Molecular Plant
Review Article

CellPress
PARTNER JOURNAL

Plant Ionomics: From Elemental Profiling to Environmental Adaptation

Xin-Yuan Huang and David E. Salt*

Institute of Biological and Environmental Sciences, University of Aberdeen, Cruickshank Building, St Machar Drive, Aberdeen AB24 3UU, UK

*Correspondence: David E. Salt (david.salt@abdn.ac.uk)

http://dx.doi.org/10.1016/j.molp.2016.05.003

ABSTRACT

Ionomics is a high-throughput elemental profiling approach to study the molecular mechanistic basis underlying mineral nutrient and trace element composition (also known as the ionome) of living organisms. Since the concept of ionomics was first introduced more than 10 years ago, significant progress has been made in the identification of genes and gene networks that control the ionome. In this update, we summarize the progress made in using the ionomics approach over the last decade, including the identification of genes by forward genetics and the study of natural ionomic variation. We further discuss the potential application of ionomics to the investigation of the ecological functions of ionomic alleles in adaptation to the environment.

Key words: nutrient homeostasis, natural variation, ionomics, *Arabidopsis thaliana*, Casparian strip, adaptation

Huang X.-Y. and Salt D.E. (2016). Plant Ionomics: From Elemental Profiling to Environmental Adaptation. Mol. Plant. **9**, 787–797.

INTRODUCTION

The ionome is defined as ''the mineral nutrient and trace element composition of an organism, and represents the inorganic component of cellular and organismal systems'' (Salt et al., 2008). Since the concept of the ionome was first proposed more than 10 years ago (Lahner et al., 2003), significant progress has been made in the field of ionomics, in which high-throughput elemental profiling is combined with genetics to identify the genes that control the ionome (Danku et al., 2013). A milestone study in the development of ionomics was carried out in the genetic model plant *Arabidopsis thaliana* (Lahner et al., 2003; with commentaries by Hirschi, 2003 and Rea, 2003). In this study inductively coupled plasma mass spectrometry (ICP-MS) was used to analyze the leaf ionome of many thousands of plants, leading to the identification of multiple ionomic mutants. Interestingly, only 11% of the mutants identified showed alterations in a single element, supporting the concept that ionomic networks in plants are coordinately regulated and need to be viewed as a whole. This was further reinforced by the discovery of ionomic regulatory networks involved in both Fe and P homeostasis (Baxter et al., 2008b), and this concept was recently reviewed by Baxter (2015). Ionomic studies have now been performed in many plant species, including rice (Norton et al., 2010; Zhang et al., 2014; Pinson et al., 2015), maize (Baxter et al., 2013, 2014; Mascher et al., 2014; Gu et al., 2015), barley (Wu et al., 2013), soybean (Ziegler et al., 2013), *Lotus japonica* (Chen et al., 2009), tomato (Sanchez-Rodriguez et al., 2010), and others (Watanabe et al., 2007; White et al., 2012; Parent et al., 2013). The concept of the ionome has also been applied to *Saccharomyces cerevisiae* with an ionomic analysis of knockout and overexpression alleles of all identified genes in the yeast genome (Eide et al., 2005; Yu et al., 2012). Recently, ionomic studies in humans (Sun et al., 2012; Malinouski et al., 2014) and other animals (Yoshida et al., 2014; Ma et al., 2015) have also been reported. By coupling genetics with high-throughput elemental profiling, ionomics has led to the identification of numerous genes controlling the ionome. Furthermore, ionomics has allowed the discovery of genes controlling natural variation in the plant ionome. The application of technical advances in genetic mapping approaches such as deletion mapping (e.g., Baxter et al., 2009) and DNA microarray-based bulk segregant analysis (BSA) (e.g., Gong et al., 2004; Chao et al., 2011) has greatly aided in the success of ionomics, and the application of mapping by sequencing (e.g., Kamiya et al., 2015) is set to further accelerate these successes. Meanwhile, technical advances in imaging the cellular and subcellular localization of elements has started to allow a cell-type and tissue-specific understanding of the ionome (reviewed by Zhao et al., 2014).

In this update, we highlight progress over the past decade in the use of ionomics to identify genes controlling the ionome (Table 1). We also discuss the potential application of this approach to characterize the functions of natural ionomic loci in adapting to the edaphic (soil) environment.

Published by the Molecular Plant Shanghai Editorial Office in association with Cell Press, an imprint of Elsevier Inc., on behalf of CSPB and IPPE, SIBS, CAS.

Mutant/ accession name	Ionomic phenotype	Causal gene	TAIR ID	Gene description	Identification approach	References
frd3	High Mn and Co	*FRD3*	AT3G08040	Citrate transporter	Forward genetics	Delhaize, 1996; Rogers and Guerinot, 2002; Durrett et al., 2007
esb1-1	Low Ca and Mn; high Na, S, K, As, Se, and Mo	*ESB1*	AT2G28670	Dirigent domain-containing protein	Forward genetics	Baxter et al., 2009; Hosmani et al., 2013
myb36-1	Low Ca, Mn, and Fe; high Na, Mg, and Zn	*MYB36*	AT5G57620	MYB domain transcription factor	Forward genetics	Kamiya et al., 2015
tsc10a-1	Low Mg, Ca, Fe, and Mo; high Na, K, and Rb	*TSC10A*	AT3G06060	3-Ketodihydrosphinganine reductase	Forward genetics	Chao et al., 2011
sgn3	Low K; high Mg	*SGN3*	AT4G20140	Receptor-like kinase	Forward genetics	Pfister et al., 2014
sgn1	High Mg	*SGN1*	AT1G61590	Kinase	Forward genetics	Alassimone, J., Fujita, S., et al., unpublished results
nakr1-1/ npcc6	High Na, K, and Rb	*NaKR1*	AT5G02600	Metal binding protein	Forward genetics	Tian et al., 2010
cpr5	Low K	*CPR5*	AT5G64930	Constitutive expression of pathogen resistance	Forward genetics	Borghi et al., 2011
Ts-1, Tsu-1	High Na	*HKT1;1*	AT4G10310	Sodium transporter	Natural variation	Rus et al., 2006; Baxter et al., 2010
Ts-1, Se-0	High Co	*FPN2*	AT5G03570	Ferroportin metal efflux protein	Natural variation	Morrissey et al., 2009
Shahdara, Hod	High sulfate, S, and Se	*APR2*	AT1G62180	5′-Phosphosulfate reductase	Natural variation	Loudet et al., 2007; Chao et al., 2014b
Bay-0	High sulfate	*ATPS1*	AT3G22890	ATP sulfurylase	Natural variation	Koprivova et al., 2013
L*er*-0, Shahdara	Low Mo	*MOT1*	AT2G25680	Molybdenum transporter	Natural variation	Tomatsu et al., 2007; Baxter et al., 2008a; Poormohammad Kiani et al., 2012
CS28181	Low Cd	*HMA3*	AT4G30120	Heavy metal ATPase	Natural variation	Chao et al., 2012
Kas-1; Kr-0	High As	*ATQ1/HAC1*	AT2G21045	Arsenate reductase	Natural variation	Sanchez-Bermejo et al., 2014; Chao et al., 2014a

Table 1. Summary of Ionomic Genes.

IDENTIFICATION OF IONOMIC GENES BY FORWARD GENETICS

A systematic screen for mutants with altered leaf ionomic profiles was carried out by Lahner et al. (2003). In this proof-of-concept screen, 338 putative mutants with alterations in the leaf accumulation of single or multiple elements were identified from a screen of 4747 M2 fast neutron (FN) mutant plants. Of these 338 putative mutants, 51 were confirmed after rescreening of all M3 families. Chen et al. (2009) also performed a similar large ionomic screen of 2000 mutagenized M2 *Lotus japonica* plants. In such large experiments, plants have to be grown in different experimental blocks (e.g., plant cultivation trays) repeatedly over many months to allow the harvesting and analysis of thousands of plant samples. It is notable that the ionome may also vary at different growth stages. To be successful it is thus essential to minimize the variation introduced from the growth media (such as different batches of soil), the growth stage, and the growth environment. In our screen of FN mutagenized *A. thaliana*, the wild-type and the known ionomic mutant *frd3* were included in each plant growth tray as controls and plants were harvested at 5 weeks old. *FRD3* encodes a citrate transporter that plays an important role in the Fe deficiency response (Rogers and Guerinot, 2002; Green and Rogers, 2004; Durrett et al., 2007; Roschzttardtz et al., 2011; Pineau et al., 2012). Mutation of *FRD3* leads to the overaccumulation of a range of metals in leaves including Mn and Co (Delhaize, 1996). Hierarchical clustering of 44 confirmed FN mutants (Lahner et al., 2003) using the leaf ionome as the phenotype demonstrates that clustering of mutants is generally independent of the plant cultivation tray or the soil batch (Figure 1A). Furthermore, *frd3* grown in different cultivation trays and soil batches are exclusively clustered together (Figure 1A),

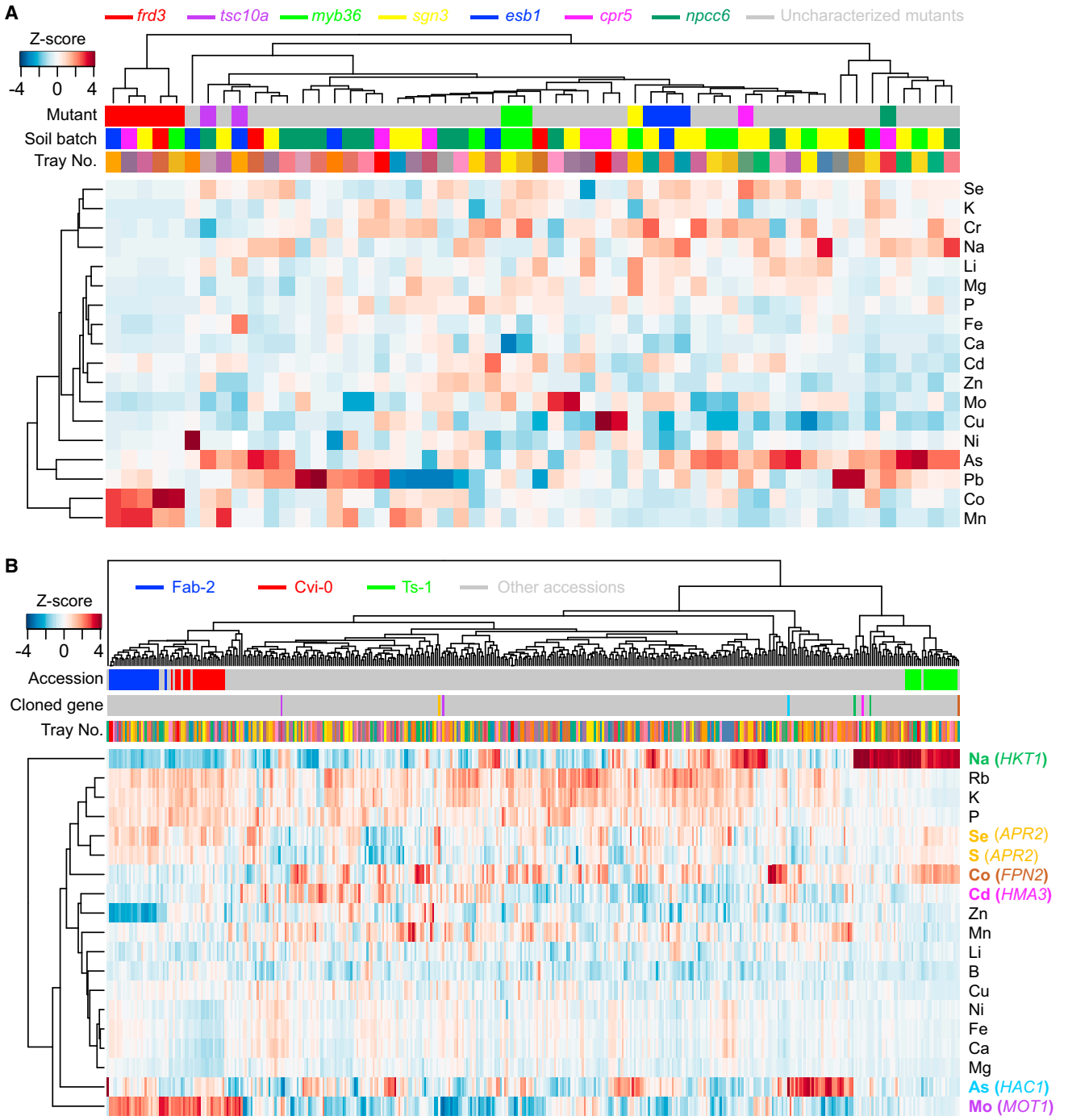

Figure 1. Hierarchical Clustering of *A. thaliana* FN Mutants and Natural Accessions Based on the Leaf Ionome.

The clustering was performed using the percentage difference from Col-0 grown in the same cultivation tray.

(**A**) Hierarchical clustering of *A. thaliana* FN mutants. The causal genes for cloned mutants are listed on the top, with the same color highlighting the mutants in the first row. Different soil batches or plant cultivation trays in which the mutants were grown are shown in different colors in the second and third rows, respectively. The remaining rows represent the elements quantified in each mutant. The wild-type Col-0 and the *frd3* mutant were used as controls and grown in all plant cultivation trays.

(**B**) Hierarchical clustering of 349 *A. thaliana* natural accessions. Three accessions, Fab-2, Cvi-2, and Ts-1, were grown in each plant cultivation tray as controls and are highlighted by various colors in the first row. The second row highlights the accessions with causal genes that have been cloned, and accessions are shown in the same colors as the elements and causal gene names in the far right column. Different colors in the third row indicate different plant cultivation trays. The remaining rows represent the elements quantified in each accession.

reinforcing the conclusion that mutants with a similar ionomic phenotype are reliably clustered together based on this phenotype.

The *enhanced suberin1-1* (*esb1-1*) mutant (Baxter et al., 2009) is the first ionomic mutant to be cloned from the forward genetic screen reported by Lahner et al. (2003), who originally named it 145:01. *ESB1* encodes a dirigent-domain-containing protein. Mutation of *ESB1* causes the disruption of the normal deposition of lignin required for Casparian strip formation, leading to ectopic deposition of lignin and suberin at the endodermis (Hosmani et al., 2013; with commentary by Halpin, 2013). The *esb1* mutant displays a multielement ionomic phenotype, with accumulation of lower concentrations of Ca, Mn, and Zn and higher levels of Na, S, K, As, Se, and Mo in leaves. A second mutant isolated by Lahner et al. (2003) named 112:50, which also shows a complex multielement ionomic phenotype, was recently identified as *myb36-1* (Kamiya et al., 2015; with commentary by Franke, 2015). Detailed analysis revealed that the transcription factor MYB36 directly regulates the expression of the genes involved in the formation of Casparian strips, including *ESB1*, *Casparian strip domain proteins* (*CASP*s), and *Peroxidase 64* (*PER64*) (Kamiya et al., 2015). Loss of function of *MYB36* leads to the absence of Casparian strips and deposition of ectopic lignin and suberin at the endodermis. These changes lead to an elevated concentration of Na, Mg, and Zn, and a decreased concentration of Ca, Mn, and Fe in the leaves (Kamiya et al., 2015). Ectopic expression of *MYB36* is sufficient to build Casparian strip-like structures in the cortex and the epidermis. Even though the Casparian strip-like structures are not perfectly formed, it would be interesting to determine whether and how such additional structures affect the leaf ionome. The *tsc10a-1* mutant (Chao et al., 2011), which alters sphingolipid biosynthesis, is another mutant showing enhanced suberin deposition in the root from the screen of Lahner et al. (2003), in which it was originally named 71:13. Even though the detailed mechanism of enhanced suberin deposition in this mutant is not clear, the *tsc10a* mutant shows a clearly altered leaf ionome, including increases in Na, K, and Rb and decreases in Mg, Ca, Fe, and Mo (Chao et al., 2011). Given the significant role the Casparian strip is now known to play in regulating the leaf ionome (Baxter et al., 2009; Hosmani et al., 2013; Pfister et al., 2014; Kamiya et al., 2015), it would be of great interest to determine the integrity of Casparian strips in the *tsc10a* mutant and investigate whether *TSC10A* is required for the formation of Casparian strips. In a forward genetic screen for endodermal barrier mutants, the *schengen3* (*sgn3*) mutant was isolated, which displayed a defect in Casparian strip formation (Pfister et al., 2014). Unlike the Casparian strip mutants *esb1*, *myb36*, and the double mutant *casp1 casp3* (Hosmani et al., 2013), *sgn3* only has a Casparian strip defect and no ectopic endodermal suberin or lignin. The *sgn3* mutant shows several ionomic alterations, including low K and high Mg in leaves (Pfister et al., 2014). Interestingly, the Lahner et al. (2003) screen also identified the 117:20 mutant with reduced K and increased Mg. This mutant has recently been identified as a new allele of *sgn3* (Hermans, C., and Salt, D.E., unpublished results) (Figure 1A), further highlighting the importance of the Casparian strip in controlling the leaf ionome. The fact that alteration of a common biological system, such as endodermal diffusion barriers, leads to similar ionomic phenotypes raises the intriguing possibility that hierarchical clustering of ionomic mutants could help to identify novel mutants in the same biological pathway. This is supported by the fact that different alleles of *esb1*, *myb36*, and *tsc10a* cluster together independent of the plant cultivation tray or soil batch in which they were cultivated (Figure 1A). Uncharacterized mutants that cluster with mutants disrupted in known processes of interest, such as Casparian strip development, could therefore be prioritized for cloning.

As discussed above, many ionomic mutants have turned out to be Casparian strip mutants, indicating the critical role of Casparian strips in maintenance of the ionome in plants. It is now becoming clear that the Casparian strip-bearing endodermis acts as a barrier in plant roots to control the selective entry of water and mineral nutrients into the vascular system and translocation to the shoot (Geldner, 2013). The endodermis not only prevents mineral nutrients entering the stele from the cortex, but also stops the leak of mineral nutrients from the stele back to the cortex. When Casparian strips are not formed correctly, ions at high concentration in the cortex and low concentration in the stele will leak into the stele, whereas ions that are high in the stele and low in the cortex will leak out of the stele. Therefore, the complex changes observed in the leaf ionome in Casparian strip mutants are likely to be dependent on the gradient of ions across the endodermis (Kamiya et al., 2015). Loss of *SGN3* function causes Casparian strips with gaps to form, leading to reduced leaf K and increased Mg, suggesting that the concentration of K is normally higher in the stele compared with the cortex, while the concentration of Mg is lower in the stele relative to the cortex. The ionomic changes of *esb1* and *myb36* are complex, which reflects the fact that these two mutants not only have defects in Casparian strip formation but also compensatory ectopic deposition of lignin and suberin at the endodermis. Such ectopic deposition of lignin and suberin would be expected to affect both apoplastic and transmembrane ion transport across the endodermis. Indeed, recent studies have shown that endodermal suberization is dynamic and responsive to mineral nutrient status (Barberon et al., 2016).

The forward genetic screen for ionomic mutants reported by Lahner et al. (2003) also identified several non-Casparian strip mutants. The *nakr1-1* (for *sodium potassium root defective 1-1*, also called *npcc6*), reported by Lahner et al. (2003) as 136:31, is one of these mutants. *NaKR1* encodes a soluble metal binding protein and is required for the loading of Na and K into the phloem (Tian et al., 2010). The mutant *nakr1-1* accumulates higher level of Na, K, and Rb, although the molecular function of *NaKR1* remains unknown. Another mutant from this screen named 126:45 was identified as a new loss-of-function allele of *CONSTITUTIVE EXPRESSER OF PATHOGENESIS RELATED GENES 5* (*CPR5*), which accumulate low K in leaves. The low-K phenotype is independent of both the elevated salicylic acid and jasmonic acid that is required for the local resistance to pathogens in the *cpr5* mutants (Borghi et al., 2011). The molecular mechanisms underlying the link between CPR5 and the low-K phenotype are currently unclear. However, increased expression of various *CYCLIC NUCLEOTIDE GATED CHANNELS* (*CNGCs*) in the shoot of *cpr5* and decreased expression of the high-affinity potassium transporter *HAK5* in the roots may constitute part of the mechanism (Borghi et al., 2011).

An additional forward genetic screen of 3162 EMS mutagenized plants (D.E. Salt, unpublished results), following the protocol of

Lahner et al. (2003), but with plants grown under either Fe or phosphate deficiency, identified a further 36 leaf ionomic mutants. Genetic analyses of these mutants has identified new alleles of *BRUTUS* (Hindt, M.N., et al., unpublished results), *SOS1* (Brazelton, J., and Salt, D.E., unpublished results), and *MOT1* (Andreatta, M.A., and Salt, D.E., unpublished results). Recent work from the laboratory of Niko Geldner (Alassimone, J., Fujita, S., et al., unpublished results) has identified *Schengen-1* as a kinase that plays a critical role in Casparian strip formation. Interestingly, analysis of two high Mg mutants identified in our recent screen revealed them both to be loss-of-function allele of *sgn1* (Kamiya, T., and Salt, D.E., unpublished results). Work on novel mutants with high leaf S and high leaf Na from this same screen is also ongoing to identify and characterize the genes involved.

Importantly, the bulk segregant approach in F2 mapping populations has also failed to work. Generally this has been due to a lack of penetrance of the ionomic mutant phenotype in F2 progeny of an outcross mapping population. This suggests that natural variation in the outcross parent can suppress the mutant phenotype. The recent use of background mutations in the mutagenized line as markers for mapping (Abe et al., 2012) means that a backcrossed F2 mapping population can now be used, overcoming issues of low mutant phenotype penetrance in an outcrossed F2 population.

IDENTIFICATION OF IONOMIC ALLELES FROM NATURAL ACCESSIONS

Genetic variation in natural accessions provides abundant resources for identification of quantitative trait locus (QTL) and causal genes. In our study of 349 *A. thaliana* accessions from the HapMap population (Baxter et al., 2010), we observed large variation in most of the 19 elements we quantified (Figure 1B). Unsupervised hierarchical clustering of these accessions based on their leaf ionomic phenotypes revealed that the clustering of accessions is largely independent of the experimental blocks (plant cultivation trays) in which they were grown. Rather, the accessions tend to be clustered together based on their ionomic profiles. This is supported by the clustering of the three accessions Fab-2 (low Zn), Cvi-2 (high Mo), and Ts-1 (high Na) with known leaf ionomic profiles that were included in each plant cultivation tray as controls (Figure 1B). The robustness of this clustering is further supported by the fact that chemical analogs, such as S and Se, and K and Rb, which are known to co-vary in *A. thaliana* (Broadley et al., 2010), are grouped together, as would be expected for elements that are likely regulated by the same processes in plants. The macronutrients Ca and Mg are also known to be co-regulated in *A. thaliana* (Broadley et al., 2010), and these elements are also grouped together in our clustering (Figure 1B). Taken together, these results afford further validation of the high-throughput ionomic approach, providing justification for the use of this set of natural accessions for identification of ionomic QTLs and causal genes.

Traditional linkage mapping or QTL mapping using biparental crosses made between accessions from the phenotypic extremes have been successful in identifying genes responsible for variation in several ionomic traits. The *HKT1;1* gene encoding an Na transporter was identified to be the causal gene controlling high leaf Na in the *A. thaliana* accessions Ts-1 and Tsu-1 (Rus et al., 2006). Reduced expression of *HKT1;1* is responsible for the overaccumulation of Na in the leaves of these accessions, possibly driven by a deletion in the promoter (Rus et al., 2006; Baxter et al., 2010). Whether variation in the coding regions of *HKT1;1* also plays a role is unclear. Using a similar approach, a polymorphism in the coding region of *FPN2* (also known as *IREG2*), a ferroportin upregulated by Fe deficiency (Schaaf et al., 2006), was identified to be causal for variation in leaf Co in accessions Ts-1 and Se-0 (Morrissey et al., 2009). Further work suggested that this variation in *FPN2* is involved in alterations in Fe homeostasis (Morrissey et al., 2009). Mapping using DNA microarray-based BSA in phenotyped F2 populations has also been successful at identifying *Adenosine 5′-Phosphosulfate Reductase2* (*APR2*) as the causal gene for variation in total leaf S and Se in the Hod *A. thaliana* accession (Chao et al., 2014b). The variation in amino acid sequence of APR2 changes the enzymatic rate, resulting in variation in sulfate and selenate reduction and higher accumulation of S and Se. Furthermore, an analysis of APR2 alleles across 855 *A. thaliana* accessions revealed 11 haplotypes with amino acid changes in conserved regions of the protein. Enzymatic analysis revealed that activity of APR2 varies by four orders of magnitude across the *A. thaliana* species. However, the reason for such variation remains unknown (Chao et al., 2014b). Interestingly, this F2 mapping approach sometimes fails to identify clear QTLs. The most striking example of this was the failure to identify the genetic basis of elevated K in the Wa-1 accession. Further analysis revealed that this was because it was the cytotype and not the genotype of Wa-1 that was driving elevated leaf K. To our surprise, the ploidy level of the root was controlling variation in leaf K (Chao et al., 2013).

A second successful approach used to identify ionomic QTLs is to use recombinant inbred lines (RILs) developed by crossing two parental lines and then selfing for multiple generations to generate essentially immortal homozygous lines. QTL analyses in the Bay-0 × Shahdara RIL population also identified *APR2* as being causal for a QTL controlling variation in leaf sulfate concentrations (Loudet et al., 2007). This RIL population was further used to identify *ATP sulfurylase 1* (*ATPS1*) as a second gene controlling variation in leaf sulfate accumulation (Koprivova et al., 2013). Unlike the variation in the coding region of *APR2*, which drives the S phenotypes (Loudet et al., 2007; Chao et al., 2014b), a deletion in the first intron of *ATPS1*, resulting in lower *ATPS1* transcript accumulation, is responsible for the ATPS1 controlled sulfate phenotype (Koprivova et al., 2013). Thus, so far, natural variation in the total S or sulfate accumulation in *A. thaliana* is controlled by APR2 and ATPS1, two key enzymes in the sulfate reduction and assimilation pathway.

Combining bulk segregant-based mapping in an F2 population with analysis of RILs has also proved to be a fruitful approach (Baxter et al., 2008a). To identify the casual locus driving low Mo in the L*er*-0 accession, we performed DNA microarray-based BSA on a phenotyped Col-0 × L*er*-0 F2 mapping population. This was followed by using selected RILs from an existing Col-0 × L*er*-0 RIL population with informative recombination break points to fine-map the causal locus. This revealed that variation at *Molybdenum Transporter 1* (*MOT1*) is responsible for natural variation of leaf Mo in L*er*-0 (Tomatsu et al., 2007; Baxter et al., 2008a). The functional

polymorphism in *MOT1* in L*er*-0 is a 53-bp deletion in the promoter (Tomatsu et al., 2007; Baxter et al., 2008a). However, further analysis of the *MOT1* promoter in 283 accessions revealed a total of six non-coding structural polymorphisms (Forsberg et al., 2015). Of these six polymorphisms, two were significantly associated with variation in leaf Mo across a species-wide collection of 340 accessions. The 53-bp deletion originally identified in L*er*-0 is associated with decreased leaf Mo, and a duplicated 330-bp insertion in the promoter that has undergone a 4-bp deletion was associated with elevated leaf Mo. These polymorphisms appear to act to control Mo in leaves by either decreasing or increasing *MOT1* expression, respectively. However, an amino acid polymorphism in *MOT1* from the *A. thaliana* Sha accessions is fully associated with the phenotypic effect of this weak allele across ~300 genotyped accessions, suggesting that polymorphisms in the coding region of *MOT1* are also important (Poormohammad Kiani et al., 2012).

The major limitation of linkage mapping or QTL mapping is that it only exploits the variation between two genotypes (biparental cross) or among a few genotypes (multiple accession intercross). However, allelic diversity is much higher in natural populations. Genome-wide association (GWA) analysis is an ideal approach for exploiting this extensive allelic variation in natural populations to uncover important genotype-to-phenotype links. GWA has become a powerful approach to isolate the QTLs or genes controlling phenotypic variation in plants, such as flowering time and disease resistance (Weigel and Nordborg, 2015). Several natural alleles controlling ionomic traits have also been identified using GWA mapping. In the first comprehensive GWA study in *A. thaliana*, 107 traits including the leaf concentration of 18 elements were analyzed in 96–192 accessions (Atwell et al., 2010). Significant single nucleotide polymorphisms (SNPs) associated with leaf Na were identified in the genomic region surrounding *HKT1;1*, a gene encoding a known Na transporter. However, significant SNPs for other elements were not identified, perhaps because of the small numbers of accessions (n = 93) used in the ionomic portion of the study. Using a larger set of 349 accessions genotyped at ~250 000 SNPs, which represents a large portion of the species-wide diversity in *A. thaliana* (Baxter et al., 2010), we have identified four ionomic loci using GWA mapping. *HKT1;1* was identified as controlling 32% of the variation in leaf Na accumulation across this species-wide collection (Baxter et al., 2010). Using GWA mapping coupled with linkage mapping and transgenic complementation, natural variation in leaf Cd accumulation in *A. thaliana* was revealed to be primarily governed by allelic diversity in the coding region of *HMA3* (Chao et al., 2012). Variation at the *HMA3* locus accounts for 30% of the total variation of leaf Cd in the 349 accessions. HMA4 is also a key protein required for Cd accumulation and tolerance in *A. thaliana* (Hussain et al., 2004; Mills et al., 2010) and *Arabidopsis halleri* (Hanikenne et al., 2008). However, genetic variation at *HMA4* appears to not be a major contributor to natural variation in leaf Cd accumulation in *A. thaliana* (Chao et al., 2014a, 2014b).

GWA analysis was also successful at identifying the *HAC1* gene in controlling variation in As concentration in leaves (Chao et al., 2014a). *HAC1* encodes an arsenate reductase, which has also been shown to control natural variation of arsenate tolerance in *A. thaliana* (Sanchez-Bermejo et al., 2014). Variation at *HAC1* accounts for 18% of the total variation in leaf As accumulation in this species-wide collection of *A. thaliana*. GWA mapping is powerful at detecting alleles that have large trait effects and occur at relatively high frequency in the population. However, this approach is not always successful if the causal allele is rare in the population. For example, *APR2* was not identified in a GWA scan for genetic associations with variation in total leaf S accumulation, most likely because the weak *APR2* allele is rare in this collection of 349 accessions (Chao et al., 2014b). Furthermore, traditional GWA mapping is based on the mean difference in phenotype between alternative genotypes at each marker and is unable to detect causal associations if the genes they tag have multiple alleles with opposite effects on traits. Such alleles can, however, be detected if GWA mapping is based on the phenotypic variance between the alternative genotypes, an analysis known as variance heterogeneity GWA, or vGWA. The identification of *MOT1* as the causal gene for variation in leaf Mo is one of the successful examples of vGWA (Shen et al., 2012). The strong vGWA signal observed at *MOT1* was further fine-mapped and determined to be driven by the presence of three independent genetic polymorphisms all in strong linkage disequilibrium (LD) with the markers displaying the genetic variance heterogeneity. Two of the three genetic polymorphisms are promoter variants of *MOT1*, and the third a variant located ~25 kb downstream of *MOT1*. The fact that one variant tags three different minor alleles with different effects on the mean Mo concentration explains the increased phenotypic variance of the group of accessions with these genotypes (Forsberg et al., 2015). It is this allelic heterogeneity around *MOT1* that makes it difficult to detect significant SNPs associated with leaf Mo in 96 accessions (Atwell et al., 2010), and to barely detect significant SNPs in the 340 accessions (Forsberg et al., 2015), when using mean-based GWA analysis. Using a multilocus mixed modeling approach to reanalyze the leaf Na data of Baxter et al. (2010) determined that the optimal model contained not one but six SNPs that together explained 52.6% of the total phenotypic variation. This was taken as evidence for allelic heterogeneity also at *HKT1;1* (Segura et al., 2012). To be comprehensive, GWA approaches will therefore require consideration of the allelic heterogeneity that may underlie ionomic traits. Given the relatively small LD of 10 kb (Kim et al., 2007) in *A. thaliana*, GWA mapping offers a method to rapidly map to only a handful of candidate genes. In our four successful examples of using GWA mapping to identify loci controlling variation in Na, Cd, Mo, and As, we found the peak associated SNPs to be within the coding region of *HKT1;1*, 3.1 kb from *HMA3*, 15.4 kb from *MOT1*, and 19.8 kb from *HAC1*. Therefore, based on our experience a combination of traditional linkage mapping in biparental crosses with parents chosen from the phenotypic extremes for rare alleles, coupled with GWA mapping (with models to detect single and multiple loci), appears to be a good approach for identification of the loci controlling natural ionomic variation.

IONOMICS AND ENVIRONMENTAL ADAPTATION

As sessile organisms, plants have evolved sophisticated mechanisms to adapt and survive in a diverse range of environments,

including highly heterogeneous soils. Plants take up most of the mineral nutrients and trace elements they require from the soil. Therefore, the ionomic profile of plants grown in a common environment may reflect adaptations to their native local environment (Baxter et al., 2010; and commentary by Anderson and Mitchell-Olds, 2010). As discussed above, several natural alleles controlling the variation of ionomic traits have been identified. The identification of such natural allelic variants may allow a new approach to investigate the molecular basis of adaptation to native soils. Recent work by Busoms et al. (2015) has identified clear signals of local adaptation in *A. thaliana* to elevated soil salinity at the coast in Catalonia, with salt-tolerant coastal populations and salt-sensitive inland populations being separated by less than 30 km. Clear selective edaphic agents that act over short distances to maintain differentiated subpopulations of *A. thaliana* therefore do exist, and it is not unreasonable that at least in some cases this local adaptation could be driven by ionomic loci.

HKT1;*1* is one of the candidate genes potentially involved in adaptation to saline soil. The Na transporter HKT1; 1 functions in Na homeostasis by removing Na^+ from the xylem sap to regulate Na accumulation in the leaves (Munns and Tester, 2008). Natural accessions with a weak allele of *HKT1*;*1* accumulate higher concentrations of Na, and this allelic variation in the strength of *HKT1;1* appears to be modulated at the level of gene expression (Rus et al., 2006; Baxter et al., 2010). Furthermore, the ability to accumulate Na in leaves was found to be strongly associated with coastal habitats and other potential saline soils (Baxter et al., 2010). Accessions with elevated leaf Na containing the weak allele of *HKT1;1* are therefore more likely to grow in potentially saline-affected soils, suggesting a role for *HKT1*;*1* in adaptation to soil salinity. Multiple haplotypes containing weak alleles of *HKT1;1* exist (Baxter et al., 2010), suggesting repeated evolution to saline-affected environments. However, since the salinity level in the native habitat where each accession was collected is not available, further fieldwork is required to confirm that allelic variation at *HKT1*;*1* is in fact linked to soil salinity. Using a multiple-year field-based reciprocal transplant experiment, we have recently shown that *A. thaliana* accessions collected near the coast generally outperform those from adjacent inland habitats when grown under high salinity, indicating a local adaptation to soil salinity (Busoms et al., 2015). The next step is to perform a high-density collection of the adjacent coastal and inland populations and the soils from the plants' native habitats. Genotyping the *HKT1*;*1* locus in the population and determining the salinity level of the local soil could reveal whether *HKT1*;*1* functions in adaptation to soil salinity. Such studies are currently ongoing.

Mo is an essential element for plants and is required for enzymes involved in nitrate assimilation, sulfite detoxification, purine catabolism, and abscisic acid biosynthesis (Mendel, 2011). The bioavailability of Mo for plant growth is largely dependent on soil pH, and Mo deficiency is often observed in acidic soils. On the other hand, excess Mo could also have an impact on plant development (Kaiser et al., 2005). Across the *A. thaliana* species there is significant functional variation of *MOT1* that is involved in controlling variation in Mo accumulation (Baxter et al., 2008a; Forsberg et al., 2015). Furthermore, this variation also controls variation in tolerance to acid soils in *A. thaliana* (Poormohammad Kiani et al., 2012). Such allelic diversity suggests that *MOT1* may play an important role in adaptation. Indeed, West Asian *A. thaliana* accessions with the weak allele of *MOT1* occur in their native habitat on soils with high water-extractable Mo content (Poormohammad Kiani et al., 2012). It is notable that *Copper Transporter 6* (*COPT6*), nearby the *MOT1* loci, is also associated with natural variation in leaf Mo accumulation (Forsberg et al., 2015). *COPT6* is thought to play a role in Cu redistribution when Cu is limited in the environment (Garcia-Molina et al., 2013), and *MOT1* has been shown to be upregulated under Cu deficiency (Billard et al., 2014). Therefore, it is possible that *MOT1* and *COPT6* may play integrated roles in environmental adaptations that involve both Cu and Mo.

It is largely unknown whether other natural ionomic alleles also play a role in adaptation to the corresponding elements in soils, such as *APR2* and *ATPS1* for S, *HMA3* for Cd, or *HAC1* for As, and further studies are required to unravel this. However, it is notable that natural ionomic variation in different species tends to be controlled by genes from the same gene family, in some cases sharing the same type of causal polymorphisms. For example, variation in shoot Na concentration is controlled by *HKT1;1* in *A. thaliana* (Rus et al., 2006; Baxter et al., 2010), *HKT1;5/SKC1* in rice (Ren et al., 2005), and *HKT1;5-A* in wheat (James et al., 2006; Munns et al., 2012), all belonging to the class 1 subfamily of HKT transporters (Platten et al., 2006). Variation in the coding region of *HKT1;5/SKC1* is responsible for variation in salt tolerance and shoot Na and K concentrations in rice (Ren et al., 2005), while variation in the promoter region of *HKT1;1* appears to drive the high leaf Na in *A. thaliana* (Rus et al., 2006). The variation of leaf Mo in *Mimulus guttatus* is controlled by the homolog of *A. thaliana MOT1* (Lowry et al., 2012). *HMA3* is responsible for controlling variation in Cd accumulation in both *A. thaliana* (Chao et al., 2012) and rice (Ueno et al., 2010). Interestingly, the functional polymorphisms in *HMA3* in both *A. thaliana* and rice are in the coding regions. Such conservation suggests that selection is acting at the same loci across species, with similar ionomic consequences. Such conservation should provide a powerful pathway to translate discoveries of ionomic loci in *A. thaliana* into crops and other plants.

IONOMIC BIG DATA AND THE IHUB

Taking advantage of high-throughput elemental profiling, we are able to analyze the leaf ionome of more than 1000 samples per week sustainably over multiple years. In the past decade, we have analyzed 205 169 unique *A. thaliana* samples for 18 to 20 elements. This represents approximately 4 million publicly accessible data points from 15 369 unique lines/accessions, and includes 11 478 FN mutagenized plants, 28 357 T-DNA mutagenized plants (representing 3452 genes), 21 960 EMS mutagenized plants, and 40 388 wild-type plants including 753 different accessions and 1474 inbred lines. All data can be downloaded from the iHUB at www.ionomicshub.org. To effectively utilize this scale of data it is essential to develop data management tools to appropriately store, retrieve, and analyze such data. We have developed the iHUB cyberinfrastructure to facilitate the management of these large ionomic datasets (Baxter

et al., 2007; Danku et al., 2013; Salt et al., 2014). The iHUB provides integrated workflow control for data acquisition and validation, data storage, data search and retrieval, and data visualization. Given that the plant ionome is highly dependent on the growth stage, tissue, and environment in which plants are cultivated, the iHUB captures all information relating to cultivation, harvesting, sample preparation, elemental analysis, and data processing. Such information includes the samples' genotype, planting and harvesting date, tissue type, growth media and watering conditions, growth environment, and ICP-MS analytical conditions. Common genotypes are also included in each plant cultivation tray. This provides the ability to compare samples from plants cultivated and analyzed years apart. The iHUB also contains tools for data discovery, data annotation, data sharing, and data publication. Public users are able to search the data based on either line/accession name or ionomic phenotype. Multiple sets of selected data can be combined into unique datasets, which can be associated with a stable digital object identifier for citation in publications. Workgroups can also be set up to share private datasets between collaborators. An Ionomics Atlas (Dragut et al., 2012) accessed via the iHUB has also been developed to connect the leaf ionome of *A. thaliana* populations with their landscape distribution, which includes ionomic data and associated genetic loci for 348 accessions of the HapMap collection, and the geographic location, climate data (temperature, humidity, pressure, and precipitation) and soil properties of the habitat where the accessions were collected. The Ionomics Atlas allows users to access and explore the interconnection of ionomic, genomic, and environmental data through a Google Maps interface. Such a tool allows an investigation of the potential role of natural ionomic variation in adaptation to the landscape. Currently the iHUB not only includes ionomic data of *A. thaliana* but also data of other species, including rice (Zhang et al., 2014; Pinson et al., 2015), soybean, maize, and yeast (Yu et al., 2012). The iHUB is useful to directly test gene function and identify potentially interesting mutants for further study. Since it first went live in August 2007, the iHUB has had 15 687 unique users from 2217 cities in 124 countries.

CONCLUSIONS AND PERSPECTIVES

Ionomics has emerged over the last decade as a powerful tool for successfully identifying genes and gene networks that control mineral nutrient and trace element homeostasis. Such success has required the timely integration of the analytical methods of high-throughput elemental profiling with modern molecular genetics. The molecular, cellular, and physiological functions of genes discovered using this approach are starting to be unraveled, although the road from gene to function (and publication) can still be a long one. Ionomics has also yielded advances in our understanding of the loci that control natural ionomic variation. However, the ecological functions of allelic variants of ionomic loci, if any, are still largely unknown. With the release of the RegMap panel of 1307 *A. thaliana* accessions (Horton et al., 2012), which combines both regional and species-wide collections with accurate information on the collection site of each accession, and the increasing availability of whole-genome sequences of more than 1000 accessions (http://1001genomes.org/), it is now becoming possible to probe the potential ecological functions of ionomic loci across the genome. Such opportunities are leading to what might be termed "landscape ionomics," which will require the coupling of ionomics, population genomics, and field-based ecological studies.

FUNDING

UK Biotechnology and Biological Sciences Research Council grant BB/L027739/1 and BB/L000113/1 (to D.E.S.), the US National Institutes of Health grant 2R01GM078536 (to D.E.S.), and the US National Science Foundation grant IOB 0419695 (to D.E.S.).

ACKNOWLEDGMENTS

We wish to thank our collaborators Mary Lou Guerinot, Niko Geldner, and Christian Hermans for kindly allowing us to incorporate in this update unpublished data on *BRUTUS*, *SGN1*, and *SGN3*, respectively. We also thank Mary Lou Guerinot, Niko Geldner, Takehiro Kamiya, and the ERA-CAPS Root Barrier project for productive discussions relating to ionomics and the plant ionome. No conflict of interest declared.

Received: March 25, 2016
Revised: May 12, 2016
Accepted: May 16, 2016
Published: May 19, 2016

REFERENCES

Abe, A., Kosugi, S., Yoshida, K., Natsume, S., Takagi, H., Kanzaki, H., Matsumura, H., Mitsuoka, C., Tamiru, M., Innan, H., et al. (2012). Genome sequencing reveals agronomically important loci in rice using MutMap. Nat. Biotechnol. **30**:174–178.

Anderson, J.T., and Mitchell-Olds, T. (2010). Beyond QTL cloning. PLoS Genet. **6**:e1001197.

Atwell, S., Huang, Y.S., Vilhjalmsson, B.J., Willems, G., Horton, M., Li, Y., Meng, D., Platt, A., Tarone, A.M., Hu, T.T., et al. (2010). Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines. Nature **465**:627–631.

Barberon, M., Vermeer, J.E., De Bellis, D., Wang, P., Naseer, S., Andersen, T.G., Humbel, B.M., Nawrath, C., Takano, J., Salt, D.E., et al. (2016). Adaptation of root function by nutrient-induced plasticity of endodermal differentiation. Cell **164**:447–459.

Baxter, I. (2015). Should we treat the ionome as a combination of individual elements, or should we be deriving novel combined traits? J. Exp. Bot. **66**:2127–2131.

Baxter, I., Ouzzani, M., Orcun, S., Kennedy, B., Jandhyala, S.S., and Salt, D.E. (2007). Purdue ionomics information management system. An integrated functional genomics platform. Plant Physiol. **143**:600–611.

Baxter, I., Muthukumar, B., Park, H.C., Buchner, P., Lahner, B., Danku, J., Zhao, K., Lee, J., Hawkesford, M.J., Guerinot, M.L., et al. (2008a). Variation in molybdenum content across broadly distributed populations of *Arabidopsis thaliana* is controlled by a mitochondrial molybdenum transporter (MOT1). PLoS Genet. **4**:e1000004.

Baxter, I.R., Vitek, O., Lahner, B., Muthukumar, B., Borghi, M., Morrissey, J., Guerinot, M.L., and Salt, D.E. (2008b). The leaf ionome as a multivariable system to detect a plant's physiological status. Proc. Natl. Acad. Sci. USA **105**:12081–12086.

Baxter, I., Hosmani, P.S., Rus, A., Lahner, B., Borevitz, J.O., Muthukumar, B., Mickelbart, M.V., Schreiber, L., Franke, R.B., and Salt, D.E. (2009). Root suberin forms an extracellular barrier that affects water relations and mineral nutrition in *Arabidopsis*. PLoS Genet. **5**:e1000492.

Baxter, I., Brazelton, J.N., Yu, D., Huang, Y.S., Lahner, B., Yakubova, E., Li, Y., Bergelson, J., Borevitz, J.O., Nordborg, M., et al. (2010). A coastal cline in sodium accumulation in *Arabidopsis thaliana* is driven by natural variation of the sodium transporter AtHKT1;1. PLoS Genet. **6**:e1001193.

Baxter, I.R., Gustin, J.L., Settles, A.M., and Hoekenga, O.A. (2013). Ionomic characterization of maize kernels in the intermated B73 x Mo17 population. Crop Sci. **53**:208–220.

Baxter, I.R., Ziegler, G., Lahner, B., Mickelbart, M.V., Foley, R., Danku, J., Armstrong, P., Salt, D.E., and Hoekenga, O.A. (2014). Single-kernel ionomic profiles are highly heritable indicators of genetic and environmental influences on elemental accumulation in maize grain (*Zea mays*). PLoS One **9**:e87628.

Billard, V., Ourry, A., Maillard, A., Garnica, M., Coquet, L., Jouenne, T., Cruz, F., Garcia-Mina, J.M., Yvin, J.C., and Etienne, P. (2014). Copper-Deficiency in *Brassica napus* induces copper remobilization, molybdenum accumulation and modification of the expression of chloroplastic proteins. PLoS One **9**:e109889.

Borghi, M., Rus, A., and Salt, D.E. (2011). Loss-of-function of *Constitutive Expresser of Pathogenesis Related Genes5* affects potassium homeostasis in *Arabidopsis thaliana*. PLoS One **6**:e26360.

Broadley, M.R., Hammond, J.P., White, P.J., and Salt, D.E. (2010). An efficient procedure for normalizing ionomics data for *Arabidopsis thaliana*. New Phytol. **186**:270–274.

Busoms, S., Teres, J., Huang, X.Y., Bomblies, K., Danku, J., Douglas, A., Weigel, D., Poschenrieder, C., and Salt, D.E. (2015). Salinity is an agent of divergent selection driving local adaptation of *Arabidopsis* to coastal habitats. Plant Physiol. **168**:915–929.

Chao, D.Y., Gable, K., Chen, M., Baxter, I., Dietrich, C.R., Cahoon, E.B., Guerinot, M.L., Lahner, B., Lu, S., Markham, J.E., et al. (2011). Sphingolipids in the root play an important role in regulating the leaf ionome in *Arabidopsis thaliana*. Plant Cell **23**:1061–1081.

Chao, D.Y., Silva, A., Baxter, I., Huang, Y.S., Nordborg, M., Danku, J., Lahner, B., Yakubova, E., and Salt, D.E. (2012). Genome-wide association studies identify heavy metal ATPase3 as the primary determinant of natural variation in leaf cadmium in *Arabidopsis thaliana*. PLoS Genet. **8**:e1002923.

Chao, D.Y., Dilkes, B., Luo, H., Douglas, A., Yakubova, E., Lahner, B., and Salt, D.E. (2013). Polyploids exhibit higher potassium uptake and salinity tolerance in *Arabidopsis*. Science **341**:658–659.

Chao, D.-Y., Chen, Y., Chen, J., Shi, S., Chen, Z., Wang, C., Danku, J.M., Zhao, F.-J., and Salt, D.E. (2014a). Genome-wide association mapping identifies a new arsenate reductase enzyme critical for limiting arsenic accumulation in plants. PLoS Biol. **12**:e1002009.

Chao, D.Y., Baraniecka, P., Danku, J., Koprivova, A., Lahner, B., Luo, H., Yakubova, E., Dilkes, B., Kopriva, S., and Salt, D.E. (2014b). Variation in sulfur and selenium accumulation is controlled by naturally occurring isoforms of the key sulfur assimilation enzyme ADENOSINE 5′-PHOSPHOSULFATE REDUCTASE2 across the *Arabidopsis* species range. Plant Physiol. **166**:1593–1608.

Chen, Z., Watanabe, T., Shinano, T., Okazaki, K., and Mitsuru, O. (2009). Rapid characterization of plant mutants with an altered ion-profile: a case study using *Lotus japonicus*. New Phytol. **181**:795–801.

Danku, J.M., Lahner, B., Yakubova, E., and Salt, D.E. (2013). Large-scale plant ionomics. Methods Mol. Biol. **953**:255–276.

Delhaize, E. (1996). A metal-accumulator mutant of *Arabidopsis thaliana*. Plant Physiol. **111**:849–855.

Dragut, E., Ouzzani, M., Madkour, A., Mohamed, N., Baker, P., and Salt, D.E. (2012). Ionomics Atlas: a tool to explore interconnected ionomic, genomic and environmental data. In Proceedings of the 21st ACM International Conference on Information and Knowledge Management (New York, NY, USA: Association for Computing Machinaery (ACM)), pp. 2680–2682.

Durrett, T.P., Gassmann, W., and Rogers, E.E. (2007). The FRD3-mediated efflux of citrate into the root vasculature is necessary for efficient iron translocation. Plant Physiol. **144**:197–205.

Eide, D.J., Clark, S., Nair, T.M., Gehl, M., Gribskov, M., Guerinot, M.L., and Harper, J.F. (2005). Characterization of the yeast ionome: a genome-wide analysis of nutrient mineral and trace element homeostasis in *Saccharomyces cerevisiae*. Genome Biol. **6**:R77.

Franke, R.B. (2015). Caspary's conductor. Proc. Natl. Acad. Sci. USA **112**:10084–10085.

Forsberg, S.K.G., Andreatta, M.E., Huang, X.-Y., Danku, J., Salt, D.E., and Carlborg, Ö. (2015). The multi-allelic genetic architecture of a variance-heterogeneity locus for molybdenum concentration in leaves acts as a source of unexplained additive genetic variance. PLoS Genet. **11**:e1005648.

Garcia-Molina, A., Andres-Colas, N., Perea-Garcia, A., Neumann, U., Dodani, S.C., Huijser, P., Penarrubia, L., and Puig, S. (2013). The *Arabidopsis* COPT6 transport protein functions in copper distribution under copper-deficient conditions. Plant Cell Physiol. **54**:1378–1390.

Geldner, N. (2013). The endodermis. Annu. Rev. Plant Biol. **64**:531–558.

Gong, J.M., Waner, D.A., Horie, T., Li, S.L., Horie, R., Abid, K.B., and Schroeder, J.I. (2004). Microarray-based rapid cloning of an ion accumulation deletion mutant in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **101**:15404–15409.

Green, L.S., and Rogers, E.E. (2004). FRD3 controls iron localization in *Arabidopsis*. Plant Physiol. **136**:2523–2531.

Gu, R., Chen, F., Liu, B., Wang, X., Liu, J., Li, P., Pan, Q., Pace, J., Soomro, A.-A., Lübberstedt, T., et al. (2015). Comprehensive phenotypic analysis and quantitative trait locus identification for grain mineral concentration, content, and yield in maize (*Zea mays* L.). Theor. Appl. Genet. **128**:1777–1789.

Halpin, C. (2013). Cell biology: up against the wall. Curr. Biol. **23**:R1048–R1050.

Hanikenne, M., Talke, I.N., Haydon, M.J., Lanz, C., Nolte, A., Motte, P., Kroymann, J., Weigel, D., and Kramer, U. (2008). Evolution of metal hyperaccumulation required cis-regulatory changes and triplication of HMA4. Nature **453**:391–395.

Hirschi, K.D. (2003). Strike while the ionome is hot: making the most of plant genomic advances. Trends Biotechnol. **21**:520–521.

Horton, M.W., Hancock, A.M., Huang, Y.S., Toomajian, C., Atwell, S., Auton, A., Muliyati, N.W., Platt, A., Sperone, F.G., Vilhjalmsson, B.J., et al. (2012). Genome-wide patterns of genetic variation in worldwide *Arabidopsis thaliana* accessions from the RegMap panel. Nat. Genet. **44**:212–216.

Hosmani, P.S., Kamiya, T., Danku, J., Naseer, S., Geldner, N., Guerinot, M.L., and Salt, D.E. (2013). Dirigent domain-containing protein is part of the machinery required for formation of the lignin-based Casparian strip in the root. Proc. Natl. Acad. Sci. USA **110**:14498–14503.

Hussain, D., Haydon, M.J., Wang, Y., Wong, E., Sherson, S.M., Young, J., Camakaris, J., Harper, J.F., and Cobbett, C.S. (2004). P-type ATPase heavy metal transporters with roles in essential zinc homeostasis in *Arabidopsis*. Plant Cell **16**:1327–1339.

James, R.A., Davenport, R.J., and Munns, R. (2006). Physiological characterization of two genes for Na^+ exclusion in durum wheat, *Nax1* and *Nax2*. Plant Physiol. **142**:1537–1547.

Kaiser, B.N., Gridley, K.L., Ngaire Brady, J., Phillips, T., and Tyerman, S.D. (2005). The role of molybdenum in agricultural plant production. Ann. Bot. **96**:745–754.

Kamiya, T., Borghi, M., Wang, P., Danku, J.M., Kalmbach, L., Hosmani, P.S., Naseer, S., Fujiwara, T., Geldner, N., and Salt, D.E. (2015). The MYB36 transcription factor orchestrates Casparian strip formation. Proc. Natl. Acad. Sci. USA **112**:10533–10538.

Kim, S., Plagnol, V., Hu, T.T., Toomajian, C., Clark, R.M., Ossowski, S., Ecker, J.R., Weigel, D., and Nordborg, M. (2007). Recombination and

linkage disequilibrium in *Arabidopsis thaliana*. Nat. Genet. **39**:1151–1155.

Koprivova, A., Giovannetti, M., Baraniecka, P., Lee, B.R., Grondin, C., Loudet, O., and Kopriva, S. (2013). Natural variation in the ATPS1 isoform of ATP sulfurylase contributes to the control of sulfate levels in *Arabidopsis*. Plant Physiol. **163**:1133–1141.

Lahner, B., Gong, J., Mahmoudian, M., Smith, E.L., Abid, K.B., Rogers, E.E., Guerinot, M.L., Harper, J.F., Ward, J.M., McIntyre, L., et al. (2003). Genomic scale profiling of nutrient and trace elements in *Arabidopsis thaliana*. Nat. Biotechnol. **21**:1215–1221.

Loudet, O., Saliba-Colombani, V., Camilleri, C., Calenge, F., Gaudon, V., Koprivova, A., North, K.A., Kopriva, S., and Daniel-Vedele, F. (2007). Natural variation for sulfate content in *Arabidopsis thaliana* is highly controlled by APR2. Nat. Genet. **39**:896–900.

Lowry, D.B., Sheng, C.C., Zhu, Z., Juenger, T.E., Lahner, B., Salt, D.E., and Willis, J.H. (2012). Mapping of ionomic traits in *Mimulus guttatus* reveals Mo and Cd QTLs that colocalize with MOT1 homologues. PLoS One **7**:e30730.

Ma, S.M., Lee, S.G., Kim, E.B., Park, T.J., Seluanov, A., Gorbunova, V., Buffenstein, R., Seravalli, J., and Gladyshev, V.N. (2015). Organization of the mammalian ionome according to organ origin, lineage specialization, and longevity. Cell Rep. **13**:1319–1326.

Malinouski, M., Hasan, N.M., Zhang, Y., Seravalli, J., Lin, J., Avanesov, A., Lutsenko, S., and Gladyshev, V.N. (2014). Genome-wide RNAi ionomics screen reveals new genes and regulation of human trace element metabolism. Nat. Commun. **5**:3301.

Mascher, M., Gerlach, N., Gahrtz, M., Bucher, M., Scholz, U., and Dresselhaus, T. (2014). Sequence and ionomic analysis of divergent strains of maize inbred line B73 with an altered growth phenotype. PLoS One **9**:e96782.

Mendel, R.R. (2011). Cell biology of molybdenum in plants. Plant Cell Rep. **30**:1787–1797.

Mills, R.F., Valdes, B., Duke, M., Peaston, K.A., Lahner, B., Salt, D.E., and Williams, L.E. (2010). Functional significance of AtHMA4 C-terminal domain in planta. PLoS One **5**:e13388.

Morrissey, J., Baxter, I.R., Lee, J., Li, L., Lahner, B., Grotz, N., Kaplan, J., Salt, D.E., and Guerinot, M.L. (2009). The ferroportin metal efflux proteins function in iron and cobalt homeostasis in *Arabidopsis*. Plant Cell **21**:3326–3338.

Munns, R., and Tester, M. (2008). Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. **59**:651–681.

Munns, R., James, R.A., Xu, B., Athman, A., Conn, S.J., Jordans, C., Byrt, C.S., Hare, R.A., Tyerman, S.D., Tester, M., et al. (2012). Wheat grain yield on saline soils is improved by an ancestral Na^+ transporter gene. Nat. Biotechnol. **30**:360–364.

Norton, G.J., Deacon, C.M., Xiong, L.Z., Huang, S.Y., Meharg, A.A., and Price, A.H. (2010). Genetic mapping of the rice ionome in leaves and grain: identification of QTLs for 17 elements including arsenic, cadmium, iron and selenium. Plant Soil **329**:139–153.

Parent, S.E., Parent, L.E., Egozcue, J.J., Rozane, D.E., Hernandes, A., Lapointe, L., Hebert-Gentile, V., Naess, K., Marchand, S., Lafond, J., et al. (2013). The plant ionome revisited by the nutrient balance concept. Front Plant Sci. **4**:39.

Pfister, A., Barberon, M., Alassimone, J., Kalmbach, L., Lee, Y., Vermeer, J.E., Yamazaki, M., Li, G., Maurel, C., Takano, J., et al. (2014). A receptor-like kinase mutant with absent endodermal diffusion barrier displays selective nutrient homeostasis defects. Elife **3**:e03115.

Pineau, C., Loubet, S., Lefoulon, C., Chalies, C., Fizames, C., Lacombe, B., Ferrand, M., Loudet, O., Berthomieu, P., and Richard, O. (2012). Natural variation at the FRD3 MATE transporter locus reveals cross-talk between Fe homeostasis and Zn tolerance in *Arabidopsis thaliana*. PLoS Genet. **8**:e1003120.

Pinson, S.R.M., Tarpley, L., Yan, W., Yeater, K., Lahner, B., Yakubova, E., Huang, X.-Y., Zhang, M., Guerinot, M.L., and Salt, D.E. (2015). Worldwide genetic diversity for mineral element concentrations in rice grain. Crop Sci. **55**:294–311.

Platten, J.D., Cotsaftis, O., Berthomieu, P., Bohnert, H., Davenport, R.J., Fairbairn, D.J., Horie, T., Leigh, R.A., Lin, H.X., Luan, S., et al. (2006). Nomenclature for HKT transporters, key determinants of plant salinity tolerance. Trends Plant Sci. **11**:372–374.

Poormohammad Kiani, S., Trontin, C., Andreatta, M., Simon, M., Robert, T., Salt, D.E., and Loudet, O. (2012). Allelic heterogeneity and trade-off shape natural variation for response to soil micronutrient. PLoS Genet. **8**:e1002814.

Rea, P.A. (2003). Ion genomics. Nat. Biotechnol. **21**:1149–1151.

Ren, Z.H., Gao, J.P., Li, L.G., Cai, X.L., Huang, W., Chao, D.Y., Zhu, M.Z., Wang, Z.Y., Luan, S., and Lin, H.X. (2005). A rice quantitative trait locus for salt tolerance encodes a sodium transporter. Nat. Genet. **37**:1141–1146.

Rogers, E.E., and Guerinot, M.L. (2002). FRD3, a member of the multidrug and toxin efflux family, controls iron deficiency responses in *Arabidopsis*. Plant Cell **14**:1787–1799.

Roschzttardtz, H., Seguela-Arnaud, M., Briat, J.F., Vert, G., and Curie, C. (2011). The FRD3 citrate effluxer promotes iron nutrition between symplastically disconnected tissues throughout *Arabidopsis* development. Plant Cell **23**:2725–2737.

Rus, A., Baxter, I., Muthukumar, B., Gustin, J., Lahner, B., Yakubova, E., and Salt, D.E. (2006). Natural variants of AtHKT1 enhance Na^+ accumulation in two wild populations of *Arabidopsis*. PLoS Genet. **2**:e210.

Salt, D.E., Baxter, I., and Lahner, B. (2008). Ionomics and the study of the plant ionome. Annu. Rev. Plant Biol. **59**:709–733.

Salt, D.E., Ouzzani, M., Dragut, E., Baker, P., and Rangarajan, S. (2014). iHUB: an information and collaborative management platform for life sciences. In WWW Companion '14 Proceedings of the Companion Publication of the 23rd International Conference on World Wide Web Companion (New York, NY, USA: Association for Computing Machinaery (ACM)), pp. 139–142.

Sanchez-Bermejo, E., Castrillo, G., Del Llano, B., Navarro, C., Zarco-Fernandez, S., Martinez-Herrera, D.J., Leo-Del Puerto, Y., Munoz, R., Camara, C., Paz-Ares, J., et al. (2014). Natural variation in arsenate tolerance identifies an arsenate reductase in *Arabidopsis thaliana*. Nat. Commun. **5**:4617.

Sanchez-Rodriguez, E., Rubio-Wilhelmi, M.D., Cervilla, L.M., Blasco, B., Rios, J.J., Leyva, R., Romero, L., and Ruiz, J.M. (2010). Study of the ionome and uptake fluxes in cherry tomato plants under moderate water stress conditions. Plant Soil **335**:339–347.

Schaaf, G., Honsbein, A., Meda, A.R., Kirchner, S., Wipf, D., and von Wiren, N. (2006). *AtIREG2* encodes a tonoplast transport protein involved in iron-dependent nickel detoxification in *Arabidopsis thaliana* roots. J. Biol. Chem. **281**:25532–25540.

Segura, V., Vilhjalmsson, B.J., Platt, A., Korte, A., Seren, U., Long, Q., and Nordborg, M. (2012). An efficient multi-locus mixed-model approach for genome-wide association studies in structured populations. Nat. Genet. **44**:825–830.

Shen, X., Pettersson, M., Ronnegard, L., and Carlborg, O. (2012). Inheritance beyond plain heritability: variance-controlling genes in *Arabidopsis thaliana*. PLoS Genet. **8**:e1002839.

Sun, L., Yu, Y., Huang, T., An, P., Yu, D.X., Yu, Z.J., Li, H.X., Sheng, H.G., Cai, L., Xue, J., et al. (2012). Associations between ionomic profile and metabolic abnormalities in human population. PLoS One **7**:e38845.

Tian, H., Baxter, I.R., Lahner, B., Reinders, A., Salt, D.E., and Ward, J.M. (2010). *Arabidopsis* NPCC6/NaKR1 is a phloem mobile metal binding protein necessary for phloem function and root meristem maintenance. Plant Cell **22**:3963–3979.

Tomatsu, H., Takano, J., Takahashi, H., Watanabe-Takahashi, A., Shibagaki, N., and Fujiwara, T. (2007). An *Arabidopsis thaliana* high-affinity molybdate transporter required for efficient uptake of molybdate from soil. Proc. Natl. Acad. Sci. USA **104**:18807–18812.

Ueno, D., Yamaji, N., Kono, I., Huang, C.F., Ando, T., Yano, M., and Ma, J.F. (2010). Gene limiting cadmium accumulation in rice. Proc. Natl. Acad. Sci. USA **107**:16500–16505.

Watanabe, T., Broadley, M.R., Jansen, S., White, P.J., Takada, J., Satake, K., Takamatsu, T., Tuah, S.J., and Osaki, M. (2007). Evolutionary control of leaf element composition in plants. New Phytol. **174**:516–523.

Weigel, D., and Nordborg, M. (2015). Population genomics for understanding adaptation in wild plant species. Annu. Rev. Genet. **49**:315–338.

White, P.J., Broadley, M.R., Thompson, J.A., McNicol, J.W., Crawley, M.J., Poulton, P.R., and Johnston, A.E. (2012). Testing the distinctness of shoot ionomes of angiosperm families using the Rothamsted Park Grass Continuous Hay experiment. New Phytol. **196**:101–109.

Wu, D., Shen, Q., Cai, S., Chen, Z.H., Dai, F., and Zhang, G. (2013). Ionomic responses and correlations between elements and metabolites under salt stress in wild and cultivated barley. Plant Cell Physiol. **54**:1976–1988.

Yoshida, S., Date, Y., Akama, M., and Kikuchi, J. (2014). Comparative metabolomic and ionomic approach for abundant fishes in estuarine environments of. Jpn. Sci. Rep. **4**:7005.

Yu, D., Danku, J.M., Baxter, I., Kim, S., Vatamaniuk, O.K., Vitek, O., Ouzzani, M., and Salt, D.E. (2012). High-resolution genome-wide scan of genes, gene-networks and cellular systems impacting the yeast ionome. BMC Genomics **13**:623.

Zhang, M., Pinson, S.R.M., Tarpley, L., Huang, X.Y., Lahner, B., Yakubova, E., Baxter, I., Guerinot, M.L., and Salt, D.E. (2014). Mapping and validation of quantitative trait loci associated with concentrations of 16 elements in unmilled rice grain. Theor. Appl. Genet. **127**:137–165.

Zhao, F.J., Moore, K.L., Lombi, E., and Zhu, Y.G. (2014). Imaging element distribution and speciation in plant cells. Trends Plant Sci. **19**:183–192.

Ziegler, G., Terauchi, A., Becker, A., Armstrong, P., Hudson, K., and Baxter, I. (2013). Ionomic screening of field-grown soybean identifies mutants with altered seed elemental composition. Plant Genome **6** http://dx.doi.org/10.3835/plantgenome2012.07.0012.