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

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Project Goals: The goal of this project is to build an integrated pipeline to characterize metabolic interactions and pathways at a cellular level, using a combination of computational prediction, metabolic network modeling, and high-throughput experimental testing. This pipeline will be divided into three stages in order to develop a high-resolution subcellular map of small molecule metabolism in Sorghum and Brachypodium: a) generating localization predictions using bioinformatic algorithms, b) testing those predictions using nanotechnology mediated transformation of fluorescently tagged target proteins and high-sensitivity confocal imaging, and c) using the experimental data to generate new compartmentalized metabolic network models as well as refining existing pathway models. This project will initiate the creation of a repository for subcellular locations of metabolic enzymes, yielding important insight into the structure and function of metabolic networks in model systems as well as economically important crop species.

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

We identified that the polymers used to load vectors onto CNTs could be toxic to plants and play a role in batch-to-batch variability. We sought to quantitatively gauge and better understand these effects using associated toxicity marker genes. We identified that *PR1*, a marker for biotic stress, is upregulated in response to infiltration. Using RT-qPCR, we are exploring a library of amine-containing polymers to identify trends in chemical structure and functionality that minimize toxicity while providing an ideal platform for biomolecule conjugation to CNTs. At the same time, we are exploring covalent attachment methods, including EDC-NHS (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, *N*-Hydroxysuccinimide) and triazine-based chemistries, to identify new avenues for the attachment of cationic polymer and subsequent electrostatic loading of biomolecular cargo.

We are exploring several alternatives to CNT-mediated leaf transformation. In order to increase throughput of transient transformation screenings, protocols for sorghum protoplast isolation and GFP delivery into protoplasts using CNTs have been tested. CNTs at the tested conditions are likely to be toxic as controls containing only CNTs also showed fluorescence. As an alternative to CNTs, we are optimizing particle bombardment parameters to deliver DNA into sorghum callus cells.

To broaden the knowledge base of plant transformation methods and to pivot our efforts from limited lab access as a result of COVID19, we have also written two articles: (1) an in-depth review on current sorghum biotechnology and challenges that need to be addressed to efficiently improve sorghum transformation and (2) a perspectives article on the use of nanotechnology to advance plant genetic engineering.

References:

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