Investigating the Inheritance of Four Domestication Traits in Five Elite Pennycress Breeding Lines

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Introduction

Pennycress (*Thlaspi arvense L.*) is a promising midwestern oilseed cover crop for potential markets in biofuel, edible oil, and animal feed due to its winter hardiness, high oil content, and high yield. However, wild pennycress is prone to seed pod shattering, contains high concentrations of erucic acid, and polyunsaturated fatty acid (lowering the oleic oil content), along with high fiber and high seed dormancy. Therefore, the UMN Pennycress Breeding Program is working to introduce four domestication genes into elite wild-type pennycress lines to combat these issues.

Table 1. The four domestication gene mutants addressing unattractive wild pennycress traits

Desired Trait	Mutant Allele	Information
Reduced Shattering	*ind1*	Increases Yield
Reduced Erucic Acid	*fae1*	Increases Suitability for Animal and Human Consumption
Reduced Polyunsaturated Fatty Acid (PUFA)	*rod1*	Increases Stability of Oleic Acid
Thinner, Transpart Seed Coat	*tt2*	Lower Fiber (Increased Animal Consumption) and Decreases Dormancy

Methods

Competitive allele specific PCR (KASP) was used to genotype five populations of BC_1F_1 plants for heterozygous or homozygous alleles for each domestication gene mutant.

- The DNA was extracted, quantified, and normalized to 20ng/µL.
- MN106 mutated lines were used for mutant and wild-type checks.
- The plates were run on a Roche LightCycler 480 (LC480) and a Roche LightCycler 96 (LC96).
- Chi-Square tests were run using Microsoft Excel.

Figure 1. BC_1F_1 populations maturing in the greenhouse

Figure 2. Process of pennycress emasculation on the female parent

Objectives

- Genotype five populations of BC_1F_1 crosses to identify the segregation ratio for the four domestication gene mutant alleles in order to find the desired four gene heterozygous stacked plants for the next backcross cycle
- Learn the process of pennycress genotyping, crossing, greenhouse, and field work in a breeding program

Results

Table 2. KASP marker segregation of four EMS induced mutations of domestication genes in five BC_1F_1 crosses using the elite lines as the recurrent parent. **P ≲ 0.05**

Cross	Elite parent	4 Stack (FAE1fae1ROD1rod1IND1ind1TT2tt2)	Expected	Expected ratio	P value
XPC23001	**MN16207**	**11**	**27**	**1:16**	**0.001*** **
XPC23002	MN17077-2	10	17	1:16	0.080
XPC23003	**X17014-185**	**2**	**7**	**1:16**	**0.051**
XPC23005	X17014-47	10	11	1:16	0.756
XPC23006	X17014-64	12	13	1:16	0.775

- The probability of a plant having all four genes being heterozygous is ½ x ½ x ½ x ½ = 1/16, so the Expected number = 1/16 x total amount of plants in the population.
- A non-significant P-value is desirable and implies that the ratio of plants we found are not far from the expected ratio.
- Population XPC23001 has a statistically significant P-value of 0.001, and population XPC23003 has a P-value of 0.051 which is very close to the threshold value.

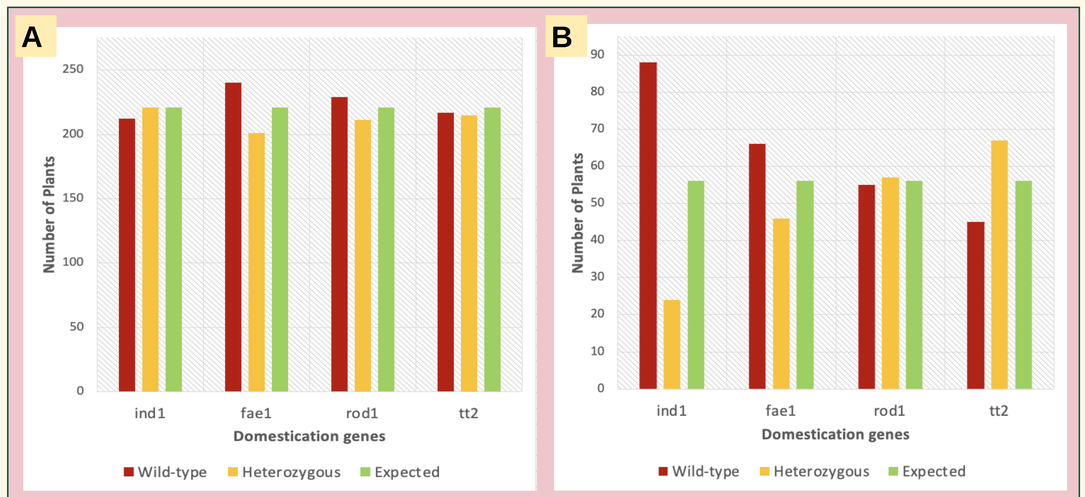

Figure 2. Segregation of individual genes in BC_1F_1 of **(A)** XPC23001 and **(B)** XP23003

- The probability of a plant having one of the genes being heterozygous or homozygous is 1:1 ratio.
- Population XPC23001 did not have statistically significant individual gene ratios.
  - -The *fae1* gene was close with a heterozygous P-value of 0.057 and a wild-type P-value of 0.071.
- Population XPC23003 was found to have distorted individual gene segregation ratios.
  - -The *ind1* gene has a statistically significant deviation with a P-value of 0.00000000147 for the heterozygous and wild-type.
  - -The *tt2* gene is also statistically significant with a P-value of 0.0376 for the heterozygous and wild-type.

Conclusions

- Most P-values are not statistically significant.
  - -Most plants followed the expected segregation ratios according to the Law of Independent Assortment.
- Population XPC23001 was found to have a statistically significant deviation from the expected for the four-stack.
  - -P-value of 0.001
  - -The individual gene segregation ratios are all non-significant which is unexpected.
  - -There is not a clear explanation why this happened.
- Population XPC23003 has distorted individual gene segregation ratios for the *tt2* and *ind1* genes.
  - -This may explain why the four-stack ratio P-value of 0.051 is almost statistically significant.
  - -It is unknown why this population is this dramatically distorted.

Future Directions

- A total of 45 four-stack heterozygous plants were identified and used as male parents to make the second backcross (BC_1F_1 x elite wild-type).
- Their progeny will be genotyped.
- NIR readings of the BC1F1 progeny will be done to gain lipid profile data and validate the KASP marker data.

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