Washington Grain Commission
Wheat and Barley Research Final Report

Project: 3389

Progress Report Year: __*3*__ *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. Scot Hulbert and Michael Pumphrey screened the Louise mutant population for loss of high temperature adult plant resistance to stripe rust in the field in 2015. Four lines segregating for loss of stripe rust resistance were identified, and backcrossed to wild-type Louise in the greenhouse. In order to clone a gene, one must prove that mutations in that gene result in loss of function (in this case loss of stripe rust resistance). These lines will be used in the process of cloning the Louise stripe rust resistance gene. Evaluation of the Jagger population revealed that it was not suitable for the originally planned screen for altered vernalization and photoperiod sensitivity. We are in the process of developing a new Norstar mutant population that Deven See will use for this screen. Norstar is a highly cold tolerant winter wheat with a strong vernalization requirement. This new TILLING population will be useful to the Skinner, Carter, and See research programs aimed at understanding cold tolerance and vernalization. Spring wheat mutant lines generated by this project were provided to Dr. Ian Burke's lab for use in forward genetic screens for altered herbicide resistance.

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 in year 1. 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. Additional JD TILLING lines were generated in year 3. This is the first club wheat mutant population. Club wheat is genetically quite different from common wheat, and contains unique genes for stripe rust resistance. This mutant population will be useful in efforts to clone genes, including stripe rust resistance genes, from club wheat.

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. The Norstar TILLING population is under construction, and should be completed in year 3.

Impact: This project created 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 enabled WSU researchers to propose the use of existing mutant populations in grant proposals with aims involving gene cloning and wheat improvement. In the long term, this should improve the ability of WSU wheat researchers to obtain federal funding. Resources have been used by the wheat researchers: Scot Hulbert, Arron Carter, Ian Burke, Camille Steber, Deven See, Dan Skinner, and Brian Beecher.

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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.
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Project year: 3 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 for screening (Year 1). Jagger did not have a strong enough vernalization requirement for use in forward mutant screens for altered vernalization and photoperiod sensitivity. Eltan was chosen as an improvement, and a larger mutant population was developed (Year 2 and 3). Finally, three labs requested a Norstar mutant population because of its excellent freezing tolerance. The new objective to develop a Norstar TILLING population was undertaken in year 3.	Year 1. The Louise population was advanced, and initial analysis of Jagger population performed. Year 2. Mutagenesis of Eltan to create 1,000 additional Eltan TILLING lines. Year 3. Eltan population was advanced and genomic DNA prepared. Norstar was mutagenized. Louise mutants that lost stripe rust resistance were identified in a field screen. These Louise mutants will be used in an effort to clone an HTAP stripe rust resistance gene.	Results were reported at the Wheat Research Review and at field day presentations.
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 Alpowa+Yr5 population and screening. Year 2 and 3. Creation of JD TILLING lines.	Results will be reported at the Wheat Research Review and at field day presentations.
3. Perform TILLING 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 were reported at the Wheat Research Review.