Title: Engineering Cyclopropane Fatty Acid Accumulation in Plant Vegetative Tissues

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Project Goals: Our overall goal is to create and build DNA designs for the synthesis of specialty fatty acids (cyclopropane, hydroxy, and variant unsaturated fatty acids) in sugarcane.

Abstract Text: Cyclopropane fatty acids (CPAs) contain a three-carbon ring, and can be used as industrial feedstocks for coatings, lubricants, plastics, and cosmetics. Our previous research showed that expression of CPA synthase in plant seeds results in low levels of CPA accumulation and we identified metabolic bottlenecks in acyl transfer between the site of synthesis (phospholipids) and deposition in TAG. Additional acyltransferases from *Sterculia* seeds increased CPA accumulation in storage oil in transgenic seeds[1,2]. To build DNA designs for synthesis of CPAs in sugarcane, we first established a rapid screening system using transient expression in *Nicotiana benthamiana* leaves for evaluating the effects of additional genes on CPA accumulation in vegetative tissues. We succeeded in accumulating about 1% CPA in *N. benth* leaves upon transient coexpression of *Escherichia coli* cyclopropane fatty acid synthase (CPS) in combination with WRI1, Oleosin and diacylglycerol acyltransferases (DGAT)2. The use of either single or double 35S promoter had no effect on CPA yield, and addition of an intron to EcCPS reduced CPA accumulation in *benth* leaves. Using the *N. benth* transient expression system, we evaluated the effects of different acyltransferases on leaf CPA production, including *Sterculia foetid*a lysophosphatidic acid acyltransferase (LPAT), *Litchi chinensis* and *Mus musculus* DGAT2, and phosphatidylcholine:diacyl glycerol cholinephosphotransferase. Indeed, coexpression of acyltransferases from plants that naturally accumulate CPA increased CPA production in leaves, with SfLPAT showing the most significant effect. Proof-of-principle constructs that increased CPA in *N. benth* were transferred to sugarcane. The effective gene combination for CPA accumulation in *N. benth* gave the strongest effect in sugarcane yielding one transgenic line that accumulates approximately 1% CPA. This is the first demonstration of accumulation of CPA, a specialty fatty acid in vegetative tissues of oilcane.

References/Publications

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