

Final Technical Report

DOE Award Number: DE-SC0020358
Institutional Name: Leland Stanford Junior University
Period of Performance: 9/15/2019 – 9/14/2023
PI Name: Jose Dinneny

Summary for PI José Dinneny, Stanford University

Through this grant, we developed a comparative framework to elucidate the mechanisms behind variations in environmental stress responses among a diverse group of species within the Brassicaceae family. Our focus was on the differences in physiological and transcriptomic responses to abscisic acid (ABA), a hormone associated with water stress. We examined the differential growth responses of four Brassicaceae species, finding that most exhibited reduced root growth correlated with smaller meristem size. In contrast, *Schrenkiella parvula* showed accelerated growth due to increased root cell elongation. We employed RNA sequencing to analyze the transcriptional responses to ABA across these species, and innovative bioinformatics techniques were used to pinpoint biological pathways with significant divergence. Additionally, we utilized DAP-seq to map the gene regulatory networks associated with ABA-responsive transcription factors, revealing that variations in the regulation of growth hormone biosynthesis play a critical role in the distinct ABA effects on root growth among the species. This research sets a new standard for comparative physiology by integrating comparative genomics and transcriptomics to uncover pathway divergences.

Verslues PE, Bailey-Serres J, Brodersen C, Buckley TN, Conti L, Christmann A, Dinneny JR, Grill E, Hayes S, Heckman RW, Hsu P-K, Juenger TE, Mas P, Munnik T, Nelissen H, Sack L, Schroeder JI, Testerink C, Tyerman SD, Umezawa T, Wigge PA (2023) Burning questions for a warming and changing world: 15 unknowns in plant abiotic stress. *The Plant Cell* doi:10.1093/plcell/koac263

Sun Y, Oh DH, Duan L, Ramachandran P, Ramirez A, Bartlett A, Dassanayake M, Dinneny JR (2022) Divergence in a stress-associated gene regulatory network underlies differential growth control. *Nature Plants*. doi 10.1038/s41477-022-01139-5

Summary for Co-PI Maheshi Dassanayake, LSU

We developed new computational pipelines and analyzed the root scRNAseq data generated for five target species (*Arabidopsis thaliana*, *Sysimbrium irio*, *Schrenkiella parvula*, *Eutrema salsugineum*, and *Camelina sativa*) under control, salt, and ABA treated conditions. New computational method development was required to assign cell types for scRNAseq data clusters across multiple species, treatments, and cells from different developmental stages. We identified cell-type, species, and stress dependent gene networks that shed insight into the evolution of stress responsive traits in the target Brassicaceae species which included an oil crop, stress-adapted species, and stress-sensitive species. While the significant results from this analysis are currently being prepared for a manuscript aimed to be submitted within three months, the tools, analyses, and insight gained from this project have led to five other published papers describing plant adaptations to environmental stress in diverse plants. We have created genome browsers for *S. parvula* and *E. salsugineum*, tissue expression atlases, and developed computational tools to study ortholog evolution, gene functional networks, and phylogenetic expression profiles among closely related species. This project partially supported four graduate students who completed their PhD thesis within the project duration, 2 post-doctoral scientists, 2 visiting graduate students from Israel and Brazil, and 8 undergraduate research students at LSU.

Tran KN, Pantha P, Wang G, Kumar N, Wijesinghege C, Oh DH, Duppen N, Li H, Hong H, Johnson JC, Kelt R, Matherne MG, Clement A, Tran D, Crain C, Adhikari P, Zhang Y, Oroozani M, Sessa G, Larkin JC, Smith AP, Longstreth D, Finnegan P, Testerink C, Barak S, Dassanayake M (2021) Balancing growth amidst salinity stress – lifestyle perspectives from the extremophyte model *Schrenkiella parvula* *bioRxiv* 2021.08.27.457575; doi:10.1101/2021.08.27.457575

Tran KN, Wang G, Oh DH, Larkin JC, Smith AP, Dassanayake M (2021) Multiple paths lead to salt tolerance - pre-adaptation vs dynamic responses from two closely related extremophytes *bioRxiv* 2021.10.23.465591; doi: <https://doi.org/10.1101/2021.10.23.465591>

Spatiotemporal gene expression atlas of the extremophyte *Schrenkiella parvula* (2022) Wijesinghege C, Wang G, Pantha P, Tran KN, Dassanayake M *bioRxiv* 2022.10.24.513627; doi: <https://doi.org/10.1101/2022.10.24.513627>

Bird, T., Nestor, B.J., Bayer, P.E. *et al.* Delayed leaf greening involves a major shift in the expression of cytosolic and mitochondrial ribosomes to plastid ribosomes in the highly phosphorus-use-efficient *Hakea prostrata* (Proteaceae). *Plant Soil* (2023). <https://doi.org/10.1007/s11104-023-06275-1>

Pramod Pantha, Dong-Ha Oh, David Longstreth, Maheshi Dassanayake, Living with high potassium: Balance between nutrient acquisition and K-induced salt stress signaling, *Plant Physiology*, Volume 191, Issue 2, February 2023, Pages 1102–1121, <https://doi.org/10.1093/plphys/kiac564>

Summary for Co-PI Song Li, Virginia Tech

We have developed machine learning tools to select candidate regulatory genes and associated regulatory interactions that are most “important” in predicting gene expression change under stress (Song et al. 2020). Following up on this direction, we have tested the model using a single cell stress response data from mouse monocytes and demonstrated the model can be applied to single cell data (Lee et al. 2021). We have also published protocols for the analysis of gene regulatory networks and multi-omics integration using single cell data (Chau, Timilsena, and Li 2023) (Li, Yan, and Lee 2021). To facilitate cell type annotation, we have published machine learning models to select informative cell type marker genes that cannot be identified by conventional statistical test (Yan et al. 2022). We also developed a computational protocol for trajectory analysis which can be used for determining cell type developmental stages and emergence of novel cell types. This protocol was first tested in the monocyte paper (Lee et al. 2021) and further tested and applied in a tomato single cell data to identify a novel cell layer endodermis (Cantó-Pastor et al. 2022). Same protocols have been used in the preliminary analysis of the single cell data generated in this project.

Cantó-Pastor, Alex, Kaisa Kajala, Lidor Shaar-Moshe, Concepción Manzano, Prakash Timilsena, Damien De Bellis, Sharon Gray, et al. 2022. "A Suberized Exodermis Is Required for Tomato Drought Tolerance." *BioRxiv*. <https://doi.org/10.1101/2022.10.10.511665>.

Chau, Tran, Prakash Timilsena, and Song Li. 2023. "Gene Regulatory Network Modeling Using Single-Cell Multi-Omics in Plants." In *Methods in Molecular Biology*, 259–75. *Methods in Molecular Biology* (Clifton, N.J.). New York, NY: Springer US.

Lee, Jiyoung, Shuo Geng, Song Li, and Liwu Li. 2021. "Single Cell RNA-Seq and Machine Learning Reveal Novel Subpopulations in Low-Grade Inflammatory Monocytes With Unique Regulatory Circuits." *Frontiers in Immunology* 12 (February): 627036.

Li, Song, Haidong Yan, and Jiyoung Lee. 2021. "Identification of Gene Regulatory Networks from Single-Cell Expression Data." *Methods in Molecular Biology* 2328: 153–70.

Song, Qi, Jiyoung Lee, Shamima Akter, Matthew Rogers, Ruth Grene, and Song Li. 2020. "Prediction of Condition-Specific Regulatory Genes Using Machine Learning." *Nucleic Acids Research* 48 (11): e62.

Yan, Haidong, Jiyoung Lee, Qi Song, Qi Li, John Schiefelbein, Bingyu Zhao, and Song Li. 2022. "Identification of New Marker Genes from Plant Single-Cell RNA-Seq Data Using Interpretable Machine Learning Methods." *The New Phytologist* 234 (4): 1507–20.

Summary for Co-PI John Schiefelbein, University of Michigan

As part of Aim 1 from the original grant proposal, the Schiefelbein lab generated single-cell RNA-seq (scRNA-seq) datasets from the roots of five Brassicaceae species under normal and stress conditions. Specifically, we used the 10X Genomics Chromium droplet-based platform to generate approximately 6,000 single-cell transcriptomes for each of two replicates from *Arabidopsis thaliana*, *Camelina sativa*, *Eutrema salsugineum*, *Schrenkiella parvula*, and *Sisymbrium irio* grown on standard media, 5 μ M ABA, and 100 mM NaCl. We used established pipelines to evaluate the characteristics of these data and conducted comparative transcriptome clustering and visualization to confirm the quality of these scRNA-seq datasets.

Farmer, A., Thibivilliers, S., Ryu, K.H., Schiefelbein, J., and Libault, M. (2021) Single-nucleus RNA and ATAC sequencing reveals the impact of chromatin accessibility on gene expression in *Arabidopsis* roots at the single-cell level. *Molecular Plant* 14:372-383. doi: 10.1016/j.molp.2021.01.001 [33422696]

Ryu, K.H., Zhu, Y., and Schiefelbein, J. (2021) Plant cell identity in the era of single-cell transcriptomics. *Annual Reviews of Genetics* 55:479-496. doi: 10.1146/annurev-genet071719-020453 [PMID: 34530637]

Yan, H., Lee, J., Song, Q., Li, Q., Zhao, B., Schiefelbein, J., and Li, S. (2022) Identification of new marker genes from plant single-cell RNA-seq data using interpretable machine learning methods. *New Phytologist* 234:1507-1520. doi: 10.1111/nph.18053 [PMID: 35211979]

Zhu, Y. and Schiefelbein, J. (2023) A conserved gene regulatory network controls root epidermal cell patterning in superrosid species. *New Phytologist* 238:2410-2426. doi: 10.1111/nph.18885