Development of Capric Acid-Rich *Camelina* Germplasm for Bio-Jet Fuel Production

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Introduction

Camelina has been shown to be a viable feedstock for bio-based jet fuel that generates up to 75% lower greenhouse gas emissions than petroleum-derived jet fuel.

In this study, we aimed to enhance the value of camelina seed oil for bio-jet fuel by introduction of seed-specific transgenes for a specialized FatB thioesterase, lysophosphatidic acid acyltransferase (LPAT), diacylgylcerol acyltransferase (DGAT) from *Cuphea* species to produce oils enriched in medium-chain fatty acids (C10-14). Levels of C10-14 were further increased by RNAi silencing of genes for acyl-acyl carrier protein synthases that limit accumulation of these fatty acids.

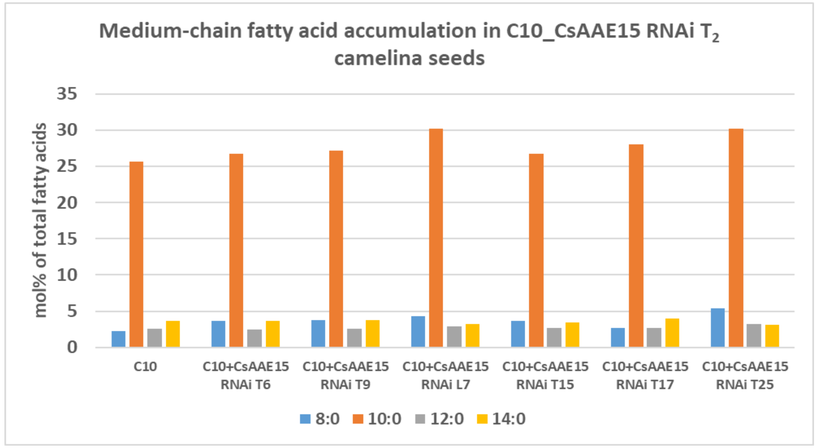
Materials and methods

Two RNAi constructs were prepared with hairpin cassettes placed under control of the seed specific glycininin promoter and a Basta herbicide selection marker. One construct was designed using *CsAAE15* sequence for the hairpin that should also target the analogous region of *CsAAE16*. The second construct contained a *CsAAE15/CsAAE16* chimeric hairpin that should target both genes. These were introduced into our 10:0 producing camelina background described above using *Agrobacterium*-based transformation.

Also these constructs were used for engineering 10:0 production in the Ukrainian camelina breeding line FEORZhYaFD and enhance 10:0 levels in seeds of this variety by silencing of *AAE15/16*.

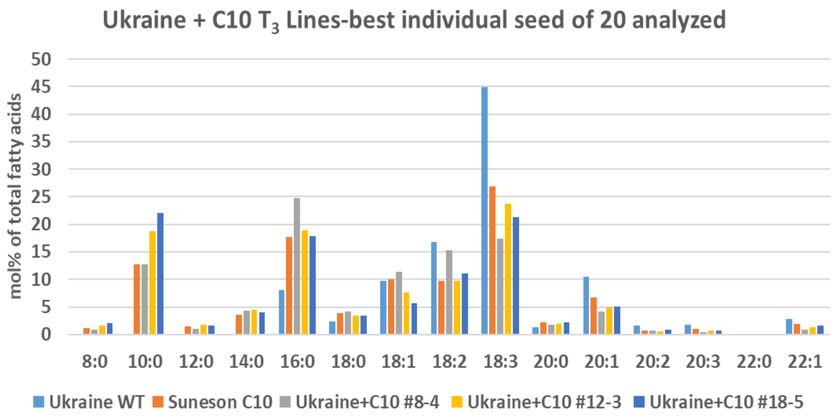
Results

Two RNAi constructs were prepared with hairpin cassettes placed under control of the seed specific glycininin promoter. As a selection marker resistance to Basta herbicide was chosen. One construct was designed using *CsAAE15* sequence for the hairpin that should also target the analogous region of *CsAAE16*. The second construct contained a *CsAAE15/CsAAE16* chimeric hairpin that should target both genes. Both constructs were introduced into our 10:0 producing camelina background (Suneson variety) using *Agrobacterium*-based transformation. As a result T_2 plants were obtained



Seeds from the top-preforming T_2 line from the *CsAAE15* construct accumulated 10:0 to amounts of up to 30 mol% of the total fatty acids, whereas seeds from the background line used for transformation contained 25 mol% of 10:0, or a ~25% increase in relative amounts of 10:0 with the *AAE* gene-suppression. In addition, seeds from this line accumulated the medium-chain fatty acids 10:0, 12:0, and 14:0 to amounts of up to 37mol% versus 32 mol% in seeds from the 10:0 background. We also detected a ~25% increase in 10:0 content in seeds of our best performing T_2 line from the *CsAAE15/16* construct.

These findings indicate that this strategy for enhancing 10:0 and total medium-chain fatty acid levels in camelina seeds is effective.



Forty-four transgenic lines were generated in the FEORZhYaFD background for production of 10:0, then five highest 10:0-producing lines were advanced to the T_2 generation. Seeds from the top 10:0-producing line accumulated 10:0 to amounts of ≤25 mol% of the total fatty acids. Total amounts of the bio-jet fuel-type medium-chain fatty acids (8:0, 10:0, 12:0, 14:0) accumulated to ~28 mol % of the seed fatty acids in this line, with a large decrease in polyunsaturated fatty acids (18:2, 18:3). Plants had no observable growth differences compared to the FEORZhYaFD non-transformed plants. Line 8-4 was determined to contain a single locus insertion of the transgenes and was chosen for further metabolic engineeringwith the *CsAAE15* and *CsAAE15/16* RNAi constructs. Beside that lines 12-3 and 18-5 could have double insertion, which now is being verified with RT-PCR.

We are currently advancing these lines to homozygosity and will more thoroughly analyze the seed fatty acid composition and seed oil content from these lines

Fig. 1. *C. sativa* before flowering

Fig. 2. *C. sativa* unmatured seed pods. Left – c. Suneson, right – c. FEORZhYaFD

Conclusions

The project demonstrated the feasibility of engineering camelina, including a Ukraine-adapted variety, for the production of oils with up to 30% of medium-chain fatty acids (C10-14) that are more readily convertible to bio-jet fuel than conventional camelina oil that is rich in C16-C22 fatty acids. Levels of medium-chain fatty acids were increased in camelina seed oil by silencing expression of genes for enzymes that restrict accumulation of these fatty acids. The camelina varieties generated by this project will promote enhanced profitability for dryland farmers and an optimized, low carbon emission feedstock for the aviation fuel industry.

Acknowledgements

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