Title: Genetic Engineering of Camelina to Improve Seed Oil Yield

Authors: Xiao-Hong Yu, [1,2] * (xhyu@bnl.gov), Sanket Anaokar,[1] Yuanheng Cai,[1,2] and **John Shanklin**[1,2]

Institutions: [1]Biology Department, Brookhaven National Laboratory, Upton, NY; and [2]University of Stony Brook, Stony Brook

Website URL: https://www.montana.edu/econproject/index.html

Project Goals: This work is part of the ECON, i.e., Enhancing Camelina Oilseed Production with Minimum Nitrogen Fertilization in Sustainable Cropping Systems project. Camelina is a Brassica oilseed crop that has great potential to become a sustainable source of bioenergy in the US. However, its low nitrogen use efficiency, seed and oil yield as compared to other major oilseed crops limit its potential. The goal of this project is to decipher the genetic and physiological mechanisms that determine the nitrogen use efficiency and oilseed yield during critical processes of the camelina life cycle: 1) how camelina, in partnership with soil microbes, maximizes its ability to absorb and assimilate nitrogen into vegetative biomass; and 2) upon the transition to reproductive growth, how nitrogen is efficiently remobilized from senescing tissues (leaves and silicles) into sinks (seeds) to optimize yield potential by increasing seed size and enhancing oil synthesis.

Abstract Text: The synthesis of fatty acids (FA), the building blocks of TAGs, is highly energy demanding and is therefore under tight multi-level homeostatic control. WRI1 is the master transcriptional activator that targets many FA synthesis genes[1], overexpression of which increase FA accumulation in seeds. Trehalose 6-phosphate (T6P) reduces the phosphorylation WRI by SnRK1, to preventing its degradation[2]. Overexpression of the *E. coli* T6P synthase, OtsA led to increased FA accumulation in Arabidopsis[3]. To improve WRI stability in Camelina, T6P synthase, under the control of the seed specific Phaseolin promoter was transformed into the wild type camelina Suneson to boost T6P level in its seeds. OstsA transgenics showed poor germination with only 3 out of 8 transgenic of the transgenic seeds germinating. Additional transformation yielded more than 300 lines of which 36 germinated. Seeds from these lines were collected for analysis of lipid composition and fatty acid content.

The first committed and rate limiting step in FA synthesis is acetyl-CoA carboxylase (ACCase), which converts acetyl-CoA to malonyl-CoA in the plastid. Excess FA inhibits the carboxytransferase (CT) ACCase half-reaction and triggers irreversible ACCase inhibition. This inhibition that is mediated by biotin attachment domain-containing (BADC) proteins, homologs of biotin carboxytransferase proteins (BCCPs)[4]. BADCs lack a critical lysine necessary for the covalently attachment of the biotin cofactor required for carboxylation in BCCPs[5]. BADCs are conditional inhibitors of FA synthesis that displace BCCP in the ACCase complex when intracellular FA accumulate. Knocking-out two of the Arabidopsis *BADC* genes, i.e., badc1badc3 resulted in an increase in TAG content of 25% with respect to that of wild type[4]. We are targeting the downregulation of BADC genes in camelina as a primary approach to increasing seed oil yield. CRISPR vectors targeting single BADC genes i.e., *BADC1, BADC2, and BADC3*

and the combination of *BADC1 and BADC3* have been constructed and transformed into camelina Suneson. 5 CRISPR-CsBADC3 lines were genotyped. One line was edited in 1 allele of each of the *BADC3* isoforms. We also obtained 8 CRISPR-CsBADC1 lines and 36 CRISPR-CsBADC1,3 lines for which genotyping is underway. Increases in FA synthesis rates are expected to result in increased levels of total FA accumulation, but we anticipate additional factors will be required to convert excess FA into TAG and to prevent its degradation. We have previously used an Arabidopsis or nasturtium diacylglycerol acyltransferase, DGAT1 and an oil droplet protecting protein, sesame OLEOSIN (cysOLE1) to increase oil accumulation[6]. We recently found that a mammalian DGAT2 (mDGAT2) is more effective than Arabidopsis DGAT1 in converting FA into TAG. We have also created variants of the sesame OLEOSIN that significantly increases its ability to protect TAG relative to cysOLE1. These "pull" and "protect" factors including *Sesame* oleosin, and several variants engineered to increase its stability, and mouse DGAT2 were employed to boost oil accumulation in camelina. Constructs containing the single gene for oleosins and/or mouse DGAT2 were transformed into camelina *suneson*. Currently we obtained 15, 13, 5, 14, 26, and 28 transformants of CysOle1, Ole1_CysDel_KR , Ole1_5_Mod, mDGAT, CysOle1+ mDGAT, Ole1_CysDel_KR + mDGAT and Ole1_5_Mod + mDGAT, respectively.

Future efforts will focus on identifying the most successful BADC suppression lines and the most effective additional gene combinations described above. The optimized approaches will be combined to create camelina seeds with significantly elevated TAG content beyond those discovered by genetic variation. Subsequently we will combine optimized oilseed accumulation factors in lines optimized for nitrogen use efficiency.

References/Publications

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Funding Statement: This research is supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomic Science Program grant no. DE-SC0021369.