PROJECT NO: New

TITLE: Wheat α-amylases and their effect on falling numbers: investigating the susceptibility of wheat starch to α-amylase isozymes

PERSONNEL:

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Co-Investigators: Daolin Fu, Assistant Professor, Department of Plant, Soil and Entomological Sciences, University of Idaho (Moscow, ID), 208-885-1542, dlfu@uidaho.edu

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JUSTIFICATION:

Unpredictable low falling number (FN) issues have generated a lot of frustration for Idaho wheat growers. In 2014, Idaho wheat growers lost more than $100 million in revenue due to the low FN caused by pre-harvest sprouting (PHS). In 2016, the low FN issues happened again and were widely spread throughout northern Idaho. It is still unclear to growers and researchers what triggered the decrease in FN and caused such an economic disaster in 2016. The measurement of FN is determined by the starch pasting property. All the factors influencing starch, either in plants or during FN measurement, contribute to changes in FN. The conventional view is that the rise of α-amylase activity is responsible for a low FN. A-amylase cleaves α-(1→4)- glucosidic linkages, which are about 95% of the total linkages in starches. The α-amylase enzyme can break molecules from the middle of molecular chains, instead of removing glucose one by one from the end of molecules. Thus, α-amylase can quickly hydrolyze starch and decrease molecule size from about a half million glucosyl units to the size of two or three glucosyl units. Therefore, the viscosity decreases dramatically in a very short time, which results in a low FN. The interaction between starch and α-amylase is not as simple as a chemical reaction. Each α-amylase isozyme has slightly different amino acid sequences, and small variants can result in diverse hydrolytic capacity and patterns. **Starch does not respond to various α-amylase isozymes in the same way, and the same wheat starch would have different FNs due to the difference of isozymes.** The proposed project will extend the research plan "Wheat α-amylase and their effect on falling number" submitted in 2016 by Daolin Fu, the co-investigator of this project at the University of Idaho. Fu's research plan was to identify the α-amylase gene family and characterize the expression profile of α-amylase genes. In this supplemental project, we plan to *identify the susceptibility of wheat starch to α-amylase isozymes*. The success of this project will strengthen Fu's proposal by adding *an important outcome – identification of the α-amylase genes that lead to quick hydrolysis of starch and subsequent decrease of FN.*

HYPOTHESIS & OBJECTIVES:

We hypothesize that α-amylase isozymes have different hydrolytic effects on wheat starch, which results in different FNs. Our objectives are to (1) express and produce α-amylase isozymes, and (2) examine the susceptibility of wheat starches to α-amylase isozymes.

PROCEDURES:

Objective 1: Expressing and purifying α-amylase isozymes.

More than 20 α-amylase genes have been identified in wheat. Based on their DNA and protein sequences, wheat α-amylases can be classified in 4 to 6 subgroups. We propose cloning at least one representative isozyme in each subgroup, for a total of 4 to 6 isozymes. Target genes will be cloned into a protein expression vector that has a Glutathione-Sepharose-Transferase (GST) or Polyhistidine (His) tag. The α-amylase fusion protein will be expressed using prokaryotic or eukaryotic expression systems and purified using affinity chromatography.

Objective 2: Examining the susceptibility of wheat starches to α-amylase isozymes.

The purified isozymes from Objective 1 will be used to hydrolyze commercial wheat starch. We will (1) analyze specific activities of each isozyme by measuring the amount of hydrolytic products (e.g., maltose and maltotriose) generated from soluble starch (a standard starch) by α-amylase, and (2) investigate the hydrolytic kinetic on hydrolyzing granular and gelatinized starch to understand the potential damage of starch in plants and during the FN measuring process, respectively. In addition, we will (3) examine the morphology change of starch granules using scanning electronic microscopy (SEM). For the gelatinized starch (mimics the FN measurement), we will (4) measure the viscosity changes using a Rapid Viscosity Analyzer (RVA), and (5) analyze residual starch structure using a high performance size-exclusion chromatography equipped with a refractive index detector (HPSEC-RI) to explore the hydrolytic pattern of each isozyme and their influence on FN.

The proposed project will be carried out at the University of Idaho (Moscow, ID). All chemical and biochemical analyses will be performed in triplicate, and viscosity and chromatography analyses will be performed in duplicate. One-way ANOVA will be applied to evaluate the significance of differences. A doctoral graduate student will be assigned to perform experiments, and Lin will use active-learning and problem-solving teaching styles to mentor the student.

DURATION: 1 year (FY18)

COOPERATION:

Lin will design and conduct chemical and biochemical assays, manage the data, present research findings at professional conferences, and communicate research progress and results to the Idaho Wheat Commission. **Fu** and **Lin** will work together to produce isozymes and communicate regularly via e-mail, phone, and face-to-face meetings. The graduate student assigned to this project will submit weekly progress reports to **Lin**.

ANTICIPATED IMPACT, EXPECTED OUTCOMES & INFORMATION TRANSFER:

This project will

1) Advance understanding the effect of α-amylase genes on FN to improve the strategy of developing wheat cultivars with consistent quality.
2) Advance knowledge of the interaction between α-amylase and starch to address issues related to unpredictable low FN.

We plan to publish our findings in research journals with a focus on biochemistry, such as the *Journal of Biological Chemistry*. We will also present our research to the wheat and cereal communities (e.g., the Wheat Quality Council meeting, the American Association of Cereal

Chemists International annual meeting) and in relevant publications, such as *Grain* magazine.

LITERATURE REVIEW - *Topic: <u>The variation of α-amylase isozymes</u>*

The enzyme α-amylase is synthesized within the cells of the aleurone layer. At germination, the synthesis of α-amylase is induced by a hormone released from the embryo[1-4]. Then, α-amylases migrate into the starchy endosperm and hydrolysis of the starch granules begins. A number of isozymes have been identified[5-7]. Two groups of isozymes were identified in winter wheat during development, sprouting, and germination[6]. Four isozymes in group I, representing 84% of the total activity, were readily adsorbed onto starch granules and degraded starch; three isozymes in group II were not adsorbed onto granules and may only hydrolyze the hydrolysates generated by group I isozymes. Eleven phenotypes of α-amylase were found in winter wheat varieties bred in Russia and Ukraine, and the distribution of phenotypes was associated with geographic origins[8]. A significant difference in α-amylase activities was also observed in the nine examined cultivars in China[9]. The same study also revealed α-amylase activities of drought-resistant cultivars were significantly higher than those of drought-sensitive cultivars under drought-stressed conditions[9]. An investigation of commodity wheat grown in the Republic of Kazakhstan demonstrated the synthesis of isoenzymes in the aleurone layer was responsible for a significant decrease in FN[10]. Taken together, research demonstrates various α-amylase isozymes exist in wheat, and the activities of isozymes and their interaction with starch granules differs and affects FN. The proposed research aims to identify the mutation of a specific α-amylase allele affecting FN, their hydrolytic capacity on degrading wheat starch granules, and their influence on viscosity during FN measurement.

BIBLIOGRAPHY:

1. Briggs, D., *Journal of the Institute of Brewing* **1963,** *69* (1), 13-19.
2. Briggs, D., *Annual Proceedings of the Phytochemical Society* **1973,** *1972* (9), 219-277.
3. Paleg, L.; Coombe, B.; Buttrose, M., *Plant Physiology* **1962,** *37*, 798-803.
4. Varner, J.; Ram Chandra, G.; Chrispeels, M., *Journal of Cellular and Comparative Physiology* **1965,** *66* (S1), 55-67.
5. Tkachuk, R.; Kruger, J. E., *Cereal Chemistry* **1974,** *51* (4), 508-29.
6. Sargeant, J. G., *Cereal Research Communication* **1980,** *8* (1), 77-86.
7. Jacobsen, J. V.; Scandalios, J.; Varner, J. E., *Plant physiology* **1970,** *45* (4), 367-371.
8. Netsvetaev, V. P.; Bondarenko, L. S.; Motorina, I. P., *Russian Journal of Genetics* **2016,** *52* (12), 1244-1252.
9. Zhao, H.; Zhang, H.; Wang, H., *Mailei Zuowu Xuebao* **2008,** *28* (4), 633-637.
10. Mamytova, N. S.; Kuzovlev, V. A.; Khakimzhanov, A. A.; Fursov, O. V., *Applied Biochemistry and Microbiology* **2014,** *50* (5), 531-537.

IDAHO WHEAT COMMISSION - BUDGET FORM

Allocated by	Idaho Wheat Commission	during FY 2016	$	-
Allocated by	Idaho Wheat Commission	during FY 2017	$	-

REQUESTED FY2018 SUPPORT:

	Salary	Temporary Help	Fringe	Travel	OE	Graduate Tuition/Fees	TOTALS
Idaho Wheat Commission	$ 18,720	$ -	$ 374	$ 4,000	$ 10,000	$ 9,004	$ 42,098

TOTAL BUDGET REQUEST FOR FY 2018: $ 42,098

BREAKDOWN FOR MULTIPLE SUB-BUDGETS:

	(PI name)	*(PI name)*	*(PI name)*	*(PI name)*
Salary		$ -	$ -	$ -
Temporary Help	$ -	$ -	$ -	$ -
Fringe Benefits	$ -	$ -	$ -	$ -
Travel	$ -	$ -	$ -	$ -
Operating Expenses	$ -	$ -	$ -	$ -
Graduate Student Tuition/Fees	$ -	$ -	$ -	$ -
TOTALS	$ -	$ -	$ -	$ -

Total Sub-budgets $ -

Explanatory Comments: (see FY2018 Guidelines for definition)

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