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Effect of *Triticum monococcum* glutenin loci on cookie making quality and on predictive tests for bread making quality

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ABSTRACT

The effects of *Triticum monococcum* glutenin loci on cookie making quality and predictive tests for bread making quality were evaluated in recombinant substitution lines (RSLs) between chromosome $1A^m$ from *T. monococcum* and chromosome 1A from Chinese Spring. All four combinations of high molecular weight (HM_r-GS) and low molecular weight glutenin alleles (LM_r-GS) were studied in a factorial design to evaluate their interactions. Grain protein content was used as a covariable to evaluate the effect of these loci independently of the variation in protein content among lines. No significant interactions were detected indicating an additive effect. RSLs carrying the HM_r-GS from *T. monococcum* showed a 13·6% increase in SDS sedimentation volume ($p=0·004$) and a significant reduction in cookie diameter (−5·2%, $p=0·02$), and cookie quality (−6·8%, $p=0·02$). RSLs carrying the LM_r-GS from *T. monococcum* showed a significant decrease in the proportion of polymeric protein (−2·8%, $p<0·0001$), SDS sedimentation volume (−8·1%, $p=0·03$) and gluten strength (−16·5%, $p=0·01$), and a significant increase in cookie quality (5·9%, $p=0·05$). The *T. monococcum* LM_r-GS allele has potential value to be used in soft wheat breeding programs. These results suggest that diploid *T. monococcum* could be a valuable source for new allelic variation for storage proteins loci and new quality characteristics.

Keywords: wheat, *Triticum monococcum*, end-use quality, glutenins.

INTRODUCTION

Improvement of bread making (BMQ) and cookie making quality (CMQ) is a major objective in breeding programs of hard and soft wheats, respectively. Both BMQ and CMQ are affected by quantity and quality of storage proteins. Glutenins and gliadins are polymeric and monomeric proteins, respectively, constitutive of the main protein fraction of the endosperm, referred to as gluten. The proportion of polymeric protein in flour, the allelic variation at the high (HM_r-GS) and low

ABBREVIATIONS USED: ANOVA = analysis of variance; ANCOVA = analysis of covariance; BMQ = bread making quality; CS = Chinese Spring; CMQ = cookie making quality; HM_r-GS = high molecular weight glutenin subunit; LM_r-GS = low molecular weight glutenin subunit; RFLP = restriction fragment length polymorphism; RSL = recombinant substitution line; SDS = sodium dodecyl sulphate; SE-HPLC = size-exclusion high performance liquid chromatography.

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molecular weight (LM_r-GS) glutenin subunits loci and the ratio between HM_r-GS and LM_r-GS are the main sources of variation in BMQ and CMQ[1–3].

HM_r-GS are encoded at the *Glu-1* loci, located on the long arms of chromosomes 1A, 1B and 1D, while LM_r-GS are controlled by genes at the *Glu-3* loci, located on the short arms of the same chromosomes and closely linked to the *Gli-1* gliadin loci[4]. Allelic variation at the three HM_r-GS loci has been extensively studied and its correlation with BMQ is well established (reviewed in refs[5–8]). However, this knowledge is more limited for the LM_r-GS allelic variants[9–12], and even scarcer for the interactions between these two loci[13–15]. Interpretation of the effect of the LM_r-GS allelic variants on quality is also complicated by the tight linkage between the LM_r-GS *Glu-3* and the gliadin *Gli-1* loci. In the few cases where these two loci were separated by recombination, effects on gluten strength were associated to the LM_r-GS locus[16,17]. Moreover, functional tests have shown that LM_r-GS polypeptides incorporated into the flour have a dough strengthening effect[18]. These results, together with correlation studies of LM_r-GS allelic variation and dough properties[9,19], suggest that differences in dough strength are mainly originated in the LM_r-GS rather than in the linked gliadin alleles.

A research area that has received relatively limited attention is the effect of the allelic glutenin variants from wild diploid species on end-use quality. Particularly interesting is *Triticum monococcum* L., that carries an A^m genome closely related to the A genome of hexaploid wheat and is the only cultivated diploid species of *Triticum*. Electrophoretic analyses of HM_r-GS[20–22] and LM_r-GS[23,24] from this species have shown allelic variants not previously reported in hexaploid wheat. Variation in the bread making performance among accessions of *T. monococcum* has also been observed[25–27] indicating that *T. monococcum* is a potential source of genetic variation for storage proteins and new quality characteristics. However, there are limited numbers of studies on the effect of specific allelic variants on quality parameters at the diploid level[21,28,29]; and even less on the effects of the *T. monococcum* alleles within a bread wheat genetic background[30].

The objective of this study was to evaluate the effects of the *T. monococcum* HM_r-GS and LM_r-GS on CMQ and predictive tests for BMQ within a bread wheat genetic background. Interactions between these two loci were also evaluated.

EXPERIMENTAL

Materials

Dr J. Dvorak *et al.*[31] (Univ. of California, Davis, CA, U.S.A.) previously produced a series of monosomic recombinant substitution lines (RSL) between chromosome $1A^m$ from *T. monococcum* (accession G1777) and chromosome 1A from bread wheat in the genetic background of Chinese Spring. The recombination points between chromosome 1A and $1A^m$ in each line were characterised with 94 RFLP markers[31]. Chromosome counts were made on the monosomic RSLs kindly provided by Dr Dvorak to select disomic RSLs from their progenies. Fourteen disomic RSLs, Chinese Spring and the original substitution line of chromosome $1A^m$ of *T. monococcum* in Chinese Spring, CS($1A^m$)[31] were selected for this study (Fig. 1). These 16 lines are identical to Chinese Spring for all chromosome pairs except 1A, reducing genetic variation among lines and increasing the sensitivity of the experiments to detect small effects of loci located on chromosome 1A on CMQ and predictive tests for BMQ.

These 16 lines included four groups of four lines. Each group has one of the four possible HM_r-GS/LM_r-GS allele combinations (Fig. 1). Plants within each group have the same HM_r-GS/LM_r-GS combination, but differ in the recombination points on chromosome 1A. Recombinant substitution lines #38 and #63 have a crossover between the same RFLP markers (Fig. 1). However, since they were produced independently it is likely that they would have different crossover points within this 3·9 cM interval. RSLs #3, #8 and #38 (Fig. 1) were replaced in the Davis trial by RSLs #14, #87 and #15 (not shown), respectively, because of limited availability of seed. These new lines have the same HM_r-GS/LM_r-GS allelic combinations as the lines they replaced and their chromosome 1A structure has been described before[32].

Allelic constitution of these lines at the HM_r-GS and LM_r-GS loci was previously determined by RFLP analysis[31] and was confirmed for each experiment by SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) after extraction of polymeric proteins using dimethyl

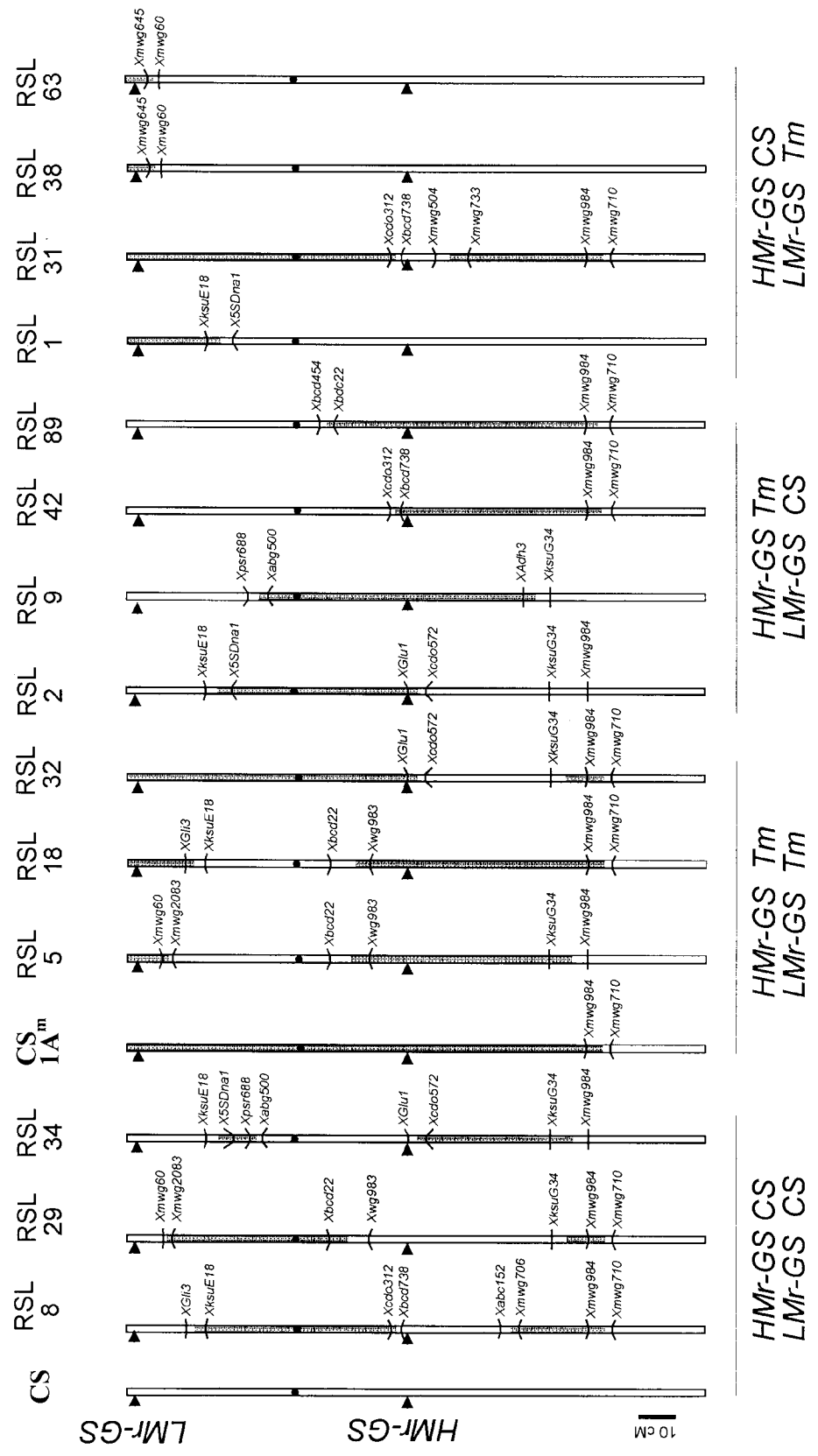

Figure 1 Genetic constitution of the 16 RSLs used in this study based on RFLP maps constructed with 94 markers[31,32]. Only markers flanking the recombination events in these lines are indicated in the figure. Grey segments represent $1A^m$-chromosome segments and white segments Chinese Spring-chromosome segments. Centromeres are indicated by circles and the postion of the LM_r-GS (*Glu-3*) and HM_r-GS (*Glu-1*) loci by arrowheads – *shown opposite*.

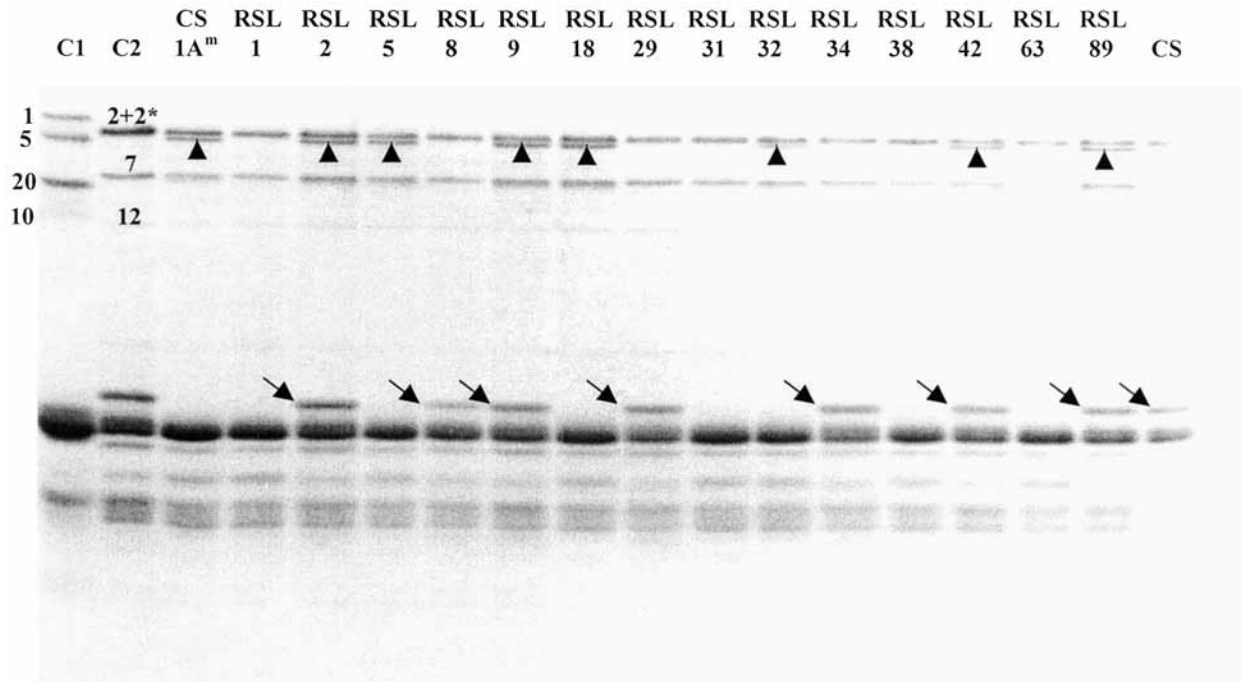

Figure 2 SDS-PAGE[33] patterns for the 16 lines used in this study including Chinese Spring (CS = Null, 7 + 8, 2 + 12). Two bread wheat varieties were used as standards: C1 = Olympic (1, 20, 5 + 10) and C2 = Line 886 (2*, 7 + 8, 2 + 12). HM_r-GS band 8 in C2 and the RSLs are not clear in this gel because of the protein extraction technique. HM_r-GS from *T. monococcum* are indicated by arrowheads and LM_r-GS from Chinese Spring *Glu-A1* by arrows.

sulphoxide to eliminate monomeric proteins[33] (Fig. 2).

Field trials

Four subsamples of each of the 16 lines were grown at two locations (Castelar, Buenos Aires, Argentina and Davis, CA, U.S.A.) in a completely randomized design. Each subsample consisted of four rows, 1·5 m long (20 plants per row).

Quality determinations

Grain yield and 500-grain weight were measured for each subsample. Protein content was determined for each subsample using Near-Infrared Method for Whole Grain Analysis (AACC 39-21)[34]. Kernel hardness and kernel moisture were determined by the Single Kernel Characterization System (Perten Model 4100). Samples were milled according to the AACC method 26-21A using a Bühler Mill model MLU-202, adjusted to 70%.

Size-exclusion high performance liquid chromatography (SE-HPLC) analyses were performed on the same subsamples to determine the relative proportion of the three main size classes of wheat endosperm proteins. Peak 1 of the chromatogram consists mainly of polymeric glutenins (HM_r-GS and LM_r-GS held together by disulphide bonds) as well as a very small proportion of gliadin-like components with odd number of cysteines[35–39] and HM_r-albumins. Monomeric proteins elute in peaks 2 and 3. Peak 2 consists of gliadins and peak 3 of albumins and globulins. Since the SE-HPLC evaluation is done under unreduced conditions, polymeric structures remain similar to their native state, without breaking into HM_r or LM_r subunits[40].

Lines included in the present study have a common Chinese Spring background, which is not appropriate for bread manufacture. Consequently, no direct bread baking methods were performed. The potential value of the *T. monococcum* alleles on BMQ was evaluated by predictive quality tests. The SDS (sodium dodecyl sulfate) sedimentation volume test (AACC 56-70)[34] was selected as an indirect method to evaluate BMQ because of its positive and significant correlation with loaf volume[41]. The differences in mixing time and mixing tolerance were determined by mixograms obtained on a 10-g mixograph following standardised pro-

cedures (AACC 54-40 A)[34]. For Chopin Alveograph test, tenacity (P, in mm), extensibility (L, in mm), P/L ratio (no units), and dough strength (W, in 10^{-4} J) were measured following standardised procedures (AACC 54-30 A)[34].

Cookie baking tests and determination of cookie diameter were performed according to AACC method 10-52. For the determination of cookie quality, four cookies were laid edge to edge to determine width (four-width) and then stacked on top of each other to determine height (four-height). The cookie factor was calculated as the ratio between four-width and four-height. Cookie quality was then determined as the ratio between the sample cookie factor and the same factor calculated from a control included in every test and expressed as a percentage[42].

SDS sedimentation and mixograph tests were performed for each subsample but alveograph and cookie baking tests were performed using pooled grain from the four subsamples from each line.

Statistical analysis

Data was organised in a three-way factorial analysis including all possible interactions between the three classification variables (location, HM_r-GS allele, and LM_r-GS allele). Protein content was used as a covariable to increase the sensitivity of the analysis and to determine the effects of HM_r-GS and LM_r-GS alleles independently of grain protein content[12].

Variation between the four RSLs carrying the same HM_r-GS/LM_r-GS combination, but different segments of the *T. monococcum* and *T. aestivum* chromosome 1A (Fig. 1) was used to estimate the error term. Averages of the four subsamples were used in the analysis. Statistical analyses were performed using SAS[43].

RESULTS

Lines included in the present study did not significantly differ in kernel texture as determined by the SKCS analysis ($p>0{\cdot}05$). This was expected because all lines have identical Chinese Spring chromosome 5D that carries the major gene responsible for variation in grain texture. The general average SKCS value of 50·4 obtained from these lines is in the limit between the medium hard and medium soft classes.

The SDS-PAGE separation of polymeric proteins from the 16 lines included in this study shows that *T. monococcum* accession G1777 has only one HM_r-GS (x-subunit) that has a higher mobility than the *Glu-A1* subunits 1 and 2* from the control varieties (Fig. 2, arrowhead). The absence of a *Glu-A1* band in the lines carrying the Chinese Spring allele correspond to the known presence of a null *Glu-A1* allele in Chinese Spring (Null, 7 + 8, 2 + 12). For the LM_r-GS *Glu-A3* locus a low mobility band is present in the B-subunits region of the SDS-PAGE in the lines carrying the Chinese Spring allele, but is absent in the lines carrying the *T. monococcum* LM_r-GS allele (Fig. 2, arrows). These last lines showed some LM_r-GS bands of higher intensity than the lines carrying the Chinese Spring low mobility band (Fig. 2).

Presence and absence of the HM_r-GS and LM_r-GS divide the 16 lines in four classes. These classes did not differ significantly in grain yield or 500-grain weight at both locations. Significant effects on protein content associated with these two loci were detected on the experiment performed in Argentina but not in the one performed in the U.S.A. Moreover, there was no significant correlation on protein content measures from the 16 lines between both locations, indicating that most of the observed variation was environmental. Therefore, total seed protein was used as a covariable in the three way factorial analyses of variance to adjust means from all parameters to a common level of protein.

No significant interactions were detected between locations and glutenin loci for any of the evaluated parameters indicating consistent effects at both locations. In addition, interactions between HM_r-GS and LM_r-GS were not significant for all the parameters analysed in this study (Table I). Since none of the interactions in the model was significant, only the main effects are discussed hereafter.

The percent of polymeric protein (peak 1, SE-HPLC) was significantly associated to the LM_r-GS locus, showing a reduction of 2·8% ($p<0{\cdot}0001$) for the *T. monococcum* allele relative to the *T. aestivum* allele (Table I). No significant differences in albumins and globulins (peak 3) were observed between LM_r-GS classes and, therefore, the association between these classes and the percent of monomeric proteins (peak 2) was complementary to the effect observed for polymeric proteins (Table I). Lines carrying the *T. monococcum* HM_r-GS allele showed a slightly higher percentage of polymeric protein (1%) than lines carrying the null *T. aestivum*

Table I Probability values (p) from the three way factorial analyses of covariance (location, HM_r-GS allele, LM_r-GS allele) using protein content as covariable. The difference between the *T. monococcum* and the *T. aestivum* allele is indicated for the significant effects as a percentage of the *T. aestivum* allele (% *T. m.*). HM_r-GS*LM_r-GS indicates interaction effects.

Variable	HM_r-GS		LM_r-GS		HM_r-GS*LM_r-GS	
	p	% *T. m.*	p	% *T. m.*	p	% *T. m.*
HPLC						
Peak 1	0·14	ns	<0·0001	−2·8%	0·40	ns
Peak 2	0·13	ns	<0·0001	+3·6%	0·31	ns
Peak 3	0·93	ns	0·55	ns	0·68	ns
BMQ						
SDS sedim. volume	0·004	+13·6%	0·03	−8·1%	0·76	ns
Mixing time	0·87	ns	0·29	ns	0·35	ns
Mixing tolerance	0·76	ns	0·22	ns	0·61	ns
Dough strength W	0·26	ns	0·01	−16·5%	0·71	ns
Alveograph P/L ratio	0·57	ns	0·57	ns	0·45	ns
CMQ						
Cookie diameter	0·02	−5·2%	0·07	ns	0·11	ns
Cookie quality	0·02	−6·8%	0·05	+5·9%	0·21	ns

ns = not significant.

allele at both locations, but the differences were not significant ($p = 0·14$).

For the BMQ predictive tests, the HM_r-GS and LM_r-GS loci were significantly associated to differences in SDS sedimentation volume and dough strength (alveograph W). No significant effects were detected for mixing time, mixing tolerance and alveograph P/L ratio (Table I).

The HM_r-GS allele from *T. monococcum* was associated to a significant 13·6% increase in SDS sedimentation volume ($p = 0·004$). Conversely, the presence of the LM_r-GS allele from the same species was associated with a significant 8·1% decrease in SDS sedimentation volume ($p = 0·03$). Presence of the LM_r-GS allele from *T. monococcum* was also associated to a significant decrease in dough strength (W), and with a reduction of 16·5% in the adjusted means compared to Chinese Spring ($p = 0·01$).

A second analysis of covariance was performed using total polymeric protein as covariable instead of total protein. This new covariable was calculated as the product between total protein content and the proportion of polymeric protein. The objective of this analysis was to determine if the significant effects observed for some of these glutenin loci were only the result of associated variation on the amount of polymeric protein (peak 1), or were also originated in intrinsic properties of the proteins from the different allelic classes. When SDS sedimentation values were adjusted by this second covariable, the 8·1% difference between the LM_r-GS alleles (Table I) was reduced to 4·9%. This difference was no longer significant ($p = 0·22$) suggesting that the significant association between SDS sedimentation volume and LM_r-GS alleles was partially explained by the association of these alleles with variation on the percent of polymeric protein. The effects of the LM_r-GS on dough strength (W) ($p = 0·01$ with 2nd covariable) and HM_r-GS on SDS sedimentation ($p = 0·009$ with 2nd covariable) were not significantly affected by the use of the modified covariable (Table I). This suggests that these effects are more related to the quality of the gluten proteins than to their effect on the amount of polymeric protein.

For the CMQ tests, HM_r-GS and LM_r-GS loci contributed to opposite effects on cookie diameter and cookie width, and therefore on cookie quality. The HM_r-GS allele from *T. monococcum* was significantly associated with a decrease of 6·8% of cookie quality, and the LM_r-GS allele with an increase of 5·9% in cookie quality (Table I).

When total polymeric protein content was used to replace protein content as covariable, a 7·9% increase in cookie quality adjusted means was associated with the presence of the *T. monococcum* LM_r-GS allele ($p = 0·02$). The *T. monococcum* HM_r-GS allele was associated with a significant decrease of 7·5% in cookie quality in this second analysis ($p = 0·02$). These results suggest that effects on CMQ are more related to qualitative differences

in the proteins determined by these alleles rather than to quantitative differences in polymeric protein.

DISCUSSION

The use of RSLs isogenic for all chromosome pairs except 1A increased the power of the genetic analyses, allowing the detection of significant effects for small differences in quality parameters. Allelic variation at chromosomes different than 1A that could have obscured associations between quality traits and storage protein alleles was eliminated in the RSL lines.

Four of the RSLs included in this study showed recombination between the LM_r-GS *Glu-A3* locus and the gliadin *Gli-A3* locus (Fig. 1, RSLs 5, 29, 38, 63). These recombinant plants were used to separate the effect of these two storage protein loci. No RSLs were available with recombination between the closely linked *Glu-A3* and *Gli-A1* gliadin locus and, therefore, it was not possible to rule out the possibility that the effects associated to *Glu-A3* were originated in allelic variation at the linked *Gli-A1* gliadin locus. However, all previous studies aimed to differentiate the effect of these two linked loci have shown that the allelic variation at the LM_r-GS is the responsible for the observed effects on gluten strength[9,16–19].

Interactions between HM_r-GS and LM_r-GS

HM_r-GS and LM_r-GS are aggregated as polymers in the gluten via intermolecular disulphide bonds. Differences in the amino acid sequence among subunits may determine differences in the molecular bindings that define the gluten network. Therefore, it is possible to speculate that there may be interactions between the HM_r-GS and LM_r-GS alleles in their effects on quality. Three previous studies reported significant interactions between HM_r-GS and LM_r-GS alleles on gluten strength, SDS sedimentation and maximum dough resistance[13–15]. In this study, however, no significant interactions were detected between these two loci for any of the studied parameters (Table I) suggesting additive effects. Similar additive effects on dough quality were found by other authors in studies in hexaploid[9,44] and tetraploid wheat[16] suggesting that interactions between HM_r-GS and LM_r-GS depend on the particular allele combinations segregating in each cross and on the quality parameters measured in each study.

Effects on BMQ predictive tests

Bread wheat cultivars carrying either of the active *Glu-A1* alleles 1 or 2* have stronger gluten and better BMQ than cultivars with the null allele. It was suggested that a quantitative increase in HM_r-GS in cultivars carrying the active *Glu-A1* alleles might account for the association of the active alleles with good BMQ[44,45]. The active HM_r-GS from *T. monococcum* accession G1777 was also associated with an increase in SDS sedimentation volume compared to the null allele of Chinese Spring. This effect was not modified by the introduction of the total polymeric protein content as covariable in the model, suggesting that this effect was not determined only by a quantitative increase in polymeric protein.

Additional experiments would be necessary to test if the *T. monococcum* G1777 HM_r-GS allele produces higher SDS sedimentation volume than bread wheat alleles 1 or 2*. Preliminary results using RSLs of cultivar Cheyenne 1A in Chinese Spring[31] and QTL analysis showed that Cheyenne allele 2* was associated with a 19% increase in SDS sedimentation volume[46] compared to the 13·6% increase observed here for the *T. monococcum* allele. This result suggests that the beneficial effect of the *T. monococcum* allele will not be superior to that of the active *T. aestivum* 2* allele.

The LM_r-GS allele from *T. monococcum* showed a negative effect on BMQ predictive tests, and its presence was associated to a decrease on SDS sedimentation volume and gluten strength. The effect of this allele on SDS sedimentation volume was mainly associated to its negative effect on the percentage of polymeric protein. On the other hand, the differences in dough strength (W index) were independent of the use of the total polymeric protein as covariable suggesting that different parameters are differentially affected by the quantitative variation in polymeric protein.

Effects on CMQ

Soft wheat flours with low protein content are preferred for pastry products. However, protein content and soft texture are not the only factors determining end-use properties. Protein quality can also influence the baking properties of soft

wheat flour[47–50]. Development of a strong gluten matrix is undesirable in cookie baking since this prevents cookie spread, as indicated by the negative correlation between cookie diameter and percentage of gluten in the protein[47].

The *T. monococcum* HM_r-GS allele, associated with an increase in SDS sedimentation volume, showed the expected negative effect on all cookie quality parameters when compared with the null *Glu-A1* allele of Chinese Spring. On the contrary, the LM_r-GS allele from *T. monococcum*, associated with weak gluten, showed a positive effect on the same parameters. This effect was not modified by the addition of the total polymeric protein content as covariable in the statistical analysis suggesting that it was determined by qualitative rather than quantitative differences in the polymeric protein between alleles. To our knowledge, this is one of the first reports on the effect of specific LM_r-GS alleles on CMQ.

The new *T. monococcum* LM_r-GS allele has potential value to be used in breeding programs for soft wheats. However, to minimise the amount of *T. monococcum* chromatin introgressed in commercial cultivars, it is important to select a small translocation as the initial source of the *T. monococcum* LM_r-GS allele because chromosome $1A^m$ from this species recombines poorly with bread wheat chromosome 1A[31]. Recombinant substitution line #38 (Fig. 1) containing only a small terminal *T. monococcum* $1A^mS$ segment would be an ideal source of the *T. monococcum* LM_r-GS allele. This recombinant chromosome 1A carries the Chinese Spring *Glu-A1* null allele, also associated to good CMQ. Selection for the *T. monococcum* LM_r-GS allele in crosses with no clear SDS-PAGE polymorphism can be done using LM_r-GS DNA clone pTdUCD1[51] and RFLPs that differentiates the *T. monococcum* and the *T. aestivum* alleles[31]. An alternative selection tool is the microsatellite marker located within the *Glu-A3* locus[52].

CONCLUSIONS

Genotypes containing the *T. monococcum* HM_r-GS allele and Chinese Spring LM_r-GS allele had the strongest gluten and the best response to BMQ predictive test. On the contrary, genotypes containing the *T. monococcum* LM_r-GS *Glu-A3* allele and Chinese Spring HM_r-GS *Glu-A1* allele had the best CMQ. This study suggests that diploid *T. monococcum* could be a valuable source of new allelic variation for storage proteins loci and quality characteristics.

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