Integrative analysis of plasticity in cell fate determination in plants

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Project overview

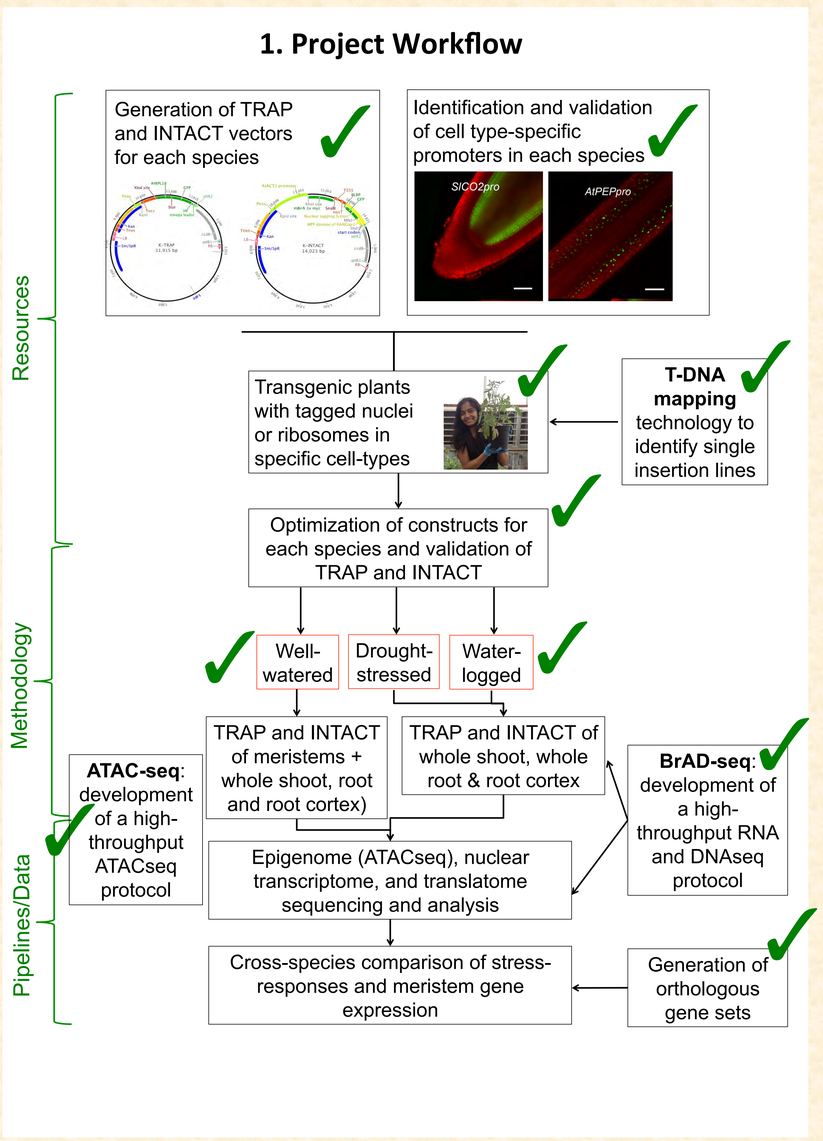
From the germination of a seed to the fertilization events that lead to the next generation, plant development is exquisitely orchestrated by genetically determined processes that are fine-tuned by environmental cues. This entails the precise regulation of networks of genes in individual cells over the course of the plant life cycle. Global and local climate change is a challenge to agricultural productivity. Of all the stresses experienced by crops, paucity or overabundance of water are particularly damaging.

In this project, **we will decipher the complex regulation of genes within specific plant cell types during development and in response to water stress in three important crops: rice, tomato and the forage legume *Medicago truncatula*.** We have refined the **INTACT** (Isolation of nuclei tagged in specific cell types) and **TRAP** (Tagged ribosome affinity purification), technologies (Figures 1-3) to examine the epigenome, transcriptome, and translatome of specific cell types in these crop species.

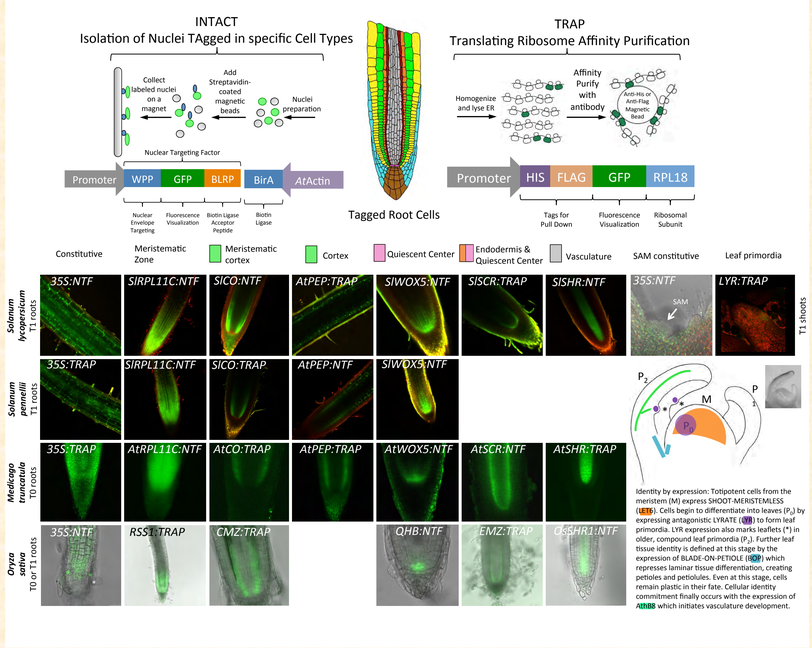
This project will address two important biological questions: **How does gene regulation in the stem cells (meristem) of roots and shoots differ across species? How does environmental stress influence the development of specialized cell types in the root?**

The project will have multiple broader impacts. First, it will establish and share resources for the evaluation of cell-type specific expression in three important crops. Second, the experiments will provide broad new insights, which will facilitate downstream improvement of abiotic stress tolerance. Third, the project will engage postdoctoral researchers and graduate students in advanced interdisciplinary training in biology and computational sciences. Finally, the project will engage high school students in the classroom and the laboratory, develop teaching tools, and foster greater understanding of the importance of plant research to humankind.

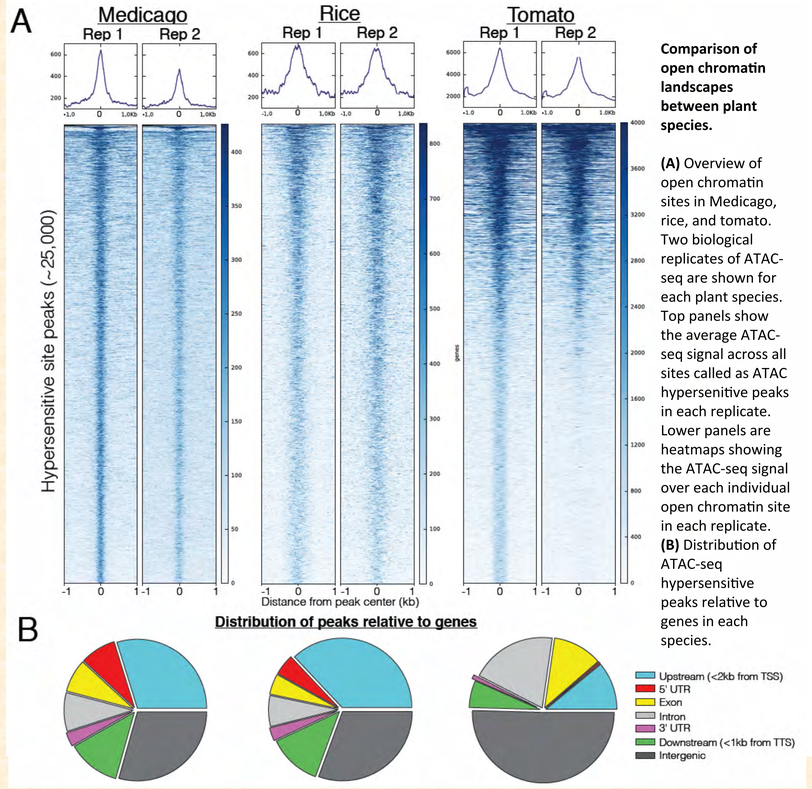
1. Project Workflow



2. Establishment of INTACT and TRAP lines in four species



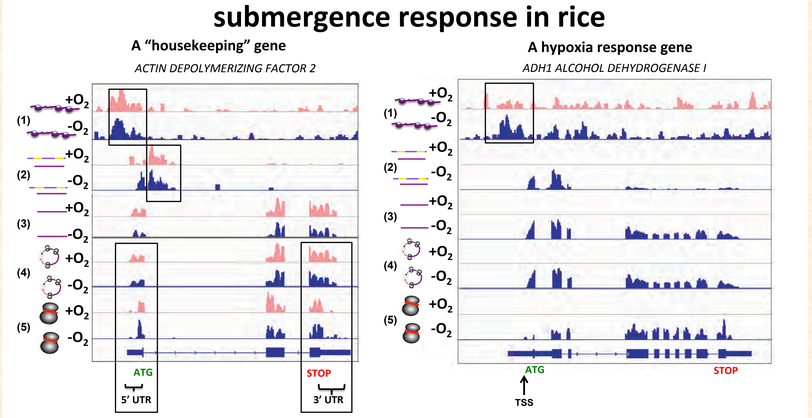
3. Identification of unique and conserved regions of chromatin availability in the root tip



Comparison of open chromatin landscapes between plant species.

(A) Overview of open chromatin sites in Medicago, rice, and tomato. Two biological replicates of ATAC-seq are shown for each plant species. Top panels show the average ATAC-seq signal across all sites called as ATAC hypersensitive peaks in each replicate. Lower panels are heatmaps showing the ATAC-seq signal over each individual open chromatin site in each replicate. **(B)** Distribution of ATAC-seq hypersensitive peaks relative to genes in each species.

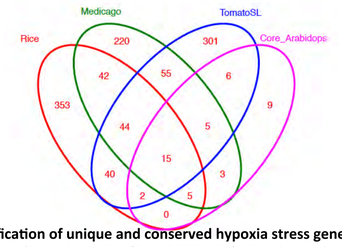
4. Breathing underwater I: A multi-tier analysis of the submergence response in rice



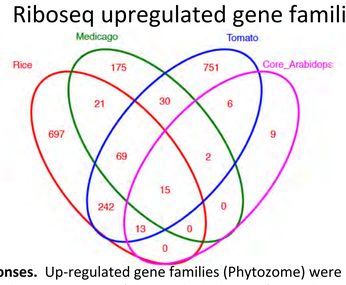
Different tiers of regulation for a houskeeping gene and for a hypoxia response gene under control conditions (top row) and submergence stress conditions (bottom row). (1)ATACseq (2)pre-mRNAseq (3) total RNAseq (4) TRAPseq (5) Riboseq

5. Breathing underwater II: Unique and conserved responses of submergence stress across species

Pre-mRNA upregulated gene families



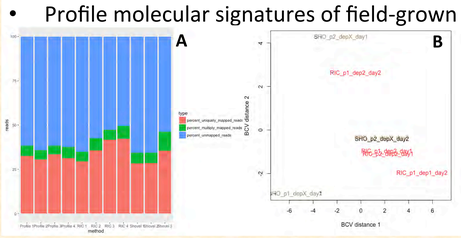
Riboseq upregulated gene families



Identification of unique and conserved hypoxia stress gene responses. Up-regulated gene families (Phytozome) were identified for each species (EdgeR FDR<.01). Overlap with the core hypoxia gene set (Mustroph et al., 2009) was also characterized.

6. Remaining analyses

- How do cortex-specific cell type molecular signatures differ in drought tolerant and drought-sensitive species?
- How do submergence and drought molecular signatures differ across species?
- Profile molecular signatures of field-grown plants



RNAseq libraries prepared from field-grown tomato roots collected using 3 different methods:

(A) provided adequate mapped read coverage for DGE analysis

(B) Profile and **soil**-grown roots did not differ in gene expression, while DE genes in root ingrowth cores (**RICs**) compared to other methods were enriched for lateral root initiation, NaCl response

7. Integration of community resources for cell-specific genomics and science education.

We emphasize the dissemination of protocols, data analysis pipelines, genomic data, vectors and the genetic resources. We provide advice on translation of these technologies. Virtual instances of our pipelines have been established for INTACT (RNAseq and H3K27me3/H3K4me3 modifications). Detailed methods have been published for TRAP-Seq and Ribo-Seq. We also incorporate education and training targeted to underrepresented and underprivileged students in STEM and plant genomics-enabled research.

Outreach Programs

1. High School Interns: Our Davis outreach has created a partnership with the AP Biology class in Pioneer High School in Woodland, CA. Our program runs throughout the academic year and supplements the AP Biology course with three student-led laboratory exercises. We take in four students to intern in the laboratory on a weekly basis to learn a wide variety of topics.

Subject	Mentor(s)
Bacterial Transformation	Kristina Zumstein
Fast Plants	Donnelly West
Root Gene Expression	Kaisa Kajala
Root Environmental Response	Sharon Grey
Jeopardy AP Review	All

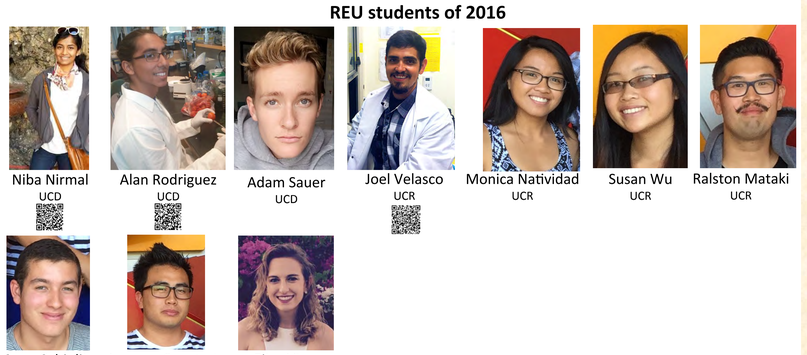
Student-led Class Laboratories
Molecular Genetics Lab
Fast Plants Genetics Lab
Jeopardy! AP review

Are you smarter than a high schooler? Play our Jeopardy game!

2. Community College: Our Riverside outreach enabled Community College students to perform TRAP and semi-q PCR in a biology lab course.

3. Research experience for undergraduates (REU): Each year we have hosted undergraduates in research internships. The interns learn laboratory skills and carry out parts of this project under the mentorship of graduate students and postdocs. REU student work includes generation of T-DNA mapping libraries and the confocal microscopy images of T1 plants (Panel 2).

REU students of 2016



Niba Nirmal, UCD
Alan Rodriguez, UCD
Adam Sauer, UCD
Joel Velasco, UCR
Monica Natividad, UCR
Susan Wu, UCR
Ralston Mataki, UCR
Sean Cabinlit, UCR
Lorenzo Sanataria, UCR
Elise Viox, Emory