Quantitative Variation of HMW Glutenin Subunits from Hard Red Spring Wheats Grown in Different Environments[1]

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ABSTRACT

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The objective of this study was to investigate the quantitative variation of HMW glutenin subunits in relation to glutenin polymers and hence breadmaking quality across different environments. Six genotypes of hard red spring (HRS) wheat were grown at seven locations in North Dakota in 1998 in a randomized complete-block experimental design with three replicates at each location. Unreduced SDS-soluble glutenins of flour were fractionated by multistacking SDS-PAGE into different sized glutenin polymers, followed by SDS-PAGE and imaging densitometry to determine the quantitative variation of HMW glutenin subunits. SDS-insoluble glutenin polymers also were examined for their quantitative composition of HMW glutenin subunits. The results showed that the percentage of HMW glutenin subunits was significantly affected by growing locations. The quantity of HMW glutenin subunits in SDS-insoluble glutenins was significantly and positively correlated with loaf volume. SDS-insoluble glutenin polymers had a higher percentage of HMW glutenin subunits than did SDS-soluble glutenins. SDS-insoluble glutenin polymers in flour were positively and significantly correlated in proportions of both total and individual HMW glutenin subunits in total SDS glutenins. SDS-insoluble glutenin polymers also were positively and significantly correlated with the combined proportion of HMW glutenin subunits 2* + 5. The results of this study indicated that either subunit 2* or 5 might be more important in forming a greater quantity of larger SDS-insoluble glutenin polymers than other subunits. SDS-insoluble glutenin polymers from different cultivars or locations could have different quantities of HMW glutenin subunits in their composition. SDS-insoluble glutenin polymers with more HMW glutenin subunits might be larger sized than those with less HMW glutenin subunits. Environment significantly influenced the quantitative variation of HMW glutenin subunits, which in turn affected the size distribution of glutenin polymers, and hence breadmaking quality.

Flour quality of hard red spring (HRS) wheat is important in determining its suitability for making breads. Understanding the mechanism of variation for flour quality would be of interest to plant breeders, wheat growers, grain traders, and end-use processors. Flour protein content has long been known to be a major factor in determining dough properties and breadmaking quality (Finney and Barmore 1948) and is significantly affected by environmental conditions (Johnson et al 1972; Cochran et al 1978; Rao et al 1993). Protein qualities, such as size distribution of glutenin polymers and ratio of gliadins to glutenins, could be significantly affected by genotype, environment, and genotype-by-environment interaction (Gupta et al 1992; Blumenthal et al 1995; Ciaffi et al 1995; Graybosch et al 1995). Previously, we investigated cultivar and environmental effects on the quantities of different protein fractions including SDS-soluble and -insoluble glutenin polymers and the size distribution of SDS-soluble glutenin polymers as determined by multistacking SDS-PAGE (Zhu and Khan 2001). Our results showed that environment affected the quantity of SDS-soluble and -insoluble glutenins and the size distribution of glutenin polymers. Quantities of high molecular weight (HMW) glutenin subunits were associated with the sizes of glutenin polymers. The results of Payne and Corfield (1979) showed that larger size glutenin aggregates contained more HMW glutenin subunits than smaller size aggregates. Gupta et al (1993) and Popineau et al (1994) also indicated that a higher ratio of HMW glutenin subunits in glutenin aggregates were more difficult to extract than in easily extractable fractions. Results of Graveland et al (1985) indicated that the smallest glutenin aggregates did not contain HMW glutenin subunits. More detailed information was obtained regarding the quantitative composition of different sizes of glutenin polymers using a technique of multistacking SDS-PAGE to separate glutenin polymers into various species of different molecular weights (Khan and Huckle 1992). Huang and Khan (1997a) showed that there was a gradual decrease of the ratio of HMW glutenin subunits to low molecular weight (LMW) glutenin subunits as the sizes of glutenin polymers decreased. The larger glutenin aggregates contained a higher proportion of HMW glutenin subunits than did the smaller aggregates.

In their study of the relationship between HMW glutenin subunits and breadmaking quality for HRS wheat, Khan et al (1989) found that the scoring system of Payne et al (1987) to predict breadmaking quality for HMW glutenin subunits did not work for HRS wheat. A large variation of quality characteristics existed but 95% of HRS wheats contained good quality subunits (Khan et al 1989). However, further studies suggested that the quantities of both total HMW glutenin subunits (Huang and Khan 1997b) and individual subunits (Huang and Khan 1997c) played an important role in determining the dough mixing strength and breadmaking performance of HRS wheats.

Therefore, to understand more about environmental variation of quality for HRS wheat, this study was conducted to investigate how quantitative variation for HMW glutenin subunits was associated with different sizes of glutenin polymers, and hence breadmaking quality across different locations.

MATERIALS AND METHODS

Experimental Design

Experimental design and information about cultivars and locations is as outlined in Zhu and Khan (2001). Six cultivars of HRS wheat were grown in seven locations representing climatic variations across North Dakota. Among the cultivars, five (Sharp, Russ, ND 705, Forge, ND 706) had the HMW glutenin composition of 2*, 7+9, and 5+10, and one (Hamer) had 2*, 7+8 and 5+10 according to the nomenclature of Payne et al (1987). Randomized complete block design was used with three replicates at each location.

Protein Extraction and Quantification

Extraction and quantitative determination of unreduced SDS-soluble and -insoluble glutenin polymers were reported previously (Zhu and Khan 1999, 2001). Unreduced SDS-soluble proteins were extracted at room temperature from 60 mg of flour using 1 mL of 0.05*M* sodium phosphate buffer containing 0.5% SDS (w/v), pH 6.8, and 10% (v/v) glycerol vortexed for 8 hr. The supernatant was determined for protein content using the micro-Kjeldahl method containing all SDS-soluble proteins including SDS-soluble glutenins. The SDS-soluble glutenins were fractionated by multistacking SDS-PAGE (Khan and Huckle 1992). The gels were stained with Comassie Brilliant Blue R-250 dye and the proteins were quantified by densitometry (Zhu and Khan 2001). The % SDS-soluble

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glutenins in flour was obtained by multiplying the percentage value of the SDS-soluble glutenins from densitometry with % SDS-soluble proteins in flour as determined by the micro-Kjeldahl method. The proteins in the residue were regarded as SDS-insoluble glutenins with their percentage determined by the difference between total flour protein content and SDS-soluble protein content. Flour protein content was determined by near-infrared reflectance spectrometry (NIR) (Approved Method 39-11, AACC 2000).

To determine and quantify the HMW glutenin subunit composition of various origins of multistacking SDS-PAGE, preparative multistacking SDS-PAGE was run with gels 3 mm thick. Subsequent quantification of HMW glutenin subunits was determined according to Zhu et al (1999). Composition of HMW and LMW glutenin subunits was determined by SDS-PAGE according to Huang and Khan (1997b). The gels were stained with Coomassie Brilliant Blue G-250 dye (Neuhoff et al 1988, 1990), whose intensity is dependent on interaction with basic amino acid residues (Lys, His, Arg) (Burnouf and Bietz 1985; Tal et al 1985) and the adjoining hydrophobic amino acid residues (Tal et al 1985). Percentage of different HMW glutenin subunits in SDS-soluble glutenins was obtained by their relative percentage from densitometry on SDS-PAGE gels of reduced SDS-soluble glutenins. Percentage of total HMW glutenin subunits was the sum of the percentage of different HMW glutenin subunits. The quantity of HMW glutenin subunits in SDS-soluble glutenins was determined by multiplying the relevant % HMW glutenin subunits from densitometry with the known % SDS-soluble glutenins in flour. The residue (containing SDS-insoluble glutenins), after extraction removal of SDS-soluble proteins, was extracted with 400 μL of Na phosphate buffer containing 0.05*M* sodium phosphate, pH 6.8, 0.5% SDS (w/v), 20% glycerol (v/v), and 1% (w/v) dithiothreitol reducing agent (DTT) at 65°C by vortexing for 1 hr. After centrifugation at 10,000 × *g* for 5 min on a minicentrifuge (Eppendorf 5415C), the supernatant was collected and the extraction was repeated once. The pooled supernatants from the two extractions were analyzed for protein concentration using a protein assay (Bio-Rad DC) compatible with the reducing agent (Hercules, CA). The assay, based on the reaction of protein with an alkaline copper tartrate solution and Folin reagent, is a modified Lowry assay developed for protein samples solubilized in detergent. The supernatants (15 μg) were loaded on an equal protein basis onto a gel 0.75 mm thick for SDS-PAGE (Huang and Khan 1997b). Each sample was run as three replicates in the same gel. The staining and subsequent quantification of HMW glutenin subunits were conducted according to Zhu and Khan (1999). The % HMW glutenin subunits in SDS-insoluble glutenin polymers was determined by densitometry on SDS-PAGE gels of reduced SDS-insoluble glutenin polymers. This percentage was used to calculate the quantity of HMW glutenin subunits in SDS-insoluble glutenin polymers by multiplying with the amount of SDS-insoluble glutenins determined previously in flour.

Baking Tests

Baking tests were performed according to Approved Method 10-09 (AACC 2000) with the following modifications. Fungal amylase (SKB 15) replaced malt dry powder. Instant dry yeast (1%) was used in lieu of compressed yeast. Ascorbic acid (10 ppm) and shortening (2%) were added as required. Doughs were mechanically punched using 6-in. rolls, and mechanically molded (Roll-R-Up, National Mfg., TMCO, Lincoln, NE). Loaf volume was determined by rapeseed displacement measurement 30 min after bread was removed from oven.

Statistical Analyses

Analyses of variance (ANOVA) was conducted and Pearson's correlation coefficients were determined using statistical software (10.51 Extra, Minitab Inc., State College, PA). Comparison was made using least significant difference (LSD) at probability of 0.01.

RESULTS AND DISCUSSION

Effect of Genotype and Environment on Quantity of HMW Glutenin Subunits

ANOVA showed that genotype (G), environment (E), and genotype-by-environment interaction (G × E) all had significant effects on % HMW glutenin subunits from either SDS-soluble glutenin poly-

TABLE I
Percentage of HMW Glutenin Subunits (GS) from SDS-Soluble Glutenins at 4% Origin of Multistacking SDS-PAGE and SDS-Insoluble Glutenins Among Cultivars from Seven Locations

	% Total HMW GS		Subunits 2*+5		Subunit 7		Subunits 9+10	
Cultivars	SDS-Soluble	SDS-Insoluble	SDS-Soluble	SDS-Insoluble	SDS-Soluble	SDS-Insoluble	SDS-Soluble	SDS-Insoluble
Hamer	9.8a[a]	18.7a	1.71a	4.2a	4.05a	7.0a	4.06a	7.4a
Sharp	9.8a	20.4a	2.09a	5.0a	3.94a	7.6a	3.74a	7.8a
Russ	10.2a	20.5a	2.28a	5.3a	3.98a	7.5a	3.92a	7.7a
ND 705	9.1a	20.0a	1.87a	4.8a	3.60a	7.1a	3.59a	8.0a
Forge	9.2a	19.5a	1.77a	4.7a	3.71a	7.0a	3.72a	7.8a
ND 706	7.6a	16.6a	1.26a	3.7a	3.11a	5.9a	3.23a	7.1a
$LSD_{0.01}$[b]	3.25	5.19	1.10	2.10	1.43	2.19	1.21	1.77

[a] Values followed by the same letter in the same column are not significantly different ($P < 0.01$)
[b] Least significant difference ($P < 0.01$).

TABLE II
Percentage of HMW Glutenin Subunits from SDS Soluble Glutenins at 4% Origin of Multistacking SDS-PAGE and SDS-Insoluble Glutenins Among Locations from Six Cultivars

	% Total HMW GS		Subunits 2*+5		Subunit 7		Subunits 9+10	
Locations	SDS-Soluble	SDS-Insoluble	SDS-Soluble	SDS-Insoluble	SDS-Soluble	SDS-Insoluble	SDS-Soluble	SDS-Insoluble
Langdon	11.8ab[a]	22.8ab	2.6ab	5.7ab	4.8a	8.3a	4.5ab	8.7a
Williston	11.2ab	21.7a–c	2.1a–c	5.3ab	4.2ab	7.7ab	4.9a	8.7a
Casselton	6.7c	16.4cd	1.2c	3.6bc	2.8c	6.0ab	2.8cd	6.7b
Hettinger	8.7bc	18.5a–d	1.5bc	4.1bc	3.4bc	6.8ab	3.9b–d	7.7ab
Dickinson	7.6c	17.4b–d	1.4bc	4.1bc	3.0c	6.3ab	3.2b–d	6.9ab
Carrington	6.1c	14.7d	0.9c	2.9c	2.6c	5.7b	2.7d	6.1b
Minot	12.7a	23.5a	3.3a	6.6a	5.3a	8.3a	4.1a–c	8.7a
$LSD_{0.01}$[b]	3.52	5.58	1.22	2.26	1.15	2.37	1.31	1.91

[a] Values followed by the same letter in the same column are not significantly different ($P < 0.01$)
[b] Least significant difference ($P < 0.01$).

mers at 4% origins of multistacking SDS-PAGE or SDS-insoluble glutenin polymers. Only the 4% origins were investigated because 4% origins contained the largest sized aggregates with a higher proportion of glutenins in the flour of good quality compared with the flour of poor quality (Huang and Khan 1997a). Among genotypes, no significant difference was observed for % total HMW glutenin subunits at the 4% origins and from SDS-insoluble glutenin polymers (Table I). In contrast, location showed a significant difference (Table II), indicating a greater environmental effect existed on the % total HMW glutenin subunits from SDS-soluble and -insoluble glutenin polymers. Evidently, environment could have a significant effect on % total HMW glutenin subunits in both SDS-soluble and -insoluble glutenin polymers.

To determine the response of individual HMW glutenin subunits to environment, the amounts of three groups of HMW glutenin subunits of different molecular weights were compared among the cultivars as well as among the locations. Subunits 5 and 2* have the largest molecular weight size among all glutenin subunits identified so far (Shewry et al 1992) and were combined for better quantification because in this study these two subunits were adjacent in the separation. Likewise, the smaller subunits 9 and 10 were quantified together, except that the cultivar Hamer had subunit 8 instead of subunit 9. The mid-sized subunit 7 was quantified individually. Coomassie dyes could produce different staining intensities depending on the content of basic amino acid residues of HMW glutenin subunits (Burnouf and Bietz 1985). The % HMW glutenin subunits 2* and 5 might be underestimated and subunit 10 might be overestimated (Wieser and Zimmermann 2000). However, scanning densitometry of Coomassie dye stained electrophoresis gels has been used and is accepted by researchers in quantifying HMW glutenin subunits (Payne et al 1981; Galili and Feldman 1983; Kolster et al 1992; Huang and Khan 1997c). In the present study, the comparison of cultivars and locations for quantification of HMW glutenin subunits by scanning densitometry of gels is further justified because almost all the cultivars had the same HMW glutenin subunit composition. The results of this study showed that there were no significant differences among cultivars for the HMW glutenin subunits from both the 4% origins of multistacking SDS-PAGE and SDS-insoluble glutenin polymers (Table I). In contrast, there were significant differences among locations for all the three groups of HMW glutenin subunits in their percentages from both SDS-soluble glutenin polymers at the 4% origins of multistacking SDS-PAGE and SDS-insoluble glutenin polymers (Table II). These results indicate that environment could have a dramatic influence on percentages of different HMW glutenin subunits in glutenin polymers.

Quantity of HMW Glutenin Subunits and SDS-Insoluble Glutenin Polymers

SDS-insoluble glutenin polymers contained a greater proportion of HMW glutenin subunits than did SDS-soluble glutenin polymers (Table III), indicating that more HMW glutenin subunits were required than LMW glutenin subunits in forming larger glutenin polymers. This is in agreement with the results of previous studies (Payne and Corfield 1979; Gupta et al 1993; Popineau et al 1994; Huang and Khan 1997a). In addition, the amount of SDS-insoluble glutenin polymers in flour was highly and positively correlated with % HMW glutenin subunits from either SDS-soluble glutenin polymers at 4% origins (0.55**, $P < 0.01$) or from SDS-insoluble glutenin polymers (0.52**, $P < 0.01$). This may indicate that the presence of more SDS-insoluble glutenin polymers might be a result of larger polymers formed from a greater quantity of HMW glutenin subunits. SDS-insoluble glutenin polymers might still contain aggregates with glutenin polymers with different molecular weight ranges. A higher % HMW glutenin subunits from 4% origins in a cultivar might be an indicator that more SDS-insoluble glutenin polymers exist in the gluten proteins of the cultivar.

The quantity of SDS-insoluble glutenin polymers in flour was positively correlated with the percentages of all three groups of HMW glutenin subunits (2*+5, 7, and 9+10) to total SDS-insoluble glutenin polymers (Table IV). However, the % SDS-insoluble glutenin polymers in flour was positively correlated only with subunits 2* and 5 as relative percentages to total HMW glutenin subunits instead of to total SDS-insoluble glutenin polymers. This suggested that more subunits 2* and 5 were needed when forming more SDS-insoluble glutenin polymers compared with subunits 7 or 9+10. Either subunit 2* or 5 might be more important than other subunits included in this study in extending chains of glutenin polymers. This is in accordance with the results of Huang and Khan (1997c) and Lafiandra et al (1993) indicating that subunit 5 seemed to play a more important role than other HMW subunits in extending polymer chains. This also supports the result of Köhler at al (1997) that molecular modeling by HMW subunit 5 was more important in forming intermolecular disulfide bonds than HMW subunit 7.

Percentage and Quantity of HMW Glutenin Subunits in Relation to Loaf Volume

The quantity of HMW glutenin subunits from SDS-insoluble glutenin polymers was positively and significantly correlated with loaf volume (r = 0.53**, $P < 0.01$). However, the % total HMW glutenin subunits in SDS-insoluble glutenin polymers had no significant correlation with loaf volume (r = 0.15, not significant) using linear regression. The data distribution tested normal for the % total HMW glutenin subunits. Because all the six HRS wheat cultivars had the same HMW glutenin subunit composition of 2*, 7+9, and 5+10 except Hamer, which had 2*, 7+8 and 9+10, qualitative differences between HMW glutenin subunit composition did not seem to play a significant role in the breadmaking quality differences of these cultivars. Therefore, the quantity of HMW glutenin subunits in SDS-insoluble glutenin polymers might be important in determining breadmaking quality of HRS wheats. The results of this study seem to support this speculation and are in agreement with previous findings that total quantity of HMW glutenin subunits was important not only in determining dough strength but also in determining loaf volume of wheat flours (Huang and Khan 1997a). However, care must be taken in interpreting the relationship between the quantity of HMW glutenin subunits and breadmaking quality because only 28% of the variation could be explained based on the correlation coefficient (r = 0.53) in this study. Our previous study indicated that other factors, such as total protein content and quantity of SDS-insoluble glutenins, also were important in determining the breadmaking quality of HRS wheat (Zhu and Khan 2001).

TABLE III
Percentage of HMW Glutenin Subunits of SDS-Insoluble Glutenins and SDS-Soluble Glutenins at 4% Origins of Multistacking SDS-PAGE

Glutenins	% Total HMW GS	% Subunits 2*+5	7	9+10
SDS insoluble	19.3a[a]	4.6a	7.0a	7.6a
SDS soluble (4% origins)	9.3b	1.8b	3.7b	3.7b

[a] Values followed by the same letter in the same column are not significantly different ($P < 0.01$).

TABLE IV
Correlation Coefficients of % SDS-Insoluble Glutenin Polymers in Flour with % of Different HMW Glutenin Subunits in Total SDS-Insoluble Glutenin Polymers and in Total HMW Glutenin Subunits from SDS-Insoluble Glutenin Polymers

	Glutenin Polymers					
	% Subunits in Total SDS-Insoluble Glutenins			% Subunits in Total HMW Glutenin Subunits		
	2*+5	7	9+10	2*+5	7	9+10
% SDS-insoluble glutenins in flour	0.50**	0.46*	0.53**	0.36*	–0.23	–0.21

[a] *,** $P < 0.01$, 0.05.

CONCLUSIONS

Our results indicated that environment could significantly affect the % total HMW glutenin subunits and individual HMW glutenin subunits from both SDS-soluble and -insoluble glutenin polymers. The % HMW glutenin subunits was a determinant in the size distribution between SDS-soluble and -insoluble glutenin polymers. This observation agrees with previous findings that more HMW glutenin subunits were associated with larger glutenin aggregates and the % HMW glutenin subunits play an important role in determining the size distributions of glutenin aggregates (Cornec et al 1994; Huang and Khan 1997a). A greater percentage of HMW glutenin subunits in SDS-insoluble glutenins might indicate that the SDS-insoluble glutenin polymers were larger than those with lower percentages. Therefore, more HMW glutenin subunits might form larger SDS-insoluble glutenin polymers, which could then give rise to better loaf volume in breadmaking.

Cultivars of HRS wheat with the same HMW glutenin subunit composition could have a significant difference in loaf volume for locations where the cultivars were grown. Variation in quantity of HMW glutenin subunits in SDS-insoluble glutenin polymers due to different environments might be a major factor in determining differences in breadmaking quality of hard red spring wheat.

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