Improving Energycane by Metabolic Engineering for Hyperaccumulation of Lipids and RNAi Suppression of Flowering

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Project Goals

The main objective of ROGUE (Renewable Oil Generated with Ultra-productive Energycane) project is to engineer the two most productive American crops—energycane and *Miscanthus*—to produce a sustainable supply of biodiesel, biojet fuel, and bioproducts.

1. Increasing oil accumulation and targeting this to the mature stem
2. Increasing photosynthetic efficiency to power oil synthesis
3. Multi-gene construct transformation of energycane and Miscanthus
4. Field testing, processing and techno-economic analysis

Abstract

Metabolic engineering to divert carbon flux from sucrose to oil in a high biomass crop like energycane has been proposed as a strategy to boost both energy density of high biomass crops and lipid yields per acre for biodiesel production. Recently, we have succeeded with metabolic engineering to drastically increase triacyl glycerol (TAG) content in vegetative tissues of sugarcane by upregulating fatty acid synthesis, TAG synthesis and optimization of TAG storage (Zale et al. 2016, Parajuli et al. 2020). Energycane is like sugarcane an interspecific hybrid in the genus Saccharum. In contrast to sugarcane, energycane has a high proportion of the ancestral species *Saccharum spontaneum* in its genome which contributes to higher tiller number, biomass yield and persistence in addition to a reduced stem diameter and sugar content. Therefore, energycane is an ideal feedstock for this approach due to its superior biomass production and persistence. However, energycane is among the most recalcitrant crops in tissue culture and genetic transformation.

In this study, a multigene expression construct for lipogenic factors and selectable marker gene was generated by modular Golden Gate assembly with the goal to elevate the production of free fatty acids, catalyze their conversion into TAG and reduce TAG hydrolysis. Culture media optimizations included media supplementation to overcome browning and necrosis in tissue culture. Following biolistic gene transfer to embryogenic callus and selection of antibiotic resistant callus, transgenic plantlets were regenerated. The presence and expression of transgenes in the

regenerated plants were confirmed by PCR and qRT-PCR analysis, respectively. Transgenic plants were vegetatively propagated in the greenhouse and were transplanted to a field site at the University of Florida Plant Science and Education Center near Citra, FL in randomized and replicated plots under USDA-APHIS permit in March 2021. TAG and total fatty acid (TFA) content for different leaf positions, stem sections and juice as well as biomass fresh and dry weight were determined at the end of the growing season. The TAG and TFA content varied depending on tissue, maturity, stem and leaf position with the highest detected TAG and TFA content reaching 9.9% and 12.3% of leaf dry weight on average of three replications, respectively.

Lipid yield per land area from high biomass crops like energycane is determined by the lipid concentration in the biomass, the total biomass yield and the extractability of the lipids from the biomass. Flowering of energycane may affect oil yield and the extractability of oil. Upon flower induction vegetative growth ceases and sucrose/oil that has accumulated in the stalks is re-mobilized for use in reproductive development. Flowering often leads to dehydration of the stalk tissues, which negatively affects stalk density, and compromises sugar extraction in conventional energycane or lipid extraction in metabolically engineered lipid cane. Therefore, we generated transgenic energycane plants harboring a construct for RNAi mediated co-suppression of multiple flowering genes. Since energycane is vegetatively propagated for establishment of plantings, suppression of flowering will not require an altered agronomic practice while improving the biocontainment of the engineered crop. Transgenic plants were planted in replicated field plots at the University of Florida Plant Science and Education Center near Citra, FL in randomized and replicated plots, following a decision letter from USDA-APHIS that these transgenic plants are not-regulated by USDA-APHIS. Data will be presented comparing expression of the RNAi construct during photo inductive period with flowering response and biomass production.

References/Publications

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