Relationships Between Gluten Protein Composition and Quality Characteristics in Four Populations of High-Protein, Hard Red Winter Wheat[1]

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ABSTRACT

High-performance liquid chromatography (HPLC) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were used to analyze the gliadin and glutenin protein composition of 286 experimental winter wheats. Tested lines were derived from four hard red winter wheat populations produced as part of a high-protein breeding program. Quality parameters (grain protein content, SDS sedimentation volume, and mixograph characteristics) were correlated with the presence and amounts of each measured protein component. Statistically significant relationships between quality characteristics and protein components were observed in all four populations, and all observed correlations were population-specific. Stepwise multiple regression analysis was used to identify the combination of protein variables that best explained variation in each quality parameter. Highly significant models were developed; however, the components of each model varied with the population. Quality characteristics also were correlated with genetic similarity indices (based on protein composition) between experimental lines and all parental lines; no meaningful relationships were identified. The results suggest that the quality characteristics of experimental wheat lines are a function of the interaction of numerous protein components, are influenced by a number of genetic factors in addition to endosperm protein composition, and cannot be predicted from the quality characteristics of parental lines.

End-use quality (including milling, baking, and dough-handling properties) of hard red winter wheats (HRWW) is influenced by the quantity and quality of the gluten proteins (Pomeranz 1973). Extensive genetic variation exists among wheats in regard to gluten protein composition. Genetic loci that encode the primary gluten proteins, gliadins and glutenin subunits are situated on nine chromosome arms (Payne and Lawrence 1983). Each locus is complex, containing several structural genes. The large number of genes, combined with extensive biochemical variation, provides for a near-infinite number of possible combinations of gliadin and glutenin subunits. Based on levels of observed variation in gliadin composition alone, Metakovsky and Sozinov (1987) estimated that at least 20 million possible genotypes exist.

Gliadins and glutenin subunits influence the structure and function of gluten and flour. Gliadins exist as monomers, imparting viscosity to doughs and interacting with lipids in trapping gas bubbles during fermentation (Nierle 1987). Glutenin subunits, through the formation of intermolecular disulfide bonds, form large aggregates that impart strength and elasticity to doughs. Thus, improving the end-use quality of HRWWs in breeding programs depends on the fixation of optimal combinations and amounts of gliadins and glutenin subunits.

Numerous researchers have reported attempts to correlate the end-use quality of wheat with gliadin and/or glutenin subunit composition. Payne et al (1987) support the concept that end-use quality is highly dependent on high molecular weight (HMW) glutenin subunit composition. Significant correlations between quality and HMW glutenin subunit composition have been determined for wheats from many countries (Sontag et al 1986, Payne et al 1987, Lukow et al 1989). However, Graybosch et al (1990) found that analysis of HMW glutenin subunit composition had limited use in predicting the end-use quality of 69 experimental lines of high-protein HRWW. Lines with poor combinations of HMW glutenin subunits generally were poor in quality; however, the presence of favorable combinations of HMW glutenin subunits did not guarantee acceptable quality. Khan et al (1989) found similar results in a sample of North American hard red spring wheats.

Other investigators have noted that gliadins and low molecular weight (LMW) glutenin subunits also contribute to variation in quality. Heubner and Bietz (1986) and Heubner (1989) found that variation in gliadin composition can account for significant variation in end-use quality. Gupta and Shepherd (1988) presented evidence for a role of LMW glutenin subunits in the determination of end-use quality of bread and durum wheats. More recently, Autran and Galterio (1989) found significant correlations between gliadins and LMW glutenin subunits and durum wheat quality. Thus, variation in all classes of gluten proteins may influence the end-use quality of wheat lines.

Correlations between the presence or absence of specific polypeptides obtained in these reports suggest that wheat breeders could use protein subunit composition to predict end-use quality of newly derived wheat lines. For example, Campbell et al (1987) and Cressey et al (1987) found significant relationships between the presence of specific HMW glutenin subunits and gliadin components and various measures of end-use quality in parental and advanced breeding lines. If such relationships were common, the lines carrying proteins correlated with either good or poor quality could be identified during early generations, increasing the efficiency of breeding programs by allowing undesirable lines to be discarded without evaluating their quality.

Our objective was to determine whether analyzing the composition of gluten protein would help identify high-quality lines in diverse wheat populations. The composition of the HMW and LMW gliadin and glutenin subunits of 286 experimental lines derived from four breeding populations was determined. Flour quality parameters of the derived lines were correlated with the presence and/or amounts of specific gliadins or glutenin subunits or their combinations. Analyses were conducted to determine whether genetic similarity to parental lines, inferred from protein subunit phenotypes, would be a means of predicting quality characteristics.

MATERIALS AND METHODS

Plant Materials

Progeny selected from four HRWW populations were evaluated. The populations were derived from single matings between individual plants selected from a three-way cross-combination and a fourth parent (Table I). Parental lines thus contributed unequally to each of the populations. The agronomic and quality characteristics of the parental lines were diverse. At least one parent of each population carried genes conditioning high grain protein content. Gluten strength characteristics of the parental lines ranged from very weak (NapHal) to exceedingly strong (Plainsman V). Each population was evaluated using at least 62

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independent F_4 derived lines. A total of 286 lines, randomly selected from each population, were tested; no prior selection for agronomic or quality characteristics was exercised. All lines were planted in a single-replication trial in 1986-87 and 1987-88 in Lincoln, NE. Lines were planted in single 8-ft rows with 12-in. spaces between rows.

End-Use Quality Evaluations

Grain samples for the 1986-87 and 1987-88 nursery trials were used for quality evaluations. Whole grain samples were ground in a Udy Cyclone sample mill. To obtain flour samples, 35 g seed was tempered to 15.2% moisture for 18 hr and milled on a Brabender quadrumat laboratory mill. Whole grain protein (WP) was determined from duplicate 25-mg samples by the Kjeldahl procedure. Protein content was calculated as percent $N \times 5.7$. Mixing characteristics were evaluated with 10 g of flour using a National Manufacture mixograph according to AACC method 54-40A (AACC 1983). Mixograph peak time (MT) was recorded in minutes; mixograph tolerance (MTO) was evaluated using a 0–7 scale. Sodium dodecyl sulfate (SDS) sedimentation value was measured using a modification of AACC method 56-61A (the flour samples used were 2 g rather than 3.2 g). Samples from each harvest year were analyzed independently.

Analysis of Gliadin Composition

To obtain gliadins, 50 mg of ground wheat was extracted with 1 ml of 70% ethanol for 30 min at ambient temperature. Samples were centrifuged at 16,000 $\times$ *g* for 5 min. Gliadin composition of the supernatants was analyzed by reversed-phase high performance liquid chromatography (HPLC). A Bio-Rad protein chromatography system including an AS-48 automatic sample injector was used. Detection of samples was based on absorption at 210 nm. The support used was an analytical (250 $\times$ 4.6 mm i.d.) Hi-Pore reversed-phase (C4) column with 300 A pores. Column temperature was 70°C. Solvent A was 0.1% (v/v) trifluoroacetic acid; solvent B was 100% acetonitrile containing 0.1% trifluoroacetic acid. Samples were eluted with a linear gradient ranging from 25 to 50% solvent B; elution time was 45 min. Data were recorded on a Hewlett Packard integrator-recorder. An Apple MacIntosh 2E with Bio-Rad GPS 2.2 software was used as a controller module. Chromatograms were standardized and peaks were numbered 1–25. Peak areas were expressed as percent of the total chromatograph area.

Analysis of Glutenin Composition

Glutenin was isolated by dimethyl sulfoxide extraction (Burnouf and Bietz 1989) of 1.5-mg samples of ground wheat. Glutenin subunit composition was analyzed by separation of reduced pyridylethylated proteins on gradient SDS-polyacrylamide gel electrophoresis (PAGE) gels followed by silver-staining (Graybosch and Morris 1990). Glutenin proteins with apparent molecular weights (MW) ranging from 30,000 to 116,000 were analyzed. Gels were photographed using Polaroid 665 film. Photographic negatives were interpreted using a Bioimage Visage 110 image analyzer equipped with Visage 4.4F software. Each lane was divided into 37 MW classes based on commonly occurring breaks in the distribution of protein bands. A qualitative estimate of glutenin composition of each line was developed by measuring the integrated intensity of protein in each MW class. The amount of protein in each class was determined as a percentage of protein per lane.

Statistical Analyses

Pearson correlation coefficients (Steel and Torrie 1980) were used to test for relationships between each gliadin peak area, the amount of measured protein in each glutenin MW class, and flour quality parameters. To estimate the contribution of variation in grain protein content to variation in the remaining quality parameters, correlation coefficients also were calculated between these variables. Possible effects of groups of biochemically similar gliadins and glutenins were analyzed. Gliadin HPLC peaks in three major areas were summed to estimate the major gliadin classes (Lookhart and Albers 1988). The three groups were peaks 1–9, 10–18, and 19–25. Glutenin electrophoregrams were subdivided into five ranges of MW classes: 79,500–116,000 (this class includes all HMW glutenin subunits), 48,500–79,500, 40,000–49,000, 34,000–39,000, 30,000–34,000. The amount of protein in each HPLC or MW class was correlated with the results of quality analyses. Possible relationships between gliadin and glutenin subunit composition were examined by developing correlation coefficients between gliadin peak areas and glutenin MW classes. Only those coefficients that showed statistically significant relationships in both harvest years are reported.

Stepwise procedures (SAS 1985) were used for multiple regression analyses to select the combinations of gliadin peaks and glutenin bands that accounted for the largest amount of variation (r^2 values) in each quality parameter. A statistical significance level of 0.01 was required for inclusion of a parameter in the model. Multiple regression models were developed independently for the 1986-87 and 1987-88 harvest years. The regression models developed from the 1986-87 quality data were evaluated by correlating predicted values to quality measurements of the subsequent year; the regression models developed from the 1987-88 quality data were evaluated in a similar manner using the quality measurements of the previous year. All statistical analyses were performed independently for each population.

Similarity indexes (SI) were calculated to estimate the resemblance of each experimental line (in terms of protein composition) to parental lines of each population (Angus et al 1988). Separate SIs were calculated from gliadin and glutenin composition and from the combination of both protein classes. SIs were calculated from the equation $SI = w/m$, where w = number of peaks (or bands) in common, and m = total number of peaks or bands. Gliadin peaks that contributed less than 0.5% of the total peak area and glutenin bands that contributed less than 1.0% of the total glutenin measured were excluded from calculation. SIs for each line were correlated with quality parameters to determine whether the quality of a given experimental line is a function of genetic similarity of a given parental line.

HMW glutenin subunits were classified according to the system of Payne and Lawrence (1983). A *Glu-1* quality score (Payne et al 1987, Lukow et al 1989) was calculated for each line. Since the experimental material consisted of F_4-derived lines, a number of lines were heterogeneous for HMW glutenin subunits arising from the same chromosomes. In such cases, *Glu-1* scores were calculated by averaging the scores of the subunits present. Pearson correlation coefficients were used to test for relationships between *Glu-1* scores and flour quality parameters. Within each population, variation in HMW glutenin composition often was limited; thus, for each pair of allelic contrasts detected, mean values for quality parameters were compared using *t*-tests (Steel and Torrie 1980).

TABLE I
Pedigrees of the Four Hard Red Winter Wheat Populations Studied

Population	Pedigree
I	GKF-8261//NapHal/CI13449/3/Plainsman V
II	Plainsman V/3/NapHal/CI13449//NE7070
III	NapHal/CI13449//NE7060/3/F26-70
IV	NapHal/CI13449//GKF-8261/3/NE78868

RESULTS

Mean values and ranges for each quality parameter were reported by population for each harvest year (Table II). In all four populations, mean values for WP were higher than those typically encountered in HRWW lines. The high protein content of these materials was a consequence of enhanced genetic potential for grain protein content and of using an agronomic environment that conditioned elevated protein production. Variation for mixograph characteristics and SDS sedimentation values was extensive in all populations. Variation in quality parameters among lines, however, was not related to variation in grain protein content.

Correlations of grain protein with mixograph and SDS sedimentation values were nonsignificant (data not presented), with one exception: a low correlation between grain protein content and MT in population II ($r = 0.25$, $P < 0.05$, in both harvest years).

A typical HPLC separation of gliadin monomers (extracted from the parental line NE78868) is shown in Figure 1. Twenty-five major peaks were differentiated and used in subsequent analyses. Correlations of single gliadin peaks and of groups of peaks with protein and quality parameters are given in Table III. Only correlations that were statistically significant in both harvest years are presented. The number and identity of peaks that correlated significantly with quality parameters varied by population. Only peak 23 was correlated with the same quality parameter in more than one population, showing significant positive relationships with increasing MTO in three of the four populations. The magnitude of the r values was similar for each harvest year for most of the correlations. Only one significant

TABLE II
Mean Values and Ranges for Quality Parameters in the Four Hard Red Winter Wheat Breeding Populations Studied

Population	Year	WP[a] (%)	SDS (cc)	MT (min)	MTO (0–7)
I	1987	20.8 (16.5–26.0)	37.1 (20.0–44.0)	5.0 (2.7–9.3)	3.1 (0–7)
	1988	22.2 (16.8–26.0)	32.3 (25.0–30.0)	5.5 (2.7–11.2)	3.0 (0–6)
II	1987	20.7 (17.7–24.6)	29.7 (24.0–36.0)	3.2 (1.3–8.0)	2.8 (0–7)
	1988	21.6 (18.6–27.6)	30.5 (22.0–29.0)	3.7 (1.3–9.5)	3.0 (0–7)
III	1987	19.4 (17.2–22.5)	29.9 (22.0–36.0)	3.8 (2.0–5.7)	2.8 (1–5)
	1989	19.8 (16.5–23.2)	32.0 (24.0–39.0)	4.7 (3.3–7.3)	2.7 (1.5–4.5)
IV	1987	19.9 (17.5–23.2)	26.1 (8.0–34.0)	3.8 (1.3–6.7)	2.7 (0–5)
	1988	21.1 (17.5–25.3)	28.9 (15.0–41.0)	4.9 (1.0–9.8)	3.1 (0–6)

[a] WP = whole-kernel protein, SDS = sodium dodecyl sulfate sedimentation volume, MT = mixograph peak time, MTO = mixograph tolerance.

TABLE III
Correlations Between Gliadin Peak Areas and Quality Parameters[a]

Population	Gliadin Peaks	WP[b] 1987	WP[b] 1988	SDS 1987	SDS 1988	MT 1987	MT 1988	MTO 1987	MTO 1988
I	7	...	...	...	...	0.35**[c]	0.26*	...	...
	23	...	...	...	...	...	...	0.51**	0.27*
II	17	...	...	−0.50**	−0.31**	−0.44**	−0.40**	−0.53**	−0.47**
	18	...	...	0.57**	0.35**	0.44**	0.44**	0.56**	0.55**
	19	...	...	...	...	−0.35**	−0.41**	−0.34**	−0.38**
	23	...	...	0.58**	0.37**	0.40**	0.32**	0.53**	0.52**
	24	...	...	−0.52**	−0.27*	−0.43**	−0.31	−0.40**	−0.42**
	10–18	...	...	...	...	0.35**	0.37**	0.34**	0.35**
III	4	0.32*	0.31*	...	...	...	...	...	...
IV	9	...	...	0.29*	0.31*	...	...	...	...
	10	...	...	...	...	−0.30**	−0.35**	−0.35**	−0.27**
	16	...	...	−0.35**	−0.42**	...	...	...	...
	23	...	...	...	...	...	...	−0.30*	0.29*
	24	−0.28*	−0.30*	...	...	...	...	...	...

[a] Statistically significant ($P < 0.05$) correlation coefficients in both harvest years are given.
[b] WP = whole-kernel protein, SDS = sodium dodecyl sulfate sedimentation volume, MT = mixograph peak time, MTO = mixograph tolerance.
[c] ** = $P < 0.01$, * = $P < 0.05$.

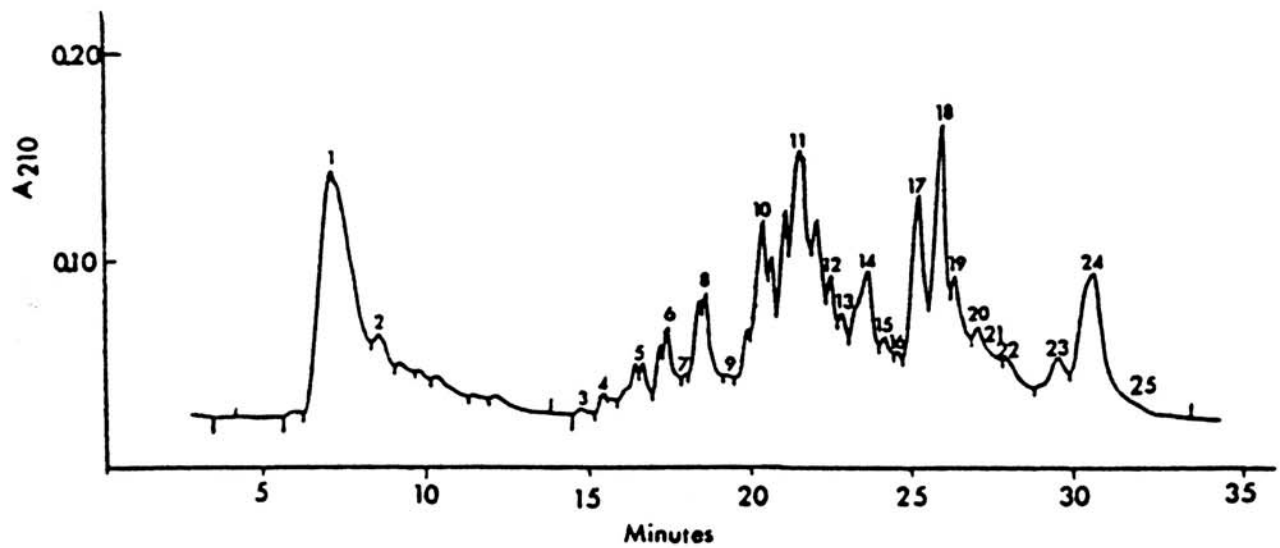

Fig. 1. High-performance liquid chromatogram of gliadins extracted from the hard red winter wheat parental line NE78868. The 25 numbered gliadin peaks were used in statistical analysis.

TABLE IV
Correlations Between Molecular Weight (MW) Classes and Quality of Glutenin[a]

Population	MW Class (in thousands)	WP[b] 1987	WP[b] 1988	SDS 1987	SDS 1988	MT 1987	MT 1988	MTO 1987	MTO 1988
I	98–100	...	...	−0.48**[c]	−0.34**	...	...	...	...
	60–62.5	...	...	...	...	0.34**	0.32*	...	...
	44.75–46	...	...	...	...	0.26*	0.33*	...	...
	43–44	...	...	...	...	0.27*	0.28*	0.33*	0.28*
	39–40	...	...	...	...	0.34*	0.28*	...	...
II	74–79.5	...	...	...	...	−0.31**	−0.28**	...	...
	68–71	...	...	...	...	−0.28*	−0.24*	...	...
	50–52	−0.28*	−0.24*	...	...	...	...	...	...
	46–46.5	...	...	...	...	...	...	−0.31**	−0.33**
	42–43	...	...	0.34**	0.28**	0.33*	0.24*	0.39**	0.46**
	48.5–79.5	...	...	...	...	0.29*	0.33**	0.26*	0.42**
III	110–113	...	...	...	...	−0.28*	−0.26*	...	...
	43–44	0.37**	0.31*	...	...	...	...	...	...
	40–42	...	...	...	...	...	...	0.46**	...
	39–40	...	...	...	...	−0.37**	0.29*	...	0.26*
	36.5–38	...	...	...	...	...	...	0.34**	...
IV	105–110	...	...	0.44**	0.55**	0.42**	0.46**	0.49**	0.31*
	88.5–92	...	...	−0.50**	−0.57**	−0.39**	−0.33**	−0.33**	0.49**
	74–79.5	...	...	...	...	0.31*	0.31*	0.26*	−0.30**
	71–74	...	...	−0.35**	−0.33*	...	...	...	0.28*
	65.5–68	...	...	−0.30**	−0.27*	−0.36**	−0.26*	−0.29**	−0.35**
	50–52	...	...	...	...	−0.37*	−0.26*	...	...
	32.5–32.75	...	...	0.35**	0.28*	...	...	...	...
	48.5–79.5	...	−0.28*	−0.38**	−0.38**	−0.37**	...	...	...

[a] Statistically significant ($P < 0.05$) correlation coefficients are given.
[b] WP = whole-kernel protein, SDS = sodium dodecyl dulfate sedimentation volume, MT = mixograph peak time, MTO = mixograph tolerance.
[c] ** = $P < 0.01$, * = $P < 0.05$.

relationship was established between groups of gliadin peaks and any quality parameter. Peaks 10–18 were positively correlated with mixograph parameters in population II; however, individual peaks in this group showed both positive and negative relationships with the same quality parameters.

Figure 2 illustrates a typical SDS-PAGE separation of glutenin subunits. Variation observed within each population was extensive.

Table IV lists correlations between glutenin MW classes and quality parameters that were statistically significant for each year of analysis. In each population, a number of glutenin MW classes showed significant correlations with quality parameters. However, as was the case for gliadin monomers, the magnitudes of the individual correlations were low. Also, few identical glutenin MW classes showed significant r values across populations. Glutenin subunits in the MW classes 39,000–40,000 and 74,000–79,500 showed significant relationships in two populations each; however, in both cases, the correlations obtained in the two populations revealed opposite trends.

Nearly all correlations between groups of glutenin MW classes and quality parameters were nonsignificant. The amount of glutenin protein in the 48,500–79,500 MW class showed significant negative correlations with mixing time in two populations.

Significant correlation coefficients between gliadins and glutenin MW classes are listed in Table V. For simplicity, correlations are given only for gliadins and glutenin subunits that also were significantly correlated with quality parameters. In each population, several significant relationships were observed; however, most of the correlations occurred between gliadins and glutenin subunits of less than 80,000 MW. Comparing Table V with Tables III and IV reveals the frequent occurrence of simultaneous correlations between gliadin and glutenin subunits and quality parameters. For example, in population II, glutenin MW class 42,000–43,000 was positively correlated with SDS sedimentation values and with both mixograph characteristics (Table IV). In the same population, gliadin peak 17 was negatively correlated and gliadin peak 18 was positively correlated with these same quality parameters. Glutenin subunits in MW class 42,000–43,000 were negatively correlated with gliadin peak 17 and positively correlated with gliadin peak 18 (Table V). The occurrence of such simultaneous relationships prevents a definitive assignment

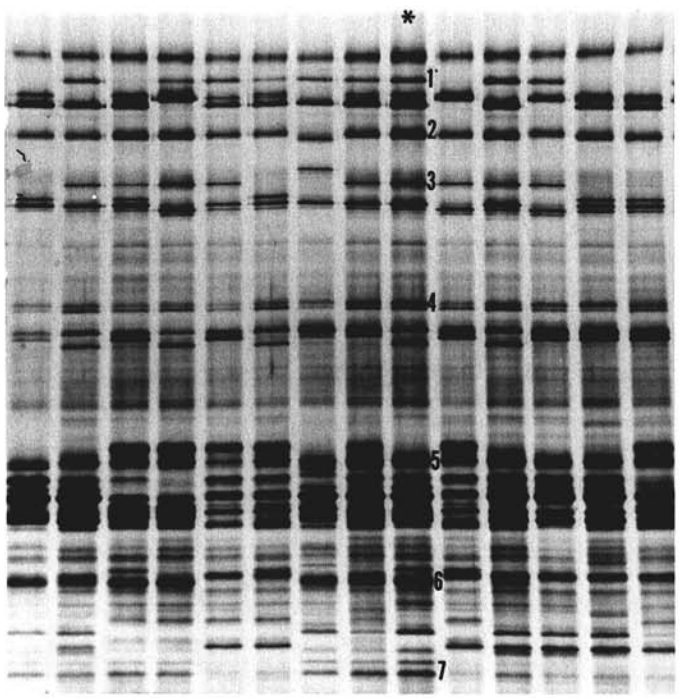

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis separation of glutenin subunits purified by dimethyl sulfoxide extraction from 13 hard red winter wheat experimental lines from population II and the cultivar Plainsman V (indicated by *). The molecular weight of the indicated reference proteins is as follows: 1 = 115,300; 2 = 99,600; 3 = 87,900; 4 = 61,300; 5 = 45,300; 6 = 37,300; 7 = 32,000.

of specific roles for gliadins and glutenin MW classes in determining quality parameters.

Stepwise multiple regression was used to identify the combination of gliadins and glutenin MW classes that explained the largest amount of variation (i.e., the highest r^2 value) in each quality parameter within each population. The derived equations contained numerous variables and are not presented. However, the components of each equation differed with the populations

and for each year evaluated. Table VI lists the r^2 value for the combination of variables that best predicted each quality parameter. The results were variable across populations; however, up to 90% of the variation in certain quality parameters could be explained through various combinations of glutenin protein bands and gliadin HPLC peaks. Averaged across populations and years, the highest r^2 values were recorded for SDS (0.70); WP had the lowest average r^2 value (0.54).

The regression model calculated from the quality results of each harvest year was used to predict the quality of the previous or successive year. The r^2 values that described the degree of relationship between the actual and predicted results were calculated (Table VI). With only three exceptions (WP in populations I and II and SDS in population III), the predicted values from one harvest year were significantly correlated with the actual values observed in the alternative year. However, the magnitude of the r^2 values was significantly lower than that observed for the models developed within each harvest year.

SIs were calculated to estimate the genetic relationships between each experimental line and its parental line. An SI value of 1.0 would indicate that an experimental line was identical in protein composition to a parental line. Calculated SIs ranged from 0.52 to 0.79 (not shown). Significant r values for the regressions of SI against quality parameters are listed in Table VII; few significant relationships were identified. Thus, recovery of parental protein phenotypes does not lead to recovery of parental quality characteristics.

Relationships between *Glu-1* scores and quality parameters are presented in Table VIII. Highly significant correlations were obtained between *Glu-1* scores, mixograph parameters, and SDS sedimentation values in two of the four populations. The magnitudes of the calculated r values were higher than those calculated for the relationships between any individual glutenin subunit or gliadins and quality parameters in these two populations. However, in populations I and III, variation in HMW glutenin subunit composition did not contribute to quality variation, whereas significant relationships were established for LMW glutenin subunit and gliadin variation.

The effect of HMW glutenin subunit variation in populations II and IV was further examined by comparing the mean quality values for each contrasting allelic pair. Table IX lists the contrasts for which statistically significant differences in mean values were identified. A number of sister lines were heterogeneous for HMW glutenin subunit composition; values for these lines are included but were not used to estimate statistical significance. In population II, lines carrying the chromosome 1D-encoded subunits 5 + 10 had significantly higher values for MT and MTO did than sister lines carrying the alternative subunits 2 + 12. However, the observed ranges in quality parameters of the contrasted lines overlapped. The quality scores for the heterogeneous types were intermediate in value. In population IV, lines that lacked 1D-encoded HMW glutenin subunits had much lower quality scores than did sister lines carrying 5 + 10; however, the comparison of means in population IV is based on a very small sample size of lines carrying null alleles. Additional allelic contrasts noted in these populations included the following: chromosome 1A-encoded subunits 1 versus 2 in populations I, II, and IV; chromosome 1B-encoded subunits 7 + 8 versus 7 + 9 in populations II and III; and 1B subunits 7 + 8 versus 13 + 16 in population IV. No significant differences in mean quality values were observed for sister lines possessing any of these alternative pairs of alleles.

TABLE V
Statistically Significant Correlations Between Glutenin Molecular Weight (MW) Classes and Gliadin Peaks

Population	Glutenin MW Class (in thousands)	Gliadin Peaks	r
I	60–62.5	1	−0.26*[a]
	44.75–46.0	19	−0.38**
	43–44	17	−0.27*
	43–44	18	0.41**
	39–40	1	0.38**
II	74–79.5	2	0.25*
	68–71	2	0.32*
	50–52	1	0.26*
	50–52	17	0.24*
	46–46.5	17	0.47**
	46–46.5	18	−0.52**
	42–43	4	0.31**
	42–43	7	−0.31**
	42–43	17	−0.63**
	42–43	18	0.72**
III	110–113	12	0.40**
	110–113	14	−0.37**
	43–44	15	0.31**
	40–42	16	0.39**
	39–40	22	0.26**
IV	88.5–92	1	0.30*
	...	88.5–92	3−0.30*
	74–79.5	11	−0.35*
	71–74	16	0.30*
	71–74	20	−0.30*
	50–52	20	−0.29*
	50–52	24	−0.27*
	32.4–32.75	17	−0.44**
	32.4–32.75	18	0.49**
	32.4–32.75	23	0.38**

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

TABLE VI
Results of Stepwise Multiple Regression Analyses

Population	Quality Parameter	No. of Variables in Regression Model: Gliadins 1987	Gliadins 1988	Glutenins 1987	Glutenins 1988	R^2 1987	R^2 1988	r^2 1987 Predicted vs. 1988 Actual	r^2 1988 Predicted vs. 1987 Actual
I	WP[a]	2	1	5	4	0.53	0.33	0.00	0.00
	SDS	4	2	10	4	0.83	0.83	0.16	0.22
	MT	4	8	6	7	0.67	0.83	0.49	0.42
	MTO	5	5	3	5	0.71	0.62	0.32	0.27
II	WP	5	5	3	9	0.41	0.64	0.02	0.05
	SDS	4	2	1	3	0.58	0.39	0.07	0.28
	MT	3	3	2	3	0.41	0.45	0.35	0.32
	MTO	2	3	1	2	0.44	0.50	0.37	0.34
III	WP	3	5	5	1	0.56	0.46	0.28	0.22
	SDS	4	5	1	9	0.46	0.78	0.02	0.01
	MT	5	1	4	3	0.66	0.39	0.28	0.18
	MTO	2	5	3	7	0.42	0.75	0.11	0.26
IV	WP	4	4	2	9	0.90	0.81	0.56	0.55
	SDS	5	2	12	9	0.90	0.81	0.56	0.55
	MT	3	4	3	3	0.50	0.55	0.48	0.41
	MTO	5	6	5	6	0.68	0.76	0.41	0.48

[a] WP = whole-kernel protein, SDS = sodium dodecyl sulfate sedimentation value, MT = mixograph peak time, MTO = mixograph tolerance.

DISCUSSION

Many studies have attempted to characterize relationships between specific gliadin or HMW glutenin subunits and end-use quality (Campbell et al 1987). Comparatively fewer studies have tried to identify the role of LMW glutenins in the determination of wheat quality. Pogna et al (1988) and Autran and Galterio (1989) showed correlations between the presence of specific LMW glutenins and quality parameters in durum wheats. Gupta and Shepherd (1988) found differences in dough strength in biotypes of two Australian bread wheat cultivars that carried different LMW glutenin subunits. In this report, several LMW glutenin classes were found to correlate with various quality parameters; however, no one LMW glutenin subunit was related to any of the quality measures in more than one segregating population.

Genetic linkage between gliadin and LMW glutenin subunit genes prevents a complete assessment of the role both types of proteins play in the structure and function of gluten. LMW glutenins and gliadins arise from complex loci on the short arms of chromosomes 1A, 1B, and 1D (Payne et al 1987). Pogna

TABLE VII
Statistically Significant Correlations Between Similarity Indexes and Quality Parameters

Similarity Index	Population	WP[a] 1987	WP[a] 1988	SDS 1987	SDS 1988	MT 1987	MT 1988	MTO 1987	MTO 1988
GKF-8261-*Glu*[b]	I	...	...	−0.35**[c]	−0.39**	...	...	...	...
GKF-8261-Total[d]	I	...	...	−0.41**	−0.39**	...	...	...	...
NapHal-*Glu*	I	...	...	−0.26**	−0.36**	...	...	...	...
CI13449-*Gli*[e]	II	...	...	...	...	0.44*	0.37**	+0.43**	0.34**
NE7060-*Gli*	II	...	...	...	...	...	...	−0.25*	−0.28*
Plainsman V-*Gli*	II	...	...	...	...	0.25*	0.34**	0.32**	0.44**
CI13449-Total	II	...	...	...	...	0.38**	0.42**	...	...
NapHal-Total	III	...	...	...	...	...	...	0.33**	0.29**
CI13449-Total	III	...	...	...	...	...	...	0.27*	0.34**
CI13449-*Gli*	IV	...	...	...	...	...	...	0.31*	0.32*
NE78868-Total	IV	−0.34**	−0.28*	...	...	...	...	...	...
NapHal-Total	IV	−0.32*	−0.26*	...	...	...	...	...	...

[a] WP = whole-kernel protein, SDS = sodium dodecyl sulfate sedimentation volume, MT = mixograph peak time, MTO = mixograph tolerance.
[b] Similarity index calculated from glutenin composition.
[c] ** = $P < 0.01$, * = $P < 0.05$.
[d] Similarity index calculated from all protein components.
[e] Similarity index calculated from gliadin composition.

TABLE VIII
Statistically Significant Correlations Between *Glu-1* Score and Quality Parameters in the Four Hard Red Winter Wheat Populations

Population	WP[a] 1987	WP[a] 1988	SDS 1987	SDS 1988	MT 1987	MT 1988	MTO 1987	MTO 1988
II	...	...	...	...	0.61**[b]	0.62**	0.49**	0.51**
III	0.26**	0.27**	...	...	...	...	...	...
IV	...	...	0.71**	0.67**	0.58**	0.61**	0.53**	0.52**

[a] WP = whole-kernel protein, SDS = sodium dodecyl sulfate sedimentation volume, MT = mixograph peak time, MTO = mixograph tolerance.
[b] ** = $P < 0.01$.

TABLE IX
Comparison of Quality Characteristics of High Molecular Weight (HMW) Glutenin Allelic Contrasts[a]

Population	HMW Glutenin Subunits	No. of Lines		WP[b] 1987	WP[b] 1988	SDS 1987	SDS 1988	MT 1987	MT 1988	MTO 1987	MTO 1988
II	2 + 12[c]	34	Mean	20.3	21.2	...	...	2.4	2.8	2.0	2.3
			SE[d]	0.19	0.27	...	...	0.11	0.14	0.23	0.22
			Range	17.7–22.0	18.9–27.6	...	...	1.5–4.5	1.8–5.0	0–5	0–5.5
	5 + 10[c]	26	Mean	21.0	21.9	...	...	4.2	4.8	3.7	3.7
			SE	0.23	0.19	...	...	0.3	0.3	0.37	0.25
			Range	18.9–24.3	20.4–25.0	...	...	1.3–8.0	1.8–9.5	0.5–7	0.5–7
	5 + 10[e]		...	...	...	...	...	...	...	...	...
	2 + 12	14	Mean	20.6	21.7	...	...	3.6	3.8	3.1	3.1
			SE	0.32	0.39	...	...	0.26	0.22	0.3	0.2
			Range	18.3–22.2	18.6–24.3	...	...	2.3–5.3	1.8–5.1	1–7	1–4.5
IV	Null[c]	3	Mean	...	...	13.3	18.0	1.2	1.3	0.0	0.8
			SE	...	...	2.9	1.5	0.14	0.16	0.0	0.0
			Range	...	...	8–18	15–20	1.3–1.7	1–1.5	0.0	0–1.5
	5 + 10[c]	48	Mean	...	...	27.7	30.2	4.2	5.3	2.9	3.4
			SE	...	...	0.49	0.52	0.17	0.24	0.18	0.19
			Range	...	...	19–34	18–41	2–6.7	2.3–9.8	0–5	0–6
	5 + 10[e]		...	...	...	...	...	...	...	...	...
	Null	4	Mean	...	...	15.5	21.3	1.6	1.8	1.0	1.0
			SE	...	...	1.3	1.4	0.15	0.34	0.57	0.46
			Range	...	...	8–20	19–25	1.3–2.0	1.3–2.8	0–2	0–2

[a] Comparisons with statistically significant ($P < 0.05$) mean differences are given.
[b] WP = whole-kernel protein, SDS = sodium dodecyl sulfate sedimentation volume, MT = mixograph peak time, MTO = mixograph tolerance.
[c] Homogeneous lines.
[d] Standard error of the mean.
[e] Heterogeneous lines.

et al (1988) demonstrated that the strong gluten characteristics associated with durum wheats carrying γ-gliadin 45 actually were due to the presence of a linked LMW glutenin subunit. We also found significant correlations between several LMW glutenins and gliadin HPLC peaks. Thus, correlations between quality parameters and gliadins (Table III) in our materials could be due to the presence of genetically linked genes encoding LMW glutenin subunits. Alternatively, the ethanol extracts analyzed by HPLC might contain LMW glutenin subunits; partial solubility of these proteins in 70% ethanol has been demonstrated (Graybosch and Morris 1990).

Application of one-dimensional SDS-PAGE separation of glutenin subunits in the prediction of end-use quality has limitations. Since the assignment of proteins to discreet classes was based on migration distances, proteins with similar molecular size but different amino acid composition (or quality effects) could have been included in the same MW class. This could alter correlations between protein subunits and quality parameters. However, appropriate statistical tests in genetic studies necessitate the objective analysis of a large number of lines. The experiments reported were designed to test the usefulness of a potential technology. Two-dimensional protein separation obviously would better allow the identification of quality-related proteins. However, the time and labor required for such analyses would limit their utility in breeding programs.

Many studies support the hypothesis that wheat dough handling and breadmaking quality largely are influenced by a small number of important proteins (Payne et al 1987, Lukow et al 1989). However, Hoseney et al (1987) argue that gluten quality is more likely determined by the interaction of all polypeptides within a flour rather than by single polypeptides alone. Our results suggest that both hypotheses are partly correct. However, the particular genetic background of the wheats included in the experiment must be considered. In certain genetic backgrounds, such as populations II and IV in the current study, the presence or absence of specific proteins clearly influences flour quality. In the other populations, no specific protein was identified as having a significantly large effect on quality; in these populations, quality was dependent on the effects of a large number of proteins.

Identification of a few specific proteins with significant effects on quality would aid in the development of higher quality wheats. However, to be useful to wheat breeders, correlations of quality with the presence or amounts of these specific proteins must be consistent across diverse genetic backgrounds. In this study, no proteins showed consistently high correlations with quality parameters across all four populations.

Predictive models based on protein subunit composition also must be relatively unaffected by environmental factors. The test of our multiple regression equations indicates that the development of such models will be difficult. Predicted and actual results showed some correlation across growing seasons. However, the magnitude of the correlations were not sufficient to allow confidence in selections based on the models. The number of parameters that must be measured (all gliadin and glutenin subunits) to achieve this level of prediction will discourage even the most adventurous wheat breeder.

SIs were calculated in an attempt to predict quality of experimental lines as a function of genetic similarity to a good-quality parent. However, the calculated SIs showed little value in the prediction of end-use quality parameters. The SIs reported here were based on proteins arising from nine chromosome arms (Payne et al 1987), or approximately 10% of the wheat genome. Thus, SIs may have been based on only a fraction of the genes that might influence quality. Actual genetic distance between progeny and parental lines will differ significantly from that estimated from protein composition alone. Cox et al (1985) reached a similar conclusion after comparing SIs based on gliadin electrophoretic patterns with similarities based on pedigree analysis.

In conclusion, HRWW quality appears to be dependent on the interaction of a large number of gene products. The identification of specific protein subunits that will be indicative of good quality across a broad range of genetic backgrounds seems unlikely. Simultaneous measurement of a number of protein components could result in the development of multiple regression models with potential value in predicting quality; however, the complexity of the required biochemical and statistical analyses will limit the application of such techniques in breeding programs.

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