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

1. Grain yield and quality prediction tests

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Summary. The high-molecular-weight glutenin subunits (HMW glutenin), encoded by alleles at homoeologous loci *Glu-A1*, *Glu-B1*, and *Glu-D1* on the long arms of chromosomes *1A*, *1B*, and *1D* of a set of F_8 random recombinant inbred lines (RIL) derived from the bread wheat cross Anza × Cajeme 71, were classified by SDS-PAGE. Anza has poor breadmaking quality and HMW-glutenin subunits (Payne numbers) null (*Glu-A1c*), 7+8 (*Glu-B1b*), and 2+12 (*Glu-D1a*); Cajeme 71 has good quality and 1 (*Glu-A1a*), 17+18 (*Glu-B1i*), and 5+10 (*Glu-D1d*). The combinations of these alleles in the RIL were examined for associations with grain yield and four indicators of grain quality – protein content, yellowberry, pearling index, and SDS sedimentation volume. Data were obtained from a field experiment with three nitrogen fertilization treatments on 48 RIL and the parents. Orthogonal partitioning of the genetic variance associated with the three HMW glutenin subunit loci into additive and epistatic (digenic and trigenic) effects showed strong associations of these loci with grain yield and the indicators of quality; however, the associations accounted for no more than 25% of the differences between the parents. Genetic variance was detected among the RIL, which had the same HMW glutenin genotype for all traits. Epistatic effects were absent for grain yield and yellowberry, but were substantial for grain protein content, pearling index, and SDS sedimentation volume. All three loci had large single-locus additive effects for grain yield, protein, and SDS sedimentation volume. Yellowberry was largely influenced by *Glu-B1* and *Glu-D1*, whereas pearling index was associated with *Glu-A1* and *Glu-B1*. Even though the observed associations of effects of HMW glutenin loci with the quantitative characters were small relative to the total genetic variability, they are of considerable importance in understanding the genetics of wheat quality, and are useful in the development of new wheat varieties with specific desired characteristics.

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

Introduction

Wheat is our major cereal crop, and of the total world wheat production, 65% is consumed by humans, mainly in the form of bread and other baked products made almost entirely from the endosperm milled into flour (Bushuk 1986). The breadmaking quality of flour is influenced both by protein content and protein type but for a given protein content, wheat quality is largely a function of the nature of the gluten protein composition (Finney and Barmore 1948). Gluten proteins are known to confer on wheat dough its unique cohesive and elastic characteristics (Wall 1979). Differences between varieties in protein quality are considered to be caused mainly by different combinations of endosperm storage protein variants (Payne et al. 1984).

Wheat gluten consists of two major protein types: glutenin and gliadin. These proteins are synthesized on the endoplasmic reticulum in the endosperm and are deposited in protein bodies (Shewry and Miflin 1985). Gliadin molecules consist of a complex mixture of polypeptides which, when fractionated according to charge by gel electrophoresis at low pH, separate into four groups designated as α, β, γ, and ω (Wall 1979). Each variety of hexaploid wheat contains about 25 major gliadin polypeptides and as many minor components (Wrigley and Shepherd 1973).

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Glutenins are made up of polypeptide chains (subunits) similar to those of gliadins, but cross-linked by disulfide bonds into higher level polymers. When treated with a reducing agent, such as sodium dodecyl sulfate (SDS), the glutenin molecules dissociate into subunits of differing molecular weight: the high-molecular-weight (HMW) subunits and the low-molecular-weight (LMW) subunits (Payne and Corfield 1979). There is wide variation among varieties in the electrophoretic patterns of HMW glutenins (Payne et al. 1981 a).

In *Triticum aestivum* L., the genes coding for the HMW glutenin subunits occur at three complex loci (*Glu-A1*, *Glu-B1*, and *Glu-D1*) on the long arms of homoeologous group 1 chromosomes (Bietz et al. 1975; Lawrence and Shepherd 1981). Wheat varieties each contain three to five HMW glutenin subunits, two coded by genes at *Glu-D1*, one or two by *Glu-B1*, and none or one by *Glu-A1* (Lawrence and Shepherd 1980; Payne et al. 1981 a). Payne and Lawrence (1983) reported 3 alleles at *Glu-A1*, 11 at *Glu-B1*, and 6 at *Glu-D1*. Later works identified more alleles in bread wheat (Payne et al. 1984; Lawrence 1986; Sontag et al. 1986; Waines and Payne 1987). LMW glutenin genes are located on homoeologous chromosomes of group 1, and there are two complex loci controlling gliadin synthesis on groups 1 and 6.

The isolation and characterization of wheat endosperm proteins and their relationship to the functionality of wheat flour have been a subject of considerable study using several approaches. Most notably, the association of particular HMW glutenin subunits and flour quality, first reported by Payne et al. (1979, 1981 b) using the SDS sedimentation test, has been substantiated by others using various methods (Moonen et al. 1982; Branlard and Dradevet 1985; Lawrence et al. 1987; Lagudah et al. 1988). The genetic materials for these studies included segregating populations (Payne et al. 1984; Moonen et al. 1982; Lorenzo et al. 1987; Lagudah et al. 1988), varieties (Burnouf and Bouriquet 1980; Branlard and Dardevet 1985), biotypes obtained from the same variety (Lawrence et al. 1987), near-isogenic lines (Payne et al. 1987 b), lines deficient in certain glutenin subunits (Lawrence et al. 1988), and single-chromosome substitution lines (Zemetra et al. 1987; Mansur et al. 1990; Krattiger et al. 1987).

Branlard and Dardevet (1985), comparing varieties, found that two types of bands of HMW glutenin subunits can be recognized: those that are positively correlated with gluten strength and tenacity (2*, 5+10, 7+9), and those that are positively correlated with extensibility of the dough (1, 13+16, 17+18). The protein content of the flour had no influence upon those correlations.

Lawrence et al. (1987) compared biotypes of the same variety, differing only in HMW glutenin subunit composition. Dough strength was measured by resistance to extension in the Brabender extensograph. Greatest contrasts in resistance were provided by pairs of biotypes that differed at locus *Glu-D1* for subunits 5+10 and 2+12. Smaller differences were found in subunits controlled by the *Glu-A1* (2*>1>null) and *Glu-B1* (7+9>20 and 7+8>7+9). Payne et al. (1984) had concluded that bands 7+8, 13+16, and 17+18 were equivalent in their contributions to quality, but Grama et al. (1987), studying hexaploid wheat derivatives from hybrids of bread wheat and emmer (*T. diccocoides*), found better quality for some allelic combinations than others: *Glu-A1* (2*>1); *Glu-B1* (7+8>17+18, 13+16>7+9); and *Glu-D1* (5+10>2+12).

Lorenzo et al. (1987), using two hybrid populations, evaluated the contribution of different HMW glutenin protein subunits to loaf volume based on the SDS sedimentation test. Bands 5+10 in a homozygous state were always associated with higher sedimentation volumes than 3+12 homozygotes or the heterozygote having both 5+10 and 3+12. The importance of individual subunits in the A and B genomes depends on the interaction with other glutenin proteins. Odenbach and Mahgoub (1988) found that the HMW glutenin subunits 2*, 7+8, 7+9, and 5+10 were associated with large sedimentation volumes, whereas their allelic variants null, 6+8 and 2+12 were associated with small sedimentation volumes.

Lagudah et al. (1988) studied the relationship of the *Glu-1* loci with Brabender farinograph and extensograph dough properties in an F_3 population. Variation in dough properties was not influenced by protein content. HMW subunits of glutenin, particularly 5+10 of the *Glu-D1* locus, were associated with greater dough stability and higher maximum resistance to extension than their allelic 2+12. Lawrence et al. (1988), studying lines deficient in expression of some *Glu-A1* alleles, found that loss of subunits 5+10 at the *Glu-D1* locus and subunits 17+18 at the *Glu-B1* locus had significantly greater effects on mixograph time to peak than loss of subunit 1 at the *Glu-A1* locus.

Because the combining of "good-quality" alleles coded at different loci has at least partially additive effects upon bread-making quality, Payne et al. (1984) developed a simple scoring system that assigns a numerical value to each HMW subunit on the basis of SDS volume. This enables the overall quality of a variety to be estimated in terms of its constituent HMW glutenin subunits. Using this method, varieties grown in different countries (Payne et al. 1987 a; 1988) were scored, and the *Glu-A1* quality scores related well to overall breadmaking quality.

Single-chromosome substitution lines have revealed genetic activity on the chromosomes having HMW glutenin alleles, but also on other chromosomes. Mansur et al. (1990) confirmed and extended the earlier work by

Morris et al. (1966), using the Cheyenne chromosomes substituted for Chinese Spring chromosomes. Chromosomes *1A, 1B, 1D, 3A, 3B, 7A,* and *7B* contributed to good bread loaf volume. Somewhat different results were obtained by Krattiger et al. (1987). They showed improved bread loaf volume of Cappelle Deprez having chromosomes *1A, 1D, 4D, 4A, 5D, 6B,* or *6D* substituted from Bezostaya I. Zemetra et al. (1987), studying Cheyenne and Wichita reciprocal substitution lines, also showed the importance of group 1 chromosomes and others. They found some evidence for interactions among genes on different chromosomes, as did Rogers et al. (1988).

The objective of this paper and the companion one (M. Rousset, J. Carrillo, C. Qualset, D. Kasarda in preparation) is to examine associations of the HMW glutenin subunit alleles with various aspects of wheat end-use properties, by means of a random population of near-homozygous lines termed recombinant inbred lines (RIL). The RIL were obtained from the hybrid Anza and Cajeme 71, two red-grained spring wheat varieties that contrast greatly in grain protein content and breadmaking quality. Both parents are high-yielding, short-statured wheats. Since wheat end-use quality is strongly influenced by environment, especially as related to grain protein content, this study was conducted under three soil nitrogen regimes to induce a range in grain protein content. This paper deals with grain yield and attributes of the grain that are implicated as influencing or predicting breadmaking quality.

Materials and methods

Genotypes

Anza and Cajeme 71 are short-statured (*Rht1* and *Rht1* + *Rht2*, respectively), photoperiod-insensitive, spring wheat cultivars with very good yield potential. Both cultivars were developed in Mexico by N. E. Borlaug and staff at CIMMYT in the 1960s. Cajeme 71 was released by the Mexican national wheat program in 1971 and Anza by the University of California, also in 1971. Cajeme 71 flour has good milling and breadmaking properties, whereas Anza has good milling and poor breadmaking properties. These two cultivars were hybridized, and random F_2-derived F_8 lines were developed at Davis/CA (Cox et al. 1985). These lines will be termed recombinant inbred lines (RIL) because they were developed by selection of random F_2 plants and, thereafter, advanced to near-homozygosity by single-plant propagation from F_2 onward. Available for study were 177 RIL, which were assayed by single seeds (several for each RIL) for HMW glutenin, using SDS-PAGE by Payne and Corfield's (1979) method as described by Fullington et al. (1983). The protein subunit bands were numbered (Table 1, Fig. 1) according to the system adopted by Payne and Lawrence (1983). Their system of allelic designation was adopted here also (Table 1). For this study we have coded the alleles by 1 and 2 for the alleles of Anza and Cajeme 71, respectively, in each of the three genomes of homoeologous chromosome group 1 (Table 1). In the population of 177 RILs, 17 were heterozygous at one or more HMW glutenin loci.

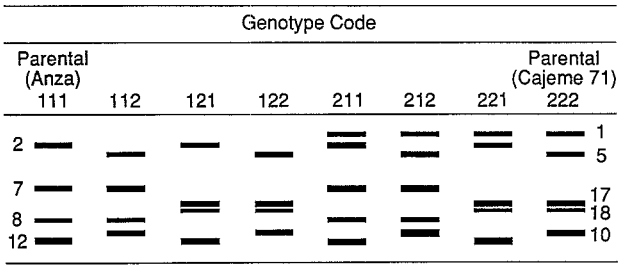

Fig. 1. SDS-PAGE patterns of HMW glutenins of Anza and Cajeme 71 and recombinants obtained from the hybrid. Genotype codes as given in Table 1, bands identified as in Payne and Lawrence (1973)

Field experiment

Fifty-two RIL, homozygous at HMW glutenin loci, were selected at random from the group of 177 for a field experiment. Six entries of Anza, one of Cajeme 71, and one of Yecora Rojo (a sib of Cajeme 71) were also included. Four RIL later proved to be heterozygous for one or more HMW glutenin loci and were deleted from the analysis.

The 60 entries were sown in a randomized block split-plot design with three nitrogen levels with three replicates at Davis on January 12, 1983. The levels of N were 40, 80, and 120 kg/ha. Ammonium nitrate was applied fro treatments N40 and N80 on March 8, and for treatment N120 on March 8 and 23. One nitrogen treatment in the third replicate was flooded, so only two replicates were used in the statistical analysis. Plots were 2.8 m long and consisted of 4 rows, 30 cm apart. The experiment received usual irrigation and weed control for wheat grown in the Sacramento Valley of California.

Grain quality

The grain nitrogen content for each entry in the field was determined by the Kjeldahl method and converted to protein percentage as N concentration $\times 5.7 \times 100$. Yellowberry was estimated visually as the percentage of grains with a yellow surface appearance in a 200-grain sample. Pearling index, a measure of hardness, was determined on 200 grains in a barley pearling machine. The index was $100\times$ the weight of grains after 30-s treatment, divided by the original weight of 200 grains.

The SDS sedimentation was used as a measure of gluten strength of the flour (Axford et al. 1979). In this method, the volume of materials that sedimented after mixing flour with a solution of SDS and lactic acid was measured in milliliters in a 100-ml cylinder. Larger values of SDS volumes indicate greater gluten strength of the flour. We followed the method proposed by Axford et al. (1978), using 6 g of whole-grain meal obtained by grinding the sample in a Udy mill with a 1.00-mm sieve. A mechanical shaker was used rather than hand-shaking as Axford et al. used (1978).

Genetic analysis

The data obtained from each field plot were subjected to analyses of variance to detect genetic variation among RIL and between the two parents, and to detect genotype $\times$ environment interaction as may have resulted from the three N management levels. The among-RIL variance was orthogonally partitioned into additive (α_A, α_B, α_D), additive $\times$ additive (α_{AB}, α_{AD}, α_{BD}),

Table 1. HMW glutenin allele and SDS-PAGE band designations with frequencies of homozygous genotypes observed among recombinant inbred lines (RIL) from the F_8 Anza × Cajeme 71

HMW glutenin allele			SDS-PAGE band			Genotype Code	No. of RIL	
Glu-A1	*Glu-B1*	*Glu-D1*	*Glu-A1*	*Glu-B1*	*Glu-D1*		Whole pop.	Field exp.
a	b	a	l	7/8	2/12	211	16	4
a	b	d	l	7/8	5/10	212	21	4
a	i	a	l	17/18	2/12	221	17	6
a	i	d	l	17/18	5/10	222[a]	19	5
c	b	a	n[c]	7/8	2/12	111[b]	20	10
c	b	d	n	7/8	5/10	112	19	7
c	i	a	n	17/18	2/12	121	35	9
c	i	d	n	17/18	5/10	122	13	3
Prob. (equal frequency)							>0.02	>0.25
a	–	–	l	–	–	1––	73	19
c	–	–	n	–	–	2––	87	29
Prob. (equal frequency)							>0.25	>0.10
–	b	–	–	7/8	–	–1–	76	25
–	i	–	–	17/18	–	–2–	84	23
Prob. (equal frequency)							>0.50	>0.75
–	–	a	–	–	2/12	––1	88	29
–	–	d	–	–	5/10	––2	72	19
Prob. (equal frequency)							>0.10	>0.10
Total							160	48

[a] Cajeme 71
[b] Anza
[c] n – null, no band expressed

and additive × additive × additive (α_{ABD}) single degree of freedom comparisons as follows:

Effect	Genotype							
	111	112	121	122	211	212	221	222
α_A	−1	−1	−1	−1	1	1	1	1
α_B	−1	−1	1	1	−1	−1	1	1
α_D	−1	1	−1	1	−1	1	−1	1
α_{AB}	1	1	−1	−1	−1	−1	1	1
α_{AD}	1	−1	1	−1	−1	1	−1	1
α_{BD}	1	−1	−1	1	1	−1	−1	1
α_{ABD}	−1	1	1	−1	1	−1	−1	1

Note that genotype 222 = Cajeme 71 was used as the base genotype since this genotype was known to have better quality than Anza (111), thus giving positive rather than negative effects if they existed. The mean value of all RIL within each HMW glutenin genotype was used to compute gene effects and sum of squares for tests of significance for each of the seven types of gene action at each N treatment and for the mean overall N levels. Table 1 shows that there were unequal numbers of RIL within each HMW glutenin genotype, but these did not differ from the expected frequency, one-eight in each class. Tests of significance were made using the mean number of lines per HMW glutenin genotype class (6). Further analysis was done to test for genetic differences among RIL within each HMW glutenin genotype. A separate ANOVA was done for each HMW glutenin group, and the test of significance utilized the error mean square from the whole experiment as the denominator in *F*-tests.

Results and discussion

N effects and relationships among characters

The RIL and N treatments showed highly significant differences among them for all characters studied (Table 2). There was little evidence for genotype × N interaction; the only significant interaction was grain yield. The two cultivars, Anza and Cajeme 71, differed greatly in all the traits (Table 3). Compared to Cajeme 71, Anza showed higher grain yield, lower grain protein, higher percent yellowberry, less kernel hardness indicated by the lower pearling index, and lower SDS sedimentation volumes. The mean values of the RIL were, on average, intermediate to the parents (Table 3) for all the characters as expected of random lines, confirming the results of Cox et al. (1985) for these same lines.

In the RIL, grain yield and quality increased as the amount of N fertilizer was increased. The effect was larger between N80 and N120 and was statistically significant for all characters, except for pearling index. The response of Anza and Cajeme 71 to the increase of nitrogen was similar to that observed in the RIL. The pearling index showed less increment than other characters as N was increased. The N treatments were sufficient to create a range of responses for evaluation of the genetic basis of grain quality in this set of RIL.

Table 2. Relevant mean squares and coefficients of variation from the analyses of variance for RIL and parents in a field experiment at three nitrogen fertilization levels

Source	*df*	Grain yield	Grain protein	Yellowberry	Pearling index	SDS sedimentation
Nitrogen levels	2	2,837**	84.05**	25,743**	56.26**	1,185**
Genotypes	59	192**	2.74**	3,222**	76.30**	247**
G × N	118	24*	0.37	200	11.12	8
Error	177	18.3	0.47	164	11.11	10
CV, %		9.0	6.6	24.5	5.4	6.0

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

Table 3. Means for RIL and parents for each nitrogen fertilization level. Standard errors are given for N level means

Genotype and nitrogen fertilization level		Character				
		Grain yield kg/ha	Grain protein %	Yellowberry %	Pearling index %	SDS sed. volume ml
RIL						
Nitrogen	40	4,090	9.8	63.5	61.7	50.1
	80	4,500	10.0	55.1	62.2	51.8
	120	5,100	11.4	33.2	63.1	56.3
	Mean	4,560	10.4	50.6	62.3	52.7
Cajeme 71						
Nitrogen	40	3,610	10.7	32.0	67.0	58.5
	80	4,050	11.3	17.8	65.5	60.0
	120	4,520	12.5	10.8	66.9	62.0
	Mean	4,060	11.5	20.2	66.5	60.2
Anza						
Nitrogen	40	5,040	9.3	89.1	56.7	41.1
	80	5,320	10.6	84.5	56.9	43.5
	120	5,790	10.7	75.7	58.9	47.0
	Mean	5,380	9.9	83.1	57.5	43.8
Standard error						
All genotypes		39	0.06	1.17	0.30	0.29
Anza or Cajeme 71		302	0.48	9.06	2.36	2.24

Table 4. Phenotypic correlations among characters at each N level, RIL and parents included ($n=60$)

Characters correlated		Nitrogen level			
		40	80	120	Mean
SDS sedimentation	– grain protein	0.55**	0.51**	0.59**	0.56**
	– grain yield	−0.46**	−0.43**	−0.42**	−0.54**
	– pearling index	0.41**	0.49**	0.29*	0.45**
	– yellowberry	−0.64**	−0.62**	−0.60**	−0.64**
Grain protein	– grain yield	−0.47**	−0.45**	−0.40**	−0.62**
	– pearling index	0.39**	0.50**	0.38**	0.53**
	– yellowberry	−0.79**	−0.76**	−0.70**	−0.82**
Pearling index	– grain yield	−0.55**	−0.51**	−0.32*	−0.64**
	– yellowberry	−0.65**	−0.63**	−0.49**	−0.69**
Yellowberry	– grain yield	0.62**	0.63**	0.61**	0.77**

* $P<0.05$
** $P<0.01$ (58 *df*)

The inverse relationship between grain protein and grain yield that is frequently found in wheat (Terman et al. 1969; Mesdag 1979; Bhatia and Rabson 1987; Johnson and Mattern 1987) was confirmed (Table 4). Grain yield was also negatively related to each of the other predictors of grain quality (Table 4); the correlation of yellowberry to grain yield was positive, but high yellowberry is an indicator of low protein content and, hence, lower quality. The correlations of grain protein with pearling index, SDS sedimentation, and yellowberry were indicative of increased quality related to grain protein content.

Significant correlations (Table 4) were found among SDS sedimentation, pearling index, and yellowberry. Therefore, these three parameters can be useful in studying gene effects on grain quality.

HMW glutenin genotypes and frequencies

Anza and Cajeme-71 differed at all three *Glu-1* loci on the long arm of chromosomes *1A*, *1B*, and *1D* (Fig. 1). Anza had no band at *Glu-A1*, 7 and 8 at *Glu-B1*, and 2 and 12 at *Glu-D1*; Cajeme 71 had band 1 at *Glu-A1*, 17 and 18 at *Glu-B1*, and 2 and 12 at *Glu-D1*. The eight different combinations of bands in the RIL (Fig. 1), their frequencies in the whole population, and frequencies in the random sample of RIL in the field experiment are shown in Table 1. There was good agreement generally between the observed frequencies of RIL and the expectation from independent random assortment (Table 1). In the case of the whole population there was some disagreement, because in two of the eight RIL, genotypes 121 and 122, differed from expected frequencies.

HMW glutenin loci effects

Gene effects were computed for each N level and for the mean over N levels (Table 5). The results were very consistent over the N levels, as expected, because the RIL × N interaction effects were generally not significant. For reference, means over N levels for each of the eight derived genotypes compared to Anza and Cajeme 71 are presented in Table 6. Significant effects of HMW loci were found for grain yield and for all of the quality-related traits measured in this study (Table 5). Considering each of the three N levels, seven gene action effects, and five characters, there were 105 tests of significance for gene action effects. Twenty-seven of 45 (60%) additive single-locus effects were significant, whereas only 11 of 60 (18%) of the epistatic two- or three-locus effects were significant. On the basis of means over N levels, 13 of 15 (87%) additive and 9 of 20 (45%) epistatic effects were significant. The epistatic effects were usually considerably smaller than the additive effects, and the per-

Table 5. Additive (α_A, α_B, α_D) and interaction (α_{AB}, α_{AD}, α_{BD}, α_{ABD}) gene effects for Anza and Cajeme 71 recombinant inbred lines, based on means of RIL lines within each HMW glutenin genotype

Character	Nitrogen	Gene effect							Σ
		α_A	α_B	α_D	α_{AB}	α_{AD}	α_{BD}	α_{ABD}	
Grain yield, kg/ha	40	−118**	−85	−113**	26	9	42	76	
	80	− 12	−43	−171**	51	−12	−13	−26	
	120	−167**	− 4	−218**	−11	69	55	2	
	Mean	−100**	−44**	−167**	20	22	28	17	−224
Grain protein, %	40	0.05	0.15*	0.18**	0.00	0.02	−0.08	0.02	
	80	0.16**	0.00	0.11	−0.04	0.19**	−0.19**	−0.11	
	120	0.05	0.20**	0.10	0.00	0.10	−0.15*	0.00	
	Mean	0.09**	0.14**	0.11**	−0.01	0.11**	−0.13**	−0.04	+0.27
Yellowberry, %	40	0.82	−5.55**	−4.72**	0.35	0.32	−0.25	−1.35	
	80	−0.29	−7.26**	−4.01**	1.96	2.96*	−1.96	−0.59	
	120	−2.26	−8.56**	−3.44**	0.14	1.01	0.91	−0.19	
	Mean	−0.55	−7.12**	−4.05**	0.82	1.45	−0.42	−0.72	−10.59
Pearling index, %	40	0.69*	−0.56	0.26	−0.79**	−0.71*	0.54	0.56	
	80	0.94**	0.21	0.26	−0.14	−0.19	−0.06	0.49	
	120	0.64	−0.91**	−0.09	−0.29	−0.26	−0.36	0.46	
	Mean	0.74**	−0.41*	0.16	−0.41*	−0.39*	0.01	0.51**	+0.21
SDS sed., ml	40	1.28**	0.88**	2.22**	−0.82**	−0.82**	−0.22	−0.42	
	80	1.65**	1.18**	1.82**	−0.45	−0.95**	−0.48	−0.85**	
	120	2.15**	1.75**	1.82**	0.12	−0.42	−0.68*	−0.49	
	Mean	1.69**	1.29**	1.99**	−0.39*	−0.74**	−0.44*	−0.59**	2.81

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

Table 6. Mean values of each HMW glutenin genotype (see Table 1 for explanation of genotype codes) for all characters, means all N levels

Character	HMW glutenin genotype										
	Anza (111)	111	112	121	122	211	212	221	222	Cajeme 71 (222)	SE mean[a]
Grain yield	5,060	4,863	4,464	4,714	4,358	4,616	4,235	4,477	4,276	4,060	174
Grain protein	10.3	10.0	10.4	10.6	10.5	10.0	10.7	10.6	10.7	11.5	0.28
Yellowberry	81.0	63,3	51.7	46.9	36.4	56.2	53.3	45.9	37.8	20.2	5.23
Pearling index	58.6	60.9	62.9	61.8	61.9	65.0	63.4	62.2	62.8	66.5	1.36
SDS sed.	44.5	47.7	53.1	50.9	56.5	52.3	56.8	56.0	56.6	60.2	1.29

[a] SE assuming mean of 6 lines included for each HMW genotype

Table 7. *F*-ratios to test for significance of genetic variation among RIL within HMW glutenin genotypes. Number of lines as given in Table 1 and error mean squares from Table 2

Character	Source of variation	HMW glutenin genotype							
		111	112	121	122	211	212	221	222
Grain yield	Among lines	12.50**	3.50**	11.50**	5.65**	4.29**	15.31**	7.00**	12.10**
	N × lines	1.74*	2.18*	0.79	1.22	1.89	0.78	1.88*	1.40
Pearling index	Among lines	6.74**	1.42	6.75**	9.54**	8.96**	16.66**	3.14**	1.63
	N × lines	1.73*	0.66	1.04	0.29	0.82	0.73	1.64	0.74
Yellowberry	Among lines	12.65**	10.89**	21.31**	16.87**	18.29**	27.38**	6.92**	22.72**
	N × lines	1.06	2.04*	0.88	1.53	0.52	1.10	1.22	2.25*
Grain protein	Among lines	2.71**	5.97**	7.22**	6.54**	4.60**	2.40**	2.24**	11.24**
	N × lines	0.63	0.78	1.22	0.59	0.35	0.26	0.76	1.09
SDS sed.	Among lines	14.39**	20.54**	11.43**	29.48**	3.68*	3.51*	21.80**	4.11**
	N × lines	0.71	0.57	1.04	0.28	0.62	0.21	1.36	1.52

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

centage of significant effects increased when the over-N treatment means were used, because of greater precision in those means.

The associations of the HMW glutenin loci to the five measured traits were variable, depending on the locus and trait. These loci by no means accounted for all of the variation in these characters. This can be seen by comparing the derived Anza and Cajeme 71 HMW glutenin genotypes to the parental varieties (Table 6). With no exceptions, the derived 111 and 222 genotypes did not reach the parental values. Viewed in another way, the sum of the gene effects, $\Sigma\alpha_i$, for each character can be compared to the difference, d, between Anza and Cajeme 71. The percentage ratios $\Sigma\alpha_i/_d$ were 22, 22, 17, 3, and 18 for grain yield, grain protein, yellowberry, pearling index, and SDS sedimentation, respectively. When these differences were computed using the derived 111 and 222 genotypes, the percentages were 38, 39, 42, 11, and 22, respectively. It must be remembered that the means for the HMW glutenin genotypes were computed over several RIL, thus, some of the lines may have attained the parental values. These results are expected, assuming that these characters are multigenic and that the associations are in part due to linkage of genes to the HMW glutenin loci and in part due to associations of genes that were not separated by recombination. Further information on this point is available from the analysis of variation among RIL within HMW glutenin lines within HMW glutenin genotypes (Table 7). All but 2 of 40 tests for genetic variance were significant. That result is expected if the variation is distributed randomly among the lines within or among a HMW glutenin genotype. In contrast, if the variation in a trait was completely associated with a HMW glutenin locus or loci, the *F*-ratios in Table 7 would approximate 1.0. The extent of variation among RIL within HMW glutenin genotypes is illustrated in Fig. 2. This figure shows the effects of allelic substitution on the genotype means, and shows that all genotypes have overlapping distributions.

These results point to the following important observations: (1) variation in grain yield and grain quality characters are associated with the major gene loci for

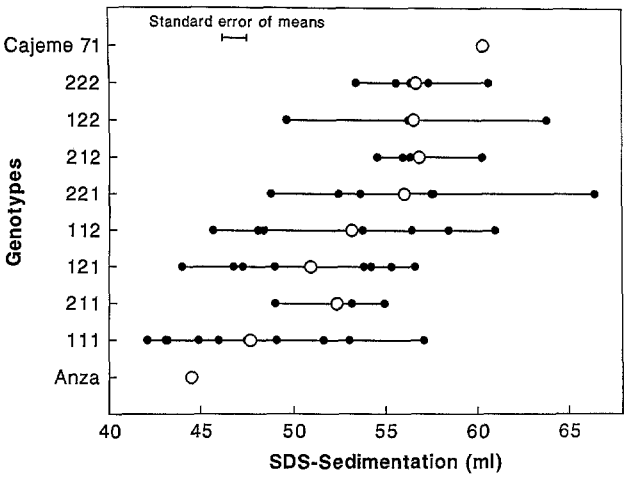

Fig. 2. Sedimentation volumes for Anza and Cajeme 71 and individual RIL (mean over three N treatments) in each HMW glutenin genotype. *Open circles* are the HMW glutenin genotype means. *Closed circles* are the RIL values

HMW glutenins; (2) these associations account for less than 25% of the differences in quality between the two parents; (3) the associations are not randomly distributed among the HMW glutenin genes on chromosomes *1A*, *1B*, and *1D*; and (4) epistatic effects, expressed as interactions among homozygous loci, are important for some characters and can be counter to additive effects of individual loci.

A few specific comments for each measured trait follow, based on Table 5.

Grain yield

All of the single-locus additive effects were negative and significant, whereas none of the epistatic effects were significant. The high grain yield of Anza was strongly associated with the HMW glutenin alleles that are attributed to low breadmaking quality. The *Glu-D1* locus had more influence on grain yield than did the other two loci.

Grain protein

The associations with HMW glutenin loci were positive as single loci, and one epistatic effect was positive and another negative; thus, even though there was epistasis, the net effect was that the *B*, *D*, and *A* loci from Cajeme 71, in that order, had substantial effects on grain protein. Note that the protein and grain yield additive effects differed in magnitude for the three loci, suggesting that yield and protein are not completely pleiotropically related.

Yellowberry

This condition is associated with low grain protein content and softness of the endosperm. These results are remarkable for the lack of association with *Glu-A1*, very strong association to *Glu-B1*, and moderately large effect of *Glu-D1*. No epistatic effects were detected. This suggests that genes from Anza associated or linked to *Glu-B1b* and *Glu-D1a* contribute to yellowberry and, hence, to lower grain quality.

Pearling index

This measurement of grain hardness mirrors the results for yellowberry, but there are important differences. The *Glu-A1* locus is associated with a strong positive effect, the *Glu-B1* locus shows a negative effect, and both positive and negative epistatic effects involved all three loci. Grain hardness genes are known to be on chromosome *5D* (Morrison et al. 1989), these results suggest genes on the group 1 chromosomes as well.

SDS-sedimentation

This measurement is widely known to have a positive relationship to breadmaking quality attributed to the glutenins. It has been used as a criterion for judging the contributions of individual HMW glutenin alleles on quality (Payne et al. 1987b). Hence, it is important to establish the genetic basis for this relationship. All three HMW glutenin loci were strongly associated with SDS sedimentation volume. The ranking of loci for positive effect was *Glu-D1* > *Glu-A1* > *Glu-B1*. However, a most important result was that all epistatic effects were significant and negative. The sum of negative effects (2.16 ml) reduced the single-locus effects (4.97 ml) by 43%; with these interactions it would be difficult to recover the high sedimentation value of Cajeme 71 from the Anza × Cajeme 71 hybrid. The single-locus effects (4.97 ml), without a reduction due to epistatis, account for only 4.97/15.7 = 32% of the difference between Anza and Cajeme 71, suggesting that other genetic factors contribute to SDS sedimentation volume. This is apparent in Fig. 2, where the wide ranges in SDS sedimentation were shown for each HMW glutenin genotype.

The SDS sedimentation volume, as related to grain protein content for the HMW glutenin genotypes (Fig. 3), shows progressively less improvement as one, two, and three Cajeme 71 alleles are substituted for the Anza alleles. This accounts for the epistatic effects and offers a suggestion for the plant breeder's common observation that grain quality is not easily recovered in hybrid populations. A second problem for plant breeders has been the recovery of high-yielding, high quality segregates. In the present study, the positive additive effects for SDS sedimentation are negatively related to the additive effects on grain yield; in both instances α_D is larger than α_A and α_B.

With knowledge about which HMW glutenin alleles are "good," the breeder can assay populations of single

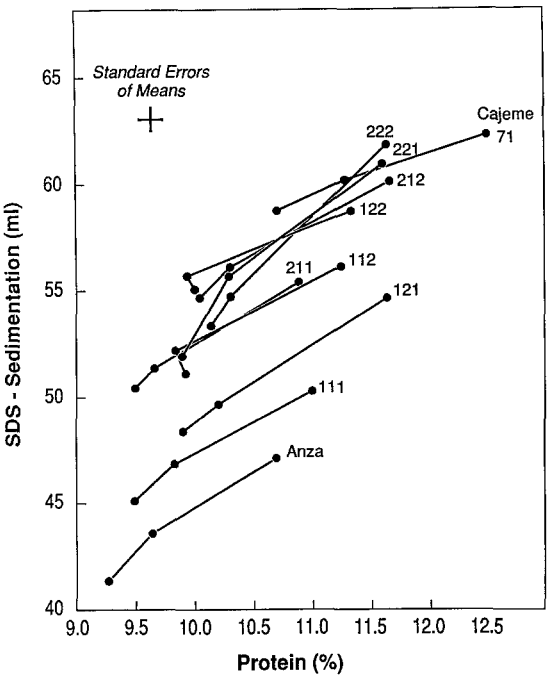

Fig. 3. Relationship of SDS sedimentation volume to grain protein percentage for Anza and Cajeme 71 and the recombinants obtained from the hybrid. Genotype codes as in Table 1. Each point on the response curve, from the left, is due to N treatments (N40, N80, and N120)

plants for SDS sedimentation and then, by single-kernel SDS-PAGE, detect the desired combinations of alleles. Based on the data presented here, it seems important to identify large numbers of plants with the desired *Glu-A1*, *Glu-B1*, and *Glu-D1* alleles so that intensive selection can then be practiced to achieve high quality, as may be contributed by other loci, including the low-molecular-weight glutenins (Gupta and Shepherd 1988), gliadins (Sozinov and Poperelya 1980), lipids (Morrison et al. 1989), and the genes on chromosomes not known to be associated with specific storage proteins.

The results of this study have revealed significant associations of HMW glutenin genes to end-use quality indicators, but at the same time have shown that the genetic system has greater interlocus interactions than previously suspected. Thus, the breeder can, to some extent, be aided by selecting for desired HMW glutenin alleles, but must also practice recurrent selection on agronomic and quality traits on a rather large scale.

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