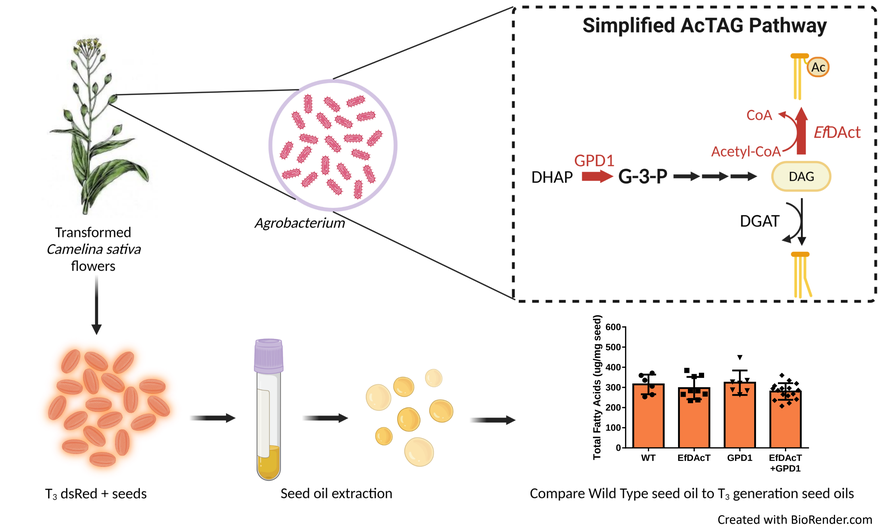
Increasing Oil Production in *Camelina sativa* Engineered to Synthesize Unusual Lipids

Isabella J. Davis, Linah Alkotami, and Timothy P. Durrett
Kansas State University Department of Biochemistry

Graphical Abstract

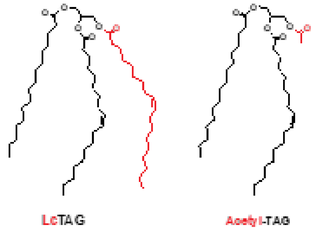


Acetyl Triacylglycerols; The Better Petroleum Alternative

Long chain triacylglycerols (LcTAG) are the main component in typical vegetable oils. LcTAG have three fatty acid chains attached to a glycerol backbone. Acetyl triacylglycerols (AcTAG) are being studied as an alternative to traditional vegetable oils due to its low viscosity at colder temperatures. These unusual properties are caused by the replacement of the third fatty acid chain with an acetyl group.

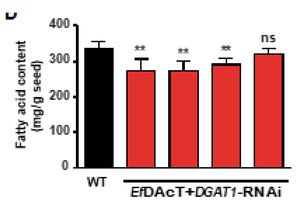
DAcT genes are responsible for the production of AcTAG in *Euonymus* species. *Ef*DAcT from the wintercreeper, *E. fortuneii,* has successfully been inserted into camelina and produces very high levels of AcTAG. However, in these transgenic lines, overall fatty acid levels are decreased when compared to wild-type camelina.

If available glycerol-3-phosphate (G3P) is the limiting factor in the number of fatty acids produced, overall fatty acid content of genetically-enhanced camelina is expected to reach wild-type production levels. Overexpression of glycerol-3-phosphate dehydrogenase (GPD1) slightly increased the production of G3P in T_2 independent lines. Using T_3 homozygous lines overexpressing GPD1, independent lines were analyzed for fatty acids and compared to fatty acid content of wild-type camelina.



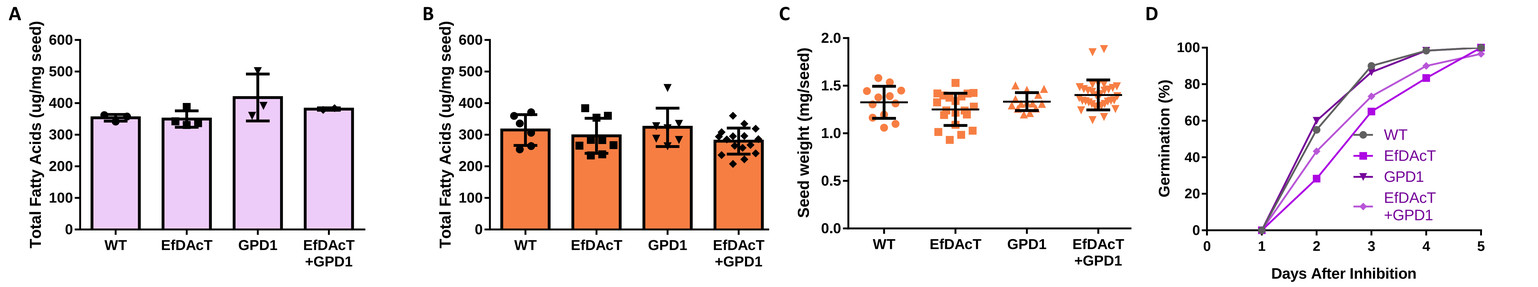
LcTAG molecules have three fatty acid chains. AcTAG molecules have two fatty acid chains and an acetyl group located on the third carbon.

*Ef*DAcT is the gene used from *Euonymus fortuneii* to synthesize AcTAG in camelina.



Fatty acid content of mature seeds from wild type and T_3 independent lines expressing *Ef*DAcT+ DGAT1-RNAi. (Alkotami et al (2021) Plant J 106: 953)

Results



(A) Total fatty acids of T_2 generation GPD1 was slightly increased while *Ef*DAcT and *Ef*DAcT + GPD1 showed no significant difference compared to wild-type plants. (B) Total fatty acids of T_3 generation of *Ef*DAcT, GPD1, *Ef*DAcT + GPD1, and wild-type plants showed no significant differences. Total fatty acids were calculated by an average of independent line replicants. (C) Seed weight of T_3 generation of *Ef*DAcT, GPD1, *Ef*DAcT + GPD1, and wild-type plants showed no significant differences. (D) Germination rates of *Ef*DAcT and *Ef*DAcT +GPD1 were slow compared to wild-type and GPD1 germination rates. Germination rates were calculated by average cotyledon emergence from three replicates.

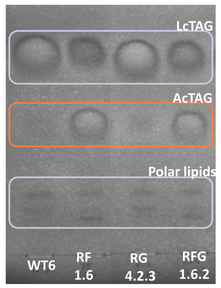
Extracting Fatty Acids from Camelina seeds

Seed selection of homozygous transgenic seed

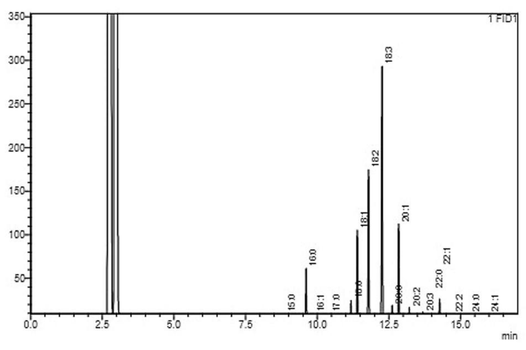
Wild-Type | dsRed+GDP1 | dsRed+*Ef*DAcT | dsRed+*Ef*DAcT+GDP1

Lipid Extraction

Thin Layer Chromatography to separate AcTAG from LcTAG



Gas Chromatography to quantify fatty acid content



Conclusions and Future Work

Total fatty acids were hypothesized to increase when GPD1 was overexpressed in camelina. After lipid analysis of WT, *Ef*DAcT, GPD1, and *Ef*DAcT +GPD1 transgenic lines, the data rejects the hypothesis. Gas Chromatography analysis showed no significant increase or decrease in *Ef*DAcT, GPD1, or *Ef*DAcT +GPD1 total fatty acid content compared to wild-type seeds. Compared to T_2 camelina lines, total fatty acid content appears to have decreased in all seed lines. It is hypothesized to have been caused by a fungal infection and aphid infestation during plant drying.

Seed weight was also expected to differ between T_3 transgenic lines. When analyzed, these were also not significantly different. Germination between seed lines shows slower germination rates in *Ef*DAcT and *Ef*DAcT +GPD1 seed lines suggesting the production of AcTAG using *Ef*DAcT may cause slower germination.

Future work will include increasing the number of independent lines and replicates in each line. Gene expression analyses will also be conducted to determine the rate of expression between independent lines. Current independent lines will also be reanalyzed since there was a decrease in total fatty acids between T_2 and T_3 wild-type lines. With increased sample size and improved growth conditions, seed total fatty acids are expected to increase.

Summary

Lipid analysis of GPD1, *Ef*DAcT, and *Ef*DAcT +GPD1 transgenic camelina seed showed no significant change in overall fatty acid content.

Acknowledgements

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