

## Systems Biology to Improve Camelina Seed and Oil Quality Traits

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**Project Goals: Camelina has great potential to become a sustainable high energy-yielding source of biofuel in the US. This project aims to address two critical needs for realizing this potential: to increase seed size and oil content for improved seedling establishment and oil yield, and to optimize oil quality for satisfactory fuel properties. Specifically, quantitative trait loci (QTLs) and molecular markers associated with these important traits will be identified using high-density genome maps and repeated field trials in Montana and Washington states. Modern genomics and biotechnological approaches will be employed to uncover novel molecular mechanisms (including genes and gene networks regulated by microRNAs and transcription factors) regulating fatty acid modification, oil accumulation and seed size in Camelina.**

Camelina (*Camelina sativa*) is a low-input, non-food oilseed plant that has great potential to become a sustainable high energy-yielding source of biofuel in the US. This project aims to address two critical needs for realizing this potential: 1) to increase seed size and oil content for improved seedling establishment and oil yield, and 2) to optimize oil quality for satisfactory fuel properties. Progresses have been made in the following areas:

1. To identify quantitative trait loci (QTL) and molecular markers associated with seed size, oil content and other important agronomic traits. We are using two complimentary populations to identify QTL controlling these traits in camelina. 1) A panel consisting 230 accessions of *Camelina sativa* and 21 camelina varieties is used for genome wide association studies. These lines were planted in the fields in Bozeman, MT and Pullman, WA in April-May 2017 and showed wide variations in several important traits including seed size, oil content and flowering time. 2) A biparental population derived from a cross between two contrasting varieties is used for linkage mapping. Using the single-seed descent (SSD) method, we developed a population comprising 361 recombinant inbred lines (RILs) from the cross between a large-seed variety Pretyzh (2.21mm<sup>2</sup>, 1.8 mg/seed, oil content 31.6%) originated from Ukraine, and Suneson (1.51mm<sup>2</sup>, 1.3 mg/seed, oil content 36.9%), a variety resulted from the selection in a German accession (PI 633192). The SSD population is now at the F6 generation. Field evaluation in Bozeman, MT showed great variation in several phenotypic traits (e.g., seed size, pod size, oil content, plant height, and flowering time). Sequencing of the parental lines and the population is underway to discover SNP markers for construction of a genetic linkage map.
2. To discover novel molecular mechanisms (including gene networks regulated by microRNAs and transcription factors) regulating fatty acid modification and seed size in camelina. In collaboration with the Joint Genome Institute (JGI), RNA samples have been collected from camelina lines that differ in seed size and fatty acid composition (e.g., high linolenic acid) for

RNAseq and microRNA profiling. Over 40 miRNAs have been selected and specifically overexpressed in camelina seeds. Preliminary data in transgenic plants suggested that several microRNAs may play important roles in developing seed in camelina. For example, overexpression of miR167a showed pleiotropic effects such as increased seed size and delayed seed maturation. Especially, the miR167a overexpressed seeds had increased 18:2 and decreased 18:3 compared to the seeds from wild-type, while other fatty acids remained unchanged. Comparative transcriptome analysis and chromatin immunoprecipitation experiments suggest that miR167a regulates fatty acid composition in camelina seeds by suppressing the expression of *FAD3* through a regulatory cascade involving AUXIN RESPONSE FACTORS (ARF6/8), the putative targets of miR167, and several transcription factors involved in lipid biosynthesis such as ABSCISIC ACID-INSENSITIVE 3 (ABI3), BASIC LEUCINE-ZIPPER 2 (bZIP2).

3. Modification of fatty acid composition in camelina seeds. 1) An effective tool using the CRISPR/Cas9 technology has been successfully developed in camelina (Ozseyhan et al., 2018). Homozygous knockout mutants were successfully created in a single generation by simultaneously targeting three *FAEI* genes using an egg cell-specific Cas9/gRNA expression. Very-long-chain fatty acids in the mutants were reduced to less than 2% of total fatty acids compared to over 22% in the wild type, and the C18 unsaturated fatty acids were concomitantly increased. 2) Artificial microRNA was used to down-regulate the expression of *FATB* in camelina seed. Over 40% reduction of saturated fatty acids (16:0+18:0) was observed in transgenic seeds compared to the non-transgenic wild type. Transgenic seeds also contained increased linolenic acid (18:3). 3) A fatty acid desaturase (DES9\*), derived from the cyanobacterial 16:0/18:0 acyl lipid desaturase by directed evolution, was used to reduce levels of 16 and 18-carbon saturated fatty acids. Saturated fatty acid levels were reduced by more than 60%, compared with control plants. Monounsaturated fatty acid products, 16:1 and 18:1, were also greatly increased in seeds expressing the desaturase. The seed from transgenic lines with the lowest saturate levels had reduced oil, but there were only mild phenotypic changes in other lines.

## Publication

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