Phenotypic and Genotypic Assessment of Experimental Pennycress Lines

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Introduction

Field pennycress, *Thlapsi arvense,* is a newly domesticated cover crop and a member of the Brassicaceae family. It is commonly known as a weed that flowers across the various regions of North America.

Pennycress is currently being studied in hopes to develop a profitable cover crop that can be used as an oilseed cash crop or potential biofuel provider. Pennycress additionally can be used to prevent erosion and can absorb excess nitrogen in the soil, preventing runoff.

Figure 1. Pennycress
Field-pennycress in the flowering stage of its lifecycle

Research Objectives

This project serves to phenotypically and genotypically characterize different experimental pennycress breeding lines for seed purification and advancement. The experimental lines are seeking to reduce pod shatter (*ind1*), low erucic acid (*fae1*), low polyunsaturated fatty acid (*rod1*), and low glucosinolate (*aop2*). For self-pollinated species, pure lines will breed true (genetic identity maintained from one generation to the next).

Methods

To determine segregation within the breeding lines, competitive allele specific PCR (KASP) was used to genotype plots for mutant vs. wild-type alleles, and select plants homozygous for the ind1, fae1, rod1 and aop2 mutations.

DNA was extracted from pennycress tissue, quantified, and normalized to 20ng/μL. PCR was run to bind the markers to the samples, and then KASP was run to determine segregation.

Experimental Lines

Experimental Line	Genotype	# Plots	# Plants Sampled	# Pure Seed Sources
Quad Mutant	MN106ind1fae1rod1aop2	14	56	1
Triple Mutant	MN106ind1fae1rod1	46	184	10
Wild Type	MN17077-2_1	1	4	WT (harvested)

Results

Main Points

- Only one of the 14 MN106ind1fae1rod1aop2 (quad stack) seed sources which was traced back to 14 individual plants was homozygote for KASP markers alleles of *fae1,ind1,rod1,aop2.* The individual plants genotyped from this seed source was harvested as pure seed for future genetic studies. The other plants were bulk harvested as breeder seed source for seed increase.
- 10 of the 43 MN106ind1fae1rod1 (triple stack) seed sources, which were tracked back to 43 individual plants were homozygote for KASP markers alleles of *ind,1fae1,rod1*. The individual plants genotyped in those single-plant derived source were harvested and will be used as pure seed for future studies which require the highest genetic purity. The other plants were bulked as pure breeder seed for seed increase.
- Overall, MN106ind1fae1rod1 was taller than MN106ind1fae1rod1aop2.
- There was no difference in the fall and spring germinate rate between the triple mutant stack and the quad mutant stack.

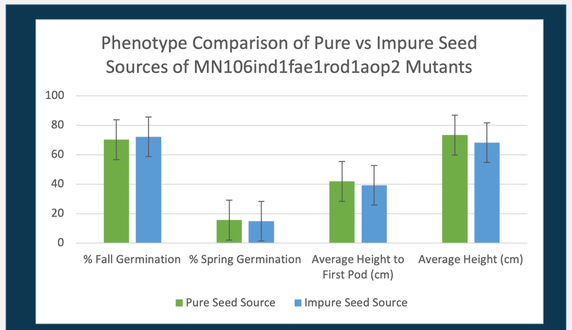


Figure 2.

The quad stack comparison chart showcases key differences between the pure and impure seed sources within the specific breeding line. The sample size is the smallest within the quad mutant stack line (save for the wild type control) which highlights the importance of a large sample size. There is only one pure seed source present within this line.

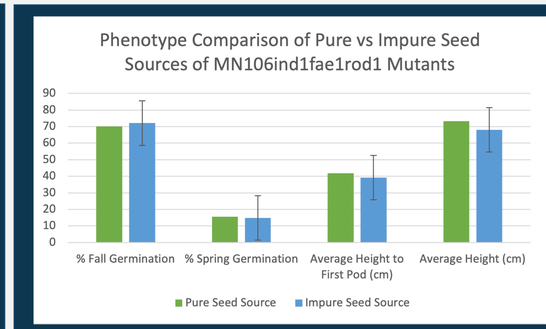
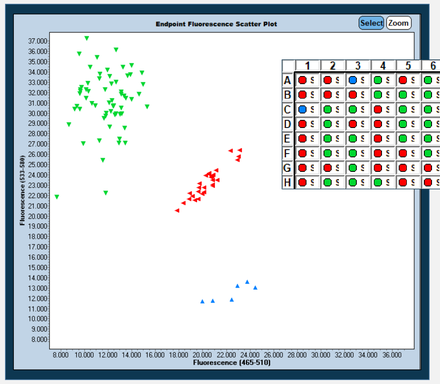


Figure 3.

The chart shows the key differences between the pure and impure seed sources within the triple mutant stack experimental breeding line. The sample size for the triple mutant stack is the largest group with 10 pure seed sources and 36 impure seed sources.



LightCycler II 480 output for genetic marker *ind1-552* for the quad mutant experimental pennycress line.

LightCycler 96 output for genetic marker *fae1-010A* for the quad mutant experimental pennycress line.

Conclusions

The genetic variation in pennycress is known to be very narrow. Reliable KASP markers shall be used to identify seed purity in pennycress. In this work, the pure seed of MN106ind1fae1rod1aop2 (quad stack) , MN106ind1fae1rod1 (triple stack) harvested based on the KASP marker and field observation will be used for domestication and breeding in the upcoming season.

Future Plans

Future plans for the domestication of pennycress by selectively breeding for favorable domestication traits like the ones shown here are to continue these lines of pennycress with their pure seed sources in the next planting season.

Moreover, the purified lines are ultimately being cultivated for the eventual release to the market, not only for the potential environmental and personal benefit of the farmer, but for the improvement of our testing accuracy.

References

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Acknowledgements

We thank Dr. David Marks for the initial seed of those lines and providing the KASP markers information. A special thank you to Dr. James Anderson for designing this internship project. I thank my mentors Dr. Julia Zhang, lab manager Dr. Emily Conley, Jay Fordham, Sarah Korth, and graduate student Nanthicha Krueger for your guidance and help with this project.

IPREFER is supported by Agriculture and Food Research Initiative Competitive Grant No. 2019-69012-29851 from the USDA National Institute of Food and Agriculture. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

USDA United States Department of Agriculture National Institute of Food and Agriculture