Advancing Field Pennycress as a New Oilseed Biofuels Feedstock that does not Require New Land Commitments

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http://www.wiu.edu/pennycress/ http://cbs.umn.edu/marks-lab/home
https://about.illinoisstate.edu/jcsedbr/Pages/Research.aspx

Project Goals: This is a collaborative project to generate and employ genetic and germplasm resources in improving the agronomic traits of Field Pennycress (*Thlaspi arvense* L.; pennycress) for its use as a winter annual oilseed/meal/cover crop grown throughout the U.S. Midwest Corn Belt. While wild strains can produce up to 2,200 kg of seeds per hectare (840 L/ha oils e.g. for biofuels and 1,470 kg/ha press-cake e.g. for animal feeds), they suffer from inconsistent seed germination and stand establishment, sub-optimal oil and meal quality, and yield loss due to pod shatter. Our aims are to 1) generate large EMS mutant populations for internal and community use; 2) employ forward genetic screens to identify mutants having improved agronomic traits; 3) identify the genetic lesions underlying those traits, focusing on those having the highest scientific and agronomic value; and 4) employ reverse genetic tools including CRISPR-Cas9 to target mutations in genes shown to improve traits in other species.

Pennycress (*Thlaspi arvense*; Field pennycress) is an oilseed plant of the Brassicaceae family that is closely related to Arabidopsis, camelina, and rapeseed canola. Pennycress is native to Eurasia and naturalized to North America, growing widespread throughout temperate regions of the world. Pennycress can be grown as a winter annual oilseed-producing cover crop, for example, planted in the fall in standing corn and harvested in the spring in time to plant full-season soybeans throughout the 80 million-acre U.S. Midwest Corn Belt. Once commercialized, elite pennycress varieties will provide additional income to farmers and agribusinesses thereby strengthening rural communities. Pennycress will also provide ecosystem services as a cover crop, for example, reducing soil and nutrients runoff and providing habitat and pollinator support on otherwise vacant farmland.

Field trials with current isolates have demonstrated that pennycress can be seeded in upper Midwest cornfields in the late summer and fall, at which time the plants begin to grow then overwinter, producing mature seed in the spring that can be harvested without disrupting soybean planting or yields. As an energy crop adopted throughout the U.S. Midwest, pennycress varieties could annually produce 1.3 billion gallons of liquid transportation fuels and 15 million tons of high-protein seed meal, once modest breeding improvements are made (facilitated by resources and germplasm from this project). Many other products could also be produced from this oilseed feedstock. Longer-term agronomic and genetic improvements have the potential to more than double this impact.

While pennycress holds much agronomic promise, economically-viable varieties remain to be developed. Current varieties are hampered by inconsistent germination and stand establishment,

un-optimized maturity for a given growth zone, suboptimal oils quality for biodiesel and jet fuel production, high seed glucosinolate content, and significant harvest loss due to pod shatter. This project is employing modern forward and reverse genetic strategies to rapidly generate and identify lines of pennycress that harbor mutations/natural gene variants conferring superior agronomic traits. These lines are being incorporated into breeding programs located in the Midwest. We envision pennycress adoption will occur throughout the U.S. and the world.

Some project highlights:
A) We have generated large EMS mutant populations (over 20,000 lines) and performed medium and high-throughput screens in identifying nearly 100 mutant lines exhibiting agronomically beneficial phenotypes including reduced pod shatter, early flowering/senescing, and larger flowers/pods/seeds. In one screen, seeds from over 13,000 individual M_3-generation lines have been subjected to NIR spectroscopic analysis. Reanalysis of M_4-generation family members confirmed possible oil content alterations. For example, M_4-generation seeds from one M_3 plant segregated for higher oil content (37.5% vs. 33.5% co grown wild type), while seeds from others segregated for fatty acid profiles more suitable than wild type for various biofuel and industrial applications. We are performing targeted DNA sequencing of these lines as well as next-generation sequencing to identify causative mutations in candidate genes. The most promising mutants are being phenotypically and genotypically characterized in detail as well as crossed with breeding lines and field tested.

B) We have used the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) genome editing approach to generate and identify pennycress plants homozygous for heritable frameshift mutations in agronomically relevant genes. These pennycress mutants are being phenotypically analyzed as well as crossed with breeding lines. Many other pennycress genes are being targeted for knockout using our streamlined genome-editing pipeline so as to rapidly improve pennycress traits including reduced glucosinolate, reduced seed coat fiber, improved oil quality and quantity, and reduced pod shatter.

C) We identified a natural single base-pair deletion in the *DELAY OF GERMINATION1* (*DOG1*) gene of pennycress variety Spring32. *DOG1* mutations confer reduced seed dormancy in canola and Arabidopsis without causing adverse phenotypes. We are assessing how widespread loss-of-function *DOG1* mutations are in pennycress varieties and are introgressing this natural gene variant into breeding lines.

D) Both our in-field and in-lab experiments have shown that the Elizabeth variety has superior seed germination, stand establishment, and yield compared to most other pennycress varieties we have tested in Central Illinois. Elizabeth was isolated by Terry Isbell at the USDA (Peoria, IL) as a natural variant within the Beecher strain. We have generated a segregating population of Elizabeth x Beecher crossed plants and will be scoring in Year 3 the reduced seed dormancy phenotype; bulk segregant analysis and next generation sequencing will be performed in order to identify the genetic basis for these agronomically superior traits.

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