



Elemental Profiles Reflect Plant Adaptations to the Environment

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Conolidine (Fig. 1) is an alkaloid first isolated from *Tabernaemontana divaricata* together with a number of other MIAs with opioid analgesic properties. The rarity of this alkaloid (0.00014% yield from stem bark) precluded its testing for biological activity, and its de novo synthesis in sufficient quantities has shown that it is an effective nonopioid analgesic (38). Strictosidine is a central precursor of over 2000 different biologically active MIAs. Expression of the strictosidine pathway in a heterologous plant system such as tobacco or in microorganisms could be used as a scaffold for producing rare alkaloids such as conolidine by metabolic engineering of the remaining few steps.

Approximately two-thirds of new drugs in the past 25 years have originated from the discovery of particular secondary metabolites derived from natural biodiversity. This success has been attributed to the structural complexity of molecules found in living organisms, which have an average of 6.2 chiral centers per molecule as compared to an average of 0.4 chiral centers found in combinatorial libraries (39). Such chemically complex molecules are very difficult and costly to produce efficiently by chemical synthesis. A chiral center is defined as a carbon atom associated with four different atoms, so that their mirror images cannot be superimposed. The creation of new pathways in microorganisms and plants (Fig. 1) and the introduction of new reactions (37) or suppression of existing ones (11, 15) in plants can be very effective for randomly generating previously unknown molecules. These could be biosynthetic

intermediates from an existing pathway, or totally novel products could be produced from these intermediates (18). This should remove the bottleneck limiting the production of sufficient quantities of thousands of previously unknown metabolites with numerous chiral centers for testing and drug discovery. The affordability of genome sequencing adds a component to pathway discovery that can be combined with expression studies, functional analyses, and engineered plants to identify gene function across plant species. Such studies promise to reveal new biologically active secondary metabolites, making use of vast aspects of plant biodiversity for new drug discovery.

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PERSPECTIVE

Elemental Profiles Reflect Plant Adaptations to the Environment

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Most mineral elements found in plant tissues come exclusively from the soil, necessitating that plants adapt to highly variable soil compositions to survive and thrive. Profiling element concentrations in genetically diverse plant populations is providing insights into the plant-environment interactions that control elemental accumulation, as well as identifying the underlying genes. The resulting molecular understanding of plant adaptation to the environment both demonstrates how soils can shape genetic diversity and provides solutions to important agricultural challenges.

The majority of the elements that make up a plant, with the exception of carbon and oxygen, are obtained from soil through the roots. These soil-derived elements are required

for plant structure, metabolism, protein function, signaling, and proper osmotic and electrochemical potential. Elemental accumulation requires the integration of processes across biological scales, including interactions with the soil matrix and biota, subcellular localization, metabolism, and gas exchange. Thus, the elemental composition of tissues [the “ionome” (*I*)] is a consequence of complex plant processes and plant-environment interactions.

“Soil” is not a homogeneous entity at any scale. To adapt to element availability differences, which can vary across distances as small as a few meters (see Fig. 1), plants must alter their uptake and storage of both nutrients and toxic elements. Ionomics phenotyping of genetically distinct plants can identify alleles that alter element concentrations in tissues (2). The distribution of these alleles in plant populations can be related back to the soil characteristics of each plant’s position on the landscape. This ecological genomics approach, comparing the spatial distribution of genetic polymorphisms affecting the ionome to soil composition, has begun to identify the genetic determinants of plant adaptation to the soils in which they grow (3, 4).

The concentrations of distinct elements are interdependent and covary between genetic backgrounds and environmental conditions. Elemental species and compounds that are sufficiently similar in size and charge can be bound, metabolized, and transported by some of the same proteins, chelators, and pathways. This results in the coordinated accumulation of these chemically similar elemental species when the shared membrane transport proteins or chelating metabolites are up- or down-regulated. Examples include transport

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Plant Metabolism

of both Fe^{2+} and Zn^{2+} by an iron transporter (5), AsO_4^{3-} and PO_4^{3-} by phosphate transporters (6), and arsenite and silicic acid by silicic acid transporters (7). Similarly, the nonspecific chelator nicotianamine affects both iron and zinc accumulation in shoot vacuoles (8), and iron limitation increases leaf concentrations of zinc in *Arabidopsis thaliana* (9). Iron limitation also alters levels of molybdenum, which indicates that this covariation can include chemically dissimilar elements. Analyzing the ionome of recombinant inbred populations in several species (10–13) revealed multielement covariation networks that include many nonsimilar element pairs. The connections between elements in the covariation networks differed between genotypes, species, and environments. Plant responses to element limitation can affect non-intuitive sets of elements, which indicates that we have much to learn about how element accumulation is regulated. A future goal of ionomics research is to identify the molecular causes of this variation and to use genome sequences to identify orthologous processes across species.

Ionic profiling of mutant populations is also identifying genes responsible for plant processes beyond membrane transport. In *A. thaliana*, the loss of either the *Enhanced Suberin 1* gene (*ESB1*) or the sphingolipid biosynthetic gene *TSC10A* increases suberin in the endodermis in roots (14, 15). Both mutants accumulate more potassium, calcium, and iron in shoots. These multielement phenotypes were only observable by using the ionomics approach and provide genetic confirmation that the Casparian strip forms a barrier to transport for some elements, as hypothesized from histological evidence 147 years ago (16).

For traits that are responsive to the environment, extrapolating findings from a few environments or a few alleles will not be sufficient to explain the distribution of extant wild individuals or to predict the effects of changes in climate or land use. Multivariate differences in genotypes, soil types, climatic factors, and nutrients prohibit testing every combination. Alternatively, association mapping and population genetics use genetic markers and recombinant genotypes in extant populations to detect alleles of phenotypic consequence. The gene-level resolution of these approaches is an ideal complement to ionomics and other high-throughput phenotyping data. If the collection sites (wild) or preferred growth sites (domesticated) of the members of the population are known, environmental descriptors can be estimated for each accession. These environmental variables can be tested for correlation with allelic data to identify candidate genes as the molecular determinants of adaptation. For example, when 200,000 single-nucleotide polymorphisms (SNPs) were used to scan for signs of past selection in 1307 *A. thaliana* accessions, SNPs associated with laboratory-generated ionic phenotypes (in 93 accessions) were overrepresented in regions that

had undergone complete or nearly complete selective sweeps (4). This suggests that soil-driven selection plays a substantial role in patterns of diversity in *A. thaliana* and that ionic profiling detects alleles with adaptive consequences across a wide range of environments.

The explanatory power of combining ionomics, association mapping, and environmental data is illustrated by studies of polymorphism in the Na^+ transporter HKT1. In *A. thaliana*, HKT1 knockouts exhibit altered sodium accumulation and sensitivity to salinity stress (17). Quantitative trait loci (QTLs) for sodium accumulation mapped to HKT1, and association mapping of sodium levels identified alleles of HKT1 that modulate leaf sodium accumulation (3, 18). Colocalization of accessions with these alleles and high predicted soil sodium concentration implicate HKT1 in adaptation to sodic soils (3). The strength of this correlation is partly derived from the proximity of collection sites to the ocean, not exclusively from observed soil profiles. The resolution of current soil maps (at best $\sim 10,000 \text{ m}^2$) and collection location metadata are likely insufficient to support most tests of soil-mediated selection; additional joint soil and accession collections may be required to obtain growth location environmental data.

Many questions regarding plant interactions with soil are best addressed in nonmodel plant species. For example, ionic profiling of locally adapted plant species could help explain how certain species thrive on soils with radically different chemistries, such as serpentines (low calcium/magnesium ratio; low nitrogen, phosphorus, and potassium; and high heavy metals) (19). Such

extreme conditions impose selective pressures on plants that result in fitness trade-offs such that sister taxa [e.g., *Lasthenia* (20)] can be found growing on either side of serpentine soil borders (Fig. 1) and even restricted to ionically distinct regions within a serpentine outcrop. Ionic study of these forms of adaptation has the potential to uncover molecular mechanisms of adaptation and speciation. Identification of the genes responsible for adaptation to the environment, the role of soil-driven selection in patterns of genetic diversity, and the consequences and constraints imposed by plant physiology is now within our reach and could yield the necessary knowledge to make agriculture resilient to abiotic stress.

Production on most agricultural land is limited by soil elemental content (21). Adapting crops to overcome this constraint through improved genetics is an essential component of the effort to improve the human condition. Deficiencies of essential nutrients such as nitrogen, phosphorus, and potassium and excesses of toxic elements such as sodium and aluminum limit production in large parts of the developed and developing world. In addition to limiting yield, poor food quality—such as deficiencies in the essential nutrients iron, zinc, and calcium or excesses of the toxic elements arsenic and cadmium—can negatively impact human health. It is predicted that human population growth, soil nutrient depletion, and salinization by irrigation of fields will increase agricultural utilization of compromised soils. Fertilizer costs, already prohibitive for most farmers, will rise as the cost of producing nitrogen fertilizer rises and known reserves of phosphorus and potassium are depleted (22).



Fig. 1. Local soil variation can determine plant communities and performance. (Left) Soils surrounding serpentine outcrops have highly varied soil chemistries and plant species distinct from the surrounding environment inhabit them. (Right) Spatial variation in wheat plant performance due to saline soil. [Photos: (left) Nishanta Rajakaruna, (right) International Maize and Wheat Improvement Center (CIMMYT)]

However, substantial progress has been made in both ameliorating important elemental limits to crop production and improving food safety by utilizing a molecular understanding of elemental accumulation (23, 24).

As an example of the former, analysis of wheat lines with an introgressed QTL for sodium tolerance identified an HKT1 allele that can extract Na^+ from xylem sap and thereby prevent sodium translocation to the shoots (25). Although a difference in leaf sodium was evident in all environments tested, yield gains were only evident where concentrations of sodium in the soil were highest. This confirms that, in the absence of meter-scale environmental information, heritable ionic phenotypes are more informative for the adaptation of crop genotypes to high-sodium environment than yield and other complex traits that integrate many biological processes.

The shared transport of compounds containing arsenic and chemically similar molecules containing the nutrients silicon and phosphorus underlies both a global food safety crisis and its solution. In rice, silicic acid and phosphate transporters can also move arsenite and AsO_4^{3-} , respectively. Particularly in regions of Southeast Asia with high arsenic concentrations in groundwater, the promiscuity of these transporters is responsible for acute toxicity, disease, and shortened life spans because of dietary intake of arsenic via consumption of rice. Even in the United States, arsenic intake from rice increases breast cancer

risk (26). Because we have a molecular understanding of arsenic uptake, breeding (27) and transgenic modification of crops with a transporter that sequesters arsenic in the root (28) have the potential to improve food safety and the health of hundreds of millions of people.

The prediction of tolerance to sodium stress by element accumulation measurements demonstrates that ionomics can accelerate crop improvement. This is complicated by the many agroecological challenges that limit yield and our insufficient understanding of the trade-offs that result from adaptation to particular soil conditions. Fortunately, the problem of local adaptation has been solved by evolution many times over. Ionomics and genetic association studies in model organisms and crops will directly identify alleles that promote element uptake or exclusion by plants. Using precise quantitative phenotyping of the ionome to characterize variation in plant-soil interactions, we are on the cusp of adding a new dimension to our understanding of why and how particular plants occupy their positions in the landscape and adapting agriculture to marginal soils.

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REVIEW

Achieving Diversity in the Face of Constraints: Lessons from Metabolism

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Metabolic engineering of plants can reduce the cost and environmental impact of agriculture while providing for the needs of a growing population. Although our understanding of plant metabolism continues to increase at a rapid pace, relatively few plant metabolic engineering projects with commercial potential have emerged, in part because of a lack of principles for the rational manipulation of plant phenotype. One underexplored approach to identifying such design principles derives from analysis of the dominant constraints on plant fitness, and the evolutionary innovations in response to those constraints, that gave rise to the enormous diversity of natural plant metabolic pathways.

Metabolism meets two seemingly conflicting needs: responding dynamically to developmental and environmental changes while maintaining the homeostasis required by a living cell, organ, or whole organism.

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This challenge is especially acute for plants, which are sessile organisms that endure constantly changing environmental conditions over life spans ranging from weeks to hundreds of years. For example, carbon fixation and allocation in leaves responds dynamically to unpredictable changes in environment, with time scales ranging from minutes to months. Consistent with a need for rapid response, the turnover time of most key metabolites of central carbon metabolism is on the order of 1 s (1, 2).

Plant metabolic phenotypes are the result of hundreds of millions of years of evolutionary history, during which some ancestral metabolic networks were restructured to meet the demands of changing environments while others remained close to their evolutionary ancient forms. For example, changes in temperature and aridity led to dozens of independently evolved variants of C4 metabolism for carbon fixation, even as the core process of the Calvin-Benson-Bassham pathway—which uses ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) for carbon fixation—remained conserved (3–5). A current challenge in metabolism is to understand the physicochemical constraints on the structure and function of the metabolic network, and thereby gain insight into how evolution worked within these restrictions to shape the characteristics of extant plants.

Beyond Tinkering: The Utility of Design Principles for Plant Metabolic Engineering

Metabolic engineering promises opportunities to increase yield in agriculture and produce chemicals at lower economic and environmental cost. Despite progress, the rate of success in moving from concept to agricultural production or microbial fermentor has fallen short of expectations. For example, tens to hundreds of millions of dollars were spent in the public and private sector in