Oil Yield and Quality Optimization of *Camelina sativa* Transformed with Avocado (*Persea americana*) PDAT1 and DGAT1 Genes

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Central Questions:

Are PDAT and DGAT genes responsible for increased expression of TAG?
Can avocado (*Persea Americana*) DGAT and PDAT genes improve fatty acids composition in *Camelina sativa*?

Introduction:

Camelina sativa is a member of the *Brassicaceae* family, and is an oilseed crop commonly grown for use in industry.
Avocado (Persea americana), has a very high content of oils in its mesocarp. In this high oil content are many fatty acids that are nutritious and healthy for humans.

In plants, fatty acids are converted into Triacylglycerol (TAG) for storage using a commonly known pathway- the Kennedy Pathway. Diacylglycerol (DAG), the precursor to TAG, can be converted to TAG through diacylglycerol acyltransferase (DGAT), or through phospholipid:diacylglyerol acyltransferase (PDAT).

The goal is to insert DGAT1 and PDAT1 genes from *P. americana* into *C. sativa* in order to increase oil yield and fatty acids composition. If it is shown possible in *C. sativa*, then these genes could be inserted into other crops in order to increase their oil yield as well as the oil composition for heart healthy oil compostions. This could be used industrially in biofuel or vegetable oil production, or as a way to increase nutritiousness of crops by providing more necessary fatty acids.

In this project, *C. sativa* had been transformed with a vector containing DsRed containing either PDAT1 or DGAT1 genes. T2 lines were harvested and analyzed, and T3 homozygous transgenic lines would be selected for further study to optimize for good qualities such as high oil and seed yield, harvest index, confirmation of gene expression, etc.

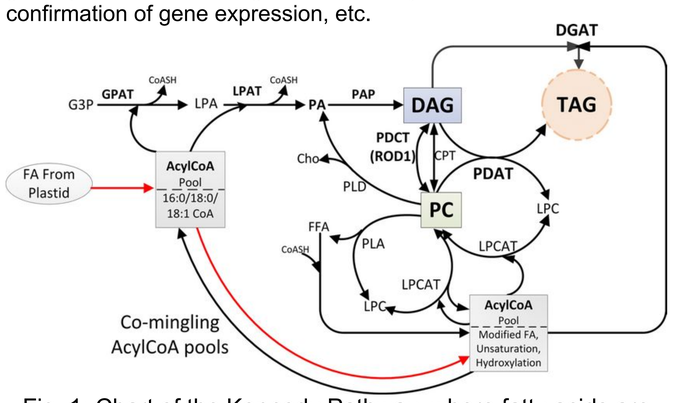


Fig. 1: Chart of the Kennedy Pathway, where fatty acids are converted to TAG. (Chapman, Ohlrogge, 2012)

References:

Chhikara, S., Abdullah, H.M., Akbari, P., Schnell, D. and Dhankher, O.P. (2018), Engineering *Camelina sativa* (L.) Crantz for enhanced oil and seed yields by combining diacylglycerol acyltransferase1 and glycerol-3-phosphate dehydrogenase expression. Plant Biotechnol J, 16: 1034-1045. https://doi.org/10.1111/pbi.12847

Chapman, K. D., Ohlrogge, J. B. (2012). Compartmentation of triacylglycerol accumulation in plants. Journal of Biological Chemistry, 287(4), 2288–2294. https://doi.org/10.1074/jbc.r111.290072

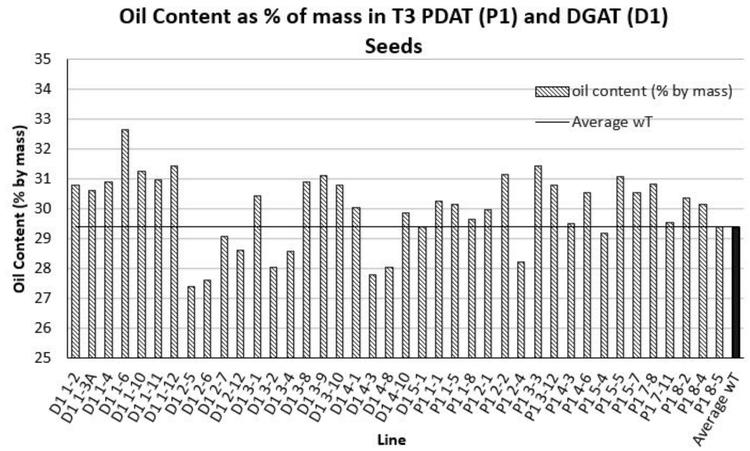
Methods:

Overview: The T2 lines were harvested. DsRed is a fluorescent protein, which allowed identification of plants with seeds which were 100% transgenic (homozygous for the transgene). These lines were measured for many characteristics including total seed yield, seed mass, dry plant biomass, and oil yield. From these measurements, ideal lines were selected and regrown to produce T3 homozygous lines. The fatty acid content of the seeds were also analyzed, though due to preparation time this occurred after the ideal lines were selected.

Measuring oil content using NMR: Three samples of T3 plant seeds were analyzed in an NMR machine to determine the oil content. In addition, oil content could be combined with seed mass to learn the total oil yield per plant.

Measuring fatty acid content using GC/FID: The fatty acids stored in TAG were converted into Fatty Acid Methyl Esters (FAME) and run on a GC/FID to get the content of individual fatty acids.

Results



samples. N = 3.

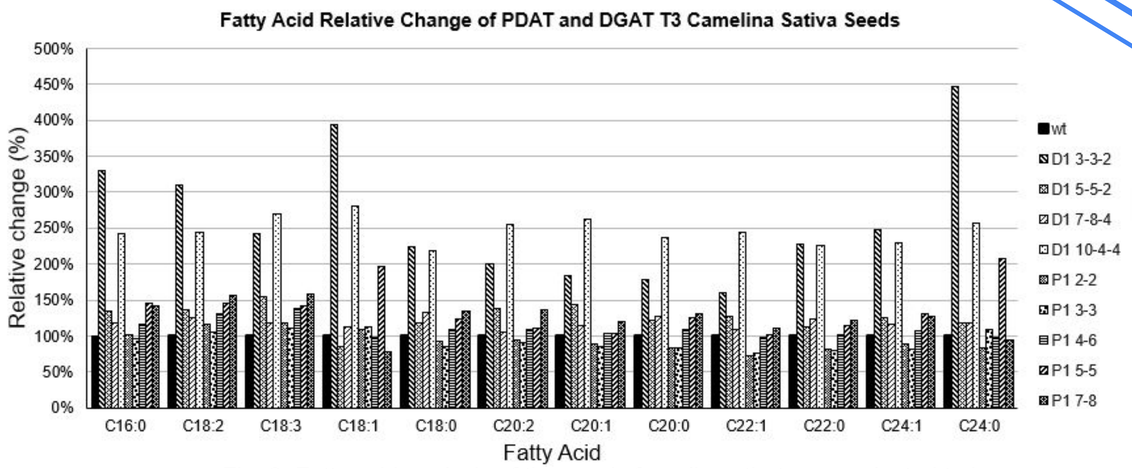


Fig. 3: Fatty acid analysis of the seeds from lines that were selected to be grown to T4 for further analysis, compared with a wild-type.

Conclusions:

The preliminary data looks promising as several PaDGAT1 and PaPDAT1 lines showed significantly increased oil yield and changed fatty acids compositions.
Further analysis of the selected lines showing higher yields is in progress.

Next Steps:

The grown T3 lines have been harvested. They will be analyzed shortly with all the same measurements as the T2 lines (with the exception of the DsRed analysis, which is not necessary as all lines are homozygous). This will provide conclusive evidence and the effect of PaDGAT1 and PaPDAT1 genes on the seed and Oil yields and fatty acids

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