Washington Grain Commission
Wheat and Barley Research Annual Progress Report

Project #: 3690
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.
PIs: Camille M. Steber, Michael O. Pumphrey, Arron H. Carter, and Kimberly Garland Campbell
Cooperators: Deven See, Craig Morris, Aaron Esser, and Drew Lyon

Executive summary: The goal 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 low FN problems through evaluation of the WSU cereal variety trials, and with sprouting and LMA problems through greenhouse and field testing. We have identified molecular markers linked to LMA and PHS resistance to allow selection in early breeding lines. We improved LMA methods.
Objective 1. Identify varieties with stable FN by performing FN tests and statistical analysis of variety trials in environments that have preharvest sprouting and/or LMA
FN data from the soft white winter WSU variety trials was analyzed using five statistical methods designed to examine the impact of genetics and the environment. FN is difficult to analyze because it is impacted by multiple environmental factors leading to preharvest sprouting or LMA. The factor analytic model provided the best fit for this complex dataset, and will be used to compare variety performance for falling number and how stable that falling number is in challenging environments.
Objective 2. Screen winter and spring wheat breeding lines for preharvest sprouting and/or LMA. In 2018, 1,335 lines were screened for LMA and 708 lfor preharvest sprouting susceptibility by spike-wetting test. In 2019, 1113 samples were screened for LMA, and 810 were screened for PHS susceptibility by spike-wetting test.
Objective 3. Identify molecular markers linked to LMA susceptibility in northwest wheat.
a. We completed a genome-wide association study of the hard red spring TCAP population (250 lines) and identified six putative genes/loci associated with LMA phenotype. Of these 6, two were previously identified in Australian wheat. We will examine if these markers predict LMA in PNW breeding lines. b. Two winter RIL populations were screened for LMA in 2019.
Objective 4. Develop molecular markers for selection of PHS resistance in northwest wheat.   Genome-wide association mapping was conducted in two related populations. Some loci linked to good seedling emergence did not correspond to loci for preharvest sprouting resistance, suggesting that we may be able to select preharvest sprouting resistant without compromising seedling emergence. A preliminary geomic selection model was developed in the hope of selecting PHS tolerance without compromising emergence.

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 enable the selection of new varieties with more stable FN. Posting of FN data on the WSU small grains website and the PNW FN website makes this data available to farmers and to breeders.

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Project PI(s): Camille M. Steber, Michael O. Pumphrey, Arron H. Carter, and Kimberly Garland Campbell

Project initiation date: July 1, 2018

Project year (X of 3-yr cycle): This is year 2 of 3 of the funding cycle.

Objective	Deliverable	Progress	Timeline	Communication
1. Identify varieties with stable FN by performing FN tests and statistical analysis of variety trials in environments that have PHS and/or LMA.	**1.** FN testing results posted on the PNW FN website (steberlab.org). **2.** Development of statistical methods to compare varieties for performance and stability of FN. **3.** A new FN comparison tool.	**1.** FN testing of spring and winter varieties has been completed for all variety trial locations that showed a low FN problem in 2017 and 2018. **2.** FN data from the WSU soft white winter variety testing in 2013, 2014, and 2016 has been analyzed using five different statistical approaches. A first article was published in Crop Science. Ongoing research using a factor-analytic model in AMSREML appears to provide the best tool for comparing both how well a variety performs for falling number, and how stable the falling number is over changing environments. **3.** We discovered that as an FN machinie ages, it can give highly inaccurate FN measurements in the FN 250-350 sec range. **4.** Based on a survey of 2019 WSU variety trial locations: LMA resulted in low FN in SWW wheat due in the lower rainfall zones (Connell, Dusty, and Lind); preharvest sprouting resulted in serious problems with low FN in SWW in Creston, Almira, and Reardan; PHS is also likely the cause of low FN in spring wheat in Fairfield, Lamont, Palouse, and Pullman.	**1.** Annually. FN testing of susceptible varieties will be used to determine which WSU Cereal Variety locations will be subject to FN testing. **2.** In 2018 and 2019, compare various methods for ranking varieties for FN. **3.** In 2019, complete statistical analysis of soft white winter FN data from 2013, 2014, and 2016. **4.** In 2020, select a method for annual analysis of variety trial FN data.	Results of annual FN testing will be made available on the PNW FN website and on the WSU small grains website. Information will be published in peer-reviewed journals, summarized in a Wheat Life article, presented during field days, and presented at the annual Wheat Review.

2. Screen winter and spring wheat breeding lines for PHS and LMA susceptibility.	Data obtained will allow selection for increased resistance to LMA and preharvest sproutng in winter and spring wheat breeding programs at WSU. This should indirectly lead to release of variaties with increased resistance to low FN.	**1.** In 2018, the LMA field-testing method was used to induce LMA in a total of 1,335 lines. This included 72 spring wheat breeding lines, 168 winter wheat breeding lines, 185 variety trial and parental lines, 426 TCAP spring wheat mapping plots, and 484 QAM winter wheat mapping lines. FN testing of this material is still in progress. **2.** Preharvest sprouting resistance was tested using greenhouse spike-wetting tests of spikes harvested at physiological maturity from the field. Testing results were obtained for 495 soft white winter and 213 spring wheat lines. **3.** Experiments were performed to optimize the temperature, humidity, and developmental timing of LMA induction. The goal of using the Chemwell-T robot to optimize alpha-amylase enzyme assays (Megazyme SD assay) met with serious problems in 2018. The programming of the robot does not allow it to maintain a consistent 5 minute reaction time, making results inconsistent. This meant that over 1000 samples had to be repeated using the Phadebas enzyme assay.	**1.** Perform field LMA testing annually of about 1000 lines annually for breeding and mapping. **2.** Perform spike-wetting tests annually. **3.** Continue improving methods to increase efficiency.	Information will be published in peer-reviewed journals, summarized in a Wheat Life article, presented during field days, and presented at the annual Wheat Review.
3. Identify molecular markers linked to LMA susceptibility in northwest wheat.	**1.** Molecular markers linked to LMA resistance allowing selection in earlier generation breeding lines. **2.** Mapped LMA genes/loci linked to LMA resistance and susceptibility in the soft white spring TCAP population. **3.** Mapped LMA genes/loci linked to LMA resistance and susceptibility in recombinant inbred line populations.	**1.** We have completed 4 greenhouse and two field LMA testing experiments for the spring TCAP population. Preliminary genome-wide association mapping was completed in 2019, and identified 6 loci associated with the LMA trait. **2.** In 2019, LMA testing was performed on 1,113 samples including 603 breeding and variety trial samples. We were unable to test the TCAP in 2019 due to problems with Hessian fly. **3.** The parents for 10 spring RIL populations and 20 winter RIL populations have been screened in a single greenhouse LMA experiment. Two promising populations for LMA mapping, Cara x Xerpha, and Xerpha x Bobtail were screened in the field in 2019. 4. LMA testing results revealed that tall wheat (rht wild-type) tend to induce LMA without a cold shock treatment. Because this LMA phenotype was much more consistent than any we've seen, future mapping efforts will focus on tallxtall crosses.	**1.** The goal to complete TCAP LMA screening in 2018 must be extended into 2019. **2.** Genome-wide association mapping in the TCAP was to be completed in 2019. **3.** RIL populations for LMA mapping will be identified by 2019, and LMA screening initiated in 2019 and 2020.	Information will be published in peer-reviewed journals, summarized in a Wheat Life article, presented during field days, and presented at the annual Wheat Review.

4. Develop molecular markers for selection of preharvest sprouting resistance in northwest wheat.	**1.** Molecular markers that can be used to select for resistance to preharvest sprouting. **2.** Identify markers that can select for sprouting resistance without compromising field emergence.	1. Mapping results for preharvest sprouting were based on FN and sprouting scores from spike-wetting tests. Mapping was also preformed for emergence based on field emergence and coleoptile/seedling elongation. Comparison found that there were some strong genes/loci lined to emergence that were not linked to preharvest sprouting susceptibility. This is a preliminary result, but suggests that this mapping approach may be used successfully in soft white winter wheat populations. **2.** A preliminary genomic selection model was developed. **3.** Spike-wetting tests were performed for 461 doubled-haploid winter wheat breeding lines derived from parents in the QAM and SNP winter wheat mapping populations. These data can be used to confirm marker-trait associations for molecular markers.	**1.** In 2018, GWAS was performed in a second population to confirm marker-trait associations. **2.** In 2018, spike-wetting tests were performed on winter doubled haploid populations. **3.** In 2019 and 2020, we will develop a genomic prediction model.	Information will be published in peer-reviewed journals, summarized in a Wheat Life article, presented during field days, and presented at the annual Wheat Review. The goal in to summarize these markers on the PNW fallng number website to make it easier for wheat breeders to access this information.