

## 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 (QTL) 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.

**Progresses** have been made in the following areas during the last reporting period:

1. To identify quantitative trait loci (QTL) and molecular markers associated with seed size, oil content and other important agronomic traits. 1) A population of recombinant inbred lines (RILs) derived from the cross of two contrasting varieties was used for linkage mapping. SNP markers were generated by genome sequencing, and a linkage map comprising nearly 2400 SNPs was constructed that spanned a genetic distance of about 2035cM covering ~78% of the camelina genome. The RIL population (at the F5 and F6 generations of single seed descent) was grown in Bozeman, MT for phenotypic evaluations. A total 38 QTL were detected for seed and pod sizes (e.g., area, length and width). Several QTL were also detected for other important traits such as oil content, plant height, and flowering time. Significant QTL were chosen for future fine mapping and candidate gene identification. 2) A panel consisting 230 accessions of *Camelina sativa* was used for genome wide association studies (GWAS). These lines were planted in the fields in Bozeman, MT and Pullman, WA in 2017 and 2018. Several important traits evaluated, including seed size, oil content and flowering time, showed wide variations. Genotyping by genome resequencing is being conducted in collaboration with the Joint Genome Institute (JGI).
2. To discover novel molecular mechanisms that regulate fatty acid modification and seed size in camelina. 1) Overexpressing microRNA167A (miR167OE) in camelina under a seed-specific promoter changed fatty acid composition and increased seed size [1]. The miR167OE seeds had a lower  $\alpha$ -linolenic acid with a concomitantly higher linoleic acid content than the wild type. This decreased level of fatty acid desaturation corresponded to a decreased transcriptional expression of the camelina fatty acid desaturase3 (*CsFAD3*) in developing seeds. Chromatin immunoprecipitation experiments combined with gene expression studies indicated that the miR167 targeted *CsARF8*, which bound to promoters of camelina *bZIP67* and *ABI3* genes. These transcription factors directly or through the ABI3-bZIP12 pathway regulate *CsFAD3* expression and affect the  $\alpha$ -linolenic acid accumulation. Also, comparative transcriptome analysis was conducted to study mechanisms that caused increased seed size in miR167OE. Expression levels of many genes were altered in miR167OE compared to wildtype, including orthologs that have previously been identified to

affect seed size in other plants. Most notably, genes for seed coat development such as suberin and lignin biosynthesis were down-regulated. These results suggested possible mechanisms that we will test to increase seed size in camelina. 2) Seed-specific suppression of ADP-glucose pyrophosphorylase (AGPase) increased seed size and weight [2]. The RNAi suppression reduced AGPase activities which concurred with moderately decreased starch accumulation during seed development. Transcripts of genes examined that are involved in storage products were not affected, but contents of sugars and water were increased in developing seeds. The transgenic seeds were larger than wild-type plants due to increased cell sizes in seed coat and embryos, and mature seeds contained similar oil but more protein contents. The larger seeds showed advantages on seedling emergence from deep soils. This study indicated that changing starch and sugar metabolism during seed development may increase the size and mass of seeds without affecting their final oil content in Camelina. Increased seed size may improve seedling establishment in the field and increase seed yield.

3. Modification of fatty acid composition in camelina seeds. 1) Artificial microRNA was used to down-regulate the expression of *FATB* in camelina seed [3]. Over 40% reduction of saturated fatty acids (16:0+18:0) was observed in transgenic seeds compared to the non-transgenic wild type. 2) A two-gene strategy for reducing oil saturates in camelina oil. To reduce saturated fatty acids (16:0) in the oil, we expressed a DES9\* glycerolipid desaturase, which we developed by directed evolution, in seeds of Camelina. As we reported in our 2018 abstract, this resulted in substantial lowering of 16:0. However, further experiments indicated that oil content and seed weight of the transgenic plants was severely compromised. We have also used seed-specific expression of the FAT5 acyl-CoA desaturase from *C. elegans*. However, by itself FAT5 resulted in only small reductions in 16:0 content of the oil. Because the FAT5 and DES9\* desaturases act on different lipid substrates and at different stages of oil synthesis, we hypothesized that more modest expression of the two enzymes together might provide for a better result. To accomplish this, we cloned the FAT5 and DES9\* coding sequences into a single binary vector under control of different seed-specific promoters, and used the vector for transformation of camelina plants. Our initial results from FAT5-DES9\* transgenic plants indicate that this strategy has worked. The double transgenic plants have only 1.8% 16:0 in the oil (compared to 7.5% in the parental line), while retaining oil content and seed weight comparable to the parental line. We are now undertaking detailed biochemical and physiological studies of seed metabolism and physiology to compare our FAT5-DES9\* lines with untransformed controls. Taken as a whole our work will not only lead to camelina lines with greatly improved oil characteristics, but will also result in an improved understanding of the metabolism and physiology of oilseed crops.

## Publications

1. Na, G., Mu, X., Grabowski, P., Schmutz, J. & Lu, C. (2019) *Plant J*, doi:10.1111/tpj.14223.
2. Na, G., Aryal, N., Fatihi, A., Kang, J. & Lu, C. (2018) *Biotechnol Biofuels* **11**, 330.
3. Ozseyhan, M. E., Li, P., Na, G., Li, Z., Wang, C. & Lu, C. (2018) *Biochem Biophys Res Commun* **503**, 621-624.

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