Overexpression of the Phosphatidylcholine: diacylglycerol cholinephosphotransferase (*PDCT*) Gene Has Increased Carbon Flux Toward Triacylglycerol (TAG) Synthesis in Camelina (*Camelina sativa*) Seeds

*Hesham M. Abdullah[1] (abdull76@msu.edu), Na Pang[1], Benjamin Chilcoat[2], Yair Shachar-Hill[1], Om Parkash Dhankher[2], and **Danny J. Schnell**[1]

[1]Department of Plant Biology, Michigan State University, East Lansing, MI 48824, USA
[2]Stockbridge School of Agriculture, University of Massachusetts Amherst, MA 01003, USA

URL: https://groecamelina.natsci.msu.edu/

Project Goals:

Our research aims to sustainably increase oilseed yields in the non-food oilseed crop plant, *Camelina sativa*, thereby making it a commercially viable alternative for biofuels and bioproducts production. Camelina has shown considerable promise as a dedicated industrial oilseed crop because it requires low agronomic inputs, is naturally more resistant to both biotic and abiotic stress than other oilseed crops, and Camelina oil-based blends have been tested and approved as liquid transportation fuels[1,2]. This project aims to increase fixed carbon allocation to triacylglycerol (TAG) by identifying metabolic bottlenecks that control seed oil production and engineering these limiting steps to increase TAG production in developing Camelina seeds. We describe here the positive impact of engineering the *PDCT* gene, encoding phosphatidylcholine:diacylglycerol cholinephosphotransferase 1, on both the levels and composition of Camelina seed oil.

Abstract

In our previous studies, we utilized metabolomic and transcriptomic profiling approaches in developing Camelina seeds to further understand the routes, rates, and regulation of pathways that determine seed and oil yields to increase Camelina's productivity[3]. We revealed the potential limiting factor(s) in oil synthesis pathways, and accordingly, we selected several candidate genes/enzymes for metabolic engineering of Camelina. Among those genes, in the described work, we targeted the overexpression of the *PDCT* gene, a homolog to the Arabidopsis Reduced Oleate Desaturation 1, *ROD1* gene (TAIR ID: AT3G15820), which encodes Phosphatidylcholine:diacylglycerol cholinephosphotransferase 1 enzyme[4].

PDCT is proposed to act as a gate keeper responsible for the interconversions of diacylglycerol (DAG) and phosphatidylcholine (PC) pools[4,5]. On this basis, we hypothesized that increased PDCT activity in developing Camelina seeds would enhance metabolic carbon flux toward increased levels of TAG and alter oil composition to make it more compatible with the proposed industrial uses of Camelina oils. To test this hypothesis, we engineered Camelina by expressing its *PDCT* gene under the control of the seed-specific phaseolin promoter. Transgenic Camelina plants exhibited significant increases in seed mass and seed oil content, overall higher seed and oil yields and altered polyunsaturated fatty acid (PUFA) content compared to their parental wild-type (WT) plants. Further, the preliminary results from embryo culturing coupled with [^{14}C]acetate labeling of developing Camelina embryos indicated increased rates of fatty acid incorporation into glycerolipids. This resulted in higher total radiolabeled lipid content in the PDCT transgenic lines, particularly in the TAG and DAG lipid classes, relative to that in WT embryos.

We conclude that overexpression of the TAG biosynthetic gene, PDCT, appears to be a positive strategy to achieve a synergistic effect on the flux through the TAG synthesis pathway, thereby further increasing oil yields in *Camelina sativa*.

References

1. United States Department of Agriculture, F.A.S. https://apps.fas.usda.gov/psdonline/app/index.html#/app/downloads (2017).

2. Rosillo-Calle, F., Pelkmans, L. & Walter, A. A global overview of vegetable oils, with reference to biodiesel. A Report for the IEA Bioenergy Task 40 (2009).

3. Abdullah, H.M., Chhikara, S., Akbari, P. et al. Comparative transcriptome and metabolome analysis suggests bottlenecks that limit seed and oil yields in transgenic Camelina sativa expressing diacylglycerol acyltransferase 1 and glycerol-3-phosphate dehydrogenase. Biotechnol Biofuels 11, 335 (2018). https://doi.org/10.1186/s13068-018-1326-2

4. Lu C, Xin Z, Ren Z, Miquel M, Browse J. An enzyme regulating triacylglycerol composition is encoded by the ROD1 gene of Arabidopsis. Proc Natl Acad Sci U S A. 2009 Nov 3;106(44):18837-42. doi: 10.1073/pnas.0908848106. Epub 2009 Oct 15. PMID: 19833868; PMCID: PMC2774007.

5. Yang W, Wang G, Li J, Bates PD, Wang X, Allen DK. Phospholipase Dζ Enhances Diacylglycerol Flux into Triacylglycerol. *Plant Physiol*. 2017;174(1):110-123. doi:10.1104/pp.17.00026

Acknowledgment: This research is funded by the Department of Energy (ARPAe DE-0000200 & BER DE-SC0018269)