PROJECT NO: New Project

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

PERSONNEL: Jianli Chen, Yueguang Wang, Junli Zhang, J. Clayton

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JUSTIFICATION: Late maturity α-amylase (LMA) is used to describe the synthesis of high pI α-amylase in the late stage of grain development in the absence of rain or sprouting (Mares and Mrva, 2008). The enzyme activity is retained in the grain at harvest, resulting in flour with low falling number that is unsuitable for bread-making and other end uses. Grain with very high levels of amylase activity 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. LMA damage has become a production problem in some of high elevation areas in southern Idaho and Pacific Northwest in recent years. Some widely grown cultivars have shown 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). Therefore, it is urgent to initiate a research program to solve this production concern using a combination of traditional breeding and molecular-assisted approaches.

HYPOTHESIS & OBJECTIVES: Based on the previous publications, expression of LMA can be induced under a cold temperature shock 25 to 30 day after flowering. Resistance to LMA can be identified when wheat plants are grown in field environments in which the cold temperature has been a problem during grain maturation, or in growth chambers with controlled cold temperature shock. LMA phenotype can be identified through assessing falling number and α-amylase activity. LMA-resistant and susceptible lines can be identified through extensive screening of varieties grown in the environments where wheat varieties often suffer cold temperature shock during the late stage of grain filling. The selected LMA-resistant varieties will be provided to wheat growers and be used as parents in the breeding program. LMA is controlled by one to two recessive genes and expression of LMA is significantly affected by environments. Molecular markers associated with LMA can be identified in recombination inbred lines (RILs) (biparental) and natural populations (association mapping). The use of biparental mapping populations exploits only the genetic diversity of the parental lines, while association mapping, also referred to as linkage disequilibrium (LD) mapping, can reveal the influence of specific sequence polymorphisms in candidate genes to phenotypic variation in a wider germplasm pool (Myles et al., 2009). The objectives of this study are: 1) to establish LMA-screening protocol for materials growing in southern Idaho; 2) to identify genetic variation in adapted cultivars and potential releases for resistance to LMA in diverse environments; 3) to identify QTL/markers associated with resistance to LMA.

PROCEDURES:

Experiment 1. Establish LMA-screening protocol

Considering the cost of testing, five lines, IDO599, IDO862, Alturas, IDO686, and IDO694 will be used in this experiment. IDO599 and IDO862 are two new releases that have high falling numbers, or resistance to LMA; while Alturas, IDO686, and IDO694 (Blanca Grande derived line) have low falling numbers, or are susceptible to LMA. Based on the protocols being used by Australian researchers, we will test the following modified protocols using our lines.

1.1 Greenhouse test. Eighteen plants of each five lines will be planted in the greenhouse and three plants of each line will be used as controls. Cold shock treatment (15/10°C, d/n) will be applied to three plants of each line for 7 days at 22, 24, 26, 28, and 30 days after flowering, respectively. We will assess α-amylase activity in two replicates using five half seeds of each plant after ripening. A total of 180 samples (18 x 5 x 2) will be assessed using a high pI α-amylase-specific ELISA kit (Mrva and Mares, 2002).

1.2 Field test at Aberdeen. Three replications of the five lines will be planted in the field in Aberdeen where LMA rarely occurs. We will apply cold shock treatment (15/10°C, d/n) for three spikes cut from each plot for 7 days at 22, 24, 26, 28, and 30 days after flowering, respectively. From each plant cut, three additional spikes cut from the same plot will be used as control and maintained in greenhouse until ripening. The spikes are cut below the third internode (retaining the flag leaf) and the stems placed in deionised water (i.e. without sucrose, nitrogen, or antiseptic) in greenhouse (for controls) or in a growth chamber for cold shock treatment with temperature at 15/10°C (d/n). After 7 days cold shock, the detached tillers are transferred to the same greenhouse where the control spikes are grown to complete ripening. We will assess α-amylase activity in two replicates of each spike using five half seeds of each spike after ripening. A total of 180 samples (6 x 3 x 5 x 2) will be assessed in the same way as the greenhouse test. An additional 15 grain samples harvested from the field will be used as the second control of each plot.

1.3 Field test at Ririe and/or Soda Spring. These two locations had LMA problems in previous years. Three replications of the five lines will be planted in the two locations, respectively. Ten spikes of each line will be cut twice a week (Monday and Thursday) for three weeks starting 30 days after flowering. Immediately dry the spikes after cutting. We will assess α-amylase activity of each plot for each cut in two replicates using five half seeds of each spike as well as grain sample after harvesting. By doing this test we can determine how the environment affects LMA expression and how each line responds to the environment. Weather conditions will be recorded at each cutting in the environments.

The results obtained from the three methods will be analyzed separately and compared to each other to determine effects of genotype, environment, testing methods, and genotype by environment interactions.

Experiment 2. Identification of genetic variation in adapted spring wheat cultivars and potential releases for resistance to LMA. We will focus on spring wheat for this experiment in the first year. 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 (EYT) from the University of Idaho Breeding and Genetics Program. Falling number of each line will be tested using grain sample from diverse locations after harvesting. The selected entries will be assessed for α-amylase activity. Temperature and precipitation of the test environments will be recorded during growing season, especially during grain filing stages.

Experiment 3. QTL mapping of LMA in natural and RIL populations

Association mapping. A total of 150 hard white spring wheat lines from the National Small Grain Collection (NSGC) will be used in this study. LMA phenotype of each line will be assessed using method developed in experiment 1.1. Over 5000 SNP data spanning the wheat genome are available for the 150 lines used and provided by TCAP project. After LMA phenotyping of the 150 lines is completed, association analysis will be conducted using JMP Genomics. QTL and SNP markers associated with resistance to LMA and resistant lines will be identified and used in the breeding program. We plan to complete this project in two years in 2012 and 2013. One MS student being supported by National Need Fellowship (NNF) will conduct this project.

RIL mapping. Two mapping populations (IDO599 x Alturas and IDO599 x IDO686) are being developed using modified single seed decent method. IDO599 is resistant to LMA; while Alturas and IDO686 are susceptible to LMA (Unpublished data). In 2013 and 2014, LMA phenotypic data of each line in the two mapping populations will be assessed using method developed in experiment 1; while SSR and SNP marker data of each line will be generated in Aberdeen lab or Wheat Genotyping Center at Pullman, WA. Phenotypic and genotypic data derived from the two populations will be used to identify QTL and markers associated with LMA and the results will be compared with those obtained from association mapping.

DURATION: three to five years

COOPERATION: N/A

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 industries.

LITERATURE REVIEW: LMA 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. In most cases, LMA expression is triggered by a cold temperature shock, but this only occurs during a window of sensitivity around 25–30 days post-anthesis (Mrva and Mares, 2001a). Expression is highly variable within and between seasons and regions, between plots in field trials, between individual plants, between spikes on individual plants and within the grains in a spike (Mares and Mrva, 2008). The high pI α-amylase (LMA-amylase) is related to wheat kernel structure. Mrva (unpublished data) observed that high pI 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 pI α-amylase is concentrated in the crease region of the grain. The explanation for this apparent difference remains unclear and need to be further elucidated.

The genetic mechanisms of LMA have been studied by Australian researchers for many years. The high pI 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 low pI isozymes that are controlled by genes at homoeologous loci located on the long arms of group 7 chromosomes (Gale, 1983; Gale and Ainsworth, 1984). QTL mapping on LMA has been conducted in DH lines and in synthetic wheat. Two QTL were identified on chromosome 7B and 3B, respectively, in a DH population, and the effect of both was 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).

We have conducted a preliminary screening for materials growing in southern Idaho and identified some LMA-resistant and susceptible lines. Recombination inbred line populations are being developed and QTL associated with resistance to LMA can be identified in our materials. In conjunction with the recently awarded Triticeae CAP project, a subset of materials from NSGC and a large amount of SNP marker data can be used in this project to accelerate identification of QTL associated with LMA and novel LMA-resistant materials that can be used in the development of LMA-resistant cultivars.

REFERENCES:

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Evers, A.D., J. Flintham, and K. Kotecha. 1995. α-amylase and grain size in wheat. Journal of Cereal Science 21:1–3.

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Tjin Wong Joe, A.F., R.W. Summers, G.D. Lunn, M.D. Atkinson, P.S. Kettlewell. 2005. Pre-maturity a-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 2011	$ -
Allocated by	Idaho Wheat Commission	during FY 2012	$ -

REQUESTED FY 2013 SUPPORT:

	Salary	Temporary Help	Fringe	Travel	OE	CO	Grad Fees	TOTALS
Idaho Wheat Commission	$ 10,500	$ 11,440	$ 4,561	$ 4,000	$ 15,000	$ 50,000	$ 7,000	$ 102,501

OTHER RESOURCES (not considered cost sharing or match):

a) Industry	$ -
b) UI (salaries, operating)	$ -
c) Other (local, state)	$ -
d) Other	$ 46,100
e)	$ -
TOTAL OTHER RESOURCES	$ 46,100

TOTAL PROJECT ESTIMATE FOR FY 2013:	$ 102,501 *(Requested)*	$ 46,100 *(Other)*	$ 148,601 *(Total)*

BREAKDOWN FOR MULTIPLE SUB-BUDGETS:

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

Total Sub-budgets $ -

10.31.2011 - Version

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CURRENT AND PENDING SUPPORT Form:

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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
	Current:				
J. Chen	IWC	$137,227	7/01/11 – 6/30/12	40	Developing wheat cultivars for Idaho and world markets
J. Chen and J. Marshall	IWC	$19,066	7/01/11 – 6/30/12	5	Marker-assisted breeding of fusarium head blight resistance in wheat
J. Marshall, J. Chen and D. Hole	IWC	$44,066	7/01/11 – 6/30/12	5	Protecting Idaho and PNW Wheat with Dwarf Bunt Resistance Development
J. Chen	IWC	$200,000	7/01/11 – 6/30/12	10	Plot Combine, Functional Equipment for Wheat Improvement
J. Chen	USDA-ARS	$246,625	02/01/11 – 12/31/15	10	Phenotyping of NSGC via TACA under T-CAP
Dubcovsky, Chen et al.	USDA-NIFA TCAP	$25M	02/01/11 – 12/31/15	15	Improving barley and wheat germplasm for changing environments
R. Zemetra, J. Chen, & D. Hole	USDA-NIFA, NNF	$249,000	01/01/11 – 12/31/14	5	Developing the Next Generation of Neoclassical Plant Breeders
J. Chen	BASF	$14,000	1/01/12 – 12/31/12	5	Development of Imazamox resistant winter cultivars

	Pending:				
J. Chen	IWC	$138,134	7/01/12 – 6/30/13	20	Developing wheat cultivars for Idaho and world markets
J. Chen	IWC	$102,501	7/01/12 – 6/30/13	15	Digging the genetic factors underlying late maturity α-amylase (LMA) in wheat

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:	Juliet M. Marshall	Juliet Marshall	1/2/12
	(Type/Print name)	(Signature)	(Date)
Reviewer 2:	Katherine O'Brien	Katherine O'Brien	1-3-12
	(Type/Print name)	(Signature)	(Date)
Dept.Head/ Unit Administrator	James B. Johnson	James B. Johnson	9 Jan. 2012
	(Type/Print name)	(Signature)	(Date)