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 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.

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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 though the WSU small grains website and WSU variety trials, Wheat Life Magazine, local industry interviews and grower meetings, and national conferences.

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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<td>2. Commercialize FN LFI and ELISA with ELX.</td>
<td>First phase rapid tests that provide a &quot;bad/mediocre/good&quot; metric based on falling number calibration.</td>
<td>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.</td>
<td>Year 1-2 Status: ongoing and anticipated to be complete by the end of Year 2.</td>
<td>Meetings: 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.</td>
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<td>4. Proteomics pipeline for LMA/PHS immunomarkers.</td>
<td>Identified protein targets specific for PHS/LMA and used for immunomarkers for breeding</td>
<td>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.</td>
<td>Year 1-2 Status: ongoing but anticipated to be complete by summer of 2024.</td>
<td>Anticipated Publications: 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.</td>
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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, publicizes/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