Systematic Identification of Subcellular Location of Sorghum Metabolic Enzymes

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Project Goals: The goal of the Sorghum Metabolic Atlas (SMA) project is to create an integrated pipeline to characterize metabolic interactions and pathways at a cellular level by mapping Sorghum enzymes using a variety of experimental approaches. This pipeline is divided into three stages: a) establishing Agrobacterium- and nanotechnology-mediated transient transformation of grasses to identify subcellular location of Sorghum enzymes through high-resolution confocal imaging; b) selecting enzymes to determine their subcellular localization; and c) using experimental data to generate new compartmentalized metabolic network models as well as refining existing pathway models. This project will create a repository for subcellular locations of metabolic enzymes, yielding important insight into the location and function of metabolic networks in Sorghum.

Plant metabolism underpins many traits that improve plant productivity. Decoding plant metabolic networks is crucial to meet the increasing demand of crop production. Although subcellular localization of enzymes is critical to understanding metabolic networks, localization of the majority of the enzymes is unknown. Fluorescently tagged enzyme localization information is available for many enzymes from the model species *Arabidopsis thaliana* but is largely lacking in most plants, including in the DOE flagship bioenergy plants such as *Sorghum bicolor*.

Due to the challenges associated with Sorghum transformation, we used two complementary monocot systems to determine the localization of Sorghum enzymes: 1) leaves of *Egeria densa*, an aquatic grass; and 2) roots of *Brachypodium distachyon*. Sorghum enzymes were heterologously expressed in both species via an Agrobacterium-mediated transformation method and the protein subcellular localization was identified through confocal microscopy.

In our pilot experiment, we examined the expression of a fluorescent reporter under a maize ubiquitin promoter in a modified pANIC5A and pGVG monocot vectors. The vector modification added a fluorescent tag up-/down-stream of a Gateway cassette into each vector. As a proof of concept, we selected 37 genes from 27 pathways to determine subcellular localization. To quantify the degree of subcellular localization conservation between Arabidopsis and sorghum, we selected 18 sorghum enzymes whose Arabidopsis orthologs have been

experimentally localized. In addition, we selected 6 sorghum-specific genes with no Arabidopsis orthologs and 8 sorghum genes with unlocalized Arabidopsis orthologs. Subsequent enzyme datasets were selected by focusing on pathways of biological interest to maximize coverage of the metabolic network and potential downstream studies to elucidate the function and regulation of the pathways.

The organelle markers were generated using the organelle localization signal sequence tagged with a fluorescent protein[1]. The markers containing the cytosol, nucleus, chloroplast, mitochondria, and peroxisomes localizing fluorescent proteins are currently being validated. Once confirmed, these constructs will be co-expressed with fluorescently tagged enzymes to determine the organelle localization of enzymes.

Overall, this project aims to improve location to function relationship of sorghum metabolic enzymes to facilitate better strategies to bioengineer pathways to tackle challenges related to global warming and food security.

References:

- Osaki, Y and Kodama, Y (2017) Particle bombardment and subcellular protein localization analysis in the aquatic plant Egeria densa. PeerJ 5 : e3779 https://doi.org/10.7717/peerj.3779