Title: Advancing field pennycress as a new oilseed biofuels feedstock that does not require new land commitments – Improving pennycress stand establishment

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Project Goals:

Pennycress (*Thlaspi arvense* L.) is being domesticated to serve as new cash/cover crop for the Midwestern United States. Currently land is left fallow approximately 8 months of the year between the fall harvest and spring sowing of traditional summer crops. During the fallow period the land is susceptible to soil erosion, nutrient leaching, and runoff pollution. Winter pennycress can be grown during the fallow period as a cover crop to provide ecosystem services to protect the land. Importantly, pennycress produces a harvestable oil seed that can be used for the production of biofuels and other products. As a weed, pennycress seeds exhibits a high degree of dormancy that results in a long-lived seed bank. In addition, the latent dormancy reduces stand establishment. The main goal of the work presented in this poster is to identify pennycress mutants that exhibit reduced dormancy and better stand establishment.

Abstract text.

As a member of the Brassicaceae, pennycress is closely related to the model plant Arabidopsis thaliana. Pennycress shares important attributes with Arabidopsis including self-compatibility and a small genome exhibiting a reduced degree of gene duplication¹. Prior work has shown that there is a mostly one to one correspondence between genes in pennycress and Arabidopsis. Further, it has been shown that the same spectrum of mutants found in Arabidopsis can be created in pennycress². In this report mutations that reduce dormancy are sought. Pennycress seeds need both light and a relatively high-water potential to germinate. Currently, the most common methods for sowing pennycress establishment dependent on fall rains that can be rare in the Midwestern United States. The seeds of most crop plants can be drilled deep enough to make contact with soil containing a sufficiently high-water potential to promote uniform germination. Therefore, pennycress mutants with reduced requirements for light and high water potential will potentially allow pennycress seeds to be more deeply drilled in order to improve stand establishment. In addition, such traits should reduce the formation of long-lived seed banks.

Dormancy is a well-studied trait in Arabidopsis³. Numerous Arabidopsis mutants have been identified that reduce dormancy. The goal of this work was to identify similar mutants in pennycress. In theory these mutants can be identified via classical mutagenesis or produced through gene editing. The work described in this report combines classical mutagenesis with modern genomics to enable rapid reverse genetic approaches to identify recessive mutations that promote pennycress domestication. This approach relies on conducting whole genome sequencing of a population of mutagenized individuals. The goal is to produce a database called a mutant gene index (MGI) that contains a list of multiple mutations in every gene in pennycress. The MGI can be used to identify individuals in the mutagenized population that contains mutations that confer useful traits. Thus, to identify pennycress mutants with reduced seed dormancy, prior work on dormancy in Arabidopsis has been used to identify gene targets. Data presented in this report is on the characterization of two pennycress mutants selected as likely targets to show reduce seed dormancy.

Three key traits are needed to improve field establishment. These include reduced dormancy to speed germination under optimal conditions, the ability to germinate in the dark to allow seeds to be more deeply drilled, and the ability to germinate at a lower water potential. Given these needs, three types of in lab experiments were designed to characterize two chosen candidate mutants named mutant A and mutant B. In summary, it was found that both mutants germinated faster than the parental controls under optimal conditions. In addition, mutant B germinated much better than either mutant A or the control in the dark. Finally, under reduced water potential mutant A germinated better than either mutant B or the control. Limited field testing has shown that mutant B, the best dark germinator, does established better than either mutant A or the control in the future additional field tests will be conducted and these studies will include the double mutant.

References

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