PROJECT NO: New

TITLE: Characterizing the cause effect for low falling number hard white spring wheat grown in 2015 and 2016

PERSONNEL: J. Chen, R. Wang, K. Isham, J. Wheeler, W. Zhao, and N. Klassen

ADDRESS: Jianli Chen, University of Idaho (UI) Aberdeen Research & Extension Center, Aberdeen, ID 83210; 208-397-4162, ext. 229; jchen@uidaho.edu.

JUSTIFICATION

Wheat grown in Idaho and Pacific Northwest (PNW) has contributed the major supplies for domestic and international trade markers. The recent outbreaks (2014 and 2016) of low falling number (LFN) wheat have been threatening the wheat industry in this area. Two major causes of LFN wheat are pre-harvest sprouting (PHS) and late maturity alpha amylase (LMA). PHS is an immerging problem for wheat production in Idaho and PNW where the most of soft white wheat grown in the US because of increased rainfall during harvest season. Soft white wheat is more vulnerable to PHS than hard white, soft red, and hard red wheat. Breeding for PHS resistance in soft white wheat has not been started until recent years. LMA is an endemic problem induced by sudden temperature reduction during grain filling stage. Little progresses have been made in variety development because the effective screening methods of LMA are not available. Screening of PHS and LMA are both very difficult and confounded by environments in field nurseries and are very expensive in controlled environments. It is urgent to develop resistant cultivars to sustain wheat production in this area. The proposed research will use recently-developed LMA and known PHS screening protocols to characterize doubled haploid lines (DHLs) derived from the cross between UI Platinum x SY Capstone and use the most update genomics tools to identify molecular markers associated with PHS and LMA. Our ultimate goal is to discovery the genes controlling both PHS and LMA in our germplasm and introgression these genes in our cultivar development using molecular marker assisted selection.

RESEARCH HYPOTHESIS AND OBJECTIVES

Both PHS and LMA can result in the increase of α-amylase activity and LFN. The PHS can be induced at plant physiological maturity and commonly assessed by germination tests, while LMA can be induced by cold temperature shock for plants during grain filling stages (25 to 35 days after anthesis) before maturity. Based on the FN data the two parents are both LMA and PHS inducible cultivars. Resistance to LMA and PHS can be assessed separately with the appropriate conditions. The objectives of this study are: 1) to characterize the cause effect for the LFN DHLs by half-seed alpha amylase test (FY18); 2) to assess PHS and LMA separately under controlled environments (FY18); 3) to identify candidate genes controlling LMA and PHS upon the objectives1 and 2 completed (FY19 and FY20).

PRELIMINARY RESULTS

A mapping population, comprised of 111 DHLs were derived from the cross UI Platinum x SY Capstone. The two parents and DHLs were grown in three field with different planting dates in Aberdeen in 2015 and in Aberdeen, Soda Springs, and Moscow, ID in 2016. The three trials in 2015 and Aberdeen trial in 2016 were irrigated, while trials in Soda and Moscow in 2016 were non-irrigated. There was no visible pre-harvest sprouting observed, while natural infection of stripe rust occurred in all trials. Grains from each line in all trials were assessed by falling number (FN) test using standard AACC methods (Table 1). Using FN and SNP data we identified four major QTLs on chromosome 1B, 2B, 5A, and 7A. The SNP markers for 5A QTL have been converted to KASP markers and used in fine mapping and QTL dissection. To identify the candidate genes associated with LFN it is necessary to firstly understand the cause effect of LFN segregated in this population so that we can dissect the QTL to either LMA or PHS or both. Based on the weather data and FN data (Table 1) we tentatively conclude that the LFN lines in SD16 trial were contributed by both LMA and PHS, while the rest of five trials were contributed mainly by LMA. The proposed research will confirm our preliminary conclusion.

Table 1. Summary of FN data in the two parents and 111 DHLs in six trails.

	DHLs				Parents	
Trials	Min	Max	Mean	No. of LFN lines	UI Platinum	SY Capstone
AB15 a	199	408	320	38	297	325
AB15 b	190	396	314	42	273	319
AB15 c	205	406	312	35	265	318
AB16	200	366	312	36	334	329
Mos1 6	in progressing				352	335
SD16	**173**	360	**272**	**87**	**256**	**174**

PROCEDURES:

Objective 1. Characterize the cause effect for the LFN lines harvested in Soda and Aberdeen in 2016 using half-seed alpha amylase method

Based on the preliminary results we choose the 40 DHLs plus the two parents in the first step to understand the cause effect of LFN occurred in the six trails in 2015 and 2016. The 40 DHLs included 36 LFN DHLs from AB16, four HFN DHLs in both SD16 and AB16. It is known that if alpha amylase (AA) content in embryo (head) part of the kernel was higher than that in the distal part of kernel (tail) we say the cause of PHS, the opposite we say the cause of LMA. We have used the AA ratio ((AAhead-AAtail)/(AAhead+AAtail)) to separate PHS and LMA. We have purchased a kernel cutter that can cut 48 kernels at a time in the previous study. All alpha amylase activities will be tested in microplate using Megazyme kit from Ceralpha Alpha-Amylase (Product code: K-CERA, http://secure.megazyme.com/Ceralpha_Alpha-Amylase).

Objective 2. Assess PHS and LMA separately under controlled environments

2.1 Assess PHS in DHLs and the two parents

We propose to screen PHS resistance in summer 2017. The 111DHLs and the two parents will be planted in 5 x 10 feet yield plots in a randomized complete block design with two replications in the field. Days to heading, flowering, physiological maturity (PMD), and daily weather data will be recorded. At PMD the DHLs will be assessed for PHS (percentage of germinated kernels) using germination method published (Lin et al. 2016). We have established a spike germination system in greenhouse that can do 200 spikes at a time. The PHS data of the DHLs will be used in QTL analysis and the identified QTL will be compared to those identified on 1B, 2B, 5A, and 7A.

2.2 Assess LMA in DHLs and the two parents

Based on where the wheat plants are grown we can screen LMA in two methods, spike-cutting and whole plant cold shock. We propose to use the whole plant method to screen LMA in the DHLs in fall of 2017 and/or spring of 2018. Because of page limit the method details will not be described here. Upon the completion of 2.1 and 2.2 we will know specifically which QTL associated with LMA or PHS. We will develop specific KASP to screen NILs in objective 3.

Objective 3. Identify candidate genes controlling LMA and PHS upon the objectives1 and 2 completed in FY19 and FY20

DURATION: three years

COOPERATION: D. Fu, Y. Wang, K. Schroder, A. Lin, J. Marshall, K. O'Brein

ANTICIPATED BENEFIT/EXPECTED OUTCOMES/INFORMATION TRANSFER:

The completion of the proposed study will help us to understand the genetic control of LMA and PHS. Through transgressive segregation we will select few DHLs that have improved resistance to LMA and/or PHS. The identified markers will accelerate cultivar development for resistance to both problems. In long term, growers will benefit from growing such resistant cultivars, milling and baking industry will use high quality grain in their products and increase their profit. Our research findings will be published in Idaho grain Magazine, Wheat Life, referred journals, presented at different growers and professional meetings. One to two graduate students will also be trained through this project.

LITERATURE REVIEW

Falling number (FN) is an important quality characteristic for wheat (Hagberg, 1960). It has been widely used as a measure of grain quality for wheat and other cereals (Belitz et al., 2004). Grain flour with low FN produces dough that is sticky and difficult to process and loaves that are discolored and poorly structured (Chamberlain et al., 1982). The FN test measures the rheological properties of starch and the degree of starch damage. PHS can increase a-amylase activity, causing starch breakdown, which results in lower FN. PHS is usually caused by a premature break in seed dormancy under humid and wet conditions before harvest, by excessive rain, and sometimes by lodging. Red-seeded wheat usually has longer seed dormancy and better PHS resistance than white wheat (Liu et al., 2008). Measuring spike germination (Kulwal et al., 2012) and testing a-amylase activity are commonly used to assess PHS, but both are time-consuming and expensive.

Low FN values can also be associated with LMA, a genetic defect involving the synthesis of high isoelectric point (pI) isozymes of a-amylase during the later stages of grain development in the absence of germination, resulting in mature grain with high pI a-amylase (Mares and Mrva, 2008). Assessing LMA is more difficult than assessing PHS. Expression of LMA is usually triggered by cool temperature shock during the middle stages of grain development (Mrva and Mares, 2001; Mrva et al., 2006). Screening for LMA requires a controlled environment such as a growth chamber. Isoelectric focusing (Gale and Ainsworth, 1984; Mares and Gale, 1990) and an Enzyme Linked Immunosorbent Assay (ELISA; Mrva et al., 2006) have been used to detect and quantify high-pI a-amylase. Both assays are time consuming and expensive. In addition, the ELISA format is patented and not yet commercially available.

Molecular markers associated with FN have been identified in several studies (Kunert et al., 2007; Zhang et al., 2014), but their use in selecting of LMA have not been evaluated. Recently, we identified two major QTL $X_{QTL.ui\text{-}2B}$ and $X_{QTL.ui\text{-}5A}$ associated with FN (manuscript is in preparation) and developed KASP markers for the two QTL. In the proposed study (FY18) we are going to discern the cause effect for the two QTL in relation to PHS and LMA.

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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	$ 8,128	$ 16,208	$ 4,916	$ 2,500	$ 11,000	$ 5,173	$ 47,925

TOTAL BUDGET REQUEST FOR FY 2018: $ 47,925

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)

11.21.2016 - Version