

Title: Cell type specific abiotic stress responses among closely related Brassicaceae species reveal how stress tolerance in extremophytes differ from stress-sensitive models

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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. (*Following abstract is focused on this objective*)

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.

Abstract:

Environmental stress tolerance is a complex trait that involves spatio-temporal regulation of large gene networks. Our current understanding of how plants regulate gene expression to achieve stress tolerance is largely limited to model plants and crops that are highly sensitive to environmental stresses. Wild plants adapted to harsh environments developed as extremophyte models offer a unique molecular genetic resource to investigate how millions of years of natural selection has resulted in innovative ways of achieving stress resilient growth. With ongoing environmental challenges to biofuel focused agriculture often compounded by climate change, new designs of smart crops are critically needed. Improved insight into genetic mechanisms regulating stress responsive gene networks for multiple abiotic stresses is needed as input for such smart crop designs to optimize balance between plant growth and stress survival. The core abiotic stress responsive networks operating at the cell-type level have been determined for *Arabidopsis thaliana* [1, 2]. However, these networks are limited in explaining how stress tolerance limits can be expanded to levels we observe in stress-adapted plants in the wild. Therefore, in our current research, we have used a comparative platform to explore and deduce flexible gene regulatory networks that respond to abiotic stresses across closely related Brassicaceae genomes that includes both extremophytes and their closely related stress-sensitive species [3, 4].

We have selected the extremophyte models, *Eutrema salsugineum* and *Schrenkiella parvula* compared to the stress-sensitive models, *Arabidopsis thaliana* and *Sisymbrium irio* to study single cell transcript profiles of root tissues in response to ABA and salt treatments. We have generated scRNAseq datasets for ~15,000 cells per condition from each species. Individual cells were assigned to ten defined root cell types separated into clusters based on their similarity to reference transcriptome profiles, presence of cell-type marker genes, and their alignment with published root scRNAseq profiles. Differential expression profiles within the same cell type across the species at control conditions allowed us to identify cell type specific basal expression level differences between extremophytes and their stress-sensitive models, undetected with bulk RNAseq data. Surprisingly, orthologs of many well-established *A. thaliana* cell type marker genes did not show the expected preferential expression in specific cell types in the other species. We identified new cell type markers that were shared among all target species previously not documented as marker genes in *A. thaliana* as well as markers that were preferentially expressed in selected cell types only at a species specific level. Ortholog expression between closely related species within an in-group in a clade generally showed higher similarity compared to those outside the clade. However, a significant proportion (~30 - 40%) of ortholog expression profiles did not match the phylogenetic signal supported by the transcript sequence similarity of a common ancestor. Clustering of transcripts into expression

modules across all cell types, conditions, and species revealed that cell identity was better predicted by both positive and negative contributors of expression instead of clusters of expressed marker genes alone. These expression modules that distinguished cell types could be supported by enriched cell-type specific functions such as xylem or endodermis development and highlighted developmental processes that differed among the species and further diverged when examined under stress treatments. These results collectively provide insights into spatio-temporal regulation of stress responsive genes at a single cell resolution unexplored before using extremophytes in a comparative study.

References/Publications

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