

Wheat Research Progress Report

Project #: 3019-3851

Title: Development of a Wheat TILLING Population

Researchers: Camille M. Steber, Deven See, and Brian Beecher

Collaborators: Kimberly Garland Campbell, Kimberlee K. Kidwell, Daniel Z. Skinner, Scot Hulbert, Michael Neff, and Ian Burke

Progress Report Year: 2008

The goal of this project is to develop TILLING populations in locally adapted cultivars for use by Washington State University researchers.

TILLING is an exciting new genetic tool for wheat. Mutant screens and TILLING are mirror images of each other (Table 1). Mutant screens, like those previously used to identify Scarlet-Rz1, are used when you don't know which gene can give you the desired trait. Plants are mutagenized, mutants identified based on phenotype, then the mutant gene must be mapped, and eventually cloned. TILLING is a "reverse genetics" tool for identifying mutations in genes previously shown to control important agronomic traits in other plants such as rice, maize, and Arabidopsis. In TILLING, you begin with a cloned gene sequence, screen for mutations in that gene, then determine if they cause the expected trait or phenotype. This approach allows us to apply information from molecular genetic studies in other plant species to wheat. This tool will be made available to all WSU wheat researchers.

Table 1. Comparison of steps in TILLING and Mutant Screens

TILLING	1. Mutagenize plants	2. Isolate genomic DNA from every plant	3. Screen for plants with mutations in the cloned gene.	4. Determine if mutations cause the expected trait
Mutant screen	1. Mutagenize plants	2. Screen every plant for desired trait (phenotype)	3. Isolate genomic DNA and map mutant genes	4. Clone gene

Accomplishments:

This project is in its early stages. To summarize our accomplishments to date:

1. Isolation of homozygous 'Louise', 'Alpowa', and 'Jagger' for mutagenesis through three generations of single plant descent.
2. Mutagenesis and advancement of 'Louise', 'Alpowa', and 'Jagger' lines in preparation for performing 2,000 genomic DNA preparations for TILLING.

Results:

TILLING has been shown to work in wheat by identifying mutations in the wheat Waxy genes, Wx-A1 and Wx-B1 (Slade et al 2005). This study by Slade et al 2005 constructed a TILLING population in hard red spring wheat cultivar 'Express' (Westbred). The current project will construct a TILLING population for soft white wheat cultivars 'Alpowa' and 'Louise'. The hard red winter, 'Jagger' has been added as a representative of winter wheat. These varieties were chosen due to 1) their economic importance in Washington, 2) the fact that Alpowa and Louise differ in drought tolerance, quality, and yield, and 3) the fact that the Wheat CAPS project has developed all of these lines as mapping parents.

1. Because TILLING identifies single base changes or mutations in a given gene, it is important to mutagenize grains that are homozygous and genetically identical to each other. In order to decrease genetic variation in the lines to be mutagenized, we have performed three generations of single plant descent for 'Alpowa' and 'Louise'.
2. The resulting wheat grain has been mutagenized with EMS at 0.4%, 0.6%, 0.8% and 1.0% to determine the concentration which gives approximately 30% killing. These plants are being advanced to the M2 generation in the greenhouse.
3. M2 grain will be planted in order to isolate leaf tissue from genomic DNA preparation. Tissue will be harvested individually from each plant for genomic DNA extraction. This DNA will be used to detect mutations by the TILLING method. Promising candidates will be sequenced. As part of this research we will test two methods for high throughput detection of mutation /single nucleotide changes, the LiCor electrophoresis and fluorescent detection system and ABI capillary separation.
4. We will begin by screening for mutations in ERA1, a drought tolerance gene in Arabidopsis and canola.