

Title: Mining Innovative Strategies for Stress-resilient Growth in Extremophyte Relatives of Brassicaceae Oil Crops

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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:

Wild relatives of crops, especially “extremophytes” naturally adapted to harsh environments, harbor traits that may have been lost during crop domestication, as well as novel innovations that enabled survival and growth under stresses. Two extremophyte oil crop-relatives, *Schrenkiella parvula* and *Eutrema salsugineum* in Brassicaceae, offer models to define gene functions contributing to physiological mechanisms that enable survival and growth under salt stresses and sub-optimal nutrient conditions. We used a comparative analysis of stress-responsive transcriptomes, ionomes, metabolomes, and the gene regulatory networks (GRN) between the extremophyte models and their stress-sensitive relatives, including *Arabidopsis thaliana*, to identify innovations in stress response strategies and genetic variations behind them.

We found that, while achieving the similar level of salt tolerance, *S. parvula* and *E. salsugineum* take distinct strategies¹. While *S. parvula* allows tissue Na⁺ accumulation similar to *A. thaliana* upon salt stresses, *E. salsugineum* appeared to limit the net Na⁺ entry. Still, both extremophytes maintained ion nutrient levels including K⁺, which *A. thaliana* failed to do so. Both extremophytes accumulate metabolites that can serve as osmo-protectants and antioxidants in response to salt treatment by following different trajectories. *E. salsugineum* metabolome showed stress readiness and pre-adaptation, coupled with its overall basal-level growth rates compared to both *A. thaliana* and *S. parvula*. On the other hand, *S. parvula* metabolome was characterized with rapid response to salt treatment, dynamically moving from pre-stress state more similar to that of *A. thaliana* to a state converging to that of *E. salsugineum* upon salt treatment. Transcriptomic responses in *S. parvula* supported the observed growth uninterrupted by salt treatment², seemingly governed by genes associated with auxin and ABA pathways.

To elucidate the innovations in stress-associated gene function in extremophyte lineages, we compared the GRNs responsive to the stress hormone ABA between the two extremophytes and two stress-sensitive relatives, *A. thaliana* and *Sisymbrium irio*³. *S. parvula*, mirroring its uniquely low disturbance of primary growth in the presence of salt^{1,2}, showed an enhanced root growth upon ABA treatment, in contrast to the inhibition of growth in the other three species. We characterized the genomic landscape of transcription factor (TF)-binding for a key stress-response TF family, ABA-RESPONSIVE ELEMENT BINDING FACTORS (AREB/ABF), by performing DNA Affinity Purification followed by sequencing (DAP-Seq) for all ABF orthologs in the four target species, as well as comparative RNA-seq analyses of ABA-responses in both root and shoot tissues. By performing Phylogenetically informed Profiling (PiP) and comparative GRN analysis, we found prevalent modifications in both transcriptome ABA responses as well as the AREB/ABF-binding landscapes in the *S. parvula* lineage. For example, expression of orthologs related to translation machinery showed strong attenuation as an early response to ABA in roots, but this response was strikingly absent in *S. parvula*. While the core ABA signaling GRN was relatively conserved, GRNs associated with the growth hormone auxin were highly divergent especially between *S. parvula* and the other species through gain-and-loss of AREB/ABF-binding. We identified *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1 (TAA1)* as a key candidate whose ortholog in *S. parvula* showed a modification in ABA-responsive GRN, which may be at least partially responsible for the distinct growth response to ABA treatment. We are currently expanding the comparative analyses to ten Brassicaceae species including the oil crop *Camelina sativa*, and developing pipelines that enable GRN comparisons across multiple species with polyploidy and lineage(s)-specific gene duplications. Our study will provide both genetic resources and strategies for developing more stress-resilient oil crops, as well as identify gene functions that cannot be explored in stress-sensitive model plants.

References/Publications

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