**Discovering Innovations in Stress Tolerance Through Comparative Gene Regulatory Network Analysis and Cell-Type Specific Expression Maps.**

**José R. Dinneny**[1*] (dinneny@stanford.edu), Maheshi Dassanayake,[2] Dong Ha Oh,[2] Song Li,[3] and John Schiefelbein[4]

[1]Stanford University, Stanford, CA; [2]Louisiana State University, Baton Rouge, LA; [3]Virginia Tech, Blacksburg, VA; and [4]University of Michigan, Ann Arbor, MI

**Project Goals:**

*Objective 1: Discover how extremophytes and stress sensitive species differ in the cell-type functions of roots and those triggered downstream of ABA*

*Objective 2: Define how changes in the wiring of gene regulatory networks produce innovations in transcriptional regulation in extremophytes and how bioenergy crops have diverged.*

*Objective 3: Establish a data driven, predictive framework for accelerating functional testing of stress resilience genes using Arabidopsis and Camelina as a chassis for engineering.*

The current post-genomics era is defined by a multitude of genome sequences that are available to study. Experimentally tractable molecular genetic model species were some of the first to have their genomes sequenced. Today, however, genomes of organisms that are difficult to culture, rare or even extinct exist in our data repositories. While their genome sequences have been unlocked, the nature of the genes contributing to this fascinating diversity in physiology and development is currently hidden due to a lack of methods available to extract significant functional meaning. Which genes allow cactus to survive the desert heat, or sea grass to grow in ocean water, or some ferns to tolerate desiccation? In agriculture, domestication has led to the breeding of rapidly growing cultivars that perform well when the climate cooperates, but often fail when water or nutrients are limiting. Bioenergy crops will be grown on soils of poor quality so as not to compete with other agricultural sectors, yet these environments will dramatically impact biomass accumulation. If the innovations that nature has selected for across plant species can be discovered, we have an opportunity to address these challenges and improve the sustainability of agriculture in ways that are simply impossible by traditional breeding.

Innovations in gene function allow wild plants to inhabit environments that are commonly stressful to domesticated crops. Our interdisciplinary team seeks to identify such innovations by defining the regulatory and physiological context that genes function across 11

sequenced Brassicaceae genomes, including bioenergy crops, and crop wild relatives, using recent advances in single-cell RNA sequencing (scRNA-seq) and DNA affinity purification sequencing (DAP-seq) technologies.  Furthermore, machine-learning algorithms will utilize the evolutionary history of gene duplication events and functional genomics data to identify innovations in gene function associated with growth control under environmental stress. Putative genes associated with extremophyte resilience will be introduced into stress sensitive species to test whether extremophyte physiological properties can be transferred to naïve genomes.  These studies will utilize a molecular genetic model that can be rapidly characterized using scalable and open source phenomics systems.  Through our investigation, we will establish an experimental and data analytics pipeline that will be broadly applicable to the study of gene function at the plant family level and result in a road map for improving plant traits for bioenergy and beyond.