

Title: Divergence in stress tolerance mechanisms across the Brassicaceae family highlight strategies for maintaining growth and physiological balance under extreme environments

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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.

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 text:

How plants achieve salt tolerance has been a focal question in our quest to develop bioenergy crops that can be grown on marginal lands for sustainable energy production. One bottleneck to overcome this challenge has been our lack of understanding in how naturally stress adapted plants, extremophytes, respond and thrive under salt stress. To address this need, we used the extremophyte models, *Schrenkiella parvula* and *Eutrema salsugineum* with the closely related premier model, *Arabidopsis thaliana* in a multi-omics experimental design. In this study, we used a comparative transcriptomic, ionomic, and metabolomic profiles to gain insight on how these extremophytes respond to salt stress differently from the stress sensitive model, *A. thaliana*.

We found that *S. parvula* and *E. salsugineum* accumulated less Na⁺ and maintained higher K⁺ in the shoots compared to *A. thaliana* under salt stress. However, Na⁺ accumulation in *E. salsugineum* was comparable to that of *A. thaliana* in the roots. Among the two extremophytes, only *S. parvula* maintained a low Na⁺ concentration in the roots upon salt exposure. Salt stress led to a reduction in the abundance of macro and micro-nutrients in *A. thaliana* roots, while both halophytes could maintain their overall nutrient balance similar to control levels at comparable salinities. Concordant with the ionomes, the shoot metabolomes of the extremophytes showed minimal changes compared to *A. thaliana*. Among the notable changes to the root metabolome, *S. parvula* increased in abundance of its primary sugars, amino acids, and other intermediates in the TCA cycle while the abundance of these metabolites decreased in *E. salsugineum* under salt stress. However, this reduction did not lead to a significant net reduction of these pools, as *E. salsugineum* had higher basal levels of these metabolites in roots compared to *S. parvula* and *A. thaliana*. Interestingly, *S. parvula* metabolomes converged with *A. thaliana* more than with *E. salsugineum* prior to salt stress and transitioned to a metabolome that was more representative of

E. salsugineum with increased salt concentrations or treatment durations. The transcriptomic responses to salt stress further supported the metabolic adjustments of each species. Up-regulated genes in all three species were enriched in stress-related pathways including ROS scavenging and osmolyte biosynthesis. In addition, differently regulated genes in *S. parvula* were indicative of uninterrupted root growth during salt stress governed by genes associated with auxin and ABA pathways, quite contrasting to the other two species. In the shoots, the transcriptomic effect on photosynthesis was significantly less in the extremophytes compared to *A. thaliana*. Overall, the extremophytes showed stress preparedness, both at the transcriptome and metabolome levels to allow sufficient nutrient uptake to promote growth and development under salt stress levels that impaired growth in *Arabidopsis*. Both extremophytes indicated successful yet different strategies for stress tolerance at the metabolome and transcriptome levels during salt stress.

While the hormonal networks controlling growth have been extensively characterized in stress-sensitive plants, it is unclear how these pathways are rewired in plants that maintain growth in extreme environments. We have compared the developmental and molecular responses of four closely related members of the Brassicaceae family including two salt-tolerant species (*S. parvula* and *E. salsugineum*) and two salt-sensitive species (*Sisymbrium irio* and *A. thaliana*) to the salt stress-induced hormone, abscisic acid (ABA). While ABA inhibits root growth in most species, we uncovered substantial growth-promoting effects in *S. parvula*, due to an enhancement in cell elongation. Comparative transcriptomics informed by phylogenetic relationships uncovered lineage and extremophile-specific differences in ABA response. DNA Affinity Purification followed by sequencing (DAP-Seq) was utilized to establish gene regulatory networks (GRNs) in each species for the entire ABA-RESPONSIVE ELEMENT BINDING FACTORS (AREB/ABF) clade. Comparative GRN analysis identified relative conservation in the core ABA signaling GRN, while the auxin growth-hormone GRN was highly divergent, revealing how patterns of gain and loss of cis-regulatory elements mediate novel physiological outcomes. Our findings demonstrate that the targets of hormone signaling pathways are highly divergent between species and that diametric inversion of growth regulation is possible, even between closely related species of the same plant family (Sun et al., 2020).

References/Publications

1. Sun Y, Oh DH, Duan L, Ramachandran P, Ramirez A, Bartlett A, Dassanayake M, **Dinneny JR** (2020) Divergence in a stress-associated gene regulatory network underlies differential growth control in the Brassicaceae family. bioRxiv preprint doi: <https://doi.org/10.1101/2020.11.18.349449>

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