Genetic Improvement of Seed Yield and Oil Content in Field Pennycress

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Project Goals:

This project is aimed at understanding the genetic control of seed size and oil content in field pennycress (*Thlaspi arvense* L.; pennycress). This knowledge will be applied to improve pennycress seed and oil yields for its use as a new winter annual cash cover crop for the U.S. Midwest. Winter annual cover crops provide a continuous living cover on otherwise fallow agricultural soil. Genetic improvement of seed yield and oil content will aide in widespread adoption of pennycress as a profitable cover crop that can serve as a source of renewable feedstock for the biodiesel and biofuels industries as we transition to cleaner and more sustainable sources of energy.

Abstract:

Pennycress possesses extreme winter hardiness (up to -30ºC), high natural seed yields (1100 – 2250 Kg/hectare), highly oil-rich seeds (27%-39% on a dry weight basis), and protein for food, feed and other end uses (Sedbrook et al. 2014). Pennycress is planted in the fall and grows into a rosette that is dormant during the winter, flowers and sets seed in the spring, and is harvested in June. Fall planted pennycress acts as a continuous living cover on the otherwise fallow soil. This reduces pollution of our water sources through nutrient run-off and soil erosion from barren farmlands. In the spring, pennycress provides an early food source for pollinators, as well as helps in suppressing weeds. Cropping systems that can leverage optimal growing conditions for both the major summer annual cash crops (e.g. corn and soybeans) and winter annual pennycress can help maximize farmer profits and environmental benefits in the future.

The widespread adoption of pennycress will depend upon its economic viability as a second cash cover crop where the benefits outweigh the cost of inputs and farmer effort. Pennycress has spent most of its evolutionary history as a weed and has only recently been the subject of domestication and breeding efforts. The first generation of domesticated pennycress with improvements in reduced pod shatter, low erucic acid and glucosinolates concentration, and earlier flowering have already been achieved (Chopra et al., 2019). However, increased seed size and higher oil content are desirable for maximum yield and productivity and to make it amenable for mechanical processing. Therefore, our current research aims to i) identify loci that control seed size and oil content in a diverse collection containing wild germplasm via association mapping, ii) identify loci that control seed size and oil content in two biparental recombinant inbred line (RIL) populations, iii) characterize genes controlling seed size and oil content in pennycress EMS mutagenesis populations, and iv) generate pennycress CRISPR-Cas9 knockouts in genes known to regulate seed size and oil content in *Arabidopsis*.

The diversity panel has been grown in four locations over three years and genotyped with genotyping-by-sequencing to produce a rich dataset for genome wide association study. Two $F_{6\text{-}7}$ RIL populations have been developed from parents divergent in seed size and oil content and planted in field conditions for the first time during fall 2020. These populations will be genotyped and phenotyped in 2021 for QTL mapping. We have generated and phenotyped over 15,000 EMS pennycress lines generated in MN106 genetic background and identified large seeded as well as high oil mutants which are currently being tested in field conditions. In addition, several single and stacked CRISPR-Cas9 mutants for both seed size and oil content have been generated in Spring32 genetic background. The results from these projects will help advance the knowledge of the genetic basis that underlies seed size and seed oil content in pennycress. Research relating to the development of large seeded and high oil pennycress can ensure the use of pennycress as a renewable feedstock for the biofuels industry, and in addition serve as a valuable resource for more translational research into other major oilseed crops that provide a significant portion of edible oil around the world.

References

1. Chopra, R., Johnson E. B., Emenecker, R., Cahoon, E.B., Lyons, J., Kliebenstein, D.J., …. Marks, M.D. Identification and stacking of crucial traits required for domestication of pennycress. *Nature Food.* 1(1): 84-91.
2. Sedbrook, J., Phippen, W.B., Marks, D.M. (2014). New approaches to facilitate rapid domestication of a wild plant to an oilseed crop: Example pennycress. *Plant Sci*. 227:123-132.

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