A phenomics approach to uncovering the molecular basis of root traits in crops

Overview:
The next generation of crops must produce more food, fiber, and fuel despite escalating environmental pressures and an acute need to reduce farming inputs. My overarching interests are to understand the mechanisms plants use to sense and respond to their environment, and to translate this knowledge to crop improvement. While intense focus has been placed on aboveground traits, belowground growth processes have been relatively ignored despite their great potential to alleviate environmental stress and increase resource use efficiency (1). Thus, my research objectives focus on phenotyping the growth and development of root and rhizome systems, and characterizing the gene networks that direct them. Meeting these objectives requires the development of enabling technologies and infrastructure that can capture and analyze morphologically complex subterranean structures over time and at high-throughput (2). I will integrate these phenotyping efforts with natural variation and modern sequencing methods to facilitate our understanding of how genotype dictates phenotype for belowground traits of agricultural importance.

My research covers two broad areas: 1) Development of systematic phenotyping capabilities, and 2) Identifying the mechanistic basis of root growth as conditioned by the environment. During my postdoctoral training with Philip Benfey, I have led a team in the development and implementation of a high-throughput 3-dimensional root imaging and analysis pipeline to dissect the genetic basis of root growth in rice (3). Currently, I am focusing on identifying specific genes controlling promising quantitative trait loci (QTLs) using transcriptomics and advanced mapping populations. I believe that this strategy of combining precision root phenotyping with quantitative genetic and next generation sequencing analysis will effectively reveal the genetic basis of agriculturally relevant traits in any given plant species. Furthermore, to provide a fine-grained view of important plant-environment interactions, I will employ an automated 3D imaging platform that will record root growth hourly over the course of several weeks. This technology will allow my lab to answer fundamental questions about how local growth decisions at each root tip result in particular root system architectures, and how roots respond to local nutrients and other heterogeneous environments.

Accomplishments:
Pre-doctoral Research
I have been a maize biologist for the past 13 years, dating back to my undergraduate research with Scott Gold, in which I developed new markers to study the maize-Ustilago maydis pathosystem (4). Subsequently in Wayne Parrott's lab, I learned plant transformation techniques in pursuit of developing an artificial maize centromere (5). This project transitioned into my dissertation project with R. Kelly Dawe, which focused on the epigenetic determinants of plant centromere identity. I made several key contributions, including pioneering RNA-ChIP in plants to discover that non-coding RNAs corresponding to centromere-localized repeat elements were preferentially bound to the maize centromere-kinetochore complex (6, 7). Later work in the field corroborated this finding in cell lines and demonstrated that a similar system was essential for human neocentromere function (8). In collaboration with Ron Okagaki and Ron Phillips, I studied maize neocentromere behavior in the oat-maize addition lines using deconvolution microscopy and an antibody I developed to the oat centromeric histone (CENH3) to show that 1) maize centromeric DNA can seed functional oat kinetochores, and 2) the stability of these kinetochores was correlated to the size of the CENH3 domain (9). In subsequent gene overexpression work in Arabidopsis, I used live confocal imaging of the root tip to show that expanding the CENH3-kinetochore domain resulted in massive chromosome mis-segregations and pleiotropic developmental defects in the plant (manuscript in preparation).

Postdoctoral Research
For my postdoctoral work with Philip Benfey, I have widened my research focus to root system architecture and its role in plant health and productivity, primarily in maize and rice. 'Root system

architecture' encompasses the spatial and temporal organization of roots in the soil, and thus greatly influences the resource capturing abilities of a plant. However, root architecture traits are notoriously difficult to measure due to the opacity of soil and a complex 3D morphology that is environmentally sensitive. Until recently, little was known about the quantitative genetic and environmental factors that condition root architecture in crop plants, nor did a comprehensive approach for analyzing them exist.

I have led teams that advanced root phenotyping and quantitative analysis in the following ways:

1. Development of a high throughput 3D root imaging platform capable of processing hundreds of plants per day ranging in size from Arabidopsis to maize. This system uses a transparent gellan gum substrate, and a similar hydroponic system will be available soon (Figure 1).

2. Development of a computational analysis pipeline capable of automatically phenotyping dozens of root architecture traits for hundreds of samples.

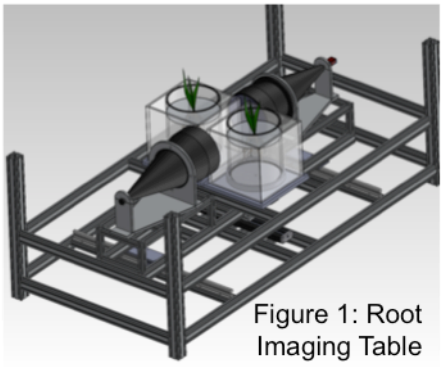
Figure 1: Root Imaging Table

3. Implementation of these tools to conduct the first quantitative genetic analysis of root architecture in 3D (3). We identified 89 QTLs that control 25 root traits in a rice bi-parental mapping population. We used a multivariate mapping technique to identify the five most significant regions of the genome controlling root architecture.

4. Working closely with another postdoc in the Benfey lab, I have transcriptionally profiled root tissue of parental lines, and combined with genotyping-by-sequencing of the mapping population, I am co-localizing differentially expressed genes to physically defined QTL regions. I am concurrently phenotyping advanced mapping populations developed at the most significant QTLs to narrow down confidence intervals and speed gene identification.

Applying these phenotyping tools, I initiated a number of independent projects while funded by a USDA-NIFA postdoctoral fellowship:

5. Quantifying root architectural differences that may account for increased nitrogen uptake efficiency in maize Illinois High Protein lines.

6. Quantifying root architectural differences and growth responses to neighboring roots in maize lines selected for yield at high planting density.

7. Studying subterranean development (e.g. rhizomes and tillers) of perennial plants switchgrass, Tripsacum, Sorghum propinquum, and teosinte to begin an effort to identify genes that control perenniality.

8. Automating the root-imaging platform to collect high-density time series datasets around the clock, and collaborated with a computer scientist who developed software to quantify the growth patterns of each root in the network.

Future Directions:

With inexpensive sequencing and ever-increasing germplasm resources in hand, high throughput precision phenotyping has become a major bottleneck to unleashing the full power of genomics and natural variation on crop improvement (2). My lab will employ integrated phenomic-genomic approaches to link important root and rhizome traits to the gene regulatory networks that control them. Although virtually any plant species or germplasm resource can be studied in the 3D imaging system, I will initially focus on the independent projects I began in which existing preliminary data suggest a high likelihood of identifying genes significant to agriculture:

Aim 1. Identify genes and gene networks that control root traits underlying key agronomic qualities

a) Combined physical and expression QTL analysis of an Illinois High x Low Protein maize mapping population. The Illinois High and Low Protein (IHP and ILP) lines have been recurrently selected for high or low seed protein content, respectively, for over 100 years (10). During this time, IHP lines have dramatically increased their capacity for nitrogen uptake, with corresponding root architectural changes hypothesized to support this trait (11). My analysis using the gel-imaging platform revealed significant architectural differences between IHP and ILP root systems (manuscript in preparation), including a striking lateral root phenotype (Figure 2). This and other phenotypes are quantifiable in the 3D imaging system and provide the basis for mapping QTLs responsible for an increased ability to mine nitrogen from the soil. However, root architecture genes controlling quantitative traits are difficult to pinpoint; in fact none have been cloned in maize. Differential gene expression analysis offers a viable way to identify causal genes within a QTL region (12), and by expression profiling the entire mapping population, a genome-wide analysis of loci affecting transcriptome regulation (expression QTLs, eQTLs) will be conducted. By focusing on eQTLs that influence candidate gene expression, I will anchor gene regulatory networks to nitrogen uptake as relates to root traits. Specifically, I will:

i) Phenotype ~150 families from an IHP x ILP mapping population to identify QTLs segregating for lateral root proliferation.

ii) Simultaneously profile the root transcriptomes of each family and conduct an eQTL analysis to identify cis-acting factors that co-localize with physical QTLs in order to identify gene candidates, as well as trans-factors that may be part of the regulatory network.

iii) Clone and validate major genes that control improved nitrogen uptake via root architecture. I will generate transgenic maize lines, employ field phenotyping methods such as 'shovelomics', and collect physiological data relating to nitrogen metabolism to support these findings.

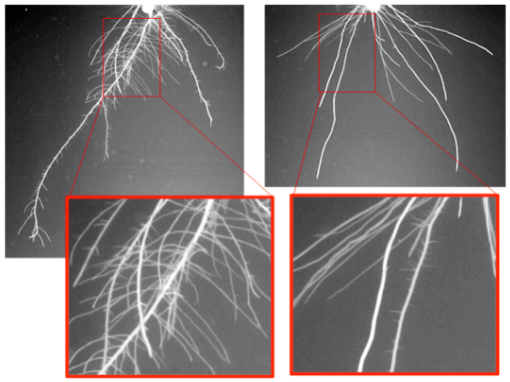

Figure 2: Lateral roots in IHP (left) and ILP (right) lines

b) Phenomic-genomic analysis of the effects of long-term selection for high yield at increasing planting density on maize root growth. A primary factor driving the approximately 8-fold increase in US maize production over the past 80 years has been the ability of modern varieties to maintain high yields at increasing planting densities (13). While canopy traits such as leaf angle have clearly contributed to this phenomenon, a recent study using historical maize lines and computer modeling indicated that root architectural changes were also likely to play an important role (13). I characterized phenotypic differences between unimproved germplasm (BSSS x Corn Borer; cycle 0) and germplasm that had been recurrently selected for yield at planting density (BSSS x Corn Borer; cycle 17) (manuscript in preparation). Ongoing studies are focused on identifying changes in growth patterns when these germplasm are grown two-to-a-pot, which should illuminate the belowground process by which cycle 17 material can thrive in close proximity to its neighbors. I will transcriptionally profile cycle 0 and cycle 17 roots, grown individually and in pairs, to identify differentially expressed genes that may contribute to these phenotypic changes. Additionally, a fine–grained analysis of root-root interactions will be undertaken in a high-density time series analysis using the automated imaging table and software outlined in Aim 2.

c) Characterize the molecular and developmental basis of perenniality in grasses. The perennial life form offers a number of desirable traits, including rapid early season growth that facilitates increased biomass, and an established root system that facilitates increased stress resistance and growth in marginal soil by providing access to greater water and nutrient resources. Despite the fact that each major crop variety has a close perennial relative, little is known about the mechanisms that confer perenniality outside of a few studies that suggest a handful of major genes condition the trait (14). As part of a large-

scale synthetic biology proposal to engineer perennial maize (PI Dawe, co-PI's Buckler, Costich, Holland, Kushner, Murray, Parrot, and Topp), I will:

i) Identify the genes underlying a large-effect perenniality QTL segregating in four F_4 heterogeneous inbred families developed by co-PI Murray from a Sorghum bicolor x S. propinquum cross. I will use the 3D imaging system to follow the development of root and rhizomes from the crown in order to determine morphological differences that segregate with the perennial trait (Figure 3). Expression profiling and eQTL analysis of relevant tissue will be conducted to identify genes and gene regulatory networks that condition perenniality in sorghum.

ii) Conduct a similar analysis in Zea mays x Z. diploperennis advanced mapping populations developed by co-PIs Buckler, Holland and Murray. Perennial genes between sorghum and maize will be compared.

Figure 3: 3D model of a perennial Tripsacum rhizome and root system

Aim 2. Imaging and analysis of fine-scale root growth dynamics

A fundamental question arises in the study of any biological network, "Is the network topology purely an emergent property of local patterns, or is there coordination at a higher level?" Using the automated imaging table and growth analysis software that were developed under my USDA-NIFA postdoc, I will investigate relationships between local root growth patterns and global architecture traits in the following ways:

a) Increase the throughput of this technology and generate several dozen high-density time series data sets by imaging hourly during approximately two weeks of growth. Map the growth rates, angles, curvatures, and branching patterns of each root back to their respective global architectures (Figure 4).

b) Extend these analyses to functional studies of root growth response to nitrogen availability in the IHP/ILP lines, as well as response to neighboring roots in the BSSS x Corn Borer material.

c) Integrate data from Aims 1 and 2 to begin to build a model of how the environment conditions root growth, which will connect molecular networks, root dynamics, and global architecture.

Figure 4: Bi-hourly 3D reconstruction of a rice root system over 24 hours of growth. Colors indicate growth at each time point.

Summary

My research will advance root phenotyping by developing novel imaging and analysis tools that will be used to characterize complex and otherwise inaccessible root morphologies. By applying these tools to selected maize and sorghum germplasm in combination with transcriptome analysis, I will identify genes that control root traits strongly correlated to efficient nitrogen uptake, the ability to grow in close proximity with other plants, and perenniality; all high value targets for crop improvement. I intend to follow gene discoveries with detailed molecular and physiological analysis to understand the genetic and environmental context in which they operate. In the longer term, I plan to translate this knowledge to the field, likely with the aid of breeders, using transgenics, marker assisted selection, or genomic selection.

Citations

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