Metabolic Engineering of Triacylglycerols with Specialized Fatty Acids in Sorghum

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Project Goals: Our research aims to enhance production of triacylglycerols (TAG) with specialty fatty acids in sorghum (*Sorghum bicolor* (L.) Moench) stems. Metabolic engineering of TAG biosynthesis with specialty fatty acids (e.g., medium-chain and hydroxy fatty acids) can increase TAG value and functionality for renewable alternatives to petroleum-based oils. Sorghum is regarded as an ideal feedstock for bioenergy because of its capacity for high biomass productivity with low agronomic inputs. Applying synthetic biology for modular and multiple gene assembly, we are introducing multigene-expression vectors in sorghum and currently evaluating the most effective transgene combinations using tobacco systems to increase the throughput of the design-build-test-learn cycle of our synthetic biology efforts.

Plants store triacylglycerols (TAG) in their seed as energy sources for germination and seedling establishment. Although TAG is principally stored in seeds for this purpose, numerous studies indicate that vegetative tissues also have the ability for TAG synthesis that can serve as energy-dense, renewable alternatives to petroleum-based oils. Sweet sorghum (*Sorghum bicolor* (L.) Moench) is regarded as an attractive feedstock for bioenergy because of its capacity for high biomass production under rain-fed conditions on marginal soils. The goal of this research is the synthetic re-design of lipid metabolism in stalks for enhanced TAG production and storage and to generate TAG with specialty fatty acids (e.g., medium-chain and hydroxyl fatty acids) to increase TAG value and functionality. Accumulation of TAG is achieved by the three major processes: push, pull, and protect (3P). Fatty acid synthesis can be pushed using master transcription factors such as WRINKLED1 (WRI1), pulled into TAG assemply by acyltransferases such as diacylglycerol acetyltransferase (DGAT), or protected from degradation by

oil body coat proteins such as oleosin. In addition to this fundamental gene combination (WRI1 + DGAT + oleosin), specific acyltransferases and acyl-ACP thioesterases are required for specialty fatty acid synthesis. We have identified and characterized the specific acyltransferases and acyl-ACP thioesterases from various plant species. We currently focus on medium-chain and hydroxy fatty acids because they are suitable for jet fuel and industrial heat-tolerant lubricants, respectively. By applying synthetic biology for modular and multiple gene assembly, we generated diverse vector constructs for TAG biosynthesis with specialty fatty acids. We are currently evaluating the most effective transgene combinations using tobacco systems to increase the throughput of the design-build-test-learn cycle of our synthetic biology efforts. The most effective combinations are then used for sorghum transformation.

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