Identification of the tyrosine- and phenylalanine-derived soluble metabolomes of sorghum

Jeffrey Simpson,[1] Jacob Olson[1*] (olson169@purdue.edu), Brian Dilkes, [1] and **Clint Chapple[1]**

[1]Purdue University, West Lafayette, IN

Project Goals: Improving our understanding of plant genomes and metabolomes is critical to understand the function of genes, unlock higher plant productivity, develop new strategies to protect crops from biotic and abiotic stress, and identify sources of new plant-based products. Progress towards these goals is limited by the fact that we do not know the identity of most plant metabolites, their biochemical origins, or the function of most of the genes involved in their synthesis and regulation. We will address these challenges through our recently developed stable isotope feeding/LC-MS/genome wide association strategy. This will identify functional gene-metabolite relationships for metabolites that are derived from amino acids in Arabidopsis and sorghum and authenticate them using reverse genetics. When complete, these data will identify known and unknown metabolites within untargeted LC-MS analyses, and characterize the genes involved in their synthesis.

The synthesis of small organic molecules, known as specialized or secondary metabolism, is one way plants resist and tolerate biotic and abiotic stress. Many specialized metabolites are derived from the aromatic amino acids phenylalanine (Phe) and tyrosine (Tyr). Improved characterization of the specialized metabolites derived from these amino acids is necessary to inform strategies for developing crops with improved stress resilience and traits for the biorefinery. *Sorghum bicolor* (L.), a drought tolerant monocot, is widely cultivated for feed and food and is an attractive crop for biofuels. Unlike dicots, sorghum and other monocots possess Phe and Tyr ammonia-lyase activity (PAL and TAL, respectively), which generate cinnamic acid and *p*-coumaric acids, respectively. Cinnamic acid can, in turn, be converted to *p*-coumaric acid by cinnamate 4-hydroxylase. Thus, Phe and Tyr are both precursors of common downstream products. Not all derivatives of Phe and Tyr are shared. Each amino acid acts as the precursor for unique metabolites relevant to sorghum adaptation, such as the anti-herbivore cyanogenic glycoside dhurrin derived from Tyr. In this study we used ^{13}C isotopic labeled precursors, and our recently developed PODIUM analytical pipeline, to identify MS-features derived from Phe and Tyr in sorghum. Over 600 MS-features were identified from Phe and/or Tyr across the roots, stems, and developing leaves of sorghum seedlings. These features comprised 20 percent of the MS signal collected. Ninety percent of the labeled mass features were derived from both Phe and Tyr. The ratio of incorporation of Phe and Tyr varied considerably between metabolites and tissues, suggesting the existence of multiple pools of *p*-coumaric acid that are fed by the two aromatic amino acids. Phe incorporation was greater for many known hydroxycinnamate esters and flavonoid glycosides. In contrast, mass features derived exclusively from Tyr were the most abundant in every tissue. The Phe and Tyr-derived metabolite library was also utilized to retrospectively annotate soluble MS-features in two *brown midrib* mutants (*bmr6* and *bmr12*), locating several MS features that change significantly in each mutant.

Funding statement. This work was supported by the U.S. Department of Energy, Office of Science (BER), Grant DE-SC0020368.