Theor Appl Genet (1994) 88:81–88

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Effect of gliadins and HMW and LMW subunits of glutenin on dough properties in the F_6 recombinant inbred lines from a bread wheat cross

Received: 16 March 1993 / Accepted: 28 June 1993

Abstract The storage proteins of 64 F_2-derived F_6 recombinant inbred lines (RILs) from the bread wheat cross 'Prinqual'/'Marengo' were analyzed. Parents differed at four loci: *Gli-B1* (coding for gliadins), *Glu-B1* (coding for HMW glutenin subunits), *Glu-A3/Gli-A1* (coding for LMW glutenin subunits/gliadins) and *Glu-D3* (coding for LMW glutenin subunits). The effect of allelic variation at these loci on tenacity, extensibility and dough strength as measured by the Chopin alveograph was determined. Allelic differences at the *Glu-B1* locus had a significant effect on only tenacity. None of the allelic differences at either the *Glu-A3/Gli-A1* or *Glu-D3* loci had a significant effect on quality criteria. Allelic variation at the *Gli-B1* locus significantly affected all of the dough properties. Epistatic effects between some of the loci considered contributed significantly to the variation in dough quality. Additive and epistatic effects each accounted for 15% of the variation in tenacity. Epistasis accounted for 15% of the variation in extensibility, whereas additive effects accounted for 4%. Epistasis accounted for 14% of the variation in dough strength, and additivity for 9%. The relative importance of epistatic effects suggest that they should be included in predictive models when breeding for breadmaking quality.

Key words Bread wheat · Gliadins · HMW and LMW subunits of glutenin · Dough properties

Introduction

Gluten proteins confer the rheological characteristics to the wheat dough (Wall 1979). Protein quality differences between varieties are considered to be caused mainly by different combinations of endosperm storage protein variants (Payne et al. 1984). These proteins consist of two major fractions: gliadins and glutenins. Gliadins are monomeric proteins and, when fractionated by gel electrophoresis at low pH, they separate into four groups, alpha-, beta-, gamma- and omega-gliadins (Woychik et al. 1961). Glutenins are multimeric aggregates of high-molecular-weight (HMW, Payne et al. 1981) and low-molecular-weight (LMW, zones B and C, Jackson et al. 1983) subunits held together by disulphide bonds. There is a wide variation among varieties in the electrophoretic patterns of gliadins (Bushuk and Zillman 1978), HMW subunits of glutenin (Payne et al. 1981) and LMW subunits of glutenin (Gupta and Shepherd 1990).

Omega- and gamma-gliadins are coded for by genes at the *Gli-1* loci located on the short arms of group 1 chromosomes (Payne et al. 1982). LMW glutenins are coded for by genes at the *Glu-3* loci (Singh and Shepherd 1988) that are very closely linked to those at the *Gli-1* loci (Payne et al. 1984; Pogna et al. 1990; Singh and Shepherd 1988). HMW glutenin subunits are coded by genes at the *Glu-1* loci found on the long arms of group 1 chromosomes (Bietz et al. 1975; Lawrence and Shepherd 1981).

Varying degrees of relationships have been reported to exist between types of gliadin components and flour quality (Sozinov and Poperelya 1980). Associations between HMW glutenin subunits and different flour quality criteria have been established (Branlard and Dardevet 1985a; Moonen et al. 1982; Payne et al. 1981). The association between some LMW glutenin subunits and quality characteristics has been recently studied (Gupta 1987; Gupta et al. 1991; Gupta and Shepherd 1988).

Combined studies of HMW glutenin subunits and gliadin composition in different wheat cultivars and progenies have revealed that their relative influence on dough properties varies (Branlard and Dardevet 1985b; Lagudah et al. 1988; Payne et al. 1987a). However, Payne et al. (1987) suggested that the effect of gliadins on dough quality should be attributed to the LMW glutenin subunits associated with them. Subsequently, Gupta and Shepherd (1988) found an additive effect of certain HMW and LMW glutenin subunits on dough resistance.

Each of the gluten protein fractions may contribute to the end-use quality of the wheat. However, only a few combined studies of the three groups of proteins have been reported (Khelifi and Branlard 1992; Payne et al. 1987a). The objectives

Communicated by F. Salamini

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of the study reported here were: (1) to analyze allelic variation at the *Glu-1*, *Glu-3* and *Gli-1* loci in F_6 recombinant inbred lines (RILs) from a cross between two bread wheat cultivars; and (2) to determine the effect of allelic variation at these loci and their interactions on the Chopin alveograph dough properties of the F_6 lines.

Materials and methods

Plant material

The intervarietal group 1 chromosome substitution lines of 'Prinqual' and 'Cappelle' in 'Courtot' were from the INRA Plant Breeding Station of Clermont-Ferrand, France. Those lines were analyzed to determine the chromosomal control of the LMW subunits of glutenin and gliadins in these cultivars.

The experimental material consisted of 104 F_2-derived F_6 RILs from the bread wheat cross 'Prinqual'/'Marengo'. 'Prinqual' is a spring bread wheat of good breadmaking quality from the USA. 'Marengo' is a winter wheat of medium breadmaking quality from France. These RILs were obtained by the selection of random F_2 plants and, thereafter, advanced to the F_6 generation by the pedigree method without selection.

Field experiment

The F_6 seeds from each F_5 row were divided into two sets and sown in a replicated randomized complete block design trial at Clermont-Ferrand in 1989–1990. Each block consisted of two rows 1.5 m long and 25 cm between rows, with 30 grains per row. Parents were included in the trial. Plants were grown under normal field conditions with fungicide application.

Alveograph test

Grains from each entry, at both replications, were milled in a Chopin-Dubois mill, and 100 g of the flour was used for a replicated micro-alveograph test (50 g per test). This test allows measurement of the rheological characteristics of tenacity (T, in mbar), extensibility (L, in mm) and strength (W, in J^{-4}) of the dough.

Electrophoresis

The proteins of 20-mg flour samples were extracted using the sequential extraction method of Marchylo et al. (1989) as modified by Singh et al. (1991). Reduced proteins (HMW and LMW subunits of glutenin) were fractionated in 12% acrylamide gels 1 mm thick, in the discontinuous system of Laemmli (1970) as modified by Payne et al. (1980). The unreduced extracts were fractionated in 10% acrylamide gels 1.5 mm thick using the same method. Electrophoresis was performed for the 12% gels at a constant current of 30 mA/gel at 15 °C for 4 h and 30 min and for the 10% gels at 40 mA/gel for 3 h and 30 min at the same temperature. The gels were stained with Coomasie Blue R and destained first with water and then with 6% trichloroacetic acid. The nomenclature given here for LMW glutenin and gliadin alleles is related to the cultivars used in this study.

Results and Discussion

Protein characterization of parents and F_6 RILs

Table 1 summarizes the allelic differences found among the parents 'Prinqual' and 'Marengo' for HMW glutenin subunits, LMW glutenin subunits and gliadins. The HMW glutenin phenotypes of the parents are shown in Figs. 1A and 2A. Both parents possessed the *Glu-A1b* and *Glu-D1d* alleles and differed at the *Glu-B1* locus. 'Prinqual' (Fig. 1A: b, f; Fig. 2A: b, h) had the *Glu-B1i* allele (bands 17 + 18) and 'Marengo' (Fig. 1A: i; Fig. 2A: a, g) the *Glu-B1b* allele (bands 7 + 8).

Table 1 Allelic differences between parents for the gluten proteins and location of the genes controlling them (Symbols for the *Glu-1* and *Gli-1* loci are according to Payne and Lawrence (1983). Symbols for the *Glu-3* loci are according to Singh and Shepherd (1988). Symbols for *Gli-1* and *Glu-3* alleles are new)

Protein type	Locus	Allelic differences in parents	
		Prinqual	Marengo
HMW glutenin	*Glu-B1*	*Glu-B1i*	*Glu-B1b*
LMW glutenin	*Glu-A3*	*Glu-A3p*	*Glu-A3m*
	Glu-D3	*Glu-D3p*	*Glu-D3m*
Gliadins	*Gli-A1*	*Gli-A1p*	*Gli-A1m*
	Gli-B1	*Gli-B1p*	*Gli-B1m*

The B-zone LMW glutenin phenotypes of the parents are shown in Figs. 1A and 2A. 'Prinqual' possessed five bands of different mobility (Fig. 1A: b, f; Fig. 2A: b, h) and 'Marengo', four (Fig. 1A: i; Fig. 2A: a, g). The three faster bands had equal mobilities in both parents and were considered to be the same. The two bands of lower mobility in 'Prinqual' (arrowed in Fig. 1A: f) are coded for by chromosome 1D, as can be seen in the 'Courtot' ('Prinqual 1D') substitution line (Fig. 1A: g), and will subsequently be considered to indicate the presence of the *Glu-D3p* allele. The slower band of 'Marengo' (arrowed in Fig. 1A: i) had the same mobility as the second slower band of 'Courtot' (Fig. 1A: h), and they were considered to be the same. This second band of 'Courtot' is coded for by the 1A chromosome, as can be seen by the 'Courtot' ('Prinqual 1A') substitution line (Fig. 1A: c), and will subsequently be considered to indicate the presence of the *Glu-A3m* allele. Since the 'Courtot' ('Prinqual 1A') substitution line did not show any extra band, 'Prinqual' was classified as null type and the allele will be named *Glu-A3p*. 'Marengo' did not possess an alternative band to *Glu-A3p*, so it was deduced that possessed a different allele, named *Glu-D3m*.

Figure 1B shows the unreduced protein patterns of the parents. 'Marengo' possesses a low mobility band (arrowed lane j) that is not present in 'Prinqual' (lanes b, f, i). This omega-gliadin and the slowest band of 'Cappelle' (lanes k, p) had the same mobility and were therefore, considered to be the same protein. The 'Courtot' ('Cappelle 1B') substitution line (lane n) shows that this protein is coded for by chromosome 1B, and the allele will be named *Gli-B1m*. 'Prinqual' possesses four bands coded for by chromosome 1B, as is shown by the 'Courtot' ('Prinqual 1B') substitution line (lane e). The slowest band (arrowed lane i) had the same mobility as the second slowest band of 'Marengo', and therefore they were considered to be the same protein. The absence of the slowest band of 'Marengo' was considered to indicate the presence of the *Gli-B1p* allele. The presence of the other three bands (marked

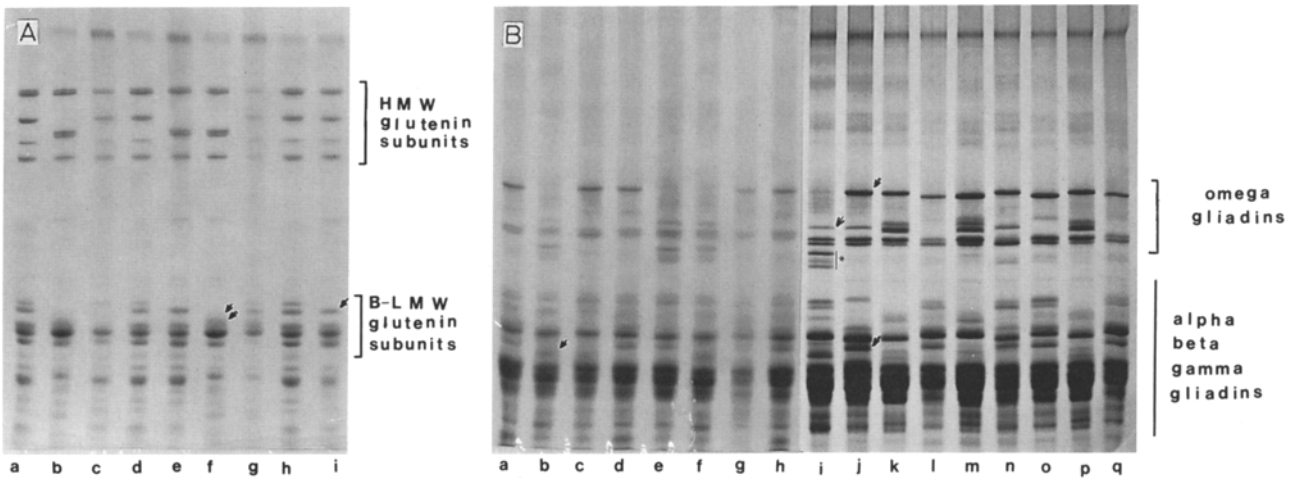

Fig. 1 A, B SDS-PAGE Patterns of **A** HMW and LMW glutenin subunits from cvs 'Courtot' (*a, d, h*), 'Prinqual' (*b, f*) and 'Marengo' (*i*) and the substituted lines 'Courtot' ('Prinqual 1A') (*c*), 'Courtot' ('Prinqual 1B') (*e*) and 'Courtot' ('Prinqual 1D') (*g*); and **B** gliadins from cvs 'Curtot' (*a, d, h, l, q*), 'Prinqual (*b, f, i*), 'Marengo' (*j*) and 'Cappelle' (*k, p*) and the substituted lines 'Courtot' ('Prinqual 1A') (*c*), 'Courtot' ('Prinqual 1B') (*e*), 'Courtot' ('Prinqual 1D') (*g*), 'Courtot' ('Cappelle 1A') (*m*), 'Courtot' ('Cappelle 1B') (*n*) and 'Courtot' ('Cappelle 1D') (*o*). B-zone LMW glutenin band and gliadin differences between 'Prinqual' and 'Marengo' are denoted by *arrows*. * indicates unidentified omega-gliadins (see text)

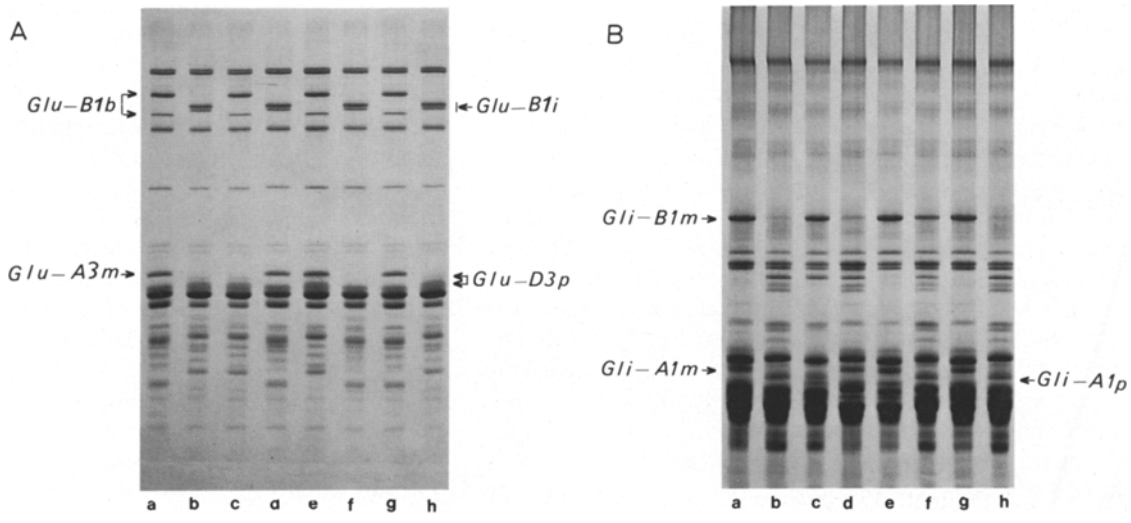

Fig. 2 SDS-PAGE patterns of **A** glutenin subunits and **B** gliadins of the parents 'Prinqual' (*b, h*) and 'Marengo' (*a, g*) and the F_6 RILs showing the four main phenotypic classes (*c, d, e, f*). Arrows *indicate* the subunits used in the identification of the alleles considered in this study

by * in lane i) will be discussed later. Another gliadin band (arrowed in lane b) was detected in 'Prinqual' and found, from the 'Courtot' ('Prinqual 1A') substitution line (lane c), to be coded for by chromosome 1A. This band is not present in 'Marengo', and the allele will be named *Gli-A1p*.

In summary, the main protein differences between 'Prinqual' and 'Marengo' arise from allelic variation at five loci: *Glu-B1*, *Glu-A3*, *Glu-D3*, *Gli-A1* and *Gli-B1*.

The protein composition of all 104 F_6 RILs at both replications was determined, and the protein phenotypes of each duplicated line compared. Those lines that were different at both replications, or out of type, were eliminated. The few segregating lines detected were also eliminated. Sixty-four F_6 duplicated lines were retained for further analysis.

Table 2 presents the occurrence of the protein alleles at the *Glu-B1*, *Glu-A3*, *Glu-D3*, *Gli-A1* and *Gli-B1* loci among the 64 F_6 RILs analyzed. At the *Glu-B1* locus, the two parental types, *Glu-Bli* and *Glu-B1b*, were found (Fig. 2A, lanes c, d, e, f), with more lines possessing the *Glu-B1b* allele from 'Marengo' (69%). Figure 2A shows the four B-zone LMW glutenin phenotypes found among the F_6 RILs: the two parental types (*Glu-A3p*, *Glu-D3p*, lane c, and *Glu-A3m*, *Glu-D3m*, lane d) and the two recombinant types (*Glu-A3m*, *Glu-D3p*, lane e, and *Glu-A3p*, *Glu-D3m*, lane f), with frequencies of 20%, 36%, 27% and 17%, respectively. At the *Gli-B1* locus both parental types were detected among the F_6 RILs (Fig. 2B: c, d, e, f), with a higher proportion of lines possessing the *Gli-B1m* allele of 'Marengo' (64%).

Table 2 Occurrence of protein alleles in the F_6 RILs of the 'Prinqual'/'Marengo' cross and goodness of fit for the expected proportions

Locus	Allele	Number of RILs	%	*P*
Glu-B1	*Glu-B1i*	20	31	0.01–0.001[a]
	Glu-B1b	44	69	
Glu-A3	*Glu-A3p*	24	37	0.05–0.01[a,b]
	Glu-A3m	40	63	
Glu-D3	*Glu-D3p*	30	47	0.50–0.10[c]
	Glu-D3m	34	53	
Gli-A1	*Gli-A1p*	24	37	0.05–0.01[a]
	Gli-A1m	40	63	
Gli-B1	*Gli-B1p*	23	36	0.05–0.01[d]
	Gli-B1m	41	64	

[a] Expected ratio 32:32
[b] The linked gliadins at the *Gli-A1* locus were used to verify homozygosity
[c] Expected ratio 33:31
[d] Expected ratio 31:33

The three 1B-encoded gliadins of 'Prinqual' (marked by * in lane i) were studied in the F_6 RILs. They will be named w1, w2 and w3 according to their increasing mobility. Twenty-seven lines possessed bands w1w2w3; 17, no bands; 14, w1 alone; 6, w2w3. This result suggested the presence of two linked loci. The joint segregation of w1 and of w2w3 with the 1B-encoded gliadin of 'Marengo' (*Gli-B1m* allele) was studied. Thirty-eight parental types and 26 recombinant types were found in the first case and 54 parental types and 10 recombinant types in the second one. These results suggested the presence of three linked loci, with the probable implication of the *Gli-B3* and *Gli-B4* loci (Galili and Feldman 1984; Radaelli et al. 1992; Dachkevitch et al. 1993). Because of the uncertain knowledge of these three proteins, they were excluded for further analysis. A study is in progress to determine their nature and chromosomal location.

The *Gli-A1p* allele was always associated to the absence of the *Glu-A3m* allele, and the presence of the *Glu-A3m* allele was always associated to the presence of the gliadin band arrowed in Fig. 1B, lane j from 'Marengo'. Consequently, it was deduced that this band is coded for by the *Gli-A1* locus (*Gli-A1m* allele, Fig. 2B, lane a). Moreover, these observations indicated a complete or close linkage between the *Glu-A3* and *Gli-A1* loci, as reported by Singh and Shepherd (1988). Since no recombination was detected between the alleles at these loci we will refer to it as the complex locus *Glu-A3/Gli-A1*. The allelic variation at the *Gli-A1* locus was used to verify homozygosity at the *Glu-A3* locus.

The frequencies of the alleles at the *Glu-B1*, *Glu-A3/Gli-A1* and *Gli-B1* loci among the F_6 RILs were significantly different from the expected ones (Table 2). Sozinov and Poperelya (1980) suggested the association between some gliadin components coded for by the homoeologous group 1 chromosomes and low-temperature tolerance. In the cross analyzed here, 'Prinqual' is a cold-sensitive wheat, and elimination through generations of cold-sensitive lines probably reduced the presence of the *Glu-A3/Gli-A1* and *Gli-B1* alleles from 'Prinqual': the *Glu-D3* locus was not involved. The low proportion of the *Glu-B1i* allele from 'Prinqual' could also be due to an association between this allele and cold-sensitivity, but this hypothesis requires confirmation.

Dough characteristics of parents and F_6 RILs

Dough tenacity was 106 mbar and 79 mbar for the 'Prinqual' and 'Marengo' parents, respectively, while the range observed in the F_6 RILS was 56-154 mbar. The mean value was 85 mbar, the frequency distribution being slightly biased towards the lowest values. Dough extensibility was 140 mm and 174 mm for 'Prinqual' and 'Marengo', respectively. The range observed in the F_6 RILs was 98–229 mm, with a mean value of 163 mm. The frequency distribution was slightly biased towards the highest values. Dough strength was 446 J^{-4} and 357 J^{-4} for 'Prinqual' and 'Marengo', respectively. The range observed in the F_6 RILs was 209–545 J^{-4} with a mean value of 332 J^{-4}, which was lower than the lowest parent. Frequency distribution was slightly biased towards the lowest values. In all cases, the bias of the distribution of the F_6 RILs was towards the 'Marengo' values, probably due, as has been suggested for the allele frequencies, to natural elimination, through generations, of low-temperature-sensitive types.

Effect of allelic variation in protein bands on dough properties

The results of the analysis of variance for the dough characteristics of the F_6 RILs are presented in Table 3. Parents were excluded from the analysis because it was considered that the only valid comparisons were those within a common background. Table 3 also includes the sum of squares as a percentage of the total sum of squares, and these can be interpreted as indications of the relevance of the various terms. It should be noted that heterozygous type at the *Glu-D3* and *Gli-B1* loci could not be differentiated from the homozygous types *Glu-D3p* and *Gli-B1m*. However, the low level of heterozygosity expected in the F_6 generation suggested that results would not be biased due to this fact.

Allelic variation at the *Glu-B1* locus had only a significant effect ($P < 0.05$) on dough tenacity, and accounted for 4% of the variation between the F_6 RILs. Lines possessing the *Glu-B1b* allele had a significant higher dough tenacity ($P < 0.05$) than those possessing the *Glu-B1i* allele. Controversial results have been reported about the influence of these alleles on dough characteristics. Lagudah et al. (1988) found no differences in maximum resistance between F_3 lines possessing bands 7 + 8 or 17 + 18, whereas Branlard and Dardevet (1985a) found a significant positive effect of bands 17 + 18 on dough extensibility. However, Lawrence et al. (1987) and Payne (1986) found no differences in dough quality between these alleles.

Allelic variations at both the *Glu-A3/Gli-A1* and *Glu-D3* loci had no significant influence on dough characteristics.

Table 3 Analysis of the variance of dough characteristics of the F_6 RILs of the 'Prinqual'/'Marengo' cross [mean squares (MS) and sum of squares as percentage of the total sum of squares (%)] (*T* Tenacity, *L* extensibility, *W* strength)

Source	*df*	T		L		W	
		MS	%	MS	%	MS	%
Replication	1	1	0	405	0	288	0
Glu-B1	1	1256*	4	1677	1	11526	2
Glu-A3/Gli-A1	1	195	1	456	0	5402	1
Glu-D3	1	3	0	210	0	3651	1
Gli-B1	1	3607***	10	4207*	3	35825**	5
*Glu-B1*Glu-A3/Gli-A1*	1	424	1	3637*	3	54227***	8
*Glu-B1*Glu-D3*	1	418	1	1501	1	25470*	3
*Glu-B1*Gli-B1*	1	790	2	431	0	7292	1
*Glu-A3/Gli-A1*Glu-D3*	1	3	0	81	0	9	0
*Glu-A3/Gli-A1*Gli-B1*	1	3765***	11	13223***	10	5134	1
*Glu-D3*Gli-B1*	1	36	0	743	1	13217	1
Error	116	207		913		4779	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Payne et al. (1987a) found a significant negative effect of the null allele at the *Glu-A3* locus on dough quality. Gupta et al. (1989) found a significant negative effect of the null type *Glu-A3e* from the bread wheat variety 'Kite' on dough quality. The negative effect of the null type *Glu-A3p* could not be confirmed in the progeny analyzed here (Tables 4, 5, 6). Singh et al. (1991) proposed the LMW glutenin subunits useful in identifying individual *Glu-A3* alleles as classified by Gupta and Shepherd (1990). The four bands they demonstrated lie in the slower moving B-zone, and their absence indicated the presence of the null allele. Although we made no attempt to apply the allele classification of Gupta and Shepherd (1990), the null type *Glu-A3p* found here could correspond to the *Glu-A3e* of Gupta et al. (1989).

The allelic variation observed at the *Gli-B1* locus had a significant effect on all three rheological parameters, T ($P < 0.001$), L ($P < 0.05$) and W ($P < 0.01$). This allelic variation accounted for 10%, 3% and 5% of the total variation on dough tenacity, extensibility and strength, respectively. Comparison of the means shows that for dough tenacity (Table 4) and strength (Table 6) lines possessing the *Gli-B1m* allele had a significant lower value ($P < 0.001$ and $P < 0.01$, respectively) than those with the *Gli-B1p* allele. These results are in agreement with those of Sozinov and Poperelya (1980) who found a high influence of gliadin alleles on quality; but they differed from those of Branlard and Dardevet (1985b), who found no correlation between gliadins and tenacity, and from those of Lagudah et al. (1988), who found that gliadins had no effect on dough characteristics. On the other hand, Payne et al. (1987a) found that allelic variation at the *Gli-A1* locus had a high effect on rheological measurements in the 'CS × Cs' ('Hope 1A') F_5 progeny. However, they attributed this effect to the LMW subunits of glutenin associated to the gliadins. Gupta and Shepherd (1988) and Gupta et al. (1989) found that allelic

Table 4 Mean differences in tenacity (T) associated with the allelic variation at the loci considered and to the allelic variation at one locus in the F_6 RILs possessing a common allele at another locus

Common allele	Alleles compared			Number		T		*t*
	X		Y	X	Y	X	Y	
	Glu-B1i	vs	*Glu-B1b*	20	44	80 ± 11	87 ± 17	*
	Glu-A3p[a]	vs	*Glu-A3m*[a]	24	40	83 ± 12	86 ± 17	ns
	Glu-D3p	vs	*Glu-D3m*	30	34	86 ± 13	84 ± 17	ns
	Gli-B1p	vs	*Gli-B1m*	23	41	91 ± 21	81 ± 10	***
Glu-B1i				9	11	83 ± 12	78 ± 10	ns
	Glu-A3p[a]	vs	*Glu-A3m*[a]					
Glu-B1b				15	29	83 ± 13	89 ± 19	ns
Glu-B1i				4	16	78 ± 9	81 ± 12	ns
	Glu-D3p	vs	*Glu-D3m*					
Glu-B1b				26	18	87 ± 14	87 ± 21	ns
Glu-B1i				8	12	82 ± 14	79 ± 10	ns
	Gli-B1p	vs	*Gli-B1m*					
Glu-B1b				15	29	96 ± 24	82 ± 10	***
Glu-A3p[a]				13	11	82 ± 11	84 ± 13	ns
	Glu-D3p	vs	*Glu-D3m*					
Glu-A3m[a]				17	23	89 ± 14	84 ± 19	ns
Glu-A3p[a]				12	12	81 ± 12	85 ± 12	ns
	Gli-B1p	vs	*Gli-B1m*					
Glu-A3m[a]				11	29	103 ± 24	80 ± 9	***
Glu-D3p				12	18	91 ± 17	83 ± 9	*
	Gli-B1p	vs	*Gli-B1m*					
Glu-D3m				11	23	92 ± 26	80 ± 10	*

* $P < 0.01$; *** $P < 0.001$; ns, not significant

[a] *Glu-A3* represents the complex locus *Glu-A3/Gli-A1*

Table 5 Mean differences in extensibility (L) associated with the allelic variation at the loci considered and to the allelic variation at one locus in the F_6 RILs possessing a common allele at another locus

Common allele	Alleles compared			Number		T		*t*
	X		Y	X	Y	X	Y	
	Glu-B1i	vs	*Glu-B1b*	20	44	168 ± 27	160 ± 30	ns
	Glu-A3p[a]	vs	*Glu-A3m*[a]	24	40	166 ± 29	161 ± 63	ns
	Glu-D3p[a]	vs	*Glu-D3m*	30	34	163 ± 32	163 ± 27	ns
	Gli-B1p	vs	*Gli-B1m*	23	41	156 ± 35	167 ± 25	ns
Glu-B1i				9	11	177 ± 21	161 ± 29	ns
	Glu-A3p[a]	vs	*Glu-A3m*[a]					
Glu-B1b				15	29	159 ± 31	161 ± 30	ns
Glu-B1i				4	16	162 ± 22	170 ± 28	ns
	Glu-D3p	vs	*Glu-D3m*					
Glu-B1b				26	18	163 ± 33	156 ± 26	ns
Glu-B1i				8	12	169 ± 23	168 ± 30	ns
	Gli-B1p	vs	*Gli-B1m*					
Glu-B1b				15	29	150 ± 40	166 ± 23	*
Glu-A3p[a]				13	11	163 ± 31	169 ± 27	ns
	Glu-D3p	vs	*Glu-D3m*					
Glu-A3m[a]				17	23	163 ± 33	160 ± 28	ns
Glu-A3p[a]				12	12	173 ± 33	159 ± 23	ns
	Gli-B1p	vs	*Gli-B1m*					
Glu-A3m[a]				11	29	138 ± 29	170 ± 25	***
Glu-D3p				12	18	155 ± 43	168 ± 21	ns
	Gli-B1p	vs	*Gli-B1m*					
Glu-D3m				11	23	158 ± 26	165 ± 28	ns

* $P < 0.05$; *** $P < 0.001$; ns, not significant
[a] *Glu-A3* represents the complex locus *Glu-A3/Gli-A1*

Table 6. Mean differences in dough strength (W) associated with the allelic variation at the loci considered and to the allelic variation at one locus in the F_6 RILs possessing a common allele at another locus

Common allele	Alleles compared			Number		W		*t*
	X		Y	X	Y	X	Y	
	Glu-B1i	vs	*Glu-B1b*	20	44	318 ± 60	338 ± 74	ns
	Glu-A3p[a]	vs	*Glu-A3m*[a]	24	40	161 ± 63	337 ± 74	ns
	Glu-D3p	vs	*Glu-D3m*	30	34	340 ± 63	325 ± 76	ns
	Gli-B1p	vs	*Gli-B1m*	23	41	351 ± 84	321 ± 58	**
Glu-B1i				9	11	348 ± 62	292 ± 46	**
	Glu-A3p[a]	vs	*Glu-A3m*[a]					
Glu-B1b				15	29	306 ± 61	355 ± 75	**
Glu-B1i				4	16	303 ± 38	321 ± 64	ns
	Glu-D3p	vs	*Glu-D3m*					
Glu-B1b				26	18	345 ± 64	328 ± 86	ns
Glu-B1i				8	12	331 ± 67	309 ± 55	ns
	Gli-B1p	vs	*Gli-B1m*					
Glu-B1b				15	29	362 ± 92	326 ± 60	*
Glu-A3p[a]				13	11	315 ± 59	330 ± 71	ns
	Glu-D3p	vs	*Glu-D3m*					
Glu-A3m[a]				17	23	358 ± 61	322 ± 79	*
Glu-A3p[a]				12	12	329 ± 71	315 ± 58	ns
	Gli-B1p	vs	*Gli-B1m*					
Glu-A3m[a]				11	29	376 ± 94	323 ± 60	**
Glu-D3p				12	18	345 ± 69	366 ± 60	ns
	Gli-B1p	vs	*Gli-B1m*					
Glu-D3m				11	23	358 ± 101	309 ± 56	*

* $P < 0.05$; ** $P < 0.01$; ns, not significant
[a] *Glu-A3* represents the complex locus *Glu-A3/Gli-A1*

variation at the *Glu-A3* locus was associated with significant differences in dough extensibility. Our results here show that while there was no significant effect of individual allelic variation at the *Glu-A3/Gli-A1* and *Glu-D3* loci, there was a significant effect at the *Gli-B1* locus. Since no allelic variation was detected for the 1B-encoded LMW glutenin bands, the significant effect of allelic variation at the *Gli-B1* locus on dough quality should be due to non-detectable differences in LMW glutenin composition or to variation in gliadin composition.

The effect of allelic variation at the loci considered on rheological differences between the F_6 RILs also depended on

the interactions between alleles at some of those loci. Thus, interactions between alleles at the *Glu-B1* and *Glu-A3/Gli-A1* loci contributed significantly to the variation in the extensibility ($P < 0.05$) and the strength ($P < 0.001$) of the dough. These interactions accounted for 3% of the extensibility and 8% of the dough strength variation. A comparison of means shows that for dough strength (Table 6) lines with the null phenotype *Glu-A3p* were superior to those possessing the *Glu-A3/Gli-A1m* allele only when the *Glu-B1i* allele was present ($P < 0.01$); the contrary occurred when the *Glu-B1b* allele was present ($P < 0.01$). The interaction between alleles at the *Glu-B1* and *Glu-D3* loci was only significant ($P < 0.05$) for dough strength and accounted for 3% of the variability between lines. Payne et al. (1987a) found an additive effect of allelic variation at the *Glu-A1* and *Glu-A3* loci on the sedimentation test, rheological measurements and loaf volume in a F_5 population from the 'CS/CS' ('Hope 1A') cross. Gupta et al. (1989) also found a cumulative effect of allelic variation at the same loci on dough resistance and extensibility in a F_6 population from a bread wheat cross, the interactions not being significant. Our results here show that variation in extensibility and dough strength did not depend on additive effects of allelic variations in LMW/gliadins and HMW glutenin subunits only and that epistasis had a significant effect.

The interaction between alleles at the *Glu-B1* and *Gli-B1* loci was not significant for all of the three quality traits considered. The comparison of means shows that the *Gli-B1p* allele was significantly superior to *Gli-B1m* for tenacity ($P < 0.001$, Table 4) and strength ($P < 0.05$, Table 6) and significantly inferior for extensibility ($P < 0.05$) only when the *Glu-B1b* allele was present. This result was consistent with that of Lagudah et al. (1988), who found a non-significant interaction between the HMW glutenin subunits and the gliadins they analyzed. Branlard and Dardevet (1985b) found that HMW glutenin subunits and gliadins interact in the expression of extensibility and strength of the dough. Our results here suggest that the interaction they found may also be due to the LMW glutenin subunits associated with gliadins.

The interaction between alleles at the *Glu-A3/Gli-A1* and *Gli-B1* loci contributed significantly ($P < 0.001$) to the variation on dough tenacity (11%) and extensibility (10%). With respect to tenacity, the comparison of means shows (Table 4) that the *Gli-B1p* allele was superior to *Gli-B1m* ($P < 0.001$) when the *Glu-A3m* allele was present. On the contrary, for extensibility (Table 5) lines possessing the *Gli-B1m* allele were superior to those with the *Gli-B1p*) allele ($P < 0.001$) in the presence, as for tenacity, of the *Glu-A3m* allele.

Our results show that both additive and epistatic effects between some of the *Glu-B1*, *Glu-A3/Gli-A1*, *Glu-D3* and *Gli-B1* loci had a significant effect on the dough characteristics of the F_6 RILs analyzed. Additive effects at the *Glu-B1* locus accounted for 4% of the total variability on tenacity and those at the *Gli-B1* locus explained 10% of the variability on tenacity, 3% on extensibility and 5% on strength of the dough. Epistatic effects between the *Glu-B1* and *Glu-A3/Gli-A1* loci explained 3% of the variability on extensibility and 8% on strength of the dough. The *Glu-B1*Glu-D3* interaction explained 3% of the variability on dough strength and that between the *Glu-A3/Gli-A1* and *Gli-B1* loci accounted for 11% of the variability on tenacity and 10% on extensibility. In total, additive and epistatic effect each accounted for 15% of the variation in tenacity. With respect to extensibility epistasis accounted for 15% of the variability, whereas additive effects accounted for 4%. For dough strength epistasis accounted for 14% of the variability and additivity for 9%.

The percentage of explanation of the total variability on dough characteristics due to the allelic variation at the four loci considered was low: 30% for tenacity, 19% for extensibility and 23% for dough strength. Other factors, such as different proteins than those analyzed here, protein content or lipids, could affect the expression of the quality parameters considered.

Epistatic effects have been reported in the analysis of the effect of HMW glutenin alleles on breadmaking quality by means of different genetical material. Carrillo et al. (1990) and Rousset et al. (1992) used F_2-derived F_8 lines from one cross, Kolster et al. (1991) analyzed breeding lines, and Payne et al. (1987b) worked with near-isogenic substitution lines. All of these researchers concluded that epistasic effects between alleles at the *Glu-1* loci had an important effect on the quality properties of the dough. The interaction between two non-homeologous loci, *Glu-A1* and *Glu-A3* has been studied by Gupta et al. (1989) in an F_6 population, and they found that it was not significant. Khelifi and Branlard (1992) studied two F_4 progenies, and they concluded that there were additive effects of the *Glu-3* and *Gli-1* alleles with those of *Glu-1*, but their data suggest more epistatic than additive effects. The results obtained in this study shows that epistasis between alleles at some of the loci considered had a significant effect on dough characteristics. Nevertheless, it should be noted that interactions found here might be specific for this cross because their effect on quality criteria can be affected by the genetical background. On the other hand, the variability found among the F_6 RILs was relatively low because parents used had medium to high dough characteristics. More studies should be done in order to quantify the relevance of epistasis in the expression of quality. The relative importance of epistatic effects found here suggests that they should be included in predictive models when breeding for breadmaking quality. Thus, when establishing the contribution of an allele in breeding for quality, one should take into account interactions with alleles at other loci as these might affect the phenotypic expression of the allele being considered on the quality of the dough.

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