Title: EPICON: From Leaves to Roots to Microbes – How Sorghum and Its Microbiome Respond to Drought

Peggy G. Lemaux[1*] (lemauxpg@berkeley.edu), Jeffrey Dahlberg,[2] Devin Coleman-Derr,[1,3] Robert Hutmacher,[4] Christer Jansson,[5] Ronan O'Malley,[6] Elizabeth Purdom,[1] John Taylor,[1] Axel Visel[6] and John Vogel[1,6]

[1] University of California, Berkeley; [2]University of California, Kearney Ag Research & Extension Center, Parlier; [3]USDA-ARS Plant Gene Expression Center, Albany CA; [4]University of California, West Side Research & Extension Center, Five Points; [5]EMSL-Pacific Northwest National Laboratory, Richland WA; and [6]DOE Joint Genome Institute, Walnut Creek CA.

Project Goals:

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

EPICON research focuses on drought, given that its frequency and severity will increase with climate change. It is broadly accepted that both transcriptomic and epigenetic changes play major roles in regulating drought responses. These are the issues driving EPICON research. Spatiotemporal responses of transcriptomic and epigenetic controls were examined in leaves and roots of field-grown, drought-tolerant *Sorghum bicolor* (L.) Moench. RNA-Seq, BS-Seq, proteomics, metabolomics, and histone profiling are used to gain a mechanistic understanding of plant acclimation to and recovery from drought. Shotgun metagenomics, metatranscriptomics, and metabolomics were also used to monitor sorghum's rhizosphere microbiome.

Sorghum bicolor was chosen because it is a widely cultivated, drought- and flood-tolerant cereal – important as a flexible bioenergy feedstock with a relatively reduced environmental footprint. Drought was imposed in fields in California's Central Valley, where rare summer rainfall permits controlled drought conditions. One pre-flowering 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. Weekly phenotypic measurements were taken during the growing season, grain and biomass yields at harvest. Most impacted yield-related phenotypes due to pre-flowering drought, were later flowering, shorter stature, lower forage/grain yields.

Year one transcriptional profiling of triplicate, weekly leaf and root samples, revealed widespread adaptations at all developmental stages, including within one week after watering pre-flowering droughted plants and after imposing post-flowering drought – with 44% of expressed genes significantly affected (Varoquaux et al., submitted). Based on 350 transcriptomes, fast, global, temporal transcriptomic responses were observed in leaves and roots, including modulation of well-known drought pathways. Roots showed greater transcriptional disruptions than leaves; pre-flowering drought had more complex temporal changes than post-flowering drought; large differences were found between genotypes.

In-depth studies were done on two drought-related transcriptional responses. (1) Genotypic differences were seen in core photosynthesis and reactive oxygen species (ROS)-scavenging pathways, suggesting possible mechanisms of drought tolerance and delayed senescence in the post-flowering, drought-tolerant, stay-green variety. Baseline gene expression differences between the two varieties may affect how equipped plants are for pre- or post-flowering drought. For example, the stay-green variety has constitutively higher mRNA expression of genes involved in ROS scavenging and osmoprotection, versus the non-post-flowering drought-tolerant variety. (2) Large-scale depletion in expression of genes critical to arbuscular mycorrhizal (AM) symbiosis occurred during drought, with corresponding drops in AM fungal root mass. That drop in mass during pre- and post-flowering drought corresponded with decreased mRNA expression in AM fungal symbiosis-induced genes, suggesting drought leads to reduced AM fungal survival and a loss in vital symbiotic interactions. These gene expression recovery differences were the largest genotype-specific drought response for a single functional category of genes, indicating AM symbiosis may explain some genotype differences during pre-flowering drought recovery.

Preliminary analysis of BS-Seq data, designed to explore DNA methylation patterns, revealed many regions in leaves where changes correlated with plant development, including varietal differences. Additionally, strong overexpression from transposable elements occurred under drought and, at times, continued after water resumption. Current efforts focus on elucidating connections between transposable elements and methylation changes. Also, LC-MS analysis of intact, untargeted leaf histones enabled discovery of novel drought- or development-related histone posttranslational modifications. Data suggest terminal clipping of histones H4 and H3 may regulate plant growth and drought tolerance differently in the varieties.

Metabolomic and proteomic changes in leaf and root samples, identical to those above, are being analyzed in samples in which proteins and metabolites are extracted from the same samples. GC-MS of root and leaf metabolites revealed greater disruptions in roots versus leaves and significant differences between the two varieties. Proline was significantly increased in both varieties during pre-flowering drought versus controls. Glycerol 3-phosphate was significantly increased in roots during pre-flowering drought, which correlated with rhizosphere monoderm enrichment. iTRAQ-labeled peptides revealed significant changes in roots with significantly different protein profiles between the varieties. At the most extreme pre-flowering drought time, ascorbate metabolism, flavonoid and carotenoid biosynthesis, and porphyrin and chlorophyll metabolism pathways were more significantly changed in the stay-green variety versus the pre-flowering drought-tolerant variety. This validates transcriptomic data where genes involved in ROS-scavenging and photosynthesis are more significantly altered in the stay-green 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. Gene function in those populations was inferred from shotgun metagenomic and metatranscriptomic analyses. Rapid changes in bacterial community composition occurred following pre-flowering drought, revealing relative enrichment in most monoderm (Gram-positive) bacterial lineages (Xu et al., 2018). After re-watering, reversion occurred within one week, leading to domination by diderm (Gram-negative) lineages. Monoderm enrichment during drought was accompanied by increases in transcriptional activity, specifically for genes related to carbohydrate and amino acid transport and metabolism. From metabolomic analyses, drought-treated roots were enriched in many of the same carbohydrate and amino acid metabolites, suggesting interplay between plant metabolism and bacterial community activity. Second year data recapitulates these findings and showed that the root microbiome returns to a diderm-dominated state within eight hours of rewatering.

From year one fungal data, both pre-flowering and post-flowering drought exerted significant effects on fungal diversity and community composition. However, the two drought conditions exerted asymmetrical changes in community composition in roots and rhizosphere. Abundance of plant pathogens, *i.e., Fusarium*, *Gibberella* and *Sarocladium*, decreased in pre-flowering drought but increased in post-flowering drought in rhizosphere, only partially in roots. The rhizosphere fungal community largely followed the root fungal community pattern, but the soil fungal community was not substantially affected. Symbiotic arbuscular mycorrhizal (AM) fungi were found in root, rhizosphere and soil (Gao et al 2018); however, diversity and community composition were not affected by drought. Instead, AM fungal biomass decreased during pre- and post- flowering drought (Varoquaux et al, submitted).

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:

1. Xu et al., 2018. *Proc Natl Acad Sci USA.* www.pnas.org/cgi/doi/10.1073/pnas.1717308115
2. Gao et al., 2018. *Int Soc Micro Ecol J* https://doi.org/10.1038/s41396-018-0264-0
3. Varoquaux et al., *Genome Biology* (submitted)

Funding: DOE Office of Biological and Environmental Research (DE-SC0014081).