



OPINION PAPER

Should we treat the ionome as a combination of individual elements, or should we be deriving novel combined traits?

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Abstract

It has been more than 10 years since the concept of the ionome, all of the mineral nutrients in a cell tissue or organism, was introduced. In the intervening years, ionomics, high throughput elemental profiling, has been used to analyse over 400 000 samples from at least 10 different organisms. There are now multiple published examples where an ionomics approach has been used to find genes of novel function, find lines or environments that produce foods with altered nutritional profiles, or define gene by environmental effects on elemental accumulation. In almost all of these studies, the ionome has been treated as a collection of independent elements, with the analysis repeated on each measured element. However, many elements share chemical properties, are known to interact with each other, or have been shown to have similar interactions with biological molecules. Accordingly, there is strong evidence from ionic studies that the elements of the ionome do not behave independently and that combinations of elements should be treated as the phenotypes of interest. In this review, I will consider the evidence that we have for the interdependence of the ionome, some of its causes, methods for incorporating this interdependence into analyses and the benefits, drawbacks, and challenges of taking these approaches.

Key words: Elemental profiling, environment, G×E, genetics, ionomics, plant nutrition.

Introduction

It has been more than 10 years since the concept of the ionome, all of the mineral nutrients in a cell tissue or organism, was introduced (Lahner *et al.*, 2003). In the intervening years, ionomics, high throughput elemental profiling, has been used to analyse over 400 000 samples from at least 10 different organisms (Broadley *et al.*, 2008; Wu *et al.*, 2008; Chen *et al.*, 2009; Sankaran *et al.*, 2009; Yu *et al.*, 2012; Ziegler *et al.*, 2013; Baxter *et al.*, 2014; Bus *et al.*, 2014; Malinouski *et al.*, 2014; Zhang *et al.*, 2014). There are now multiple published examples where an ionomics approach has been used to find genes of novel function, find lines or environments that produce foods with altered nutritional profiles, or define gene by environmental effects on elemental accumulation. In

almost all of these studies, the ionome has been treated as a collection of independent elements, with the analysis repeated on each measured element. However, many elements share chemical properties, are known to interact with each other, or have been shown to have similar interactions with biological molecules. Accordingly, there is strong evidence from ionic studies that the elements of the ionome do not behave independently and that combinations of elements should be treated as the phenotypes of interest. In this review, I will consider the evidence that we have for the interdependence of the ionome, some of its causes, methods for incorporating this interdependence into analyses and the benefits, drawbacks, and challenges of taking these approaches.

Evidence and cause

The strongest evidence that the elements of the ionome are interconnected is the identification of mutants in multiple organisms with multi-element phenotypes (Lahner *et al.*, 2003; Eide *et al.*, 2005; Chen *et al.*, 2009; Yu *et al.*, 2012; Ziegler *et al.*, 2013; Malinouski *et al.*, 2014). In *Arabidopsis*, many of these phenotypes have been shown to segregate as a single locus and quite a few have been cloned, verifying that a single genetic polymorphism can affect multiple elements (Fig. 1). The nature of the genes underlying these multi-element phenotypes reveals the wide variety of mechanisms that can affect multiple elements.

Transporters

Transport of elements across membranes is a critical step in their movement and regulation. While some transporters appear to be specific for single elements, studies of plant transporter mutants or heterologous expression of plant transporters in yeast have demonstrated multi-element specificity. Examples include the Fe/Mn/Cd specificity of IRT1 and the Fe/Co specificity of FPN2 (Korshunova *et al.*, 1999; Connolly *et al.*, 2002; Morrissey *et al.*, 2009). There are several examples where the plants will express a transporter that is less specific in order to increase the uptake of a scarce elemental nutrient, leading to the accumulation of deleterious elements (Rogers *et al.*, 2000; Zhao *et al.*, 2009, 2010; Mitani-Ueno *et al.*, 2011). Variation in FRD3, which encodes a citrate transporter, affects the interplay between Fe and Zn

homeostasis (Pineau *et al.*, 2012). This suggests that transporters of chelators, which have multi-element specificities, can affect multiple elements by controlling the quantity and localization of the chelators.

Alterations of plant physiological properties

Several mutants have been cloned that affect the physiological properties of a plant and thereby have a significant effect on the elemental content. One of the first ionic mutants to be cloned was the *esb1* gene (Baxter *et al.*, 2009), which affects the structure of the casparian strip (Hosmani *et al.*, 2013). Across dozens of independent experiments, as many as nine elements were significantly different between the mutant and the wild type. Further analysis of other mutants that modify the structure of the casparian strip has confirmed that the permeability of this key structure has a major effect on the balance of elements that make it to the shoot (Pfister *et al.*, 2014). For example, loss of the SGN3/GASSHO1 receptor-like kinase, which is critical for assembling the microdomains to build the casparian strip, leads to a K/Mg phenotype, although, surprisingly, most other elements are unaffected. Altering root structure (Hermans *et al.*, 2010) and phloem (Tian *et al.*, 2010) function have both been shown to affect multiple elements.

The co-ordinated response of several processes to an environmental stimulus can create a multi-element response in the ionome. Both low-Fe and low-P concentrations in the growth medium induced multi-element signatures that could be used to predict the soil condition. Mutants with a constitutive

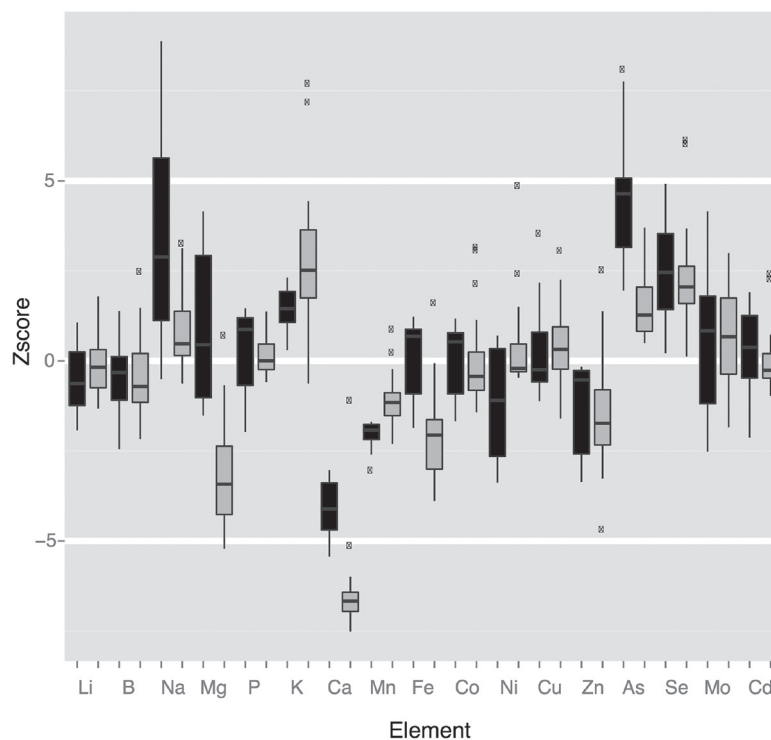


Fig. 1. Multi-element phenotype for the *esb1-2* mutant. Boxplots showing the distributions of Z-scores (number of standard deviations from the Col-0 mean) from trays 1095 (black) and 1146 (grey) at www.ionomicshub.org (last accessed: 15 December 2014). Note that Na, K, Ca, Mn, Zn, As, and Se are different in both trays, while Mg and Fe are only different in tray 1146.

low-Fe response have the same multi-element profile as plants grown in low-Fe conditions. Mutants can also alter several processes to create a multi-element phenotype. The TSC10A mutant, for example, alters sphingolipid biosynthesis, resulting in changes to endodermal suberin deposition (and probably casparian strip function) and Fe homeostasis that lead to a seven element phenotype that overlaps with the signatures of those perturbations (Chao *et al.*, 2011).

Studies of quantitative genetics populations provide additional, if indirect, evidence that the elements of the ionome are not independent of each other. Studies in multiple species have shown that many significant correlations between elements are observed across the lines of a population (Broadley *et al.*, 2008; Ghandilyan *et al.*, 2009a, b; Sankaran *et al.*, 2009; Buescher *et al.*, 2010; Bus *et al.*, 2014; Zhang *et al.*, 2014; Pinson *et al.*, 2015). However, which elements are correlated with each other changes across environments, populations, and species, suggesting that different processes are involved depending on the genotypes and environments involved. These studies have also identified quantitative trait loci (QTLs) for different elements that co-locate to the same region of the genome, suggesting that the same causal gene variant is driving the phenotypes. With the number of elements measured and the number of genes underlying a QTL, it is possible but not likely that these co-localizations are due to random chance. This question will be resolved in the next few years as studies utilizing association mapping studies, with much narrower genetic resolution, are likely to be published.

Several other processes, including interactions with symbiotic organisms, alteration of the rhizosphere pH, and alterations of cell wall biochemistry, are likely to affect multiple ions. As the total number of cloned mutants is small, little can be inferred from the absence of mutants affecting these processes. However, an alternative hypothesis would be that there are mechanisms to buffer changes in some elements, resulting in no phenotype or single element phenotypes. Indeed nothing presented here should be taken as evidence that the elements never act independently. There are several clear examples where polymorphisms in a gene affect a single element, even when the affected process, such as cell size in the root or the pleiotropic response to perceived pathogen attack, might reasonably be expected to affect multiple elements (Borghi *et al.*, 2011; Chao *et al.*, 2013).

Solutions

Given all of these examples, we are left with the question of how best to conceptualize and analyse the ionome: as independent elements, or as an interdependent network?

There are several advantages to treating the elements independently, the simplest being that it reflects their fundamental chemical properties and the way that they are measured using modern spectroscopy techniques. This approach also allows for simple comparisons across tissues, experiments, and species. Given that sometimes the elements do act independently, we should continue to analyse the elements independently in every experiment.

We should not, however, assume that we are getting the full understanding of the underlying biology if we limit our analysis to the independent element frame. The examples detailed above demonstrate that there are many cases where several elements are acting in a dependent manner, and treating them as independent can muddle the thinking and interpretation. There is a temptation to attempt to define ‘the phenotype’ and then use statistical tests to see if the stated phenotype repeats. As a result, the phenotype can be defined down to those elements that are most reproducible, while eliminating elements that are more responsive to genetic or environmental variation and might provide novel insights into function. A more integrated understanding of the ionome might enable the detection of gene by environment interactions that modify the elemental signatures.

Another reason to move beyond thinking of individual elements is that multi-element approaches might allow for more sensitivity in detecting significant variation in the ionome. The change in a process that has small effects on four elements might not be detectable in any single element, but a pooled trait could boost the signal-to-noise ratio to allow detection. Combining the elements can also allow for the detection and removal of artefacts such as weighing or machine errors that affect multiple elements, improving the remaining signal for each element.

When thinking of combining elemental traits, there are two possible broad approaches, those that use directed knowledge of chemical or biological relatedness and those that use an undirected approach. Which approach is appropriate depends on the questions being asked and the level of prior knowledge about the elemental relationships in the relevant system.

The simplest way of combining elements is to create ratios to see where two elements differ, or average their normalized concentrations to determine where they are acting in concert. Elemental ratios have been used to diagnose issues in crop nutrition (Walworth and Sumner, 1987), and appear to be important signifiers of adapting to serpentine soils (Bradsahw, 2005). When this approach gets expanded beyond the small number of obvious ratios to the whole ionome, however, it greatly expands the number of traits that need to be tracked. Just 10 elements can have 45 dual element ratios and up to 405 (non-independent) amalgamated ratios, a problem that gets even worse when dealing with >20 elements (Parent *et al.*, 2013b). There is also a danger when the measured ionome gets expanded to include trace elements that ratios of well-measured elements to elements around the limit of detection could be prone to artefacts.

In contrast to the ratio approaches, undirected approaches such as principal component analysis (PCA) can act to reduce the dimensionality of the ionome. PCA identifies orthogonal vectors through the n -dimensional elemental space that account for major components of the variation in the dataset examined. The PCAs can reflect artefacts, environmental variables or processes affecting the ionome. Since it is undirected, there is an opportunity to identify

hidden factors that might not have been found in a directed approach. Indeed there are at least two unpublished examples of PCA statistics leading to the identification of strong QTLs affecting the ionome in genomic regions where no signal was detected for individual elements (Asaro and Baxter, unpublished data: BP Dilkes, personal communication). The limitations of this approach include that the PCAs are calculated within the universe defined by a given dataset, so comparison with other datasets, either within or across species, is problematic, making follow-up and interpretation difficult.

An intriguing new approach uses the nutrient balance concept to create hierarchical sets of relationships between elements based on prior knowledge (Parent et al., 2013a, b). This approach allows for an element to have different specified relationships with different elements and to test these balances in the context of all the other relationships. A simple example of this approach is detailed in Parent et al. (2013a, b), where they model four balances for five elements as shown in Fig. 2. An obvious disadvantage of this approach is that it relies on prior knowledge to define the relationships, but the authors state that unbiased ways of defining the balances are possible and under development.

Each dataset of 20+ elemental traits is already difficult to comprehend. Attempting to take all of these approaches would transform them into dozens if not hundreds of composite traits, making interpretation of the data within a biological context intractable. So we are left with the question of how to sort through these approaches to prioritize those that are most useful. From my perspective, genetics holds the key to resolving these questions. The approaches that we should use going forward are those that produce heritable results, which will allow for the detection of novel loci, leading to the identification of new genes and gene by environment interactions underlying elemental accumulation. The other important principle is that we should publish, curate, and maintain repositories of ionic data so that researchers, statisticians, and bioinformaticians can develop, test, and publish their new ideas for analysing the data. The data exchange at www.ionomicshub.org was designed with this exact purpose in mind and contains >250 000 samples worth of data.

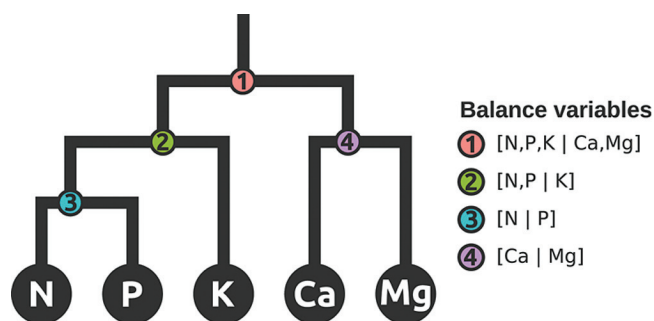


Fig. 2. Mobile-and-fulcrums at mass equilibration point illustrates four hierarchically nested balances that represent a subcomposition or subspace of nutrients in the ionome. Fig. 1 from Parent et al. (2013a; *The plant ionome revisited by the nutrient balance concept. Frontiers in Plant Science* 4, 39, doi: 10.3389/fpls.2013.00039).

Conclusion

I hope that this review has demonstrated that the ionome is not simply a collection of 20+ independent elements. There are clear relationships between the elements, some with known causes and some that are yet to be discerned. While there are several promising approaches, and more under development, we have certainly not arrived at the best solution, and different questions may require different solutions.

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