

**Washington Grain Commission  
Wheat and Barley Research Annual Progress Report**

**Project #: 3690**

**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.

**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 two main causes of low FN. The project identified cultivars with low FN problems through FN testing of the WSU cereal variety trials. Preharvest sprouting and LMA testing results were provided to the WSU and USDA breeding programs to enable selection from improved FN. Genes or loci associated with preharvest sprouting and LMA tolerance were identified by mapping using greenhouse and field-testing results. We have identified molecular markers linked to LMA and PHS resistance to allow selection in early breeding lines. We improved LMA testing methods. This is the final report for the FN project. A new proposal was not submitted. However, Dr. Steber would like to continue to present research plans and achievements during the annual Wheat Research Review.

**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.

FN testing was performed in all three years of the project (data posted on <http://steberlab.org/project7599.php>). There were only mild problems with low FN in 2020. If we look at soft white winter wheat, in 2020 only 5 of the 18 locations had mild to moderate problems with low FN whereas in 2019, 15 of 20 locations had FN problems and four of these locations had severe problems. In a preliminary survey of susceptible checks in 2020, only 3 hard winter, 4 soft white spring, and 2 hard spring locations had low FN.

**Objective 2. Screen winter and spring wheat breeding lines for preharvest sprouting and/or LMA.** PHS and LMA testing were performed both on elite breeding lines and on mapping populations in order to improve selection for higher FN varieties and identify important loci associated with these traits. In 2018, 1335 lines were screened for LMA and 708 for 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. In 2020, 1440 samples were tested for LMA and 3,229 for PHS.

**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,

*QLMA.wsu.3B* , *QLMA.wsu.6B* , and *QLMA.wsu.7B* were located close to LMA QTLs previously mapped in Australian or CIMMYT wheat lines (Mrva and Mares, 2001; Tan et al., 2004; McNeil et al., 2009; Emebiri et al., 2010). We will examine if these markers predict LMA in PNW breeding lines by examining their association with LMA in the WSU Cereal Variety Trials (collaboration with Deven See). b. Two winter RIL populations, Cara x Xerpha and Xerpha x Bobtail, were screened for LMA in 2019 and 2020. A genetic map was developed by K. Garland Campbell for the Cara/Xerpha population. This will allow us to perform a preliminary QTL analysis in 2021. A genetic map will need to be developed for the Xerpha/Bobtail population obtained from R. Zemetra in collaboration with Deven See.

Table 1. Putative significant QTL from GWAS for LMA.

<sup>1</sup> GH QTL	Marker	Chr	Position	$-\log_{10}(p)$	<sup>2</sup> Effect	MAF	<sup>3</sup> Fav allele	n
<i>QLMA.wsu.3B</i>	IWB63008	3B	801300	8.6	0.08	0.10	A/G	242
<i>QLMA.wsu.1A</i>	IWB35476	1A	1512200	6.4	-0.05	0.33	T/C	120
<i>QLMA.wsu.6B</i>	IWB26762	6B	1104500	7.0	-0.07	0.11	T/C	120
Field QTL								
<i>QLMA.wsu.3A</i>	IWB11852	3A	207400	6.9	0.16	0.26	A/C	206
<i>QLMA.wsu.7B</i>	IWB25774	7B	1335900	9.8	0.16	0.25	A/G	206
<i>QLMA.wsu.7D</i>	IWB48862	7D	861900	9.9	0.17	0.24	T/C	206

<sup>1</sup>GH = greenhouse

<sup>2</sup>The positive Effect values indicate elevated risk of LMA phenotype in N. America hard red spring wheat TCAP association panel

<sup>3</sup>The major allele that reduce the susceptibility of LMA in N. America hard red spring wheat TCAP association panel

**Objective 4. Develop molecular markers for selection of PHS resistance in northwest wheat.** Genome-wide association mapping was conducted in two related populations based on spike-wetting tests and falling numbers after artificial or natural rain (see Martinez et al., 2018). 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 genomic 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 300 sec). Moreover, a consistent problem with low FN could damage the reputation of Washington wheat in foreign markets. Screening for increased genetic resistance to low FN, LMA, and preharvest sprouting will enable the selection of new varieties with more stable FN. Currently, screening breeding lines for PHS and LMA is a labor-intensive process that is only feasible for mid to late breeding lines. The identification of molecular markers associated from preharvest sprouting and LMA tolerance will allow selection for improved genetic resistance in earlier breeding material and should greatly improve the FN of new varieties. Posting of FN data on the WSU small grains website and the PNW FN website (<http://steberlab.org/project7599.php>) makes this data available to farmers and to breeders.

## Publications:

1. Steber, C. M., Carter, A. H., & Pumphrey, M. O. (2018, January). Hunting for genes: falling numbers project seeks to reduce risk by breeding for genetic resistance. *Wheat Life*, 61(1), 50–51.
2. Martinez, S. A., Godoy, J., Huang, M., Zhang, Z., Carter, A. H., Garland Campbell, K. A., & Steber, C. M. (2018). Genome-Wide Association Mapping for Tolerance to Preharvest Sprouting and Low Falling Numbers in Wheat. *Frontiers in Plant Science*, 9, 1–16, <https://doi.org/10.3389/fpls.2018.00141>.
3. Delwiche, S. R., Higginbotham, R. W., & Steber, C. M. (2018). Falling number of soft white wheat by near-infrared spectroscopy: A challenge revisited. *Cereal Chemistry*, 95(3), 469–477.
4. Steber, C. M., & Garland Campbell, K. A. (2019, April). Rising optimism fuels falling numbers research. *Wheat Life*, 62(4), 40–52.
5. Sjoberg, S., Carter, A. H., Steber, C. M., & Garland-Campbell, K. A. (2019). Unravelling Complex Traits in Wheat: Approaches for Analyzing Genotype × Environment Interactions in a Multi-environment Study of Falling Numbers. *Crop Science*, 60, 3013–3026.
6. Martinez, S. A., Shorinola, O., Conselman, S., See, D., Skinner, D. Z., Uauy, C., & Steber, C. M. (2020). Exome sequencing of bulked segregants identified a novel TaMKK3-A allele linked to the wheat ERA8 ABA-hypersensitive germination phenotype. *Theoretical and Applied Genetics*, 133, 719–736.
7. Sjoberg, S. M., Carter, A. H., Steber, C. M., & Garland-Campbell, K. A. (2020) Application of the factor analytic model to assess wheat falling number performance and stability in multi-environment trials. *Crop Science*, doi:10.1002/csc2.20293.
8. Liu, Chang, KM Tuttle, KA Garland Campbell, M O. Pumphrey, C.M. Steber. Investigating Conditions That Induce LMA Using Northwestern U.S. Spring Wheat (*Triticum aestivum L.*). *Seed Science Research*. Submitted and accepted with minor revision.
9. Liu, Chang, RS. Parveen, SR. Revolinski, KA Garland Campbell, M O. Pumphrey, C.M. Steber. The Genetics of LMA in North American Spring Wheat (*Triticum aestivum L.*). *Seed Science Research*. Submitted and accepted with minor revision.

**WGC project number:** 3690  
**WGC project title:** Developing Washington Wheat with Stable Falling Numbers (FN) through resistance to preharvest sprouting and LMA  
**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 3 of 3 of the funding cycle.

Objective	Deliverable	Progress	Timeline	Communication
<p><b>1.</b> Identify varieties with stable FN by performing FN tests and statistical analysis of variety trials in environments that have PHS and/or LMA.</p>	<p><b>1.</b> FN testing results posted on the PNW FN website (steberlab.org). <b>2.</b> Development of statistical methods to compare varieties for performance and stability of FN. <b>3.</b> A new FN comparison tool.</p>	<p><b>1.</b> FN testing of spring and winter varieties has been completed for all variety trial locations that showed a low FN problem in 2017, 2018, and 2019. <b>2.</b> The 2020 field season did not have wide-spread problems with low FN. FN testing is underway. <b>3.</b> Two articles by Sjoberg et al were published in Crop Science exploring statistical methods to analyze FN data based on the WSU soft white winter variety testing in 2013, 2014, and 2016. The factor-analytic model in AMSREML provided the best tool for comparing varieties based on overall FN performance and stability under pressure in challenging environments. <b>4.</b> We discovered that as an FN machine ages, it can give highly inaccurate FN measurements in the FN 250-350 sec range. <b>5.</b> An FN comparison tool has been added to the small grains variety selection tool based on FN in 2019. This tool will be improved to include data over the years analyzed using a factor analytic model. This will require enhanced curation and analysis of the data.</p>	<p><b>1.</b> Annually, FN testing of susceptible varieties will be used to determine which WSU Cereal Variety locations will be subject to FN testing. <b>2.</b> In 2018 and 2019, compare various methods for ranking varieties for FN. <b>3.</b> In 2019, complete statistical analysis of soft white winter FN data from 2013, 2014, and 2016. <b>4.</b> In 2020, select a method for annual analysis of variety trial FN data.</p>	<p>Results of annual FN testing have been made available on the PNW FN website (steberlab.org) and on the WSU small grains website. Information has been published in peer-reviewed journals, summarized in Wheat Life articles, presented in the FN workshop at the PNW-Wheat Quality Council, during field days, and at the annual Wheat Review.</p>
<p><b>2.</b> Screen winter and spring wheat breeding lines for PHS and LMA susceptibility.</p>	<p>Data obtained will allow selection for increased resistance to LMA and preharvest sprouting in winter and spring wheat breeding programs at WSU. This should indirectly lead to release of varieties with increased resistance to low FN.</p>	<p><b>1.</b> LMA field-testing was performed in 2018, 2019, and 2020, screening about 1400 lines per year including winter and spring wheat breeding lines, variety trial and mapping populations. Enzyme assays of 2020 material are still in progress. <b>2.</b> Preharvest sprouting tolerance was tested using greenhouse spike-wetting tests of spikes harvested at physiological maturity from the field. The PHS testing was expanded in 2020 to include 1,683 plots of breeding lines and 1,534 mapping lines. <b>3.</b> Experiments were performed to optimize the conditions for LMA induction. <b>4.</b> In 2020, doubled haploid populations were screened both for fall emergence and for preharvest sprouting tolerance at maturity. This will be used to generate a genomic selection model.</p>	<p><b>1.</b> Perform field LMA testing annually of about 1000 lines annually for breeding and mapping. <b>2.</b> Perform spike-wetting tests annually. <b>3.</b> Continue improving methods to increase efficiency.</p>	<p>Information has been published in peer-reviewed journals, summarized in Wheat Life articles, presented in the FN workshop at the PNW-Wheat Quality Council, during field days, and at the annual Wheat Review.</p>
<p><b>3.</b> Identify molecular markers linked to LMA susceptibility in northwest wheat.</p>	<p><b>1.</b> Molecular markers linked to LMA resistance allowing selection in earlier generation breeding lines. <b>2.</b> Mapped LMA genes/loci linked to LMA resistance and susceptibility in the soft white spring TCAP population. <b>3.</b> Mapped LMA genes/loci linked to LMA resistance and susceptibility in recombinant inbred line populations.</p>	<p><b>1.</b> Six loci/genes associated with LMA in spring wheat were identified in 2020 on chromosomes 1A, 3A, 3B, 6B, 7A and 7B. The loci on 3B, 6B, and 7B are close to LMA loci previously mapped by Australian researchers. Ongoing research will determine if these 6 molecular markers or nearby markers can select for LMA resistance in PNW breeding lines. <b>2.</b> In another project, the parents for 10 spring RIL populations and 20 winter RIL populations were screened for LMA. Two promising winter 2-parent populations were chosen for LMA mapping, Cara x Xerpha, and Xerpha x Bobtail. <b>3.</b> LMA field testing was performed on Cara/Xerpha and Xerpha/Bobtail in 2019 and 2020. A preliminary QTL analysis of Cara/Xerpha will be conducted in 2021. <b>4.</b> LMA testing results revealed that tall wheat (<i>rht</i> 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.</p>	<p><b>1.</b> The goal to complete TCAP LMA screening in 2018 must be extended into 2019. <b>2.</b> Genome-wide association mapping in the TCAP was to be completed in 2019. <b>3.</b> RIL populations for LMA mapping will be identified by 2019, and LMA screening initiated in 2019 and 2020.</p>	<p>The Association Mapping study has been provisionally accepted for publication in the journal Seed Science Research. Information has been summarized in Wheat Life articles, in presentations at the Wheat Quality Council, Western and Eastern wheat workers meetings, presented during field days, and presented at the annual Wheat Review.</p>

<p>4. Develop molecular markers for selection of preharvest sprouting resistance in northwest wheat.</p>	<p>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.</p>	<p>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 in 2020. 3. In 2021, spike-wetting tests and field emergence scoring were performed for 800 doubled-haploid winter wheat breeding lines derived from parents in the QAM and SNP winter wheat mapping populations. These data will be used to confirm marker-trait associations for molecular markers.</p>	<p>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.</p>	<p>Information has been published in the peer-reviewed journal TAG, summarized in Wheat Life articles, presented in the FN workshop at the PNW-Wheat Quality Council, during field days, and at the annual Wheat Review. The goal is to summarize these markers on the PNW falling number website to make it easier for wheat breeders to access this information.</p>