Metabolic Engineering of Energycane for Hyperaccumulation of Triacylglycerol and Improved Biomass Production

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Project Goals

Renewable Oil Generated with Ultra-productive Energycanes—or ROGUE—is engineering the two most productive American crops—energycane and Miscanthus—to produce a sustainable supply of biodiesel, biojet fuel, and bioproducts.

Project goals are to:
1) Engineer energycane to produce an abundance of lipids in the form of triacylglycerol which can be converted into biodiesel, biojet fuel, and bioproducts.
2) Altered expression of flowering genes, pyruvate Pi dikinase and proteins involved in chloroplast division in energycane to enhance biomass yield and cold tolerance.

Abstract

Metabolic engineering to achive hyper-accumulation of lipids [e.g. triacylglycerol (TAG)] in the vegetative tissues of high biomass crops is a promising strategy to improve lipid yields for biofuel production. Energycane is an ideal feedstock for this approach due to its superior biomass production and persistence.

In this study, a multigene expression construct was used to elevate the production of free fatty acids, catalyze their conversion into TAG and prevent TAG hydrolysis. This construct was transferred into energycane callus, using the biolistic particle delivery system. Presence of transgenes in the regenerated plants were confirmed by PCR. A combination of TLC and GC-MS analysis revealed that the TAG contents in transgenic leaf tissue was elevated more than 18-fold compared to wild-type energycane. Currently, we are propagating the highest TAG producing energucane plants for field trials which are scheduled to begin under USDA-APHIS permit in April 2021 and will allow analysis of lipid and biomass production.

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 is expected to 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. Often flowering also leads to dehydration of the stalk tissues, which negatively affects stalk density, and also compromises sugar extraction in conventional sugarcane or lipid extraction in metabolically engineered lipid cane. Therefore, we recently generated transgenic energycane plants harboring a construct for RNAi mediated 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 biosafety of the engineered crop. Transgenic plants are currently being propagated in the greenhouse in preparation of field testing and will be characterized for target gene suppression during photo inductive period as well as for flowering and biomass production.

Genetic improvement of photosynthetic efficiency could potentially be achieved by developing a photosynthetically more effective canopy. To evaluate the effect of chloroplast size on light penetration into the canopy and biomass production, we intend to modify the expression of the cytoskeletal Filamenting temperature-sensitive Z (FtsZ) protein, which is critical for chloroplast division. Overexpression and RNAi constructs of FtsZ were introduced into energycane callus and regenerated through somatic embryogenesis. Pyruvate orthophosphate dikinase (PPDK) has been proposed as rate limiting enzyme in C4 photosynthesis. It regenerates the substrate phosphoenol pyruvate (PEP) for the initial carbon-fixation step. C4 plants are also severely limited by low temperature, possibly because PPDK is highly cold-labile and partially dissociates below 14 ºC. Therefore, we decided to explore the over-expression of Miscanthus x giganteus PPDK in energycane. MxgPPDK with its native regulatory sequences were introduced into energycane callus by biolistic gene transfer. The regenerated plants will be evaluated for the effect of PPDK overexpression on photosynthetic efficiency, cold tolerance and biomass accumulation.

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