Metabolic Engineering of Triacylglycerols in Vegetative Tissues of Sorghum

Kiyoul Park, Truyen Quach, Hyojin Kim, Tara J. Nazarenus, Shirley Sato, Edgar B. Cahoon, and Tom E. Clemente

United States Department of Energy Center for Advanced Bioproducts and Bioenergy Innovation, University of Nebraska, Lincoln, Nebraska, USA

Triacylglycerols (TAG) provide for energy-dense carbon storage of fatty acids (FA) in plants. TAG (or "oil") naturally accumulates to high levels in seeds to provide energy and carbon-skeletons needed for germination. By contrast, vegetative tissues typically only store TAG transiently, in part to protect cells from cytotoxic free FA. Here, we have redesigned the biomass crop sorghum (*Sorghum bicolor*) to accumulate TAG to high levels in vegetative tissues, including leaves and stalks. The goal of this work is to provide a TAG-derived co-product value stream in a Midwest-adapted, drought tolerant crop for advanced biofuels and bioproducts. Benefiting from our ability to transform sorghum with multi-transgene constructs, we introduced gene combinations to: (1) push photosynthetic carbon into FA biosynthesis using a Wrinkled1 transcription factor transgene; (2) pull of FA into TAG storage using a novel diacylglycerol acyltransferase (DGAT) transgene; and (3) protect TAG from turnover using an oleosin oil body coat protein transgene. After extensive primary transformants, we identified T_0 lead events that accumulate TAG in leaves to ≥2% DW, compared to ≤0.1% DW of TAG found in non-transformed plants in a Tx430 grain sorghum background. We advanced the T_1 generation of the lead events in the University of Nebraska-Lincoln Biotech Field Facility in Mead, NE. For field-grown Tx430 lines, TAG levels of ≥6.6% DW were detected in leaves after flowering (99 days after sowing) and 2.5% DW in stalks after harvest (127 days after sowing). No obvious differences in biomass of engineered lines were observed. We also generated events in the Ramada sweet sorghum background that has greater biomass and sucrose stem content compared to Tx430. These events accumulated ≥5.6% DW TAG in leaves under greenhouse

conditions versus 0.1% DW TAG in leaves of non-transformed plants. We have initiated collaborative fluxomics and root-soil metagenomics experiments. A field trial for summer of 2022 is also planned to more fully assess the traits. Our results to date show the feasibility of sorghum as a vegetative oil production platform. Information gained from our current lines will guide iterative development of the vegetative TAG trait to address biosynthetic and catabolic factors that limit further increases in oil production and the balance of photosynthetic carbon partitioning to support growth and storage.