

Systems Biology to Improve Camelina Seed and Oil Quality Traits

Chaofu Lu^{1*} (clu@montana.edu) and John Browse²

¹Montana State University, Bozeman; ²Washington State University, Pullman

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.

The available genomic resources (including genome, transcriptome and microRNA sequences) of Camelina and its close relative Arabidopsis and our efficient biotechnological research tools will ensure our success to achieve the following specific objectives:

1. To identify quantitative trait loci (QTLs) and molecular markers associated with seed size, oil content and other important agronomic traits. Association studies of a collection of over 250 Camelina accessions and molecular mapping using recombinant inbred line (RIL) populations will be conducted. Agronomic traits will be evaluated in repeated field trials in Montana and Washington states.
2. To discover novel molecular mechanisms (including gene networks regulated by microRNAs and transcription factors) regulating fatty acid modification and seed size in Camelina. MicroRNAs affecting seed traits will be obtained by a high-throughput screen in transgenic Camelina for seed-specific overexpression. Bioinformatics analyses, genetics

and biochemical experiments will be conducted to decipher the mechanisms through which miRNAs affect seed development and oil accumulation.

3. To test functions of known genes, as well as newly identified candidates from 1&2 for their effects on improving seed size, oil content and fatty acid composition. Novel desaturases and transcription factors that have shown promising preliminary results will be vigorously tested for their roles in improving fatty acid composition and increasing seed size and oil content. These efforts are facilitated by our efficient biotechnological tools such as an *Agrobacterium*-mediated transformation protocol and the CRISPR/Cas9 method that allow for efficient gain/loss-of-function testing of beneficial genes in a semi-high-throughput fashion. Improved traits will be validated in field conditions following USDA APHIS guidelines.

This project will result in several natural and engineered *Camelina* lines with advanced traits like large seed and high content of high-quality oil. Increased oil content in larger *Camelina* seeds will greatly boost harvestable oil yield and improve the ability to establish seedlings particularly under unfavorable field conditions. *Camelina* oils containing ultrahigh oleic (>80%) and enhanced palmitoleic acids will greatly improve fuel properties such as enhanced oxidative stability and cold flow. Because novel approaches will be explored to engineering beneficial traits in *Camelina*, this program will also contribute to understanding fundamental mechanisms regulating oilseed metabolism and physiology. Specifically, identification of genes and molecular markers associated with seed size, oil content and other important agronomic traits will facilitate the development of the next generation *Camelina* varieties. Novel gene regulatory networks uncovered by studying microRNAs will greatly advance the understanding of plant lipid metabolism, and provide new tools for improving oil qualities of many oilseed crops. In addition, this project will provide training opportunities for several young scientists such as graduate students and postdocs in plant genomics and biotechnology.

This research project is supported by the DOE Office of Science, Office of Biological and Environmental Research (BER), grant no. DE- SC0016400.