Theor Appl Genet (1992) 83:403–412

Use of recombinant inbred lines of wheat for study of associations of high-molecular-weight glutenin subunit alleles to quantitative traits

2. Milling and bread-baking qualitiy

M. Rousset [1], J.M. Carrillo [2], C.O. Qualset [3,*], and D.D. Kasarda [4]

[1] Station for the Improvement of Plants, INRA, Clermont-Ferrand Cedex, Domaine de Crouelle, F-63039, France
[2] Department of Genetics, E.T.S.I. Agronomy Polytechnic University E-28040 Madrid, Spain
[3] Department of Agronomy and Range Science, University of California, Davis, CA 95616, USA
[4] U.S. Department of Agriculture, Western Regional Research Center, Albany, CA 94710, USA

Received December 11, 1990; Accepted April 18, 1991
Communicated by A.L. Kahler

Summary. Recombinant inbred lines (RILs) derived by single plant descent to F_8 from a hybrid of Anza, a low-quality cultivar, and Cajeme 71, a high-quality cultivar, differed in alleles at three high-molecular-weight glutenin (HMW-glu) seed storage protein loci. The 48 RILs were classified by SDS-PAGE for the Anza alleles *Glu-A1c* (null), *Glu-B1b* (subunits 7+8), and *Glu-D1a* (subunits 2+12) and for Cajeme 71 alleles *Glu-A1a* (subunit 1), *Glu-B1i* (subunits 17+18), and *Glu-D1d* (subunits 5+10). All RILs and parents were grown in a replicated field trial with three levels of nitrogen (N) fertilization. Additive and additive × additive gene effects for the three loci were detected by orthogonal comparisons of means for each of six wheat end-use quality traits. Each HMW-glu genotype was represented by three to ten RILs so that variability among RILs within each HMW-glu genotype could be examined. N effects were consistently small. All traits except flour yield were highly correlated with predictor traits studied earlier. Flour protein content, baking water absorption, dough mixing time, bread loaf volume, and bread loaf crumb score were all correlated, suggesting similar gene control for these traits; however, specific additive locus contributions were evident: α_B for flour yield; α_B and α_D for flour protein; and α_B for absorption, but differing in sign; all three loci for mixing time, but α_B was negative; and all three loci were positively associated with loaf volume. Digenic epistatic effects were significant for flour yield (α_{AD}), flour protein (α_{AB}), and absorption and mixing time (α_{AD}, α_{BD}). Only flour yield showed a trigenic epistatic effect. Six of seven epistatic effects were negative, thus showing how progress in breeding for high quality may be impeded by interaction of genes which, by themselves, have strong positive additive effects. Considerable genetic variance among RILs within a HMW-glu genotype was detected for all traits, and the summation of α effects accounted for a mean of 13% of the parental differences for the six traits examined in this study. Clearly, further resolution of the genetics of wheat quality would be desirable from a plant breeding point of view.

Key words: *Triticum aestivum* L. – Protein content – Additive gene action – Epistasis

* To whom correspondence should be addressed

Introduction

Improved flour quality is a major goal in wheat (*Triticum aestivum* L.) breeding programs. A clearer understanding of the genetic control of flour and bread-making quality would increase the probability of identifying superior genotypes and make possible the subsequent development of improved cultivars. Earlier we reviewed previous research on the relationships of wheat quality to gluten proteins (Carrillo et al. 1990). We studied random recombinant inbred lines (RILs) from a cross of two spring wheat cultivars having widely divergent flour quality factors (Carrillo et al. 1990). Several predictor tests were examined in relation to HMW glutenin protein subunits. Other workers used small-scale prediction tests, including the Brabender extensograph (Lawrence et al. 1987) and the Chopin alveograph (Branlard and Dardevet 1985). Lagudah et al. (1988) studied the relationship of

the *Glu-1* loci with Brabender farinograph and extensograph dough properties in a F_3 population. Lawrence et al. (1987) found that HMW glutenin subunits 5+10 at the *Glu-D1* locus and subunits 17+18 at the *Glu-B1* had significantly greater effects on mixograph time to peak than loss of subunit 1 at the *Glu-A1* locus. Only a few studies have evaluated actual bread-baking performance in relation to HMW glutenin subunit composition (Campbell et al. 1987; Cressey et al. 1987).

While there are limitations of all approaches to the evaluation of HMW glutenin subunit effects on quality, the effects of heterozygosity in segregating populations and comparisons of unrelated cultivars having different HMW glutenin alleles present rather severe confounding effects. Whole chromosome substitution lines (Zemetra et al. 1987; Mansur et al. 1990) and homozygous backcross-derived lines congenic for HMW glutenin subunit alleles (Payne et al. 1987) provide direct comparisons and thus inferences about allelic effects, at least to the extent that linkage blocks are unimportant. Intracultivar variants also have been used (Lawrence et al. 1987), but the biotypes may not be isogenic for the background genotype, thereby confounding comparisons. An additional approach, used in the present study, is to examine RILs from a hybrid between two inbred cultivars. Such lines permit the estimation of genetic effects of particular loci in numerous related lines. Mean effects over all lines most probably estimate associations or direct effects of major loci, such as the HMW glutenin loci on the long arms of chromosomes 1A, 1B, and 1D of hexaploid wheat. At the same time, these gene effects or associations can be examined among a group of genetically different lines with the same major gene allelic composition, to infer whether or not the traits under investigation are controlled by widely dispersed loci. Thus, the congenic line approach provides an accurate assessment of direct effects of marker alleles, while the random recombinant inbred line approach detects average effects of marker loci as well as an estimate of the influence of additional unknown genes. Further refinements in this approach call for a large number of marker loci, preferably mapped throughout all of the chromosomes. Thus, the restriction fragment length polymorphism approach (Beckman and Soller 1986) may give much improved resolution of the genetics of quantitative traits. This will be especially useful for genetic analysis of wheat flour quality.

The present paper is an interim approach to that goal. A set of recombinant inbred lines (RILs) have been well characterized for numerous flour quality and environmental factors (Carrillo et al. 1990; W. Kelman and C. Qualset, unpublished results), which have been used to increase our understanding of the genetic basis of wheat flour quality, as well as to examine a method of assessing quantitative genetic variation.

Materials and methods

Genotypes

Anza (poor quality) and Cajeme 71 (good quality) were hybridized and recombinant inbred lines were derived from single-plant progenies in the F_8 generation. After the last generation of selfing, each RIL was bulk-harvested to provide seed for replicated field trials and for electrophoretic analysis, to classify each RIL and the parents for high-molecular-weight glutenin (HMW-glu) protein subunits. The parents had subunit composition as follows, according to Payne and Lawrence's (1983) designations of alleles and subunits (in parentheses): Anza *Glu-A1c* (null), *Glu-B1b* (7/8), and *Glu-D1a* (2/12); Cajeme 71 *Glu-A1a* (1), *Glu-B1i* (17/18), and *Glu-D1d* (5/10). Anza and Cajeme 71 were assigned the codes 111 and 222, respectively, for the alleles on the homoeologous chromosomes 1A, 1B, and 1D. Each RIL was similarly coded for specific allelic composition by SDS electrophoresis (Carrillo et al. 1990). All eight digenic genotypes were represented among the 48 RILs in the frequencies: 10, 7, 9, 3, 4, 4, 6, and 5 for the respective genotypes 111, 112, 121, 122, 211, 212, 221, and 222. Cajeme 71 alleles were assigned code 2 because, for most traits, this cultivar had the most favorable values.

Field experiment

Details were presented earlier (Carrillo et al. 1990) and only the main features are recounted here. The parents and RILs were grown in the winter crop cycle, in a three-replicate field experiment at Davis, California, using common field-plot management techniques. The experiment included 56 entries (48 RILs, Anza repeated six times in each replicate, and Cajeme 71 and Yecora Rojo). The experimental area had been previously cropped with Sudan grass to deplete soil nitrogen (N). Three N fertilization levels were included as main plots (N40, N80, N120) for 40, 80, and 120 kg ha^{-1} N applied. One replicate was atypical because of winter flooding damage and was not included in the analysis. After individual plot data were recorded, the grain from the two replicates was composited for each parent and RIL for quality evaluation.

Milling and baking tests

All wheat quality factors reported in this paper were evaluated at the USDA Western Wheat Quality Laboratory at Pullman/WA, according to AACC (1983) approved methods. Rationale for the methods used was reviewed by Finney et al. (1987) and details are presented in annual laboratory reports. Six of the most important parameters evaluated were selected for presentation here. Flour yield, as percentage recovery of flour from the whole-grain sample, was taken as an indicator of milling performance. Flour protein on 14% moisture basis was determined by near-infrared spectroscopy and can be compared to Kjeldahl-method, whole-grain protein concentrations presented in the previous paper. Water absorption (%) during dough mixing and mixing time (min) are indicators of dough handling properties. Finally, bread-baking performance was evaluated as volume of a baked loaf of bread (cc) and a visual estimate of bread crumb quality based on a 1 to 9 score, where 1 is desirable and 9 is undesirable. Analyses of variance were conducted to detect differences among genotypes and N levels, using the genotypes × N levels mean square as an error term. Because grain samples from all replicates were pooled, no plot error mean square could be estimated and, hence, neither could tests for genotypes × N levels interactions. This interaction proved to be negligible for the grain parameters studied previously (Carrillo et al. 1990), and is likely to be the situation for the data presented here, since

Table 1. Mean squares and coefficients of variation from the analysis of variance for recombinant inbred lines (RILs) and parents in a field experiment at three nitrogen fertilization levels

Source	*df*	Flour yield	Flour protein	Baking absorption	Mixing time	Loaf volume	Crumb score
Nitrogen levels	2	8.15**	2.08**	9.54**	0.60	52,275**	44.79**
Genotypes	55	4.14**	1.07**	26.03**	5.44**	8,654**	12.29**
Error (G × N)	110	0.24	0.11	0.81	0.20	1,105	2.18
CV, %		0.7	4.1	1.5	13.2	4.1	21.6

** $P<0.01$

Table 2. Means over all RILs and parents in the experiment for each nitrogen fertilizaiton level. Standard errors are given for N level means

Genotype	N level	Flour yield %	Flour protein %	Baking absorption %	Mixing time min.	Loaf volume cc	Crumb score
RILs	N40	70.3[ab]	7.8[b]	59.9[a]	3.3[a]	786[b]	7.6[a]
	N80	69.9[b]	8.1[a]	59.5[a]	3.4[a]	800[b]	7.1[a]
	N120	70.7[a]	8.2[a]	59.1[a]	3.6[a]	847[a]	5.7[b]
	Mean	70.3	8.0	59.5	3.4	811	6.8
	SE	0.1	0.1	0.1	0.1	5	0.2
Anza	N40	71.6[b]	7.4[a]	56.6[a]	2.3[a]	725[ab]	9.0[a]
	N80	71.2[b]	7.5[a]	55.2[a]	2.2[a]	704[b]	9.0[a]
	N120	72.2[a]	7.4[a]	55.5[a]	1.9[a]	770[a]	8.0[b]
	Mean	71.7	7.4	55.8	2.1	733	8.7
	SE	0.2	0.1	0.4	0.2	13	0.6
Cajeme 71	N40	69.6[a]	8.6[a]	64.0[a]	7.2[a]	907[a]	2.0[a]
	N80	69.2[a]	9.0[a]	63.6[a]	7.1[a]	892[a]	2.5[a]
	N120	69.2[a]	8.8[a]	62.9[a]	7.0[a]	902[a]	2.5[a]
	Mean	69.3	8.8	63.5	7.1	901	2.3
	SE	0.4	0.2	0.6	0.3	24	1.0

[a, b] For comparisons among N level means, different letters represent significant differences at 5% protection level, Duncan's multiple range test

we found that the prediction tests and direct quality measurements were highly correlated.

Genetic effects

The variance among RILs was partitioned into single-degree-of-freedom orthogonal comparisons for the three HMW-glu loci as presented by Carrillo et al. (1990), to detect and estimate the additive (α_A, α_B, α_D) and additive × additive (α_{AB}, α_{AD}, α_{BD}, α_{ABD}) genetic effects. The genetic effects were estimated as regression coefficients $\alpha = \Sigma\, c_i G_i / \Sigma\, c_i^2$, where the c_i are othogonal coefficients and G_i the means for each of the $i=1, \ldots, 8$ HMW-glu genotypes. The G_i were each based upon the mean of the respective number of RILs identified for each HMW-glu genotype. Tests for genetic variation among the RIL within each HMW-glu genotype were made by computing an analysis of variance for each group. The among-RIL within-HMW-glu mean squares were tested for significance against the whole-experiment genotypes × N levels mean square.

Results and discussion

Genotype and N effects

All milling and bread-making quality characters studied showed highly significant differences among RILs (Table 1). The effects of N treatments were also significant, except for mixing time (Table 1). The two parents, Anza and Cajeme 71, differed substantially in all traits (Table 2). As expected from prior research, Cajeme 71 showed lower flour yield, higher flour protein, baking absorption, mixing time, loaf volume, and lower bread crumb score than Anza. The flour protein contents of the parent cultivars, 7.4 and 8.8%, respectively, were low, but high enough to discriminate among genotypes. Finney et al. (1987) reported quality evaluation on wheat flour rang-

Table 3. Phenotypic correlation coefficients between grain and flour quality tests in a field experiment of Anza × Cajeme 71 RILs and the parents for each N level and mean over all N levels ($n = 56$)

Characters correlated:		Nitrogen level			
Grain	Flour	N40	N80	N120	Mean
Grain protein	Flour yield	−0.21	−0.11	−0.21	0.00
	Flour protein	0.75**	0.80**	0.79**	0.67**
	Baking absorption	0.55**	0.58**	0.52**	0.34*
	Mixing time	0.28*	0.25	0.36**	0.26
	Loaf volume	0.38**	0.56**	0.62**	0.61**
	Crumb score	−0.39**	−0.46**	−0.64**	−0.55**
Pearling index	Flour yield	−0.54**	−0.50**	−0.51**	−0.46**
	Flour protein	0.47**	0.48**	0.35**	0.45**
	Baking absorption	0.60**	0.69**	0.58**	0.59**
	Mixing time	0.39**	0.41**	0.43**	0.40**
	Loaf volume	0.33*	0.51**	0.33*	0.42**
	Crumb score	−0.27*	−0.39**	−0.32	−0.34*
Yellowberry	Flour yield	0.40**	0.31*	0.30*	0.22*
	Flow protein	−0.78**	−0.83**	−0.72**	−0.76**
	Baking absorption	−0.60**	−0.66**	−0.53**	−0.49**
	Mixing time	−0.41**	−0.46**	−0.35**	−0.39**
	Loaf volume	−0.49**	−0.63**	−0.61**	−0.64**
	Crumb score	0.45**	0.56**	0.56**	0.58**
SDS sedimentation	Flour yield	−0.32*	−0.19	−0.24	−0.16
	Flour protein	0.55**	0.55**	0.54**	0.56**
	Baking absorption	0.70**	0.71**	0.59**	0.58**
	Mixing time	0.68**	0.69**	0.57**	0.62**
	Loaf volume	0.71**	0.81**	0.80**	0.80**
	Crumb score	−0.61**	−0.56**	−0.64**	−0.65**

* $P < 0.05$; ** $P < 0.01$ ($df = 54$)

ing from 6 to 17% protein. The mean values for the RILs were generally intermediate to the parents (Table 2), except for mixing time and crumb score, where the mean values of the RILs were closest to the Anza values.

The parents and RILs showed relatively little response to N fertilization (Table 2); thus, the N levels served as additional replications in this study. For the RILs, flour yield, loaf volume, and crumb score showed small but significant improvement at N120 over the other two N levels.

Relationships among grain and flour quality parameters

In the study of genetics of wheat quality and in wheat breeding, the interrelationship of quality estimated from unmilled grain with milled flour is of great interest. Carrillo et al. (1990) reported that grain quality parameters were under rather strong genetic control. Protein content is a strong determinant of end-use quality and is usually estimated to be 1 to 2% higher in whole grain than in milled flour. This is because the embryo and outer portions of the kernel that are removed by milling are higher in protein than the endosperm. In this study, grain and flour protein content were strongly correlated over all genotypes at each N level (Table 3). Remarkably, however, the flour protein content showed no response to N fertilization (Fig. 1). Thus, it appeared that N fertilization more strongly influenced N uptake in the embryo and outer layers of the kernel than in the endosperm.

The grain characteristics were generally well correlated with flour performance in mixing and baking (Table 3). An exception is flour yield, which was unrelated to grain protein or SDS sedimentation. Milling yield was related to both pearling index and yellowberry, both of these traits being indirect measures of grain hardness and therefore to bran cleaning during milling. As quality predictors, pearling index was a satisfactory indicator of flour yield, while yellowberry was more strongly related to mixing and bread loaf characteristics. Finally, SDS sedimentation proved, on the average, to be a good estimator of desirable mixing and baking properties, while grain protein content was not a good indicator of mixing time. Thus, the four predictor tests evaluated previously are complementary in their usefulness for milling and bread quality evaluation. It must be pointed out that these analyses were based on wheat of low protein content, and the discriminating properties of the predictor parameters may not be the same at higher protein contents.

Table 4. Phenotype correlation coefficients among flour quality traits in a field experiment at three N levels for Anza × Cajeme 71 RILs and parents ($n = 56$)

Characters correlated:		Nitrogen level			
		N40	N80	N120	Mean
Flour yield	Flour protein	−0.20	−0.10	−0.14	−0.09
	Baking absorption	−0.57**	−0.48**	−0.65**	−0.56**
	Mixing time	−0.44**	−0.41**	−0.64**	−0.48**
	Loaf volume	−0.01	−0.13	−0.16	−0.02
	Crumb score	−0.11	0.03	0.25	0.16
Flour protein	Baking absorption	0.60**	0.57**	0.55**	0.52**
	Mixing time	0.34*	0.29*	0.30*	0.31*
	Loaf volume	0.59**	0.60**	0.70**	0.62**
	Crumb score	−0.45**	−0.55**	−0.54**	−0.53**
Baking absorption	Mixing time	0.72**	0.73**	0.75**	0.72**
	Loaf volume	0.45**	0.73**	0.61**	0.51**
	Crumb score	−0.34*	−0.53**	−0.59**	−0.43**
Mixing time	Loaf volume	0.44**	0.58**	0.45**	0.47**
	Crumb score	−0.42**	−0.46**	−0.44**	−0.44**
Loaf volume	Crumb score	−0.77**	−0.75**	−0.79**	−0.79**

* $P < 0.05$; ** $P < 0.01$ ($df = 54$)

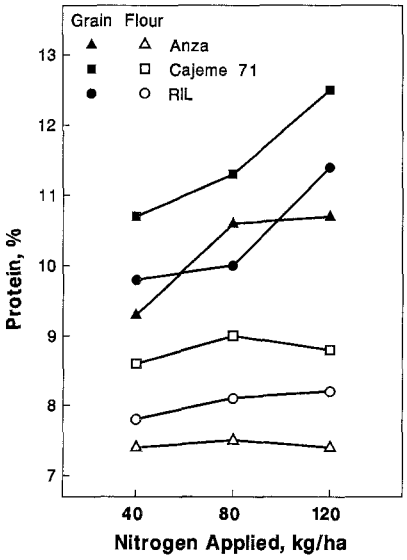

Fig. 1. Relationship of bread wheat grain and flour protein content to rate of nitrogen fertilization. Standard errors: for Anza grain (0.48) and flour (0.10); Cajeme 71 grain (0.48) and flour (0.20); and RIL grain (0.06) and flour (0.10)

Correlations among the flour quality parameters (Table 4) showed that flour yield was unrelated to protein content or bread loaf quality, but it was related to both water absorption and mixing properties of the dough. In these genetic materials, these relationships were probably related to grain hardness and yellowberry factors. Flour protein content was more strongly correlated to loaf volume than to mixing properties. Loaf volume and crumb score are the ultimate end-use quality parameters, neither of which were strongly related to baking water absorption or mixing time. Thus, there are indications that the various flour quality parameters are controlled by different gene systems in Anza and Cajeme 71.

Associations with HMW-glutenin loci

The RILs were grouped according to HMW-glutenin genotype and the means over all RILs within a HMW-glu type were computed for each flour quality parameter (Table 5). The HMW-glu means showed a range for each of the six parameters which was within the parental range. The only exception was flour yield, where the derived 222 genotype mean exceeded Cajeme 71. Of course, the individual RIL within a HMW-glu class showed a range of expression, as illustrated for mixing time and loaf volume in Figs. 2 and 3. Few of the individual lines reached or exceeded the values of the high-quality parent, but several lines had the same quality performance attributes as Anza, the lower quality parent (e.g., genotype 111 in Fig. 2).

The HMW-glu gene effects are given for each N level and the mean over N levels in Table 6. The effects for each N level were remarkably consistent for the three N levels, lending support to our hypothesis that RIL × N interaction effects were small in this experiment. Significant genetic effects were found for all parameters estimated. In all, 126 tests of significance were made for the three N levels, six traits, and seven gene action effects. Of these, 23 of 54 (43%) of the single-locus effects were significant and only 8 of 72 di- or trigenic epistatic effects

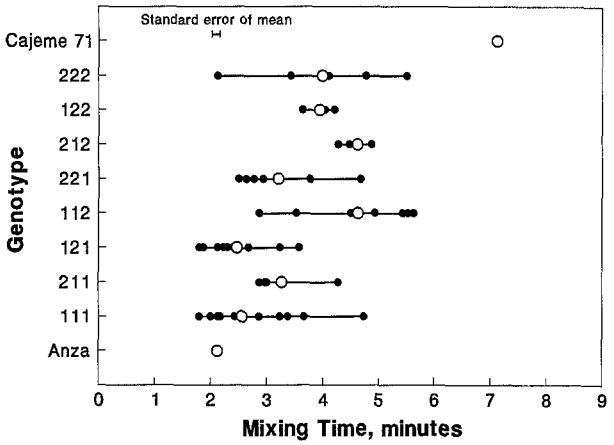

Fig. 2. Dough mixing times for Anza and Cajeme 71 and individual RILs (mean over three N treatments) in each HMW-glutenin genotype. *Open circles* are the HMW-glutenin genotype means. *Closed circles* are the RIL values

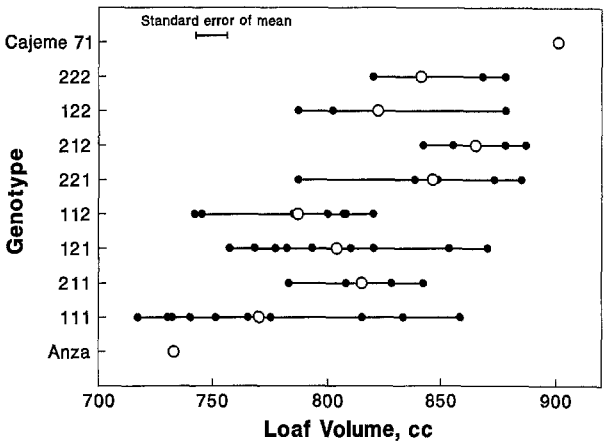

Fig. 3. Bread loaf volumes for Anza and Cajeme 71 and individual RILs (mean over three N treatments) in each HMW-glutenin genotype. *Open circles* are the HMW-glutenin genotype means. *Closed circles* are the RIL values

Table 5. Mean values for quality characters and HMW-glutenin subunit identification for each HMW-glutenin genotype using means over three N levels

Character	HMW-glutenin genotype										
	Anza (111)	111	112	121	122	211	212	221	222	Cajeme 71 (222)	SE*
Flour yield	71.7	70.4	69.3	70.5	70.5	69.2	70.6	71.2	70.7	69.3	0.2
Flour protein	7.4	7.7	7.9	8.3	8.3	7.8	8.4	8.1	8.3	8.8	0.1
Baking absorption	55.8	57.5	61.5	58.2	60.7	59.6	62.1	59.3	60.3	63.5	0.4
Mixing time	2.1	2.5	4.6	2.6	4.0	3.3	4.6	3.2	4.0	7.1	0.2
Loaf volume	733	772	787	804	822	815	865	846	841	901	13.6
Crumb score	8.7	7.8	8.1	7.6	5.1	7.1	5.2	5.5	5.4	2.3	0.6
HMW-glutenin subunit											
chromosome 1A	null	null	null	null	null	1	1	1	1	1	
1B	7/8	7/8	7/8	17/18	17/18	7/8	7/8	17/18	17/18	17/18	
1D	2/12	2/12	5/10	2/12	5/10	2/12	5/10	2/12	5/10	5/10	

* Standard error, assuming means of six lines included for each HMW-glu genotype

were significant. The significance tests based on the means computed over the three N levels are more reliable and, of these, 11 of 18 single-locus effects and 7 of 24 epistatic effects were significant.

As in the previous study of grain quality traits, we have computed the sum of the genetic effects, $\Sigma\,\alpha_i$, and compared then to the difference, d, between the two parents. The ratios $\Sigma\,\alpha_i/d$, as percentages, were 17 for flour yield, 13 for flour protein, 9 for baking absorption, 8 for mixing time, 13 for loaf volume, and 17 for crumb score. When the α_i were compared to d', the difference between the derived 111 and 222 parental HMW-glu genotypes, the percentages were 136, 30, 24, 27, 32, and 45%, respectively. The anomalous result for flour yield was due to a high value for the derived genotype 222 and because of substantial epistasis for this trait. These results were similar in magnitude to those of the previous study; that is, both the quality predictor parameters (Carrillo et al. 1990) and the parameters more directly related to quality examined in this paper showed that the HMW-glu loci accounted for an average of 13%, when compared to the parents, and 32%, when compared to the derived parental genotypes (flour yield excluded) of the total difference between the extreme phenotypes. These results, obtained from a population of lines from a biparental cross, indicated that less of the variation in baking quality is associated with the *Glu1* loci than observed in comparisons of varieties (Payne et al. 1987a, 1988;

Table 6. Additive (α_A, α_B, α_D) and interaction (α_{AB}, α_{AD}, α_{BD}, α_{ABD}) gene effects based on means of all RILs within each HMW-glutenin genotype

Character	Nitrogen level	Gene effect						
		α_A	α_B	α_D	α_{AB}	α_{AD}	α_{BD}	α_{ABD}
Flour yield	N40	0.20	0.38**	−0.08	0.18	0.26	−0.05	−0.35**
	N80	0.13	0.48**	0.20	0.00	0.28**	−0.08	−0.45**
	N120	0.05	0.40**	−0.20	0.18	0.38**	−0.18	−0.30**
	Mean	0.14	0.41**	−0.04	0.11	0.26**	−0.11	−0.36**
Flour protein	N40	0.06	0.04	0.06	−0.13	0.03	−0.09	−0.02
	N80	0.02	0.21**	0.16**	−0.19**	0.06	−0.01	−0.09
	N120	0.10	0.18**	0.17*	−0.04	0.13	−0.10	0.01
	Mean	0.06	0.14**	0.13**	−0.12**	0.07	−0.07	−0.03
Baking absorption	N40	0.41	−0.34	1.39**	−0.26	−0.44	−0.44	0.04
	N80	0.49	−0.09	1.24**	−0.21	−0.34	−0.26	0.01
	N120	0.48*	−0.33	1.25**	−0.18	−0.30	−0.30	0.05
	Mean	0.46*	−0.24*	1.29**	−0.21	−0.34**	−0.34**	0.04
Mixing time	N40	0.26*	−0.16	0.70**	−0.05	−0.11	−0.06	0.00
	N80	0.13	−0.09	0.67**	−0.01	−0.25**	−0.13	0.01
	N120	0.20	−0.31**	0.75**	0.09	−0.18	−0.25**	0.01
	Mean	0.19**	−0.18**	0.71**	0.01	−0.18**	−0.15**	0.00
Loaf volume	N40	28.75**	10.50	−2.25	−0.50	8.75	−5.50	−7.00
	N80	22.63**	8.88	18.13**	−13.38	−6.13	−7.88	−8.13
	N120	18.50**	9.25	14.25	−9.50	1.00	−6.75	−6.00
	Mean	23.00**	9.50**	10.00**	−7.75	1.25	−6.75	−7.00
Crumb score	N40	−0.64	−0.77*	−0.24	−0.14	−0.06	0.16	0.69
	N80	−0.66	−0.54	−0.46	0.56	0.34	−0.34	0.41
	N120	−0.76*	−0.41	−0.81**	0.26	−0.24	−0.19	0.64
	Mean	−0.68	−0.58	−0.53	0.23	0.03	−0.13	0.58

*,** $P<0.05$; ** $P<0.01$

Rogers et al. 1989). Thus, bread-making quality is truly a complex multigenic phenomenon that cannot be resolved by the simple effects of the HMW-glu loci.

Flour yield

This character showed very strong counteracting epistatic effects (α_{AD} and α_{ABD}), with the result that α_B was the most important single-locus effect in contributing to higher flour yield (Table 6). The results are enigmatic since Cajeme 71 had lower flour yield than Anza, and the derived 222 (=Cajeme 71), 212, and 221 genotypes had higher flour yield than Cajeme 71. There is no *a priori* reason to expect tht HMW-glu alleles are directly related to flour yield. These results may be due to complex interrelations to kernel hardness and/or yellowberry or other associations, as will be dicussed later.

Flour protein content

Flour protein was positively influenced by *Glu-B1* and *Glu-D1* loci, but reduced by the significant α_{AB} epistatic effect. The α_B and α_D effects were expressed only at the highest N level, one of the few gene effect × environment interactions that was observed in this study. The combined association of protein to HMW-glu loci was small and probably of little significance for selection of high quality in a breeding program.

Baking absorption and mixing time

The two parents differed substantially for these traits, both of which are regarded as very important in bread manufacture. The traits were strongly correlated (Table 4) and the associations with HMW-glu effects were parallel in amount and sign (Table 6). The α_A and α_D single-locus effects positively influenced the traits, while the additive effect α_B and the epistatic effects, α_{AD} and α_{BD}, contributed negatively. These results point to similar genetic control of water absorption by flour and its subsequent mixing properties. The *Glu-1D* locus with subunits 5 and 10 from Cajeme 71 had an effect that was approximately three times stronger than the *Glu-1A* (null) locus. The *Glu-1B* locus subunits 17 and 18 of Cajeme 71 were detrimental or, alternatively, from these results it can be inferred that subunits 7 and 8 from Anza contributed positively to water absorption and mixing time. The nature of gene action revealed by RILs for these traits clearly points to a potential gain in plant breeding by recombining alleles from these two parents.

Bread loaf volume and crumb score

These two traits were highly correlated and both represent the ultimate evaluation of wheat flour for bread making. Crumb score is a subjectively evaluated visual score that typically gives a high coefficient of variation. Loaf volume is more precisely measured, but is somewhat dependent upon the bread-baking process used and on the skills of the technologist. All three alleles from Cajeme 71 contributed additively to the improvement of bread loaf volume (Table 6). In contrast to the other traits examined here, epistatic effects were not important. The *Glu-A1* alleles of Cajeme 71 had about twice the effect of the *Glu-B1* and *Glu-D1* alleles on loaf volume. These results suggest that bread-making quality is simply inherited and therefore easy to manipulate by direct selection on loaf volume and crumb texture or by selection for specific HMW-glu alleles. The situation is not so simple, however, because all of the other traits examined are interrelated and complexly inherited. For example, SDS sedimentation and loaf volume were strongly correlated (Table 3), but the additive effects of the HMW-glu loci were more important for SDS sedimentation volume (Carrillo et al. 1990) than loaf volume. Furthermore, *Glu-D1* was more strongly associated with SDS sedimentation and *Glu-A1* was more strongly associated with loaf volume. The *Glu-D1* association with SDS sedimentation volume decreased with higher N fertilization. The *Glu-A1* effect on loaf volume also decreased at higher N fertilization.

The relationship of quality and specific gene effects to grain protein content is also a confounding factor in the interpretation of the genetics of quality. This can be illustrated in the present study by the covariance of flour protein and loaf volume. Figure 4 shows a very strong relationship of the HMW-glu genotypes to flour protein and loaf volume. When this result is assessed in a different way (Fig. 5) the gene effects, both single-locus additive and epistatic, also show a tendency for a linear relationship of genetic effects from negative epistatic effects to positive, single-locus additive effects for these two traits. Flour protein content of the grain can be directly modified by nitrogen fertilization and other crop management factors, but the genic composition of the flour sets limits on the variation end-use quality. Unpredictable environmental effects, such as rainfall and temperature variation, obscure both protein content and the action of specific genes.

Genetic variation among RILs within HMW-glu classes

The *F*-values are presented in Table 7 for tests for genetic differences among lines within each MWM-glu genotype. There were multiple replicates of Anza and Cajeme 71 (and its sib Yecora Rojo) in the experiment, and these were analyzed for within-Anza and within-Cajeme 71

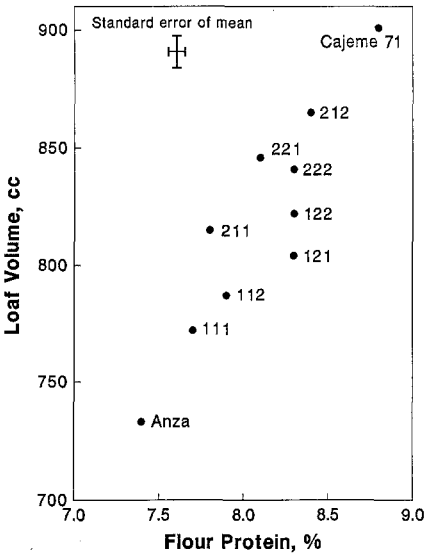

Fig. 4. Relationship of flour protein and bread loaf volume for the parents and HMW-glutenin genotype means

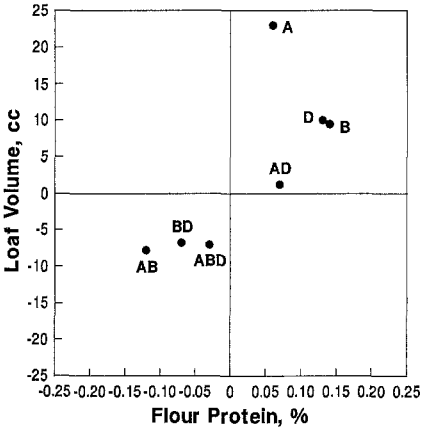

Fig. 5. Relationship of flour protein and bread loaf volume for additive and epistatic gene effects (α symbol omitted)

differences to give a degree of measure of chance effects, since no genetic variability exists among the Anza or Cajeme 71 parental entries. Three of 12 (25%) of the parental tests were significant at $P=0.05$ (Table 7). For the HMW-glu genotypes, 35 of 48 (73%) tests were significant, thus showing that genetic variation for the quality traits was distributed among the RILs independently of the HMW-glu alleles. These results were especially notable for flour yield and flour protein, where among-RIL effects were significant for all HMW-glu genotypes, except for flour yield for genotype 211. It could be argued that these traits are not directly influenced by HMW-glu subunit size, composition, or configuration so that the genes should be randomly distributed among the HMW-glu genotypes, even though significant α_B (flour yield) and α_B and α_D (flour protein) effects were detected.

Table 7. *F*-ratios for tests of significance of genetic variation among RILs in each HMW-glutenin genotype group and among replicates for Anza and Cajeme 71 within HMW-glu genotypes. Significance tested against error mean squares from Table 1

Character	HMW-glutenin genotype									
	111	112	121	122	211	212	221	222	Anza (111)	Cajeme 71 (222)
Flour yield	22.46**	6.58**	10.46**	6.33**	1.92	22.23**	18.50**	12.96**	1.26	0.03
Flour protein	4.09**	8.18**	10.80**	18.27**	5.64*	13.13**	4.27*	16.36**	0.93	0.14
Baking absorption	24.35**	11.99**	24.51**	24.00**	14.40**	16.30**	30.90**	28.60**	3.46*	1.00
Mixing time	7.25**	17.20**	5.32**	1.25	6.60*	1.35	10.60**	24.90**	0.69	21.67*
Loaf volume	6.35**	2.66	4.06**	6.56	1.75	1.18	3.18	2.35	3.54*	0.46
Crumb score	1.83	0.91	4.17**	7.52*	0.55	1.89	6.65**	5.77**	0.18	0.00

* $P<0.05$; ** $P<0.01$

For baking absorption and mixing time, genetic variation also was detected among RILs for eight of eight and seven of eight HMW-glu genotypes, respectively. For these traits, the properties of HMW-glu subunits might be expected to be directly related to flour handling properties. The results in Table 6, which show strong α_D effects and lesser, but significant, α_A and α_B effects, support that suggestion. Finally, loaf volume and bread crumb score gave different results, where only 6 of 12 (50%) of the among-RIL within-HMW-glu variances were significant. The α_A, α_B, and α_D effects for loaf volume and crumb score were all significant and quite large If the *Glu-1* loci effects are pleiotropic with these properties, then little among-RIL variation would be observed.

Conclusion

Taken together, these results clearly show that: (1) HMW-glu loci account for only a part of the variation in quality traits, (2) both additive and epistatic effects are important, and (3) there are substantial associations of traits with the *Glu-1* loci which may be due to linkage of genes to the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci, to random chance associations, or to pleiotropy. In the latter case, pleiotropy could be incomplete because of environmental influence on genes having small effects on the measured traits. These various issues may be resolved by studying marked segments of the whole wheat genome by RFLP analysis. This is a goal of current research.

The rather weak relation of the HMW-glu loci to quality is still large enough that breeders can make substantial gains by using electrophoretic selection for specific glutenin protein subunits. Further refinement by the classifications of the RILs for low-molecular-weight glutenins and gliadins (Payne et al. 1987b; Gupta et al. 1989; Metakovsky et al. 1990) may improve the genetic resolution of wheat quality as an aid in plant breeding.

Acknowledgements. Special appreciations is extended to G. L. Rubenthaler and staff at the USDA Western Wheat Quality Laboratory, Pullman/WA for their timely and extensive evaluation of milling and baking quality. This work was supported by a NATO scholarship to M. R. and grants from the W-132 Regional Project and the California Crop Improvement Association.

References

AACC (1983) Approved methods of the American Association of Cereal Chemists. American Association of Cereal Chemists, Inc., St. Paul/MN

Beckman JS, Soller M (1986) Restriction fragment length polymorphisms and genetic improvement of agricultural species. Euphytica 35:111–124

Branlard G, Dardevet M (1985) Diversity of grain protein and bread wheat quality. II. Correlation between high-molecular-weight subunits of glutenin and flour quality characteristics. J Cereal Sci 3:345–354

Campbell WP, Wrigley CW, Cressey PJ, Slack CR (1987) Statistical correlations between quality attributes and grain-protein composition for 71 hexaploid wheats used as breeding parents. Cereal Chem 64:293–299

Carrillo JM, Rousset M, Qualset CO, Kasarda DD (1990) Use of recombinant inbred lines of wheat for study of association of high-molecular-weight glutenin subunit alleles to quantitative traits. 1. Grain yield and quality prediction tests. Theor Appl Genet 79:321–330

Cressey PJ, Campbell WP, Wrigley CW, Griffin WB (1987) Statistical correlations between quality attributes and grain-protein composition for 60 advanced lines of crossbred wheat. Cereal Chem 64:299–301

Finney KF, Yamazaki WT, Youngs VL, Rubenthaler GL (1987) Quality of hard, soft, and durum wheats. In: Wheat and wheat improvement, 2nd edn. Agronomy Monograph No. 13, ASA-CSSA-SSSA, Madison/WI, pp 677–740

Gupta RB, Singh NK, Shepherd KW (1989) The cumulative effect of allelic variation in LMW and HMW glutenin subunits on dough properties in the progeny of two bread wheats. Theor Appl Genet 77:57–64

Lagudah ES, O'Brien L, Halloran GM (1988) Influence of gliadin composition and high-molecular-weight subunits of glutenin on dough properties in an F_3 population of a bread wheat cross. J Cereal Sci 7:33–42

Lawrence GJ, Moss HJ, Shepherd KW, Wrigley CW (1987) Dough quality of biotypes of 11 Australian wheat cultivars that differ in high-molecular-weight glutenin subunit composition. J Cereal Sci 6:99–101

Mansur LM, Qualset CO, Kasarda DD, Moris R (1990) Effects of 'Cheyenne' chromosomes on milling and baking quality of 'Chinese Spring' wheat in relation to glutenin and gliadin storage proteins. Crop Sci 30:593–602

Metakovsky EV, Wrigley CW, Bekes F, Gupta RB (1990) Gluten polypeptides as useful genetic markers of dough quality in Australian wheats. Aust J Agric Res 41:289–306

Payne PI, Lawrence GJ (1983) Catalogue of alleles for the complex gene loci, *Glu-A1*, *Glu-B1*, and *Glu-D1* which code for the high-molecular-weight subunits of glutenin in hexaploid wheat. Cereal Res Commun 11:29–35

Payne PI, Nightingale MA, Krattiger AF, Holt LM (1987a) The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. J Sci Food Agric 40:51–65

Payne PI, Seekings JA, Worland AJ, Jarvis MG, Holt LM (1987b) Allelic variation of glutenin subunits and gliadins and its effect on bread-making quality in wheat: analysis of F_5 progeny from Chinese Spring × Chinese Spring (Hope 1A). J Cereal Sci 6: 103–118

Payne PI, Holt LM, Krattiger AF, Carrillo JM (1988) Relationships between seed quality characteristics and HMW glutenin subunit composition determined using wheats grown in Spain. J Cereal Sci 7:229–235

Rogers WJ, Payne PI, Harindes K (1989) The HMW glutenin subunit and gliadin compositions of German-grown wheat varieties and their relationship with bread-making quality. Plant Breed 103:89–100

Zemetra RS, Morris R, Mattern PJ, Seip L (1987) Gene locations for flour quality in winter wheat using reciprocal chromosome substitutions. Crop Sci 27:677–681