Refining Metabolic Engineering Strategies for Hyperaccumulation of Triacylglycerol in Oilcane

Viet Cao[1,2]* (caodangviet@ufl.edu), Baskaran Kannan[1,2], Shelby Korynta[1,2], Sofia Cano[1,2], Hui Liu[1,3], Steve Long[1,4], John Shanklin[1,3], and **Fredy Altpeter[1,2]**

[1]DOE Center for Advanced Bioenergy and Bioproducts Innovation, Gainesville FL; [2]University of Florida, Gainesville, FL; [3]Brookhaven National Laboratory, Upton, NY; [4]University of Illinois, Urbana, IL.

Project Goals:

In this project, we:

1) Explore the field performance of oilcane, a sugarcane that was engineered to produce an abundance of lipids in the form of triacylglycerol which can be converted into biodiesel, biojet fuel, and bioproducts.
2) Refine strategies for multi-gene engineering to elevate lipid yields per land area.

These project goals support CABBI's "plants-as-factories" paradigm, in which biofuels, bioproducts, high-value molecules, and foundation molecules for conversion are synthesized directly in plant stems.

Abstract:

Sugarcane is an ideal target crop to fuel the emerging bioeconomy. It combines superior biomass production and photosynthetic efficiency with hyperaccumulation of sucrose in its stem, which offers great prospects for diversion to alternative products. We recently reported the generation of oilcane, a sugarcane which has been metabolically engineered for hyperaccumulation of triacylglycerol (TAG) in its vegetative biomass [1,2].

Using constitutive promoters for metabolic engineering approaches has the advantage that analysis can be performed early in plant development. This is beneficial when hyperaccumulation of target products like TAG depend on combined expression and catalytic performance of four or more lipogenic factors to (1) increase fatty acid synthesis, (2) increase triacylglycerol (TAG) synthesis from diacyl-glycerol and acyl-CoA, (3) optimize TAG storage and (4) minimize TAG hydrolysis in vegetative tissues. However, high level constitutive expression of lipogenic factors may also lead to a reduction in field performance, while developmentally regulated or stem specific promoters will slow the Design-Test-Build-Learn (DTBL) cycles, which are required for step changes in lipid accumulation. In contrast, inducible promoters will accelerate the selection of most promising gene variants and synergistic gene combinations for complex multi-gene engineering. Inducible promoters prevent potentially deleterious effects of hyperaccumulated target products on regeneration of transgenic plants from tissue culture or plant vigor and allow us

to explore synergistic effects of alternative gene combinations by expressing one factor under inducible promoter and the other factors under constitutive promoter.

Oilcane plants expressing all lipogenic factors under constitutive promoters were evaluated in replicated and randomized field plots at the University of Florida-IFAS Plant Science Research and Education Unit, near Citra, FL under USDA-APHIS authorization. Data will be presented detailing transgene expression, TAG content, total fatty acid content and biomass yield. Lipid accumulation varied depending on position and maturity of leaves and stems, and reached more than 10% of TAG per leaf dry weight at the time of biomass harvest, averaged over 15 biological replications. The oilcane plants grew vigorously, ratooned successfully and produced 52% of the biomass of the non-engineered sugarcane.

Inducible promoters may allow lipid production at will, at a time when tissue culture or critical stages of plant development are already completed. Since sugarcane tolerates elevated temperatures between 40° and 45°C for an extended period of time, we explored different heat shock promoters (HSP) and the combination of heat inducible and constitutively expressed lipogenic factors to accelerate DTBL cycles. When using HSP promoters to drive the most critical lipogenic factors, we noted an elevated production of transgenic events and rapid establishment of transgenic plants in soil with vigor comparable to non-transgenic sugarcane. A time course experiment evaluating heat induced transgene expression informed a treatment protocol comparing 2, 4 and 8 days of heat exposure with two 4h cycles at 40°C each day. The results showed that sugarcane leaves and stems can be induced for hyperaccumulation of TAG in both leaves and stems within 4 to 8 days of transgene expression. This strategy has great potential to accelerate DTBL cycles in sugarcane.

References

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