

**Washington Grain Commission**  
**2023 Wheat and Barley Research Annual Progress Reports and Final Reports**

**Project #:**144176; AWD005706

**Progress Report Year:**     \_1\_\_\_ of \_3\_\_\_

**Title:** Development of 21<sup>st</sup> Century alpha-amylase immunoassays to replace the archaic, much criticized Hagberg-Perten Falling Number method, to preserve wheat grain value and accelerate breeding for climate resilience.

**PI:** Amber Hauvermale

**Cooperators WSU:** Andrew G. McCubbin, Drew Lyon, and Clark Neely. **EnviroLogix:** Rob McPheeters, Adam Johnson, and Larry Pessolano. **USDA:** Byung-Kee Baik, Camille Steber, and Xianran Li. **Other Industry Partners:** HighLine Grain Growers; Paul Katovich, Geoff Schultz, The McGregor Co.; Alex McGregor, Leslie Druffel, and Cat Salois, The Wheat Marketing Center; Jayne Bock, and The Washington Grain Commission; Mary Palmer Sullivan, and Lori Maricle.

**Executive summary:** The current WGC project, supports through cash and in-kind matching, a successfully funded FFAR project awarded in 2023. First year WGC project accomplishments include: 1) the purification of alpha-amylase proteins which may be used as internal controls for rapid tests, 2) development and testing of three additional antibodies for use in rapid tests, 3) delivery of hundreds of curated grain samples to our industry collaborator for rapid test calibration and pilot-lot scale up, 4) recruitment of a new graduate student and initiation of a proteomics objective to identify immuno-markers for breeding, and 5) outreach and extension efforts; 2 Wheat Life articles, 2 Wheat Beat podcasts, 2 Timely Topics (WSU small grains), 18 PNW grain industry interviews, WSU variety trial field days, invited presentations at the Spokane Ag Expo, the PNW and Wheat Quality council, the Cereals and Gains Conference, and the Washington State Crop Improvement Association meeting, and 1 peer-reviewed publication associated with the WGC project objectives.

**Introduction** Meeting the falling number export standard without unnecessary agronomic or economic losses throughout the ‘grain chain’ is difficult because the Falling Number test is low-throughput, expensive, difficult to perform accurately, and is logistically impossible to perform in real-time at receival stations. These factors limit grain segregation at harvest. In years when weather related low falling number events are localized, the impact to the industry may be minor. However, when events are widespread, as in 2016, the economic impacts are devastating. Improvements to the timely identification of compromised grain in a given year in combination with the development of new wheat varieties with fewer problems will increase on-farm profitability resulting in significant positive agronomic and economic impacts on the Washington small grain industry.

**Approach:** A four-pronged approach has been implemented to address the need for rapid inexpensive tests that better facilitate grain sorting from farms to export terminals, the need

to better understand and differentiate between different causes of elevated alpha-amylase (preharvest sprouting, and late maturity alpha-amylase), and the need to empower and accelerate breeding for higher falling numbers. Supporting project objectives include: 1) the development and commercialization new rapid tests, 2) identifying new proteins to be used as breeding markers for increased resistance to low falling numbers caused by PHS and LMA, 3) creating weather prediction models to be used as an early warning system for low falling number events in the field, and 4) the construction of a robust extension and education pipeline to end users.

**Results:** First year results include: 1) production of tagged (6XHIS) alpha-amylase proteins for use as controls in immunoblots and lateral flow immunoassays (Figure 1), as well as three new alpha-amylase antibodies that will help to distinguish between LMA and PHS; 2) acquisition and testing of hundreds of grain samples used for calibration and scale-up of pilot lot rapid tests in collaboration with EnviroLogix with larger scale stakeholder beta-testing to begin in 2024; 3) successful organization of extension/outreach networks to disseminate information about new technology through the WSU small grains website and WSU variety trials, Wheat Life Magazine, local industry interviews and grower meetings, and national conferences.

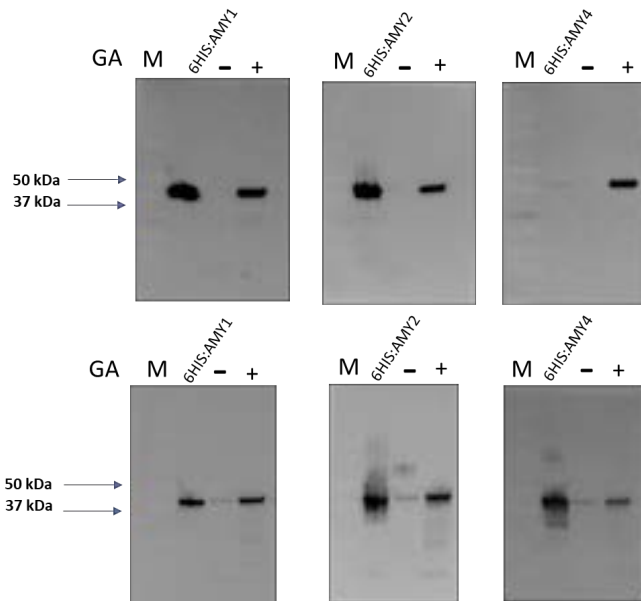


Figure 1: Immunoblots with purified alpha-amylase proteins 6XHIS:AMY1, 2 & 4 (lane 2) and proteins from wheat aleurones with or without (-/+) gibberellin treatment (GA). The top panel was detected using TaAMY1-C antibody described in Hauvermale et al., 2023. The bottom panel was detected with a newly developed alpha-amylase antibody. M = lane containing protein marker standards for determining protein sizes. Alpha-amylase proteins migrate between the 37 and 50 kDa standards.

**Impact:** In the last year measurable project impacts include: 1) successful leveraging of WMC funds for national funding through FFAR; 2) in collaboration with our collective grain industry team members, the acquisition, testing, and curation of hundreds of samples sent to EnviroLogix for rapid test calibration, and shared with USDA and university collaborators to develop different approaches to eliminate logistical and genetic issues associated with low falling numbers; 3) synthesis of specific tools (alpha-amylase protein standards, and immuno-markers for breeding) to improve rapid test performance and create other methods for pre- and post-harvest management of low falling numbers. Longer-term, deployment of innovative technologies will 1) reduce waste in the grain industry, 2) increased on-farm profitability, and 3) enhanced food production efficiency.

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**WGC project title:** Development of 21st Century alpha-amylase immunoassays to replace the archaic, much criticized Hagberg-Perten Falling Number method, to preserve wheat grain value and accelerate breeding for climate resilience.  
**Project PI(s):** Amber Hauvermale  
**Project initiation date:** February, 2023  
**Project year (X of 3-yr cycle):** 1 of 3-yr cycle

Objective	Deliverable	Progress	Timeline	Communication
1. Develop/produce purified $\alpha$ -amylases.	A renewable supply of protein standards used for immunoblots and rapid test.	Heterologous expression and purification of the four classes of alpha-amylases is complete (Figure 1).	Year 1: 6-9 mos. Status: COMPLETE	<b>Publications:</b> 1. Development of Novel Monoclonal Antibodies to Wheat Alpha-Amylases Associated with Grain Quality Problems That Are Increasing with Climate Change. Hauvermale et al., 2023. 2.The Development of Simple Wheat Meal Purification Methods for Diverse Testing Environments. Cereal Chemistry; intended submission, 2024.
2. Commercialize FN LFI and ELISA with ELX.	First phase rapid tests that provide a "bad/mediocre/good" metric based on falling number calibration.	This year EnviroLogix was able to calibrate pilot tests and is in the process of scaling up pilot lots for broad scale beta-testing. It is anticipated that beta-testing will begin early in 2024, and through harvest 2024.	Year 1-2 Status: ongoing and anticipated to be complete by the end of Year 2.	<b>Meetings:</b> 1. EnviroLogix visited the PNW in April as part of a series of industry interviews. 2. EnviroLogix has delivered three research meetings and one broader stakeholder meeting and will continue to communicate as best-tests are ready and available.
3. Organization of extension/outreach networks.	Communication tools for reporting, teaching and training including podcasts, extension or popular media publications, presentations at conferences, and training sessions.	In year 1, progress achieved included two Wheat Life articles (WMC), two Wheat Beat Podcasts and two Timely Topics (WSU small grains),	Years 1 Status: COMPLETE Annual extension and outreach efforts will occur throughout the course of the project in conjunction with Objective 6.	<b>Conference Presentations:</b> 1. 2023 Spokane Ag Expo, Spokane, WA: CM Steber, AL Hauvermale, and AL Thompson. Low Falling Numbers in Wheat, An Update. 2. 2023 Cereals and Grains Conference, Chicago IL: AL Hauvermale. Tools to Enhance Rapid Testing Platforms for Wheat Alpha-amylase Detection. 3. 2023 WSCIA, Moscow, ID: AL Hauvermale and AL Thompson. Developing Strategies and Tools To Manage and Mitigate Loss Due To low Falling Numbers. <b>Wheat Life Articles:</b> 1. Reaching a new landmark: The development of an immunoassay to manage low falling numbers post harvest. Amber L. Hauvermale, Alison L. Thompson, Camille M. Steber. 2. A Falling Numbers Fact Finding Mission. Alison L. Thompson and Amber L. Hauvermale. <b>Wheat Beat Podcasts:</b> 1. Evaluating Grain Quality and Mitigating Economic Loss. AL Hauvermale and AL Thompson. 2. The Upside of a Falling Numbers Rapid Test. AL Hauvermale. <b>WSU Timely Topics and Media:</b> 1. A new grant to help grain farmers find low falling numbers faster. 2. A new project aimed at combating low falling numbers. <b>WSU Variety Trials:</b> Walla Walla and Pullman WA. <b>Other Outreach:</b> 18 interviews conducted across the grain industry.
4. Proteomics pipeline for LMA/PHS immunomarkers.	Identified protein targets specific for PHS/LMA and used for immunomarkers for breeding	1. Pilot proteomics studies were performed to determine best conditions for large-scale experiments. 2. Samples were been submitted to TIMPL in December for large scale proteomics analysis of the differences between LMA and PHS in three different wheat varieties. Results are anticipated in the next few weeks, and data analysis will continue over the next 3 months.	Year 1-2 Status: ongoing but anticipated to be complete by summer of 2024.	<b>Anticipated Publications:</b> 1. A wheat proteomics review paper with anticipated submission in Summer/Fall 2024. 2. One to three proteomics research papers that describe differential expression of proteins with LMA and PHS to be submitted in 2024-2025.

5. Validate LMA/PHS-specific breeding LFI/ELISAs	Rapid tests that differentiate between LMA and PHS	Greenhouse and field studies with LMA and PHS are being collected in collaboration with WSU Variety Trials, and USDA scientists Camille Steber and Byung-Kee Baik. Once rapid tests are available for beta-testing these samples will be evaluated.	Years 1-3 Status: ongoing.	Nothing yet reported
6. Implement extension, education, outreach programs	Facilitation of LFI/ELISA product adoption, publicize/educate about the differing impacts of LMA and PHS on grain quality, develop market expansion for LMA grain, and train the next generation of agricultural scientists.	First year efforts have been to evaluate early adopters and to then work with EnviroLogix, the WMC, McGregor Co, the WGC, and HighLine grain growers, and other to build beta-testing platforms.	Years 1-3 Status: ongoing.	Nothing yet reported

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