

EPICON: Epigenetic Control of Drought Response in Sorghum

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Project Goals: EPICON researchers will explore epigenetic control mechanisms in the temporal response to controlled field-based, water-limiting conditions in pre-flowering and post-flowering drought-tolerant *Sorghum bicolor* varieties, and investigate changes in their associated rhizospheric bacterial and fungal microbiomes. EPICON's efforts will focus on unraveling the role epigenetic signals play in acclimation to and recovery from drought through effects on individual transcription factors or transcriptional networks that direct entire metabolic pathways. This research will utilize a wide portfolio of analytical tools, *i.e.*, RNA-Seq, smRNA-Seq, ChIP-Seq, BS-Seq, Orbitrap MS, MALDI-ToF MS, nano-DESI, metagenomics and metatranscriptomics. In performing this work we will identify genes and markers to improve genetic strategies for sorghum and other crops, particularly with respect to drought response. From the cumulative data, we will develop a model to better understand the role of epigenetics and the phytobiome in sorghum's response to drought.

Genetic manipulation of crops to increase the presence or activity of desirable traits has focused primarily on modifications of the plant's DNA sequence. However, there is increasing public research that indicates plant development and environmental responses are also mediated by epigenetics, the process by which heritable changes in phenotype or gene expression are accomplished without changes in the 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, which is likely to be an increasing problem for world agriculture due to climate change. In general, exposure of plants to abiotic stresses, including water limitation, triggers cascades of epigenetic changes, which include remodeling of chromatin, the network of DNA, RNA and various proteins making up chromosomes, coupled with related changes in regulatory mechanisms, including the involvement of small non-coding RNAs.

This project's research efforts will focus on unraveling the temporal role that epigenetic signals play in acclimation to and recovery from drought through effects on individual transcription factors or transcriptional networks that direct entire metabolic pathways. To achieve these goals, responses to water deprivation will be studied in two sorghum cultivars that differ in their drought responses, pre-flowering versus post-flowering drought tolerance. Sorghum, a widely cultivated cereal, noted 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, corn. The study of sorghum's response to drought will be conducted in California's Central Valley; this region's lack of summer rainfall will make well-controlled drought studies in the field possible. Phenotypic analyses will be conducted to chart growth, flowering, grain and biomass yield, and other observable characteristics. Leaf and root samples will be taken at regular intervals during sorghum development to perform molecular phenotyping to track spatiotemporal changes in epigenetic, transcriptomic, metabolomic and proteomic footprints, using RNA-Seq, smRNA-Seq, ChIP-Seq, BS-Seq, Orbitrap MS, MALDI-ToF MS and nano-DESI. As potential molecular mechanisms are identified, targeted engineering will be used to validate suggested findings.

Shifts in the composition and activity of sorghum-associated bacterial and fungal community composition throughout the drought period will also be monitored to determine if changes in membership or functional

capacity within the rhizosphere, root endosphere, and phyllosphere correlate with epigenetic, transcriptional or metabolomic variation in the plant. These investigations into the sorghum microbiome will be done using Illumina itag sequencing of 16S rRNA and ITS2 (internal transcribed sequence) amplicons, specific to prokaryotic and fungal microbes respectively, and via shotgun metagenomic and metatranscriptomic sequencing of rhizosphere communities.

Analysis of the entire data set will provide a better understanding of the epigenetic processes responsible for restructuring the metabolic and regulatory landscape of the sorghum genome, and the relationship of these processes to drought tolerance. These efforts will lead to the achievement of our ultimate goal, which is to identify key transcriptional regulators and pathways that control drought tolerance and to characterize their mechanisms of action, both in the plant and in associated microbial communities. Additionally, these efforts will uncover biomarkers that are associated with drought tolerance, which can be used to monitor and follow phenotypic changes in large populations. The 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.

The EPICON project is supported by the Office of Biological and Environmental Research in the DOE Office of Science.