Washington Grain Commission
Wheat and Barley Research Annual Progress Report

Project #: 5389

Progress Report Year: *_3__ 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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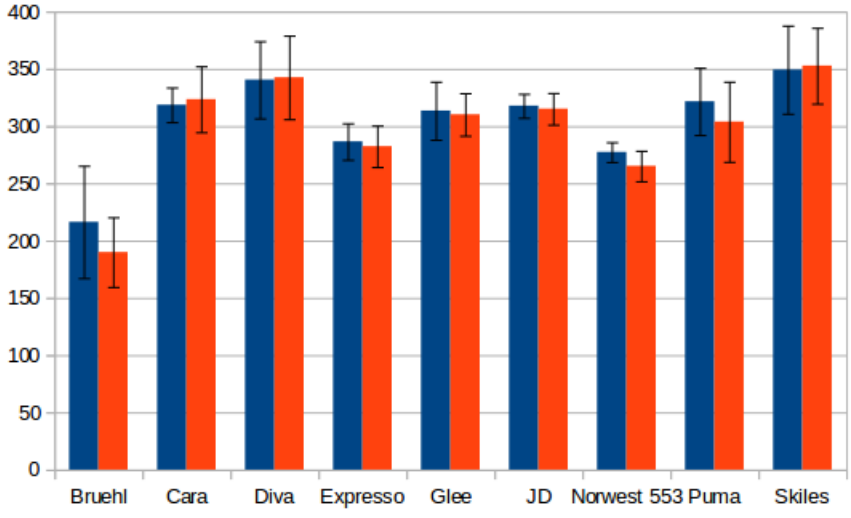
Executive summary: The goal of this project is to breed for stable Falling Numbers (FN) in Washington wheat through selection for genetic resistance to preharvest sprouting and late maturity alpha-amylase (LMA). The project identified cultivars with sprouting and LMA problems through evaluation of the WSU cereal variety trials. Whereas preharvest sprouting due to rain was the major cause of low FN in 2013, LMA due to large temperature fluctuations during late grain filling was the major cause of low FN in 2016. Analysis of the 2016 crop revealed that there are problems with LMA susceptibility in all market classes, including both red and white wheat. The project developed LMA and PHS field screening systems, identified molecular markers linked to PHS tolerance genes in northwest winter wheat, and initiated mapping of LMA susceptibility genes.

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. FN was evaluated for the winter and spring wheat variety trial locations in 2015 and 2016. Over 4000 2016 variety trial datapoints were reported on (http://steberlab.org/project7599.php). This does not include wheat breeding lines and mapping lines evaluated for FN.
B. The effect of fungicide treatment on FN. Preliminary data from the 2016 field season suggests that fungicide treatment with Quilt does not cause a significant decrease in FN (Figure 1). Grain from plants grown in Pullman WA with and without Quilt fungicide treatment (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. Given that the standard deviation of the FN test can be 30 to 50 seconds, multiple repetitions are needed to see a significant upward trend. Previous work showed that FN increased significantly only at higher temperatures (Ji and Baik, 2016; Adams and Ross, personal communication). So storing grain over the winter may not appreciably increase FN. Careful consideration is needed before choosing to store grain over the winter months.

D. Near-infrared (NIR) spectrometry as an alternative to FN. NIR is used to measure protein levels in intact grains of wheat. A collaboration was arranged with Dr. Stephen Delwiche (USDA-ARS, Beltsville MD) to examine whether an NIR calibration could be developed as a nondestructive method to estimate FN at the elevator. This could allow grain with low FN to be segregated from high FN grain, thereby preventing the low FN grain from degrading the quality of all of the grain going into a bin. We sent 544 grain samples for NIR measurement that were selected to cover a wide range of falling numbers, and to represent multiple locations from the WSU Cereal Variety Trials. Unfortunately, while it was possible to develop a calibration for one location or set of samples, the calibrations did not fit samples from other locations. This was tested using both whole grain samples and milled samples in the NIR. This means that NIR cannot reliably be used to estimate NIR at the elevator.

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. LMA induction was used to detect LMA-susceptible breeding lines in 2015 and 2017. Because there was a natural LMA event in 2016, LMA susceptible breeding lines were identified using half-seed assays of field-harvested grain. 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 developed as a method to determine if low FN in the field was due to LMA or to preharvest 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, whereas LMA causes fairly similar levels at the embryo and brush ends of the grain (Mares et al., 2006). Grains were cut, and 10 embryo ends were ground and used in one assay, while 10 brush ends were used in the other. Using the Phadebas alpha-amylase assay method in 96-well format, we were able to characterize the cause of low FN in the eleven soft white winter 2016 Cereal Variety Trial locations. 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. Half-seed assays also revealed that most of the low FN of hard red winter trials in Ritzville were due to LMA.

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. Association mapping identified preharvest sprouting susceptibility/tolerance loci in white winter wheat. Mapping was performed using both spike-wetting tests and FN on the same mapping population of 469 lines representing seven northwest breeding programs (Table 1, collaboration with Dr. Z. Zhang). Spike-wetting tests did not detect any 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 tests in addition to spike-wetting tests. One of the strong FN loci identified in 2016, *QFN.wsu.7B.1* is closely linked to a location on wheat chromosome 7B previously mapped for LMA resistance in Australian wheat. This suggests that some of the low FN problem in 2016 may be due to a known LMA susceptibility locus/gene. Table 1 below shows some of the molecular markers significantly associated with resistance to preharvest sprouting based on the spike-wetting tests and with higher FN. QTL on chromosome 3A were located close to the clone sprouting resistance gene *TaMFT1* and on 4D were close to the resistance gene *TaMKK3*. Interestingly, a strong PHS resistance gene on chromosome 2D is close to the gene giving club-shaped kernels. This may be one reason that many club varieties like ARS Crescent, Cara, and Coda have higher FN.

B. Genome-Wide Association Mapping for LMA. Preliminary examination of spring 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 of 250 lines was subjected to LMA-induction in the greenhouse in 2016 and in the field in 2017. The winter QAM panel of 469 lines was subjected to field LMA induction. These samples are currently being evaluated using alpha-amylase enzyme assays. Results will be used to identify molecular markers associated with LMA resistance and susceptibility in northwest wheat varieties.

Table 1. Loci associated with Falling Numbers (FN) and preharvest sprouting (PHS).

QTL[a]	Marker	Chr[b]	cM[b]	-log10(*p*)	maf	Effect[c]	r^2
*QFN.wsu-4A**	IWB1884	4A	152	6.63	0.48	10.28	0.00
*QFN.wsu-5A.1**	IWB60191	5A	23	7.27	0.27	7.53	0.00
QFN.wsu-5A.2	IWB9800	5A	141	7.77	0.20	7.43	0.00
QFN.wsu-5D	IWB36060	5D	202	6.11	0.35	11.70	0.08
QFN.wsu-7A.1	IWB22966	7A	35	8.34	0.06	26.09	000
QFN.wsu-7A.2	IWA334	7A	126	12.36	0.41	7.99	0.01
QFN.wsu-7B.1	IWB39063	7B	162	7.91	0.48	10.88	0.01
QPHS.wsu-1A.1	IWB2320	1A	82	6.73	0.15	-0.04	0.00
QPHS.wsu-3A.2	IWB50719	3A	68	6.71	0.14	-0.29	0.04
QPHS.wsu-4A.1	IWA7535	4A	58	8.57	0.05	-0.07	0.03
*QPHS.wsu-4B.3**	IWB22055	4B	101	6.57	0.08	-0.37	0.00
QPHS.wsu-4A.2	IWB54609	4A	66	7.30	0.17	-0.35	0.01
QPHS.wsu-2D	**IWB7652**	**2D**	**52**	**12.69**	**0.37**	**-0.85**	**0.12**
QPHS.wsu-3A.1	IWB32631	3A	15	6.63	0.26	-0.31	0.02
QPHS.wsu-1D*	**IWB71680**	**1D**	**163**	**7.22**	**0.06**	**-0.03**	**0.10**
*QPHS.wsu-7B.2**	IWB7099	7B	133	8.63	0.00	-0.02	0.01
	IWB7099	7B	133	7.58	0.01	-0.02	0.00

[a] QTL in bold explained 10% (r^2 >0.1) or more of the phenotypic variation. QTL underlined were significant in 2 environments. Loci considered to be novel are indicated with ***.**

[b] Chromosome and position according to Wang et al. (2014). Positions are not reported if the location was identified on the GrainGenes database.

[c] The allelic effect is shown in FN seconds or sprouting score.

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.

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WGC project title: Developing Washington Wheat with Stable Hagberg Falling Numbers through Resistance to Preharvest Sprouting and LMA
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Project year: year 3 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.** Performed FN testing of the 2016 WSU Cereal Vairety trail with the help of supplemental funding from project 5333. Examined the effect of fungicide treatment and storage on FN. **Year 3**. Examined the utilitity of NIR for as a nondestructive method to estimate FN, and were unable to generate a calibration that would work across all locations and years. Are in the process of examining FN for the 2017 Cereal Variety Trial locations that had low FN in susceptible cultivars.	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: a) the project website: steberlab.org/project7599.php, b) Wheat Life articles published in 2016 and 2017, c) Timely Topic articles on the Small Grains website in 2016 and 2017, d) an extension facts article published at pubs.wpdev.cahnrs.wsu.edu/pubs/fs242e , abstracts submitted to the Lind and Spillman Field Days, e) an extension review article published in Crops and Soils, f) talks at the Wheat Research Review in 2015 and 2016, g) 2015 and 2016 Wheat Academy presentations, h) a presentation to WSCIA in 2016, h) talks at 2016/17 growers meetings in Spokane, Connell, Colfax, 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. Developed a 96-well method for alpha-amylase enzyme assays using the Phadebas reagent. **Year 2.** Developed a 96-well method for alpha-amylase enzyme assays based on the Megazyme Ceralpha method. Developed half- seed assays to ascertain the contribution of LMA to the low FN problem of 2016. 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 3.** Performed field LMA testing of breeding lines. Continued to refine and develop standardized controls for the 96-well alpha-amylase enzyme assays.	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 3. Completed a genome-wide association mapping study for preharvest sprouting. Performed LMA field screening of the 469 line winter QAM assocation mapping panel and of the 250 line TCAP spring association mapping panels. Alpha-amylase enzye assays of these mapping populations are currently in process.	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