

Metabolomics as a Molecular Tool to Understand the Mechanism of Nitrogen Use Efficiency and Interactions with the Soil Microbiome in Energy Sorghum.

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Project URL: <http://sorghumsysbio.org/>

The overall goal of the project is to 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.

Metabolomics is a powerful analytical approach that can be utilized to explore changes at the molecular scale occurring in plants as a response to stress (e.g. nitrogen availability) or interaction with the soil microbiome. In the first year of our project, a preliminary field study was performed in which 10 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 mass spectrometry platforms to increase coverage of both primary and secondary metabolites including key energy substrates in carbon and nitrogen metabolic pathways and phytohormones important in the plant stress response. 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 in lignification and plant defense, reduced availability of these compounds could compromise plant defenses. Levels of phytohormones (in roots) with known roles in plant defense also varied in July compared to September, further supporting that reduced flux through the Shikimate pathway may be altering the plant defense response. However, it is unclear if this effect is in response to low nitrogen conditions or the interaction with *Pseudomonas*.

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.

This research was supported by the Office of Science (BER), U.S. Department of Energy, Grant no DE-SC0014395