Washington Grain Commission
Wheat and Barley Research Annual Progress Reports and Final Reports

Project: 3389

Progress Report Year: __*2*__ *of* __*3*__

Title: Building a Mutation Breeding Toolbox for Washington Wheat

Project PIs: Camille Steber, Brian Beecher, Daniel Z. Skinner, Deven See, and Michael Pumphrey

Cooperators: Michael Neff, Scot Hulbert, Kimberly Garland Campbell, and Arron Carter

Executive summary:

Objective 1. Use forward genetics to screen mutagenized populations derived from the TILLING populations of Alpowa, Louise, Jagger, and Eltan for mutations involved in stripe rust resistance, heat tolerance, vernalization, and photoperiod sensitivity.

The Louise population was advanced in the field and in the greenhouse to provide an M3 population for use in screening for altered stripe rust resistance and heat tolerance. Evaluation of the Jagger population revealed that it was not suitable for the screen for altered vernalization and photoperiod sensitivity. A new Norstar population will be generated this screen.

Objective 2. Generate a club wheat TILLING population in JD and generate a new forward genetics mutant population in soft white spring Alpowa+Yr5 for use to identifying and cloning stripe rust resistance genes..

Both JD and Alpowa+Yr5 seeds (M1 generation) were mutagenized and advanced. Screening of the Alpowa+Yr5 population recovered 20 mutants showing loss of the stripe rust resistance that will be used by M.Pumphrey's program for identifying the Yr5 gene. The JD population was advanced for generation of a new club wheat TILLING population. Additional JD lines will be generated in year 3.

Objective 3. Perform TILLING in the soft white winter Eltan population for mutations in cold tolerance genes *ICE1* and *ICE2*. Assist Washington researchers in TILLING for mutations in specific genes involved in coleoptile emergence, as well as cold, heat, drought, and preharvest sprouting tolerance.

TILLING was performed on the *ICE-7A1* and *ICE-7B1* genes to identify mutations that alter (improve or decrease) cold tolerance. These experiments were conducted in spring tetraploid wheat because initial screening suggested that the original Eltan population did not have a high enough density of mutations in all lines. These experiments are useful as an initial proof of concept, but ideally they should be performed in winter wheat. Successful TILLING requires a very high density of mutations (1 mutation in every 24,000 bp). New TILLING lines were generated in soft white winter Eltan using higher levels of mutagen. Several WSU researchers requested a new TILLING population in the highly freezing tolerant hard red winter line, Norstar. A seed increase of a doubled haploid-Norstar was increased in year 2, and this population will be generated in year 3.

Impact: This project provides resources for forward and reverse genetics that can allow Washington wheat researchers to transfer knowledge about gene function into superior wheat cultivars. Federal grant funding has become highly competitive, and requires extensive preliminary results. The existence of this resource has allowed WSU researchers to propose the use of existing mutant populations in grant proposals with aims involving gene cloning and wheat improvement.

WGC project number: 3389
WGC project title: **Building a Mutation Breeding Toolbox for Washington Wheat**
Project PI(s): Camille Steber, Brian Beecher, Daniel Z. Skinner, Deven See, Michael Pumphrey.
Project initiation date: 1-Jul-13
Project year: 2 of 3

Objective	Deliverable	Progress	Timeline	Communication
1. Use forward genetics to screen mutagenized populations derived from the TILLING populations of Alpowa, Louise, Jagger, and Eltan for mutations involved in stripe rust resistance, heat tolerance, vernalization, and photoperiod sensitivity.	Mutant resources that can be used to understand stripe rust resistance, heat tolerance, vernalization, and photperiod sensitivity.	The Louise population was advanced to M3 in the field and greenhouse to create M3 mutant families that can be screened for altered stripe rust resistance and heat tolerance (Year 1). Initial screening showed that Jagger did not have a strong enough vernalization requirement for use in forward mutant screens for altered vernalization and photoperiod sensitivity. Eltan is better, but it was decided that a larger mutant population with a higher density of mutations would be needed. Finally, three labs requested a Norstar mutant population because of its excellent freezing tolerance. The Norstar TILLING population was added to the objectives for year 3.	Year 1. The Louise population was advanced, and initial analysis of Jagger population performed. Year 2. Mutagenesis of Eltan for use in making 1,000 additional Eltan TILLING lines. Year 3. Mutagenesis of Norstar and construction of a TILLING population.	Results will be reported at the Wheat Research Review, at field day presentations, and Wheat Life Articles.
2. Generate a club wheat TILLING population in JD. Generate a new forward genetics mutant population in soft white spring Alpowa+Yr5 for use to identifying and cloning stripe rust resistance genes.	New TILLING population in JD. A new mutant population in Alpowa+Yr5.	JD was mutagenized and advanced for use in making a TILLING population. Alpowa+Yr5 was mutagenized for use in forward genetics, and 20 mutants showing loss of stripe rust resistance were recovered. These mutants will be used in mapping and cloning of the Yr5 stripe rust resistance gene.	Year 1 and 2. The JD TILLING population construction. Year 2. Creation of 1,000 new Eltan TILLING lines. Year 3. Creation of a Norstar TILLING population.	Results will be reported at the Wheat Research Review, at field day presentations, and Wheat Life Articles.
3. Perform TILLING in the soft white winter Eltan population for mutations in cold tolerance genes *ICE1* and *ICE2* . Assist Washington researchers in TILLING for mutations in specific genes involved in coleoptile emergence, as well as cold, heat, drought, and preharvest sprouting tolerance	Mutations in the *ICE1* and *ICE2* genes of wheat for use in understanding and improving cold tolerance in wheat. Mutations for altered photoperiod sensitivity in the Ppd-D1 gene of wheat.	Gene-specific primers to *ICE1* , *ICE2*, and to two as yet unpublished *ICE* gene homologs. TILLING recovered 33 mutations in one *ICE* gene and 23 mutations in another that are expected to result in amino acid changes. If ICE functions in wheat freezing tolerance, then these mutations will most likely result in reduced freezing tolerance. However, some of these alleles may result in a gain-of-function increase in freezing tolerance. Preliminary screening of the original Eltan population indicated that some of the lines did not contain a high enough density of mutations for TILLING. Thus, additional Eltan lines were generated in Year 2 using a higher concentration of mutagen.	Year1. Develop ICE-gene-specific primers. Year 2 and 3. Perform TILLING of ICE genes in wheat.	Results will be reported at the Wheat Research Review, at field day presentations, and Wheat Life Articles.