Metal Hyperaccumulation in Plants

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Abstract

During the history of life on Earth, tectonic and climatic change repeatedly generated large territories that were virtually devoid of life and exhibited harsh environmental conditions. The ability of a few specialist pioneer plants to colonize such hostile environments was thus of paramount ecological importance for the continuous maintenance of primary production over time. Yet, we know very little about how extreme traits evolve and function in plants. Recent breakthroughs have given first insights into the molecular basis underlying the complex extreme model trait of metal hyperaccumulation and associated metal hypertolerance. This review gives an introduction into the hyperaccumulator research field and its history; provides an overview of hyperaccumulator germplasm; describes the state of the art of our understanding of the physiological, molecular, and genetic basis underlying metal hyperaccumulation and its evolution; and highlights future research needs and opportunities.

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INTRODUCTION

Every day, we take pleasure in the overwhelming diversity of the plants around us—this is as much about our aesthetic nature as it is an assertion of the prerequisites of the survival of the human species. Plants have evolved a multitude of life forms and, though somewhat less visible, of physiological realizations. Over the past 20 years, our fundamental molecular and functional understanding of plants has been revolutionized, taking advantage of a focus on a few model plants. Recent advances in sequencing technologies, bioinformatics, and molecular biology are progressively allowing researchers to expand their scope into the phylogenetic neighborhood of the classical model organisms and to address the adaptive relevance, molecular basis, and evolution of natural phenotypic diversity. These developments are likely to bring about a transformation of our understanding of ecology and evolution in a time characterized by accelerating anthropogenic global change. This review focuses on naturally selected metal hyperaccumulation and associated hypertolerance as emerging model traits in this expanding research field.

Nickel (Ni), zinc (Zn), cadmium (Cd): used here to refer to oxidation state +II of these elements, which is the biologically relevant oxidation state for these transition metals in plants

Metal excluder: a plant pursuing the metal tolerance strategy of restricting metal movement into shoots

In a few plant taxa, the concentrations accumulated in aboveground biomass of the transition metals or metalloids Ni, Zn, Cd, Se, As, Mn, Co, Cu, Pb, Sb, or Tl are more than one, and up to four, orders of magnitude higher than in other adjacent plants (**Table 1**) (6, 88). Such a unique extent of accumulation of potentially toxic trace elements, for which concentrations can approach and even exceed the concentrations of the macronutrient cations K and Ca, has been reported in a total of approximately 500 plant taxa (~0.2% of all angiosperms) to date (6, 88). Inevitably, metal[1] hyperaccumulation is associated with a strongly enhanced ability to detoxify the metal accumulated in aboveground tissues, and thus with metal hypertolerance.

Begining in the 1950s, the first extensive studies of the vegetation on metal-contaminated soils raised some excitement among ecologists because of the very high selection pressures at these sites and the apparent rapidity of evolution of metal hypertolerant races, e.g., of the grasses *Festuca ovina* and *Agrostis tenuis* (1). This led Antonovics et al. (1) to conclude: “Heavy metal tolerance in plants is probably an example of more powerful evolution in action than industrial melanism in moths” (p. 30). In subsequent years, e.g., individuals of *Silene vulgaris* from nontolerant populations were compared to individuals collected from local populations on differently contaminated soils exhibiting Cu, Ni, Zn, Cd, or Co hypertolerance, respectively (96, 97). Hypertolerance to one particular metal was found to have arisen repeatedly in *S. vulgaris* and to be governed by only a few major loci, as in metal-hypertolerant races of several other species (70). All of these metal-hypertolerant plants pursue the most widespread metal excluder strategy of metal tolerance (5), i.e., physiological tolerance mechanisms act to minimize metal accumulation in aboveground tissues. Ni hyperaccumulation, which was first discovered in 1948 in *Alyssum bertolonii*/Brassicaceae (75), and Zn hyperaccumulation, first discovered in

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[1] For simplicity, the generic term metal here includes the metalloids and semimetals As, Se, and Sb.

Table 1 Hyperaccumulation of trace elements in land plants

Element	Critical deficiency level[1] (μg g^{-1})	Critical toxicity level[1] (μg g^{-1})	Hyperaccumulation concentration criterion (μg g^{-1}): To date	Hyperaccumulation concentration criterion (μg g^{-1}): Newly suggested[2]	Taxa (No.)	Families (No.)
Antimony	n. r.	<2	>1000		2	2
Arsenic	n. r.	<2–80	>1000		15	2
Cadmium	n. r.	6–10	>100		5	2
Cobalt	n. r.	0.4–several	>1000	>300	(26)[3]	(11)
Copper	1–5	20–30	>1000	>300	(35)	(15)
Lead	n. r.	0.6–28	>1000		(14)	(7)
Manganese	10–20	200–3500	>10,000		10	6
Nickel	0.002–0.004	10–50	>1000		390	42
Selenium	n. r.	3–100	>1000		20	7
Thallium	n. r.	20	>1000		1	1
Zinc	15–20	100–300	>10,000	>3000	15	6

[1] From (10, 40, 44, 72).

[2] This refers to metal concentrations detected in two or more specimens collected at their natural sites of growth. The adoption of this definition would require a reassessment of a number of species, including those designated metal hyperaccumulators based on single specimens.

[3] Parentheses indicate that contamination with soil particles or minerals is likely to have resulted in overreporting.

Abbreviation: n. r., no known requirement.

Updated from A. J. M. Baker, personal communication, and from (105).

1865 in *Noccaea caerulescens* (formerly, *Thlaspi caerulescens*)/Brassicaceae (8, 88, 93), began to attract increasing attention in the early 1990s as incidences of the alternative metal accumulator strategy. One reason for this was the very high level of metal hypertolerance observed in hyperaccumulator species. Because of their close phylogenetic relationship with *Arabidopsis thaliana*, the Zn/Cd hyperaccumulator species *Arabidopsis halleri* and *N. caerulescens* have been most successfully used in advancing our molecular understanding of metal hyperaccumulation and hypertolerance.

Metal hyperaccumulation and associated hypertolerance are naturally selected, extreme, complex physiological model traits, which are easy to score. In addition, soil metal concentrations—a proxy of the primary relevant selective pressure on metalliferous soils—are comparably persistent over time and quantitatively accessible through simple analytical techniques.

A further important motivation fueling research on metal (hyper)accumulators has been the intention to use these plants, or the molecular mechanisms operating therein, for the development of phytoremediation, phytomining, or bio-fortification technologies, or for the improvement of crop nutrient efficiency (19). Researchers interested in plant metal homeostasis take advantage of metal hyperaccumulators to reveal key positions of the metal homeostasis network that, when altered, lead to major changes in metal accumulation. Metal hyperaccumulators will be instrumental in the development of systems biology approaches towards an integrated understanding of plant metal homeostasis.

Metal hypertolerance is an example of an extreme abiotic stress resistance trait. However, the selective advantage of metal hyperaccumulation, in excess of physiological needs, has remained elusive (16). Most likely, metal hyperaccumulation acts as an elemental defense against herbivory and/or pathogen attack (15, 31). Future research can take advantage of the pronounced accessibility of links between biotic stresses and metal homeostasis in metal hyperaccumulators. These links are likely to be of broad relevance in plants in general.

Metal (hyper)accumulator: a plant accumulating metals primarily in the shoots and maintaining low metal concentrations in the roots

Metal homeostasis network: the complex, tightly controlled functional network orchestrating delivery of the correct amount of the correct micronutrient metal to each metal-requiring protein at the correct time while avoiding metal overaccumulation in sensitive locations

Finally, metals such as Cd and As are known as potent toxins and carcinogens in humans and animals and, as pollutants that accumulate throughout the food chain, affect rapidly increasing numbers of people (71, 74). A molecular understanding of the highly effective detoxification machinery that allows the survival of hyperaccumulators at uniquely high internal metal concentrations may facilitate the discovery of remedies for the debilitating effects of metal pollution on human health.

METAL HYPERACCUMULATORS IN THE BRASSICACEAE

Metal hyperaccumulation has evolved multiple times (**Table 1**) and is of particularly high occurrence in the Brassicaceae family (**Figure 1**).

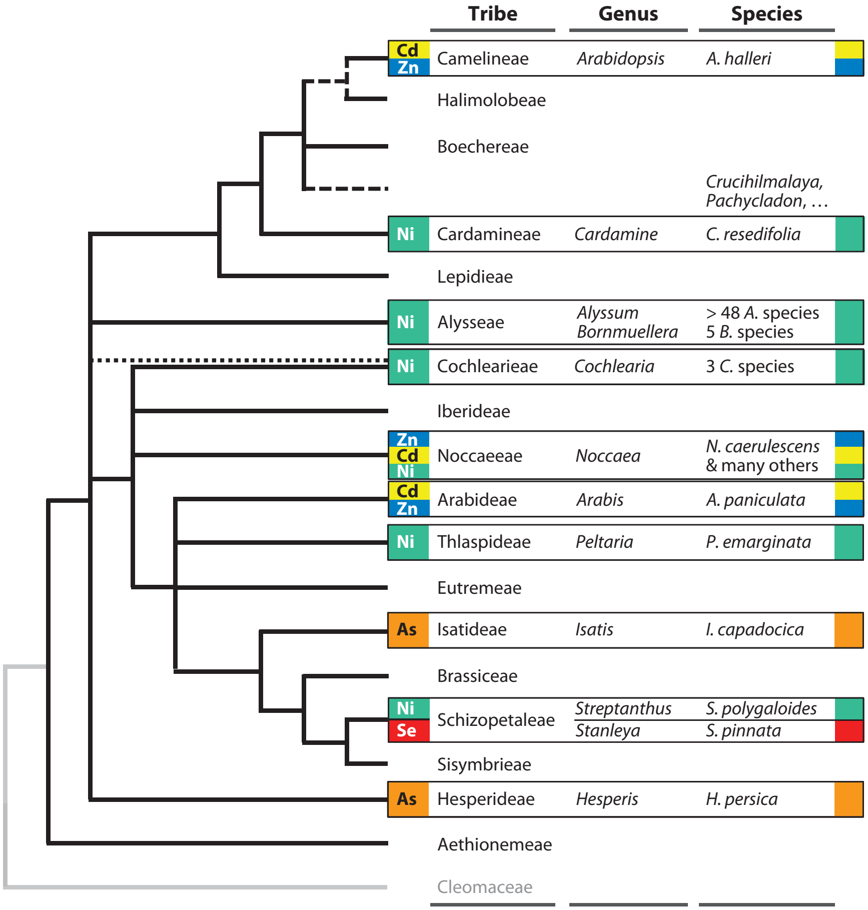

Figure 1

Occurrence of metal hyperaccumulation at the tribe level in the Brassicaceae family overlaid on the current hypothesis of phylogenetic relationships. The occurrence of metal hyperaccumulation (7, 88, 100) in distinct tribes of the Brassicaceae is an estimate of the minimum number of times metal hyperaccumulation has independently evolved in this family. The tree was redrawn in a much simplified form based on (48). *Alyssum* Ni hyperaccumulators are almost exclusively in the section Odontarrhena. *N. caerulescens* and *N. praecox* are the two Zn/Cd hyperaccumulator *Noccaea* species (88, 106). At least 4 additional *Noccaea* species are hyperaccumulators of Zn. *N. caerulescens*, along with *N. goesingense* and at least 21 additional *Noccaea* taxa, are considered Ni hyperaccumulators. Some of the *Noccaea* hyperaccumulators may in fact belong to other genera. *Sedum alfredii* (Crassulaceae) is the only known Cd hyperaccumulator outside the Brassicaceae (21).

It will soon become feasible to compare the genomic changes underlying independent incidences of the evolution of metal hyperaccumulation in different Brassicaceae species. Thus, metal hyperaccumulators could become important models for comparative genomics of adaptation.

The prevalence of the hyperaccumulation of Ni (**Table 1**) coincides with a large number of geogenically Ni-enriched ultramafic (serpentine) outcrops worldwide and in particular, e.g., in the Mediterranean region and in New Caledonia. Containing approximately 25% of the known Ni hyperaccumulator species each, the Euphorbiaceae and Brassicaceae families contribute by far the largest number of Ni hyperaccumulators (A. J. M. Baker, personal communication). Ni hyperaccumulation has evolved independently at least six times in the Brassicaceae (**Figure 1**), most likely on ultramafic soils.

Among the known Zn hyperaccumulators, a majority of nine species belong to the Brassicaceae family (**Table 1**), representing at least three independent incidences of the evolution of Zn hyperaccumulation (**Figure 1**). Zn hyperaccumulator species were identified on anthropogenically contaminated soils around historical and contemporary mine sites. Several Zn hyperaccumulator species were also reported to hyperaccumulate Cd and/or Pb, both of which are related to Zn in their chemical properties. The three metals often occur together in minerals, and hyperaccumulation and hypertolerance mechanisms of Zn and Cd involve overlapping sets of loci (20, 22, 38, 110). Zn/Cd hyperaccumulation might have evolved in rare and small areas of calamine outcrops, where Zn/Cd/Pb-rich minerals reach the surface, before the onset of anthropogenic metal pollution. Alternatively, it is possible that Zn/Cd/Pb hyperaccumulation evolved on nonmetalliferous soils containing low levels of geogenic metals, and that metalliferous soils (including mine sites) were colonized later, accompanied by an increase in metal tolerance.

Apart from a number of fern species (28, 50, 69, 107), the hyperaccumulation of arsenic (As) has so far been reported in only two angiosperm species of the Brassicaceae (45), further highlighting the uniqueness of this family with respect to metal hyperaccumulation (**Figure 1**). Se hyperaccumulators were first identified as the cause of alkali disease in cattle in areas of high soil Se contents of geogenic origin. Se hyperaccumulation has evolved once in the Brassicaceae (**Figure 1**) (88). Hyperaccumulators of other metals have been described elsewhere (6, 88, 105).

METAL HYPERACCUMULATOR MODEL PLANTS

Arabidopsis halleri

Arabidopsis halleri (formerly *Cardaminopsis halleri*) is a metal hypertolerant Zn/Cd hyperaccumulator and a self-incompatible, perennial, stoloniferous diploid species, with $2n = 16$ chromosomes and a genome size of approximately (2C) 0.56 pg, approximately 1.65 times that of *A. thaliana* (82). It diverged from *A. lyrata*, its nontolerant nonaccumulator sister species, between 1.5 and 2 Mya, and from *A. thaliana*—with which it shares on average, 94% nucleotide identity within coding regions—between approximately 3 and 5.8 Mya (17). As metal hyperaccumulation and associated hypertolerance are common to all known *A. halleri* subspecies, it is likely that this trait evolved with the divergence from the *A. lyrata* lineage.

A. halleri is distributed in and around central and eastern Europe (subspecies *halleri*), with ssp. *ovirensis* in the Eastern Alps, and ssp. *gemmifera* in Japan and Taiwan on the margins of the species distribution (56). Two further subspecies are not well described to date (49). Populations of *A. halleri* ssp. *halleri* occur on soils contaminated with Zn and Cd, e.g., in the Harz Mountains of Germany. In addition, there are numerous natural populations of *A. halleri* on nonmetalliferous soils, often on noncalcareous soils in mountain regions, e.g., in the Bohemian forest and the central Swiss Alps. There are no reports concerning elemental composition or metal tolerance in *A. kamchatika*, a proposed natural hybrid of *A. halleri* with the closely related nonaccumulator *A. lyrata* (49), to date.

All subspecies and populations of *A. halleri*—on both noncontaminated and metalliferous soils—are considered to exhibit constitutive (used in the sense of species-wide) Zn and Cd hyperaccumulation. Sixteen to 40% of individuals from natural populations contained leaf Zn concentrations $>10{,}000$ μg g^{-1} dry biomass (12). Between-individual variation in leaf Cd concentrations was even more pronounced than for Zn concentrations. Ten to 33% of all individuals contained hyperaccumulator levels of >100 μg g^{-1} Cd, including a number of individuals from noncontaminated soils containing Cd concentrations below the detection limit of 1 μg g^{-1} dry soil mass (12).

Root elongation tolerance tests suggested that *A. halleri* plants tolerate at least 76-fold higher Zn concentrations (110) and 8-fold higher Cd concentrations (13) in a hydroponic medium, when compared to *A. thaliana* or *A. lyrata*. In a comparison of 33 European accessions of *A. halleri*, the most Zn-tolerant accession from a metal-contaminated site in Silesia, Poland, tolerated 1.8-fold higher Zn concentrations than the most sensitive accession from a nonmetalliferous site in Tyrol, Austria (79).

Noccaea caerulescens

Noccaea caerulescens (formerly *Thlaspi caerulescens*) is a self-compatible, biannual, diploid Zn/Cd hyperaccumulator with $2n = 14$ chromosomes, and a genome size (2C) of approximately 0.7 pg, about twice that of *A. thaliana* Col-0 (0.34 pg), with which it shares, on average, approximately 88% nucleotide sequence identity within coding regions (81, 82). Divergence from the *A. thaliana* lineage is estimated at about 20 Mya (17). With a center of distribution in northwestern and western Europe, reports of the species *N. caerulescens* are particularly abundant in Scandinavia, the British Isles, and France (**http://data.gbif.org/welcome.htm**). Zn hyperaccumulation is considered a species-wide trait in *N. caerulescens* (7, 14, 76, 90). The highest leaf Zn concentration ever reported in a plant of 53,450 μg g^{-1} dry biomass was measured in an individual collected at a mine site north of St. Félix de Pallières, France (90). This population of *N. caerulescens* is also among a small group of populations in southern France in which extraordinarily high leaf Cd concentrations of up to 2908 μg g^{-1} (Viviez, France) were detected (90).

In comparison to *A. halleri*, *N. caerulescens* exhibits substantially more between-population phenotypic differentiation with respect to metal-related traits. A number of populations on ultramafic soils hyperaccumulate either Ni only, or both Zn and Ni (90). Populations on noncontaminated and Zn/Cd/Pb-contaminated soils are all Zn hyperaccumulators. Cd hyperaccumulation has been reported in the majority of populations on contaminated soils (7, 65, 90). At least some accessions from ultramafic and noncontaminated soils appear to lack Cd hypertolerance (4). Higher levels of Zn or Cd tolerance are associated with lower levels of shoot Zn or Cd accumulation, respectively (2, 43, 65, 73). Shoot Zn and Cd accumulation are correlated (76, 92) across different accessions of *N. caerulescens* in comparisons excluding the high-Cd hyperaccumulating accessions from southern France (92). A few major genetic loci appear to account for between-accession differences in the extent of Zn and Cd hyperaccumulation, respectively, in *N. caerulescens* (3, 22).

Similar to *A. halleri*, there is substantial variation in leaf Cd, Zn, or Ni concentrations within populations of *N. caerulescens* (65, 90). Characteristically, metal hyperaccumulation and hypertolerance are less metal-specific in *N. caerulescens* than in the Zn/Cd hyperaccumulator *A. halleri*, which lacks the ability for cotolerance or coaccumulation of Ni, and in *Alyssum* Ni hyperaccumulators, which exhibit only minor cotolerance and coaccumulation of the chemically similar Co and no cotolerance or coaccumulation of Zn (51, 53). By contrast, most *N. caerulescens* accessions can accumulate hyperaccumulator concentrations of Ni in their leaves (81, 82) when Ni is present in elevated concentrations in the soil, although Zn is generally accumulated preferentially when both metals are supplied at equal concentrations (2, 101) even in Ni hyperaccumulating accessions.

In both hyperaccumulator model species described above, the respective contributions of colonization of metalliferous sites from local populations on nonmetalliferous sites and from distant populations on other metalliferous sites are currently under investigation (14, 80).

PHYSIOLOGY OF METAL HYPERACCUMULATION

The discussion of the physiological and molecular basis of metal hyperaccumulation is restricted here to Zn/Cd and Ni. Among these transition metal cations, accumulation and detoxification pathways are similar and better understood than for As and Se (84, 112).

Characteristic of the hyperaccumulation of Ni and Zn, and, to a somewhat lesser extent, Cd, is a fundamentally altered partitioning of these metals. In most plants, metals are predominantly accumulated in the roots. As a consequence, shoot:root ratios of metal concentrations are generally substantially below unity in these plants. By comparison, in hyperaccumulators metal concentrations are substantially higher in the leaves and much lower in the roots. Accordingly, shoot:root ratios of metal concentrations are abnormally high in hyperaccumulators, and generally above unity (7, 53, 99). This is a result of the alteration of the following processes in hyperaccumulators.

(*a*) There is a strongly enhanced rate of loading of the hyperaccumulated metal(s) from the root symplasm into the apoplastic xylem for root-to-shoot transport with the transpiration stream (38, 53, 61). This also renders accessible high-capacity metal storage sites in the shoot (see item *b*).

(*b*) Metal detoxification and sequestration in the leaves, mostly inside the vacuoles of leaf cells, are highly effective (54, 57, 59, 68).

(*c*) The mobility of metals within the root symplasm for movement towards the vasculature is strongly enhanced, for example, as a result of reduced sequestration of metals in root vacuoles (61, 99).

(*d*) Root metal uptake rates are increased, generally through an increase in V_{MAX}, without major changes in K_M, of root metal uptake rates (60, 67).

(*e*) In the shoot, cellular metal uptake rates are enhanced (61), and there is a highly effective system for cell-to-cell metal distribution (52, 57–59). As a result, high-capacity metal storage sites are rendered accessible and excessive accumulation is prevented in any given cell type along the pathway of the metal from the xylem to the storage sites.

(*f*) A strongly enhanced ability to protect roots from metal toxicity (13, 110) operates partially through efficient shuttling of metals to the shoot (see item *a*), but additional mechanisms are likely to make important contributions.

Accordingly, metal hyperaccumulation requires complex alterations in the plant metal homeostasis network.

GENETIC AND MOLECULAR BASIS OF METAL HYPERACCUMULATION

Membrane Transporters of Metals

A segregating BC1 population obtained through a cross between *A. halleri* and the nonaccumulator *A. lyrata*, followed by a subsequent back-cross of an F1 individual to *A. lyrata*, has been used for the identification of chromosomal segments cosegregating with Zn and Cd hypertolerance. Three QTL for each Zn and Cd hypertolerance, respectively, were mapped to chromosomal regions of an estimated number of between 3000 and 800 genes (20, 25, 110). In independent approaches, candidate genes were identified based on transcriptome comparisons between *A. halleri* and *A. thaliana* (9, 99, 108),

BC1: back-cross 1

heterologous screening of cDNA libraries (25), and functional genomics results from the related nonaccumulator species *A. thaliana* (35, 41, 104). A high degree of synteny of both *A. halleri* and *A. lyrata* with *A. thaliana* and the design of markers for candidate gene loci were used to genetically map many of these candidate genes.

Overlapping QTL for Zn and Cd hypertolerance both contain the candidate gene *AhHMA4* (*Heavy Metal ATPase 4*) (20, 110). Through silencing of *AhHMA4* by RNAi interference upon stable transformation with an intron-spliced hairpin construct, it was demonstrated that *AhHMA4* is required for the full extent of Cd and—to a slightly lesser degree—Zn hypertolerance in *A. halleri* (38). Moreover, *AhHMA4* was shown to be required for Zn hyperaccumulation and for normal levels of shoot Cd accumulation in an accession of *A. halleri* that is not Cd-hyperaccumulating under laboratory conditions (38) (**Figure 2**). *AhHMA4* encodes a plasma membrane protein of the heavy metal pump family of P-type ATPases

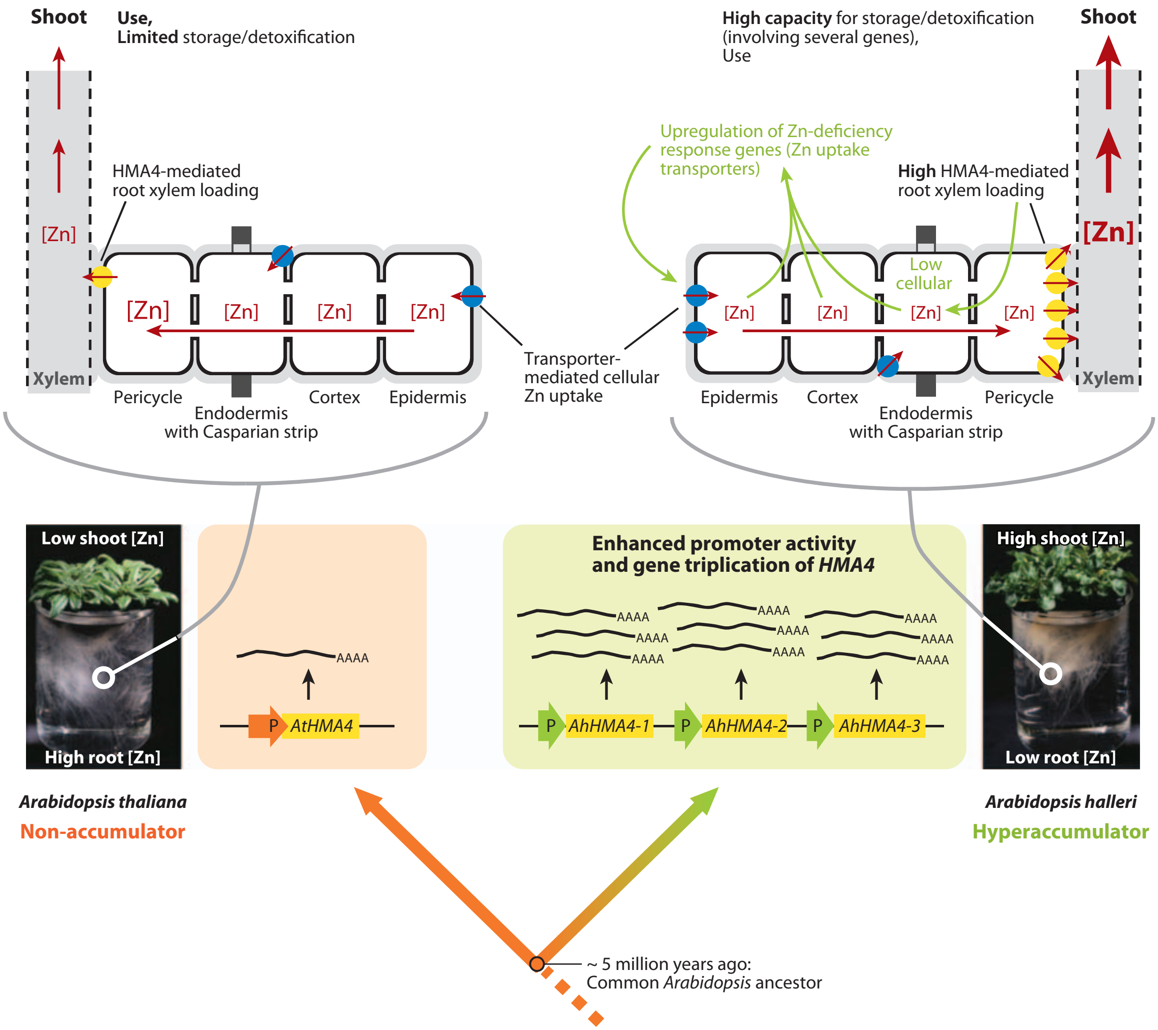

Figure 2

Metal hyperaccumulation in *Arabidopsis halleri* evolved through *cis*-regulatory changes and triplication of *HMA4*. Figure reprinted from (38). See text for details. In the top panel, the apoplast is shown in gray, the intracellular symplast in white.

(20), which is capable of conferring Cd and Zn tolerance to metal-sensitive yeast mutants (99). To date, there is no evidence for a difference between the protein functions of *Ah*HMA4 and the homologous *At*HMA4. The latter is known to mediate xylem loading of Zn and Cd in the roots of *A. thaliana*. In an *A. thaliana* double mutant of *hma4* and the homologous *hma2*, shoot Zn concentrations are reduced by approximately 50%, root Zn concentrations are increased, and shoots exhibit Zn deficiency symptoms under normal growth conditions (41).

The key difference between *AhHMA4* and *AtHMA4* is a between 6- and 53-fold higher transcript abundance of *AhHMA4* in *A. halleri* (99), with only subtle differences in the localization of transcripts (38). This indicates that, as a major step in the evolution of metal hyperaccumulation, a function existing in the common ancestor was strongly enhanced in *A. halleri*. High *HMA4* transcript levels in *A. halleri* are a result of a combination of gene copy number expansion to three, almost identical, intact and expressed *AhHMA4* gene copies present in tandem in the genome of *A. halleri*, and promoter mutations enhancing promoter strength of all three *AhHMA4* gene copies (38). When introduced into *A. thaliana* or *A. halleri*, the promoters of all three *AhHMA4* genes conferred much higher expression levels of reporter genes than the *AtHMA4* promoter.

AhHMA4 affects other components of the metal homeostasis network that are characteristically altered in hyperaccumulators. High *AhHMA4* expression accounts at least partly (38) for the strongly increased transcript levels of Zn deficiency response genes observed in *A. halleri* in the steady state irrespective of Zn supply (9, 99, 108). This is likely to contribute to the observed high Zn uptake rates into the roots of hyperaccumulators (60), and possibly to low levels of sequestration of metals in root vacuoles or enhanced mobilization from root vacuoles.

The work on *AhHMA4* showed that alterations in a single key process in metal homeostasis can contribute to hypertolerances to several metals, as well as to hyperaccumulation, and can lead to secondary alterations of other processes through existing pathways of regulatory coupling within the metal homeostasis network. These secondary alterations, in turn, contribute further to metal hyperaccumulation. This implies a reduction in the number of loci requiring mutations for metal hyperaccumulation to evolve, when compared to expectations based on the required complex physiological alterations. Similar to *A. halleri*, transcript levels of *NcHMA4* of *N. caerulescens* are substantially higher than those of *AtHMA4* in *A. thaliana* in both roots and shoots (11). The available evidence is consistent with a central role for *HMA4* in metal hyperaccumulation and hypertolerance in *N. caerulescens* as well as in *A. halleri* (78).

RNA interference showed that high *AhHMA4* transcript levels are necessary for the full extent of Zn and Cd hypertolerance in *A. halleri* (see above). The expression of *AhHMA4* in *A. thaliana* suggested that *AhHMA4* is not sufficient and needs to operate in conjunction with other genes to enhance metal tolerance (38), although this was not apparent from the genetic approaches (20, 110). Important candidates are genes encoding vacuolar metal transporters capable of sequestering metals inside leaf cells. Transcript levels of a gene encoding a vacuolar membrane Zn/H^+ antiporter, *AhMTP1*, are about 20-fold higher in the leaves of *A. halleri* than in *A. thaliana* (9, 25). Interestingly, genomic copy number is also expanded for this gene, and each of two highly expressed gene copies of this gene from *A. halleri* cosegregate with Zn hypertolerance in the BC1 generation of a cross between *A. halleri* and *A. lyrata*, respectively (25).

AhMTP1: *Metal Transport Protein 1*; *Metal Tolerance Protein 1*; also termed *CDF1*, *Cation Diffusion Facilitator 1*; a close homologue of *AtMTP1* also known as *ZAT*, *Zn Transporter of Arabidopsis thaliana*

Similar to *HMA4*, *MTP1* transcript levels are higher in the Zn/Cd hyperaccumulator *N. caerulescens* (2) and in the Ni hyperaccumulator *N. goesingense* (37), when compared to closely related nonaccumulators. As for *HMA4*, there is no conclusive evidence to date for a functional difference between hyperaccumulator and nonaccumulator MTP1 proteins. Heterologous expression of *AhMTP1* or *AtMTP1* in yeast (9, 25) and of *AtMTP1* and *NgMTP1* in *A. thaliana* (37, 104) enhanced Zn

Tonoplast: vacuolar membrane

tolerance in these organisms. Evidence from *AtMTP1* RNAi lines (23) and shoot-specific expression of *NgMTP1* in *A. thaliana* (37) suggested that MTP1 can generate a metal sink in the shoot that results in an increase in shoot Zn concentrations. Shoot-specific overexpression of *NgMTP1* has been reported to systemically trigger enhanced expression of Zn deficiency response genes in the root (37). Grafting experiments between *N. caerulescens* and the less Zn-accumulating *Thlaspi perfoliatum*, which was originally identified as a Ni hyperaccumulator (89), rather provide evidence against a major contribution of shoot Zn sequestration to metal hyperaccumulation (36).

NcZNT1 (*Zn Transporter 1*) encodes a putative Zn and Cd transporter most homologous to AtZIP4 of the ZIP (Zinc-regulated transporter, Iron-regulated transporter-related Protein) protein family (83) and was identified based on high transcript levels in *N. caerulescens* when compared to *Thlaspi arvense* and the ability to complement a Zn-uptake defective yeast mutant, making it a good candidate for a plasma membrane root metal uptake system. Later, genes encoding several additional ZIP family members were found to be highly expressed in *N. caerulescens* (2, 103) and *A. halleri* (9, 63, 99, 108) in the steady state under Zn-sufficient growth conditions, whereas most of them form part of the transcriptional Zn deficiency response in *A. thaliana* (99). The responsiveness of transcript levels of these genes to Zn status is nevertheless largely conserved in *A. halleri* and *N. caerulescens*. In comparison to *A. thaliana*, the high steady-state transcript levels of these genes are likely to be—at least partly—a consequence of a physiological Zn depletion in the roots of *A. halleri*, for example, through the enhanced expression of *HMA4* (38, 99) (see above). A GFP fusion of *Ah*IRT3 of the ZIP family has been shown to locate to the plasma membrane, and overexpression of *AhIRT3* in *A. thaliana* led to increased Zn and Fe accumulation (63). It can be considered as certain that ZIP family genes contribute to metal hyperaccumulation, but their individual functions remain to be identified.

In the F2 of a cross between *A. halleri* and *A. lyrata*, markers on several chromosomes were found to cosegregate with leaf Zn accumulation (27). Markers designed in the previously identified candidate gene loci *ZIP6* and *NRAMP3* (*Natural Resistance-Associated Macrophage Protein 3*) (26) were not found to be associated with any of the three to four detected QTL for leaf Zn accumulation, whereas it remains possible that the previously identified candidate genes *ZIP9* (108) and *HMA3* (9) colocate with QTL for leaf Zn accumulation. Surprisingly, no QTL was detected around the *AhHMA4* locus on *A. halleri* chromosome 3.

Additional candidate genes have been identified, and some of them partially characterized, but their functions in metal hyperaccumulation remain unclear (66, 67, 77, 87, 105).

Metal Chelation

In *Alyssum* Ni hyperaccumulator species, in the Ni hyperaccumulator *N. goesingense*, and in *N. caerulescens*, which is capable of Ni hyperaccumulation, steady-state concentrations of the free amino acid histidine are substantially higher than in closely related nonaccumulators (46, 53, 85). Histidine was shown to have a key role as a Ni chelator in both Ni hypertolerance and high root-to-shoot Ni flux in the xylem in *Alyssum lesbiacum*. Enhanced production of histidine is primarily a result of constitutively elevated transcript levels of the two genes encoding ATP-phosphoribosyl transferase, the enzyme catalyzing the first, rate-limiting step of histidine biosynthesis (42). Xylem Ni and histidine concentrations show a proportional relationship in *Alyssum* hyperaccumulators (also addressed as the histidine response), which was interpreted as indirect evidence suggesting that a Ni-histidine complex is transported into the xylem (46).

Using Mg-ATP-energized root tonoplast vesicles, the chelation of Ni with histidine was shown to suppress Ni transport into root vacuoles of *T. caerulescens*, whereas citrate chelation enhanced net Ni transport (91). This could be an alternative or additional mechanism in which histidine acts to enhance root-to-shoot Ni flux. Interestingly, histidine chelation enhanced the transport of Ni into root tonoplast vesicles of *T. arvense*. These findings might explain earlier results from *Arabidopsis thaliana* metabolically engineered to contain high concentrations of histidine. In comparison to wild-type *A. thaliana*, the transgenic plants exhibited a higher level of Ni tolerance, but shoot Ni accumulation was not increased (42, 111).

Nicotianamine (NA) is a low-molecular-weight metal chelator, which exhibits very high stability constants for the binding of all transition metal cations and is present in higher concentrations in the hyperaccumulators *A. halleri* and *N. caerulescens* than in closely related nonaccumulators (102, 108). In *A. thaliana* and other plants, NA is required to maintain Fe, Zn, and Cu homeostasis, in particular, by ensuring cell-to-cell mobility of metals (47, 98). One NA molecule is synthesized from three molecules of *S*-adenosyl-L-methionine by the enzyme nicotianamine synthase (NAS), with three molecules of *S*-methyl-5′-thioadenosine arising as a by-product (39, 64). Transcript levels of *NAS2*, *NAS3*, and *NAS4* are substantially higher in *A. halleri* (9, 99, 108), and those of *NAS2* and *NAS4* in *N. caerulescens* (103), than in closely related nonaccumulators. The expression of *NAS* cDNAs in yeast cells was reported to confer Zn tolerance (9, 108) and Ni tolerance (102). Ectopic overexpression of various *NAS* cDNAs in plants increased Ni tolerance (24, 86).

Metal-NA complexes are substrates of membrane transporters of the yellow stripe-like (YSL) and possibly other protein families, for some of which transcript levels are higher in hyperaccumulator plants than in nonaccumulators (33, 99). Metal toxicity can manifest itself through the interference between a metal present in excess and other micronutrient metals (34). Several candidate genes identified based on their high expression in metal hyperaccumulators, including *NAS* genes, may function to maintain the homeostasis of nonaccumulated micronutrient metals in the presence of high fluxes or high amounts of the hyperaccumulated metal(s) (9, 77, 108).

At the site of maximum metal accumulation inside leaf vacuoles, metals are chelated by organic acids such as malate or citrate (54, 62, 94), which are present in high concentrations in hyperaccumulators. The capacity for organic acid accumulation in a plant may determine its maximum divalent cation accumulation. High organic acid concentrations are unlikely to make major specific contributions to metal hyperaccumulation or hypertolerance, but they may be a prerequisite.

Phytochelatins (PC): low-molecular-weight, metal-chelating compounds known to be required for basic metal tolerance found in all plants. PCs are synthesized nontranslationally from glutathione by the enzyme phytochelatin synthase to form molecules of (γ-EC)$_n$G ($n \sim 2$ to 11)

Antioxidants in Metal Hypertolerance

Several metal hyperaccumulators, in particular, Ni-hyperaccumulating *Noccaea* species and *N. caerulescens*, contain elevated concentrations of glutathione (GSH), cysteine, and *O*-acetylserine when compared to closely related nonaccumulators (30). Salicylic acid or a degradation product was proposed as an upstream signal resulting in increased serine acetyltransferase (SAT) activity and higher steady-state GSH levels (29). In *N. goesingense*, this correlates with high expression levels of genes encoding SAT and glutathione reductase. Overexpression of *NgSAT* in *A. thaliana* was sufficient to increase GSH levels and Ni, Co, Zn, and—to a small extent—Cd tolerance. This increase in metal tolerance was proposed to result from a GSH-mediated reduction in oxidative damage arising under metal exposure (30, 32), although GSH can also act as a metal chelator and as a substrate for the biosynthesis of phytochelatins (PCs) (18). Treatment with L-buthionine-[S,R]-sulfoximine, an inhibitor of the enzyme γ-glutamylcysteine synthetase in the biosynthetic pathway of GSH, did not cause a reduction in Cd, Zn, or Ni hypertolerance of different accessions of *N. caerulescens* (95), thereby providing evidence against a role for GSH or PCs in metal hypertolerance.

OUTLOOK

In *A. halleri* and *N. caerulescens*, largely overlapping sets of candidate genes for metal hyperaccumulation and hypertolerance were identified, based on their high expression levels in the hyperaccumulator species when compared to closely related nonaccumulators and their predicted functions in metal homeostasis. Thus, highly similar gene expression patterns associated with metal hyperaccumulation appear to have evolved independently in *A. halleri* and *N. caerulescens* (55). In *A. halleri*, a surprisingly high proportion of candidate genes appear to have undergone gene copy number expansions (25, 38, 99, 100). Further direct functional and genetic evidence, as well as genome sequencing, will help to gain more insight into these emerging patterns of genomic changes underlying metal hyperaccumulation.

SUMMARY POINTS

1. Metal hyperaccumulation occurs in approximately 0.2% of all angiosperms and is particularly well represented in the Brassicaceae.
2. *A. halleri* and *N. caerulescens* are emerging model hyperaccumulator species.
3. Metal hyperaccumulation involves strongly enhanced metal partitioning from the root into the shoot and very high levels of metal tolerance.
4. High expression of *AhHMA4* encoding a cellular metal export pump is required for Zn hyperaccumulation and for the full extent of Zn and Cd hypertolerance in *A. halleri*. Compared to *A. thaliana*, high *HMA4* expression in *A. halleri* is the result of a combination of gene copy number expansion and mutations in *cis* that enhance the strengths of the promoters of *AhHMA4* genes.
5. There is strong experimental support for a function of high expression levels of *MTP1* encoding a vacuolar membrane Zn transporter in Zn hypertolerance through metal sequestration in leaf vacuoles.
6. Plasma membrane Zn uptake systems of the ZIP family are likely to contribute to high root metal uptake rates in hyperaccumulators.
7. High transcript levels and gene copy number expansion are common among *HMA4*, *MTP1*, and ZIP family genes in *A. halleri*.
8. *A. halleri* and *N. caerulescens* share a common core set of highly expressed candidate genes for metal hyperaccumulation and metal hypertolerance.

FUTURE ISSUES

1. It will be important to increase the resolution and accuracy of the genetic mapping of loci for metal hyperaccumulation and hypertolerance, including the following:
    a. Fine mapping, ideally to individual gene(s);
    b. Development of physiologically guided, more integrated phenotyping approaches to account for the strong interactions between metal accumulation/partitioning and tolerance; and
    c. An attempt to establish separate phenotyping methods for shoot and root metal tolerance.

2. The causes of the enormous between- and within-population differences in leaf metal concentrations observed in the field need to be determined in order to better understand the phenomenon of metal hyperaccumulation.
3. To facilitate genetic mapping and as a first basis for comprehensive comparative genomics and population genetic studies, the assembled and annotated genome sequences of several hyperaccumulators and closely related nonaccumulators will be required.
4. The clarification of the phylogenetic position and taxonomy of metal hyperaccumulator taxa will be necessary for comparative genomics and to prioritize among possible additional future model metal hyperaccumulators.
5. The contributions to metal hyperaccumulation and hypertolerance need to be experimentally determined for additional genes using genetic approaches so that robust and more complete scientific models of these traits can be developed (109).

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Antonovics J, Bradshaw AD, Turner RG. 1971. Heavy metal tolerance in plants. *Adv. Ecol. Res.* 7:1–85
2. Assunção AG, da Costa Martins P, de Folter S, Voojis R, Schat H, Aarts MGM. 2001. Elevated expression of metal transporter genes in three accessions of the metal hyperaccumulator *Thlaspi caerulescens*. *Plant Cell Environ.* 24:217–26
3. Assunção AG, 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 Phytol.* 170:21–32
4. Assunção AGL, Ten Bookum WM, Nelissen HJM, Vooijs R, Schat H, Ernst WHO. 2003. Differential metal-specific tolerance and accumulation patterns among *Thlaspi caerulescens* populations originating from different soil types. *New Phytol.* 159:411–19
5. Baker AJM. 1981. Accumulators and excluders—strategies in the response of plants to heavy metals. *J. Plant Nutr.* 3:643–54
6. Baker AJM, Brooks RR. 1989. Terrestrial higher plants which hyperaccumulate metallic elements—a review of their distribution, ecology and phytochemistry. *Biorecovery* 1:81–126
7. Baker AJM, Reeves RD, Hajar ASM. 1994. Heavy metal hyperaccumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. & C. Presl (Brassicaceae). *New Phytol.* 127:61–68
8. Baumann A. 1885. Das Verhalten von Zinksalzen gegen Pflanzen und in Böden. *Die landwirtsch. Versuchsstat.* 31:1–53

9. 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 J.* 37:251–68
10. Beckett PHT, Davis RD. 1977. Upper critical levels of toxic elements in plants. *New Phytol.* 79:95–106
11. Bernard C, Roosens N, Czernic P, Lebrun M, Verbruggen N. 2004. A novel CPx-ATPase from the cadmium hyperaccumulator *Thlaspi caerulescens*. *FEBS Lett.* 569:140–48
12. Bert V, Bonnin I, Saumitou-Laprade P, de Laguerie P, Petit D. 2002. Do *Arabidopsis halleri* from nonmetallicolous populations accumulate zinc and cadmium more effectively than those from metallocolous populations? *New Phytol.* 155:47–57
13. Bert V, Meerts P, Saumitou-Laprade P, Salis P, Gruber W, Verbruggen N. 2003. Genetic basis of Cd tolerance and hyperaccumulation in *Arabidopsis halleri*. *Plant Soil* 249:9–18
14. Besnard G, Basic N, Christin PA, Savova-Bianchi D, Galland N. 2008. *Thlaspi caerulescens* (Brassicaceae) population genetics in western Switzerland: Is the genetic structure affected by natural variation of soil heavy metal concentrations? *New Phytol.* 181:974–84
15. Boyd RS. 2007. The defense hypothesis of elemental hyperaccumulation: status, challenges and new directions. *Plant Soil* 293:153–76
16. Boyd RS, Martens SN. 1992. The *raison d'être* for metal hyperaccumulation in plants. In *The Vegetation of Ultramafic (Serpentine) Soils*, ed. AJM Baker, J Proctor, RD Reeves, pp. 279–89. Andover, Hampshire: Intercept Ltd.
17. Clauss MJ, Koch MA. 2006. Poorly known relatives of *Arabidopsis thaliana*. *Trends Plant Sci.* 11:449–59
18. Clemens S. 2001. Molecular mechanisms of plant metal tolerance and homeostasis. *Planta* 212:475–86
19. Clemens S, Palmgren MG, Krämer U. 2002. A long way ahead: Understanding and engineering plant metal accumulation. *Trends Plant Sci.* 7:309–15
20. Courbot M, Willems G, Motte P, Arvidsson S, Roosens N, et al. 2007. A major quantitative trait locus for cadmium tolerance in *Arabidopsis halleri* colocalizes with *HMA4*, a gene encoding a heavy metal ATPase. *Plant Physiol.* 144:1052–65
21. Deng DM, Shu WS, Zhang J, Zou HL, Lin Z, et al. 2007. Zinc and cadmium accumulation and tolerance in populations of *Sedum alfredii*. *Environ. Pollut.* 147:381–86
22. 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*. *Theor. Appl. Genet.* 113:907–20
23. Desbrosses-Fonrouge AG, 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 Lett.* 579:4165–74
24. Douchkov D, Gryczka C, Stephan UW, Hell R, Bäumlein H. 2005. Ectopic expression of nicotianamine synthase genes results in improved iron accumulation and increased nickel tolerance in transgenic tobacco. *Plant Cell Environ.* 28:365–74
25. Dräger DB, Desbrosses-Fonrouge AG, Krach C, Chardonnens AN, Meyer RC, et al. 2004. Two genes encoding *Arabidopsis halleri* MTP1 metal transport proteins co-segregate with zinc tolerance and account for high *MTP1* transcript levels. *Plant J.* 39:425–39
26. 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. *Mol. Ecol.* 15:3045–59
27. Filatov V, Dowdle J, Smirnoff N, Ford-Lloyd B, Newbury HJ, Macnair MR. 2007. A quantitative trait loci analysis of zinc hyperaccumulation in *Arabidopsis halleri*. *New Phytol.* 174:580–90
28. Francesconi K, Visoottiviseth P, Sridokchan W, Goessler W. 2002. Arsenic species in an arsenic hyperaccumulating fern, *Pityrogramma calomelanos*: a potential phytoremediator of arsenic-contaminated soils. *Sci. Total Environ.* 284:27–35
29. Freeman JL, Garcia D, Kim D, Hopf A, Salt DE. 2005. Constitutively elevated salicylic acid signals glutathione-mediated nickel tolerance in Thlaspi nickel hyperaccumulators. *Plant Physiol.* 137:1082–91
30. Freeman JL, Persans MW, Nieman K, Albrecht C, Peer W, et al. 2004. Increased glutathione biosynthesis plays a role in nickel tolerance in Thlaspi nickel hyperaccumulators. *Plant Cell* 16:2176–91

31. Freeman JL, Quinn CF, Marcus MA, Fakra S, Pilon-Smits EAH. 2006. Selenium-tolerant diamondback moth disarms hyperaccumulator plant defense. *Curr. Biol.* 16:2181–92
32. Freeman JL, Salt DE. 2007. The metal tolerance profile of *Thlaspi goesingense* is mimicked in *Arabidopsis thaliana* heterologously expressing serine acetyl-transferase. *BMC Plant Biol.* 7:63
33. Gendre D, Czernic P, Conejero G, Pianelli K, Briat JF, et al. 2007. *TcYSL3*, a member of the YSL gene family from the hyperaccumulator *Thlaspi caerulescens*, encodes a nicotianamine-Ni/Fe transporter. *Plant J.* 49:1–15
34. Ghasemi R, Ghaderian SM, Krämer U. 2009. Interference of nickel with copper and iron homeostasis contributes to metal toxicity symptoms in the nickel hyperaccumulator plant *Alyssum inflatum*. *New Phytol.* 184:566–80
35. Grotz N, Fox T, Connolly E, Park W, Guerinot ML, Eide D. 1998. Identification of a family of zinc transporter genes from Arabidopsis that respond to zinc deficiency. *Proc. Natl. Acad. Sci. USA* 95:7220–24
36. Guimarães MD, Gustin JL, Salt DE. 2009. Reciprocal grafting separates the roles of the root and shoot in zinc hyperaccumulation in *Thlaspi caerulescens*. *New Phytol.* 184:323–29
37. Gustin JL, Loureiro ME, Kim D, Na G, Tikhonova M, Salt DE. 2009. MTP1-dependent Zn sequestration into shoot vacuoles suggests dual roles in Zn tolerance and accumulation in Zn-hyperaccumulating plants. *Plant J.* 57:1116–27
38. Hanikenne M, Talke IN, Haydon MJ, Lanz C, Nolte A, et al. 2008. Evolution of metal hyperaccumulation required *cis*-regulatory changes and triplication of *HMA4*. *Nature* 453:391–95
39. Higuchi K, Kanazawa K, Nishizawa N-K, Chino M, Mori S. 1994. Purification and characterization of nicotianamine synthase from Fe-deficient barley roots. *Plant Soil* 165:173–79
40. Hong CL, Jia YB, Yang XE, He ZL, Stoffella PJ. 2008. Assessing lead thresholds for phytotoxicity and potential dietary toxicity in selected vegetable crops. *Bull. Environ. Contam. Toxicol.* 80:356–61
41. Hussain D, Haydon MJ, Wang Y, Wong E, Sherson SM, et al. 2004. P-type ATPase heavy metal transporters with roles in essential zinc homeostasis in *Arabidopsis*. *Plant Cell* 16:1327–39
42. Ingle RA, Mugford ST, Rees JD, Campbell MM, Smith JAC. 2005. Constitutively high expression of the histidine biosynthetic pathway contributes to nickel tolerance in hyperaccumulator plants. *Plant Cell* 17:2089–106
43. Ingrouille MJ, Smirnoff N. 1986. *Thlaspi caerulescens* J. & C. Presl (*Thlaspi alpestre* L.) in Britain. *New Phytol.* 102:219–33
44. Kabata-Pendias A, Pendias H. 1992. *Trace Elements in Soils and Plants*. Boca Raton, FL: CRC Press. 365 pp.
45. Karimi N, Ghaderian SM, Raab A, Feldmann J, Meharg AA. 2009. An arsenic-accumulating, hypertolerant Brassica, *Isatis cappadocica*. *New Phytol.* 184:41–47
46. Kerkeb L, Krämer U. 2003. The role of free histidine in xylem loading of nickel in *Alyssum lesbiacum* and *Brassica juncea*. *Plant Physiol.* 131:716–24
47. Klatte M, Schuler M, Wirtz M, Fink-Straube C, Hell R, Bauer P. 2009. The analysis of Arabidopsis nicotianamine synthase mutants reveals functions for nicotianamine in seed iron loading and iron deficiency responses. *Plant Physiol.* 150:257–71
48. Koch MA, Al-Shehbaz IA. 2009. Molecular systematics and evolution of "wild" crucifers (Brassicaceae or Cruciferae). In *Biology and Breeding of Crucifers*, ed. SK Gupta, pp. 1–19. Boca Raton, FL: CRC Press/Taylor & Francis Group
49. Koch MA, Matschinger M. 2007. Evolution and genetic differentiation among relatives of *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 104:6272–77
50. Koller CE, Patrick JW, Rose RJ, Offler CE, MacFarlane GR. 2007. *Pteris umbrosa* R. Br. as an arsenic hyperaccumulator: accumulation, partitioning and comparison with the established As hyperaccumulator *Pteris vittata*. *Chemosphere* 66:1256–63
51. Krämer U. 1996. *Nickel hyperaccumulation in the genus Alyssum L.* D. Phil. thesis. Oxford Univ., Oxford. 215 pp.
52. Krämer U, Baker AJM, Hawes CR, Smith JAC, Grime GW. 1997. Micro-PIXE as a technique for studying nickel localisation in leaves of the hyperaccumulator plant *Alyssum lesbiacum*. *Nucl. Instrum. Methods B* 130:346–50

53. Krämer U, Cotter-Howells JD, Charnock JM, Baker AJM, Smith JAC. 1996. Free histidine as a metal chelator in plants that accumulate nickel. *Nature* 379:635–38
54. Krämer U, Pickering IJ, Prince RC, Raskin I, Salt DE. 2000. Subcellular localization and speciation of nickel in hyperaccumulator and non-accumulator Thlaspi species. *Plant Physiol.* 122:1343–53
55. Krämer U, Talke IN, Hanikenne M. 2007. Transition metal transport. *FEBS Lett.* 581:2263–72
56. Kubota H, Takenaka C. 2003. *Arabis gemmifera* is a hyperaccumulator of Cd and Zn. *Int. J. Phytoremediation* 5:197–201
57. Küpper H, Lombi E, Zhao FJ, McGrath SP. 2000. Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. *Planta* 212:75–84
58. Küpper H, Lombi E, Zhao FJ, Wieshammer G, McGrath SP. 2001. Cellular compartmentation of nickel in the hyperaccumulators *Alyssum lesbiacum*, *Alyssum bertolonii* and *Thlaspi goesingense*. *J. Exp. Bot.* 52:2291–300
59. Küpper H, Zhao FJ, McGrath SP. 1999. Cellular compartmentation of zinc in leaves of the hyperaccumulator *Thlaspi caerulescens*. *Plant Physiol.* 119:305–12
60. 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 Physiol.* 112:1715–22
61. Lasat MM, Baker AJM, Kochian LV. 1998. Altered Zn compartmentation in the root symplasm and stimulated Zn absorption into the leaf as mechanisms involved in Zn hyperaccumulation in *Thlaspi caerulescens*. *Plant Physiol.* 118:875–83
62. Lee JH, Reeves RD, Brooks RR, Jaffré T. 1978. The relation between nickel and citric acid in some nickel-accumulating plants. *Phytochemistry* 17:1033–35
63. Lin YF, Liang HM, Yang SY, Boch A, Clemens S, et al. 2009. Arabidopsis IRT3 is a zinc-regulated and plasma membrane localized zinc/iron transporter. *New Phytol.* 182:392–404
64. Ling H-Q, Koch G, Baeumlein H, Ganal MW. 1999. Map-based cloning of *chloronerva*, a gene involved in iron uptake of higher plants encoding nicotianamine synthase. *Proc. Natl. Acad. Sci. USA* 96:7098–103
65. Lloyd-Thomas DH. 1995. *Heavy metal hyperaccumulation by Thlaspi caerulescens J. & C. Presl*. PhD thesis. Univ. Sheffield, Sheffield. 155 pp.
66. Lombi E, Tearall KL, Howarth JR, Zhao FJ, Hawkesford MJ, McGrath SP. 2002. Influence of iron status on cadmium and zinc uptake by different ecotypes of the hyperaccumulator *Thlaspi caerulescens*. *Plant Physiol.* 128:1359–67
67. 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 Phytol.* 149:53–60
68. Ma JF, Ueno D, Zhao FJ, McGrath SP. 2005. Subcellular localisation of Cd and Zn in the leaves of a Cd-hyperaccumulating ecotype of *Thlaspi caerulescens*. *Planta* 220:731–36
69. Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, Kennelley ED. 2001. A fern that hyperaccumulates arsenic. *Nature* 409:579
70. Macnair MR. 1993. Tansley Review No. 49: The genetics of metal tolerance in vascular plants. *New Phytol.* 124:541–59
71. Madejón P, Murillo JM, Marañón T, Cabrera F, López R. 2002. Bioaccumulation of As, Cd, Cu, Fe and Pb in wild grasses affected by the Aznalcóllar mine spill (SW Spain). *Sci. Total Environ.* 290:105–20
72. Marschner H. 1995. *Mineral Nutrition of Higher Plants*. London: Academic
73. Meerts P, Van Isacker N. 1997. Heavy metal tolerance and accumulation in metallicolous and non-metallicolous populations of *Thlaspi caerulescens* from continental Europe. *Plant Ecol.* 133:221–31
74. Meharg AA. 2005. *Venomous Earth—How Arsenic Caused The World's Worst Mass Poisoning*. Basingstoke, UK: Macmillan. 256 pp.
75. Minguzzi C, Vergnano O. 1948. Il contenuto del nichel nelle ceneri di *Alyssum bertolonii* Desv. *Atti Soc. Tosc. Sci. Nat. Ser. A* 55:49–77
76. 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 Phytol.* 165:503–12
77. Oomen RJ, Wu J, Lelievre F, Blanchet S, Richaud P, et al. 2009. Functional characterization of NRAMP3 and NRAMP4 from the metal hyperaccumulator *Thlaspi caerulescens*. *New Phytol.* 181:637–50

78. Papoyan A, Kochian LV. 2004. Identification of *Thlaspi caerulescens* genes that may be involved in heavy metal hyperaccumulation and tolerance. Characterization of a novel heavy metal transporting ATPase. *Plant Physiol.* 136:3814–23
79. Pauwels M, Frerot H, Bonnin I, Saumitou-Laprade P. 2006. A broad-scale analysis of population differentiation for Zn tolerance in an emerging model species for tolerance study: *Arabidopsis halleri* (Brassicaceae). *J. Evol. Biol.* 19:1838–50
80. Pauwels M, Saumitou-Laprade P, Holl AC, Petit D, Bonnin I. 2005. Multiple origin of metallicolous populations of the pseudometallophyte *Arabidopsis halleri* (Brassicaceae) in central Europe: the cpDNA testimony. *Mol. Ecol.* 14:4403–14
81. Peer WA, Mahmoudian M, Freeman JL, Lahner B, Richards EL, et al. 2006. Assessment of plants from the Brassicaceae family as genetic models for the study of nickel and zinc hyperaccumulation. *New Phytol.* 172:248–60
82. 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 Phytol.* 159:421–30
83. Pence NS, Larsen PB, Ebbs SD, Letham DL, Lasat MM, et al. 2000. The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. *Proc. Natl. Acad. Sci. USA* 97:4956–60
84. Persans MW, Salt DE. 2000. Possible molecular mechanisms involved in nickel, zinc and selenium hyperaccumulation in plants. *Biotechnol. Genet. Eng. Rev.* 17:389–413
85. Persans MW, Yan X, Patnoe J-MML, Krämer U, Salt DE. 1999. Molecular dissection of the role of histidine in nickel hyperaccumulation in *Thlaspi goesingense* (Hálácsy). *Plant Physiol.* 121:1117–26
86. Pianelli K, Mari S, Marques L, Lebrun M, Czernic P. 2005. Nicotianamine over-accumulation confers resistance to nickel in *Arabidopsis thaliana*. *Transgenic Res.* 14:739–48
87. Plaza S, Tearall KL, Zhao FJ, Buchner P, McGrath SP, Hawkesford MJ. 2007. Expression and functional analysis of metal transporter genes in two contrasting ecotypes of the hyperaccumulator *Thlaspi caerulescens*. *J. Exp. Bot.* 58:1717–28
88. Reeves RD, Baker AJM. 2000. Metal-accumulating plants. In *Phytoremediation of Toxic Metals—Using Plants to Clean Up the Environment*, ed. I Raskin, BD Ensley, pp. 193–229. New York: Wiley
89. Reeves RD, Kruckeberg AR, Adigüzel N, Krämer U. 2001. Studies on the flora of serpentine and other metalliferous areas of western Turkey. *South Afr. J. Sci.* 97:513–17
90. Reeves RD, Schwartz C, Morel JL, Edmonson J. 2001. Distribution and metal-accumulating behaviour of *Thlaspi caerulescens* and associated metallophytes in France. *Int. J. Phytoremediation* 3:145–72
91. Richau KH, Kozhevnikova AD, Seregin IV, Vooijs R, Koevoets PL, et al. 2009. Chelation by histidine inhibits the vacuolar sequestration of nickel in roots of the hyperaccumulator *Thlaspi caerulescens*. *New Phytol.* 183:106–16
92. Roosens N, 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 Environ.* 26:1657–72
93. Sachs J. 1865. Handbuch der Experimentalphysiologie der Pflanzen. In *Handbuch der Physiologischen Botanik*, ed. W Hofmeister, pp. 153–54. Leipzig: Engelmann
94. Sarret G, Saumitou-Laprade P, Bert V, Proux O, Hazemann JL, et al. 2002. Forms of zinc accumulated in the hyperaccumulator *Arabidopsis halleri*. *Plant Physiol.* 130:1815–26
95. 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. *J. Exp. Bot.* 53:2381–92
96. Schat H, Vooijs R. 1997. Multiple tolerance and co-tolerance to heavy metals in *Silene vulgaris*: A cosegregation analysis. *New Phytol.* 136:489–96
97. 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* 50:1888–95
98. Takahashi M, Terada Y, Nakai I, Nakanishi H, Yoshimura E, et al. 2003. Role of nicotianamine in the intracellular delivery of metals and plant reproductive development. *Plant Cell* 15:1263–80

99. Talke IN, Hanikenne M, Krämer U. 2006. Zinc-dependent global transcriptional control, transcriptional deregulation, and higher gene copy number for genes in metal homeostasis of the hyperaccumulator *Arabidopsis halleri*. *Plant Physiol.* 142:148–67
100. Tang YT, Qiu RL, Zeng XW, Fang XH. 2005. A new found Pb/Zn/Cd hyperaccumulator—*Arabis paniculata* Franch. *Acta Sci. Nat. Univ. Sun Yatseni* 44:135–36
101. Taylor SI, Macnair MR. 2006. Within and between population variation for zinc and nickel accumulation in two species of Thlaspi (Brassicaceae). *New Phytol.* 169:505–13
102. Vacchina V, Mari S, Czernic P, Marques L, Pianelli K, et al. 2003. Speciation of nickel in a hyperaccumulating plant by high-performance liquid chromatography-inductively coupled plasma mass spectrometry and electrospray MS/MS assisted by cloning using yeast complementation. *Anal. Chem.* 75:2740–45
103. Van de Mortel JE, Almar Villanueva L, Schat H, Kwekkeboom J, Coughlan S, et al. 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 Physiol.* 142:1127–47
104. van der Zaal BJ, Neuteboom LW, Pinas JE, Chardonnens AN, Schat H, et al. 1999. Overexpression of a novel Arabidopsis gene related to putative zinc-transporter genes from animals can lead to enhanced zinc resistance and accumulation. *Plant Physiol.* 119:1047–55
105. Verbruggen N, Hermans C, Schat H. 2009. Molecular mechanisms of metal hyperaccumulation in plants. *New Phytol.* 181:759–76
106. Vogel-Mikus 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. *Environ. Pollut.* 133:233–42
107. Wang HB, Wong MH, Lan CY, Baker AJM, Qin YR, et al. 2007. Uptake and accumulation of arsenic by 11 Pteris taxa from southern China. *Environ. Pollut.* 145:225–33
108. Weber M, Harada E, Vess C, 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 J.* 37:269–81
109. Weigel D, Nordborg M. 2005. Natural variation in Arabidopsis. How do we find the causal genes? *Plant Physiol.* 138:567–68
110. Willems G, Dräger DB, Courbot M, Godé C, Verbruggen N, Saumitou-Laprade P. 2007. The genetic basis of zinc tolerance in the metallophyte *Arabidopsis halleri* ssp. *halleri* (Brassicaceae): an analysis of quantitative trait loci. *Genetics* 176:659–74
111. Wycisk K, Kim EJ, Schroeder JI, Krämer U. 2004. Enhancing the first enzymatic step in the histidine biosynthesis pathway increases the free histidine pool and nickel tolerance in *Arabidopsis thaliana*. *FEBS Lett.* 578:128–34
112. Zhao FJ, McGrath SP, Meharg AA. 2010. Arsenic as a food chain contaminant: mechanisms of plant uptake and metabolism and mitigation strategies. *Annu. Rev. Plant Biol.* 61:In press

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