## Winter Annual Oilseed Quarterly Report: Summer 2017

Pennycress Genomics Research Update

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Field pennycress (*Thlaspi arvense L.*) is a new, innovative winter annual cover crop. Similar to traditional cover crops, pennycress provides ecosystem services such as erosion control and reduced nutrient leaching from the fall through early spring. However, pennycress also yields a valuable oilseed crop. Pennycress is winter hardy in Minnesota, and the oil produced from pennycress seed can be used as a feedstock for biofuels or biopolymers. Despite these ecosystem and economic benefits, the use of pennycress as a cash cover crop may be limited by its weedy characteristics. These characteristics include seed shattering that leads to the development of a seed bank in the soil and seed mixing due to small seed size that causes contamination of elite breeding lines with unimproved genetics.

As part of the Forever Green Initiative, the University of Minnesota has initiated a pennycress genomics program with the goal of domesticating pennycress. Through the domestication process, a weedy species like pennycress can be developed into a valuable crop that will not develop a seed bank and can be distinguished from wild pennycress. The genomics program started in 2012 with the development of genome (Dorn et al. 2015) and transcriptome databases (Dorn et al. 2013), followed by the generation of mutagenized pennycress lines (Marks et al. 2017). Mutagenesis of wild-type pennycress has led to the identification of mutants which can help resolve the problems associated with seed shattering and seed contamination. These mutants were subject to whole-genome sequencing to identify the causative genes in the sequenced lines (Figure 1). Once the causative genes are identified, the breeding process can be expedited through marker-assisted selection.

We have identified several reduced shatter mutants (Figure 2). Both non-shatter mutants had independent events in the same candidate gene. Mutation in this gene results in lack of lignified patches at the valve margins which influences the fruit dehiscence process. The fact that two different mutations in the same gene were found provides evidence that this gene is involved in the shattering phenotype in pennycress. Molecular markers have been developed for these two mutations and are being used to introduce these reduced shatter traits into the breeding program. This same process of gene identification is being used to determine the causative genes for seed color mutants (Figure 3). This seed color trait will be useful for the breeding program to include in future domesticated pennycress varieties, because black



undomesticated seeds can be easily sorted out and discarded from the lighter colored domesticated varieties.

We will continue to use marker-assisted breeding to introduce the reduced seed shatter and light seed coat color to high yielding pennycress lines. Performance evaluations of these breeding lines in field conditions will begin in fall 2018.

> Whole Genome Sequence - M<sub>3</sub> or M<sub>4</sub> or M<sub>5</sub> (10X coverage) Filter the raw reads with trimmomatic pipeline Alignment of raw reads to the Thlaspi v1.0 using bwa-mem Convert sam to bam files using samtools Mark duplicates using picard tools Rename the read groups and index the files using picard tools Call SNPs using the GATK haplotype caller Filter against the known SNPs from natural variation + Group A-D indentified thru NIKS Amino acid prediction of the SNPs using Thlaspi v1.0 protein database

Figure 1: A pipeline developed to identify the causal mutations in the pennycress mutants through whole-genome sequencing.



Figure 2. Typical seed shatter phenotype in check line MN106 compared to reduced shatter mutants A7129 and A7236 after a wind storm in June 2016.



Figure 3. Range of seed coat color variation from the normal black color exhibited by check line MN106 to the very light yellow of mutant line A7187.

## References

Dorn KM et al. (2013), De novo assembly of the pennycress (Thlaspi arvense) transcriptome provides tools for the development of a winter cover crop and biodiesel feedstock. Plant Journal 75, 1028-1038.

Dorn KM et al. (2015), A draft genome of field pennycress (Thlaspi arvense) provides tools for the domestication of a new winter biofuel crop. DNA Research 22, 121-131.

Marks et al. (2017), Arabidopsis as a Guide for the Rapid domestication of Thlaspi arvense as a new ecofriendly oilseed cover crop. (In preparation)