

<u>Triacylglycerol, Total Fatty Acid, and Biomass Accumulation of Metabolically</u> <u>Engineered Energycane Grown under Field Conditions Confirms its Potential</u> <u>as Feedstock for Drop-in Fuel Production</u>

Background/Objective

Metabolic engineering for hyperaccumulation of lipids in vegetative tissues of high biomass crops promises a step change in oil yields for the production of advanced biofuels. Energycane is the ideal feedstock for this approach due to its exceptional biomass production and persistence under marginal conditions. In this study, we evaluated metabolically engineered energycane with constitutive expression of the lipogenic factors WRINKLED1 (WRI1), DIACYLGLYCEROL ACYLTRANSFERASE1 (DGAT1), and OLEOSIN1 (OLE1) for the accumulation of triacylglycerol (TAG), total fatty acid (TFA), and biomass under field conditions.

Approach

Here, we explored field performance for both oil and biomass yield in metabolically engineered energycane lines L2 and L13, each constitutively expressing WRI1 at similar levels but DGAT1, and OLE1 at different levels.

Result

Dry biomass yield was similar in WT (36.97 t ha⁻¹) and line L2 (38.32 t ha⁻¹) with moderately expressed *DAGT*1 and *OLE*1 and TAG and TFA accumulation of 12- and 1.6-fold that of WT leaves, respectively. In contrast, line L13, with intron-mediated enhancement of *DGAT*1 expression, displayed a 245- to 330-fold increase in TAG (up to 9.9% of DW) and a 4.75- to 6.45-fold increase in TFA content (up to 12.9% of DW) compared with WT leaves and yielded 17.65 t ha⁻¹ dry biomass.

(A) 9.00 c†# (B) 12.00 WT L2 L13 ■WT ■L2 ■L13 7.00 10.00 5.00 8.00 % Leaf total FA 3.00 % Leaf TAG FA 6.00 1.00 b# 0.40 4.00 0.30 0.20 2.00 0.10 0.00 September October October August August September

TAG (A) and total FA (B) contents in first dewlap leaves of lines L2 and L13 and wild-type during plant development (August-October 2021).

Significance/Impacts

This study provides the basis for developing novel feedstocks to expand plant lipid production and point to new prospects for advanced biofuels. Our findings represent the first field study of metabolically engineered energycane and establish this crop as a promising production platform for lipid derived biofuels.

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