

Genetic improvement of seed yield and oil content in field pennycress, a non-food oilseed feedstock species

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Project Goals: Our goal is to identify and characterize lines having traits that will improve pennycress efficiency and utility as a biofuel feedstock species and make the seed easier for producers to handle, namely: 1) Increased seed size and 2) Increased seed oil content. To advance towards this goal, we are: 1) Investigating the genetic control of these traits in wild-germplasm collections using quantitative trait loci (QTL) and association mapping; 2) Identifying and characterizing EMS-induced lines for these traits using high-throughput NIRS and seed analyzer screening in combination with genomics tools; 3) Generate CRISPR-Cas9 knockouts in the genes known and found to regulate these traits in Brassica species.

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 1,500 kg/ha on average (Mitich, 1996). Pennycress seeds contain approximately 33% oil by weight, and the oil is an excellent biofuels feedstock. After pressing, the remaining pennycress meal can be used as animal feed. 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 pennycress seed size would also increase the efficiency of oil extraction. In addition to improving the seed size, increased oil content in the seed would improve the economics of growing and processing of pennycress as a biofuels feedstock. With previous USDA NIFA funding, we developed several EMS-induced pennycress mutant lines exhibiting key domestication traits such as reduced seed pod shatter, earlier flowering, and improved fatty acid profiles (Chopra et al., 2018a; McGinn et al., 2018). We have also developed and demonstrated the utility of pennycress *Agrobacterium*-mediated plant transformation and CRISPR-Cas9 genome editing, generating pennycress lines with undetectable levels of erucic acid in seed oil (McGinn et al., 2018). With pennycress domestication well under way, our new goal is to identify, characterize, and introgress into breeding lines traits that will improve pennycress efficiency and utility as a biofuel feedstock species and make the seed easier for producers to handle.

The goals of this project are to identify the genetic controls that positively or negatively regulate seed size and seed oil content of pennycress. For this purpose, we have started developing necessary populations required to perform QTL mapping in F₅ or F₆ generations. We currently are propagating F_{3:4} plants for seed increase. We have more than 15,000 M₃ mutant families derived through EMS mutagenesis and are available to screen for these traits. To effectively characterize these populations, we are optimizing and calibrating phenotypic tools such as a Marvin seed analyzer and NIRS (Chopra et al., 2018b) for high-throughput screening of the pennycress seeds. Preliminary analysis of EMS populations has helped us identify a number of lines showing variation in seed oil content and seed size. These lines were planted again to verify their inheritance of the trait. With previous USDA NIFA funding, we have generated several lines for reduced seed fiber content using EMS mutagenesis and CRISPR-Cas9 editing. Interestingly, *transparent testa 8 (tt8)* alleles from these events have shown ~10% increase in total seed oil content; we are currently verifying these results in field trials. This poster will highlight the approaches we are using to identify lines and corresponding genetic markers for the above-mentioned traits. The long-term goal of this research is to use different genetic tools to identify mutations and natural variants along with molecular markers to enhance seed size and seed oil content of pennycress for introgression into elite breeding lines.

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