How have plants adapted to be able to thrive on soils that have remarkably different chemical compositions, either deficiencies in essential elements or excesses in toxic elements? Answering this question requires an in-depth understanding of how plants mobilize, uptake, transport, sequester, and metabolize elements. However, because elements interact both chemically and biochemically, experiments focused on single elements will not fully reveal regulatory networks involved in mineral nutrient and trace element homoeostasis. These interactions among elements necessitate understanding how the entire elemental composition of a cell, tissue, or organism (the ionome) responds to genetic and environmental perturbations. **To accomplish this, I will use high-throughput elemental profiling, coupled with genetics and genomics, to identify the genetic determinants and environmental factors that regulate the ionome of crop and model plant species.** Working with a talented team of statisticians, analytical chemists, and biologists in the Salt lab at Purdue University, I have taken advantage of advances in both mass spectroscopy and genomics to overcome the barriers to such a systems-based investigation of the genetics and physiology of the ionome.

Understanding how plants regulate element composition of tissues is critical for agriculture, the environment and human health. Sustainably meeting the increasing food and biofuel demands of the planet will require growing crops with fewer inputs such as the primary macronutrient phosphorus (P). P in fertilizer is non-renewable, too expensive for subsistence farmers (*1*), and inefficiently utilized by crops, leading to runoff and severe downstream ecological consequences (*2*). Plants comprise the major portion of the human diet, and improving their elemental nutrient content can greatly affect human health. However, efforts directed at a single element can have unforeseen deleterious effects. For example, limiting iron (Fe) or P can lead to increased accumulation of the toxic elements cadmium (Cd) and arsenic (As) (*3*).

These multi-element responses to a change in the environment allow for the possibility that the ionome can be used as a probe of the physiological state of a plant. My work has recently demonstrated that changes in the shoot ionome are better predictors of the response of *Arabidopsis* to Fe or P deficiency, respectively, than the shoot concentrations of Fe or P themselves (*3*). This demonstrates that even though the growth environment is changing (despite our best efforts to keep it constant), these models and others like them will allow us to account for this variation within our data sets. For example, we are able to use the published Fe model to predict when plants grown in our experiments are responding to Fe-deficiency due to changes in soil batch or fertilization (Figure 1). These studies were

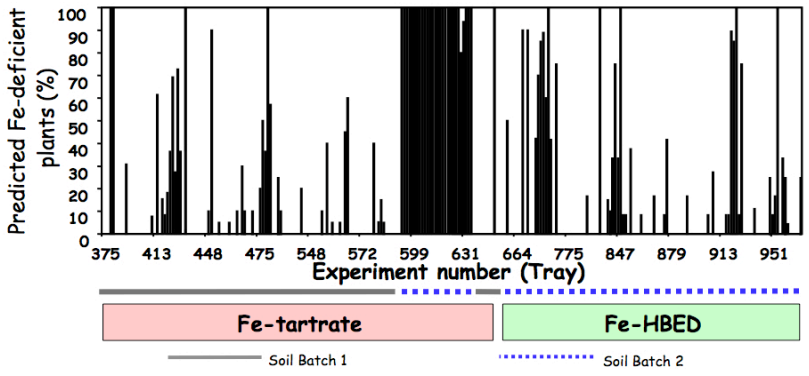

Figure 1: Fe status of Col-0 (wt) plants in 2+ years of experiments predicted by five-element Fe model. 100% is all plants predicted to be experiencing Fe deficiency. Alterations in soil batch and the form of chelated Fe in the watering solution explain a large amount of the variation.

possible because inductively coupled plasma mass spectroscopy (ICP-MS) can rapidly assay the concentrations of 17 or more elements in thousands of *Arabidopsis* samples (*4*) and because the data management system I helped create (*5*) manages all the data and meta-data (data about the data, for example, soil type, watering, light, temperature) associated with these experiments.

Using this ICP-MS phenotyping pipeline and data management system, we have taken several complementary approaches to identify the genetic determinants that control the elemental composition in *Arabidopsis*. We have used reverse genetics screens to identify lines with interesting ionomic phenotypes. In addition, we have screened mutagenized populations and natural variants, and for several ionomic mutants and QTLs we have identified the causal locus (*6, 7*). Though it can take more than 5000 samples, a screen for mutants that assays for 17 different elemental concentrations produces a large number of potentially interesting lines. Identifying the underlying mutation, however, can require analyzing thousands of additional samples for each interesting line. I have been working with high-throughput genotyping and sequencing platforms to improve the efficiency with which we identify causal mutations. Massively parallel genotyping platforms, like microarrays, can be used to rapidly rough map a particular locus by analyzing the distribution of alleles in pools of samples, a strategy we have successfully used to map mutants and QTLs (*6, 7*). We are also exploring ways to use microarray sequence capture of these rough mapped regions followed by second generation sequencing to rapidly identify the causal mutations, a process that would require the analysis of only a couple hundred samples for each interesting mutant.

Association mapping, which has the potential to be an even more powerful mapping approach, utilizes the massive amount of genotype data obtainable for accessions in a given species to perform simultaneous mapping experiments on each trait (element) measured (*8*). I have collaborated with the Borevitz and Nordborg labs to phenotype a pilot population of 96 *Arabidopsis* accessions with genotyping data from a microarray interrogating 250,000 SNPs. Several alleles, such as HKT1, shown in Figure 2, that were previously demonstrated to alter the ionome using conventional methods (*6, 7*) have significant associations in this data. Not only were these alleles mapped to very narrow intervals (~50 kb, see Figure 2), but all of the common alleles of strong effect for each element should be detectable in the same experiment. We are in the process of analyzing a larger population of 360 lines (~2500 samples) for an NIH-funded project on which I am a co-PI. To date, the project has focused on identifying genes that are responsible

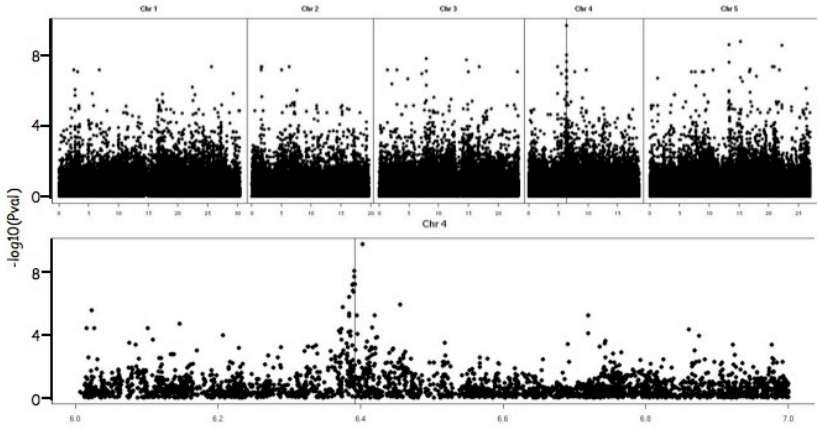

Figure 2: Genome wide association mapping of Na accumulation in *Arabidopsis* leaves. Top: The –log10(pval) for significant association between leaf Na accumulation and genetic variation at 250k snps in 96 accessions. Each peak of association is a potential lead for an allele affecting Na accumulation. Bottom: Focus on 1Mb surrounding the HKT1 locus showing that the LD signal rapidly decreases for a very small mapping window. Vertical line denotes the start codon of HKT1.

for alterations in a single environment. In preparation for the 2009 renewal of this grant, I will extend this work to perform association mapping on the same population grown under multiple different environmental conditions. This will identify genes and gene by environment interactions that alter the elemental composition of the plant, such as genes critical for ion homeostasis when plants are P limited. Following up on these alleles to understand the biochemical basis for the phenotypes will lead us to a better understanding of the fundamental biology of the plant that underlies how plants regulate their ionome in response to environmental change.

While we are gaining important information from *Arabidopsis*, it is critical to investigate elemental accumulation in monocots, which have very different mechanisms of uptake and transport for several elements (*9*) and include many of the important food and fuel crops. I have been working to adapt the approaches developed in *Arabidopsis* to monocot species. Germplasm and genotyping resources for the important monocot crop maize and the emerging monocot model species *Brachypodium distachyon* have recently been made available. The maize nested association mapping (NAM) population, 25x200 line RIL populations, will potentially enable the mapping of alleles to the resolution of 1–3 genes (*10*). In collaboration with the maize geneticist Torbert Rocheford, I measured the levels of 16 elements in single kernels from five of the NAM populations and was able to identified 20–35 QTLs, including 2–5 P QTLs, per population. This preliminary experiment demonstrates the translational potential of these ionomic methods to identify genes and gene by environment interactions important for elemental accumulation in maize. While the association mapping panel shows great promise for gene identification, maize is still a difficult species to work with in the laboratory. *Brachypodium* is similar to *Arabidopsis* in that it has a compact growth habit, short life cycle, and a sequenced genome, which make it ideal for extensive controlled environment experiments. I will screen an already developed EMS population of *Brachypodium* to establish baseline data for how this model grass regulates its ionome. This will enable us to test hypotheses developed from our maize genetic experiments and perform biochemical and cell biology experiments on a monocot model grown under controlled conditions.

These projects take an integrated approach to understanding how plants regulate their elemental composition both genetically and biochemically in response to changing environments. Since P nutrition is of critical importance for agriculture, for follow-up I will prioritize loci identified in altered P environment screens or affecting elements of the P model in all three species. In summary, I propose to use high-throughput, elemental profiling to understand the genetic and biochemical responses plants use to alter their elemental content in response to their environment. I believe that my training and skills make me an ideal candidate to successfully implement this strategy.

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