Characterization of an F_2 Population Derived from 2032 x MN106

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ABSTRACT

MN-106 and 2032 are two unique accessions of pennycress that differ in several agriculturally significant traits, including height at several benchmarks, maturation and shattering rates, and seed size. There are also genetic differences based on internal data, which influence flowering time and regulation. MN-106 and 2032 were crossed, and a phenotypic and PACE analysis was performed on the F_2 population.

The F_2 population had skewed distributions across heights at the noted benchmarks. There were also skewed distributions in the number of tillers and number of days between transplant and first flower and first dry pod. There is positive correlation between the height from the first stem to the top of the plant (FT height) and the total height, as well as positive correlation between the time to first flower and the time to first dry pod. Segregation is also evident across both the markers, with double mutants having their first flower and first dry pod earlier than wild-types. Marker1 with mutant allele have a later first dry pod than wild-types, while Marker2 with mutant allele have an earlier first dry pod than wild-types. Future projects intend to build off this research, including identifying quantitative trait loci (QTL), producing a linkage map after a continuation of single-seed descent breeding.

INTRODUCTION

MN-106 and 2032 are two unique accessions of pennycress that differ in phenotypic traits, including shattering rates, seed size, and plant height (Table 1), that have been focuses of germplasm development[1]. Furthermore, several mutations are known and characterized across these accessions, which are designated as Marker1 and Marker2. It is possible these markers have a major role in floral transition.

2032-WT and MN106-WT were crossed to form an F1 population. One hundred and thirty-eight seeds were then randomly selected from this population and grown into an F_2 population[2]. Phenotyping notes were recorded while the F_2 plants were being grown. Five 2032-WT and five MN106-WT phenotypes were also grown as controls during phenotyping.

	MN106-WT	2032-WT
Shattering Rates	Higher	Lower
Seed Size	Smaller	Larger
Plant Height	Taller	Shorter
Other Traits	Reference Genome, Cold-Tolerant	Early Flowering, Increased Germination

Table 1. Phenotypic differences between 2032-WT and MN106-WT.

METHODOLOGY

Phenotypic Analysis

The individual F_2 plants were observed across several months and measured for several phenotypic traits, including first open flower and first dry pod dates, and number of tillers and shoots (Figure 1). Several height measurements were also recorded along the central meristem, including the height from the base to the first stem (BFS), the height from the first stem to the first pod (FP), and the height from the first pod to the top (FT) (Figure 1). The seeds were harvested and weighed for yield measurements.

Genotypic Analysis

The F_2 population was genotyped with a PACE Genotyping Assay. Plant tissue was collected from each of the 138 individual F_2 plants, and DNA was extracted through a column-based DNA extraction method. Two primer mixes were created to genotype Marker1 and Marker2. Each primer mix combined three oligonucleotides: a wild-type-specific forward primer attached to a FAM fluorophore, a mutant-specific forward primer attached to a HEX fluorophore, and a common reverse primer. These primer mixes were used to run a PACE assay in a qPCR. The results were compared to the phenotypic data.

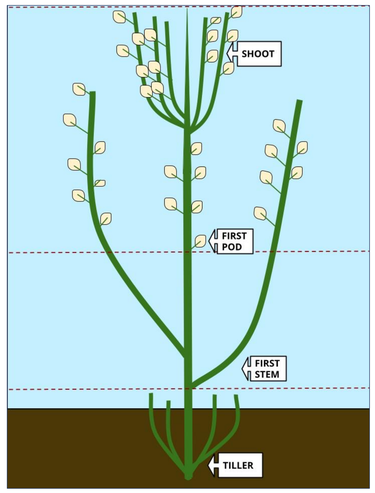
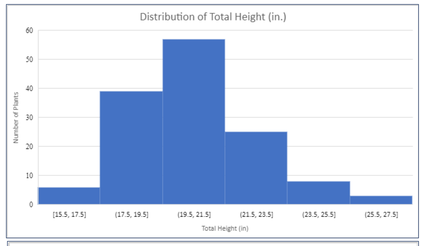
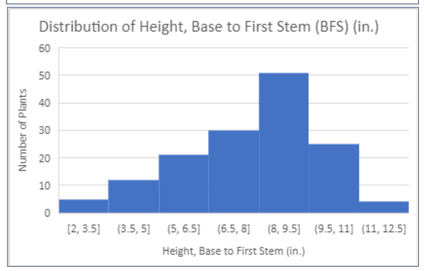
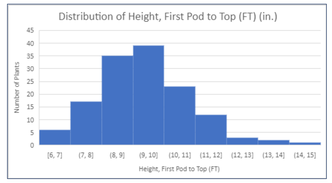


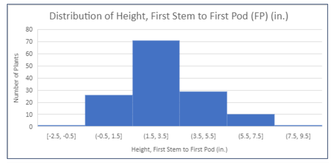
Figure 1. Measuring points for individual F_2 plants. The dotted red lines denote height at first stem, height at first pod, and total height, from bottom to top.

RESULTS









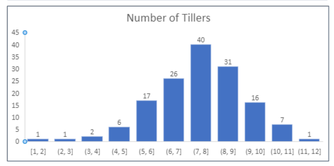


Figure 2. Segregation of height and number of tillers across 2032 x MN106 F_2 population.

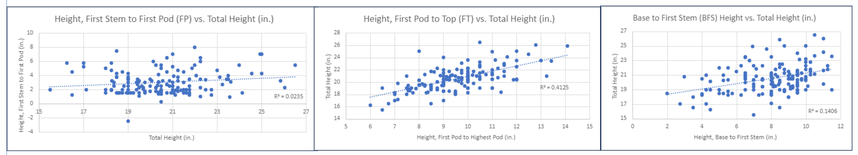


Figure 3. Total height compared to FP, FT, and BFS heights.

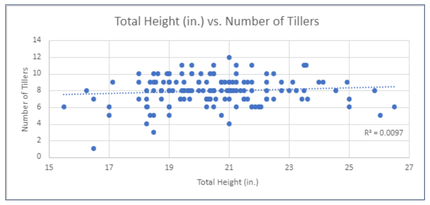
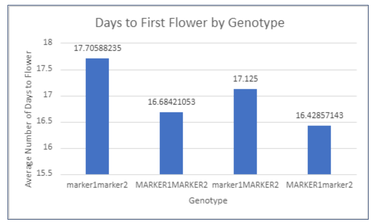


Figure 4. Total height (in.) compared to number of tillers.



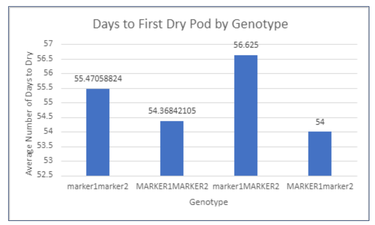
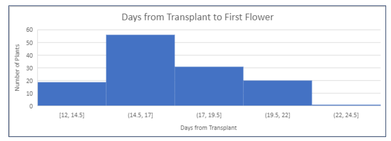


Figure 6. Average number of days to first flower and first dry pod for each genotype.



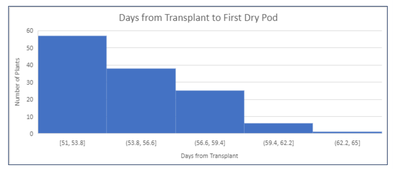


Figure 5. Segregation of days to first flower and days to first dry pod.

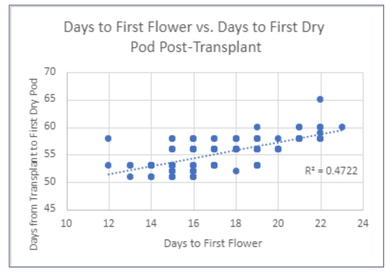


Figure 7. Days to first flower compared to days to first dry pod post-transplant.

CONCLUSIONS

There is phenotypic distribution across the 2032 x MN106 F_2 population. The total height has a skewed distribution towards MN106 (Figure 2), and the number of tillers (offshoots at the base of the stem) has a skewed distribution towards 2032 (Figure 2). The BFS height has a skewed distribution towards 2032 (Figure 2), while the FT and FP height have a skewed distribution towards MN106 (Figure 2). There is positive correlation with the FT height and the total height of the plant ($R^2 = 0.4125$) (Figure 3). However, there is no correlation with the BFS height and the total height of the plant ($R^2 = 0.1406$), and there is no correlation with the FSP height and the total height of the plant ($R^2 = 0.0235$) (Figure 3). There is also no correlation between the total height of the plant and the number of tillers ($R^2 = 0.0097$) (Figure 4).

Segregation is evident across both the Marker1 and Marker2 mutations. The number of days to first flower and the number of days to first dry pod have a skewed distribution towards 2032 (Figure 5). On average, double mutants in both Marker1 and Marker2 have their first flower and dry pod earlier than wild-type genotypes (Figure 6). Genotypes with only singular mutations (MARKER1marker2 or marker1MARKER2) have their first flower later than wild-type genotypes, but more slowly than double mutants. However, Marker2 mutants have their first dry pod later than plants without this mutation on average (Figure 6). Contrarily, Marker1 mutants will have their first dry pod earlier than plants without this mutation on average (Figure 6). There is positive correlation between days to first flower and days to first dry pod post-transplant ($R^2 = 0.4722$) (Figure 7). While some correlations are insignificant, this may be due to a small number of replicates.

FUTURE PLANS

Single-Seed Descent

Approximately one hundred more seeds will be randomly selected from the F_2 population and grown into an F_3 population (Figure 8)[2]. This process will be repeated until an F_6 population, when the population is adequately homozygous, to form a recombinant inbred line[3].

Quantitative Trait Loci (QTL) for Linkage Mapping

After 2032 x MN106 is grown out into an F6 population, further QTL analysis will identify chromosomal regions that influence flowering and drying rates of pennycress and enable identification of recombination frequency for linkage mapping[4].

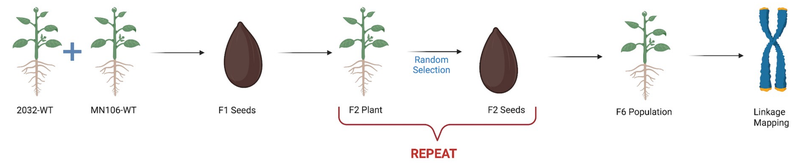


Figure 8. Graphic demonstrating the single-seed descent methodology. Graphic created on BioRender.

LITERATURE CITED

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