

Germplasm Development for Sustainable Production of *Camelina sativa* Oilseed

John K. McKay,^{1,*} (jkmckay@colostate.edu), Jack Mullen,¹ Ed Cahoon,³ Luca Comai,⁴ and Tim Durrett⁴

¹Colorado State University; ²University of Nebraska; ³UC Davis; and ⁴Kansas State University

Project Goals:

The semi-arid west is capable of making significant contributions to the production of bioenergy if appropriate feedstocks are utilized. *Camelina sativa* is poised to become an important biofuel oilseed crop for the extensive dryland farming regions of the west. We focus on development of *Camelina* as a crop adapted for growth on marginal farmland with relatively low inputs under dry conditions, as part of the DOE effort to create “regionally adapted oilseed feedstocks with enhanced yields and desirable oil qualities for biofuels”. The Great Plains and intermountain West have historically lacked rotation crops that perform well and have good market value. *Camelina* is suited for rotation with wheat, and could also reduce erosion and increase amounts of soil carbon. In our current project we have: 1) created 3 mutant populations and demonstrated their utility using a subset of the lines for forward and reverse genetics, 2) identified genomic regions controlling natural variation in oil profile and abiotic adaptation 3) engineered oil profile using a combination of mutants and transformation to create genotypes optimized for a liquid fuel supply chain.

Abstract:

Objective 1: Development of functional genomic resources of *Camelina*

A TILLING population of *Camelina* var. Ames 1043 was developed in the Comai lab. The population DNA has been re-standardized pooled and arrayed. As a result 2,048 individual DNAs are being TILLED for loci involved in abiotic stress tolerance and lipid biosynthesis. In addition we have created 2 additional mutant populations. These two genotypes, Lindo and Licalla, are the parents of a mapping population that we have used to map drought adaptation QTL. One M2 seed per family was planted to produce M2 tissue and M3 seed. Together, these populations will ensure that we reach the target of 1000 mutant lines per accession. Excess M2 seed will be banked and used for forward genetics screens (see below). In a population of this size we expect a minimum of 90 mutations in the average 1.5kb target gene with a corresponding probability of over 97% of finding a predicted Knock Out.

Objective 2: Development of germplasm for improved drought and heat tolerance and oil

Replicated field trials were performed in Fort Collins and Greeley, Colorado, under differential irrigation treatments at each site to collect phenotypic data on a variety of traits. Sixteen new QTL were discovered from this data, along with nine QTL using data from Colorado trials of the same population in previous years. Seven QTL were discovered for yield. We are moving forward with fine mapping these QTL. We also have identified QTL affecting oil composition, of interest for objective 3 below. To identify QTL associated with heat tolerance, we have also grown this Lindo x Licalla mapping population in growth chambers under a 35°C daytime temperature regime. We have observed substantial variation in growth

under heat stress. While measurements of yield are still being collected, we have identified a QTL for biomass under heat-stress conditions.

QTL and candidate gene validation. QTLs of major importance were chosen for fine mapping. We are developing near isogenic lines (NILs) in which small genomic regions containing the QTL of interest are introgressed between genotypes. This will include fine mapping of major QTLs for oil content. NIL creation is currently in progress, with the completion of backcrossing several existing RIL lines to the parents (Lindo and Licalla) as the introgression recipient. F1 plants have been backcrossed again, and now the resulting crosses are currently growing for selfing. Recombinants will be identified by genotyping at least 384 BC1S1 progeny using SNP markers flanking the QTL interval. All of these recombinants will be selfed and the progeny genotyped, and again we will select the rare recombination events so that small homozygous Lindo introgressions are captured in a homogeneous Licalla background, and vice versa. Once QTL are fine mapped to small intervals, we will use the TILLING population to identify knockouts of candidate genes in those intervals. These knockouts will be tested for effects on the relevant phenotype and then used for complementation studies with the parental alleles.

Mutant screens for drought and heat tolerance. To identify important loci not captured by the variation in the mapping population, we have begun screening the existing mutant population to identify lines with improved tolerance to heat and drought. In 2015 we evaluated 1008 M3 families in field plots under both well-irrigated and drought stress conditions at the CSU Agricultural Research, Development and Education Center. This identified 36 mutants with differential sensitivity to water stress.

Objective 3: Genetic improvement of Camelina oil quality

Seed from mutagenized lines was screened using electrospray ionisation mass spectrometry (ESI-MS) for alterations in the molecular species composition of neutral lipids and phospholipids. Seed from interesting lines was grown to the next generation, with three replicate plants per line. The seed from these lines has been harvested and is currently being analyzed to confirm the mutant phenotypes.

In addition, 993 mutagenized lines from the Ames 1043 TILLING population were analyzed by GC (gas chromatography) to identify fatty acid compositions that are ideal for biofuel production. We have identified 8 lines with possible FAD2 mutations, 19 with FAD3, 4 with FAE1 and 24 with FATB. The 993 mutagenized lines were also analyzed by HPLC (high performance liquid chromatography) for increased tocopherol levels. 16 lines had increased tocopherol levels compared to wild type.

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