PROJECT NO: BJKQ25

TITLE: Wheat alpha-amylases and their effect on falling numbers

PERSONNEL: Daolin Fu, Assistant Professor, Department of Plant, Soil and Entomological Sciences, University of Idaho

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JUSTIFICATION:

Wheat is the second most important cereal crop in the United States. The annual wheat production in the US Pacific Northwest (PNW) is ca. 6.5 million metric tons, and the PNW wheat accounts for a large portion of the US wheat exported abroad.

However, high levels of α-amylase activity in wheat grains compromise the end-use quality and causes substantially lower prices for wheat growers (2). The level of α-amylase is reflected in falling numbers (FN). The FN method is a viscometric assay involving gelatinization of flour or meal in boiling water and subsequent liquifaction of starch by α-amylase, which is an international measure for grain receival and trade. Preharvest sprouting (PHS) and late maturity α-amylase (LMA) are two major causes of unacceptably high levels of α-amylase (or low FN) in ripe wheat grain (2).

α-Amylases (EC 3.2.1.1) hydrolyze α-1,4 internal glucoside linkages in starch and related carbohydrates. In vascular plants, α-amylases are divided into three families according to cellular localization and gene structure (3). Both barley and rice have ten α-amylase isoform genes (4, 5). All α-amylases have a conserved active site at the C terminus of the protein. Family one α-amylases, such as cereal grain α-amylases that mobilize starch endosperm, is targeted to the secretory pathway. Family two α-amylases have no predicted targeting peptide, and their function remains largely unknown. Family three α-amylases have a large N-terminal extension (about 400–500 residues in length), which may play a role in leaf starch breakdown (6). Each member might have different substrate specificity and target different compartments within the cell. Different α-amylase genes have specific expression in germinating seeds, developing caryopses, and/or leaf sheaths (4, 7). Some α-amylase genes may also generate different isoforms due to alternate splicing or different transcription start sites.

Over the past 30 years, wheat α-amylases have been intensively studies. Due to the lack of the whole genome sequence of wheat, the majority of past studies had no chance to look at all α-amylase genes in wheat. Recently, wheat genome sequence becomes publically accessible, which permits us to discover all wheat α-amylase genes. However, whether there is a small subset of α-amylases that affects wheat FN is still unknown and requires further investigation.

HYPOTHESIS & OBJECTIVES:

We hypothesize: 1) wheat α-amylase genes display spatiotemporal expression during wheat development and grain filling, 2) a subset of α-amylase genes are specifically expressed in

developing caryopses and/or germinating seeds, and 3) a mutation of a specific *a*-amylase allele affects flour end-use quality as measured by falling number.

Our objectives are to: 1) identify the entire *a*-amylase gene family in wheat, 2) characterize the expression profile of different *a*-amylase genes in wheat, and 3) determine the effect of caryopsis/seed-specific *a*-amylase genes on reducing falling numbers in wheat.

PROCEDURES:

Characterize the expression profiles of the a-amylase gene family. Based on the Year-one data, we know that *a*-amylase genes are specifically expressed, and there are only a subset of *a*-amylase genes are active in the grain stages. Therefore, we are interested in discovering genes specifically expressed in grains, especially those at the late maturity stage. Using RNA-seq, we are going to investigate global transcription of *a*-amylase genes in wheat 'Brundage'. We will perform RNA-seq in developing caryopses at the watery stage (2 days after anthesis = 2 DAA), medium milk stage (14 DAA), and soft dough state (30 DAA). Three biological replicates will be processed for each developmental stage.

Determine the role of Group IV a-amylases on affecting wheat falling numbers. Based on Year-one data, we plan to prioritize our research on the Group IV *a*-amylase genes. First, we will screen for mutant lines on A and B alleles. There are ninteen mutation lines for the 5B allele, but we will use TILLING to screen mutants of the 5A allele. When both A and B mutants are identified, we plan to generate double mutation lines by combining the A and B mutations. Homozygous mutant lines will be obtained for single mutation on A or B, and for double mutations on both A and B. Their effect on falling numbers will be compared to the wild genotype Kronos. Data acquired here will be used to advance common wheat.

DURATION: 2 years, Objective 1 (FY 16-17); Objective 2 (FY 16-18); Objective 3 (FY17-18)

COOPERATION: Fu will lead the project and work with visiting scholars and/or graduate students on the gene search, expression profiles and data mining. Fu will present research findings at professional conferences, and communicate research progress and results to the Idaho Wheat Commission. Visiting scholars, graduate students and other personnel working on data collection will reports progress to Fu. The research team will regularly communicate via e-mail, phone, and face-to-face meetings. Falling Number (FN) will be measured in facilities accessible to the University of Idaho.

ANTICIPATED BENEFITS/EXPECTED OUTCOMES/INFORMATION TRANSFER:

1) Provide wheat research community with information on the relationship between specific *a*-amylase genes and wheat falling numbers.
2) Provide wheat growers with information on planting wheat cultivars that have less *a*-amylase activity during late maturity and help growers earn more profit on high FN grains.
3) Provide wheat breeders with information about which *a*-amylase may affect FN and help breeders develop cultivars with good end-use quality.

4) Provide the wheat industry with strategic information on how to solve low FN in wheat production.

LITERATURE REVIEW:

Preharvest sprouting causes the production and activation of α-amylase inside the wheat kernel, which causes low falling numbers (FN) in wheat, However, late maturity α-amylase (LMA) in wheat can cause low FN instead of preharvest sprouting (8). Wheat LMA involves the untimely synthesis of high pI α-amylase during the middle to later stages of grain development and ripening (8). LMA-prone wheat tends to produce high levels of α-amylase when exposed to cool temperatures during grain development (8). It has been suggested that LMA is caused by the activation of the *α-Amy1* genes on wheat chromosomes 6A, 6B, and 6D (9), but never convincingly proved. Therefore, it will be interesting to look at mutants of the *α-Amy1* genes in the homeologous group 6 in wheat.

Based on the currently known α-amylase genes in rice and barley, we retrieved multiple α-amylase genes in wheat. Based on the diversity of α-amylase genes in other species, the wheat family may be incomplete in current searches.

Using wheat α-amylase genes, we were able to identify the existing mutants in the Kronos population. In addition, we will use Targeting Induced Local Lesions in Genomes (TILLING) to recover mutations of a specific α-amylase copy that has no sequence data in the Kronos population.

BIBLIOGRAPHY AND REFERENCE CITED:

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2. Mares DJ & Mrva K (2014) *Planta* 240:1167-1178.
3. Stanley D, Fitzgerald AM, Farnden KJF, et al. (2002) *Biologia* 57:137-148.
4. Sugimura Y, Michiyama H, & Hirano T (2015) *Plant Prod. Sci.* 18:277-283.
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10. Whan A, *et al.* (2014) *J. Exp. Bot.* 65:5443-5457.
11. Ral J-P, *et al.* (2016) *Plant Biotech. J.* 14:364-376.

IDAHO WHEAT COMMISSION - BUDGET FORM

Allocated by	Idaho Wheat Commission	during FY 2016	$	-
Allocated by	Idaho Wheat Commission	during FY 2017	$	40,000

REQUESTED FY2018 SUPPORT:

	Salary	Temporary Help	Fringe	Travel	OE	Graduate Tuition/Fees	TOTALS
Idaho Wheat Commission	$ -	$ 31,425	$ 2,325	$ 3,000	$ 13,250	$ -	$ 50,000

TOTAL BUDGET REQUEST FOR FY 2018: $ 50,000

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

ANNUAL REPORT

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ACCOMPLISHMENTS:

Identification of wheat a-amylase genes

Using known *a*-amylase genes, we searched genome and transcriptome databases of polyploid wheat. In total, we identified twenty genomic and eleven cDNA copies in hexaploid wheat 'Chinese Spring', and nine genomic copies in tetraploid wheat 'Kronos' (Figure 1). Based on their protein sequence, we divided them into four groups. Groups I and II are close in homology, but they are quite different from the Groups III and IV. While, the Group IV displays significant variation when compared to other groups. Within groups, different copies have high similarity at cDNA level, but whether they share similar expression patterns remains to be answered.

cDNA Expression of a-amylase genes in common wheat

To understand the epxression of each groups/genes, we searched the WheatExp database, which is a homoeologue-specific database of gene expression profiles for polyloid wheat. We identified eleven active genes of *a*-amylase, which again fit four major groups of *a*-amylase. G1-6, G2-2, and G3-1 displayed high expression in spikes (Z65) and early caryopsis (Z71), several Group III genes (G3-1, G3-1b, and G3-3c) had ubiquitous expression in all tested tissues, however the Group IV genes (G4-1b and G4-1c) were specifically epxressed in grains at the soft dough stage (Z85) (Figure 2). These data agree with our assumption: *a*-amylase genes may display spatiotemporal expression during wheat development and grain filling. We plan to confirm their expression in wheat 'Brundage'.

Identification of a-amylase mutants in durum wheat

As planned in original proposal, we will use Kronos mutants to validate the function of selected *a*-amylase genes. By blasting the Kronos database, we identified nine genomic copies of the *a*-amylase genes (Figure 1). At least one copy was assigned to each of four *a*-amylase groups. For the identified Kronos genes, we discovered 264 point mutations that either introduce a different animino acid or cause a premature stop codon (Table 1). As for the Group IV *a*-amylase, we identifed ninteen mutations on the 5B allele. We have requested mutant lines and planted them in greenhouse.

PROJECTIONS:

Characterize the expression profiles of the a-amylase gene family

Based on the Year-one data, we know that *a*-amylase genes are specifically expressed, and there are only a subset of *a*-amylase genes are active in the grain stages. Therefore, we are interested in discovering genes specifically expressed in grains, especially those at the late maturity stage. Using RNA-seq, we are going to investigate global transcription of *a*-amylase genes in wheat 'Brundage'. We will perform RNA-seq in developing caryopses at the watery stage (2 days after anthesis = 2 DAA), medium milk stage (14 DAA), and soft dough state (30 DAA). Three biological replicates will be processed for each developmental stage.

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Based on Year-one data, we plan to prioritize our research on the Group IV *a*-amylase genes. First, we will screen for mutant lines on A and B alleles. There are ninteen mutation lines for the 5B allele (Table 1), but we will use TILLING to screen mutants of the 5A allele. When both A and B mutants are identified, we plan to generate double mutation lines by combining the A and B mutations. Homozygous mutant lines will be obtained for single mutation on A or B, and for double mutations on both A and B. Their effect on falling numbers will be compared to the wild genotype Kronos. Data acquired here will be used to advance common wheat.

Table 1: Mutations of the *a*-amylase genes in Kronos

Index	Gene Groups	Gene ID	Point Mutations		
			Missense	Nonsense	Silent
1	G1-2	UCW_Kronos_U_jcf7180000459809	33	1	33
2	G1-3	UCW_Kronos_U_jcf7180000459537	35	2	24
3	G1-5	UCW_Kronos_U_jcf7180000428306	15	2	9
4	G1-7	UCW_Kronos_U_jcf7180000426487	25	2	24
5	G2-1	UCW_Kronos_U_jcf7180000454774	12	0	60
6	G3-1a	UCW_Kronos_U_jcf7180000440383	19	1	12
7	G3-2a	UCW_Kronos_U_jcf7180000457019	41	3	36
8	G3-2b	IWGSC_CSS_6AL_scaff_5759140	51	3	48
9	G4-1b	IWGSC_CSS_5BL_scaff_10862015	18	1	23
		Total	249	15	269

Note: A missense point mutation results in a codon that codes for a different amino acid, a nonsense point mutation results in a premature stop codon, and a silent point mutation results in a codon that codes for the same amino acid.

Figure 1 Phylogenetic tree of *a*-amylase genes in wheat and barley
Trimmed proteins of common wheat ('Chinese Spring', without label), durum wheat ('Kronos', red circles), and barley (blue diamonds) were aligned using the Muscle module in MEGA 6.06 (1); a neighbor-joining tree was constructed using 1000 bootstrap iterations.

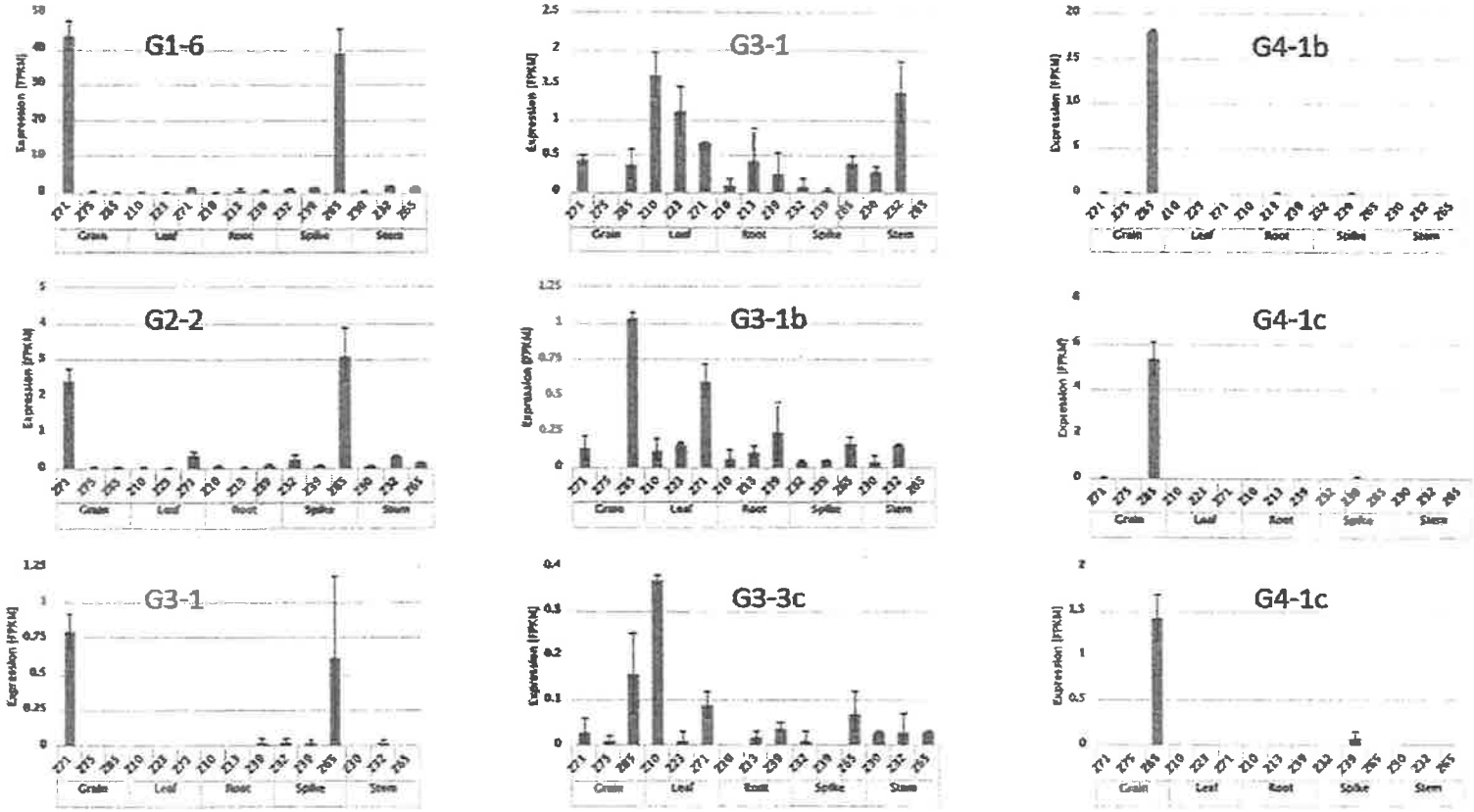

Figure 2 Expression profiles of α-amylase groups/genes in wheat

Nine α-amylase genes are from Groups I (G1), II (G2), III (G3), and IV (G4). Growth stages are at Zadoks scales.

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