

EPICON: Epigenetic Control of Drought Response in Sorghum

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Project Goals: EPICON researchers are studying epigenetic control mechanisms in the temporal and spatial response to water-limiting conditions in field-grown, pre-flowering and post-flowering drought-tolerant *Sorghum bicolor* varieties. Investigators are also searching for changes in microbial communities, including bacterial and fungal microbiomes, in soil, rhizosphere, roots and leaves associated with drought-stressed sorghum. The primary focus of these efforts is to understand the roles that epigenetic signals play in acclimation to and recovery from drought. We will investigate effects of this abiotic stress on transcriptional networks and molecular profiles *in planta*, using a wide array of analytical tools, *i.e.*, RNA-Seq, smRNA-Seq, ChIP-Seq, BS-Seq, LC-Orbitrap-MS, and GC-MS. Similarly, function of microbial populations will be inferred from results of metagenomics and metatranscriptomics. Based on outcomes, we will identify genes and molecular markers to devise genetic strategies for improving drought tolerance in sorghum and other crops. From the cumulative data, we will develop models to better understand, predict and control the coordinated role of epigenetics and the phytobiome in sorghum's response to drought.

Genetic manipulation of crops via classical breeding and mutagenesis has focused primarily on using modifications of the plant's DNA sequence to increase the presence or activity of desirable traits. However, there is increasing published research indicating that environmental responses and plant development are also mediated by epigenetics, the process by which heritable changes in phenotype and/or gene expression are accomplished without changing the underlying DNA sequence. With particular relevance to the EPICON project, epigenetic changes have been shown to play a major role in regulating plant responses to drought, an abiotic stress likely to increase in frequency and severity in the future due to climate change, resulting in major challenges for world agriculture. We hypothesize that exposure of plants to abiotic stresses, including water limitation, triggers cascades of epigenetic changes. These include remodeling of chromatin, the network of DNA, RNA and proteins making up chromosomes, coupled with related changes in regulatory mechanisms, including small non-coding RNAs.

Efforts of EPICON researchers are concentrated on discovering the temporal and spatial influence epigenetic signals play in acclimation to and recovery from drought by identifying effects on individual transcription factors and/or transcriptional networks. To achieve this goal, we are studying the responses of two *Sorghum bicolor* cultivars that differ in their tolerance to pre-flowering and post-flowering drought stress. Sorghum, a widely cultivated cereal recognized for its drought and flood tolerance, offers notable advantages as a bioenergy feedstock because of its relatively reduced environmental footprint compared to its close relative, maize. In our experimental design, drought conditions are being imposed on sorghum in the field in California's Central Valley, where lack of summer rainfall makes well-controlled studies in drought nurseries possible. In the first year of field experiments, the two cultivars were planted in a split plot design in triplicate, along with a fourth fail-safe replicate; both varieties were subjected to a normal watering regime, as well as pre-flowering and post-flowering drought treatments. Phenotypic measurements were conducted throughout the growth cycle (early June - late September) to chart, for example, the flowering time, growth, and grain and biomass yield. Initial statistical analyses of agronomic data indicate that pre- and post-flowering drought stress impacts various agronomic characteristics,

compared to plants experiencing normal watering conditions. Pre-flowering drought stress impacted the yield of both BTx642 and RTx430; however, RTx430 showed a greater tolerance to pre-flowering stress compared to BTx642. This finding is consistent with the reported pre-flowering drought stress tolerance in RTx430. BTx642, a post-flowering, stay-green variety, had yields equal to or slightly higher in drought-stressed plots than BTx642 under controlled watering conditions, while RTx430 showed a reduction in yield. Pre-flowering and post-flowering stress negatively impacted both varieties, compared to the plants grown under normal watering conditions, with regard to other agronomic measurements, including height, flowering and biomass.

For molecular analyses, leaf, root and soil samples were taken weekly at precise intervals during sorghum development. Leaf and root samples were collected to track spatiotemporal changes in epigenetic, transcriptomic, metabolomic and proteomic footprints, that are currently being analyzed using RNA-Seq, smRNA-Seq, ChIP-Seq, BS-Seq, LC-Orbitrap MS, and GC-MS. As potential molecular mechanisms and specific targets are identified *in planta*, engineering of genes and pathways in sorghum will be used to validate suggested findings. In addition, shifts in the composition and activity of sorghum-associated and soil-based bacterial and fungal communities throughout the drought period are being monitored to determine if changes in membership or functional capacity of microbes in the soil, rhizosphere, roots and leaves correlate with epigenetic, transcriptional or metabolomic variation in the plant. Changes in the microbiome are being identified using Illumina iTag, amplicon sequencing of ribosomal repeats (16S rRNA for bacteria and ITS2 for fungi). Function is being inferred via shotgun metagenomic and metatranscriptomic analysis of rhizosphere communities.

Once compiled, analysis of the entire data set, year by year and over three years, will provide a more thorough understanding of the processes responsible for restructuring the metabolic and regulatory landscape of sorghum during drought and the relationship of these processes to drought tolerance. These efforts will lead to achievement of our ultimate goal – to identify key transcriptional regulators and pathways that control drought tolerance and to characterize their mechanisms of action, both *in planta* and potentially their relationship with the associated microbial communities. Additionally, these efforts should uncover phenotypic biomarkers associated with drought tolerance, which can be used to monitor and to correlate with phenotypic changes in large breeding populations. Identified genetic targets and their regulatory pathways will be utilized in future efforts to improve growth and biomass production of sorghum and other crops under water-limiting conditions, using transgenic and genome-editing approaches.

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