

Fortified Foods and Phytoremediation. Two Sides of the Same Coin¹

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The global population is expected to reach 7 billion by the year 2013. How are we going to feed the world, prevent further degradation of our environment, and begin to reverse the damage that our increasingly industrialized society has already caused to the biosphere? We argue that plants with enhanced mineral acquisition and storage strategies can help us to achieve these goals. For example, we can use crop plants with an augmented capacity to accumulate minerals to aid sustainable agriculture and to improve human health through balanced mineral nutrition. We can also use plants to accumulate toxic metals from polluted soils and waters for cleanup purposes. Each of these goals requires understanding how plants accumulate and store minerals. This includes understanding mineral element bioavailability in the rhizosphere and root uptake, as well as translocation to and processing in the above ground parts of the plant.

Were people worrying about these topics 25 years ago? The field of plant mineral nutrition has been around for a long time, but the idea of fortifying foods pre-harvest with the 17 essential minerals required for a healthy diet is relatively new (10). With iron deficiency the leading nutritional disorder in the world today (<http://www.who.int/nut/>) and most of the world getting their iron from eating plants, increasing the iron content of crop plants could vastly improve human health. In a similar manner, although it has been known since the late 1800s that some plants can accumulate extraordinary levels of metals (Fig. 1), the idea of phytoremediation, using plants that hyperaccumulate metals in clean-up efforts, only appeared in the literature in the last 20 years. At present, at least 45 plant families are known to contain metal-accumulating species (22). Such plants can accumulate Cu, Co, Cd, Mn, Ni, Se, or Zn up to levels that are 100 to 1,000 times those normally accumulated by plants. A number of these species are members of the Brassicaceae, including a species of *Arabidopsis*, *A. halleri*, which can accumulate Zn in its shoots to concentrations of >1% of dry matter

(22). With the completion of the *Arabidopsis* genome sequence, we are now well positioned to exploit the ability of its close relatives to accumulate metals. As many of the metals that can be hyperaccumulated are also essential nutrients, it is easy to see that food fortification and phytoremediation are two sides of the same coin. In this short essay, we will choose examples highlighting Fe, Se, and Zn, all essential nutrients that can also be problematic if present in excess.

MOBILIZATION OF MINERALS IN THE RHIZOSPHERE

In the rhizosphere, a plant's ability to absorb nutrients is often limited by the availability of nutrients at the surface of the root. However, the plant is not a completely passive player, having the ability to release compounds that alter the solubility and availability of nutrients. For example, in response to phosphate-limiting conditions, some plants increase secretion of organic acids (18). At the same time, some Al tolerant plants release organic acids as part of their tolerance mechanism (18). Thus it was quite satisfying to see that plants engineered to overproduce citrate have improved phosphate nutrition, as well as increased resistance to Al (7, 17). Early results with *Arabidopsis* and papaya demonstrate that plants engineered to release citrate are capable of mobilizing iron as well (L. Herrera-Estrella, personal communication). Another successful release strategy to aid in Fe mobilization is exemplified by the grasses. When starved for Fe, the world's major grain crops release phytosiderophores that chelate soluble Fe present at low concentrations in soils (18). Genes encoding the key enzymes in the biosynthetic pathway for the mugineic acid family of phytosiderophores, nicotianamine synthase, and nicotianamine aminotransferase were recently cloned from maize and barley (13, 14, 16, 26). This paves the way for the engineering of plants with the capacity to overproduce phytosiderophores. In the event that transport of phytosiderophores proves limiting, a gene encoding a putative phytosiderophore transporter has also been recently identified in maize (E. Walker, personal communication). Of course, we also have the option of engineering the plant rhizosphere to contain mi-

¹ This paper is dedicated to the memory of Horst Marschner, a pioneer in the field of plant mineral nutrition.

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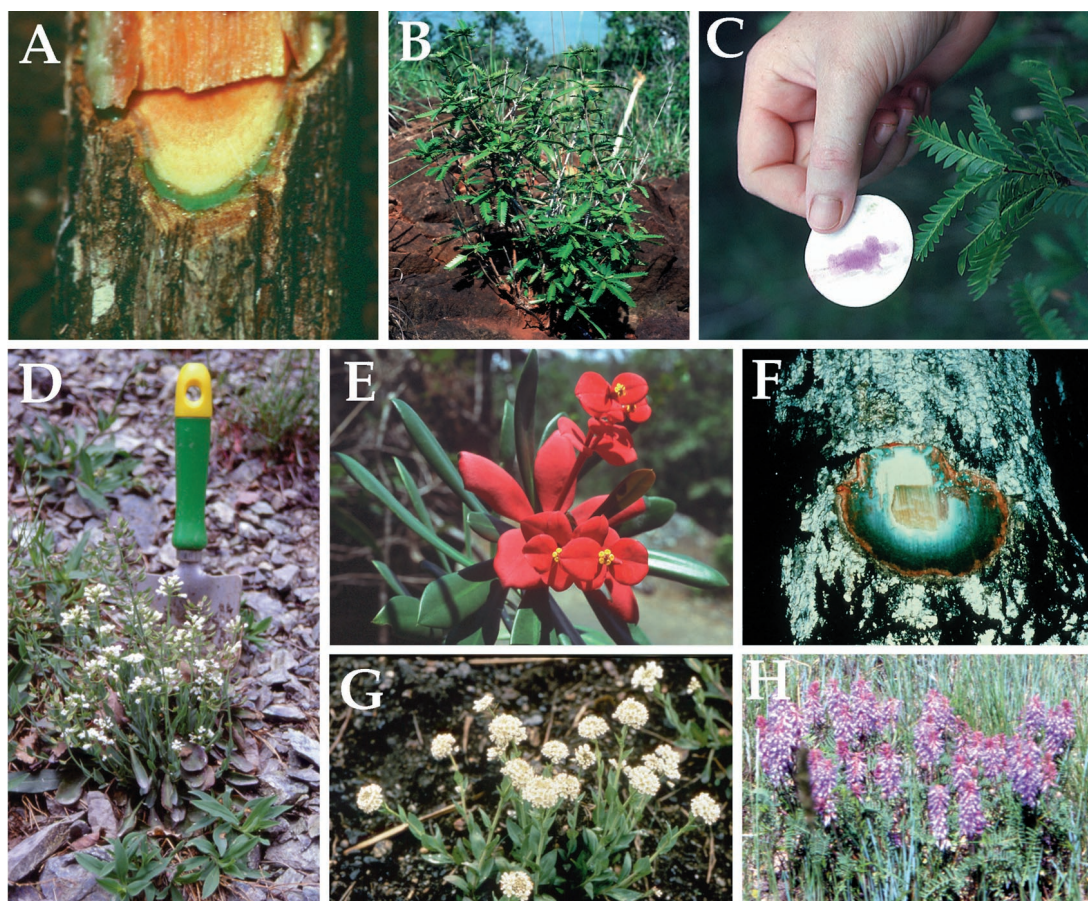


Figure 1. Examples of metal hyperaccumulating plants. A through C, *Phyllanthus* “palawanensis” (Euphorbiaceae), a shrub found in open areas of stunted forest, Palawan, Republic of the Philippines. A, Cut stem exuding a jade-green liquid that contained $88,580 \mu\text{g Ni g}^{-1}$ dry weight; B, leaves containing $16,230$ and stems containing $5,440 \mu\text{g Ni g}^{-1}$ dry weight; C, leaves crushed onto filter paper soaked with dimethylglyoxime, showing the vivid purple color of the dimethylglyoxime-Ni complex. D, *Thlaspi goesingense*, found in Redschlag, Austria, contains up to $9,490 \mu\text{g Ni g}^{-1}$ dry weight. E, *Euphorbia helena*, found in Cuba, contains $3,160$ to $4,430 \mu\text{g Ni g}^{-1}$ dry shoot biomass; F, *Serbertia acuminata*, a tree endemic to serpentine soils of New Caledonia, showing the cut stem exuding latex which contains over 25% Ni on a dry weight basis. Leaves of this species also contain $11,700 \mu\text{g Ni g}^{-1}$ dry weight. G, *Thlaspi caerulescens*, growing on an abandoned lead mine in Bradford Dale, Derbyshire, England contains up to $29,465 \mu\text{g Zn g}^{-1}$ dry weight. H, *Astragalus bisulcatus* growing in Big Hollow, Wyoming contains up to $6,530 \mu\text{g Se g}^{-1}$ dry weight. Pictures courtesy of Alan J.M. Baker, University of Sheffield, Sheffield, UK (*Phyllanthus*, *Euphorbia helena*, *Serbertia acuminata*); Walter W. Wenzel, University of Agriculture, Vienna (*Thlaspi goesingense*), and Catherine Skinner, University of Wyoming, Laramie (*Astragalus bisulcatus*).

croorganisms with an enhanced capacity to solubilize trace elements. Such “biased rhizospheres” can also help protect plants from toxic elements. It is unfortunate that a review of the microbial literature is beyond the scope of this essay.

TRANSPORT FROM THE RHIZOSPHERE INTO THE ROOT

Once mobilized in the rhizosphere, mineral elements need to be taken up across the root cell plasma membrane. Rapid progress in this area has been achieved by supplementing *Arabidopsis* genetics and genomics with the power of yeast and bacterial genetics. Consider iron, for example. All plants except the grasses must first reduce Fe(III) to Fe(II) before trans-

porting it into the cell. Identification of *Arabidopsis* mutants lacking this activity allowed the cloning of the *FRO2* gene encoding the enzyme responsible for catalyzing this rate-limiting step in iron acquisition (23). Cloning of plasma membrane transporters capable of Fe transport via functional complementation of yeast Fe uptake mutants has identified genes belonging to two different families of transporters (6, 8, 27). The first of these transporter genes identified, *IRT1*, is the founding member of what is now a large family (the ZIP family) of genes encoding divalent cation transporters with representatives in protists, fungi, plants and animals (11). *IRT1* is only expressed in the roots of iron deficient plants. However, in yeast, the *IRT1* protein is capable of transporting Mn, Zn, and Cd in addition to Fe (11). The other gene family im-

plicated in Fe transport, *Nramp*, also encodes proteins that mediate the transport of a variety of divalent cations, including Fe, Mn, and Zn (6, 27).

It should soon be possible to control the rate of trace element uptake in the root by manipulating the expression of transporter genes. The Zn hyperaccumulator *Thlaspi caerulescens* overexpresses a ZIP family root plasma membrane transporter, *ZNT1*, which is 88% identical to the Zn transport gene *ZIP4* from *Arabidopsis* (19). In the closely related nonaccumulator species, *T. arvense*, high external Zn concentrations suppress expression of this Zn transporter, indicating that metal regulation of gene expression is altered in the hyperaccumulator. One can presume that other genes have altered regulation in the hyperaccumulator to cope with potentially toxic metal levels. Because *Thlaspi* genes examined to date show 85% to 90% identity to those in *Arabidopsis*, we should be able to determine how many genes have altered expression patterns in a hyperaccumulating species such as *Thlaspi* through the use of DNA microarray or DNA chip technology. It seems unlikely that regulation of single genes will be sufficient to convert nonaccumulators into metal hyperaccumulators, although the possibility of one or two key regulatory loci remains. If whole suites of genes must be transferred, then somatic hybridization between *Thlaspi* and the high biomass crop oilseed rape offers another route to understanding which genes are involved in hyperaccumulation. Such hybrids have an intermediate morphology and show significantly higher Zn resistance and Zn accumulation than the nonaccumulating oilseed rape parent (2).

MOVING MINERAL ELEMENTS TO THE ABOVE-GROUND PARTS OF THE PLANT

So now we have the mineral in the root, but we really need to get it to the shoot, to aid either food fortification or phytoremediation (obvious exceptions to this being food crops such as potatoes). Improvements in our ability to measure ions and determine their speciation are revolutionizing our understanding of metal movement in plants. For example, the application of x-ray absorption spectroscopy to measure the chemical form of trace elements such as As and Cd in the roots versus during translocation to the shoot has revealed significant differences in the chemistry of the two processes (20, 25). Both As and Cd appear to be coordinated by thiol groups in the root, but are coordinated by oxygen atoms for transport to the shoot. A better understanding of the processes controlling these changes in the chemical speciation of trace elements should allow us to control the partitioning of various trace elements between root and shoot tissues. In the Ni hyperaccumulator, *Alyssum lesbiacum*, the free amino acid His promotes the translocation of Ni from root to shoot tissues, presumably by forming a Ni-His complex that moves in the xylem (15).

RESISTANCE AND/OR STORAGE MECHANISMS

For the sustained accumulation of potentially toxic mineral elements, it will be important to engineer various resistance and/or storage mechanisms into plants. This will be true for essential elements such as Fe and for nonessential elements such as Cd. The recent cloning of genes encoding phytochelatin synthase from *Arabidopsis*, *Schizosaccharomyces pombe*, and wheat (for review, see 5) now opens the door to the engineering of plants with the capacity to overproduce phytochelatins, enzymatically synthesized peptides known to be involved in binding Cd and other heavy metals in plants. Not surprisingly, genetics proved key in identifying the phytochelatin synthase genes. One group conferred Cd resistance on wild-type yeast (4), another group suppressed the Cd-sensitive phenotype of a particular yeast mutant (29), and the third identified a Cd-sensitive *Arabidopsis* mutant and cloned the gene using a map-based approach (12). Of course, metal complexes have to be stored and a number of metals appear to be stored in the vacuole, including phytochelatin-Cd complexes (for review, see 5). An *Arabidopsis* Zn transporter gene belonging to the cation diffusion facilitator family recently has been identified whose product may play a role in Zn sequestration in the vacuole (28). Iron, which can react with oxygen to form damaging hydroxyl radicals, is not sequestered in the vacuole but rather in plastids as ferritin. Ferritin can store up to 4,500 Fe atoms in its central cavity, making it a likely target for improving the iron content of plants. Transgenic rice plants expressing the soybean ferritin gene contained three times as much iron in its seeds as untransformed plants (9). As one-half of the world eats rice everyday, genetically engineered rice with higher levels of ferritin and lower levels of phytic acid, which impedes iron absorption, would be a significant achievement.

For certain trace elements such as Hg and Se, volatilization of the element provides a possible pathway for resistance. The Meagher laboratory has developed Hg-resistant transgenic yellow poplar trees with the ability to volatilize approximately 10-fold more Hg than wild-type plants (24). This shows that high biomass plants can be engineered to remove pollutant ionic Hg from soils and waters by volatilization. This feat was achieved by overexpressing the bacterial *merA* gene encoding a mercuric ion reductase, having first established proof of concept in *Arabidopsis*. Taking the work one step further, transgenic *Arabidopsis* plants have now been constructed which overexpress the mercuric ion reductase and a bacterial gene encoding an organomercurial lyase (1). Such plants have the capacity to convert highly toxic methylmercury, a biomagnified form of Hg, into the much less toxic elemental form. This is the first example of using pathway engineering in plants to manipulate the ecotoxicology of a pollutant metal. The use of these types of plants should provide a

very powerful tool for the removal of highly toxic organomercury compounds from the environment, especially from aquatic sediments where methylmercury can be generated from ionic mercury by bacteria. It is anticipated that the amount of mercury volatilized by engineered plants will be small relative to the atmospheric mercury load.

Although Se is toxic in high concentrations, low doses have recently been observed to play a significant role in cancer prevention (3). *Astragalus* species accumulate up to 6,000 $\mu\text{g Se g}^{-1}$ dry weight in their shoot tissues, mainly as Se-methylseleno-Cys, a compound shown to have anti-carcinogenic properties. *Astragalus* species provide an attractive source of genetic material for designing plants with enhanced concentrations of chemo-preventative Se compounds or for use in remediating Se-rich soils and waters. Due to their chemical properties, certain forms of Se are volatile, again offering dilution of less toxic forms into the atmosphere as a way to remove this potentially toxic trace element from soils and waters. Because of the chemical similarity of Se to S, it is bio-transformed in plants in the same way as S. The first step in this biotransformation is activation of selenate to adenosine 5' phosphoselenate by the enzyme ATP sulfurylase. Overexpression of this enzyme in Indian mustard-enhanced Se tolerance (21). The authors hypothesize that this increased tolerance may be due to increased assimilation of Se into volatile forms in the plants. It is interesting that these plants also appeared to accumulate 2- to 3-fold more Se in shoots than wild-type plants. However, the mechanism of this enhanced accumulation is not clear.

It is obvious that we have come along way since Justus von Liebig (1803–1873) established mineral nutrition as a scientific discipline and early plant biologists first discovered trace element accumulating plants such as *Thlaspi*. We still have a long way to go before we completely understand the mechanisms involved in mineral acquisition and homeostasis. We have, however, started along the pathway to discovery, and our future endeavors will undoubtedly produce rewards for the environment, agriculture, and human health. We should be able to construct plants that require reduced applications of fertilizers, that can grow on marginal lands, that accumulate nutrients, and that can be used to clean up contaminated sites.

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