

Title: Lessons from the Field: How Sorghum and Its Microbiome Respond to Drought

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Project Goals:

Analysis of transcriptomic and epigenetic control mechanisms during spatiotemporal responses to water-limiting conditions is being performed on leaves and roots of field-grown *Sorghum bicolor* (L.) Moench, under control and pre- and post-flowering drought. Also investigated are changes in associated bacterial and fungal communities in bulk soil, rhizosphere, leaves and roots of drought-stressed sorghum. Goals of these efforts are to understand in sorghum mechanisms functioning in acclimation to and recovery from pre- and post-flowering drought, using RNA-Seq, BS-Seq, proteomics, metabolomics, and histone profiling. Additional insights into sorghum's responses are gleaned from studying impacts on microbial populations, using metagenomics, metatranscriptomics, and metabolomics. Cumulative data are being used to devise models to better predict and control roles and interactions of transcriptional regulation and of the microbiome in sorghum's response to drought. We hope to identify genes, molecular markers and microbes to develop strategies for improving drought tolerance in sorghum and other crops.

EPICON researchers aim to better understand impacts of drought on crops, due to increased frequency and severity of that stress with climate change. Both transcriptomic and epigenetic changes play major roles in regulating reactions to drought. To better understand spatiotemporal responses, samples of leaves and roots of field-grown *Sorghum bicolor* (L.) Moench were collected weekly over the growing season. RNA-Seq, BS-Seq, metabolomics, proteomics, and histone profiling were used to gain a mechanistic understanding of plant acclimation to and recovery from drought. Shotgun metagenomics, metatranscriptomics, and metabolomics were used to monitor sorghum's rhizosphere bacterial and fungal microbiome communities.

As a widely cultivated, drought- and flood-tolerant cereal, sorghum is a flexible bioenergy feedstock with a relatively reduced environmental footprint. EPICON studies were done in fields in California's Central Valley, where rare summer rainfall permitted controlled drought conditions during the four years of field trials. One pre-flowering drought-tolerant and one stay-green, post-flowering drought-tolerant variety were planted in a replicated, split plot design, with normal watering and pre- and post-flowering drought. Phenotypic observations were taken during the growing season, grain and biomass yields at harvest. Most impacted phenotypes during pre-flowering drought were later flowering, shorter stature and lower forage/grain yields; for post-flowering drought premature plant death, lodging and reduction in seed size were seen.

Year one transcriptional profiling of triplicate, weekly leaf and root samples revealed widespread adaptations at all developmental stages, as well as after watering pre-flowering droughted plants and after imposing post-flowering drought – with 44% of expressed genes significantly affected (Varoquaux et al., 2019). Based on 350 transcriptomes, fast, temporal transcriptomic responses were seen in leaves and roots, including changes in well-known drought pathways. Roots had greater transcriptional disruptions than leaves. Pre-flowering drought had more complex temporal changes than post-flowering drought; large differences were found between genotypes. Efforts now focus on detailed analyses of Year 2 and 3 data.

In-depth studies were done on two drought-related transcriptional responses. (1) Qualitative differences in transcriptomic and proteomic data were seen in responses of photosynthesis-related genes to pre- and post-flowering drought, suggesting mechanisms of (i) enhanced photoprotection in pre-flowering drought, (ii) changes to photosynthate partitioning, specific to pre- and post-flowering drought, and (iii) altered progression of leaf senescence in post-flowering drought. Quantification of photosynthetic traits in the field confirmed genotypic and drought-induced differences in photosynthetic performance, predicted based on transcriptomic and proteomic data. (2) Large-scale reduction in expression of sorghum genes critical to arbuscular mycorrhizal (AM) fungi symbiosis occurred in both pre- and post-flowering drought and co-occurred with a drop in AM fungal mass, suggesting drought leads to loss of AM fungi and their vital symbiotic interactions. Upon re-watering, plant gene expression and AM fungal mass increased to control levels. Gene expression differences were the largest genotype-specific drought responses for a single functional gene category, indicating AM symbiosis may explain some genotype differences during pre-flowering drought recovery (Varoquaux et al 2019).

Preliminary analysis of BS-Seq data, designed to explore DNA methylation patterns, revealed many regions in leaves where changes correlated with plant development. Also, strong expression from transposable elements occurred under drought, often continuing after water resumption. Further study awaits high accuracy genome sequencing and annotation of the two genotypes. Preliminary data from year 1 leaf samples suggest terminal clipping of histones H4 and H3 may regulate plant growth and drought tolerance differently in the two genotypes (Zhou

et al. 2019). LC-MS analysis of year 2 intact histones from leaves will enable discovery of novel drought- or development-related posttranslational modifications.

Distinct, differential protein profile changes between genotypes suggest protein level changes may relate to differential drought tolerances, especially in pre-flowering drought. Comparisons of protein ratios revealed functional categories distinct to each variety. During pre-flowering drought, identity of leaf proteins, significantly increased in the pre-flowering, drought-tolerant variety versus the post-flowering, drought-tolerant variety, suggest chaperones may play key roles in stabilizing key protein or enzyme functions involved in drought tolerance in the pre-flowering, drought-tolerant variety. Conversely, the post-flowering, drought-tolerant variety showed significantly higher enrichment in beta-glucoside metabolism and photosynthesis-related proteins. Regarding metabolites, increases in proline, glyceraldehyde, shikimic acid, sugar pentoses and hexoses, were seen during pre-flowering drought. During post-flowering drought, increases in 3-hydroxycinnamic acid, mevalonic acid, quinic acid and sugar monosaccharides were seen in leaves of both varieties. Significant increases in proline, glycine, betaine, arginine, lysine and serine were seen in roots of the post-flowering, drought-tolerant variety.

Using soil, root, leaf and rhizosphere samples, collected weekly from the same plants, dramatic shifts in bacteria and fungi followed drought and re-watering (Xu et al. 2019). Genome-resolved metagenomics within rhizosphere and soil samples allowed binning of shotgun metagenomic and metatranscriptomics reads into the most abundant organisms. Results show that rapid changes in bacterial community composition, occurring after pre-flowering drought, correlate with shifts in transcriptional activity of specific biological functions, i.e., genes related to carbohydrate and amino acid transport and metabolism. Also, organisms, enriched under drought stress, contain a larger repertoire of genes in the same categories. These data, coupled with root metabolomics, suggest interplay between plant metabolism and bacterial community activity that is in part sensitive to shifts, following drought stress, in nutrient profiles within the root and rhizosphere.

Total fungal diversity and community composition are significantly affected by both pre- and post-flowering drought (Gao et al 2020). Importantly, abundance of the fungal plant pathogens, i.e., *Fusarium*, *Gibberella* and *Sarocladium*, decreases in pre-flowering drought but increases in post-flowering drought, more strongly in rhizosphere than root (Gao et al 2020). AM fungi behavior was discussed above (Gao et al 2018, Varoquaux et al 2019). Expression of sorghum genes involved in communication with AM fungi (both strigolactones and Myc-LCOs) were also found to correlate with abundance of early or late AM fungal species. Lastly, the cross-kingdom network of fungal-bacterial co-occurrence is disrupted by drought, recovering after re-watering.

Scale and scope of EPICON data provide unprecedented platforms for in-depth exploration of molecular mechanisms of drought tolerance and its interplay with the plants' biotic environment. Data generated provide many avenues for future research on sorghum and drought – likely relevant to other crops. Ultimately, genes, molecular markers and microbes causally associated with drought tolerance will be identified that improve yield and fitness under drought.

References:

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