Functional Genetics to Analyze Effects of Genes Involved in Drought Tolerance and Seed Composition in *Arabidopsis thaliana* and Maize

Climate change has involved a 20-40% reduction in mean precipitation rates[1], increased heat stress, and an overall decrease in staple crop viability in portions of Latin America. These factors have significantly reduced agricultural productivity with crop losses reaching $13 billion[2] throughout Latin America and the Caribbean. This poses a threat to many rural farmers who rely primarily on subsistence farming as well as the global food market relying on their exports. I will use functional genetics approaches to examine mechanisms of drought tolerance and potential effects on nutritive quality. Favorable pairing of these trait sets can help to mitigate the severity of food insecurity in these regions.

I have identified three genes in maize, *ZmASR3*[3], *ZmPYL12*[4], and *zmm28*[5], which upon overexpression in *Arabidopsis thaliana* result in increased drought tolerance. The mechanism of *ZmASR3* has been characterized to relate to stomatal closure, decreased reactive oxygen species accumulation, and (somewhat relatedly) regulation of abscisic acid (ABA)/stress-responsive genes, similar to that of *ZmPYL12* which has been found to relate to ABA signaling. *Zmm28* is a MADS-box transcription factor. I will use a floral dip transformation method[6] and constitutive promoter to overexpress these genes across multiple plant tissues and developmental stages. While examining drought stress response (whole-plant physiological) traits that we hypothesize will be significantly correlated with yield under drought conditions, I will also examine seed macronutrient levels (protein, fat, and starch) via AOCC wet-chemistry reference methods.

By using functional genetics and molecular cloning in transformed Arabidopsis lines (in two genotypes to examine effects in multiple backgrounds), I will measure the effects of drought on plant physiological traits and seed nutritive quality compared to wild-type. I will be using the Snapgene software platform to plan and document the molecular cloning and verification steps. I will also conduct statistical analyses of seed yield and harvest index, physiological traits, and seed composition in the R software platform.

In the summer of 2020, I will conduct the floral dip transformation experiments (with molecular design taking place and plant material generated in Spring 2020) and characterization of transformed plants. I will analyze the effects of overexpressing each of these genes in *Arabidopsis* by quantifying yield, physiological traits, and seed nutritive quality. I will then conduct a drydown experiment with transformed and non-transformed (‘wild-type’/control) plants in a shared-pot setting, and with separate pots representing well-watered and drought treatments. My goal by the end of this project is to apply the findings of these physiological, biochemical, and molecular characterizations in *Arabidopsis thaliana* to maize in Fall 2020. I will design guide RNAs to be used in maize to conduct CRISPR/Cas9 for certain of the tested genes that show promising effects on both crop productivity and quality, to determine whether these genes will confer similar directionality, magnitude, and nature of response as in *Arabidopsis*.

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[1] Garreaud, René D., et al. "The Central Chile Mega Drought (2010–2018): A Climate Dynamics Perspective." *Royal Meteorological Society (RMetS)*, John Wiley & Sons, Ltd, 21 July 2019, rmets.onlinelibrary.wiley.com/doi/abs/10.1002/joc.6219.

[2] "22 Billion Dollars in Ten Years: The Cost of Agricultural Losses from Disasters in Latin America and the Caribbean." *FAO*, 2018, www.fao.org/americas/noticias/ver/en/c/1112321/.

[3] Liang, Yani, et al. "ZmASR3 From the Maize ASR Gene Family Positively Regulates Drought Tolerance in Transgenic *Arabidopsis." International Journal of Molecular Sciences*, MDPI, 8 May 2019, www.ncbi.nlm.nih.gov/pmc/articles/PMC6539908/.

[4] He, Zhenghua, et al. "The Maize ABA Receptors ZmPYL8, 9, and 12 Facilitate Plant Drought Resistance." *Frontiers in Plant Science*, Frontiers Media S.A., 4 Apr. 2018, www.ncbi.nlm.nih.gov/pmc/articles/PMC5893742/.

[5] Wu, Jingrui, et al. "Overexpression of zmm28 Increases Maize Grain Yield in the Field." *PNAS*, National Academy of Sciences, 19 Nov. 2019, www.pnas.org/content/116/47/23850.

[6] Zhang, Xiuren, et al. "Agrobacterium-Mediated Transformation of *Arabidopsis Thaliana* Using the Floral Dip Method." *Nature Protocols*, U.S. National Library of Medicine, 2006, www.ncbi.nlm.nih.gov/pubmed/17406292/.

Learning Potential and Relationship of Project to Educational Goals

Food security has been threatened in underdeveloped countries, even more so with the volatile effects of climate change hindering staple crop viability in these regions. My focus is on the mitigation of food insecurity in Latin America and the Caribbean (LAC), with one of my long-term objectives being the engineering of abiotic resistant staple crops, such as maize and sorghum, that can also be biofortified for nutritive gain through genome editing. These techniques would be pivotal in improving diets and reducing malnutrition rates in rural regions of LAC. I plan to continue my education and obtain a PhD in genetics and genomics, in order to aid in the development of these crop breeding technologies. With my international perspective, I hope to work with the United Nations Food and Agricultural Organization to develop and integrate programs for LAC to mitigate poverty and food insecurity as defined in their Sustainable Development Goals.

The project I have proposed for the PUF entails overexpression of drought resistant genes found in maize and examination of effects on yield, physiological traits, and seed nutritive quality. These are formative steps in addressing food insecurity in drought-prone regions of LAC, allowing for a deeper understanding of the synergies and tradeoffs of using functional genetics strategies to create viable crops in the face of adverse precipitation effects from climate change. This experience will further my knowledge in the field of plant functional genetics, giving me the opportunity to network and to participate in future research projects that will positively impact food sustainability in the world.

Budget Outline
Abelina Jackson
Diepenbrock Lab

Seed Compositional Analysis = $576.00

To quantify seed compositional traits, certain samples would be sent to the UC Davis Analytical Lab, a core facility on campus. These samples will be analyzed for the 'Feed Group 2' set of nutrients offered by the Analytical Lab, which includes dry matter (DM), protein, acid detergent fiber (ADF), total digestible nutrients (TDN), ash, and fat. For the three genes I will be testing and for wild-type (control) plants, I will submit three replicates for analysis, each of which represents a bulked set of plants. The service cost is $48 per sample, so for the 12 samples it would be $576.00 to complete the Feed Group 2 analysis. Inclusion of three replicates will be useful in determining standard errors in the quantitative measurements, which I will analyze in RStudio, an open-source software platform.

Megazyme kit for total starch quantification = $336.15

Given that the analyses above include protein and fat but not starch, I will also quantify total starch through the use of a Megazyme colorimetric kit (K-TSTA, AOAC Method 996.11), so that I can more comprehensively analyze seed macronutrient composition.

Vectors = $144.00

A pCAMBIA1301 overexpression vector will be used when conducting the floral dip transformation method in *Arabidopsis thaliana*. This will enable analysis of the functions of my selected genes under drought vs. well-watered conditions. The vector will cost $144.00.

Total requested amount = $1,056.15