek S, Somssich IE. 2000. The WRKY superfamily of plant transcription factors. *Trends in Plant Science* **5**: 199–206.

Filatov V, Dowdle J, Smirnoff N, Ford-Lloyd B, Newbury HJ, Macnair MR. 2006. Comparison of gene expression in segregating families identifies genes and genomic regions involved in a novel adaptation, zinc hyperaccumulation. *Molecular Ecology* **15**: 3045–3059.

Flowers TJ, Yeo AR. 1992. *Solute transport in plants.* Glasgow, UK: Blackie Academic. & Professional.

Foy CD, Chaney RL, White MC. 1978. The physiology of metal toxicity in plants. *Annual Review of Plant Physiology* **29**: 511–566.

Frérot H, Lefèbvre C, Petit C, Collin C, Dos Santos A, Escarré J. 2005. Zinc tolerance and hyperaccumulation in F_1 and F_2 offspring from

intra and interecotype crosses of *Thlaspi caerulescens*. *New Phytologist* **165**: 111–119.

Frérot H, Petit C, Lefèbvre C, Gruber W, Collin C, Escarré J. 2003. Zinc and cadmium accumulation in controlled crosses between metallicolous and nonmetallicolous populations of *Thlaspi caerulescens* (Brassicaceae). *New Phytologist* **157**: 643–648.

Frey B, Keller C, Zierold K, Schulin R. 2000. Distribution of Zn in functionally different leaf epidermal cells of the hyperaccumulator *Thlaspi caerulescens*. *Plant, Cell & Environment* **23**: 675–687.

Friedland AJ. 1990. The movement of metals through soils and ecosystems. In: Shaw AJ, ed. *Heavy metal tolerance in plants: evolutionary aspects.* Boca Raton, FL, USA: CRC Press, 7–19.

Ghandilyan A, Vreugdenhil D, Aarts MGM. 2006. Progress in the genetic understanding of plant iron and zinc nutrition. *Physiologia Plantarum* **126**: 407–417.

Gong SL, Barrie LA. 2005. Trends of heavy metal components in the Arctic aerosols and their relationship to the emissions in the Northern Hemisphere. *Science of the Total Environment* **342**: 175–183.

Graham RD, Ascher JS, Hynes SC. 1992. Selecting zinc-efficient cereal genotypes for soils of low zinc status. *Plant and Soil* **146**: 241–250.

Graham RD, Welch RM, Bouis HE. 2001. Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: principles, perspectives and knowledge gaps. *Advances in Agronomy* **70**: 77–142.

Gravot A, Lieutaud A, Verret F, Auroy P, Vavasseur A, Richaud P. 2004. AtHMA3, a plant P_{1B}-ATPase, functions as a Cd/Pb transporter in yeast. *FEBS Letters* **561**: 22–28.

Gregory RPG, Bradshaw AD. 1965. Heavy metal tolerance in populations of *Agrostis tenuis* Sibth. and other grasses. *New Phytologist* **64**: 131–143.

Grusak MA, Cakmak I. 2005. Methods to improve the crop-delivery of minerals to humans and livestock. In: Broadley MR, White PJ, eds. *Plant nutritional genomics*. Oxford, UK: Blackwell, 265–286.

Guerinot ML, Salt DE. 2001. Fortified foods and phytoremediation. Two sides of the same coin. *Plant Physiology* **125**: 164–167.

Hacisalihoglu G, Hart JJ, Kochian LV. 2001. High- and low-affinity zinc transport systems and their possible role in zinc efficiency in bread wheat. *Plant Physiology* **125**: 456–463.

Hacisalihoglu G, Kochian LV. 2003. How do some plants tolerate low levels of soil zinc? Mechanisms of zinc efficiency in crop plants. *New Phytologist* **159**: 341–350.

Haines BJ. 2002. Zincophilic root foraging in *Thlaspi caerulescens*. *New Phytologist* **155**: 363–372.

Hall JL. 2002. Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Experimental Botany* **53**: 1–11.

Hall JL, Williams LE. 2003. Transition metal transporters in plants. *Journal of Experimental Botany* **54**: 2601–2613.

Hammond JP, Bowen HC, White PJ, Mills V, Pyke KA, Baker AJM, Whiting SN, May ST, Broadley MR. 2006. A comparison of the *Thlaspi caerulescens* and *T. arvense* shoot transcriptomes. *New Phytologist* **170**: 239–260.

Hammond JP, Broadley MR, Craigon DJ, Higgins J, Emmerson Z, Townsend H, White PJ, May ST. 2005. Using genomic DNA-based probe-selection to improve the sensitivity of high-density oligonucleotide arrays when applied to heterologous species. *Plant Methods* **1**: 10.

Hantke K. 2005. Bacterial zinc uptake and regulators. *Current Opinion in Microbiology* **8**: 196–202.

Harmens H, Den Hartog PR, Ten Bookum WM, Verkleij JAC. 1993. Increased zinc tolerance in *Silene vulgaris* (Moench) Garcke is not due to increased production of phytochelatins. *Plant Physiology* **103**: 1305–1309.

Hart JJ, Norvell WA, Welch RM, Sullivan LA, Kochian LV. 1998. Characterization of zinc uptake, binding, and translocation in intact seedlings of bread and durum wheat cultivars. *Plant Physiology* **118**: 219–226.

Hart JJ, Welch RM, Norvell WA, Kochian LV. 2002. Transport interactions between cadmium and zinc in roots of bread and durum wheat seedlings. *Physiologia Plantarum* **116**: 73–78.

Hassan MM, van Hai T. 1976. Kinetics of zinc uptake by citrus roots. *Zeitschrift für Pflanzenphysiologie* **79**: 177–181.

Hoagland DR. 1948. *Lectures on the inorganic nutrition of plants, 2nd edn.* Waltham, MA, USA: Chronica Botanica Company.

Hobbs RJ, Streit B. 1986. Heavy metal concentrations in plants growing on a copper mine spoil in the Grand Canyon. *Arizona American Midland Naturalist* **115**: 277–281.

Hodson MJ, White PJ, Mead A, Broadley MR. 2005. Phylogenetic variation in the silicon composition of plants. *Annals of Botany* **96**: 1027–1046.

Hoffland E, Wei C, Wissuwa M. 2006. Organic anion exudation by lowland rice (*Oryza sativa* L.) at zinc and phosphorus deficiency. *Plant and Soil* **283**: 155–162.

Homma Y, Hirata H. 1984. Kinetics of cadmium and zinc absorption by rice seedling roots. *Soil Science and Plant Nutrition* **30**: 527–532.

Hussain D, Haydon MJ, Wang Y, Wong E, Sherson SM, Young J, Camakaris J, Harper JF, Cobbett CS. 2004. P-type ATPase heavy metal transporters with roles in essential zinc homeostasis in *Arabidopsis*. *Plant Cell* **16**: 1327–1339.

Inan G, Zhang Q, Li P, Wang Z, Cao Z, Zhang H, Zhang C, Quist TM, Goodwin SM, Zhu J, Shi H, Damsz B, Charbaji T, Gong Q, Ma S, Fredricksen M, Galbraith DW, Jenks MA, Rhodes D, Hasegawa PM *et al.* 2004. Salt cress. A halophyte and cryophyte arabidopsis relative model system and its applicability to molecular genetic analyses of growth and development of extremophiles. *Plant Physiology* **135**: 1718–1737.

Ingrouille MJ, Smirnoff N. 1986. *Thlaspi caerulescens*. J. & C. Presl. (*T. alpestre* L.) in Britain *New Phytologist* **102**: 219–233.

Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. 2003. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **4**: 249–264.

Johnston WR, Proctor J. 1977. Comparative study of metal levels in plants from two contrasting lead-mine sites. *Plant and Soil* **46**: 251–257.

Katayama A, Tsujii A, Wada A, Nishino T, Ishihama A. 2002. Systematic search for zinc-binding proteins in *Escherichia coli*. *European Journal of Biochemistry* **269**: 2403–2413.

Kim D, Gustin JL, Lahner B, Persans MW, Baek D, Yun D-J, Salt DE. 2004. The plant CDF family member TgMTP1 from the Ni/Zn hyperaccumulator *Thlaspi goesingense* acts to enhance efflux of Zn at the plasma membrane when expressed in *Saccharomyces cerevisiae*. *Plant Journal* **39**: 237–251.

Klug A. 1999. Zinc finger peptides for the regulation of gene expression. *Journal of Molecular Biology* **293**: 215–218.

Knight B, Zhao FJ, McGrath SP, Shen ZG. 1997. Zinc and cadmium uptake by the hyperaccumulator *Thlaspi caerulescens* in contaminated soils and its effects on the concentration and chemical speciation of metals in soil solution. *Plant and Soil* **197**: 71–78.

Kobae Y, Uemura T, Sato MH, Ohnishi M, Mimura T, Nakagawa T, Maeshima M. 2004. Zinc transporter of *Arabidopsis thaliana* AtMTP1 is localized to vacuolar membranes and implicated in zinc homeostasis. *Plant and Cell Physiology* **45**: 1749–1758.

Koch M, Al-Shehbaz IA. 2004. Taxonomic and phylogenetic evaluation of the American '*Thlaspi*' species: identity and relationship to the Eurasian genus *Noccaea* (Brassicaceae). *Systematic Botany* **29**: 375–384.

Koch M, Mummenhoff K. 2001. *Thlaspi* s.str. (Brassicaceae) versus *Thlaspi* s.l. morphological and anatomical characters in the light of ITS nrDNA sequence data. *Plant Systematics and Evolution* **227**: 209–225.

Koch M, Mummenhoff K, Hurka H. 1998. Systematics and evolutionary history of heavy metal tyolerance *Thlaspi caerulescens* in Western Europe: evidence from genetic studies based on isozyme analysis. *Biochemical Systematics and Ecology* **26**: 823–838.

Krämer U. 2005a. Phytoremediation: novel approaches to cleaning up polluted soils. *Current Opinion in Biotechnology* **16**: 133–141.

Krämer U. 2005b. MTP1 mops up excess zinc in *Arabidopsis* cells. *Trends in Plant Science* **10**: 313–315.

Küpper H, Lombi E, Zhao F-J, McGrath SP. 2000. Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. *Planta* **212**: 75–84.

Küpper H, Mijovilovich A, Meyer-Klaucke W, Kroneck PMH. 2004. Tissue- and age-dependent differences in the complexation of cadmium and zinc in the cadmium/zinc hyperaccumulator *Thlaspi caerulescens* (Ganges ecotype) revealed by X-ray absorption spectroscopy. *Plant Physiology* **134**: 748–757.

Küpper H, Zhao FJ, McGrath SP. 1999. Cellular compartmentation of zinc in leaves of the hyperaccumulator *Thlaspi caerulescens*. *Plant Physiology* **119**: 305–311.

Lasat MM, Baker AJM, Kochian LV. 1996. Physiological characterization of root Zn^{2+} absorption and translocation to shoots in Zn hyperaccumulator and nonaccumulator species of *Thlaspi*. *Plant Physiology* **112**: 1715–1722.

Lasat MM, Kochian LV. 2000. Physiology of Zn hyperaccumulation in *Thlaspi caerulescens*. In: Terry N, Bañuelos G, eds. *Phytoremediation of contaminated soil and water*. Boca Raton, FL, USA: CRC Press LLC, 159–169.

Lehto NJ, Davison W, Zhang H, Tych W. 2006. Analysis of micro-nutrient behaviour in the rhizosphere using a DGT parameterised dynamic plant uptake model. *Plant and Soil* **282**: 227–238.

Lindsay WL. 1979. *Chemical equilibria in soils*. New York, NY, USA: John Wiley & Sons, Inc.

Lloyd-Thomas DH. 1995. Heavy metal hyperaccumulation by *Thlaspi caerulescens* J. & C. Presl. Sheffield, UK: University of Sheffield. *PhD thesis*.

Lombi E, Zhao FJ, McGrath SP, Young SD, Sacchi GA. 2001. Physiological evidence for a high-affinity cadmium transporter highly expressed in a *Thlaspi caerulescens* ecotype. *New Phytologist* **149**: 53–60.

López-Millán A-F, Ellis DR, Grusak MA. 2004. Identification and characterization of several new members of the ZIP family of metal ion transporters in *Medicago truncatula*. *Plant Molecular Biology* **54**: 583–596.

Ma JF, Ueno DF-J, Zhao McGrath SP. 2005. Subcellular localisation of Cd and Zn in the leaves of a Cd-hyperaccumulating ecotype of *Thlaspi caerulescens*. *Planta* **220**: 731–736.

Macnair MR. 1990. The genetics of metal tolerance in natural populations. In: Shaw AJ, ed. *Heavy metal tolerance in plants: evolutionary aspects*. Boca Raton, FL, USA: CRC Press, 235–253.

Macnair MR. 1993. The genetics of metal tolerance in vascular plants. *New Phytologist* **124**: 541–559.

Macnair MR. 2003. The hyperaccumulation of metals by plants. *Advances in Botanical Research* **40**: 63–105.

Macnair MR, Bert V, Huitson SB, Saumitou-Laprade P, Petit D. 1999. Zinc tolerance and hyperaccumulation are genetically independent characters. *Proceeedings of the Royal Society of London Series B-Biological Sciences* **266**: 2175–2179.

Maret W. 2005. Zinc coordination environments in proteins determine zinc functions. *Journal of Trace Elements in Medicine and Biology* **19**: 7–12.

Marquès L, Cossegal M, Bodin S, Czernic P, Lebrun M. 2004. Heavy metal specificity of cellular tolerance in two hyperaccumulating plants, *Arabidopsis halleri* and *Thlaspi caerulescens*. *New Phytologist* **164**: 289–295.

Marschner H. 1995. *Mineral nutrition of higher plants, 2nd edn*. London, UK: Academic Press.

Mäser P, Thomine S, Schroeder JI, Ward JM, Hirschi K, Sze H, Talke IN, Amtmann A, Maathuis FJM, Sanders D, Harper JF, TchieuJ, Gribskov M, Persans MW, Salt DE, Kim SA, Guerinot ML. 2001. Phylogenetic relationships within cation transporter families of *Arabidopsis*. *Plant Physiology* **126**: 1646–1667.

Mathys W. 1977. The role of malate, oxalate, and mustard oil glucosides in the evolution of zinc-resistance in herbage plants. *Physiologia Plantarum* **40**: 130–136.

Mazé P. 1915. Détermination des éléments minéraux rares nécessaires au développement du maïs. *Comptes Rendus Hebdomadaires des Séances de L'académie des Sciences* **160**: 211–214.

McIntosh KB, Bonham-Smith PC. 2006. Ribosomal protein gene regulation: what about plants? *Canadian Journal of Botany* **84**: 342–362.

Meerts P, Duchêne Ph, Gruber W, Lefèbvre C. 2003. Metal accumulation and competitive ability in metallicolous and non-metallicolous *Thlaspi caerulescens* fed with different Zn salts. *Plant and Soil* **249**: 1–8.

Meerts P, Van Isacker N. 1997. Heavy metal tolerance and accumulation in metallicolous and non-metallicolous populations of *Thlaspi caerulescens* from continental Europe. *Plant Ecology* **133**: 221–231.

Meyer FK. 1973. Conspectus der '*Thlaspi*'-arten Europas, Afrikas und Vorderasiens. *Feddes Repertorium* **84**: 449–470.

Meyer FK. 1979. Kritische Revision der '*Thlaspi*'-arten Europas, Afrikas und Vorderasiens. I. Geschichte, Morphologie und Chorologie. *Feddes Repertorium* **90**: 129–154.

Miller WP, McFee WW. 1983. Distribution of cadmium, zinc, copper, and lead in soils of industrial northwestern Indiana. *Journal of Environmental Quality* **12**: 29–33.

Mills RF, Krijger GC, Baccarini PJ, Hall JL, Williams LE. 2003. Functional expression of AtHMA4, a P_{1B}-type ATPase of the Zn/Co/Cd/Pb subclass. *Plant Journal* **35**: 164–176.

Mirouze M, Sels J, Richard O, Czernic P, Loubet S, Jacquier A, François IEJA, Cammue BPA, Lebrun M, Berthomieu P, Marquès L. 2006. A putative novel role for plant defensins: a defensin from the zinc hyper-accumulating plant, *Arabidopsis halleri*, confers zinc tolerance. *Plant Journal* **47**: 329–342.

Misson J, Thibaud M-C, Bechtold N, Raghothama K, Nussaume L. 2004. Transcriptional regulation and functional properties of *Arabidopsis* Pht1; 4, a high affinity transporter contributing greatly to phosphate uptake in phosphate deprived plants. *Plant Molecular Biology* **55**: 727–741.

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F. 2004. Reactive oxygen gene network of plants. *Trends in Plant Science* **9**: 490–498.

Molitor M, Dechamps C, Gruber W, Meerts P. 2005. *Thlaspi caerulescens* on nonmetalliferous soil in Luxembourg: ecological niche and genetic variation in mineral element composition. *New Phytologist* **165**: 503–512.

Moroney JV, Bartlett SG, Samuelsson G. 2001. Carbonic anhydrases in plants and algae. *Plant, Cell & Environment* **24**: 141–153.

Morris PC. 2001. MAP kinase signal transduction pathways in plants. *New Phytologist* **151**: 67–89.

van de Mortel JE, Villanueva LA, Schat H, Kwekkeboom J, Coughlan S, Moerland PD, van Themaat EVL, Koornneef M, Aarts MGM. 2006. Large expression differences in genes for iron and zinc homeostasis, stress response, and lignin biosynthesis distinguish roots of *Arabidopsis thaliana* and the related metal hyperaccumulator *Thlaspi caerulescens*. *Plant Physiology* **142**: 1127–1147.

Mullins GL, Sommers LE. 1986. Cadmium and zinc influx characteristics by intact corn (*Zea mays* L.) seedlings. *Plant and Soil* **96**: 153–164.

Mummenhoff K, Franzke A, Koch M. 1997. Molecular data reveal convergence in fruit characters used in the classification of *Thlaspi* s.l (Brassicaceae). *Botanical Journal of the Linnean Society* **125**: 183–199.

Mummenhoff K, Koch M. 1994. Chloroplast DNA restriction site variation and phylogenetic relationships in the genus *Thlaspi* sensu lato (Brassicaceae). *Systematic Botany* **19**: 73–88.

Nicolls OW, Provan DMJ, Cole MM, Tooms JS. 1965. Geobotany and geochemistry in mineral exploration in the Dugald River Area, Cloncurry District. *Australia Transactions of the Institution of Mining and Metallurgy* **74**: 695–799.

Nriagu JO. 1996. A history of global metal pollution. *Science* **272**: 223–224.

Olczak M, Morawiecka B, Watorek W. 2003. Plant purple acid phosphatases – genes, structures and biological function. *Acta Biochimica Polonica* **50**: 1245–1256.

Outten CE, O'Halloran TV. 2001. Femtomolar sensitivity of metalloregulatory proteins controlling zinc homeostasis. *Science* **292**: 2488–2492.

Papoyan A, Kochian LV. 2004. Identification of the *Thlaspi caerulescens* genes that may be involved in heavy metal hyperaccumulation and tolerance. Characterization of a novel heavy metal transporting ATPase. *Plant Physiology* **136**: 3814–3823.

Paton A, Brooks RR. 1996. A re-evaluation of *Haumaniastrum* species as geobotanical indicators of copper and cobalt. *Journal of Geochemical Exploration* **56**: 37–45.

Peer WA, Mamoudian M, Lahner B, Reeves RD, Murphy AS, Salt DE. 2003. Identifying model metal hyperaccumulating plants: germplasm analysis of 20 Brassicaceae accessions from a wide geographical area. *New Phytologist* **159**: 421–430.

Pence NS, Larsen PB, Ebbs SD, Letham DLD, Lasat MM, Garvin DF, Eide D, Kochian LV. 2000. The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. *Proceedings of the National Academy of Sciences, USA* **97**: 4956–4960.

Persans MW, Nieman K, Salt DE. 2001. Functional activity and role of cation-efflux family members in Ni hyperaccumulation in *Thlaspi goesingense*. *Proceedings of the National Academy of Sciences, USA* **98**: 9995–10000.

Piñeros MA, Shaff JE, Kochian LV. 1998. Development, characterization, and application of a cadmium-selective microelectrode for the measurement of cadmium fluxes in roots of *Thlaspi* species and wheat. *Plant Physiology* **116**: 1393–1401.

Pollard AJ, Baker AJM. 1996. Quantitative genetics of zinc hyperaccumulation in *Thlaspi caerulescens*. *New Phytologist* **132**: 113–118.

Pollard AJ, Powell KD, Harper FA, Smith JAC. 2002. The genetic basis of metal hyperaccumulation in plants. *Critical Reviews in Plant Sciences* **21**: 539–566.

Qian XD, Eguchi T, Yoshida S, Chikushi J. 2005. Analytical model for zinc uptake by root system of *Thlaspi caerulescens*. *Journal of the Faculty of Agriculture, Kyushu University* **50**: 443–458.

Reeves RD. 1988. Nickel and zinc accumulation by species of *Thlaspi* L., *Cochlearia* L. & other genera of the Brassicaceae. *Taxon* **37**: 309–318.

Reeves RD, Baker AJM. 1984. Studies on metal uptake by plants from serpentine and non-seprentine populations of *Thlaspi goesingense* Hálácsy (Cruciferae). *New Phytologist* **98**: 191–204.

Reeves RD, Baker AJM. 2000. Metal-accumulating plants. In: Raskin I, Ensley BD, eds. *Phytoremediation of toxic metals: using plants to clean up the environment*. New York, NY, USA: John Wiley & Sons, 193–229.

Reeves RD, Brooks RR. 1983. European species of *Thlaspi* L. (Cruciferae) as indicators of nickel and zinc. *Journal of Geobotanical Exploration* **18**: 275–283.

Reeves RD, Macfarlane RM, Brooks RR. 1983. Accumulation of nickel and zinc by western North American genera containing serpentine-tolerant species. *American Journal of Botany* **70**: 1297–1303.

Reeves RD, Schwartz C, Morel JL, Edmondson J. 2001. Distribution and metal-accumulating behaviour of *Thlaspi caerulescens* and associated metallophytes in France. *International Journal of Phytoremediation* **3**: 145–172.

Regvar M, Vogel K, Irgel N, Wraber T, Hildebrandt U, Wilde P, Bothe H. 2003. Colonization of pennycresses (*Thlaspi* spp.) of the Brassicaceae by arbuscular mycorrhizal fungi. *Journal of Plant Physiology* **160**: 615–626.

Reid RJ, Brookes JD, Tester MA, Smith FA. 1996. The mechanism of zinc uptake in plants. Characterisation of the low-affinity system. *Planta* **198**: 39–45.

Rengel Z. 2001. Genotypic differences in micronutrient use efficiency in crops. *Communications in Soil Science and Plant Analysis* **32**: 1163–1186.

Rengel Z, Wheal MS. 1997. Kinetic parameters of Zn uptake by wheat are affected by the herbicide chlorosulfuron. *Journal of Experimental Botany* **48**: 935–941.

Reyes JC, Muro-Pastor MI, Florencio FJ. 2004. The GATA family of transcription factors in Arabidopsis and rice. *Plant Physiology* **134**: 1718–1732.

Rigola D, Fiers M, Vurro E, Aarts MGM. 2006. The heavy metal hyperaccumulator *Thlaspi caerulescens* expresses many species-specific genes, as identified by comparative expressed sequence tag analysis. *New Phytologist* **170**: 753–766.

Riley R. 1956. The influence of the breeding system on the genecology of *Thlaspi alpestre* L. *New Phytologist* **55**: 319–330.

Robson F, Costa MMR, Hepworth SR, Vizir I, Pineiro M, Reeves PH, Putterill J, Coupland G. 2001. Functional importance of conserved domains in the flowering-time gene CONSTANS demonstrated by analysis of mutant alleles and transgenic plants. *Plant Journal* **28**: 619–631.

Roosens NH, Leplae R, Bernard C, Verbruggen N. 2005. Variations in plant metallothioneins: the heavy metal hyperaccumulator *Thlaspi caerulescens* as a study case. *Planta* **222**: 716–729.

Roosens NH, Verbruggen N, Meerts P, Ximénez-Embún P, Smith JAC. 2003. Natural variation in cadmium tolerance and its relationship to metal hyperaccumulation for seven populations of *Thlaspi caerulescens* from western Europe. *Plant, Cell & Environment* **26**: 1657–1672.

Saito K, Murai J, Kajiho H, Kontani K, Kurosu H, Katada T. 2002. A novel binding protein composed of homophilic tetramer exhibits unique properties for the small GTPase Rab5. *Journal of Biological Chemistry* **277**: 3412–3418.

Sakamoto H, Maruyama K, Sakuma Y, Meshi T, Iwabuchi M, Shinozaki Yamaguchi-Shinozaki K. 2004. Arabidopsis Cys2/His2-type zinc-finger proteins function as transcription repressors under drought, cold, and high-salinity stress conditions. *Plant Physiology* **136**: 2734–2746.

Salt DE, Prince RC, Baker AJM, Raskin I, Pickering IJ. 1999. Zinc ligands in the metal hyperaccumulator *Thlaspi caerulescens* as determined using X-ray absorption spectroscopy. *Environmental Science and Technology* **33**: 713–717.

Salt DE, Smith RD, Raskin I. 1998. Phytoremediation. *Annual Review of Plant Physiology and Plant Molecular Biology* **49**: 643–668.

Santa-Maria GE, Cogliatti DH. 1998. The regulation of zinc uptake in wheat plants. *Plant Science* **137**: 1–12.

Sarret G, Saumitou-Laprade P, Bert V, Proux O, Hazemann J-L, Traverse A, Marcus MA, Manceau A. 2002. Forms of zinc accumulated in the hyperaccumulator *Arabidopsis halleri*. *Plant Physiology* **130**: 1815–1826.

Sattelmacher B. 2001. The apoplast and its significance for plant mineral nutrition. *New Phytologist* **149**: 167–192.

Scaife A, Turner M. 1983. Diagnosis of mineral disorders in plants, 2. Vegetables London, UK: Her Majesty's Stationary Office.

Schat H, Llugany M, Vooijs R, Hartley-Whitaker J, Bleeker PM. 2002. The role of phytochelatins in constitutive and adaptive heavy metal tolerances in hyperaccumulator and non-hyperaccumulator metallophytes. *Journal of Experimental Botany* **53**: 2381–2392.

Schat H, Vooijs R. 1997. Multiple tolerance and co-tolerance to heavy metals in *Silene vulgaris*: a co-segregation analysis. *New Phytologist* **136**: 489–496.

Schat H, Vooijs R, Kuiper E. 1996. Identical major gene loci for heavy metal tolerances that have independently evolved in different local populations and subspecies of *Silene vulgaris*. *Evolution* **50**: 1888–1895.

Schmid WE, Haag HP, Epstein EE. 1965. Absorption of zinc by excised barley roots. *Physiologia Plantarum* **18**: 860–869.

Schwartz C, Morel JL, Saumier S, Whiting SN, Baker AJM. 1999. Root development of the zinc-hyperaccumulator plant *Thlaspi caerulescens* as affected by metal origin, content and localization in the soil. *Plant and Soil* **208**: 103–115.

Searcy KB, Mulcahy DL. 1985a. Pollen-tube competition and selection for metal tolerance in *Silene dioica* (Caryophyllaceae) and *Mimulus guttatus* (Scrophulariaceae). *American Journal of Botany* **72**: 1695–1699.

Searcy KB, Mulcahy DL. 1985b. Pollen selection and the gametophytic expression of metal tolerance in *Silene dioica* (Caryophyllaceae) and *Mimulus guttatus* (Scrophulariaceae). *American Journal of Botany* **72**: 1700–1706.

Searcy KB, Mulcahy DL. 1985c. The parallel expression of metal tolerance in pollen and sporophytes of *Silene dioica* (L.) Clairv, Silene alba (Mill) Krause and *Mimulus guttatus* DC. *Theoretical and Applied Genetics* **69**: 597–602.

Sharma CP. 2006. *Plant micronutrients*. Enfield, NH, USA: Science Publishers.

Shen ZG, Zhao FJ, McGrath SP. 1997. Uptake and transport of zinc in the hyperaccumulator *Thlaspi caerulescens* and the non-hyperaccumulator *Thlaspi ochroleucum*. *Plant, Cell & Environment* **20**: 898–906.

Skoog F. 1940. Relationships between zinc and auxin in the growth of higher plants. *American Journal of Botany* **27**: 939–951.

Smith RAH, Bradshaw AD. 1979. The use of metal tolerant plant populations for the reclamation of metalliferous wastes. *Journal of Applied Ecology* **16**: 595–612.

Sommer AL, Lipman CB. 1926. Evidence on the indispensable nature of zinc and boron for higher green plants. *Plant Physiology* **1**: 231–249.

Symeonidis L, McNeilly T, Bradshaw AD. 1985. Differential tolerance of three cultivars of *Agrostis capillaris* L. to cadmium, copper, lead, nickel and zinc. *New Phytologist* **101**: 309–315.

Talke IN, Hanikenne M, Krämer U. 2006. Zn-dependent global transcriptional control, transcriptional de-regulation and higher gene copy number for genes in metal homeostasis of the hyperaccumulator *Arabidopsis halleri*. *Plant Physiology* **142**: 148–167.

Taylor SI. 2004. Evolution of zinc hyperaccumulation in *Thlaspi*. Exeter, UK: University of Exeter. *PhD thesis*.

Taylor SI, Macnair MR. 2006. Within and between population variation for znic and nickel accumulation in two species of *Thlaspi* (Brassicaceae). *New Phytologist* **169**: 505–514.

van Tieghem, Ph. 1887. Réseau sus-endodermique de la racine des Cruciférés. *Bulletin de la Societé botanique de France*. Séance du 25 Mars 1887. pp. 125–131.

Tiwari A, Kumar P, Singh S, Ansari SA. 2005. Carbonic anhydrase in relation to higher plants. *Photosynthetica* **43**: 1–11.

Tolrà RP, Poschenrieder C, Barceló J. 1996. Zinc hyperaccumulation in *Thlaspi caerulescens*. II. Influence on organic acids. *Journal of Plant Nutrition* **19**: 1541–1550.

Tuomainen MH, Nunan N, Lehesranta SJ, Tervahauta AI, Hassinen VH, Schat H, Koistinen KM, Auriola S, McNicol J, Kärenlampi SO. 2006. Multivariate analysis of protein profiles of metal hyperaccumulator *Thlaspi caerulescens* accessions. *Proteomics* **6**: 3696–3706.

Ülker B, Somssich IE. 2004. WRKY transcription factors: from DNA binding towards biological function. *Current Opinion in Plant Biology* **7**: 491–498.

Vázquez MD, Barceló J, Poschenrieder C *et al.* 1992. Localization of zinc and cadmium in *Thlaspi caerulescens* (Brassicaceae), a metallophyte that can hyperaccumulate both metals. *Journal of Plant Physiology* **140**: 350–355.

Vázquez MD, Poschenrieder Ch, Barceló J, Baker AJM, Hatton P, Cope GL. 1994. Compartmentation of zinc in roots and leaves of the zinc hyperaccumulator *Thlaspi caerulescens* J & C Presl. *Botanica Acta* **107**: 243–250.

Veltrup W. 1978. Characteristics of zinc uptake by barley roots. *Physiologia Plantarum* **42**: 190–194.

Verkleij JAC, Koevoets PLM, Blake-Kalff MMA, Chardonnens AN. 1998. Evidence for an important role of the tonoplast in the mechanism of naturally selected zinc tolerance in *Silene vulgaris*. *Journal of Plant Physiology* **153**: 188–191.

Verret F, Gravot A, Auroy P, Leonhardt N, David P, Nussaume L, Vavasseur A, Richaud P. 2004. Overexpression of AtHMA4 enhances root-to-shoot translocation of zinc and cadmium and plant metal tolerance. *FEBS Letters* **576**: 306–312.

Verret F, Gravot A, Auroy P, Preveral S, Forestier C, Vavasseur A, Richaud P. 2005. Heavy metal transport by AtHMA4 involves the N-terminal degenerated metal binding domain and the C-terminal His_{11} stretch. *FEBS Letters* **579**: 1515–1522.

Vogel-Mikuš K, Drobne D, Regvar M. 2005. Zn, Cd and Pb accumulation and arbuscular mycorrhizal colonisation of pennycress *Thlaspi praecox* Wulf. (Brassicaceae) from the vicinity of a lead mine and smelter in Slovenia. *Environmental Pollution* **133**: 233–242.

Vreugdenhil D, Aarts MGM, Koornneef M. 2005. Exploring natural genetic variation to improve plant nutrient content. In: Broadley MR, White PJ, eds. *Plant nutritional genomics*. Oxford, UK: Blackwell Publishing, 201–219.

Vreugdenhil D, Aarts MGM, Koornneef M, Nelissen H, Ernst WHO. 2004. Natural variation and QTL analysis for cationic mineral content in seeds of *Arabidopsis thaliana*. *Plant, Cell & Environment* **27**: 828–839.

Webb EC. 1992. *Enzyme nomenclature, recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology*. New York, NY, USA: Academic Press.

Weber M, Harada E, Vess C, van Roepenack-Lahaye E, Clemens S. 2004. Comparative microarray analysis of *Arabidopsis thaliana* and *Arabidopsis halleri* roots identifies nicotianamine synthase, a ZIP transporter and other genes as potential metal hyperaccumulation factors. *Plant Journal* **37**: 269–281.

Weber M, Trampczynska A, Clemens S. 2006. Comparative transcriptome analysis of toxic metal responses in *Arabidopsis thaliana* and the Cd^{2+}-hypertolerant facultative metallophyte *Arabidopsis halleri*. *Plant, Cell & Environment* **29**: 950–963.

Weiss DJ, Mason TFD, Zhao FJ, Kirk GJD, Coles BJ, Horstwood MSA. 2005. Isotopic discrimination of zinc in higher plants. *New Phytologist* **165**: 703–710.

Welch RM, Graham RD. 2004. Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany* **55**: 353–364.

Wheal M, Rengel Z. 1997. Chlorsulfuron reduces rates of zinc uptake by wheat seedlings from solution culture. *Plant and Soil* **188**: 309–317.

White PJ. 1998. Calcium channels in the plasma membrane of root cells. *Annals of Botany* **81**: 173–183.

White PJ. 2001. The pathways of calcium movement to the xylem. *Journal of Experimental Botany* **52**: 891–899.

White PJ, Bowen HC, Demidchik V, Nichols C, Davies JA. 2002a. Genes for calcium-permeable channels in the plasma membrane of plant root cells. *Biochimica et Biophysica Acta-Biomembranea* **1564**: 299–309.

White PJ, Broadley MR. 2005. Biofortifying crops with essential mineral elements. *Trends in Plant Science* **10**: 586–593.

White MC, Chaney RL, Decker AM. 1979a. Differential cultivar tolerance in soybean to phytotoxic levels of soil Zn. 2. Range of Zn additions and the uptake and translocation of Zn, Mn, Fe, and P. *Agronomy Journal* **71**: 126–131.

White MC, Chaney RL, Decker AM. 1979b. Role of roots and shoots of soybean in tolerance to excess soil zinc. *Crop Science* **19**: 126–128.

White MC, Decker AM, Chaney RL. 1979c. Differential cultivar tolerance in soybean to phytotoxic levels of soil Zn. 1. Range of cultivar response. *Agronomy Journal* **71**: 121–126.

White PJ, Whiting SN, Baker AJM, Broadley MR. 2002b. Does zinc move apoplastically to the xylem in roots of *Thlaspi caerulescens*? *New Phytologist* **153**: 201–207.

White JG, Zasoski RJ. 1999. Mapping soil micronutrients. *Field Crops Research* **60**: 11–26.

Whiting SN, Broadley MR, White PJ. 2003. Applying a solute transfer model to phytoextraction: Zinc acquisition by *Thlaspi caerulescens*. *Plant and Soil* **249**: 45–56.

Whiting SN, Leake JR, McGrath SP, Baker AJM. 2000. Positive responses to Zn and Cd by roots of the Zn and Cd hyperaccumulator *Thlaspi caerulescens*. *New Phytologist* **145**: 199–210.

Whiting SN, Reeves RD, Richards DG, Johnson MS, Cooke JA, Malaisse F, Paton A, Smith JAC, Angle JS, Chaney RL, Ginocchio R, Jaffré T, Johns B, McIntyre T, Purvis OW, Salt DE, Schat H, Zhao FJ, Baker AJM. 2005. Use of plants to manage sites contaminated with metals. In: Broadley MR, White PJ, eds. *Plant nutritional genomics*. Oxford, UK: Blackwell Publishing, 287–315.

Williams LE, Mills RF. 2005. P_{1B}-ATPases – an ancient family of transition metal pumps with diverse functions in plants. *Trends in Plant Science* **10**: 491–502.

Williams LE, Pittman JK, Hall JL. 2000. Emerging mechanisms for heavy metal transport in plants. *Biochimica et Biophysica Acta-Biomembranea* **1465**: 104–126.

Wissuwa M, Ismail AM, Yanagihara S. 2006. Effects of zinc deficiency on rice growth and genetic factors contributing to tolerance. *Plant Physiology* **142**: 731–741.

Wójcik M, Skórzyńska-Polit E, Tukiendorf A. 2006. Organic acids accumulation and antioxidant enzyme activities in *Thlaspi caerulescens* under Zn and Cd stress. *Plant Growth Regulation* **48**: 145–155.

Wu L, Antonovics J. 1975. Zinc and copper uptake by *Agrostis stolonifera*, tolerant to both zinc and copper. *New Phytologist* **75**: 231–237.

Yamasaki K, Kigawa T, Inoue M, Tateno M, Yamasaki T, Yabuki T, Aoki M, Seki E, Matsuda T, Nunokawa E, Ishizuka Y, Terada T, Shirouzu M, Osanai T, Tanaka A, Seki M, Shinozaki K, Yokoyama S. 2004. A novel zinc-binding motif revealed by solution structures of DNA-binding domains of Arabidopsis SBP-family transcription factors. *Journal of Molecular Biology* **337**: 49–63.

Yanagisawa S. 2004. Dof domain proteins: plant-specific transcription factors associated with diverse phenomena unique to plants. *Plant and Cell Physiology* **45**: 386–391.

Yang X, Long XX, Ni WZ, Fu CX. 2002. *Sedum alfredii* H: a new Zn hyperaccumulating plant first found in China. *Chinese Science Bulletin* **47**: 1634–1637.

Young SD, Zhang H, Tye AM, Maxted A, Thums C, Thornton I. 2006. Characterizing the availability of metals in contaminated soils. I. The solid phase: sequential extraction and isotopic dilution. *Soil Use and Management* **21**: 450–458.

van der Zaal BJ, Neuteboom LW, Pinas JE, Chardonnens AN, Schat H, Verkleij JAC, Hooykaas PJJ. 1999. Overexpression of a novel *Arabidopsis* gene related to putative zinc-transporter genes from animals can lead to enhanced zinc resistance and accumulation. *Plant Physiology* **119**: 1047–1055.

Zelko I, Lux A, Czibula K. in press. Difference in the root structure of hyperaccumulator *Thlaspi caerulescens* and non-hyperaccumulator *Thlaspi arvense. International Journal of Environment and Pollution.*

Zha HG, Jiang RF, Zhao FJ, Vooijs R, Schat H, Barker JHA, McGrath SP. 2004. Co-segregation analysis of cadmium and zinc accumulation in *Thlaspi caerulescens* interecotypic crosses. *New Phytologist* **163**: 299–312.

Zhang YJ, Wang LJ. 2005. The WRKY transcription factor superfamily: its origin in eukaryotes and expansion in plants. *BMC Evolutionary Biology* **5**: 1.

Zhang H, Young SD. 2006. Characterizing the availability of metals in contaminated soils. II. The soil solution. *Soil Use and Management* **21**: 459–467.

Zhao FJ, Hamon RE, Lombi E, McLaughlin MJ, McGrath SP. 2002. Characteristics of cadmium uptake in two contrasting ecotypes of the hyperaccumulator *Thlaspi caerulescens. Journal of Experimental Botany* **53**: 535–543.

Zhao FJ, Lombi E, Breedon T, McGrath SP. 2000. Zinc hyperaccumulation and cellular distribution in *Arabidopsis halleri. Plant, Cell & Environment* **23**: 507–514.

Zhao FJ, Shen ZG, McGrath SP. 1998. Solubility of zinc and interactions between zinc and phosphorus in the hyperaccumulator *Thlaspi caerulescens. Plant, Cell & Environment* **21**: 108–114.

Zimeri AM, Dhankher OP, McCaig B, Meagher RB. 2005. The plant MT1 metallothioneins are stabilized by binding cadmiums and are required for cadmium tolerance and accumulation. *Plant Molecular Biology* **58**: 839–855.

Supplementary Material

The following supplementary material is available for this article online:

Table S1 Two thousand and forty-two *Arabidopsis thaliana* proteins (TAIR6) containing one or more of the 120 putative domains with observed or predicted capabilities for binding Zn, identified from the Pfam database (http://www.sanger.ac.uk/Software/Pfam/ 25 July 2006)

Table S2 One thousand two hundred and forty-five *Arabidopsis thaliana* proteins (TAIR6) containing the words 'zinc' or 'Zn' in their annotation and corrected for false positives

Table S3 One thousand six hundred and thiry-five *Arabidopsis thaliana* proteins (TAIR6) implicated in Zn homeostasis, as compiled by hand

Table S4 Two thousand three hundred and sixty-seven unique *Arabidopsis thaliana* proteins (TAIR6) contained in Tables S1–S3

Table S5 One hundred and eighty-one gene families represented in Table S4

Table S6 Zinc concentrations reported in leaf or nonwoody shoot tissues from 1108 studies, contained in 204 published papers and three unpublished datasets. These primary data define the largest set of interlinked studies, in which $[Zn]_{shoot}$ are reported for more than two species in controlled experiments where each study contains more than one species common to another study (3873 data points)

Table S7 As Table S6, but with dataset modified to exclude *Thlaspi* and *Arabidopsis* species to remove the influence of Zn hyperaccumulation on the analysis (3706 data points)

Table S8 Literature sources used to compile Tables S6 and S7

Table S9 Variance components analysis output and $\log_e$ shoot Zn concentrations for species, family and key clades of angiosperms. Mean data for phylogenetic groups were estimated after excluding *Thlaspi* and *Arabidopsis* to remove the possible influence of Zn hyperaccumulation on the analysis

Table S10 Sixty genes whose expression differs significantly between hyperaccumulators and nonhyperaccumulators (based on a re-analysis of five *Thlaspi caerulescens*, eight *Arabidopsis halleri*, five *T. arvense*, six *A. lyrata* ssp. *petraea*, and two *A. thaliana* GeneChip arrays)

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