Oilcane: Metabolic Engineering and Genome Editing to Improve Energy Density, Agronomic and Conversion Performance of Sugarcane

Fredy Altpeter[1,2*] (altpeter@ufl.edu), Baskaran Kannan[1,2], Guangbin Luo[1,2], Tufan Oz[1,2], Duoduo Wang[1,2], Saroj Parajuli[2], Ratna Karan[2], Hui Liu[1,3], Yunjun Zhao[1,3], Chang-Jun Liu[1,3], and John Shanklin[1,3]

[1]DOE Center for Advanced Bioenergy and Bioproducts Innovation; [2]University of Florida, Gainesville, Fla.; [3]Brookhaven National Laboratory, Upton, N.Y.

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Project Goals: Boosting the accumulation of extractable triacylglycerol (TAG) in sugarcane stems for production of biodiesel; developing and deploying enabling biotechnologies for precision breeding of oilcane; and elevating the agronomic and conversion performance of oilcane postharvest residues.

Metabolic engineering to divert carbon flux from sucrose to oil in a high biomass crop like sugarcane 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 succeeded with metabolic engineering to drastically increase TAG content in vegetative sugarcane tissue (Zale et al. 2016) by upregulating fatty acid synthesis, TAG synthesis and optimization of TAG storage. Current efforts focus on the identification of optimal regulatory elements and transgene variants. A library of stem specific and constitutive promoters, variants of transcription factors involved in fatty acid biosynthesis, as well as transcription terminators was generated and assembled into multi-gene expression constructs using Golden Gate cloning to evaluate the contribution of different regulatory elements and gene variants to lipid accumulation in vegetative sugarcane tissues.

We are also deploying genome editing with CRISPR/Cas9 for genetic improvement using both targeted mutagenesis and/or homology directed repair of targeted double strand breaks to generate “loss” and/or “gain of function” mutants with improved agronomic or feedstock performance. We have successfully developed an efficient HDR mediated genome editing approach conferring herbicide resistance by introduction of mutations in the endogenous acetolactate synthase (ALS) gene in highly polyploid sugarcane. Transgenic sugarcane plants were vegetatively propagated and grown in the greenhouse. Molecular characterization of vegetative progeny indicated the CRISPR/Cas9 mediated multi-allelic mutations was faithfully transmitted to the vegetative progeny. We are also generating a rapid readout system to explore alternative delivery options for the editing reagents, which provide opportunities for increased efficiency and specificity of genome modifications in sugarcane while producing transgene-free events. For this approach, target genes for “loss of function mutations” include those that are well known to create albino or chlorotic plantlets following loss of function mutations like phytoene desaturase (PDS). PDS, an essential plant carotenoid biosynthetic enzyme, is involved in the biosynthesis pathway of β-carotene which provides photoprotection of chlorophyll.

Flowering affects both sugar and biomass yields in sugarcane, since vegetative growth ceases upon flower induction and sucrose 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 plant weight, and may also compromise sugar extraction in

conventional sugarcane or lipid extraction in metabolically engineered lipid cane. Therefore, we will focus on suppression of flowering in sugarcane by CRISPR-Cas9 mediated genome editing. We have identified and sequence confirmed multiple alleles from several candidate flowering inducing genes from sugarcane cultivar CP 96-1252. Several guide RNA's were designed in silico for multi-allelic cleavage of the target genes and we selected superior gRNA's with the help of an in-vitro assay. A vector for delivery of selected gRNA's along with the Cas9 nuclease and selectable marker is currently being constructed and will be transferred to sugarcane.

Lignin is a recalcitrance factor for the conversion of sugarcane processing residues to advanced biofuels like butanol. We have recently demonstrated that reducing lignin content and/or monolignol ratio in sugarcane by genome editing or RNAi translates into improved saccharification efficiency (Jung et al. 2013; Kannan et al. 2018). Currently we are exploring alternative approaches to lignin modification including the expression of enzymes that interfere with the normal process for cell wall lignification. We are currently evaluating the overexpression of an artificial monolignol 4-O-methyltransferase to compromise lignin polymerization. Vector containing different versions of the lignin modification genes were introduced into sugarcane callus by biolistic gene transfer and will be regenerated on the media containing selection agents. Regenerated plants will be characterized for the transgene expression and lignin modification at maturity.

References

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