

How does Aerial Root Morphological Diversity Contribute to Physiology?

Overview

Aerial (adventitious) roots, which emerge from plant stems above the soil, are proposed to play an important role in structural stability and late-stage nutrient/water acquisition. Yet, how aerial roots develop, integrate environmental cues and contribute to whole plant physiology remains a poorly understood area of plant biology. To address this deficiency, my independent research aims to **determine how aerial root morphology contributes to plant physiology and the response to environmental cues**. This supports my long-term goal of increasing plant productivity and resilience through the engineering of aerial root systems tailored for specific environments.

Doctoral and Postdoctoral Research

Branching structures have always fascinated me. Specifically, I am intrigued by the way that branching generates a modular and expansive morphology, which increases surface area without a large investment into mass. As a graduate student, I developed a resin casting technique that established the first visualization of 3D bile duct architecture in mice. I used this technique to identify a dose-dependent requirement for Notch signaling in bile duct branching (Sparks et al., 2010). I went on to quantify the temporal changes in branching using x-ray microCT (Sparks et al., 2011) and identified a progressive obstructive phenotype in Notch loss-of-function mice coinciding with liver dysfunction.

To broaden my expertise in the molecular and morphological control of branching, I transitioned to investigating the multi-scale regulation of plant root development as a postdoc with Philip Benfey. At the molecular scale, I generated a tissue-specific gene regulatory network by enhanced yeast-1-hybrid assays (Sparks and Benfey, 2015). I used this network to dissect the regulation of the *SHORTROOT-SCARECROW* transcriptional cascade, which is central to cell fate decisions in the *Arabidopsis* root cortex and endodermis. I developed and validated a model for the establishment of *SHORTROOT* expression through combinatorial regulation by both activators and repressors (Sparks et al., *accepted at Dev Cell*). At the tissue scale, I worked in collaboration with another postdoc to identify and characterize MYB36, a transcription factor that promotes endodermal differentiation and inhibits proliferation (Lieberman et al., 2015). At the organ scale, I worked on the identification of genes underlying rice root architecture QTL (Topp et al., 2013). Specifically, I adapted the Multiplex Shotgun Genotyping (MSG) pipeline (Andolfatto et al., 2011) for high-resolution genotyping and generated root-specific RNA-seq datasets.

Future Research

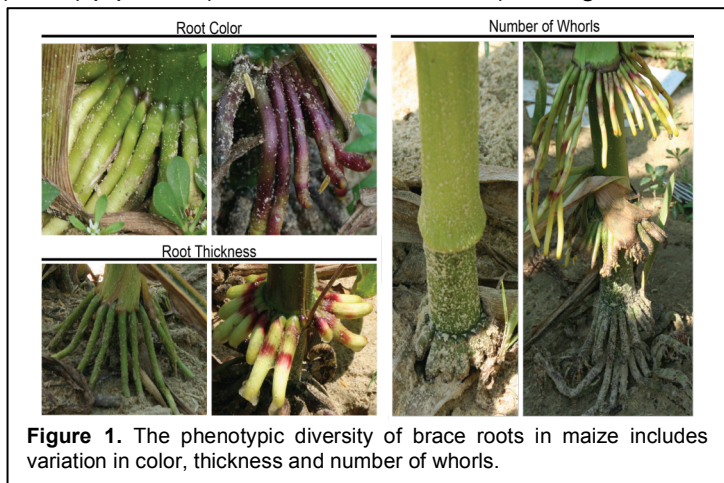
The aerial roots of maize (brace roots) show vast morphological diversity, yet it is unclear how this diversity is regulated or the functional consequences. To address this issue, my research will be driven by the following questions.

How does aerial root structure impact function?

What are the genes regulating maize brace root diversity? I have acquired field-

based phenotyping data from a diverse germplasm of 44 hybrid and 60 inbred lines (collaboration with Jim Holland, NCSU) (Fig. 1). I plan to focus on this population for the following analyses:

- Assay brace root physiology including photosynthetic potential, water, nutrient, and ion fluxes.
- Select inbred lines with opposing phenotypes for both morphological and physiological traits and generate F2 and F2:3 populations.



- Phenotype populations for the targeted brace root trait and generate high-resolution genotyping markers.
- Perform QTL analyses and RNA-seq for gene identification.
- Develop structural models to predict how phenotypic variation contributes to plant stability.

What is the role of phytohormones in maize brace root emergence? My preliminary results from the dwarf *brachytic-2* (*br2*) mutant, which is defective in polar auxin transport (Multani et al., 2003), indicate a defect in brace root emergence. Based on these results, I plan to:

- Use histological analyses to pinpoint the spatiotemporal defects in primordia initiation and patterning in the absence of auxin transport.
- Define the auxin-responsive networks regulating brace root emergence by temporal RNA-seq analyses following the exogenous application of auxin.

Develop a high-throughput imaging approach for aerial root phenotyping in the field. I will develop a platform to enable the high-throughput phenotypic characterization of aerial roots. Images will be acquired with Raspberry Pi modules fitted with side-mounted cameras and geolocation trackers. For initial testing, modules will be built onto remotely operated robotic platforms.

How does the environment influence aerial root phenotypes?

What is the effect of light quality and intensity on maize brace root phenotypes? Light intensity is one factor that has been suggested to promote maize brace root emergence (Hébert et al., 2001). In collaboration with Ullas Pedmale (CSHL) I plan to:

- Develop greenhouse-based robotic imaging and analysis platforms to characterize the brace root response to shading in the germplasm identified above.
- Assay brace root physiology in response to shading.
- Develop unbiased (mutagenesis) and targeted (known light mutants) approaches to define the mechanisms by which light signaling affects brace root emergence.

What is the effect of elevated ozone on maize brace root phenotypes? I have acquired field-based brace root phenotyping data from 44 maize hybrids that have been fumigated with ozone in the free-air concentration enrichment (FACE) fields at the University of Illinois (collaboration with Andrew Leakey), which indicate a genotype-specific morphological response to elevated ozone. To investigate this effect further, I plan to:

- Analyze the response of the diverse germplasm identified above to elevated ozone.
- Map the environmental response to elevated ozone and identify the underlying genes.
- Assay brace root physiology in response to elevated ozone.

Research Impact

The research outlined here is the basis for **a program that will identify how aerial root form and function contributes to the plant response to the environment.** In the long-term these investigations can be expanded to include additional plant species. Lodging is a significant concern in many crop species, yet only a subset of grasses forms brace roots as a part of normal development. Ultimately, I would like to identify the core brace root program and introduce this into non-brace root grasses for improved lodging resistance. In addition, the environmental influence on aerial root phenotypes can be expanded to include abiotic and biotic stresses.

Despite their proposed importance, the development and physiology of aerial roots is a relatively unexplored topic. Thus my proposed work has the potential to provide significant results in the short-term and facilitate long-term investigations. The most immediate applications are in agriculture to promote plant productivity by improving structural stability and late-stage nutrient acquisition. The preliminary data I have acquired will ensure the possibility of applying for grants from the NSF and the USDA.

Sparks et al., Hepatology 2010
Sparks et al., DMM 2011
Sparks and Benfey, MMB 2015
Sparks et al., accepted at Dev Cell
Lieberman et al., PNAS 2015

Topp et al., PNAS 2013
Andolfatto et al., Gen Res. 2011
Multani et al., Science 2003
Hébert et al., Crop Sci 2001