Trace elements: too little or too much and how plants cope

Joe Morrissey and Mary Lou Guerinot*

Address: Department of Biological Sciences, Dartmouth College, Hanover, NH 03755, USA

*Corresponding author: Mary Lou Guerinot (mary.lou.guerinot@dartmouth.edu)

F1000 Biology Reports 2009, 1:14 (doi: 10.3410/B1-14)

The electronic version of this article is the complete one and can be found at: <http://F1000.com/Reports/Biology/content/1/14>

Abstract

Plants acquire essential trace elements from the rhizosphere and must adapt to conditions that can range from deficiency to excess. Knowledge of how trace elements move from root to shoot to seed is critical for agriculture and human nutrition.

Introduction and context

Plants require an assortment of trace elements for growth, yet the properties that make these minerals essential also make them toxic at increasing concentrations. As plants primarily acquire their micronutrients from the rhizosphere, an ability to adapt to soil conditions that can range from deficiency to excess is critical for optimal growth. Ultimately, human nutrition is dependent on how plants respond to trace element availability in terms of both nutrient accumulation and yield. Deciphering trace element transport and tolerance allows the breeding of crops that are more nutrient rich and more tolerant of extreme soil conditions. Currently, many aspects of metal homeostasis in plants remain unknown. These include how plants sense metal deficiencies and respond and whether chaperones exist for all redox-active metals. Although these core questions remain unanswered, our understanding of metal tolerance and detoxification has increased greatly in the past two years with the publication of several insightful papers.

Major recent advances

Advances in understanding metal tolerance

It is well known that metal hyperaccumulating species increase their tolerance to metals by dispersing them from the root to aerial tissues. *Arabidopsis halleri* is closely related to *Arabidopsis thaliana*, yet *A. halleri* grows readily in soils polluted with zinc and cadmium, accumulating high concentrations of these metals in its leaves [1]. A series of microarray experiments had previously found

significant increases in the expression of zinc transporters in *A. halleri* relative to *A. thaliana* [2–4], but the regulatory and evolutionary basis of these changes remained unknown.

Hanikenne *et al.* [5] shed light on this question by showing that the ability of *A. halleri* to move large amounts of zinc and cadmium from root to shoot (Figure 1) is mediated by a triplication of the gene encoding the vascular-loading transporter HMA4, as well as by changes to *cis*-regulatory sequences controlling the expression of HMA4. The authors used RNA interference to knockdown HMA4 levels, resulting in zinc accumulation in the roots, reduced expression of other zinc transporters, and elimination of zinc tolerance. This showed that HMA4 is not only essential for zinc tolerance, but also serves as a 'physiological master switch' to control expression of other zinc transporters. They also found that the *AhHMA4* promoter expressed in *A. thaliana* drove much higher levels of expression than the endogenous *AtHMA4* promoter in the leaves and root vasculature. Interestingly, when *AhHMA4* was overexpressed in *A. thaliana*, the plants became more sensitive to zinc and cadmium, indicating that additional genes in *A. halleri* are also required for hyperaccumulation. Quantitative trait locus (QTL) analyses now underway in *A. halleri* may provide new candidates, in addition to those suggested from microarray studies, that can be tested [1].

Figure 1. Examples of metal tolerance mechanisms in plants

In the past two years, several genes required for metal tolerance in plants have been identified. In roots, excess metal can be loaded into vesicles and is likely exocytosed into the apoplast (e.g. HvBOR1 and boron). Tolerance is also increased by dispersing metal from the roots to the shoot. This is achieved by increasing the expression of xylem-loading metal transporters, like zinc-transporter AhHMA4. Once in the shoot, the metal can be sequestered (e.g. AhMTP1 pumps zinc into the vacuole), or pumped into vesicles and exocytosed into the hydathode (e.g. HvBOR1 and boron). CS, Casparian strip; V, vacuole.

Another means of metal tolerance, exocytosis, had been documented in humans and yeast. This mechanism has now been reported in plants, with the discovery of what is likely exocytosis of metal-loaded vesicles in both *Arabidopsis* and barley. Manganese (Mn) detoxification in *Arabidopsis* can be conferred in part by sequestration in the vacuole [6] and endoplasmic reticulum [7]. Further elucidating Mn homeostasis in *Arabidopsis*, Peiter *et al.* [8] showed that Mn detoxification also involves exclusion via exocytosis. The transporter MTP11 was localized to *trans*-Golgi vesicles, and rescued manganese sensitive yeast via microsomal loading; at the same time, over-expression of MTP11 in *Arabidopsis* improved manganese tolerance, while the loss-of-function mutant was hypersensitive to manganese. Additionally, Delhaize *et al.* [9] found that an intermediate expression level of MTP11-GFP resulted in intermediate manganese tolerance (although this construct appeared to localize to pre-vacuolar compartments). Peiter *et al.* [8] found MTP11 expressed in the hydathodes and root tips, suggesting it loads vesicles with excess manganese that are then

exocytosed, with the manganese being removed via guttation in the shoots, and via efflux into the root apoplast. Interestingly, the vesicular MTP11 was expressed in different tissues than the vacuolar manganese transporter MTP1; this suggests tissue-specific, tissue-specialized roles in Mn homeostasis for different members of the MTP family.

Boron is needed for cross-linking of plant cell walls, yet too much boron can be toxic [10]. It is only recently that boron transporters have been identified. NIP5;1, a member of the aquaporin protein family, acts to take up boron [11] and BOR1 was identified as a boron effluxer required for efficient xylem loading of boron for transport to the shoot [12]. Boron efflux also plays an important role in boron tolerance. QTL analysis in barley between the highly tolerant Sahara cultivar and the susceptible Clipper cultivar revealed that the genomic region of interest contained a *BOR1*-like gene [13], which the authors designated *Bot1*. This is another great example of where basic research in *Arabidopsis* facilitated the identification of an agronomically significant gene in barley. They discovered that boron tolerance was mediated by elevated expression of the effluxer Bot1 (Figure 1); when compared to a boron-sensitive variety, Sahara had four times as many copies of *Bot1*, much like the triplication of *HMA4* found in *A. halleri*. Sahara Bot1 also contained two amino acid changes that significantly increased its boron transport capability compared to Clipper Bot1 when expressed in yeast. And like AtMTP11, Sahara Bot1 was also highly expressed in the root tips and hydathodes, likely expelling boron and allowing the landrace Sahara to thrive in boron-rich soils. The authors note that boron toxicity causes a 17% decrease in barley yield in Australia, and the discovery of the *BOR1*-like genes will speed the breeding of boron-tolerant crops.

Sometimes the pathway for the transport of an important nutrient can also be used to transport toxic elements. Two silicon transporters have recently been implicated in arsenite transport in rice [14]. Human arsenic intake from rice consumption can be substantial because rice is particularly efficient in assimilating arsenic from paddy soils. The silicon/arsenite transporters belong to the nodulin-like intrinsic protein (NIP) subfamily.

Finally, a phenotype long attributed to phosphorous deficiency was found to be caused by iron toxicity. Root elongation in *Arabidopsis* ecotype Col-0 is inhibited during phosphorous deficiency, and was believed to be caused by a phosphorous-sensing regulatory pathway. Instead, Ward *et al.* [15] found they could restore root growth during phosphorous deficiency by simply

removing iron from the growth medium – the inhibition was actually caused by the toxic effects of iron that was likely no longer in complex with phosphate, greatly increasing its bioavailability.

Future directions

In the past year, several interesting large-scale experiments have shed light on previously unknown aspects of metal homeostasis in plants, and may serve as a preview of the style and scale of experiments to come.

Dinney *et al.* [16] undertook the task of isolating individual root cell types and measuring the transcriptional changes in response to iron deficiency and salt stress. The authors found that the majority of the transcriptome was altered in response to environmental conditions, especially in the epidermis. At the same time, they identified a small core set of stress-resistant genes that likely define the essential features of each cell layer, and appear to regulate the environment-specific expression of the rest of the transcriptome. Their dataset is a valuable resource for both deducing the role of individual iron-regulated genes, and understanding the broad spatial and temporal changes that make up the iron-deficiency response. Previous studies with salt stress in particular seem to have only identified semi-ubiquitous responses, showing the power of using a genome scale, high-resolution map of gene expression changes.

Baxter *et al.* [17] analyzed the shoot metal accumulation data of over 880 *Arabidopsis* mutants and ecotypes from the Purdue Ionomics Information Management System (PiiMS) database to identify a metal accumulation signature for iron-deficiency. Because iron is highly reactive, its concentration in shoots is tightly regulated and does not fluctuate, even in chlorotic plants. The authors found that iron-deficient plants do exhibit signature changes in other metals in the shoot: increased manganese, zinc, cobalt, and cadmium, and decreased molybdenum. The increases of all but molybdenum are explained by the transport of these metals by the iron-regulated root transporter IRT1 [18]; although they enter the root with iron, their localization is not as highly regulated, and they are able to accumulate in the shoot. The decrease in molybdenum is likely due to the acidification of the rhizosphere by the iron-regulated H⁺ATPases, which increases access to iron, but reduces molybdenum solubility.

Finally, the use of association mapping is quickly proving an invaluable tool in identifying novel genes. *Arabidopsis* is well suited for association mapping because of its rich pool of variation between natural populations. Baxter *et al.* [19] examined 98 ecotypes for

variation in shoot molybdenum, and mapped a polymorphism in seven low molybdenum ecotypes. The source of the phenotype was a 54 base-pair deletion in the promoter region of what is now known as MOT1 - a mitochondrial molybdenum (Mo) transporter. Again, like HMA4 in *A. halleri*, metal accumulation was greatly affected by changes in the promoter. MOT1 is likely just one of many genes that will soon be identified using association mapping.

Abbreviations

Mn, manganese; Mo, molybdenum; NIP, nodulin-like intrinsic protein; PiiMS, Purdue Ionomics Information Management System; QTL, quantitative trait locus.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

We would like to thank the Guerinot lab members for many constructive discussions about metals. Work in the Guerinot lab is supported by grants from the National Science Foundation (IOB 0419695; DBI-070119), the Physical Biosciences Program of the Department of Energy (DE-FG02-06ER15809), the National Institutes of Health (R01 GM 078536) and the National Institute of Environmental Health Sciences (5 P42 ES007373).

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