

PROJECT NO: BJKX25, BJKX39

TITLE: Digging the genetic factors underlying late maturity α -amylase in wheat

PERSONNEL: Jianli Chen, Yueguang Wang, and Juliet Marshall

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JUSTIFICATION:

The term late maturity α -amylase (LMA) is used to describe the synthesis of high pl α -amylase in the late stage of grain development in the absence of rain or sprouting (Mares and Mrva, 2008). Enzyme activity is retained in the grain at harvest, resulting in flour with low falling number (LFN) that is unsuitable for some of end uses. Grain with LFN (FN < 300 seconds) may be rejected as food products and downgraded to feed grain in the market. LMA damage in wheat has been a production concern in southern Australia and some areas in the UK for many years. Recently, LMA damage has become a production problem in some high elevation areas of southern Idaho and the Pacific Northwest. Some widely grown cultivars have shown consistent low falling numbers, including the soft white spring wheat 'Alturas', hard white spring wheat 'Blanca Grande', hard red spring wheat 'WestBred 936' and 'Jefferson', hard red winter wheat 'Boundary' and 'Promontory', and soft white winter wheat 'Brundage' (Gallup and Brown, personal communication). LMA also presents a significant challenge to breeders because, unlike preharvest sprouting, there are no physical signs of damage and at present there is no way to predict its occurrence (Mares and Mrva, 2008). Genetic control of LMA is complex and poorly understood. Therefore, it is essential to address this production concern using a combination of traditional breeding and molecular-assisted approaches.

HYPOTHESIS & OBJECTIVES:

We hypothesize that LLMA-inducible wheat can be identified when wheat plants are grown under a cold temperature shock 25 to 30 day after flowering. The cold shock treatment can be manipulated in growth chambers. We also hypothesize that genetic variation of LMA among wheat lines can be associated with specific molecular markers and mapped on specific chromosomal regions. The objectives of this study in FY15 are to: 1) optimize LMA-screening protocol; 2) screen LMA-inducible lines grown in diverse environments; 3) validate QTL associated with FN in a RIL population.

Procedures:

Experiment 1. Optimize LMA-screening protocol established in 2013

A total of fifteen spring wheat lines used in 2013 will be used in this experiment, including five each of the soft white, hard white, and hard red classes. Two soft white lines, Alturas and IDO686, are known to be sensitive to cold shock treatment and two soft white spring cultivars, UI Stone and UI Pettit, are insensitive to the cold shock treatment based on a previous study. The four cultivars or lines will be used as controls in the present study.

The four controls and 11 test lines will be planted in a non-replicated field trial in Aberdeen, ID in the spring of 2014. Days to heading and flowering will be recorded 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 and stem will be divided into three bundles of 60 each and immediately placed in a 5 gallon bucket half filled with distilled water. One bundle will be placed in a newer growth chamber and another in an older growth chamber. Both chambers will be set for 10°C and 12 hours light for three days for cold treatment. The third bundle will stay in greenhouse until mature. After the cold shock treatments the first two buckets will be moved to the greenhouse with the third bucket and remain until harvest. Grain from the 60 heads will be harvested and tested for FN and alpha amylase content. Iso-electric gel (IEF) electrophoresis will also be used when it is established. 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.

Experiment 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 Variety Testing Program and entries in the Elite Yield Trials (YET) from the University of Idaho Breeding and Genetics Program. The VYT will be planted in diverse environments including Idaho Falls, Ashton, Soda Springs, Kimberly, and Aberdeen, while the YET will be planted in four environments in Tetonia, Kimberly, and Aberdeen in Southern Idaho. The FN of each line will be tested using grain samples collected from these locations after harvest. FN testing will be conducted using standard protocol suggested by the manufacturer and AACC protocol developed by wheat marketing center. The FN will be used as a primary screening parameter for LMA damage.

Experiment 3. Validate QTL associated with FN in a RIL population

A total of 13 SNPs from 9 chromosomal regions were identified which associated with FN in an association panel in 2013 (unpublished data). These SNPs are being genotyped and saturated in the linkage maps derived from the UI Stone x Alturas RIL population. In 2014, we will plant the RILs of this population in replicated trials in two environments Aberdeen and Kimberly and assess the FN of each RIL after harvest. FN data from the two years will be used in QTL analysis to confirm QTL identified in the association panel and identify new QTL associated with FN in this population.

DURATION: We propose to take three years to establish a series of effective methods to identify wheat cultivars for resistance to LMA and to identify molecular markers that can be used as an alternative method for phenotypic selection. This is the second year of the project.

COOPERATION: Idaho Wheat Quality lab, UI Extension program, USDA-ARS NIFA TCAP

ANTICIPATED BENEFIT/EXPECTED OUTCOMES/INFORMATION TRANSFER

LMA damage significantly affects the wheat end-use quality. This project will establish the effective methods both in traditional and MAS to screen resistance to LMA which can be widely

used in the future. LMA-resistant cultivars selected or developed would benefit producers and wheat industry. Results obtained will be published in refereed journals and presented at national and international meetings.

LITERATURE REVIEW

LMA was first observed in a UK cultivar Professeur Marchal (Bingham and Whitemore, 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 identified (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 Pacific Northwest (PNW).

Methodology using Hagberg falling number is a simple and rapid measurement for determining α -amylase activity (Hagberg, 1960 and 1961; Perten, 1964). It is used widely in grain classification, quality control and marketing (Mares and Mrva, 2008). However, grain with a low falling number can be the result of not only LMA but also from other causes of α -amylase activity such as pre-harvest sprouting and high temperature shock (Mares and Mrva, 2008). High α -amylase activity included by warm temperature shock was also reported by the UK scientists (Major et al., 1996). In addition to temperature, nitrogen fertilizer application (Svensson, 1990; Kettlewell, 1999; Kindred et al., 2005), late application of fungicide (Svensson, 1990), and grain size (Evers et al., 1995) have been associated with variation in grain α -amylase levels and falling number. The high pl α -amylase is related to wheat kernel structure. Mrva (unpublished data) observed that high pl α -amylase is located in the aleurone tissue and adjacent starchy endosperm at the dorsal and lateral surfaces of the endosperm, while Evers et al. (1995) and Tjin Wong Joe et al. (2005) suggested that the high pl α -amylase is concentrated in the crease region of the grain. The explanation for this apparent difference remains unclear and needs to be further elucidated. Iso-electric focusing (IEF) gel electrophoresis was used to detect high pl α -amylase before Ceralpha Alpha Amylase Kit (not commercially available) was recently developed (Mrva and Mares, 2002).

LMA expression is triggered by a temperature shock, but this only occurs during a window of sensitivity around 25-30 days post-anthesis (Mrva and Mares, 2001a). Variation in LMA expression in wheat is strongly influenced by the environment and is highly variable within and between seasons and regions and is highly unpredictable (Mares and Mrva, 2008). It also varies between plots in field trials, between individual plants, between spikes on individual plants and within the grains in a spike (Gale et al., 1987 and Mares and Mrva, 2008). LMA expression was reduced in the presence of dwarfing genes such as *Rht-B1b* (*Rht1*), *Rht-D1B* (*Rht2*) and *Rht-B1c* (*Rht3*) that confer insensitivity of vegetative tissues of wheat plant to gibberellins (Mrva and Mares, 1996; Mrva et al., 2001b; Mrva et al., 2004).

The genetic mechanisms of LMA have been studied by Australian researchers for many years. The high pl isozymes in normal grain development are controlled by genes at homoeologous loci located on the long arms of chromosomes 6A, 6B and 6D in contrast to pl isozymes that are

controlled by genes at homoeologous loci located on the long arms of group 7 chromosomes (Gale, 1983; Gale and Ainsworth, 1984).

Due to the recessive nature of inheritance, selection of homozygous individuals can only be done at later generations in the breeding process. Therefore, phenotyping of LMA can be very expensive. Identifying molecular markers associated with LMA may be an alternative method to accelerate selection of LMA-resistant cultivars. At present, few mapping studies have been conducted and only in Australian materials. Two QTL were identified on chromosome 7B and 3B, respectively, in a DH population, and the effects of both were reduced in the presence of *Rht1* (Mrva and Mares, 2001b; Mrva et al., 2004). However, more recently, two major QTL on 6B and 7B were identified by an association mapping study in spring synthetic wheat (Emebiri et al., 2010). Considering overall technical problems for assessing LMA, additional mapping studies need to be conducted, especially using the LMA-resistant and susceptible materials identified in southern Idaho and in PNW wheat production areas.

REFERENCES:

- Bingham, J. and E.T. Whitmore. 1966. Varietal differences in wheat in resistance to germination in the ear and α -amylase content of grain. *Journal of Agricultural Sciences* 66:197–201.
- Emebiri, L.C, J.R. Oliver, K. Mrva, and D. Mares. 2010. Association mapping of late maturity α -amylase (LMA) activity in a collection of synthetic hexaploid wheat. *Mol Breeding* 26:39–49.
- Evers, A.D., J. Flintham, and K. Kotecha. 1995. Alpha-amylase and grain size in wheat. *Journal of Cereal Science* 21:1–3.
- Gale, M.D., A.M. Salter, and J.R. Lenton. 1987. The induction of germination alpha-amylase during wheat grain development in unfavourable weather conditions. In: Mares, D.J. (Ed.), *Fourth International Symposium on Pre-Harvest Sprouting in Cereals*. Westview Press Inc., Boulder, Co., USA, p. 273–282.
- Gale, M.D. 1983. Alpha-amylase genes in wheat. In: Kruger, J.E., LaBerge, D.E. (Eds.), *Third International Symposium on Pre-Harvest Sprouting in Wheat*. Westview Press Inc. Boulder, Co, USA, p.105–110.
- Gale, M.D. and C.C. Ainsworth. 1984. The relationship between α -amylase species found in developing and germinating wheat grain. *Biochemical Genetics* 22:1031–1036.
- Hagberg, S. 1960. A rapid method for determining α -amylase activity. *Cereal Chemistry* 37:218.
- Hagberg, S. 1961. Simplified method for determining α -amylase activity. *Cereal Chemistry* 38:202–203.
- Kettlewell, P.S. 1999. The response of alpha-amylase activity during wheat grain development to nitrogen fertilizer. *Annals of Applied Biology* 134:241–249.
- Kindred, D.R., M.J. Gooding, and R.H. Ellis. 2005. Nitrogen fertilizer and seed rate effects on Hagberg falling number of hybrid wheats and their parents are associated with α -amylase activity, grain cavity size and dormancy. *Journal of the Science of Food and Agriculture* 85:727–742.
- Major, B.J., P.S. Kettlewell, and G.D. Lunn. 1996. The effects of a period of high temperature during grain development on α -amylase activity in winter wheat (*Triticum aestivum*) in the absence of sprouting. In: Noda, K., Mares, D.J. (Eds.), *Seventh International Symposium on Pre-harvest sprouting in cereals*. Center for Academic Societies, Japan, Osaka, p. 441–447.

- Mares, D. and K. Mrva. 2008. Late-maturity α -amylase: Low falling number in wheat in the absence of preharvest sprouting (Review). *Journal of Cereal Science* 47:6–17.
- Mares, D.J. and M.D. Gale. 1990. Control of α -amylase synthesis in wheat grains. In: Ringlund, K., Mosleth, E., Mares, D.J. (Eds.), *Fifth International Symposium on Pre-Harvest Sprouting in Cereals*. Westview Press, Boulder, Co., USA, p. 183–194.
- Mrva, K., D.J. Mares, K.J. Williams, and J. Cheong. 2004. Molecular markers associated with late maturity α -amylase (LMA) in wheat. In: Black, C.K., Panozzo, J.F., Rebetzke, G.J. (Eds.), *Proceedings of the 53rd Australian Cereal Chemistry Conference*. RACI Cereal Chemistry Division, North Melbourne, Australia, p. 150–151.
- Mrva, K. and D.J. Mares. 2002. Screening methods and identification of QTLs associated with late maturity α -amylase in wheat. *Euphytica* 126:55–59.
- Mrva, K. and D.J. Mares. 2001a. Induction of late maturity α -amylase in wheat by cool temperature. *Aust. J. Agric. Res.* 52:477–484.
- Mrva, K. and D.J. Mares. 2001b. Quantitative trait locus analysis of late maturity α -amylase in wheat using the doubled haploid population Cranbrook x Halberd. *Australian Journal of Agricultural Research* 52:1267–1273.
- Mrva, K. and D.J. Mares. 1996. Inheritance of late maturity α -amylase in wheat. *Euphytica* 88:61–67.
- Perten, H. 1964. Application of the falling number method for evaluating α -amylase activity. *Cereal Chemistry* 41:127–140.
- Svensson, G. 1990. Linkages between sprouting resistance and some agronomic traits in wheat. In: Ringlund, K., Mosleth, E., Mares, D.J. (Eds.), *Fifth International Symposium on Pre-Harvest Sprouting in Cereals*. Westview Press, Boulder, Co, USA, p. 227–232.
- Tjin Wong Joe, A.F., R.W. Summers, G.D. Lunn, M.D. Atkinson, P.S. Kettlewell. 2005. Pre-maturity α -amylase and incipient sprouting in UK winter wheat, with special reference to the variety Rialto. *Euphytica* 143:265–269.

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 2015 SUPPORT:

	Salary	Temporary Help	Fringe	Travel	OE	CO	Grad Fees	TOTALS
Idaho Wheat Commission	\$ 15,458	\$ 16,931	\$ 6,672	\$ 4,000	\$ 11,500	\$ -	\$ 9,644	\$ 64,205

OTHER RESOURCES (not considered cost sharing or match):

a) Industry	\$ -
b) UI (salaries, operating)	\$ -
c) Other (local, state)	\$ -
d) USDA-TCAP (75% of Post Doc salary)	\$ 43,885
e)	\$ -
TOTAL OTHER RESOURCES	\$ 43,885

TOTAL PROJECT ESTIMATE FOR FY 2015:	\$ 64,205 (Requested)	\$ 43,885 (Other)	\$ 108,090 (Total)
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BREAKDOWN FOR MULTIPLE SUB-BUDGETS:

	Chen	Marshall	(PI name)	(PI name)
Salary	\$ 11,536	\$ 3,922	\$ -	\$ -
Temporary Help	\$ 16,931	\$ -	\$ -	\$ -
Fringe Benefits	\$ 4,614	\$ 2,058	\$ -	\$ -
Travel	\$ 4,000	\$ -	\$ -	\$ -
Operating Expenses	\$ 10,000	\$ 1,500	\$ -	\$ -
Capital Outlay	\$ -	\$ -	\$ -	\$ -
Graduate Student Fees	\$ 9,644	\$ -	\$ -	\$ -
TOTALS	\$ 56,725	\$ 7,480	\$ -	\$ -
			Total Sub-budgets	\$ 64,205

Budget:

* \$11536 is requested to cover 25% of post doctorate salary to do protocol development.

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

** \$16931 is requested to cover a MS student fiscal year stipend and \$9644 for graduate fee and health insurance.

***Travel to off-station sites to collect sample for LMA testing and attend one professional meeting.

****Cost for falling number test, alpha amylase test, PCR, containers, land charge, harvest and storage bags.

CURRENT AND PENDING SUPPORT Form:

Name: Jianli Chen

NAME (List PI/PD #1 First)	SUPPORTING AGENCY AND AGENCY NUMBER	TOTAL \$ AMOUNT	EFFECTIVE AND EXPIRATION DATES	% OF TIME COMMITT- ED	TITLE OF PROJECT
J. Chen	Current: Idaho Wheat Commission	\$240,665	7/1/13 – 6/30/14	20	Developing wheat cultivars for Idaho and world markets
J. Chen, J. Marshall	Idaho Wheat Commission	\$44,973	7/1/13 – 6/30/14	5	Digging the genetic factors underlying late maturity α -amylase (LMA) in wheat
J. Chen	USDA-ARS	\$262,620	2/1/2011 – 12/31/2015	5	Phenotyping of NSGC via TACA under T- CAP
Dubcovsky, Chen et al.	USDA-NIFA TCAP	\$510,492 subcon- tracted from 25M	2/1/2011 – 12/31/2015	10	Improving barley and wheat germplasm for changing environments
R. Zemetra, J. Chen, & D. Hole	USDA-NIFA, NNF	\$80,000 subcon- tracted from \$249,000	1/1/11 – 12/31/14	10	Developing the Next Generation of Neoclassical Plant Breeders
J. Chen	BASF	\$15,000	1/1/13 – 12/31/13	5	Development of Imazamox resistant winter cultivars
J. Chen	Idaho Wheat Commission	\$12,888	7/1/13 – 6/30/14	1	Endowment Fund
J. Chen	Pending: Idaho Wheat Commission	\$166,254	7/1/14 – 6/30/15	20	Developing wheat cultivars for Idaho and world markets
J. Chen, J. Marshall	Idaho Wheat Commission	\$56,725 subcon- tracted from \$64,205	7/1/14 – 6/30/15	5	Digging the genetic factors underlying late maturity α -amylase (LMA) in wheat

D. Strawn, J. Chen, P. McDaniel, J. Marshall	Idaho Wheat Commission	\$9,973 subcontracted from \$73,907	7/1/14 – 6/30/15	5	Field-based study of factors affecting cadmium uptake by wheat from Idaho soils
J. Chen	BASF	\$15,000	1/1/14 – 12/31/14	5	Development of Imazamox resistant winter cultivars
J. Chen	Idaho Wheat Commission	\$66,000	7/1/14 – 6/30/15	1	Combine weighing system upgrade
J. Chen	Idaho Wheat Commission	\$12,888	7/1/14 – 6/30/15	1	Endowment Fund

CURRENT AND PENDING SUPPORT Form:

Name: Juliet Marshall

NAME (List PI/PD #1 first)	SUPPORTING AGENCY AND AGENCY NUMBER	TOTAL \$ AMOUNT	EFFECTIVE AND EXPIRATION DATES	% OF TIME COMMITTED	TITLE OF PROJECT
	Current:				
Marshall, J.M., and Johnson (Schroeder)	Idaho Wheat Commission	\$29,090	7/1/13 - 6/30/14	10	Extension Wheat Nurseries
Marshall, J.M. and Johnson (Schroeder)	Idaho Barley Commission	\$13,000	7/1/13 - 6/30/14	8	Education for Barley Production / Extension Nurseries
Marshall, J.M. and Patterson, P.	Idaho Wheat Commission	\$9,746	7/1/13 - 6/30/14	7	Production Systems and Wheat Varieties for Dryland Grain
Marshall, J.M.	USDA-ARS SCA	\$9,000	10/1/13 - 9/31/14	10	Management of Wheat and Barley Root Pathogens in Idaho
Marshall, J.M.	Monsanto, Syngenta, Limagrain, etc	\$28,250	7/1/13 - 6/30/14	2	Private breeding company entries into the Extension Variety Trials

Marshall, J.M.	Bayer Crop Sciences, BASF, Syngenta, etc	\$53,092	8/1/13-7/31/14	8	Seed Treatment / Specialty Trials / Product Evaluation
Marshall, J.M.	Federal 047	\$1800		1	South Idaho Crop Management
Marshall, J.M.	Idaho State Funding	\$17,847	7/1/13 – 6/30/14	10	Barley Enhancement
Marshall, J.M.	Hatch Funding	\$1118	10/1/13 - 9/31/14	10	Foot Rot
Rashed, A. and Marshall, J.M.	Idaho Wheat Commission	\$7,800	7/1/13 – 6/30/14	2	Variety Screening for BYDV Resistance in Idaho
Marshall, J.M. and Schroeder, K.	Idaho Wheat Commission	\$9,000	7/1/13 – 6/30/14	5	Collaborative Nitrogen by Variety Interaction Study with LCS
Marshall, J.M. and Schroeder, J.	Idaho Wheat Commission	\$14,000	7/1/13 – 6/30/14	5	Biostimulant Efficacy Field Trial
Moore, A., and Marshall, J.M.	Idaho Barley Commission	\$16,000 (SA \$547)	2013-2014	1	Long-Term Impacts of Manure Application on Production of Barley and Other Crops
Moore, A. and Marshall, J.M.	Idaho Wheat Commission	\$18,210 (SA \$547)	2013-2014	1	Long-Term Impacts of Manure Application on Production of Wheat and Other Crops
Chen J., Wang, Y., and Marshall, J.M.	Idaho Wheat Commission	\$44,973 (SA \$7462.35)	7/1/13 – 6/30/14	2	Digging the genetic factors underlying LMA in wheat
Murray, T., Carter, A., and Marshall, J.M.	Idaho Wheat Commission	\$52,980 (SA \$4000)	7/1/13 – 6/30/14	1	Enhancing Resistance to Snow Mold Diseases in Winter Wheat
Marshall, J.M.	Idaho Wheat Commission	\$12,888	7/1/13-6/30/15	1	Endowment funding

	Pending:				
Marshall, J.M., and Schroeder, K.,	Idaho Wheat Commission	\$31,437	7/1/14 - 6/30/15	10	Extension Wheat Nurseries
Marshall, J.M. and Schroeder, K.	Idaho Barley Commission	\$14,672	7/1/14 - 6/30/15	8	Education for Barley Production / Extension Nurseries
Marshall, J.M. and Patterson, P.	Idaho Wheat Commission	\$9,746	7/1/14 - 6/30/15	7	Production Systems and Wheat Varieties for Dryland Grain
Marshall, J.M.	USDA-ARS SCA	\$6,042	10/1/14 - 9/31/15	10	Management of Wheat and Barley Root Pathogens in Idaho
Rashed, Marshall, Bosque-Perez, Pappu, Wallis, Eigenbrode	Idaho Wheat Commission	\$19,069	7/1/14 - 6/30/15	2	Wheat variety response to BYDV infection at different developmental stages
Rashed, A and Marshall, J.M.	Idaho Wheat Commission	\$36,400	7/1/14 - 6/30/15	2	A survey of central and eastern Idaho wireworm species and evaluating ecological and chemical approaches to maximize cereal production
Rashed, A and Marshall, J.M.	Idaho Barley Commission	\$15,540	7/1/14 - 6/30/15	2	A survey of central and eastern Idaho wireworm species and evaluating combinations of ecological and chemical approaches to limit damage to barley crops
Marshall, J.M. and Schroeder, K.	Idaho Wheat Commission	\$9,000	7/1/14 - 6/30/15	5	Collaborative Nitrogen by Variety Interaction Study with LCS

Marshall, J.M. and Schroeder, K.	Idaho Wheat Commission	\$14,000	7/1/43 – 6/30/15	5	Biostimulant Efficacy Field Trial
Moore, A. and Marshall, J.M.	Idaho Wheat Commission	\$19,110 (SA \$547)	2014-2015	5	Long-Term Impacts of Manure Application on Production of Wheat and Other Crops
Moore, A. and Marshall, J.M.	Idaho Barley Commission	\$16,000 (SA \$547)	2014-2015	5	Long-Term Impacts of Manure Application on Production of Barley and Other Crops
Rashed, Marshall, Bosque-Perez, Pappu, Wallis, Eigenbrode	Idaho Wheat Commission	\$19,069	2014-2016	3	Wheat variety response to BYDV infection at different developmental stages
Strawn, D., Chen, J., McDaniel, P., and Marshall, J.M.	Idaho Wheat Commission	\$73,907	7/1/14- 6/30/15	2	Field-based study of factors affecting cadmium uptake by wheat from Idaho Soils
Chen, J., Wang, Y., and Marshall, J.M.	Idaho Wheat Commission	\$64,205	7/1/14 – 6/30/15	2	Digging the genetic factors underlying LMA in wheat

INTERNAL PEER REVIEW/PRINCIPAL INVESTIGATOR VERIFICATION FORM

INTERNAL PEER REVIEW VERIFICATION

Commodity commissions/organizations require internal peer review by colleagues familiar with the subject matter. This proposal has been peer reviewed by the following individuals:

Reviewer 1: J.M. Boyman JM Boyman 1/6/14
(Type/Print name) (Signature) (Date)

Reviewer 2: Katherine O'Brien Katherine O'Brien 1/6/14
(Type/Print name) (Signature) (Date)

Dept. Head/ Rene McDaniel R McDaniel 1/8/14
Unit Administrator (Type/Print name) (Signature) (Date)

PROGRESS REPORT

PROJECT NO: BJKX25, BJKX39

TITLE: Digging the genetic factors underlying late maturity α -amylase (LMA) in wheat

PERSONNEL: Jianli Chen (Project Leader), Yueguang Wang (Post Doc), Junli Zhang (Ph.D student, graduated), Gabriel Garcia (MS student), Juliet Marshall (Collaborator), Katherine Obrien (Collaborator)

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ACCOMPLISHMENTS: The most important accomplishment this year is that we established a LMA-screening protocol in growth chamber and obtained useful information for LMA resistance or susceptibility in some of widely grown cultivars and new experimental lines. The results showed that the most sensitive growth stages for screening LMA inducible materials are 25 to 30 days after anthesis. The tested wheat lines were classified into three categories: LMA resistant, LMA-inducible, and LMA-susceptible.

We also assessed 215 elite lines harvested from four diverse environments Kimberly, Tetonia, Aberdeen irrigated, and Aberdeen terminal drought. We confirmed the 2012 findings on environmental and genetic effects: Kimberly is the most inducible environments and Alturas and derived lines are the most sensitive genotypes responsive to moisture and temperature changes in the growing environments.

The detailed progresses and plan for 2014 are described below:

Objective 1. Optimize LMA-screening protocol. The primary plan was to do a cold shock treatment for ten plants of five lines in five different growth stages. We modified the original plan and did a cold shock treatment for **50 plants of fifteen lines** in five different growth stages in **two growth chamber environments**. This significantly **increased amount of work**, but **allowed us** to have enough grain to do falling number and other tests, to compare if there is an effect on wheat market classes, to compare if there is an effect on growth chamber environments. Hiring the new MS student under National Need Fellowship allowed us to complete such modification. Gabriel Garcia spent whole summer conducting the field sampling and growth chamber treatment before he went to Moscow for classes this fall. Dr. Wang completed falling number and alpha amylase tests this fall. The results from the growth chamber experiment suggest that the desirable growth stage for screening LMA is likely the 25 to 30 days after anthesis. UI Stone, UI Pettit, and Lassik are the three cultivars showing FN > 300 sec. in five growth stages treated, while the remaining 12 lines showed FN < 300 sec. in two to five growth stages treated. There was no significant effect on wheat market classes, growth chamber environments. **In order to publish the research findings from this study it is necessary to repeat this experiment in FY15.**

Objective 2: Screen lines grown in diverse environments from breeding and extension programs for falling number (FN), alpha amylase (AA) activity, and germination starting rate (GSR) using protocols established in FY13

A total of 215 lines from three spring elite trials (SWS, HWS, and HRS) were screened for FN, which were harvested from four environments (Aberdeen irrigated and terminal drought, Kimberly, and Tetonia). Based on the number of lines with low falling number (LFN < 300 sec), 15 (14 SWS, 1HRS), 12 (8SWS, 3HRS, 1HWS), and 11 (10SWS, 1HRS) LFN lines were identified in Kimberly, Tetonia, and Aberdeen irrigated, respectively. Most of LFN lines are soft white spring wheat. We didn't found any LFN lines in Aberdeen terminal drought environment. The number of LFN lines reflected to the rainfall and irrigation water in these environments. The LFN lines may be caused by moisture induced alpha amylase (Similar to PHS). Some of LFN lines in this screening are consistent in the above growth chamber study, while a few LFN lines were only showed in this screening. **It is necessary to develop a protocol to separate LMA and PHS.** The half seed alpha amylase previously used cannot tell this difference. I had one student working on the isoelectric focusing gel (IEF) protocol this summer. **We need to have additional time to establish this in FY15.**

We haven't got chance to do AA and GSR in this set of materials because of the extensive falling number tests conducted in objective 1 and this experiment.

Objective 3: Validate SNP markers associated with FN

Dr. Junli Zhang completed the association mapping analysis for FN in materials from NSGC and submitted a manuscript to Crop Science. The thirteen SNPs from 9 chromosomal regions identified by Junli are being genotyped in in the mapping population derived from UI Stone x Alturas. These SNPs will saturate the genetic maps being constructed by a M.S student Santosh Nayak. Dr. Wang completed a falling number test for the RILs and parental lines in Stone x Alturas population using grain harvested this year. In 2014, we are going to plant this population in in two locations in Kimberly and Aberdeen and test for FN of each line after harvest.

PROJECTIONS:

1. Conduct the second cold shock experiment in growth chamber to repeat the 2013 experiment
2. Continue screening lines grown in diverse environments
3. Explore and try to establish a biochemical method (Isoelectric focusing gel electrophoresis) to separate LMA-induced and pre-harvest sprouting-induced alpha amylase
4. Conduct the second year phenotyping FN for UI Stone x Alturas RIL population
5. Have one MS student finish his thesis and degree program and submit manuscripts being derived from his thesis
6. Present research finding at CSSA and ASA meetings and Tri-state Growers Conference

PUBLICATIONS:

Zhang, J. Chen, J., Y. Wang, B. Bonman, J. Wheeler, W, Zhao, K. O'Brien, J.M. Marshall, H. Bockelman, and J. Bonman. 2013. Association mapping of Hagberg falling number in hard white spring wheat collection materials. Crop Science (Accepted).