Investigating Seed Size and Oil Content in Pennycress, *Thlaspi arvense*

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Project Goals: Our goal is to characterize and improve seed size and oil content in pennycress to improve its efficiency and utility as a biofuel feedstock and make the seed easier for producers to handle. To advance towards this goal, we aim to: 1) Investigate the genetic control of these traits in a wild-germplasm collection using association mapping and biparental linkage mapping to identify quantitative trait loci (QTL); 2) Identify and characterize these traits in EMS induced mutation lines using high-throughput screening tools in combination with next generation sequencing techniques; and 3) Generate pennycress CRISPR-Cas9 knockouts in the genes known to regulate these traits in *Arabidopsis*.

Pennycress is a winter hardy cover crop that provides ecosystem services such as reduced soil erosion and nutrient loss in between fall corn harvest and spring soybean planting. Unlike traditional cover crops, field pennycress produces a mature oilseed in late spring, allowing farmers to harvest two cash crops in one year. Wild-derived pennycress lines have been shown to yield >1,000 kg ha^{-1} [1]. Pennycress seeds contain on average 33% oil by weight, and the oil is an excellent biofuel feedstock. However, despite these environmental and economic benefits, pennycress is currently limited by its small seed size (1 mg/seed), which can complicate planting, harvesting, and handling of the seed. Increasing seed size would also increase the efficiency of oil extraction. In conjunction with improved seed size, increased oil content in the seed would also improve the economics of growing and processing of pennycress as a biofuels feedstock. We have collected wild pennycress accessions representing genetic diversity from North America, Europe, and western Asia[2]. By characterizing these accessions for seed size and oil content, we can identify useful variants for improvement. With USDA NIFA funding, we previously developed several EMS-induced pennycress mutant lines exhibiting key domestication traits such as reduced seed pod shatter, earlier flowering, and improved fatty acid profiles[3]. We have also developed and demonstrated the utility of pennycress *Agrobacterium*-mediated plant transformation and CRISPR-Cas9 genome editing by generating pennycress lines with undetectable levels of erucic acid in seed oil[4]. Using these recently developed techniques and germplasm, our goal is to identify and characterize traits that will improve pennycress efficiency and utility as a biofuel feedstock species and make the seed easier for producers to handle. Finally, we will introgress these traits into our elite breeding lines to develop new pennycress varieties with increased oil and seed yield.

To complete these objectives, we have compiled a pennycress association mapping panel with 319 genotypes (267 winter-type, and 52 spring-type individuals). The panel was planted in St.

Paul, MN for the 2018-2019 growing season. Harvested seed was screened for size using a Marvin Seed Analyzer and for oil content using NIRS. Phenotypic variation for both seed size and oil content was observed and will be combined with genotyping-by-sequencing derived genetic markers to identify marker-trait associations. We expect that the genotypic analysis will help us identify QTL associated with seed size and oil content and that these QTL can be used for marker assisted selection to develop improved breeding lines with larger seeds and higher oil content. In addition to using natural variation, we have also employed mutagenesis and gene editing techniques to rapidly improve seed size and oil content. We screened a pennycress ethyl methanesulphonate (EMS) mutagenesis population containing approximately 15,000 M_2 plants for larger seed size. One thousand mutant lines were identified for further screening, and 15 lines were identified as large seed mutants without lethal embryo phenotypes. Thousand seed weight for these lines ranged from 1.3 to 1.7 g compared to the wild type average of 1.1 g. These lines will be grown in the field to test the trait heritability and if the increase in seed size causes a reduction in seeds per pod under normal growing conditions. If the large seed size is heritable, we will work to identify the causative gene and develop a genetic marker for use in marker assisted trait introgression. We have generated high oil pennycress mutants using CRISPR gene editing and confirmed the phenotype in field trials. The *tt8* mutant has 10% higher oil content and has shown growth and yield characteristics agronomically indistinguishable from wild-type pennycress.

References

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