

## **Using Metabolomics to Uncover Metabolic Pathways Utilized in Nitrogen Stress and Plant-Microbe Interactions in Energy Sorghum.**

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### **Project Goals:**

- 1. Conduct deep census surveys of root microbiomes concurrent with phenotypic characterizations of a diverse panel of sorghum genotypes to define the microbes associated with the most productive lines under drought and low nitrogen.**
- 2. Utilize metabolomics to define the metabolic phenotype of a diverse panel of sorghum genotypes to explore changes at the molecular scale occurring in plants as a response to stress (e.g. drought, nitrogen availability) or plant-microbe interactions.**
- 3. Establish a foundational, systems-level understanding of plant, microbial, and environmental interactions that will lead to strategies for enhancing growth and sustainability of sorghum through genetic and microbial adaptations to water and nitrogen limited environments.**

**Methods:** In the first year of our project, a preliminary field study was performed in which 9 sorghum genotypes were grown under conditions of both low and high nitrogen. Root samples (collected at 2 time points: July and September 2015) were harvested for metabolomics analysis. Molecular profiles were characterized using a combination of targeted and non-targeted mass spectrometry platforms to increase coverage of both primary and secondary metabolites. Targeted LC-MS analyses included key energy substrates in carbon and nitrogen metabolic pathways and phytohormones important in the plant stress response. Non-targeted GC-MS and LC-MS analysis was also performed for broad capture of metabolic changes across various metabolic pathways.

**Preliminary data:** Results of this analysis reflect alterations within pathways related to the metabolic storage of nitrogen in root cells, an expected result given that plants receiving high nitrogen treatment would likely be storing available reserves. Metabolomics results also suggest incomplete flux through the Shikimate pathway in plants grown under low nitrogen conditions. Complementary investigation of the rhizosphere microbiome revealed a bloom of *Pseudomonas* in samples from the July harvest that was absent in samples collected in September. Integration of metabolite and microbiome data revealed interesting correlations that also point to incomplete

Shikimate pathway metabolism, co-occurring with the *Pseudomonas* bloom. Since metabolic end products of this pathway are important precursors for lignification and plant defense hormones, availability could compromise plant defenses. Salicylic acid was significantly decreased with low versus full nitrogen, further supporting that reduced flux through the Shikimate pathway may be altering the plant defense response. Levels of other phytohormones (in roots) with known roles in plant defense did not vary by nitrogen treatment, but were increased in July compared to September. However, it is unclear if defense hormones are elevated in July as a result of interaction with *Pseudomonas* or as a normal progression in plant development over the season.

In summary, the molecular results from this pilot experiment indicate the value of metabolite analysis in understanding mechanistic responses to stress and changes in the soil microbiome. The 2016 field study was designed with increased biological replication (to improve statistical power) as well as the inclusion of a water stress environment. Analysis of these samples is underway and will enable a closer evaluation of these pathways and integration of the molecular results with both microbiome and phenotypic field data.

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