Nanoparticle-Mediated Transformation of Sorghum towards the Determination of a Subcellular Metabolic Network Map

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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.

Understanding plant metabolic networks is essential to enable the efficient engineering of resilient and sustainable bioenergy crops. Although model species such as *Arabidopsis thaliana* have extensive resources from which to draw, there remains a lack of information in species such as *Sorghum bicolor*. Sorghum is a challenging species to work with, as it has very poor transformation efficiencies. Here, we are implementing new transformation methods using carbon nanotubes (CNTs) which will allow us to rapidly test bioinformatic predictions of enzyme subcellular locations. Initial tests using vectors with fluorescent proteins (FP) under the control of *CaMV35S* and maize *Ubiquitin* promoters have shown transient expression of FPs in sorghum leaves, indicating successful carbon nanotube-mediated transformation. However, optimization is necessary due to inconsistencies between CNT batches, leaf infiltration challenges, and validation of subcellular localization.

We found that the polymers used to load vectors onto CNTs play a key role in construct durability, DNA loading capability, and plant toxicity. To minimize hydrolysis of the polymer from the CNT surface, we tested various polymers and storage conditions. Different cationic polymers adsorbed DNA with different electrostatic strengths, largely as a function of the polymer size and structure. To identify trends in polymer structure that minimize toxicity while maintaining DNA loading capability, we measured the upregulation of *PR1*, a marker for biotic

stress, using RT-qPCR from infiltrated *Nicotiana benthamiana* leaves. With these insights, we are actively exploring new chemistries for the loading of biomolecular cargo.

A significant bottleneck in sorghum leaf transformation is the efficient infiltration of plasmid-loaded CNTs into the silica-rich leaves, additionally hindered by a thick epidermal cuticle. To enhance CNT uptake, we tested several leaf abradement and infiltration approaches. Using a combination of leaf abradement and vacuum infiltration, we successfully transformed sorghum epidermal cells. We are currently optimizing this method for reproducibility and enhanced expression of introduced genes.

Following transformation, a key component of this project lies in validation of the subcellular localizations we see in sorghum. Our primary considerations in identifying a suitable test species is the presence of prior localization studies, leaf infiltration efficiency, and close relation to sorghum. To address these criteria, we are testing several well-studied monocot species with different leaf properties in parallel, including rice, maize, and Brachypodium. As an added layer of assurance, we are also exploring alternatives to CNT-mediated leaf transformation, including Agrobacterium-mediated transformation and sorghum callus transformation.

References:

- Jha SG, Borowsky AT, Cole BJ, Fahlgren N, Farmer A, Huang SSC, Karia P, Libault M, Provart NJ, Rice SL, et al (2021) Vision, challenges and opportunities for a plant cell atlas. Elife 10: 1–27. https://doi.org/10.7554/eLife.66877.
- Jackson CT, Wang JW, González-Grandío E, Goh NS, Mun J, Krishnan S, Geyer FL, Keller H, Ebert S, Molawi K, et al (2021) Polymer-Conjugated Carbon Nanotubes for Biomolecule Loading. ACS Nano. https://doi.org/10.1021/acsnano.1c06343.
- Silva TN, Thomas JB, Dahlberg J, Rhee SY, Mortimer JC (2021) Progress and challenges in sorghum biotechnology, a multi-purpose feedstock for the bioeconomy. Journal of Experimental Botany. https://doi.org/10.1093/jxb/erab450.
- Demirer GS, Silva TN, Jackson CT, Thomas JB, Ehrhardt D, Rhee SY, Mortimer JC, Landry, MP (2021) Nanotechnology to advance CRISPR/Cas genetic engineering of plants. Nature Nanotechnology. https://doi.org/10.1038/s41565-021-00854-y.

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