SCSB Quarterly Report

General Information

Principal Investigator(s) Name(s): Sruthi Narayanan
Organization: Clemson University
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Quarter: 4

Proposal Information

Title: Exploring the Differences in Gene Expression of Drought Sensitive and Drought Tolerant Genotypes Using RNA-Seq
Amount Expended to Date: 80%. This project has three funding sources, excluding funds needed to pay salary and fringe benefits of a graduate student. Fifty percent of the experiment is funded by the SCSB and 50% by two other grants. So far, 80% of the funds needed to complete the project have been expended from all funding sources. Re-extractions of the samples that did not produce good results have been repeated and extractions are now complete. Only a small percentage of funds have been spent since the last report due to further COVID-19 delays.

Progress Assessment

RNA was collected from PI471938 (slow wilting) and NC-ROY (fast wilting), grown in both a greenhouse and growth chamber:, using 3 biological replicates samples, per genotype. Purification is set to take place in the coming weeks using the Ambion PureLink mini kit (ThermoFisher Scientific) following the manufacturer's recommended procedures. Stranded mRNA sequencing libraries will also be prepared in the coming weeks using standard TruSeq kits (Illumina) and sequenced to a depth of at least 20M reads (2x150bp PE) per replicate sample using the latest chemistry release on an Illumina NovaSeq. We had hoped to have this complete by the time the last report was submitted, but there was a last minute, unexpected delay in receiving supplies due to COVID-19. Despite these setbacks we have been able to identify genes of interest based on previous research. The expression of genes involved in starch and sugar metabolism, cell wall synthesis, photosynthesis and chlorophyll synthesis are all possible candidates regulating drought stress. Through our research we hope to validate some of the genes that have been previously identified and provide insight into possible mechanisms underlying drought tolerance in soybean. We have also started to investigate key antioxidants that are known to accumulate in plants to protect against reactive oxygen species, which are over generated under drought conditions. This is something new we had not previously planned on evaluating, but after observations that were made in both the greenhouse and in the field this year, this might be an important aspect in breeding for drought tolerance. In addition, breeding for improved antioxidant concentrations in soybean could produce healthier end products. Lastly, all yield plots have been harvested. Processing of all field samples is currently underway. In addition to what was collected in the field (yield and wilting scores), we also plan to analyze each sample for test weight, seed size and seed quality.

Key Performance Indicators

All extractions have been completed. Any samples that needed to be redone have been completed. So, we now have good quality RNA for all samples collected during the experiment. To recap, we have successfully been able to carry out the greenhouse portion of the project, which we were able to correlate to data collected in the field in 2020 and we have been able to successfully extract RNA from leaf samples collected from the greenhouse. Now the samples need to purified, sequenced and analyzed. Our timeline has been delayed several times due to COVID-19. After a delay in getting started with the project, we were able to catch back up to our projected timeline, but additional shipping delays related to COVID-19 caused further set backs. We still hope to have all lab work complete by the project deadline, but data analysis will not be completed until after the deadline. Once all data analysis is complete we will present the results to the board. The results of this experiment are important to us and we do plan to carry out the project until it is completed.

Next Steps

After RNA purification and sequencing libraries have been prepared, raw sequence reads will be preprocessed for adapters and low-quality bases with the Trimmomatic software (Bolger et al. 2014). Trimmed sequence reads will be aligned to the latest release of the Williams 82 soybean reference assembly. We will conduct pairwise comparisons of gene expression over the time course within the contrasting genotypes using the edgeR differential analysis tool. We will analyze these data for gene regulation using a variety of approaches that includes Gene Set Enrichment Analysis (GSEA), pairwise comparisons, GO enrichment, mapping to KEGG pathways, and hierarchical /k-means clustering. We will evaluate the drought tolerance profile of both genotypes and identify critical driver genes involved in water use efficiency. These data will be translated to prognostic biomarkers (SNPs or gene expression profiles) that will be used for Objective 3 (screening germplasm for these biomarkers).