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# Molecular Markers Associated with Differences in Bread-making Quality in a Cross Between Bread Wheat Cultivars with the Same High $M_r$ Glutenins

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## ABSTRACT

The association between molecular markers and bread-making quality (BMQ) was investigated in a cross between two wheat cultivars with the same high  $M_r$ -glutenin subunits but significantly different BMQ. A segregant  $F_2$  population was generated after crossing Klein 32 and Chinese Spring, and the BMQ of each  $F_2$ -derived  $F_3$  family was estimated using sodium dodecyl sulfate (SDS) sedimentation and mixograms. The same families were characterized for 11 polymorphic loci using restriction fragment length polymorphisms (RFLP) and single sequence repeats (SSR). These loci were specifically selected for their complete or close linkage to storage protein gene families. No significant differences in BMQ were detected at *XGlu-B1* and *XGlu-A1* loci using RFLP markers. Highly significant ( $P < 0.01$ ) differences were detected for *XGli-B1* and *XGlu-B3* loci on chromosome arm 1BS. The increase in the number of Klein 32 alleles at these loci determined a linear increase in sedimentation and mixogram values. It was not possible to differentiate the effect of *XGli1* from that of *XGlu3* because of the close linkage between these two loci. These two loci, considered together, explained from 11 to 15% of the variation in BMQ observed in this cross. The inclusion of the protein content of each sample as a covariable in the model increased the proportion of variation in SDS sedimentation explained by the analysis up to 46% and the precision of the statistic analysis up to 180%. Mixing parameters showed a lower correlation with protein content than SDS sedimentation parameters, and the increase in precision obtained by the use of the protein content as a covariable was non-significant.

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*Keywords:* wheat, breadmaking quality, low molecular weight glutenins, gliadins, SSR, RFLP.

## INTRODUCTION

**ABBREVIATIONS USED:** ANOVA = analysis of variance; ANCOVA = analysis of covariance; BMQ = bread-making quality; CIMMYT = Centro Internacional de Mejoramiento de Maíz y Trigo; HMr-GS = high relative mobility glutenin subunits; LMr-GS = low relative mobility glutenin subunits; RFLP = restriction fragment length polymorphisms; SDS-PAGE = sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SSR = single sequence repeats.

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The quantity and composition of seed storage proteins affect bread-making quality (BMQ). The quantity of protein in the grain is mainly affected by differences in growing conditions, particularly the availability of nitrogen. A reduction in the level of protein in the seed of a cultivar is often associated with a decrease in BMQ independently of the protein composition<sup>1</sup>. However, for a given protein content the composition of seed storage

proteins will be the major determinant of BMQ. Since composition of seed storage proteins is less affected by the environment than protein content, it is easier to manipulate in wheat breeding programs.

The composition of gliadins and glutenins is particularly important for BMQ because they impart the viscoelasticity to dough. Glutenins have a polymeric structure, determined by the presence of intermolecular disulfide bonds that allow retention of CO<sub>2</sub> and have a major effect on BMQ. This protein fraction has been categorized according to the relative mobility ( $M_r$ ) of the subunits following their reduction in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) into high- $M_r$ -glutenin subunits (HMr-GS) and low- $M_r$ -glutenin subunits (LMr-GS). Correlations between specific HMr-GS and the bread-making performance of wheat flour have been extensively studied. These studies have shown that variation in HMr-GS composition can account for up to 50% of the variation in BMQ<sup>2-4</sup>. The genetic origin of the variation not explained by the HMr-GS is an area of active research.

Although LMr-GS are present in larger proportions than the HMr-GS, correlations between BMQ and these polypeptides have been studied only recently, largely for technical reasons. Studies using SDS-PAGE and sequential extraction procedures for separating LMr-GS from gliadins<sup>5,6</sup> showed that allelic variation at loci encoding LMr-GS can have effects on quality approaching those of the HMr-GS<sup>7-10</sup>.

Gliadins are monomeric proteins with intramolecular disulfide bonds and have a relatively lower effect on quality than do glutenins. The reported association between quality and presence of some gliadin alleles was generally explained by the tight genetic linkage between the *Gli-1* locus encoding for gliadins and the *Glu-3* locus encoding for LMr-GS<sup>11,12</sup>. However, it is difficult to rule out the possibility of a direct effect of gliadins on BMQ because mutant  $\alpha$ -type and  $\gamma$ -type gliadin subunits can form intermolecular disulfide bonds. These mutant gliadins can function as chain terminators and modify the average length of the gluten polymers<sup>13</sup>.

In this paper we investigate the effect of different alleles at the LMr-GS and gliadin loci on BMQ in a segregating population from a cross between two cultivars with the same HMr-GS subunits and contrasting quality characteristics.

## EXPERIMENTAL

### Materials

Chinese Spring and the Argentine wheat cultivar Klein 32 were selected because of their identical HMr-GS and significantly different ( $P < 0.0001$ ) sedimentation and mixogram characteristics [Fig. 1(a), (b), (c) and (d)]. These two cultivars differ also in endosperm texture. Chinese Spring is a soft wheat and Klein 32 is a hard wheat. The availability of ditelocentric genetic stocks in Chinese Spring<sup>14</sup> facilitated the assignment of molecular markers to specific chromosome arms. A segregant population of 96 F<sub>2</sub> derived F<sub>3</sub> families (F<sub>2:3</sub>) was sown in a randomized complete block design with two replications.

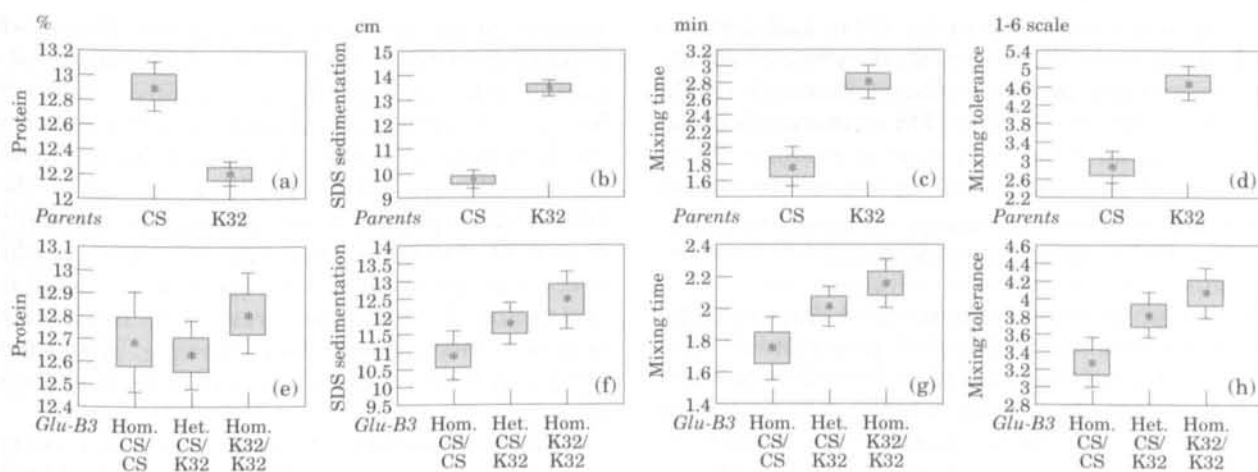
### Molecular markers

HMr-GS and LMr-GS were electrophoretically separated by SDS-PAGE using a sequential extraction method to eliminate the gliadin fraction<sup>15</sup>. To confirm the identity of the HMr-GS of Klein 32 and Chinese Spring, this fraction was separated by two-dimensional electrophoresis<sup>16</sup>.

Nuclear DNAs were extracted from the leaves of 10 to 15 plants within each F<sub>2:3</sub> family following the procedures published by the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT)<sup>17</sup>.

Restriction fragment length polymorphisms (RFLP): Chinese Spring and Klein 32 DNAs were screened for polymorphisms using restriction enzymes *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hind*III, *Sst*I, and *Xba*I. Detailed RFLP procedures have already been published<sup>18</sup>. Briefly, restriction endonuclease products were electrophoresed in 1% agarose gel and transferred to nylon membranes (Hybond N+) by capillary transfer. Probes were [ $\alpha$ -<sup>32</sup>P]-labeled with the random hexamer primer method, and hybridization was performed in a rotary hybridization chamber at 65°C. After hybridization membranes were washed at 65°C for 30 min in 2 × SSC, 30 min in 1 × SSC, and 30 min in 0.5 × SSC. Clones used in this work are listed in Table I.

Single sequence repeats (SSRs): Polymerase chain reactions (PCR) were carried out in a DNA Thermal Cycler (Perkin Elmer) on 25 ng of template DNA in 12  $\mu$ L final volume reaction, 100 nM of each primer, 200  $\mu$ M of each deoxyribonucleotide (Pharmacia), 1 × *Taq*I PCR Buffer (Promega),



**Figure 1** Variation in protein content (a, e), SDS sedimentation (b, f), mixing time (c, g) and mixing tolerance (d, h) between Chinese Spring (CS) and Klein 32 (K32) (a, b, c, d) and between  $F_2$  derived  $F_3$  families grouped by their allelic constitution at *Glu-B3* (e, f, g, h). The central black square represents the mean, the gray rectangle the standard error of the mean and the whiskers an approximate 95% confidence interval (1.96 \* standard error of the mean).

**Table I** RFLP and SSR markers

RFLP loci and references	Clones	
<i>Gli1</i> <sup>19</sup>	pcP387	
<i>XGli2</i> <sup>20</sup>	pTul	
<i>XGlu1</i> <sup>21</sup>	pDY10A/KS <sup>-</sup>	
<i>XGlu3</i> <sup>22</sup>	pTdUCD1	
<i>Xbdc</i> <sup>23</sup>	BCD (barley cDNAs)	
<i>Xcdo</i> <sup>23</sup>	CDO (oat cDNAs)	
<i>Xwg</i> <sup>23</sup>	WG (wheat genomic clones)	
<i>Xksu</i> <sup>24</sup>	KSU ( <i>T. tauschii</i> genomic clones)	
<i>Xmsu433(Lec)</i> <sup>25</sup>	pNVR20	
<i>Xpsr(Cxp3)</i> <sup>26</sup>	2473	
<i>XcsIH</i> <sup>27</sup>	CSIH ( <i>T. tauschii</i> genomic clones)	
SSR loci	Repeat	Primers
<i>Xpsp2(Gli1)</i> <sup>28</sup>	(CAA) <sub>n</sub>	F: GCA GAC CTG TGT CAT TGG TC R: GAT ATA GTG GCA GCA GGA TAC G
<i>Xpsp1(Glu3)</i> <sup>28</sup>	(CAG) <sub>n</sub> (CAA) <sub>n</sub>	F: TCC CGC CAT GAG TCA ATC R: TTG GGA GAC ACA TTG GCC
<i>Xcml5</i> <sup>29</sup>	(CT) <sub>n</sub>	F: GGT GAT GAG TGG CAC AGG R: CCC AAC AGT TGC AGA AAA TTA G

2.5 mM Mg<sup>++</sup>, and 0.5 units of *TaqI* DNA Polymerase (Promega). After an initial denaturation step at 94 °C for 2 min, the samples were subjected to 30 cycles of 1-min denaturation at 94 °C, 1-min annealing at 55 °C (*Glu-A3* and *Gli-B1*) or 60 °C (*Xcml5*), and 2-min extension at 72 °C. The cycle was finished with a 5-min extension at 72 °C. Products were analyzed in 6% acrylamide sequencing gels stained with silver (Promega). Two wheat SSRs located within the LMr-GS in *Glu-*

*A3* (*Xpsp1*) and the  $\gamma$ -gliadins in *Gli-B1* (*Xpsp2*)<sup>28</sup> and one SSR located in chromosome arm 1AL (*Xcml5-1A*)<sup>29</sup> were analyzed. The primers and internal repeats sequences are indicated in Table I.

### Bread-making quality parameters

A modification of the original SDS sedimentation test<sup>30</sup> was used in this study. The modified method<sup>31</sup> was very similar to the original one but the amount

of flour was reduced from 6 g to 1 g, and the 100-mL graduated cylinder replaced by one of 25 mL. Both methods provided almost identical results ( $r=0.97$   $P<0.001$ )<sup>32</sup>. The SDS sedimentation was selected as an indirect method to evaluate BMQ because of its positive and significant correlation with loaf volume<sup>30</sup>.

The difference in mixing properties was determined by mixograms obtained on a 10-g Mixograph following standardized American Association of Cereal Chemists' procedures<sup>33</sup>. The mixing requirement was characterized by the time to peak, and the mixing tolerance by the slope and width after peak, and by the stability of mixogram height on either side of the peak. Mixing tolerance was scored using a scale ascending from 1 to 6 with the improvement of mixing characteristics.

Protein content was determined using near-infrared reflectance spectroscopy (NIRS). Two samples from each replication of the  $F_{2:3}$  families were analyzed for protein content and SDS sedimentation. Values from these replications were highly correlated and the four values were averaged. Seeds from the two replications of each family were bulked for mixogram analysis. Protein content and SDS sedimentation tests were performed at the CIMMYT Quality Laboratory, and mixograms were performed at the Industrial Quality Laboratory of Wheat and Soybean, E.E.A. Marcos Juárez, Argentina.

### Data analysis

The genotype of each  $F_2$  plant was determined and the number of Klein 32 alleles recorded (0 = homozygous Chinese Spring, 1 = heterozygous, 2 = homozygous Klein 32). Independent analyses of variance were performed for protein content, SDS sedimentation, mixing time and mixing tolerance at each locus. Since sedimentation and mixogram values are significantly correlated with protein content<sup>1</sup>, the protein content of the sample was used as a covariable in an analysis of covariance. The increase in precision due to the inclusion of the covariable in the model was also calculated<sup>34</sup>.

For those loci showing a significant effect on the BMQ parameters the degree of dominance was calculated using the dominance/additivity ( $d/a$ ) statistic. This statistic describes the degree to which the heterozygous genotype resembles the

average of the parental homozygotes. Degree of dominance =  $d/a$ , where:  $d$  = dominance deviation =  $ck - (cc + kk)/2$ ;  $a$  = additive effect =  $(kk - cc)/2$ ;  $kk$  = mean of plants homozygous for the K32 alleles;  $cc$  = mean of plants homozygous for the Chinese Spring allele;  $ck$  = mean of the heterozygous plants. To test the significance of the degree of dominance the sum of squares of the effect was partitioned into the linear (additive) and quadratic (dominance) responses. Interaction between independent loci showing significant effect was tested using a two-way analysis of variance.

Linkage maps were constructed with the aid of the computer program Mapmaker/EXP 3.0<sup>35,36</sup> using the Kosambi function<sup>37</sup>. The goodness of fit of segregation for each pair of alleles was tested with  $\chi^2$  tests.

## RESULTS

### Screening and mapping of molecular markers

Wheat cultivars Klein 32 and Chinese Spring display identical HMr-glutenin electrophoretic patterns in SDS-PAGE. Both cultivars have alleles Glu-A1c (null); Glu-B1b (7+8); and Glu-D1a (2+12). Moreover, no different patterns for this fraction were observed when using two-dimensional gel electrophoresis (data not shown). In spite of the similar electrophoretic mobility of HMr-GS in SDS-PAGE, Klein 32 showed significantly higher ( $P<0.0001$ ) values than Chinese Spring in SDS sedimentation, mixing time and mixing tolerance [Figs 1(b), (c), and (d)]. Chinese Spring showed significantly higher ( $P<0.0001$ ) protein content than Klein 32 [Fig. 1(a)].

Fifteen loci, known to be linked to storage protein loci in homoeologous groups 1 and 6, were screened for polymorphisms between Klein 32 and Chinese Spring (Table II). A low level of polymorphism was observed in this cross, and only 10 B-genome loci, four A-genome loci, and one D-genome locus were polymorphic for at least one of the eight restriction enzymes tested (Table II). Chinese Spring bands were assigned to chromosome arms using ditelocentric stocks for homoeologous groups 1 and 6.

Two of the three SSR loci analyzed were polymorphic in this population. The SSR marker located within the LMr-GS of *Glu-A3* was not polymorphic and could not be mapped. Two fragments of 133 bp/142 bp were amplified by PCR

**Table II** RFLP loci and restriction enzymes showing polymorphisms between Klein 32 and Chinese Spring

Locus	1AS	1BS	1DS
<i>XGli1</i>	NP	<i>XbaI</i>	NP
<i>XGlu3</i>	NP	<i>XbaI</i>	NP
<i>XksuE18</i>	NP	<i>HindIII</i>	<i>HindIII</i>
<i>XksuD14</i>	NP	<i>EcoRI</i>	NP
<i>XcsIH69</i>	NP	NP	NP
<i>Xpsr540</i>	NP	NP	NP
<i>Xbcd1434</i>	NP	<i>DraI*</i>	NP
<i>Xmwg920</i>	NP	<i>DraI*</i>	NP
Locus	1AL	1BL	1DL
<i>XGli1</i>	NP	<i>XbaI</i>	NP
<i>Xwg983</i>	<i>HindIII</i>	NP	NP
<i>Xmsu433(Lec)</i>	NP	<i>DraI*</i>	NP
<i>Xcdo572</i>	NP	<i>EcoRV*</i>	NP
Locus	6AS	6BS	6DS
<i>Xpsr10(Gli2)</i>	<i>DraI</i>	NP	NP
<i>Xpsr8(Cxp3)</i>	<i>EcoRI</i>	<i>XbaI</i>	NP
<i>XksuG48</i>	<i>BamHI*</i>	NP	NP

NP, not polymorphic.

\*Indicates that the polymorphic band for this loci was not mapped because of its close linkage with other loci already present in the map.

in both cultivars. Absence of any amplification product in double ditelocentric 1AL indicates that both fragments are located in chromosome 1AS. Primers for the SSR located within the  $\gamma$ -gliadin gene *Gli-A1* amplify a fragment of 285 bp in Chinese Spring and a fragment of 252 bp in Klein 32. The difference in length between these two fragments was large enough to be scored in 2% agarose gels stained with ethidium bromide. The third SSR locus, *Xcni5*, was located by ditelocentric analysis on the long arm of chromosome 1A. No significant segregation distortion ( $\chi^2$  tests:  $P > 0.05$ ) was found for any RFLP or SSR locus.

Linkage relationships among RFLP and SSR markers are shown in Figure 2. RFLP loci *XksuD14* and *XGli-B1* were completely linked on the distal region of the map of chromosome arm 1BS. Complete cosegregation was also observed between these two RFLP markers and the SSR *Xpsp2(Gli1)* located within a  $\gamma$ -gliadin gene. The LMr-GS locus *XGlu-B3* was mapped 1.6 cM proximal to the *XGli-B1* locus (Fig. 2) and 25 cM distal to the *XksuE18* locus. Close linkage was also found between *XGli-A2* and *Xpsr8(Cxp3)* on the short arm of chromosome 6A (1.9 cM, Fig. 2). SSR marker *Xcni5*

was mapped on the long arm of chromosome 1A, 8.3 cM from the *Xwg983* locus. The orientation of these two markers relative to the centromere is currently not known.

### Effect of the allelic variation at different loci on bread-making quality

No significant differences were found in protein content for any of the 10 loci analyzed [analysis of variance (ANOVA)  $P > 0.15$ , Table III, Fig. 1(e)], indicating that allelic variation at these loci has no effect on this character. However, the protein content of each sample was significantly correlated ( $r = 0.62$ ,  $P < 0.001$ ) with the SDS sedimentation value, indicating that 38% of the variation in SDS sedimentation can be explained by variation in protein content. A lower correlation ( $r = 0.23$ ,  $P = 0.04$ ), representing only 5% of the variation, was found between protein content and mixing time, and no significant correlation ( $r = 0.10$ ,  $P = 0.38$ ) was detected with mixing tolerance. A highly significant correlation was observed between mixing time, and mixing tolerance ( $r = 0.82$ ,  $P < 0.01$ ). Regression coefficients between protein content and each BMQ parameter were homogeneous within the allelic classes for each locus ( $P > 0.05$ ).

The previous characteristics indicated that the protein content of each sample could be used as a covariable to increase the precision of the statistic analysis. ANOVA and analysis of covariance (ANCOVA) were performed for each locus, and the relative increase in precision was calculated (Table III). The relative precision of the ANCOVA for SDS sedimentation adjusted by protein content relative to the ANOVA of the unadjusted values varied from 156 to 180% among the 10 loci analyzed (average 163%). A lower increase in precision of the ANCOVA relative to the ANOVA was detected for mixing time (104%) and no increase at all was found for mixing tolerance as expected from the low correlation values between the last two parameters and protein content. These results suggest that the ANCOVA using protein content as a covariable is a valuable tool to estimate allelic differences in BMQ only for those parameters showing correlations of  $r = 0.50$  or higher with protein content.

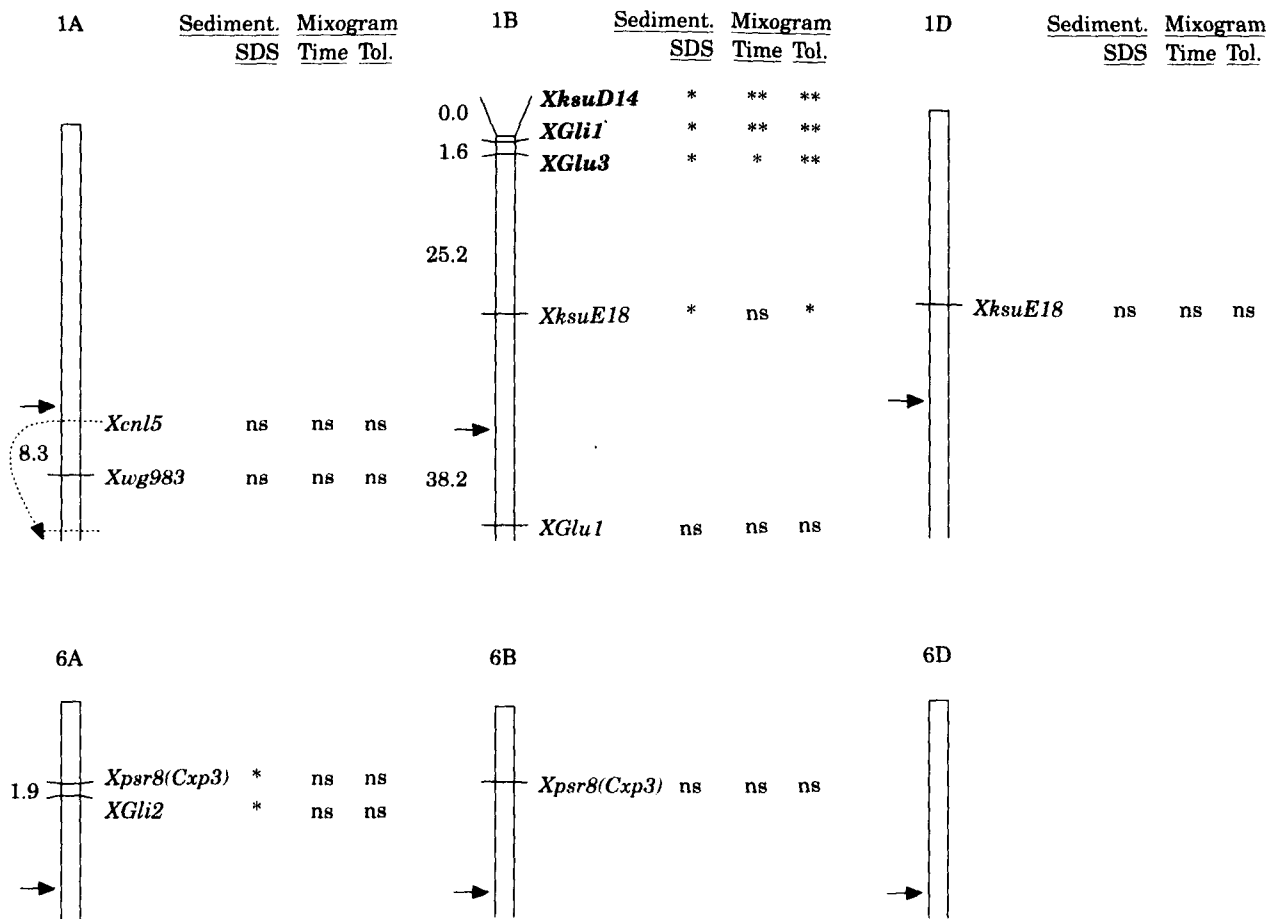
No significant difference in BMQ parameters was detected with the ANOVA or ANCOVA between Chinese Spring and Klein 32 alleles for any of the loci on the long arm of homoeologous

group I (*XGlu-B1*, *Xwg983-1A* or *Xcn15-1AL*) (Table III). Locus *Xwg983* was included in the analysis because of its close linkage (13 cM) with *XGlu-A1*<sup>38</sup>. The effect of the *XGlu-D1* loci on BMQ could not be studied because of the lack of polymorphism for the closely linked molecular markers *Xwg983*, *Xmsu433(Lec)*, and *Xcdo572* (Table II).

Highly significant (ANOVA and ANCOVA  $P < 0.01$ ) effects on all BMQ parameters were detected for *XGli-B1* and *XGlu-B3* on chromosome arm 1BS [Figs 1(e), (f), (g) and (h), Table III]. However, it was not possible to differentiate the effect of *XGli-B1* from that of *XGlu-B3* because of the close linkage between these two loci (Fig. 2). When these two loci were considered together by multiple regression they explained from 11 to 15% of the variation in BMQ observed in this cross. The percentage of variation in SDS sedimentation

explained by the model increased to 42% (*XGli-B1*) and 46% (*XGlu-B3*) when the protein content was included as a covariable in the ANCOVA. A similar analysis for mixing time explained 19% of the variation. The positive effect on sedimentation values, mixing time and mixing tolerance was associated with the presence of Klein 32 alleles at *XGli-B1* and *XGlu-B3* (Figs 1(f), (g) and (h)).

A significant effect on SDS sedimentation ( $P < 0.01$ ) was also observed at the *XksuE18* locus located in the middle of the short arm (Fig. 2). However, the effect of this locus is lower ( $P < 0.05$ ) for mixing tolerance and not significant ( $P = 0.09$ ) for mixing time. RFLP locus *XksuE18-1D* located in the middle of the short arm of chromosome 1D showed no effect on any of the quality parameters analyzed. No polymorphism was detected in the distal region of chromosome arms 1AS or 1DS



**Figure 2** Chromosomal location of the molecular markers analyzed. Symbols, \* ( $P < 0.05$ ), \*\* ( $P < 0.01$ ), and NS ( $P > 0.05$ ), indicate the significance of the analysis of variance for each locus for SDS sedimentation (SDS), mixing time (Time) and mixing tolerance (Tol.). Arrows indicate the approximate position of the centromere based on Dubcovsky *et al.* (1995, 1996).<sup>33,38</sup> Distances are in cM.

**Table III** Analysis of variance and covariance of the effect of different loci on bread-making quality parameters

Chromosome	1B	1B	1B	1B	1A	1A	1D	6A	6A	6B
	<i>XGli1</i>	<i>XGlu3</i>	<i>XksuE18</i>	<i>XGlu1</i>	<i>Xcni5</i>	<i>Xwg983</i>	<i>XksuE18</i>	<i>XGli2*</i>	<i>Xpsr8</i>	<i>Xpsr8*</i>
<b>Sedimentation</b>										
<i>P</i> ANOVA	0.023	0.036	0.034	ns	ns	ns	ns	0.051	0.036	ns
<i>P</i> ANCOVA	0.003	0.019	0.001	ns	ns	ns	ns	0.079	0.099	ns
Lineal	0.003	0.009	0.0002	ns	ns	ns	ns	–	0.038	–
Quadratic	0.083	0.214	0.985	ns	ns	ns	ns	–	0.516	–
<i>d/a</i> **	0.81 ns	0.63 ns	–0.002 ns	ns	ns	ns	ns	–	–0.430 ns	–
Relative effic. %	64.4%	57.2%	73.3%	59.4%	80.0%	63.9%	57.0%	62.3%	56.2%	59.8%
<b>Mixing time</b>										
<i>P</i> ANOVA	0.009	0.011	ns	ns	ns	ns	ns	ns	ns	ns
<i>P</i> ANCOVA	0.003	0.006	ns	ns	ns	ns	ns	ns	ns	ns
Lineal	0.001	0.001	ns	ns	ns	ns	ns	–	ns	–
Quadratic	0.856	0.727	ns	ns	ns	ns	ns	–	ns	–
<i>d/a</i>	0.08 ns	0.15 ns	–0.69 ns	ns	ns	ns	ns	–	ns	–
Relative effic. %	5.3%	6.6%	4.6%	6.1%	0.4%	4.2%	1.8%	2.3%	4.0%	3.7%
<b>Mixing tolerance</b>										
<i>P</i> ANOVA	0.003	0.009	0.023	ns	ns	ns	ns	ns	ns	ns
<i>P</i> ANCOVA	0.004	0.009	0.023	ns	ns	ns	ns	ns	ns	ns
Lineal	0.001	0.003	0.011	ns	ns	ns	ns	–	ns	–
Quadratic	0.471	0.371	0.444	ns	ns	ns	ns	–	ns	–
<i>d/a</i>	0.30 ns	0.40 ns	–0.44 ns	ns	ns	ns	ns	–	ns	–
Relative effic. %	–2.2%	–1.7%	–1.0%	–4.0%	–4.4%	–2.5%	–2.2%	–2.2%	–4.0%	2.2%
<b>Protein content</b>										
<i>P</i> ANOVA	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

\**XGli2-6A* and *Xpsr8(Cxp3)* were scored as presence absence and consequently there is only one degree of freedom for the effect.

\*\*Dominance/additivity statistic *d/a* was calculated using adjusted treatment means from the ANCOVA.

ns = not significant.

for *Xpsr540*, *XksuD14*, *Xbcd1434*, *XcsiH69*, or *Xmwg920* loci.

The effect of allelic variation at the *XGli2* locus was investigated only for chromosomes 6A and 6B because no polymorphism was detected for chromosome 6D (Table II). No significant effect on any BMQ parameter was detected for *Xpsr8(Cxp3)-6B*, a locus closely linked to *XGli-B2* (Table III). A significant effect ( $P=0.04$ ) on SDS sedimentation was detected for *Xpsr8(Cxp3)-6A* locus in the ANOVA but not in the ANCOVA ( $P=0.10$ ). However, when the sum of squares of the analysis of covariance was partitioned into the linear and quadratic effects, a significant ( $P<0.04$ ) linear response was detected (Table III). The positive effect on sedimentation values was associated with the presence of Chinese Spring alleles at *XGli-A2*.

### Gene action

The dominance/additivity (*d/a*) statistic was calculated for the adjusted means (ANCOVA) of

those loci showing a significant effect on BMQ (Table III). The observed degree of dominance was intermediate between additivity ( $d/a=0$ ) and complete dominance ( $d/a=1$ ). Positive values were observed for *XGli-B1* and *XGlu-B3*, and negative values for *XksuE18* and *Xpsr8(Cxp3)-6A*. However, partitioning of the sum of squares of the significant effects into linear and quadratic responses showed that only the linear responses were significant (Table III).

The four possible combinations of two-way analyses of variance including one of the loci of chromosome 1B (*XGli-B1*, *XGlu-B3*) and one of the loci of chromosome 6A (*XGli-A2*, *Xpsr8(Cxp3)*) detected no significant interactions. This indicates that the effect of these loci is largely additive.

### DISCUSSION

The SSR located within the storage protein genes<sup>28</sup> have some interesting properties for their use as molecular markers of multigene families in a poly-



ploid species such as wheat. On the one hand, they amplify fragments from a single locus, providing markers that are easier to interpret than those from protein gels or RFLP autoradiographs where multiple and sometimes overlapping bands originated in the three different genomes are observed. On the other hand, the SSR alleles can be precisely identified by the length of the amplified fragments in base pairs, facilitating communication of results among laboratories. Studies of the relationship between microsatellite alleles and protein bands are in progress to assess more accurately the usefulness of these SSRs for the classification of storage-protein gene variants.

The SSR located within the  $\gamma$ -gliadin gene (*Xpsp2(Gli-1)*) was completely linked to the RFLP locus *XGli-B1* and closely linked (1.6 cM) to *XGlu-B3* in the Chinese Spring-Klein 32 segregating population. Since both loci are usually inherited together, the SSR within *XGli-B1* can be used as a molecular marker for the *Gli-B1/Glu-B3* chromosome region. A survey of 110 Argentine bread-wheat cultivars<sup>39</sup> and 80 California bread-wheat cultivars (J. Dubcovsky, M. Manifesto and A. R. Schlatter, unpublished) for the *Xpsp2(Gli-1)* SSR locus showed that the 252-bp allele from Klein 32 associated with better BMQ in this study, was also present in eight other Argentine wheat cultivars (Klein Granador, Buck Atlántico, Buck Cencerro, Buck Cimarrón, Buck Manantial, Buck Napostá, Buck Pangaré, and Oncativo INTA). This allele was not found in any of the California bread-wheat cultivars analyzed. It is interesting to note that some of the Argentine cultivars with the 252-bp allele, like Buck Manantial, Buck Cencerro<sup>40</sup>, and Buck Pangaré<sup>11</sup>, are known for their excellent BMQ.

Allelic differences at the storage protein loci *Gli-B1/Glu-B3* are responsible for a major portion of the variation in BMQ detected in this cross. When the protein content was included as a covariable in the analysis of the effect of *Gli-B1/Glu-B3* loci, the statistic model accounted for almost half of the variation in SDS sedimentation observed in this cross. The significant effect of the *XksuE18 1B* locus on BMQ can be explained either by its linkage with *XGlu-B3* or by the effect of allelic variation at the *Gli-B3* or *Glu-B2* loci<sup>12</sup> located 8 to 9 cM proximal to *XksuE18*<sup>38,13,11</sup>. The large effect of the storage protein loci located on the distal part of the map of chromosome arm 1BS (Fig. 2) on BMQ is in agreement with results from other authors<sup>7,10,12,15</sup>. These results are not unexpected

because LMr-GS synthesized at the *Glu3* loci are included in the polymeric structure of the gluten by disulfide bonds and are even more abundant than the HMr-GS.

No effect of the HMr-GS on BMQ was expected in this cross because Chinese Spring and Klein 32 have similar patterns of HMr-GS in SDS-PAGE. However, it is possible to speculate that differences between HMr-GS that cannot be detected by SDS-PAGE can be responsible for the proportion of the variation in BMQ not explained by the variation at the *Gli-B1/Glu-B3* loci. This explanation can be ruled out for the HMr loci on chromosomes 1A and 1B because polymorphic RFLP markers for *XGlu-B1* and for Xwg983 (closely linked to *XGlu-A1*) showed no significant association with BMQ parameters.

A small but significant part of the variation in BMQ detected in this cross was explained by variation at the *Gli-A2* locus. The proteins coded at the gliadin loci are usually not part of the polymeric structure of the gluten. However, up to 20% of the bands detected within the LMr-G region in SDS-PAGE, after a differential extraction of monomeric gliadins, are mutant  $\alpha$ -type and  $\gamma$ -type gliadin subunits<sup>13</sup>. These mutant proteins, coded at the gliadin loci, differ from the normal gliadins in that a serine residue has been mutated to a cysteine. The resulting odd number of cysteines in the mutant  $\alpha$ -type and  $\gamma$ -type gliadins determines the incorporation of the protein into glutenin where they can function as chain terminators, affecting the average length of the polymeric fraction<sup>13</sup>. The interactions of some of the proteins coded at the gliadin loci with the gluten can explain the significant positive effects of the Chinese Spring alleles at *XGli-A2* on SDS sedimentation observed in this study. This result should be interpreted with caution because the significance of the difference between the *XGli-A2* alleles was only  $P=0.04$  and could be a chance occurrence because many independent tests were performed. However, an independent preliminary study, using four substitution lines homozygous for chromosomes 6A of Chinese Spring and four substitution lines homozygous for chromosomes 6A of Klein 32 in a random heterogeneous background (75% Chinese Spring, 25% Klein 32), also suggested a significant positive effect (ANOVA,  $P<0.02$ ) of Chinese Spring chromosome 6A on SDS sedimentation<sup>32</sup>.

The differences in hardness between Chinese Spring (soft) and Klein 32 (hard) may account

for part of the observed variation in BMQ not explained by the previously described loci. Differences in hardness affect energy consumption during grinding, the degree of endosperm/bran separation, the extraction rate, the shape and size distribution of flour particles, and the water-absorbing ability of the starch granules<sup>46</sup>. A single gene located on the distal part of the short arm of chromosome 5D is known to be the major, although not the only, determinant of endosperm texture<sup>46</sup>. Unfortunately, the Grain Softness Related Protein clone GSP<sup>47</sup>, located in the distal part of chromosome 5DS<sup>48</sup>, showed no polymorphism between Chinese Spring and Klein 32 precluding the study of the effect of the hardness locus on the BMQ parameters measured in this study.

A better understanding of the genetic factors that determine BMQ can provide breeders with a basis for more intelligent planning and selection methods in the task of breeding for grain quality. The past decade of research has focused on the HMr glutenins and has provided a ranking of the contribution of the subunits to BMQ that has been extensively used in bread-wheat breeding programs around the world. This has resulted in a significant increase in the frequency of those alleles with high ranks among the modern cultivars. For example, a survey of HMr-GS including 112 Argentine bread-wheat cultivars showed that 63% of the cultivars have the maximum ranking (Glu-1 index = 10) and 23% the second highest value (Glu-1 index = 9) (M. Echaide and J. Dubcovsky, unpublished). However, significant differences in BMQ are still present among cultivars with the maximum Glu-1 index<sup>32,40,41</sup>. This study shows that a significant part of that variation can be explained by allelic differences at the LMr loci. A better understanding of the effects of allelic variation at the LMr loci, the hardness locus, and of the interactions between HMr-GS, LMr-GS and hardness will be necessary to be able to manipulate efficiently a large proportion of the variation in BMQ.

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