Relationships Between Protein Solubility Characteristics, 1BL/1RS, High Molecular Weight Glutenin Composition, and End-Use Quality in Winter Wheat Germ Plasm[1]

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ABSTRACT

End-use quality parameters, protein solubility characteristics, and high molecular weight (HMW) glutenin subunit composition of 69 diverse wheat (*Triticum aestivum* L.) experimental lines and varieties in two experimental trials were determined. The experimental lines varied widely in total protein concentration and end-use quality characteristics. Variation in protein solubility characteristics showed little relation to quality parameters among 27 wheat lines in experiment I. Relationships between protein solubilities and quality parameters were highly significant among the genetically more diverse lines in experiment II. The presence of the 1BL/1RS translocation was identified on the basis of ω-secalins detected by sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis of 0.04 *M* NaCl-soluble proteins. The effect of 1BL/1RS on end-use quality and protein solubilities was highly dependent on genetic background. Above average levels of KOH-soluble proteins and/or average to below average levels of salt-water-soluble proteins improved the quality of 1BL/1RS lines. Significant correlations were obtained between *Glu-1* quality scores and mixing characteristics and loaf volumes of the lines. Adjusting the *Glu-1* score for the presence of 1BL/1RS improved most correlations with quality parameters. Combinations of *Glu-1* scores and protein solubility characteristics were evaluated by stepwise multiple regression to predict end-use quality parameters. However, no variable or combination of variables accounted for more than 56% of the total variation in any one quality parameter. The usefulness of *Glu-1* scores, even in conjunction with protein solubility characteristics, was limited to the identification of very poor quality wheats.

Variation in end-use quality characteristics of wheat (*Triticum aestivum* L.) varieties is highly dependent upon flour protein quantity and quality (Pomeranz 1973). Flour protein quality is a function of protein solubility characteristics and subunit composition (MacRitchie 1989). The relative amounts of protein found in each of the classical solubility classes (albumins, globulins, gliadins, and glutenins [Osborne 1907]), and variation in high molecular weight (HMW) glutenin subunit composition, have been shown to correlate with end-use quality in wheat (Orth and Bushuk 1972; Orth et al 1972; Booth and Melvin 1979; Payne et al 1979, 1981; Moonen et al 1983; Chakraborty and Khan 1988; Ng and Bushuk 1988).

Results from protein solubility studies often are difficult to compare and summarize, largely due to the absence of uniform methods of preparing protein fractions. In general, the amount of protein in gliadin-containing fractions is related to loaf volumes, dough mixing times, and various measures of water absorption; levels of glutenin-containing fractions most often correlate with dough mixing tolerance and loaf volume (Orth and Bushuk 1972, Booth and Melvin 1979, Chakraborty and Khan 1988, Khan et al 1989, MacRitchie 1989). Though it is known from reconstitution studies that salt-water extracts of flour proteins contain components that influence dough properties (Mattern and Sandstedt 1957, Chakraborty and Khan 1988, Yoshida and Danno 1989), the importance of salt-water-soluble proteins (albumins and globulins) in the determination of breadmaking properties is unclear. When comparisons are made across a number of varieties, correlations between levels of salt-water-soluble proteins and various quality parameters are either weak or insignificant (Orth and Bushuk 1972, Orth et al 1972).

Payne et al (1987) developed the *Glu-1* quality score as a means of predicting the breadmaking potential of a given wheat variety, based on HMW glutenin subunit composition. Payne et al (1987) accounted for 47-60% of the quality variation in British-grown wheat varieties through use of this index. When a correction factor was included that modified the score based upon the presence or absence of the wheat/rye translocation 1BL/1RS, the predictive value of the *Glu-1* score improved. Subsequent application of the *Glu-1* score to the analysis of Finnish (Sontag et al 1986), Spanish (Payne et al 1988), and Canadian (Lukow et al 1989) wheats provided additional support for the usefulness of this tool. However, limited variation in HMW glutenin subunit composition among hard red spring wheats (Khan et al 1989) may restrict the application of the *Glu-1* score to certain classes of wheat or breeding programs.

A high frequency of wheat lines in modern breeding programs carries genetic material from rye in the form of wheat-rye chromosomal translocations. The presence of one such translocation, 1BL/1RS (originally found in the Soviet lines Kavkaz and

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Aurora), often has been associated with diminished breadmaking quality (Zeller and Hsam 1983, Moonen and Zeven 1984, Dhaliwal et al 1987), perhaps through the elevation of salt-water soluble proteins (Dhaliwal et al 1988). Breeders of hard red winter wheats are interested in identifying means of predicting end-use quality in breeding materials. To determine whether analyses of protein solubilities and subunit composition can aid in achieving this goal, analyses of protein solubility and HMW glutenin subunit composition were conducted for experimental wheat lines derived from a current wheat breeding program. Based on examination of the pedigrees of the tested lines, a high frequency of 1BL/1RS was expected. Therefore, the effects of 1BL/1RS on protein solubilities also were assessed. In order to achieve this latter goal, a method to rapidly identify 1BL/1RS in wheat lines was developed.

MATERIALS AND METHODS

Plant Materials

Sixty-nine wheat lines from two experimental nursery trials were evaluated in this study; each was grown in a three-replication yield trial at Lincoln, NE, in 1987 using standard agronomic practices. Six released cultivars, Brule, Colt, Lancota, Plainsman V, Redland, and Siouxland, were included as checks. Siouxland and Lancota were the only check varieties present in both experiments; Siouxland was the only one that possesses the 1BL/1RS translocation. The remaining 63 were experimental lines developed by the USDA-ARS and University of Nebraska as part of a continuous effort to develop hard red winter wheat germ plasm with elevated levels of endosperm storage proteins. They were derived from intermatings of adapted, unadapted, and high-protein wheats. Separate analyses were performed for each nursery experiment. Experiment I contained 23 F_4-derived F_7 experimental lines plus four check varieties. The experimental lines had undergone preliminary selection for grain yield potential, enhanced protein content, and mixing characteristics. Experiment II contained 40 F_4-derived F_6 lines and four check varieties. The experimental lines entered in Experiment II were genetically more diverse and had undergone preliminary selection for grain yield and protein content, but not mixing characteristics. None of the experimental lines had been previously screened for baking characteristics.

End-Use Quality Analyses

One gram of sample (dry basis) from each replication was analyzed for nitrogen using the macro-Kjeldahl procedure (method 46-12, AACC 1983) prior to compositing samples over replications. Protein was calculated using 5.7 as the factor to convert N to protein. Grain samples were composited for each entry to provide adequate seed for mill and bake analyses. Samples were tempered to 15.2% moisture prior to milling on a Buhler laboratory mill. Flour protein was measured on a 12% moisture basis using near-infrared reflectance spectroscopy. Approved methods of the AACC (1983) were used for evaluation of mixing and breadmaking quality. Mixing time, mixing tolerance, farinograph peak time, and farinograph mixing tolerance index were determined on flour samples using a National Manufacturing mixograph and Brabender farinograph. Water absorption potential was estimated from the farinograph curve. A standard 100-g pup loaf bake test was used at optimal oxidation levels for each entry for determination of loaf volume potential (method 10-09, AACC 1983).

Protein Fractionations

Protein fractionation studies were conducted using whole-grain samples. Ten-gram samples were ground in a Udy cyclone mill. One hundred milligram (dry weight) samples were sequentially extracted with 0.04*M* NaCl (salt-water solubles), 70% ethanol (alcohol solubles), and 0.1% KOH (alkali solubles). Extractions were carried out at room temperature with constant agitation. Samples were extracted three times (20 min each) with 3 ml of each solvent before extraction with the next solvent in the series. The three extractions with each solvent were pooled for analyses. Three aliquots from each pooled solvent sample were used for protein determinations. Protein values were determined in solution by use of the bicinchoninic acid (BCA) procedure (Smith et al 1985). Reagents for the BCA assay were obtained from Pierce (Rockford, IL). Solvents employed were selected for their lack of interference with the BCA reaction. Protein values were determined by comparing absorbance values at 562 nm to those of wheat protein standards derived from large volume extractions. Separate standard curves were prepared for each solvent. Protein amounts in the standard solutions were determined by the Kjeldahl procedure.

Identification of 1BL/1RS Lines and Determination of HMW Glutenin Subunit Composition

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with a modified silver staining procedure was used to characterize salt-water soluble proteins from each experimental line (Graybosch and Morris 1990). Protein characteristics of known 1BL/1RS-containing wheats were compared with non-1BL/1RS wheats and rye cultivar Rymin to identify potentially unique proteins. 1BL/1RS lines were identified by the presence of rye proteins in the dilute saline extracts. Proteins extracted with KOH were not amenable to analysis by SDS-PAGE due to degradation. Thus, in order to determine HMW glutenin subunit composition, glutenins were extracted from ground wheat samples as per Burnouf and Bietz (1989) and analyzed by SDS-PAGE (Graybosch and Morris 1990). HMW glutenin subunits were classified according to the system of Payne and Lawrence (1983). A *Glu-1* quality score and a score adjusted for the presence of 1BL/1RS (Payne et al 1987, Lukow et al 1989) were calculated for each line and variety. Some lines were found to be heterogeneous for HMW glutenin subunit composition. For such lines, the *Glu-1* scores were obtained by averaging the values assigned to the observed HMW glutenin subunits.

Statistical Analyses

For statistical analyses, protein fractionation values were expressed as percent of total extracted protein. Means were calculated from two fractionations per line or variety and triplicate protein determinations for each fraction and were used to calculate standard error of the laboratory procedure. Simple correlations were used to measure relationships between protein fractionations and mixing and baking quality parameters. Separate analyses were performed for each nursery experiment.

Simple correlations also were determined between *Glu-1* scores (and adjusted scores) and end-use quality, and between *Glu-1* scores (and adjusted scores) and protein fractionation data. Again, separate analyses were performed for each nursery experiment. We then attempted to improve on the applicability of the *Glu-1* scores by combining them with results from the protein fractionation studies. Stepwise multiple regression was used to select the set of variables that explained the largest amount of variation (r^2 values) for each end-use quality parameter. Separate analyses were performed for *Glu-1* and adjusted *Glu-1* scores.

RESULTS

Identification of Lines Carrying 1BL/1RS

SDS-PAGE analysis of 0.04*M* NaCl soluble proteins from eight entries (two check varieties and six experimentals) is shown in Figure 1. Lines carrying 1BL/1RS (e.g. Siouxland, N86L090, N86L096, N86L238, N86L265, N86L266) displayed a prominent protein band at M_r 43,000; lines without 1BL/1RS (Plainsman V and N86L085) lacked this protein. This protein comigrated with ω-secalins purified from the rye cultivar Rymin (not shown). Rye genes encoding ω-secalins have been previously mapped to 1RS (Shewry et al 1986). A minority of the wheat lines displayed proteins of similar molecular weight, but the bands were not as prominent, nor did they demonstrate the yellow-orange color we found to be characteristic of the 43K ω-secalins after silver staining. Such lines were classified as non-1BL/1RS. Eleven 1BL/

TABLE I
Means and Ranges for Baking Parameters[a] and Protein Solubilities for Wheat Lines in Experiments I and II

Line or Variety	WP (%)	FP (%)	MT (min)	MTO (0–9)	LV (cm^3)	ABS (%)	FPK (min)	FMTI (Brabender units)	% of Total Protein Soluble in 0.04*M* NaCl	% of Total Protein Soluble in 70% Ethanol	% of Total Protein Soluble in 0.1% KOH
Experiment I											
Mean	18.8	15.3	5.4	3.2	1,037	64.1	11.6	23	33.5	22.0	44.5
Minimum	15.9	12.3	2.3	1.0	885	57.1	5.0	10	24.4	13.7	37.1
Maximum	21.2	16.9	10.7	4.8	1,140	67.8	23.0	50	40.1	27.5	54.5
SE	0.6	...	...	...	...	...	...	...	2.0	1.4	1.5
Experiment II											
Mean	17.6	13.6	3.8	3.3	1,005	58.8	8.7	45	32.0	19.2	48.8
Minimum	14.4	11.2	0.7	0.0	570	51.6	1.5	10	19.4	10.5	39.6
Maximum	20.2	16.1	7.0	5.5	1,140	63.7	19.0	170	42.8	26.9	63.2
SE	0.6	...	...	...	...	...	...	...	3.6	3.2	2.0

[a] WP = grain protein; FP = flour protein; MT = mixograph time; MTO = mixograph tolerance; LV = loaf volume; ABS = farinograph absorption; FPK = farinograph peak time; FMTI = farinograph tolerance index.

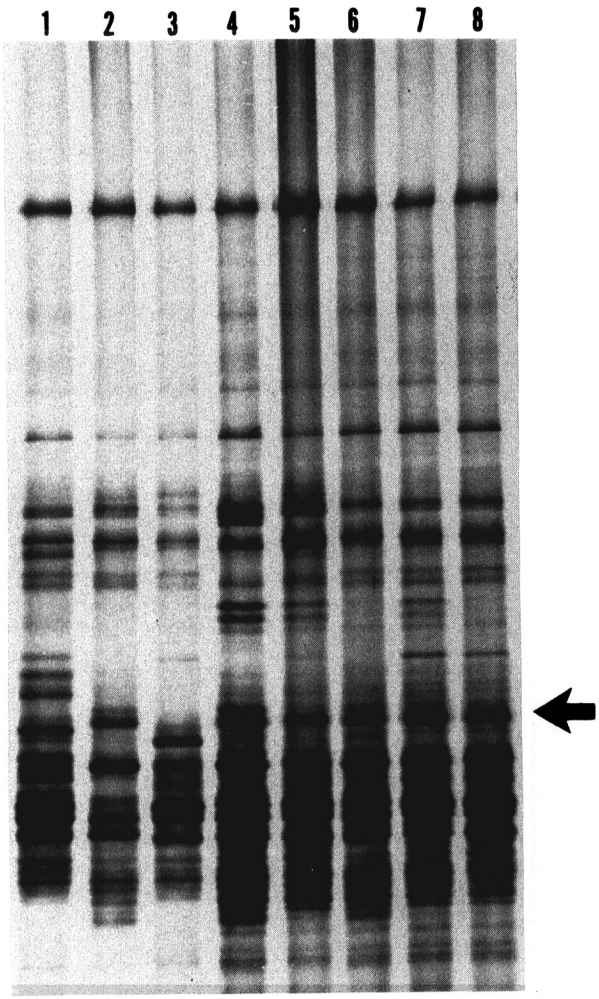

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of grain proteins extracted with 0.04*M* NaCl from selected wheat lines. The 43K ω-secalin used to identify 1A/1B lines is indicated by the arrow. Lane: **1,** Plainsman V; **2,** Siouxland; **3,** N86L085; **4,** N86L090; **5,** N86L096; **6,** N86L238; **7,** N86L265; **8,** N86L266.

1RS lines were identified in our experiments; two from experiment I and nine from experiment II. All lines classified as possessing 1BL/1RS were confirmed to be derived from a 1BL/1RS-containing parent. Presence or absence of 1BL/1RS in the experimental lines was later confirmed using cytological procedures (Lukaszewski, *personal communication*).

Variation in End-Use Quality and Protein Solubilities

Wheat lines in experiments I and II showed a wide variation in mixing and baking quality (Table I) with many lines of unacceptable end-use quality. Wheat and flour proteins were high for hard red winter wheats as a consequence of both genetic factors and optimal growing conditions. Many lines had unacceptable mixing times and tolerances; times ranged from 2.3 to 10.7 and 0.7 to 7.0 min in experiments I and II, respectively. Ranges in loaf volumes also were substantial. The variation shown here is representative of that encountered in unselected early generation lines from comparable wheat breeding programs. Lines in experiment II showed a wider range in nearly all quality parameters as expected from the lack of prescreening and from the larger genetic diversity in the trial.

TABLE II
Correlation Coefficients Between Baking Quality Parameters and Protein Concentrations of Solubility Classes

Quality Parameter[a]	Protein Solubility Class: 0.04*M* NaCl	70% Ethanol	0.1% KOH
Experiment I			
WP	−0.27	0.53**[b]	−0.43*
FP	0.06	0.53**	−0.48*
MT	0.22	0.02	−0.22
MTO	0.00	0.05	−0.04
LV	0.03	−0.21	0.13
ABS	0.01	0.38*	−0.31
FPK	0.25	0.16	−0.36
FMTI	0.16	0.01	−0.16
Experiment II			
WP	−0.42**	0.60**	−0.11
FP	−0.52**	0.71**	−0.11
MT	−0.32*	−0.03	0.39**
MTO	−0.25	−0.13	0.41**
LV	−0.57**	0.21	0.45**
ABS	−0.58**	0.60**	0.07
FPK	−0.63**	0.40**	0.32*
FMTI	0.41**	−0.04	−0.43**

[a] Parameters: WP = grain protein; FP = flour protein; MT = mixograph time; MTO = mixograph tolerance; LV = loaf volume; ABS = farinograph absorption; FPK = farinograph peak time; FMTI = farinograph tolerance index.
[b] * = Significant at $\alpha = 0.05$, ** = significant at $\alpha = 0.01$.

Significant variation in protein solubilities was found among the experimental lines (Table I). KOH-soluble protein (glutenin and residue protein) ranged from 37.1 to 54.4 and 39.6 to 63.2% of total protein in experiments I and II, respectively, with means of 44.5 and 48.7%. Salt-water-soluble and ethanol-soluble protein fractions also were highly variable, ranging from approximately 19 to 43 and 10 to 27% of total protein, respectively, over the experiments.

Protein solubilities were related to mixing and baking quality by simple correlations for each experiment (Table II). In experiment I, increases in protein content were associated with an increase in ethanol-soluble proteins and a decrease in KOH-soluble proteins. A similar response to protein concentration among

TABLE III
Results of Baking Quality Evaluations and Protein Solubilities for Selected Wheat Lines and Checks in Experiments I and II

Line or Variety	Parameter[a] WP (%)	FP (%)	MT (min)	MTO (0–9)	LV (cm^3)	ABS (%)	FPK (min)	FMTI (Brabender units)	% of Total Protein Soluble in 0.04*M* NaCl	70% Ethanol	0.1% KOH	1B/1R (+ or −)
Experiment I												
Brule	15.9	12.3	4.5	4.5	1,085	57.1	7.0	25	37.4	16.0	46.7	−
Lancota	18.5	14.7	4.5	3.5	1,090	64.2	11.0	20	28.0	17.5	54.5	−
Plainsman V	20.6	16.8	10.7	4.8	1,140	66.2	23.0	15	30.9	23.8	45.3	−
Siouxland	16.9	13.2	6.0	1.8	995	62.1	10.0	20	33.7	13.7	52.3	+
N87U112	19.2	14.6	5.0	3.8	895	60.2	13.0	20	32.1	26.7	41.2	+
N87U113	19.2	15.6	6.5	3.0	1,055	65.3	15.0	35	36.0	21.7	42.4	+
Experiment II												
Colt	16.3	12.7	2.8	3.5	970	56.1	6.5	45	35.1	21.3	43.7	−
Lancota	18.1	14.5	3.7	3.0	1,090	62.9	9.0	35	29.2	23.7	47.1	−
Redland	14.8	11.2	4.3	3.8	970	53.8	6.5	40	35.0	17.6	47.4	−
Siouxland	15.5	13.9	3.7	2.8	955	57.9	6.5	50	42.4	12.9	44.7	+
N86L028	17.9	13.8	4.3	3.0	910	58.8	7.0	40	38.7	17.2	44.1	+
N86L031	19.3	15.1	4.5	3.5	850	60.8	7.5	40	38.8	16.7	44.5	+
N86L040	18.2	14.2	3.5	4.0	915	58.8	6.5	35	36.4	17.3	46.3	+
N86L085[b]	15.8	11.2	3.5	4.5	985	51.6	3.5	60	36.0	10.5	53.5	−
N86L090[b]	18.6	13.9	0.7	0.0	570	55.1	1.5	170	41.8	18.5	39.6	+
N86L096[b]	16.1	11.2	3.0	2.5	805	52.8	3.0	70	42.8	16.6	40.6	+
N86L238[c]	17.8	12.5	4.3	4.0	880	55.5	9.5	40	30.4	20.9	48.7	+
N86L250[c]	18.4	13.0	4.8	4.0	930	57.2	12.0	30	28.4	21.6	50.0	+
N86L265[c]	19.7	15.0	1.0	0.5	755	59.1	2.5	100	31.0	26.2	42.8	+
N86L266[c]	18.3	13.1	2.8	1.5	880	55.0	5.5	80	29.3	26.3	44.4	+

[a] Parameters: WP = grain protein; FP = flour protein; MT = mixograph time; MTO = mixograph tolerance; LV = loaf volume; ABS = farinograph absorption; FPK = farinograph peak time; FMTI = farinograph tolerance index.
[b] Sister lines.
[c] Sister lines.

TABLE IV
Observed Frequencies of High Molecular Weight (HMW) Glutenin Subunit Combinations and *Glu-1* Scores in Two Winter Wheat Trials[a]

HMW Glutenin Subunits 1A[b]	1B	1D	1B/1R (+ or −)	No. Lines or Varieties	*Glu-1* Score	Adjusted *Glu-1* Score
Experiment I						
2*	7 + 9	5 + 10	−	9	9	9
2*	7 + 9	5 + 10	+	3	9	6
1,2*[c]	7 + 8	5 + 10	−	1	10	10
1	7 + 9	2 + 12	−	1	7	7
2*	6 + 8	5 + 10	−	5	8	8
2*	7 + 9	5 + 10[c] 2 + 12	−	1	8	8
2*	7 + 8	2 + 12	−	2	8	8
2*	7 + 9	2 + 12	−	1	7	7
2*	7 + 8[c]	5 + 10 7 + 9	−	1	9.5	9.5
2*	7 + 9[c] 13 + 16	5 + 10	−	1	9.5	9.5
2*	7 + 8	5 + 10	−	1	10	10
Experiment II						
2*	7 + 9	5 + 10	−	20	9	9
2*	7 + 9	5 + 10	+	5	9	6
...	7 + 9	2 + 12	−	4	4	4
1,2*[c]	7 + 9	5 + 10	+	1	9	6
1	7 + 9	5 + 10	+	1	9	6
...	7 + 9	5 + 10	−	1	6	6
1	7 + 9	5 + 10	−	3	9	9
2*	7 + 9	...	+	2	5	3
2*	7 + 9	5 + 10[c] 2 + 12	−	1	8	8
2*	7 + 8	5 + 10	−	1	10	10
2*	7 + 8	5 + 10	+	2	10	7
2*	13 + 16	5 + 10	−	1	10	10
1	13 + 16	5 + 10	−	1	10	10
1	7 + 8[c] 13 + 16	5 + 10	−	1	10	10

[a] Chromosomal assignments are based on Payne and Lawrence (1983).
[b] Chromosomal location of genes encoding HMW glutenin subunits.
[c] Mixtures: lines heterogeneous for HMW glutenin subunits.

high-protein cultivars was shown by Ulmer (1973). There was no influence of variation in protein solubility classes on mixing or baking quality among the lines in experiment I, other than a positive correlation of ethanol-soluble proteins with water absorption.

Variation in protein solubilities had substantial influence on mixing and baking quality of wheat experimental lines in experiment II (Table II). Ethanol-soluble protein levels again were positively associated with increased protein content, with a corresponding decrease in levels of salt-water-soluble proteins. Increased levels of KOH-soluble proteins were associated with increases in mixing times and tolerances and with loaf volume. Increasing levels of ethanol-soluble proteins were correlated with increases in water absorption and farinograph peak time. Enhanced levels of salt-water-soluble proteins were associated with marked reductions in overall mixing and baking quality. Significant negative correlations of salt-water-soluble proteins were found with mixing times and tolerances, loaf volumes, and water absorption.

The large differences in the relative influences of protein solubilities on mixing and baking quality in the experiments were primarily a reflection of the greater genetic variability found in experiment II for overall quality characteristics. This would suggest that inconsistencies of past research attempts to determine importance of protein solubility characteristics in mixing and baking quality may be, in part, a reflection of ranges in genetic variability, or sample variability, examined by each study.

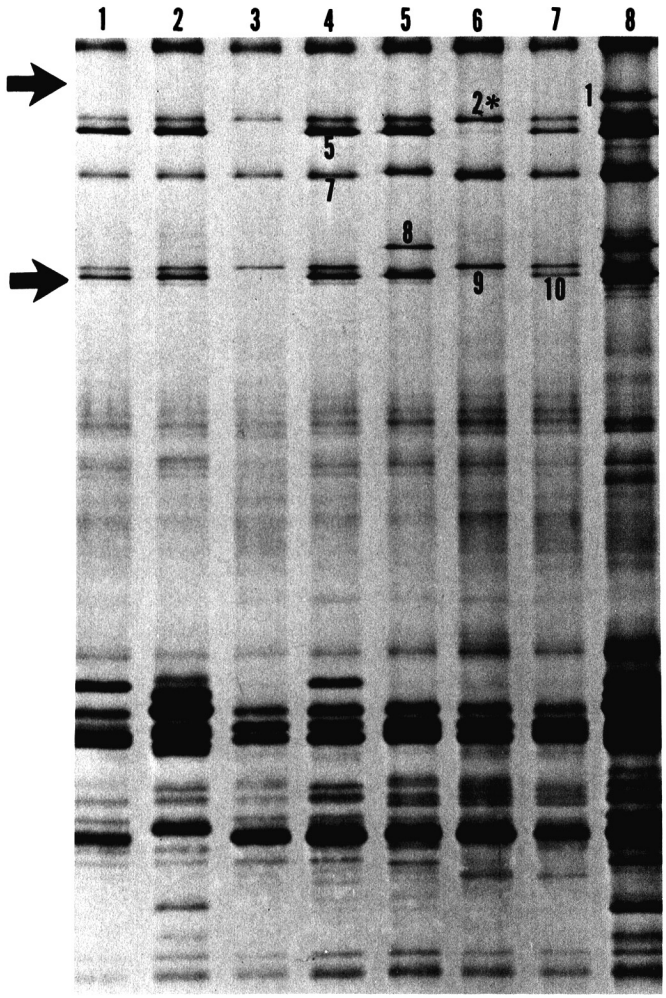

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of glutenins extracted from whole grain of selected wheat lines. HMW glutenin subunits are located in region between arrows and designated on the figure. Lines and high molecular weight glutenin subunits are shown in lanes: **1,** Siouxland (2*, 7+9, 5+10); **2,** 86L085 (2*, 7+9, 5+10); **3,** 86L090 (2*, 7+9); **4,** 86L096 (2*, 7+9, 5+10); **5,** 86L238 (2*, 7+8, 5+10); **6,** 86L265 (2*, 7+9); **7,** 86L265 (2*, 7+9, 5+10); **8,** Plainsman V (1,2*, 7+8, 5+10).

Influence of 1BL/1RS on Quality and Protein Solubilities

Quality and protein solubility characteristics of 1BL/1RS-containing lines were highly variable (Table III). These characteristics indicated a substantial influence of genetic background on 1BL/1RS effects. Experimental lines N86L085, N86L090, and N86L096 were sisters, having been derived from the same parental cross. N86L090, which carries 1BL/1RS, had extremely poor quality with very short mixing time and tolerance and low loaf volume. N86L096 also had unacceptable quality and carries 1BL/1RS, but was intermediate in quality between N86L090 and its non-1BL/1RS sister N86L085. Levels of salt-water-soluble proteins were lower in N86L085 than in N86L090 and N86L096. N86L238, N86L250, N86L265, and N86L266, another group of sister lines in this study, each possessed 1BL/1RS. Their quality characteristics suggested that certain background genetic effects may help to alleviate many of the deleterious characteristics of 1BL/1RS. N86L238 and N86L250 had much better mixing tolerances than N86L265 and N86L266. There were no differences in levels of salt-water-soluble proteins among this group of sister lines; however, N86L238 and N86L250 had higher levels of KOH-soluble proteins.

No general conclusions can be drawn regarding protein solubility characteristics of 1BL/1RS lines. Levels of salt-water soluble (0.04*M* NaCl) proteins from 1BL/1RS lines of experiment I were similar to the experimental mean, and lower than those of the high-quality cultivar Brule. In experiment II, six 1BL/1RS lines exceeded the experimental mean for concentrations of salt-water-soluble proteins and four 1BL/1RS lines had values below the mean value. There was a trend toward below average levels of 70% ethanol-soluble proteins in 1BL/1RS lines, but it was not ubiquitous. The most consistent characteristic of 1BL/1RS lines in the study was below-average levels of KOH-soluble proteins. Only two 1BL/1RS entries, Siouxland in experiment I and N86L250 in experiment II, had levels of KOH-soluble proteins that exceeded the experimental means.

HMW Glutenin Subunit Composition

Examples of SDS-PAGE HMW glutenin subunit separations from selected lines and varieties are shown in Figure 2. Table IV lists the observed combinations of HMW glutenin subunits, their frequencies, and the corresponding *Glu-1* score for each combination. One cultivar, Lancota, appeared to possess the HMW glutenin subunits 2, 5, 12, 13, and 16. However, bands 2 and 5 are allelic (Payne and Lawrence 1983) and, hence, should not occur together. The subunit that appeared to migrate as band 5 in our 11–16.5% gradient gel system was found to have a slower mobility than band 5 in 7.5% gels (not shown) and thus may represent an undescribed protein, the nature of which is now under investigation. The HMW glutenin subunit combination 2*, 7+9, 5+10 (with or without 1BL/1RS) was present in over 50% of all lines and varieties.

Glu-1 scores and adjusted *Glu-1* scores were correlated with end-use quality and protein solubility parameters for each experiment (Table V). In experiment I, mixograph scores (mixograph time and tolerance) were significantly correlated to both *Glu-1* and adjusted *Glu-1* scores. Correlations with mixograph time were notably higher with the unadjusted *Glu-1* score. Farinograph peak time was correlated only with the unadjusted *Glu-1* score, and loaf volume correlated only with the adjusted score. In experiment II, both *Glu-1* and adjusted *Glu-1* scores were significantly correlated with mixograph time, mixograph tolerance, farinograph peak time, and farinograph tolerance index, and the adjusted scores significantly correlated with loaf volume. With the exceptions of the correlation of *Glu-1* with mixograph time and farinograph peak time in experiment I, correlations of quality parameters with *Glu-1* scores were higher in experiment II. This is, at least in part, a reflection of the greater genetic diversity among lines in experiment II.

In both experiments, *Glu-1* and adjusted *Glu-1* scores were

independent of measures of total protein (grain and flour protein) and absorption. *Glu-1* scores also were independent of protein solubility characteristics. The adjusted *Glu-1* score showed only slight correlation in experiment II to decreasing levels of proteins soluble in 0.04*M* NaCl (salt-water solubles) and increasing KOH-soluble proteins (glutenin). Grain and flour protein levels and absorption values were more closely related to protein solubilities than either adjusted or unadjusted *Glu-1* scores.

The limitations of the *Glu-1* scores to explain variation in end-use quality are demonstrated in Figures 3–6. Plots of mixing time with *Glu-1* scores and adjusted *Glu-1* scores show that the variables primarily isolate those lines with extremely poor mixing characteristics. Among the majority of lines with adequate *Glu-1* scores (8 or above), there exists extensive mixing time variation. The correlations presented appear primarily to result from the presence of lines at the extremely poor end of the quality scale. Analysis of HMW glutenin subunit composition did identify two lines that lacked 1D encoded subunits. This deletion had a dramatic negative affect on mixing characteristics. Both lines were much poorer than the respective sister lines with active 1D HMW glutenin subunit genes. Each was derived from a cross that included the landrace Nap Hal as a parent. Nap Hal carries a null allele for 1D HMW glutenin subunits that is highly deleterious (Lawrence et al 1988).

The independence of *Glu-1* scores and protein solubilities, along with significant relations of each with end-use quality parameters, suggested that a combination of the qualitative *Glu-1* score and quantitative protein solubility characteristics could better explain

TABLE V
Correlation Coefficients (*r*) Between *Glu-1* Scores, Protein Fractionations, and Various Quality Parameters (experiments I and II)

	Experiment I		Experiment II	
Parameter[a]	*Glu-1*	Adjusted *Glu-1*	*Glu-1*	Adjusted *Glu-1*
WP	0.17	0.21	−0.21	−0.24
FP	0.30	0.38	−0.006	0.03
MT	0.73**[b]	0.45*	0.62**	0.61**
MTO	0.43*	0.42*	0.64**	0.69**
LV	0.23	0.40*	0.26	0.63**
ABS	−0.10	0.13	−0.03	0.14
FPK	0.62**	0.37	0.46**	0.56**
FMTI	−0.33	−0.32	−0.49**	−0.62**
NaCl	0.26	0.16	−0.11	−0.33**
EtOH	−0.05	−0.05	−0.02	−0.07
KOH	−0.21	−0.22	0.14	0.39**

[a] Parameters: WP = grain protein; FP = flour protein; MT = mixograph time; MTO = mixograph tolerance; LV = loaf volume; ABS = farinograph absorption; FPK = farinograph peak time; FMTI = farinograph tolerance index; NaCl = percent of grain protein soluble in 0.04*M* NaCl; EtOH = percent of grain protein soluble in 70% ethanol; KOH = percent of grain protein soluble in 0.1% KOH.

[b] *, ** = Significant at $\alpha = 0.05$ and $\alpha = 0.01$, respectively.

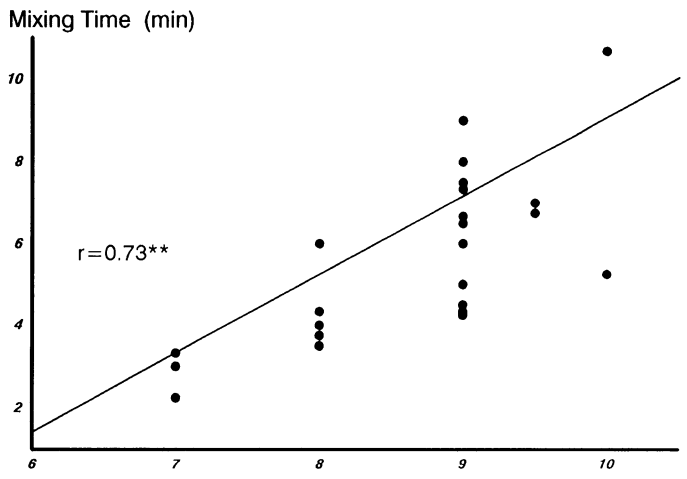

Fig. 3. Relationship between mixograph mixing time and *Glu-1* score, experiment I. Multiple observations with identical values are not plotted.

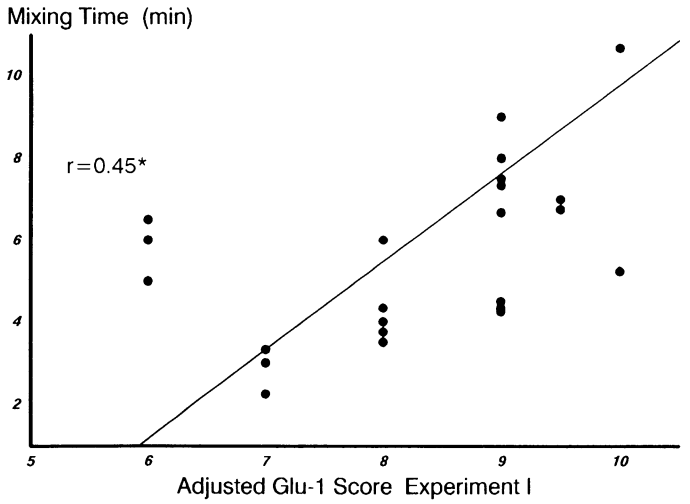

Fig. 4. Relationship between mixograph mixing time and adjusted *Glu-1* score, experiment I. Multiple observations with identical values are not plotted.

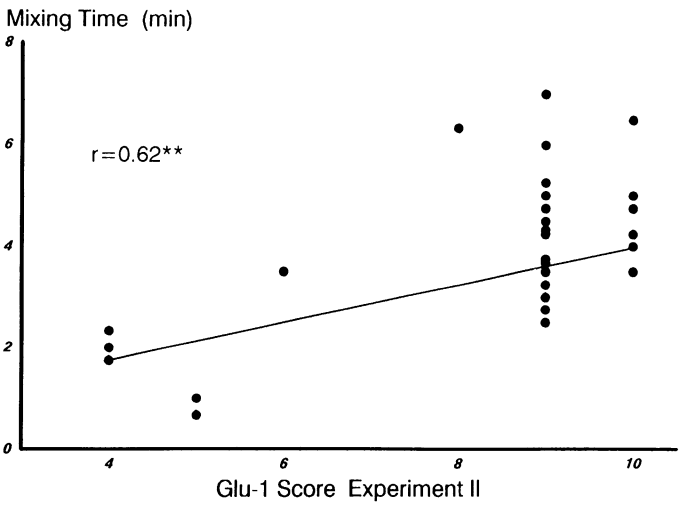

Fig. 5. Relationship between mixograph mixing time and *Glu-1* score, experiment II. Multiple observations with identical values are not plotted.

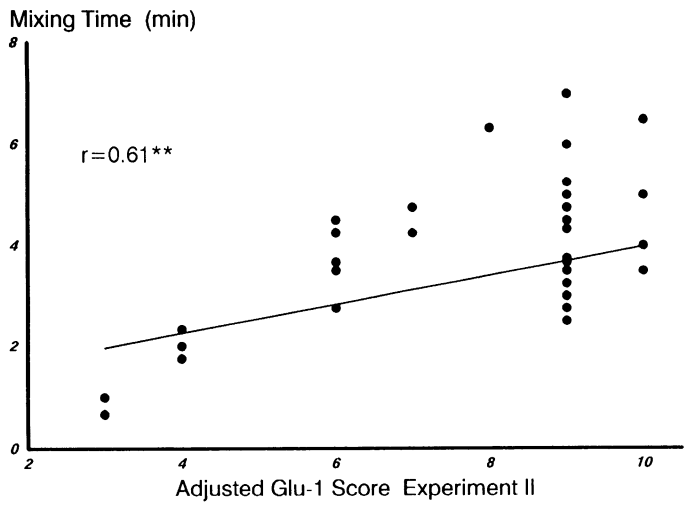

Fig. 6. Relationship between mixograph mixing time and adjusted *Glu-1* score, experiment II. Multiple observations with identical values are not plotted.

a larger proportion of variation in quality parameters. Stepwise multiple regression was used to test this hypothesis. Table VI lists the variable or combination of variables that explains the highest degree of variation (r^2) for each quality parameter. Only parameters for which significant regressions were obtained are given. While several combinations increased r^2 values, the increases were relatively small. No more than 56% ($r^2 = 0.56$) of the total variation in any quality parameter could be explained by *Glu-1* scores, protein solubilities, or their combination.

DISCUSSION

Substantial variation in quality characteristics was found among wheat lines in the two experiments. Protein solubility characteristics were highly correlated with mixing and baking quality parameters for the highly diverse lines in experiment II. However, few significant relationships were shown among parameters in experiment I. Usefulness of protein solubility in characterizing potential mixing and baking quality of germ plasm likely will depend on initial diversity of experimental materials. Also, environmental modifications of protein solubilities need to be assessed.

The effects of 1BL/1RS on wheat end-use quality and protein solubilities were highly dependent on genetic background. Many of the 1BL/1RS lines we identified did have above average levels of salt-water-soluble protein. However, elevated levels of salt-water-soluble proteins were not unique to wheats carrying 1BL/1RS. High salt-water solubles may be associated with a general reduction in overall mixing and baking quality in diverse germ plasm. The results presented herein suggest that the diminished quality attributes associated with 1BL/1RS also might result from a deficiency of KOH-soluble proteins associated with enhanced levels of salt-water-soluble proteins. The short arm of 1B is known to carry genes encoding both low-molecular-weight glutenins and gliadins (Gupta and Shepherd 1988). Koebner and Shepherd (1988) suggested that deleterious effects of 1RS are not the result of the presence of secalins, but rather, arise from the lack of gene products (perhaps contributing to levels of KOH-soluble proteins) of loci on 1BS. Both levels of KOH-soluble proteins and HMW glutenin subunit composition may influence end-use quality of 1BL/1RS possessing lines. The 1BL/1RS lines identified in this study with the best overall baking quality (N86L238 and N86L250) had the highest *Glu-1* scores (2*, 7+8, 5+10) and the highest levels of KOH-soluble protein. Further experimentation is needed to determine whether these factors might be useful in improving the quality of 1BL/1RS lines.

The correlations of quality parameters with *Glu-1* scores were slightly below those of previous reports (Payne et al 1987, Sontag et al 1986, Payne et al 1988, Lukow et al 1989). Results also differed when the two experiments reported herein were compared. The wider genetic variation in quality among lines in experiment II contributed to the higher correlations. Results of future experiments with *Glu-1* scores and protein fractionations also will be governed by the extent of the variation in quality of the experimental materials.

Glu-1 scores and protein solubilities were shown to be mostly independent. *Glu-1* scores provided higher *r* values than protein solubilities in correlations with parameters that estimate dough strength. Protein solubilities more closely related to wheat and flour protein concentrations and water absorption. Combining *Glu-1* scores with protein solubility characteristics improved the correlations with quality parameters slightly.

Thus, there are significant limitations in using either *Glu-1* scores or protein solubility characteristics in hard red winter wheat breeding. When working with wheats with average to above average quality, these predictors have little useful value. *Glu-1* scores can eliminate unacceptable lines, although some lines with acceptable quality also could be lost (Lorenzo and Kronstad 1987). The high frequency of varieties with high *Glu-1* scores among Canadian (Lukow et al 1989) and American bread wheats (G. Lookhart, *personal communication*) also will limit the usefulness of *Glu-1* scores. However, hard wheat breeders using more exotic materials, or wheats of other end-use classes, might benefit by use of protein fractionations and *Glu-1* scores.

Although major genes (e.g., those encoding HMW glutenin subunits) influencing quality clearly exist, selection schemes based on these aspects alone will be inefficient. Protein quantity and quality explain only a portion of the observed variability in end-use quality. Additional factors, such as pentosan content (Shogren et al 1988) and lipid composition (Chung et al 1980) will need to be evaluated and incorporated with protein information before a complete model of end-use quality is achieved.

TABLE VI
Stepwise Multiple Regression Analysis Between End-Use Quality Parameters and Combinations of *Glu-1* (and adjusted *Glu-1*) Scores and Protein Fractionations[a]

Parameter[b]	Variables	r^2
Experiment I		
WP	EtOH	0.28
FP	EtOH, adjusted *Glu-1* score	0.40
MT	*Glu-1* score	0.53
MTO	*Glu-1* score	0.18
LV	Adjusted *Glu-1* score	0.16
ABS	EtOH	0.15
FPK	*Glu-1* score, KOH	0.44
FMTI	*Glu-1* score	0.11
Experiment II		
WP	EtOH, adjusted *Glu-1* score, KOH	0.46
FP	EtOH	0.51
MT	*Glu-1* score, KOH	0.47
MTO	*Glu-1* score, KOH	0.49
LV	NaCl, *Glu-1* score	0.36
ABS	EtOH, KOH	0.45
FPK	NaCl, adjusted *Glu-1* score, EtOH	0.56
FMTI	Adjusted *Glu-1* score, NaCl	0.44

[a] For each parameter, only the highest significant variable or combination of variations is given.

[b] Parameters: WP = grain protein; FP = flour protein; MT = mixograph time; MTO = mixograph tolerance; LV = loaf volume; ABS = farinograph absorption; FPK = farinograph peak time; FMTI = farinograph tolerance index; NaCl = percent of grain protein soluble in 0.04*M* NaCl; EtOH = percent of grain protein soluble in 70% ethanol; KOH = percent of grain protein soluble in 0.1% KOH.

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