Washington Grain Commission
Wheat and Barley Research Annual Progress Report

Project #: 5389

Progress Report Year: *_2__ of _3__ (maximum of 3 year funding cycle)*

Title: Developing Washington Wheat with Stable Falling Numbers (FN) through Resistance to Preharvest Sprouting and LMA.

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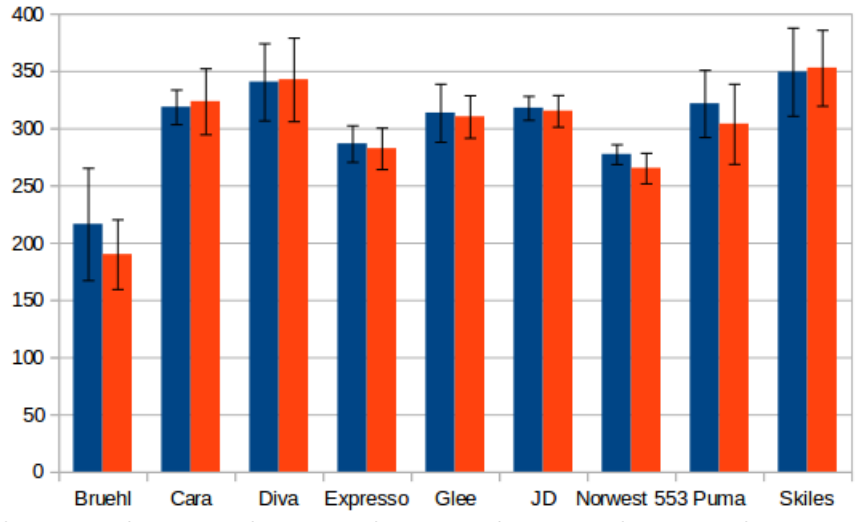
Executive summary: The goal of this project is to breed for stable Falling Numbers (FN) in Washington wheat through selection for genetic resistance to preharvest sprouting and late maturity alpha-amylase (LMA). The previous project identified existing cultivars with sprouting and LMA problems through evaluation of the WSU cereal variety trials. In order to help growers and breeders to make informed decisions, data was made available through a website showing FN relative to yield data from 2013, 2014, 2015, and 2016 (http://steberlab.org/project7599.php). The current project will enable breeders to select for resistance to LMA and PHS by developing and using phenotypic screens and molecular markers.

Objective 1. Screen spring and winter wheat cultivars, breeding, and mapping lines for preharvest sprouting tolerance using the spike wetting test and the Falling Number test.
Please note that many of the Objective 1 accomplishments were funded in part by 5389 and in part by the nonrenewable supplemental project 5333.

A. Characterization of FN. Because FN and spike-wetting tests were not well correlated in 2015, a decision was made to emphasize FN. Locations showing low FN were identified for the winter and spring wheat variety trial locations in 2015, and FN was determined for complete trials at these locations. We are in the process of analyzing FN of the 2016 WSU variety trials (over 2000 data points reported on http://steberlab.org/project7599.php), wheat breeding lines, and mapping lines for association mapping.
B. The effect of fungicide treatment on FN. Preliminary data from the 2016 field season suggests that fungicide treatment with Qulit does not cause a significant decrease in FN (Figure 1). Grain from plants grown in Pullman WA with and without Quilt fungicide treat (14 oz, applied twice and early and late jointing) were obtained from Dr. X. Chen. Based on ANOVA analysis, fungicide treatment did not have a statistically significant effect on FN in five winter wheat cultivars and in four spring wheat cultivars. The chart below shows average FN (n=4, error bars show standard deviation) for the untreated control (blue bar) and the fungicide treated (red bar) samples. This experiment was performed using varieties with stripe rust resistance. Previous publications suggested that fungicide treatment may increase the likelihood of lower FN due to LMA in stripe-rust-susceptible cultivars because dead plants don't respond to temperature shock.

Figure 1. FN with (red) and without fungicide (blue) treatment.



C. The effect of storage on FN. The FN of winter wheat breeding lines was measured in July and again in December following storage at room temperature. On average over 19 varieties, there was an increase of 38 seconds in FN over 5 months. All but one cultivar showed an increase in FN. That one line showed a 2 second decrease.

Objective 2. Improve screening for LMA susceptibility to prevent release of susceptible spring and winter varieties.

A. Cut-spike LMA testing. Greenhouse LMA testing is slow and requires considerable growth chamber space for cold treatment of whole wheat plants. LMA experiments were performed with a more efficient "cut spike" protocol for field-grown wheat. Screening detected LMA-susceptible winter breeding lines. Field cut spike experiments detected known LMA susceptible lines, but appeared to over-predict the number of LMA susceptible lines. Field cut spike assays will enable us to perform the large scale screening needed for association mapping, but will need to be confirmed by other methods.

B. 96-well alpha-amylase tests. In the greenhouse LMA test, alpha-amylase activity is detected using the Phadebas assay. In 2016, this assay was adapted into a 96 well format which greatly improves the speed of LMA testing. This also allowed us to perform faster half-seed assays to determine if low FN in the field trials were due to sprouting or to LMA.

C. Half seed assays. Half-seed alpha-amylase assays were used to determine if low FN in the field is due to LMA or sprouting. When grain is sprouted, the alpha-amylase levels are much higher at the germ/embryo end of the grain than at the brush end. Using the 96-well method, we were able to characterize the cause of low FN in the Pullman and in the Anatone 2016 Cereal Variety Trials. Surprisingly, it appears that most of the low FN was due to LMA rather than to sprouting at these two locations. For example, only 4 of 16 low-FN varieties from Anatone appeared to be sprouted based on half seed assays.

Objective 3. Identify molecular markers linked to sprouting and LMA resistance and susceptibility genes by association mapping.

A. Genome-Wide Association Mapping for Preharvest Sprouting. Preliminary association mapping identified molecular markers linked to preharvest sprouting susceptibility/tolerance in winter wheat. Mapping was performed using both spike wetting tests and FN on the same mapping population. Spike wetting tests did not detect many of the sprouting tolerance loci

detected by FN. This suggests that if we want to breed for stable FN we will need to continue running FN assays instead of relying on the less labor-intensive spike-wetting tests. The table below shows molecular markers significantly associated with resistance to preharvest sprouting based on the spike-wetting test, and with higher FN. A single locus on chromosome 6A accounting for 12% of the variation was associated BOTH with higher FN and with sprouting tolerance based on the spike wetting test.

Trait	Marker	Chrom	Position cM	Nominal p-value	MAF	Allelic effect	Environment
Spike Wetting Test	64868	1B	431.21	3.20E-07	0.15	-0.31	CF 2014
	41233	2A	158.02	1.53E-06	0.40	-0.11	Pul 2015
	7652	2D	140.14	2.10E-09	0.37	-0.42	CF 2014
	75777	3A	355.57	2.45E-08	0.07	-0.40	CF 2014
	48693	3B	250.27	1.73E-07	0.47	-0.21	CF 2014
	22512	6A	190.32	8.39E-06	0.34	0.12	Pul 2015
Falling Number	81042	1B	240.55	1.10E-08	0.17	6.93	Pul 2015
	65377	2B	308.30	7.24	0.01	-46.82	Pul 2013
	47123	5D	514.36	6.28	0.37	11.28	Pul 2013
	46863	6A	190.39	6.71	0.42	-12.24	Pul 2013
	75568	7A	372.48	12.62	0.41	-10.26	CF 2014
	75568	7A	372.48	12.31	0.41	-7.64	Pul 2015

B. Genome-Wide Association Mapping for LMA. Preliminary examination of variety trial lines did not find a significant association of LMA resistance with known SSR molecular markers on chromosomes 3B and 7B. Based on this it is important to perform association mapping using not SSR markers, but SNP markers. The spring TCAP population was subjected to LMA-induction in the field in 2016. We are currently performed greenhouse LMA experiments with the same TCAP population. Results should be available at the end of year 3.

Impact: Wheat in all market classes is dramatically discounted for low falling numbers (below 300s). Moreover, a consistent problem with low FN could damage the reputation of Washington wheat in foreign markets. Screening for low FN, LMA, and sprout-susceptibility will the selection of new varieties with more stable FN. Posting of FN data on the pacific northwest FN website makes this data available to farmers and to breeders.

WGC project number: 5389
WGC project title: Developing Washington Wheat with Stable Hagberg Falling Numbers
Project PI(s): C. Steber, M. O. Pumphrey, A.H. Carter
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Project year: year 2 of 3

Objective	Deliverable	Progress	Timeline	Communication
1. Screen spring and winter wheat cultivars, breeding, and mapping lines for preharvest sprouting tolerance using the spike wetting test and the Falling Number test.	Knowledge about the susceptibility of spring and winter wheat cultivars and breeding lines to preharvest sprouting based on the spike-wetting test and on to FN (2015, 2016, 2017). Selection of breeding lines with higher resistance to preharvest sprouting compared to current varieties.	**Year 1**. Completed FN testing for the 2014 variety trials (see steberlog.org/project 7599). Conducted spike wetting tests of winter wheat breeding lines from the 2015 field season. Identified locations with low FN problems in 2015 by FN testing of known susceptible spring and winter wheat lines. Generated 571 FN datapoints for locations with low FN problems. **Year 2.** Performing FN testing of the entire WSU Cereal Vairety trail with the help of supplemental funding from project 5333. We have generated 2340 datapoints since August. We have also examined the effect of fungicide treatment and storage on FN.	Year 1, 2, and 3. Spike-wetting tests and FN testing of breeding lines, association mapping lines, and affected variety trial locations.	Results were communicated through the project website: steberlab.org/project7599.php, a Wheat Life article published in 2016, Timely Topic articles on the Small Grains website, an extension facts article published at pubs.wpdev.cahnrs.wsu.edu/pubs/fs242e , abstracts submitted to the Lind and Spillman Field Days, talks at the Wheat Research Review in 2015 and 2016, 2015 and 2016 Wheat Academy presentations, a presentation to WSCIA in 2016, talks at 2016/17 growers meetings in Spokane, Connell, and Fairfield WA, and in Pendleton OR.
2. Improve screening for LMA susceptibility to prevent release of susceptible spring and winter varieties.	Knowledge about the susceptibility of spring and winter wheat varieties and breeding lines to LMA. Breeding of LMA resistant wheat.	**Year 1**. Compared the field cut-spike LMA testing with greenhouse LMA tests. The field cut spike assay detects known LMA susceptible lines, but may score mistake soem LMA resistant lines for susceptible lines. **Year 2.** Performed LMA screening of winter and spring wheat breeding lines in the field. Based on FN and LMA testing data, winter LMA suspects include, Jasper, SY-Ovation, Bruehl, WA8202, 4J071246-1C, and Rosalyn. Spring wheat LMA suspects include Alturus, ARS504174, WB6341, IDO851, IDO854, Nick, UI-Stone, and WA8124.	Year 1. Perform LMA testing using both the established greenhouse and new field-based technique. Determine if the field technique gives the similar results to greenhouse. Year 2 and 3. LMA testing of breeding lines and spring association panel.	same
3. Identify molecular markers linked to preharvest sprouting resistance and susceptibility genes by association mapping	Molecular markers for use in early selection for increased preharvest sprouting tolerance.	**Year 1**. Molecular markers linked to preharvest sprouting tolerance were identified based on Falling Number and spike wetting test data. The genes/loci identified by Falling Number were not identical to those identified by spike wetting test. This suggests that we need to continue to emphasize FN data when making selections in the breeding programs. **Year 2.** Identified a locus on chromosome 6A linked to preharvest sprouting as measured both by FN testing and spike-wetting tests. Performing LMA testing of the spring wheat association mapping panel to identify LMA-susceptibility genes.	Year 1. Perform association mapping to identify loci linked with PHS tolerance. Year 2 and 3. Perform field LMA tests in preparation for association mapping. Year 3 perform LMA association mapping.	same