Developing Celiac Friendly Wheat

Karl D. Glover and Stacy Lindblom-Dreis
Reporting period: July 1, 2015 – June 30, 2016
Total project period: July 1, 2015 – June 30, 2016
Report type: Final report

Research Summary:

The α-gliadin subfraction of the prolamine family has been implicated as the most-immunogenic (stimulator of the immune response) of these storage proteins. Accumulation of these storage proteins is influenced by genotype-by-environment (GxE) interactions. It has been suggested that older varieties may have as much immunogenic protein, with greater natural variation in protein motifs, as contemporary varieties, which has been debated in the popular press. The aim of this study was to assess the accumulation of α-gliadin in hard red spring wheat (HRSW) lines developed by the SDSU Spring Wheat Breeding Program. Inadvertent positive selection for traits has been described in other crops, which prompted the question regarding possible indirect selection on α-gliadin during wheat cultivar development.

Introduction:

To address the question, three objectives were proposed: 1) to develop an economical high-throughput method to assess α-gliadin accumulation in 200 genotypes of HRSW; 2) to choose 40 varieties (20 high- and 20 low-accumulating lines) to further test with the commercially-available RidaScreen® ELISA; and 3) to confirm the unique protein profiles of the 40 lines using a protein-separation method.

Description of Accomplishments:

Objective 1: Develop a high-throughput method to assess gliadin amounts in 200 genotypes.

An indirect Enzyme-Linked ImmunoSorbent Assay (ELISA) was developed in-house using commercially-available detection reagents. This step was completed prior to the beginning of this study. Varieties grown in 2013 and 2014 were assessed using the original version of the test. However, due to a change in vendor offerings, the ELISA method had to be revised in 2015. Aside from the change in cost ($10.27 per genotype per year to $14.45 per genotype per year), the revision could have led to differences between the original and revised tests that returned a marked change in the measurements. For this reason, a Bland-Altman evaluation was performed on the results of samples analyzed by both tests. The Bland-Altman evaluation did not decisively find the formats dissimilar, although the revised ELISA reported a slightly lower amount of α-gliadin per test. The measurements were considered similar enough to compare 2013-2014 and 2015 measurements.

Over 200 varieties were screened with the described ELISAs between 2013 and 2015. Of these, 195 varieties were retained for α-gliadin analysis; 61 were grown in 2 or more years. These varieties included 13 performance check varieties and SDSU experimental lines used in the Advanced Yield Trials. Only varieties selected for quality evaluation at the USDA Wheat Quality Lab in Fargo were included in the analysis reported here. An additional 40 curated varieties were obtained from the National Germplasm Repository (GRIN). These varieties were released between the late 1800s-1990s. Only a few grams were obtained, so no USDA quality assessments have been performed on these varieties at this time. Sufficient grain was obtained for the α-gliadin assessments and for planting larger plots of 37 varieties in a future trial.

Analysis of variance indicated that environmental effects strongly influenced α-gliadin accumulation. This was investigated further using a stability analysis method (Additive Mean effects and Multiplicative Interactions, or AMMI) on the 61 varieties grown over two or more years. Due to hail damage that led to a complete loss of the Groton location in 2015, a trial location near Watertown was substituted and the environmental component

was changed to location-years. The strongest influence on α-gliadin accumulation identified by AMMI was environment (F = 12.29, df = 8, p < 0.0001). The trial block (nursery) also had a strong effect on α-gliadin accumulation (F = 3.15, df= 14, p = 0.0199). Genotype had a less pronounced effect.

An indication of a similar trend in older released varieties might be seen in Figure 1. The α-gliadin accumulation of the 37 GRIN accessions, grown at the Brookings location and harvested in 2015, show little difference from the 2015 Brookings AYT lines in α-gliadin accumulation. Statistical analysis with the Mann-Whitney and Levene tests indicates that the average and variance in accumulations are similar in the GRIN accessions and contemporary varieties (U = 1005, p = 0.304; F = 3.238, p = 0.072 on 1,85 df). These results are derived from one season's data from one location, and should be interpreted with caution until the 2016 samples have been evaluated.

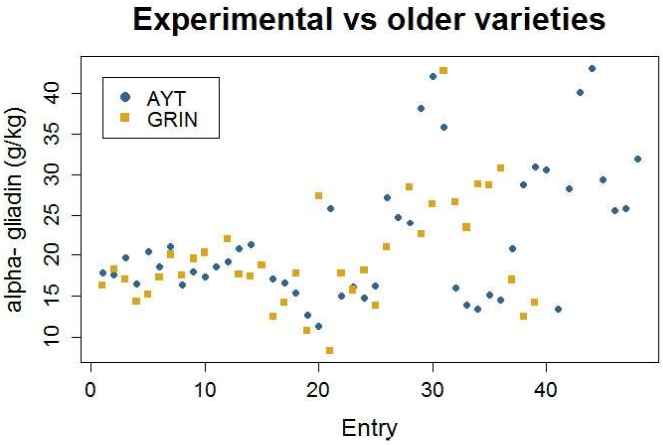

Figure 1. Comparison of the 37 GRIN accessions with 48 AYT entries grown in Brookings, 2015. The x-axis represents entry numbers for each set of cultivars, and not equivalent genotypes.

The high- and low-gliadin cultivars are summarized in Tables 1 and 2. The average α-gliadin accumulation of the 61 cultivars grown in three locations over two or more years was used to determine the 25th and 75th quartiles of this group. The corresponding bottom and top 20 lines were chosen by rank. Nine of the 12 performance check varieties grown in two or more years fell into these two groups. The α-gliadin accumulations of the check cultivars are summarized in Table 4 in the Supplementary section.

Table 1. Twenty low-gliadin accumulating HRSW varieties identified in Objective 1. Performance check varieties are highlighted in bold text.

Ranks	Name	average α-gliadin
1	SD4557	18.39
2	SD4576	18.86
3	SD4584	19.68
4	SD4578	20.76
5	SD4575	21.15
6	SD4559	21.29
7	SD4580	21.31
8	SD4579	21.50
9	SD4492	21.66
10	SD4552	22.27
11	**PREVAIL**	**23.04**
12	SD4518	25.03
13	SD4465	25.12
14	SD4493	25.20
15	SD4582	25.22
16	SD4606	25.56
17	SD4451	25.80
18	SD4532	25.82
19	SD4416	25.89
20	**KNUDSON**	**26.64**

Table 2. Twenty high-gliadin accumulating HRSW varieties identified in Objective 1. Performance check varieties are highlighted in bold text.

Rank	Name	average α-gliadin
42	SD4470	29.56
43	SD4514	29.61
44	**FOREFRONT**	**29.68**
45	**TRAVERSE**	**29.74**
46	SD4472	30.18
47	SD4529	30.42
48	SD4520	30.94
49	**OXEN**	**31.11**
50	SD4330	31.53
51	SD4321	31.57
52	SD4506	31.65
53	SD4403	31.70
54	**BRICK**	**31.76**
55	SD4595	32.23
56	SD4587	32.45
57	**FALLER**	**32.57**
58	**BRIGGS**	**32.96**
59	SD4299	33.35
60	SD4589	33.63
61	**STEELE-ND**	**34.50**

Objective 2: Use a commercial kit to evaluate high- and low-gliadin accumulating lines.

The previously-described ELISAs were used as a screening tool to identify candidate lines for further testing. The first α-gliadin measurements were evaluated to identify the most appropriate analysis method. The two test groups were selected from the high and low quartiles of the 61 HRSW varieties grown in two or more years. A two-sample t-test was used to compare 1) the means α-gliadin accumulation in the low and high lines as measured by both tests, and 2) the means as measured by each test. These two groups accumulated significantly different levels of α-gliadin as measured by the ELISAs developed for Objective 1, but no difference between the groups was identified by the commercial RidaScreen® ELISA. Ultimately seven varieties were dropped from the analyses performed in Objective 3 (three and four varieties were removed from the low and high groups, respectively) due to unacceptable within-sample variance in the commercial assay results.

These results are likely due to the difference in detection format. The in-house assays were developed using a polyclonal antibody, or mixture of antibodies naturally released by the mammalian immune system, that can detect any portion of the α-gliadin protein regardless of its condition. The polyclonal antibody permits an estimate of total α-gliadin accumulation, rather than a measure of true quantity or of a specific protein variant. The commercial assay uses a monoclonal antibody, which is composed of a single type of antibody capable of detecting a specific portion of the α-gliadin protein. This enables the commercial kit to quantify the amount of immunogenic gliadin in a sample, but is also limits it by the properties of its target. The α-gliadin protein is ethanol-soluble and tends to aggregate in aqueous solutions. The target portion is then "hidden" within a clump of protein, blocking the monoclonal antibody from binding. In addition, ethanol may affect the ability of the monoclonal antibody to bind to its target. The commercial assay is designed to detect trace amounts (as little as 2 parts per million) of α-gliadin in a complex prepared-food sample. This requires a straight flour sample—which may contain around 22 parts per thousand α-gliadin—to be heavily diluted before the test can be used. The in-house ELISAs permitted the use of up to 50% ethanol in the final step and fewer dilution steps to perform the measurements than the commercial test. The commercial test was originally used to calibrate the in-house ELISAs, leading to the similarity in final measurement levels.

Objective 3: Confirm unique protein profiles using Acid-PAGE.

The low- and high-gliadin accumulating lines were evaluated for unique banding patterns using polyacrylamide gel electrophoresis. Eighteen protein bands were identified from the Acid-PAGE performed (Figure 2). Low to moderate correlations were calculated between the α-gliadin protein patterns and accumulation quantities measured in Objectives 1 and 2. A linear model was generated using stepwise selection of candidate protein bands for use as predictors of α-gliadin accumulation as measured by the in-house ELISAs. The best model chosen used the 10 protein bands that contributed most highly to the model fit (Table 3), and explained approximately 65% of the variation observed between the low- and high-accumulation groups.

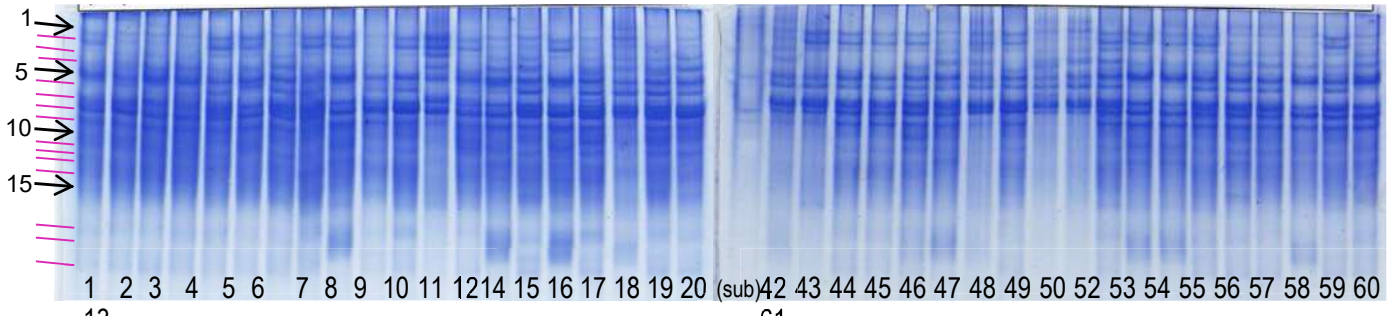

Figure 2. Acid-PAGE patterns of the 20 low- and 20-high accumulating varieties. The patterns of α-gliadin are typically consistent within cultivars, regardless of the quantity accumulated. Seven varieties were ultimately removed from the analysis of this work, and will be re-tested. The relative ranks of the varieties increase from left to right (lowest (SD4557) at far left, to highest (Steele-ND) at far right). Another variety (sub) was included in place of SD4506 (51) on this gel. The band identities are summarized in the key on the left. Nine performance check varieties were included in the low and high groups.

Table 3. Summary of the linear regression model developed using stepwise regression. The α-gliadin accumulation measured by the in-house ELISA was used as the response variable. These protein bands contributed most significantly to the model fit. One protein band, band 8, was identified by the variable selection procedure but was left out of this model due to its low significance to model fit. The residual squared error on 22 degrees of freedom was 2.583, and this model explained approximately 64.82% of the variation between the low and high variety groups. (F = 6.896, df = 10, 22, p < 0.0001). Random error = 20.71, p < 0.0001). The k-fold validation (k = 3) of this model indicated that the band names highlighted with bold text most highly contributed to model fit, with an overall mean squared error of 14.7 (n = 11).

Protein band	Coefficient	SE	t	Sig.
Band 1	4.1085	1.3047	3.149	0.0046
Band 3	-5.6354	1.7238	-3.269	0.0035
Band 4	3.1237	1.3622	2.293	0.0318
Band 5	-2.4312	0.9946	-2.445	0.0230
Band 11	-2.7219	1.2725	-2.139	0.0440
Band 12	2.7311	1.0158	2.689	0.0134
Band 13	2.7492	1.2441	2.210	0.0378
Band 14	3.3526	1.2980	2.583	0.0170
Band 15	-3.1033	1.4756	-2.103	0.0471
Band 16	7.0070	1.6527	4.240	0.0003

Projections

The remaining genotypes will be screened during the 2016 harvest; samples for quality assessment will be submitted to the USDA quality lab in Fargo. Weak correlations were found between the current panel of quality traits and α-gliadin accumulation in the 2013-2015 dataset (Figure 3). This indicates that indirect selection is unlikely responsible for α-gliadin accumulation in this HRSW population.

The model developed in Objective 3 will be validated by resampling and tested against the 2016 data. Environmental factors such as latitude, longitude, temperature, and planting dates will be included in the revised stability analysis to evaluate environmental influence on this trait. The varieties listed in Tables 1 and 2 will be assessed using DNA markers for the α-gliadin genetic locus.

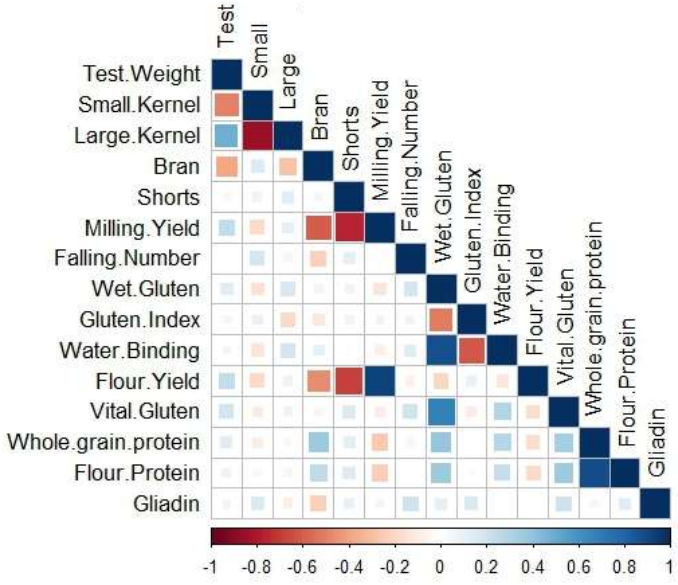

Figure 3. Correlations between wheat quality traits and α-gliadin. The color scale and square size indicate the strength of the relationship between the traits. 0 = no correlation. 1 = strong correlation.

Publications:

No publications have been submitted at this time.

Acknowledgements

This research was supported by the South Dakota Wheat Commission, the South Dakota Board of Regents and the National Institute of Food and Agriculture through the South Dakota Agricultural Experiment Station at South Dakota State University. We wish to acknowledge the assistance of the Spring Wheat Breeding Program staff and students.

Supplementary data

Table 4. Accumulation of α-gliadin at each location-year for the 13 performance check cultivars. Hail damage at the Groton location in 2015 led to substitution of data from the Watertown location. (--) indicates no data for a location-year. These measurements include samples taken from the advanced, preliminary, and parallel preliminary yield trials (AYT, PYT, and PPY).

	Brookings			Groton			Selby			Watertown		
Variety	2013	2014	2015	2013	2014	2015	2013	2014	2015	2013	2014	2015
ADVANCE	39.51	22.58	17.9	18.71	27.15	--	27.03	16.09	40.66	--	--	34.78
BRICK	70.21	20.23	17.57	25.15	28.71	--	25.80	24.55	42.26	--	--	31.35
BRIGGS	39.41	23.06	19.73	24.74	31.26	--	43.17	21.02	41.42	--	--	28.27
FALLER	35.86	35.86	16.49	31.42	28.41	--	61.28	19.45	40.68	--	--	23.72
FOCUS	--	--	20.38	--	--	--	--	--	45.73	--	--	21.67
FOREFRONT	60.68	28.40	25.35	27.01	24.70	--	64.21	22.28	35.34	--	--	31.07
GRANGER	28.10	20.89	21.03	27.46	27.2	--	42.94	20.67	35.01	--	--	19.79
KNUDSON	22.63	23.82	16.41	25.67	20.72	--	54.07	31.72	31.21	--	--	13.48
OXEN	40.83	26.46	18.02	27.89	23.11	--	24.01	35.61	41.55	--	--	16.79
PREVAIL	--	25.27	23.74	--	26.08	--	--	20.05	28.07	--	--	29.06
SELECT	25.38	22.6	18.61	34.17	22.74	--	46.66	21.23	38.12	--	--	20.07
STEELE-ND	66.05	23.97	19.15	46.98	25.07	--	48.07	18.79	37.99	--	--	24.43
TRAVERSE	35.71	21.57	20.76	44.86	19.25	--	45.86	20.67	41.14	--	--	17.88