

Genetic Designs Targeting Accumulation of Vegetative Lipids, Multiplexing of Editing Reagents for “In Context Promoter Bashing” and Gaining Insight on Roots Exudates Impact on Shaping the Root Microbiome in Sorghum (*Sorghum bicolor* (L.) Moench

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<https://cabbi.bio/research/feedstocks-theme/>

Project Goals

The goals of this project are to: introduce novel genetic variation into sorghum as a means to synthesize value-added, lipid-based co-products, to ascertain the role of the sorghum root exudate, sorgoleone, on shaping the microbiome about the rhizosphere and its relationship to inhibition of nitrification in soil, and to test a multi-plex Cas9-based delivery of editing reagents for “in context promoter bashing” as a means of perturbation of expression for specific gene calls associated with the PEPCK C4 photosynthetic pathway.

Abstract

Sorghum (*Sorghum bicolor* (L.) Moench) has high biomass potential, and sweet genotypes can accumulate sugar reserves of up to 15% in stalks. These phenotypic attributes, coupled with its capacity to perform C4 photosynthesis and its relatively low agronomic input requirements, make sorghum an ideal feedstock for the bioeconomy. A transdisciplinary team of researchers has been assembled to take a holistic approach to addressing selected input and output traits to improve the sustainability of this feedstock for the production of vegetative lipid co-products in stalks, to gain insight on the role of root exudates, namely sorgoleone, in shaping the rhizosphere microbiome, and to implement genome editing platforms for “in context promoter bashing” in the crop. A priority output trait being targeted is the shifting of metabolic flux in parenchyma cells of stalks to alter carbon reserves from sucrose to lipids within the cells. To address these target output traits, genetic designs are being assembled employing a modular assembly approach in a build-test-learn process through the introduction of novel genetic variation implementing the tools of synthetic biology, in both grain and sweet genotypes of sorghum, as a means to assess impact on sucrose flux post-flowering on lipid accumulation in stalks. We target three processes of lipid (triacylglycerol) production and storage, regarded as push, pull, and protection, using key variants of enzymes (WRINKLED1, DGAT, and OLEOSIN) regulating these processes. To gain insight on the role of sorgoleone on modulating the root microbiome, sorghum events were generated that significantly reduce the synthesis of sorgoleone, and the putative sorgoleone biosynthetic pathway has been introduced into maize, leading to the synthesis of this novel exudate in corn. These novel sorghum and maize biologicals position our team to address what impact this exudate has on shaping the soil microbiome and its relationship

to biological inhibition of nitrification (BNI). Lastly, we designed and introduced a multi-plex guide approach into sorghum targeting promoter regions of selected gene calls in the PEPCK C4 photosynthesis pathway, referred to as “in context promoter bashing”, as a way to alter expression of the respective downstream gene calls. Data gathered from the phenotypic characterizations of these biologicals will form the basis of future genetic designs to improve both quantity and functional quality of vegetative lipid accumulation in sorghum, revealing what, if any, role the sorghum exudate sorgoleone has on the BNI. This information can be exploited as an indirect means to improve nitrogen use efficiency and mitigate nitrogen run-off. Lastly, this program is expanding the genetic toolkit through deliberate edits in regulator regions as a way to perturb gene expression in C4 feedstocks.

References

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