From Leaves to Roots to Microbes: How Sorghum Responds to Drought

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Project Goals: Transcriptomic and epigenetic control mechanisms during temporal and spatial responses to water-limiting conditions are being studied in leaves and roots of field-grown, pre-flowering and post-flowering drought-tolerant *Sorghum bicolor* (L.) Moench varieties. Changes in bacterial and fungal communities, associated with drought-stressed sorghum, are also being studied in bulk soil, rhizosphere and roots. Our goal is to understand roles transcriptomic and epigenetic signals play in acclimation to and recovery from pre- and post-flowering drought, revealed through transcriptional networks and molecular profiles *in planta*, using RNA-Seq, ChIP-Seq, BS-Seq, proteomics, metabolomics, and histone profiling. Also, impact of microbial populations is being inferred from metagenomics, metatranscriptomics and metabolomics. Ultimately, we will identify genes and molecular markers to develop genetic strategies for improving drought tolerance in sorghum and other crops. Cumulative data will be used to devise models to better predict and control the roles and interactions of transcriptional regulation, epigenetics and the microbiome in sorghum's response to drought.

Based primarily on phenotype, classical breeding and mutagenesis have focused mainly on changing a plant's DNA to modify desired traits. Increasing published data, however, show that environmental responses and plant development are also mediated by epigenetics that does not involve a change in DNA sequence. Critical to EPICON research, both transcriptomic and epigenetic changes play major roles in regulating drought responses. Thus, impact of our studies will increase given the likelihood that frequency and severity of drought will increase with climate change, posing major challenges for world agricultural productivity.

Plant exposure to abiotic stresses triggers cascades of transcriptomic and epigenetic changes. EPICON efforts focus on discovering the temporal and spatial influences that transcriptomic, epigenetic, proteomic, metabolomic and microbial signals play in acclimation to and recovery from drought. Our studies focus on *Sorghum bicolor* (L.) Moench, a widely cultivated cereal noted for drought and flood tolerance, that also offers advantages as a bioenergy feedstock because of its relatively reduced environmental footprint and its flexibility in bioenergy uses. For our experimental design, drought conditions are imposed in the field in California's Central Valley, where summer rainfall is rare. In two years of field experiments, one pre-flowering and one post-flowering drought-tolerant sorghum cultivars was planted in a replicated split plot design. Both varieties were subjected to three watering treatments: normal watering and pre- and post-flowering drought treatments. Phenotypic measurements, like grain and biomass yields and flowering times and growth rates, were taken from early June to late September. Analyses of years 1 and 2 data indicate that, compared to control plants, those exposed to pre- and post-flowering drought stress had notable differences in drought effects, particularly comparing the pre-flowering drought tolerant variety to the post-flowering drought-tolerant variety.

For molecular analyses, leaf and root samples, collected weekly, were used to track spatiotemporal changes in transcriptomic, epigenetic, metabolomic and proteomic footprints. From transcriptional profiling of year 1 samples, widespread adaptations were seen in all developmental stages, along with rapid transcriptional changes after watering of droughted plants. Also, imposing drought after flowering also caused similarly rapid transcriptional changes. Genes, with developmental changes in transcription during pre-flowering drought, were classified into distinct sets of temporal patterns, revealing varied reactions to pre-flowering drought. Ongoing work is attempting to identify biological function of these responses and their relation to other epigenetic and phenotypic properties of the plant. Analysis of such responses to post-flowering drought is underway. Initial analysis of BS-Seq data in leaves revealed many regions where methylation changes are in concert with plant development, including differences between the two varieties. Drought effects on such methylation patterns is under investigation.

Corresponding metabolomic and proteomic analyses are in progress, employing a method where proteins and metabolites are extracted from the same samples. Global proteomic analyses with extensive multiplexing and fractionation enable deep proteome coverage; metabolites from the same samples are also being analyzed. LC-MS was used to analyze histones, purified in untargeted fashion, enabling discovery of novel drought-related histone posttranslational modifications. A comprehensive molecular map of soil organic matter was generated to assess differences in chemical composition across the field and rhizosphere-mediated processes.

Using soil, root and rhizosphere samples collected as above, microbiome changes were studied, following drought and re-watering treatments. Gene function was inferred fro m shotgun metagenomic and metatranscriptomic analyses. Using year 1 bacterial data, pre-flowering drought was shown to lead to rapid changes in community composition, with relative enrichment of most Gram-positive bacterial lineages. This enrichment is reversible, leading to reversion, within one week after re-watering, to a state dominated by Gram-negative lineages. The enrichment in Gram-positive lineages was accompanied by increases in transcriptional activity, specifically for gene functions related to carbohydrate and amino acid transport and metabolism. Through metabolomic analyses, drought-treated roots were shown to be enriched in many of the same carbohydrate and amino acid metabolites, suggesting interplay between plant metabolism and bacterial community activity. From year 1 fungal data, both pre-flowering and post-flowering drought exerted significant effects on fungal diversity and community composition. Pre-flowering drought induced an ~100-fold enrichment of an *Acremonium* fungus in roots; post-flowering drought had an ~2-fold enrichment of a *Gibberella* fungus. Rhizosphere fungal community largely followed patterns of the root fungal community, but, the soil fungal community was not substantially affected. The leaf fungal community was not affected by pre-flowering drought, but it was affected following post-flowering drought. Symbiotic arbuscular mycorrhizal fungi were found in root, rhizosphere and soil; however, their diversity and community composition were not affected by drought.

Over the entire project period, collected data will provide a deeper understanding of the restructuring of the metabolic and regulatory landscape during drought, including impacts of microbes. We hope to identify key transcriptional and epigenetic regulators, controlling drought tolerance, and the relationship between sorghum and its associated microbes. Identified genetic targets, regulatory pathways and beneficial microbial symbionts will be used to improve growth and biomass production of sorghum and other crops under water-limiting conditions.

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