IMPROVING BREEDING EFFICIENCY OF LOCALLY-ADAPTED CEREAL RYE VARIETIES PROGRESS REPORT FOR 2023-2024

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INTRODUCTION (objective)

Breeding for regionally adapted cereal rye varieties for Kentucky supports regional industries, like distillers, and sustainable agriculture practices through diversity in crop rotations and cover crop seed production. This work has augmented ongoing breeding efforts of the University of Kentucky to develop rye open-pollinated varieties. Specifically, we have continued breeding efforts and are developing tools to improve efficiency throughout the breeding cycle. This work addresses the KYSGGA goals of reducing winter fallow and developing new small grain varieties. Our objectives were:

Objective 1. Breeding for larger seeds and improved agronomic performance.

Objective 2. Developing new stocks and tools to breed dwarfing varieties.

Objective 3. Testing approaches for isolation.

Objective 4. Developing methodologies and estimating heritability of grain fill.

MATERIALS AND METHODS

Objective 1. Breeding for larger seeds and improved agronomic performance.

Dr. Phillips conducts rye breeding at the University of Kentucky through selection of half-sib families, polycrosses and recurrent selection populations. Selection is conducted at the University of Kentucky research farm, North Farm, in Lexington KY. In this past year, Dr. Phillips selected plants with largest seed size towards increased yield and to meet the needs of local distillers. Dr. Phillips also selected in four populations, including one population made by blending largest seed from 15 diploid populations, and an early, shorter population (KYSC1806C0), in addition to the two proposed populations. These populations were evaluated in yield plots.

Objective 2. Developing new stocks and tools to breed dwarfing varieties.

Kentucky growers have expressed that inconsistent yields and large amounts of straw are key challenges in rye production. Reducing plant height may alleviate issues with lodging and straw residue that can complicate harvest and no-till planting of soybeans following harvest. We sought to establish molecular markers that could be used to screen for rye with reduced height. We began by reviewing the scientific literature for known dwarfing genes, established lab protocols, and then tested if the largest effect dwarfing gene, *Ddw1*, was present in UKY rye germplasm.

Objective 3. Testing approaches for isolation.

The need for isolation distances to maintain rye breeding lines limits the amount of germplasm that can be screened. We tested if coleoptile color could be used as an easy-to-screen indicator of pollen contami-

nation. Rye coleoptiles are purple or green, where purple is dominant to green. We first sought to understand the genetics of coleoptile color, with the goal of moving to field trials. Ultimately, lab work showed found that the genetic inheritance of coleoptile color was too complex for use as an indicator phenotype for pollen contamination, and so this work was not brought to field scale.

Objective 4. Developing methodologies and estimating heritability of grain fill.

Kernel weight reduction negatively influences grain yield. Rye kernel weight is reduced under high temperature and drought conditions, thus rye with shorter grain filling period would increase yield stability in Kentucky. We sought to understand the heritability of grain fill so that this trait could be included in breeding programs. We proposed to measure grain maturity by color (loss of green color) and grain fill period on a large sample of rye accessions, where this research would be conducted by Dr. Szuleta in Schochoh, KY. We unfortunately were not able to complete this objective after Dr. Szuleta relocated, and thus no results are reported.

RESULTS & DISCUSSION

Objective 1. Breeding for larger seeds and improved agronomic performance.

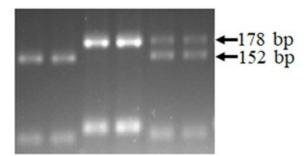
University of Kentucky rye breeding lines have been evaluated in multiple yield trials. A survey of selected populations showed that the Kentucky breeding lines have yields on par with some open pollinated commercial checks and are earlier to head (**Tables 1-2**).

In addition, a few of the top breeding lines were evaluated in a variety trial managed by Dr. Szuleta, where KYSC1701 C1 SP1 was shown to be especially promising, and thus seed will be increased for yield trials next year (**Table 3**).

Objective 2. Developing new stocks and tools to breed dwarfing varieties.

We identified *Ddw1* as our first target for developing molecular tools. We refer to alternate form of a gene as alleles. In triticale, the dominant allele is reported to result in a 30% reduction in plant height. A molecular marker was developed for triticale, where alleles have different sized bands in the assay (Figure 1; from Litvinov et al, 2020).

Figure 1. Demonstration of molecular marker for *Ddw1* in triticale; the dominant allele is larger after cutting with a restriction enzyme (178 bp) than the recessive allele (152 bp) and thus can be visualized by gel electrophoresis.



ddw1 Ddw1 Ddw1 ddw1 (Type 1) (Type2) (T1&T2)

We tested these protocols to determine if this marker would work in rye in addition to triticale, and were successfully able to evaluate the *Ddw1* marker in our lab (**Figure 2**).

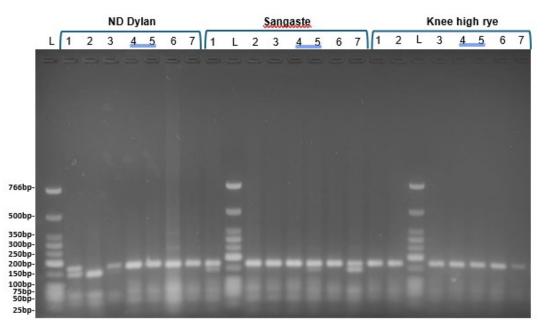


Figure 2. Gel imag from the *Ddw1* marker screened in different rye populations in our lab. Different sizes of DNA fragments indicate that we can evaluate both the dominant and recessive allele.

We then assessed the frequency of the different alleles in rye varieties: we expected that the taller rye varieties would have higher frequencies of the dominant (short) *Ddw1* allele, but did not know how common it would be across accessions. We found most rye varieties had a high frequency of homozy-gous dominant (short) alleles (DD), although some varieties like Danko, Sangaste and Aroostook also harbored heterozygotes and homozygous recessive (short) alleles (dd) (**Table 4**). These results show that the dominant (short) allele of *Ddw1* is already common in some of our Kentucky germplasm, but that the Kentucky breeding material may be further improved by selection to reduce frequency of the allele in populations.

Next Steps

- We are continuing breeding efforts for new open-pollinated rye varieties, including increased selection for reduced height, as well as increasing seed from promising lines for variety release.
- We will continue our work with the dwarfing markers by assessing the degree to which these markers reduce plant height in Kentucky varieties (e.g., if the effect is as large as is reported for triticale), selecting for the dwarfing alleles in Kentucky breeding lines to eliminate sources of tall alleles, and testing additional dwarfing markers like *Ddw3*, *Ddw4*, and *Ddw9*. The long term goal is to have a suite of molecular markers that can be used for screening for height.
- Due to the high levels of Fusarium head blight observed this year, we will screen breeding lines and varieties in the FHB nursery.
- We will continue multi-location rye variety trials
- Three promising OP lines will be increased in 1–2-acre plots for larger on-farm evaluation in 2025-2026.

ACKNOWLEDGEMENTS

We would like to thank the Kentucky Small Grain Growers' Association for their funding support of this project. Additional funding was provided by Hatch funding (KY006145) and University of Kentucky start-up funding to LB, and from the Dendri Fund to DVS. Multiple undergraduate students contributed to this project, including some funded fully or in part by this project, including Ignacio Sanguinetti, Harrison Albright, Jayla Franklin, Connor Griese and Dylan Crawford.

<u>TABLES</u>

Table 1. Results from 2023 selection yield trials in Lexington and Versailles. Yield is in 56 lb bu/ac, test weight in lbs, and heading date is reported as days after April 1

| Genotype† | Lexington | | | Versailles | | |
|--------------------|-----------|------|----|------------|------|----|
| | Yield | TW | HD | Yield | TW | HD |
| CHECK_F1_Serafino | 97.5 | 53.2 | 32 | 88.5 | 51.6 | 32 |
| CHECK_F1_TayoF1 | 102.7 | 53.0 | 32 | 80.1 | 52.2 | 32 |
| CHECK_OPV_AVENTINO | 68.7 | 51.6 | 29 | 64.6 | 51.1 | 29 |
| CHECK_OPV_ND_DYLAN | 67.1 | 51.3 | 30 | 51.3 | 50.4 | 30 |
| KYSC1503_C2 | 54.8 | 51.2 | 15 | 49.4 | 50.9 | 15 |
| KYSC1705_C2 | 67.3 | 52.0 | 18 | 52.4 | 50.0 | 16 |
| KYSC1706_C2 | 55.2 | 49.5 | 25 | 55.8 | 47.7 | 23 |
| KYSC1707_C2 | 59.1 | 51.8 | 16 | 55.2 | 50.7 | 17 |
| KYSC1710_C2 | 58.7 | 51.8 | 22 | 47.6 | 50.2 | 22 |
| KYSC1802_C2 | 42.3 | 47.1 | 24 | 70.2 | 50.8 | 22 |
| KYSC1807_C2 | 55.6 | 51.4 | 16 | 44.9 | 46.2 | 16 |
| KYSC1811_C2 | 85.8 | 51.3 | 24 | 53.2 | 50.0 | 26 |
| KYSC1812_C2 | 62.3 | 51.6 | 18 | 58.9 | 51.2 | 16 |

⁺ F1 signifies a hybrid variety; OPV signifies an open pollinated variety; C2 signifies 2 cycles of selection for larger kernels.

| | Lexington | | | Versailles | | |
|--------------------|-----------|------|----|------------|------|----|
| Genotype† | Yield | TW | HD | Yield | TW | HD |
| CHECK_F1_Serafino | 68.1 | 55.1 | 33 | 86.5 | 58.2 | 31 |
| CHECK_F1_TayoF1 | 49.6 | 51.8 | 33 | 84.9 | 56.2 | 31 |
| CHECK_OPV_AVENTINO | 21.7 | 41.2 | 31 | 43.4 | 56.5 | 29 |
| CHECK_OPV_ND_DYLAN | 28.9 | 50.8 | 32 | 31.3 | 47.2 | 29 |
| KYSC1503_C2 | 40 | 51.1 | 22 | 19.5 | 31.3 | 22 |
| KYSC1705_C2 | 36.6 | 46 | 26 | 29.7 | 42.2 | 24 |
| KYSC1706_C2 | 21.1 | 33.5 | 28 | 28.9 | 41.8 | 26 |
| KYSC1707_C2 | 34.4 | 50.3 | 26 | 36 | 47.5 | 23 |
| KYSC1710_C2 | 36.2 | 51.1 | 28 | 34.6 | 46.7 | 26 |
| KYSC1802_C2 | 33.4 | 47.4 | 29 | 39.6 | 50.4 | 26 |
| KYSC1807_C2 | 42.2 | 47.9 | 24 | 25.8 | 37.6 | 22 |
| KYSC1811_C2 | 22.3 | 33.4 | 31 | 39.4 | 54.7 | 27 |
| KYSC1812_C2 | 33.7 | 48.3 | 27 | 25.1 | 38 | 22 |

Table 2. Results from 2024 selection yield trials in Lexington and Versailles. Yield is in 56 lb bu/ac, test weight in lbs, and heading date is reported as days after April 1.

⁺ F1 signifies a hybrid variety; OPV signifies an open pollinated variety; C2 signifies 2 cycles of selection for larger kernels.

Table 3. Results from 2024 variety trial in Lexington, Ky

| Variety | Yield (bu/acre) |
|-----------------|-----------------|
| KWS Serafino | 69.5 |
| KYSC1707 | 63.9 |
| SH05 | 61.7 |
| SH06 | 59.0 |
| KYSC1705 | 58.8 |
| Aventino | 58.7 |
| KYSC1701 C1 SP1 | 56.3 |
| KYSC1503 C3 | 55.8 |
| KYSC1710 C1 SP1 | 54.8 |
| KYSC1806 C0 | 52.1 |
| KWS Tayo | 51.8 |
| SH03 | 50.9 |
| SH07 | 50.7 |
| Danko C0 | 48.8 |
| Aroostook C0 | 46.3 |
| ND Dylan | 44.0 |
| Balbo (2114C0) | 44.0 |
| AC Hazlet | 40.2 |

Table 4. Results of *Ddw1* allele frequency in six rye populations. The number of scoreable markers are shown, as well as the percent that were scoreable of the total run. Then the percent of individuals with the DD, Dd, or dd genotypes are shown.

| Variety | Scoreable markers (<i>n</i>) | Scoreable (%) | DD (%) | Dd (%) | dd (%) |
|---------------|-----------------------------------|------------------|--------|--------|--------|
| Knee high rye | 74 | 100 | 100.0 | 0.0 | 0.0 |
| Sangaste | 76 | 86.8 | 86.8 | 10.5 | 2.6 |
| Aroostook | 76 | 96.1 | 96.1 | 3.9 | 0.0 |
| Danko | 93 | 83.9 | 83.9 | 14.0 | 2.2 |
| ND Dylan | 96 | 99 | 99.0 | 1.0 | 0.0 |
| KYSC1710C1Sp1 | 64 | 96.9 | 96.9 | 3.1 | 0.0 |