

PROJECT NO: New

TITLE: *Systems for selecting resistance/tolerance to pre-harvesting sprouting and late maturity α -amylase in wheat*

PERSONNEL: Jianli Chen, (Amy) Hui-Mei Lin, and Juliet Marshall

ADDRESS: Jianli Chen, University of Idaho (UI) Aberdeen Research & Extension Center,

Aberdeen, ID 83210; 208-397-4162, ext. 229; jchen@uidaho.edu; (Amy) Hui-Mei Lin,

Associate Professor, Bi-State School of Food Science, University of Idaho and Washington State

University Agricultural science building 118, 606 Rayburn Street, Moscow, ID83844-2312; 208-

885-4661; amylin@uidaho.edu.

JUSTIFICATION:

Nationally, Idaho wheat ranks top eight states in total production and the number one in hard white (HW) production. In addition to HW, soft white (SW) is another important class to the Idaho state, accounts for 50 to 60% of state production. However, both HW and SW are more vulnerable to pre-harvest sprouting (PHS) damage than hard red (HR) and soft red (SR) wheat, and PHS has resulted in over \$100 million dollars lost in Idaho in 2014 (Blaine, personal communication). PHS caused elevated α -amylase activity to over-hydrolyze starch and affect baking quality. Another threat to Idaho wheat, late maturity alpha amylase (LMA), also associated with the elevated α -amylase activity. To approach the problems, *we initiated the project to identify genetic factors controlling LMA in 2012 and characterized fifteen spring wheat cultivars and lines in response to a cold shock treatment in growth chamber experiments.* However, very little work has been conducted for PHS in wheat lines grown in Idaho and PNW. Furthermore, though both PHS and LMA are associated with α -amylase activity and poor baking quality, it is not clear how the current gold standard -falling number (FN) presents the true α -amylase activity, the change of starch structure due to elevated α -amylase activity and climate change, and their associated baking quality to present a fair market value to growers. The proposed project, in our opinions, is critical to the Idaho wheat industry to prevent the lost caused by PHS and LMA.

HYPOTHESIS & OBJECTIVES:

FN is a gold standard to evaluate the market value of wheat based on overall rheological properties. The working hypothesis in the proposed project is that PHS and LMA caused the increase of α -amylase activity and the change of starch structure due to the growing condition and elevated enzyme activity that result in poor baking quality. The variety differences can be tagged by molecular markers associated with PHS, LMA, and starch structure related traits. The objectives of this study in FY16 are to: 1) Conduct the third year cold-shock experiment in growth chamber to optimize LMA-screening protocol; 2) Screen LMA-inducible lines grown in diverse environments; 3) Screen PHS tolerant lines via molecular marker assisted selection and germination index; 4) Investigate the influence of PHS and LMA in the starch structural characters, α -amylase activity and gluten associating with baking quality.

PROCEDURES:

Objective 1. Conduct the third year growth chamber cold shock experiment and validate sensitive growth stages, LMA-resistant and susceptible genotypes

A total of 15 spring wheat lines including five of each SWS, HWS, and HRS classes used in 2013 will be applied in the proposed study. Two SW lines, Alturas and IDO686, are sensitive to cold shock treatment; two SWS cultivars, UI Stone and UI Pettit, are insensitive in our previous studies. The four cultivars or lines will be the controls in the proposed study.

The four controls and other 11 lines will be planted in a non-replicated trial in Aberdeen, ID in spring of 2015. Days to heading and flowering will be taken to determine the time for the cold shock treatment. Five growth stages (G1 to G5) were defined as 20, 25, 30, 35, and 40 days after flowering (DAF), respectively. At each growth stage, about 180 heads of each line will be cut at the base of the plant and all leaves of each stem are removed except for the flag leaf. The 180 heads with stem are divided evenly in three bundles of 60 each and immediately placed in a 5 gallon bucket half full of distilled water. One bundle is placed in a newer growth chamber and another in an older growth chamber. Both chambers are set for 10°C and 12 hours light for three days for cold treatment. The third bundle with a water bucket will stay in greenhouse until mature. After the cold shock treatment the first two buckets are moved to the greenhouse where the third bucket stays until harvest. Grain from the 60 heads will be harvested and tested for FN and alpha amylase content. Effects of treatment (T), genotype (G), growth stage (S), T x G, T x S, G x S, G x T, and T x G x S will be analyzed using SAS.

Objective 2. Screen LMA-inducible lines grown in diverse environments

This experiment will include spring wheat entries in the *Variety Yield Trials (VYT)* from the University of Idaho (UI) Variety Testing Program and entries in the *Elite Yield Trials (EYT)* from the UI Breeding and Genetics Program. The VYT will be planted in diverse environments including Idaho Falls, Ashton, Soda Springs, Kimberly, and Aberdeen, while the EYT will be planted in Tetonia, Kimberly, and Aberdeen in Southern Idaho. The FN of each line will be examined according to AACC Method 56-81B.

Objective 3. Screen PHS tolerant lines via molecular marker assisted selection and germination index

This experiment will focus on PHS screening but build upon materials that have been tested by FN in objective 1 and 2 above in previous two years. The selected lines will be assessed using germination index and molecular markers associated with seed dormancy genes *TaPHS1* on 3A (Liu et al., 2013) and *TaSdr-B1* on 2B (Zhang et al., 2014). These data will be used to determine which lines have resistance/tolerance to PHS or LMA, or both.

Objective 4. Investigate the influence of PHS and LMA in the starch structural characters, α -amylase activity and gluten associating with baking quality

This experiment is to test the hypothesis that PHS and LMA can influence starch structure, α -amylase activity in flours, and gluten and result in poor baking quality. Twelve cultivars or lines, four from each of the three market classes (SWS, HWS and HRS) are selected for the proposed study (Table 1). To investigate the starch structure, we will isolate starch and examine the granule size distribution, morphology, average amylose content, and individual molecular weight distribution of A- and B-type granules. To investigate the change and influence from α -amylase, we will measure the activity

Table 1. Selected cultivars/lines

Classes	Cultivars/lines*	FN
SWS	Alturas, M12001	Low
	UI Pettit, UI Stone	High
HWS	Blanco Grande**	Low
	UI Platinum	Varied
	IDO1202S, LCS Star	High
HRS	Bullzeye	Low
	IDO862E, Jefferson, Lassik	Varied

* The twelve lines harvested in 2012, 2013, 2014 and 2015 will be used for starch structure study.

** Known LMA susceptible cultivar

of α -amylase of selected flours and the pasting properties of pure starch and flours, respectively. To reveal the influence from protein, we will quantify the protein amount of selected flours and characterize the glutenin gene compositions using molecular markers tightly linked to the genes.

DURATION: Objective 1 & 2: 2015 (one year); Objective 3 & 4: 2015-2016 (two years)

COOPERATION: Katherine O'Brein, Idaho Wheat Quality lab, UI Extension program

ANTICIPATED BENEFIT/EXPECTED OUTCOMES/INFORMATION TRANSFER

The completion of the proposed study will present the cultivars/line that are resistant to LMA and PHS identified by the comprehensive studies of enzyme, starch structure, and gluten composition incorporated with the conventional measurements such as FN and germination. The contribution to the Idaho wheat industry will be significant, in our views, for preventing the loss of market value caused by PHS and LMA. Our research findings will be presented to the wheat industry in wheat related meetings and conferences and publish in referred.

LITERATURE REVIEW

PHS is one of the major problems in Idaho wheat industry. The resistance to PHS is a complex trait affected by both genotype and environment (Imtiaz et al.2008). Genes for grain color and seed dormancy have been regarded as two major factors affecting PHS resistance (Gfeller and Svejda 1960; Bewley 1997; Groos et al. 2002). White grain wheat is usually more susceptible to PHS than red grain wheat (Gale and Lenton 1987; Groos et al. 2002; Himi et al. 2002). Recently, two breakthrough have been reported, one is the cloning of dormancy gene *TaPHS1* on 3A (Liu et al., 2013), another is the cloning of dormancy gene *TaSdr-B1* on 2B (Zhang et al., 2014). Molecular markers associated with the two genes will accelerate breeding for PHS resistance.

LMA was first observed in a UK cultivar Professeur Marchal (Bingham and Whitmore, 1966) and thereafter observed in two genotypes Spica and Lerma52 in Australia (Mares and Gale, 1990). Lerma52 was extensively used for crossing by breeders at CIMMYT in Mexico. More recently, substantial numbers of commercial cultivars and breeding lines prone to LMA have been indentified (Mrva and Mares, 2001). At present LMA issues have been reported in the UK, Australia, Japan, Canada, South Africa, China, Mexico, and the U.S. In the U.S, LMA was mainly reported in high elevation areas in Southern Idaho and some areas in PNW.

Hagberg falling number (FN) is used widely in grain classification, quality control and marketing (Mares and Mrva, 2008). However, it measures an overall pasting property that many intrinsic and extrinsic factors can influence the value. For example, protein can absorbs water and affect the viscosity and FN (Oliete, Pérez et al. 2010). Starch, depends on its granular distribution and molecular weight distribution of individual type of starch, absorb water in different manners, especially amylose will generate more viscosity (Geera, Nelson et al. 2006). Thus, there are concerns about using FN to evaluate the quality change cuased by LMA and PHS. For example, high temperature shock (Mares and Mrva, 2008), nitrogen fertilizer application (Svensson, 1990; Kettlewell, 1999; Kindred et al., 2005), late application of fungicide (Svensson, 1990), and grain size (Evers et al., 1995) affect FN.

Starch structure is highly associated with baking quality. For example, bread loaf volume is related to protein and amylose amount (Wilson, Bechtel et al. 2008). The relative proportion of A- and B-type starch granule within a starch greatly affects wheat product properties. A-type has

higher amylose content depends on its genotype, and amylose directly affects the pasting properties (Geera, Nelson et al. 2006, Singh, Singh et al. 2010). Thus, starch granule distribution and molecular characters have an important role in baking quality.

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COMMODITY COMMISSION BUDGET FORM

Allocated by	Idaho Wheat Commission	during FY 2013	\$102,501
Allocated by	Idaho Wheat Commission	during FY 2014	\$44,973

REQUESTED FY 2014 SUPPORT:

	Salary*	Temporary Help**	Fringe	Travel***	OE****	CO	Grad Fees**	TOTALS
Idaho Wheat Commission								
Jianli Chen	\$ 9,927		\$ 4,604	\$ 1,000	\$ 4,000	\$ -		\$ 19,531
Hui-Mei Lin		\$ 16,931	\$ 508		\$ 1,000			\$ 18,439
Juliet Marshall	\$ 3,922		2,058		1,500			7,480
								\$ 45,450

OTHER RESOURCES (not considered cost sharing or match):

a) Industry (BASF)								
b) UI (salaries, operating)								
c) USDA-TCAP								
d) Start-up (Lin)						4000	9644	\$ 13,644
e)								\$ -
TOTAL OTHER RESOURCES								\$ 13,644

TOTAL PROJECT ESTIMATE FOR FY 2014:	\$ 44,561 (Requested)	\$ 13,644 (Other)	\$ 58,205 (Total)
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BREAKDOWN FOR MULTIPLE SUB-BUDGETS:

	Jianli Chen	Hui-Mei Lin	Marshall	(PI name)
Salary	\$ 9,927		\$ 3,922	\$ -
Temporary Help		\$ 16,931	\$ -	\$ -
Fringe Benefits	\$ 4,604	\$ 508	\$ 2,058	\$ -
Travel	\$ 1,000	\$ -	\$ -	\$ -
Operating Expenses	\$ 4,000	\$ 1,000	\$ 1,500	\$ -
Capital Outlay	\$ -	\$ -	\$ -	\$ -
Graduate Student Fees				\$ -
TOTALS	\$ 19,531	\$ 18,439	\$ 7,480	\$ -

* \$9927 is requested for two full-time IH, one to do falling number and one to do growth chamber experiment and genotyping for breeding program

\$3922 is requested for Linda to do falling number test of samples from extension variety trials.

** \$16931 is requested to cover a MS student fiscal year stipend.

***Partial travel cost for one professional meeting.

****Cost for land charge, genotyping, harvest bags for Chen; partial cost for starch analysis for Lin.