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Comparison of low- and high molecular-weight wheat glutenin allele effects on flour quality

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Abstract Five crosses were made, using a set of New Zealand wheat cultivars, to measure the effect of glutenin allele differences on baking quality parameters. The alleles involved were: *Glu-A1* (2*, 1 and n), *Glu-D1* (5+10, 2+12), *Glu-A3* (c, d and e), *Glu-B3* (Sec-12, Sec-13, b and g), *Glu-D3* (a and b). The allelic variation of F_3 individual plants was identified by SDS-PAGE, and plants with the same HMW-GS and LMW-GS patterns were grouped. Quality parameters were then measured on the grouped F_4 bulks. Quality parameters measured for this study were wholemeal flour protein content (WFP), grain hardness (HAR), SDS sedimentation volume (SED), Pelshenke time (PEL), mid-line peak value (MPV) and the mid-line peak time (MPT) of a mixograph. The results showed there were significant quality differences within most populations associated with the possession of a particular allele, reaching magnitudes of up to 42% for the range between populations. Most glutenin allelic comparisons showed significant differences for at least one of the resultant measured quality parameters. Allelic differences of *Glu-A1* significantly influenced all characters except MPT, with the null allele apparently inferior; possession of 5+10 at *Glu-D1* significantly increased Pelshenke time and SED volumes relative to allele 2+12; WFP, SED and MPV were significantly affected by the *Glu-A3* alleles tested. *Glu-B3* alleles significantly affected all characters except hardness and the *Glu-D3* alleles tested significantly affected all characters other than hardness and SDS sedimentation volume.

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Introduction

Two major classes of glutenin polypeptides have been identified in wheat endosperm, designated as HMW-GS and LMW-GS; both classes occur in flour as cross-linked proteins resulting from inter-polypeptide disulphide linkages. The genes coding for HMW-GS subunits are located on the long arms of chromosomes 1A, 1B and 1D at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci respectively (Payne 1987). The genes coding for LMW-GS occur on the short arms of group-1 chromosomes at the *Glu-A3*, *Glu-B3* and *Glu-D3* loci which are tightly linked to the *Gli-1* locus (Singh and Shepherd 1988; Pogna et al. 1990). It is generally accepted that glutenins are mainly responsible for bread-making quality.

The work of Payne *et al*. (1980) provided evidence of a strong association between the presence of certain alleles coding for HMW-GS and bread-making quality. Branlard and Felix (1994) observed an 18–55% variation of strength and tenacity while the Pelshenke result could be explained by HMW-GS, and less than 20% by LMW-GS. Sontag-Strohm et al. (1996) found that progeny carrying allele *Glu-A1*b (i.e. 2*) had significantly greater SDS sedimentation volumes than the null (n) allele, and that adding a HMW-GS affected extensograph dough strength more than adding a LMW-GS, although both increased the sedimentation volumes. Griffin (1989) found that HMW-GS played only a minor role in regulating environmental variability for bread making, the whole gluten protein fraction appearing to be important, and not just the HMW-GS. Other studies have also shown that allelic variation of HMW-GS and LMW-GS are both associated with differences in the technological qualities of wheat flour (Autran et al. 1987; Payne 1987; Gupta et al. 1989b; Nieto-Taladriz et al. 1994).

As LMW-GS are present in a much greater amount than HMW-GS, great effort has been made to establish

their role in bread-making quality (Payne 1987; Gupta and Shepherd 1987, 1988; Boggini and Pogna 1989; Gupta et al. 1989a; Metakovsky et al. 1990; Pogna et al. 1990). LMW-GS have a pronounced effect on dough viscoelastic properties in both bread wheat and durum wheat. Largely additive effects of individual *Glu-3* alleles (Gupta et al. 1989b; Pogna et al. 1990) and significant interactions (Gupta et al. 1994) have been found.

Several alleles at the *Glu-3* loci have been ranked with respect to their effect on dough resistance and extensibility (Gupta and Shepherd 1988; Gupta et al. 1989b, 1990a, b, 1991, 1994; Metakovsky, et al. 1990). In Australian wheat cultivars, LMW-GS provided better predictions than HMW-GS for Rmax (maximum dough resistance). For Rmax, the alleles of *Glu-A3* showed b>d>e>c; the alleles of *Glu-B3*: i>b=a>e=f=g=h>c; the alleles of *Glu-D3*: e>b>a>c>d. In particular, allele b of *Glu-B3* was shown to increase dough strength when compared to allele c, and allele b of *Glu-A3* and *Glu-D3* was present in all the more-extensible wheats. It was also shown that dough strength could be improved without increasing grain protein levels and without reducing grain yield. They concluded that HMW-GS alone are insufficient to account for differences in quality, and that breeding lines should not be selected or discarded based only on their HMW-GS composition. LMW-GS must also be taken into consideration.

Cornish et al. (1993) catalogued information about the gluten alleles of nearly 600 wheats in GENEJAR and summarised that null alleles were detrimental to extensibility; the *Glu-3* pattern b b b (at loci *Glu-A3*, *Glu-B3* and *Glu-D3* respectively) gave the best extensibility, particularly in combination with *Glu-1* alleles b b a (at loci *Glu-A1*, *Glu-B1* and *Glu-D1* respectively). *Glu-3* b b c also had excellent extensibility. *Glu-A3* e is a null allele; *Glu-B3* c, d and g alleles had medium to weak dough properties, and should be avoided at the early stages of a bread wheat breeding programme. For *Glu-3*, the best combinations for bread baking are b b b, b b c, and c b c.

Vazquez et al. (1996) reported that allelic variation at the *Glu-A3* locus did not have a significant influence on gluten strength, whereas allelic variation at the *Glu-B3* locus did significantly affect gluten strength, as measured by sedimentation volume on durum wheat. Null alleles also did not negatively affect quality, despite their presence, implying a lower level of glutenin polymerisation (Payne et al. 1987).

Biochemical (Autran et al. 1987) and genetic (Pogna et al. 1990) studies indicated that the positive effects associated with the *Gli-1*/ *Glu-3* complex were due to the *Glu-3* alleles. However, the omega gliadins, encoded at *Gli-1* locus can influence dough extensibility (Branlard and Felix 1994). The alleles at the *Glu-A3* locus affected both the quantity and the size of the polymers. The positive effects of the glutenin subunits could be attributed primarily to their capacity to form inter-molecular disulphide linkages. LMW-GS affected the quantity and/or size distribution of the polymers due to differences in the amount and type of their subunits.

The relative quantity of total glutenin is a prime factor determining dough strength, and the positive effect of HMW-GS and LMW-GS might be due to increased total glutenin rather than qualitative superiority of specific subunits. Using near-isogenic bread wheat lines, Lawrence et al. (1988) showed that the percentage of densitogram area under HMW-GS (in SDS-PAGE gels) was strongly associated with dough strength. There are many exceptions to the qualitative basis of allele superiority. Some bread wheat cultivars contain HMW-GS 5+10, but produce weak doughs. Furthermore, 1BL-1RS wheat-rye translocation lines consistently produced weak-sticky doughs irrespective of their HMW-GS composition. In contrast, many good bread-making quality cultivars possess HMW-GS 2+12.

The effects of the *Glu-1* and *Glu-3* alleles in a wider range of genotypes are needed before their use in predicting dough properties can be fully justified. A better understanding of the effect of individual alleles on quality parameters will provide clearer information for the bread-making quality breeders.

The major objective of this work was to develop a set of recombinant inbred lines (RILs) showing allelic variation at the *Glu-1* and *Glu-3* loci in a set of NZ wheat populations with varing bread-making qualities. These RILs presented an opportunity to study the allelic effect on bread-making quality parameters in a common genetic background.

The aim of this study was to: (1) find out the effect of the glutenin alleles on the baking quality parameters; and (2) clarify differences between the alleles for different quality parameters, in order to provide information for wheat breeders and complement the general information relating specific glutenin alleles to bread-making quality.

Materials and methods

Material – crossing progeny

From six New Zealand lines, five crosses were made in 1995 and their progenies grown at the Crop and Food Research Institute under the supervision of Dr. W.B. Griffin. F_3 populations were analysed for individual plant variation by SDS-PAGE. The F_3 SDS-PAGE results were used to determine the banding patterns in the resulting F_4 families. The F_4 progeny families which had the same band patterns were then bulked for quality tests. Fifty two F_4 bulks originating from 193 F_4 families were tested for wholemeal flour protein (WFP), hardness, SDS sedimentation and Pelshenke time; and 50 by mixograph analysis. The SDS-PAGE analyses of F_3 plants and the quality tests of F_4 materials were carried out in 1997 and 1998, at INRA, Clermont-Ferrand, France.

Table 1 lists parental glutenin alleles and the value of measured quality parameters; Table 2 lists the number of analysed F_3 plants and F_4 bulk, and F_4 bulk mean value of the quality parameter.

Analysing methods

Analyses of the materials were carried out in INRA, Station D'Amelioration des Plantes, Clermont-Ferrand, France.

Protein separation by SDS-PAGE

One hundred and ninety three F_3 plants were tested by SDS-PAGE. The protocol is based on Singh et al. (1991). In order to have better resolution for both HMW-GS and LMW-GS, the acryl-

Table 1 Parental glutenin alleles and the value of measured quality parameters

Parent	Quality[a]	*Glu-A1*[b]	*Glu-B1*	*Glu-D1*	*Glu–A3*	*Glu-B3*	*Glu-D3*	Wholemeal Flour Protein (%)	Hardness	Sedimentation	Pelshenke (min)	Md-line peak time (min)	Md-line peak value (%)
Tui	D	2*	7+9	5+10	e	Sec-12	b	11.83	83	43.1	156.8	2.8	44.2
Morahi	A	2*	7+9	5+10	d	Sec-13	b	12.19	49	50.8	193.1	3.7	49.3
Rongotea	B	2*	7+9	5+10	e	b	b	12.25	78	48.8	156.2	3.0	51.5
Otane	A	2*	7+8	2+12	d	b	a	10.85	58	52.2	159.8	4.4	46.9
Pernel	D	1	7+8	5+10	c	g	b	12.33	73	58.3	175.2	3.9	48.0
Karamu	C	n	7+8	2+12	c	b	b	10.85	70	57.3	38.0	2.2	45.8
Involved allele No.		3	2	2	3	4	2	The quality results for each cultivar are the average of 12 samples from the same environment					

[a] Rankings of the quality classes (A=best, D=worst) were based on trial results, commercial baking data and experience, and were subjectively ranked by Dr. W.B. Griffin (wheat breeder with Crop and Food Crown Research Institute, Lincoln, New Zealand)

[b] The name of alleles for HMW-GS based on Payne and Lawrence (1983), and LMW-GS on Gupta and shepherd (1990c). Sec-12 and Sec-13 are encoded on chromosome 1B in 1BL-1RS wheat-rye translocation lines, in which *Glu-B3* is absent; in this study they are tested as alternatives of *Glu-B3*

Table 2 The number of analysed F_3 Plants and F_4 bulks, and mean value of the tested quality parameters for F_4 bulks

Cross	F_3 plant	F_4 group[a]	Possible groups	Wholemeal flour protein (%)	Hardness	Sedimentation (ml)	Pelshenke (min)	Mid-line peak time (min)	Mid-line peak value (%)
Morahi×Tui (pop5)	34	7	9	14.8	61.3	51.7	172	2.22	59.1
Morahi×Rongotea (pop6)	31	6	9	16.0	71.4	57.2	190	2.34	64.3
Otane×Karamu (pop7)	33	14	27	15.5	82.2	64.1	119	1.97	67.7
Otane×Pernel (pop9)	51	23	243	13.9	75.6	63.0	178	3.12	60.4
Rongotea×Tui (pop10)	44	3	3	14.2	72.0	52.0	150	2.25	59.6

[a] The possible number of F_4 groups is based on the theoretical calculation that would result from the cross to give a fully balanced factorial design including all the glutenin profiles

amide/bisacrylamide concentration was constant and the gel concentration (T) and the cross linker (C) were modified as follow, T=12.8%, and C=0.99%, by the laboratory of Dr. G. Branlard, INRA, Clermont-Fd, France.

Allele reading of HMW-GS and LMW-GS on SDS-PAGE

The bands of HMW-GS and LMW-GS on SDS-PAGE were read with the standardised HMW-GS methodology and nomenclature described by Payne and Lawrence (1983), and for LMW-GS using the methodology and nomenclature described by Gupta and Shepherd (1990c).

Quality tests

A Near-Infrared Reflectance (NIR) instrument (Inframatic 8620 Perten Instruments, Hamburg, Germany) was used to estimate the wholemeal flour protein content and hardness, white flour protein content, and moisture level. This result was also used for calculating the amount of added water to the flour for the mixograph test (Martinant et al. 1998).

For the SDS sedimentation test, 5 g of wholemeal flour was used, according to the procedure described by Axford et al. (1979).

The Pelshenke test was carried out by using 10 g of wholemeal flour. The original procedure (Pelshenke 1933) was employed for determining the dough swelling time. A temperature-controlled cabinet was used to provide better control for the condition of dough swelling.

A 10-g mixograph was performed according to the American Association of Cereal Chemist (1988), approved method AACC 54–40 A. Dough hydration took into account flour protein content, flour moisture and grain hardness (Martinant et al. 1998). The mixograph curves were computed by Mixsmart software. Mid-line Peak Value (MPV) and Mid-line Peak Time (MPT) were the major parameters used in this study.

Percentage ranges were calculated to determine the effect of a change in a particular allele on the value of each quality parameter. The formula used for each population was;

$$100[(\text{maximum mean allele value})-(\text{minimum mean allele value})]/\{[(\text{maximum mean allele value})+(\text{minimum mean allele value})]/2\}$$

Statistical analysis

Statistical analysis was by the computer statistics software Minitab and SAS 6.12 for Windows. ANOVA contrast models in SAS were used for the allelic comparison (Table 3), the data were grouped and analysed for each pair of alleles at each locus (Table 2).

Table 3 Population and allele code used for the allele's contrast model

Locus		*Glu-A1*		*Glu-D1*	*Glu-A3*		*Glu-B3*		*Glu-D3*
Population		7	9	9	5+6[a]	7+9	5+6[a]	9	7+9
Allele name		2*, n	2*, 1	5+10, 2+12	d, e	d, c	b, Sec-12, Sec-13	b, g	a, b
In model	x	n	1	2+12	e	c	See Tables 5–7	g	a
	y	2*	2*	5+10	d	d		b	b

Two-allele models Model	Allele designation (x, y)	Three-allele models Model	b, Sec-12 (S12) and Sec-13 (S-13)
Over-dominant	xy>y>x[b]	One recessive	b>S12=S13=S12S13=bS13
Over-dominant	xy>x>y	Two equal alleles	S12>bS13=S12S13=S12=S13
Over-dominant	xy>y=x		S13>bS13=S12S13=S12=b
Dominant	y=xy>x	One dominant	b=bS13>S12=S12S13=S13
Dominant	x=xy>y	Two equal recessive	S12=S12S13>b=S13=bS13
Additive	y>xy>x	Alleles	S13=S12S13=bS13>b=S12
		One allele over-dominant	S12>b=S13>bS13=S12S13
		Three additive alleles	b>bS13>S13>S12S13>S12
		Two equal alleles additive	b>bS13>S13=S12S13=S12
		with the third allele	S12>S12S13>b=bS13=S13
			S13>bS13=S12S13>b=S12

* The data used for population (5+6) were strictly balanced; the rest of the analyses used incompletely balanced data

[b] The direction indicators could apply either way (>or<) in a model as long as they are all consistently in the same direction in any contrast model. All models are simplified by assuming in models with more than one difference that all neighbouring differences within a model were of the same size

It can be seen from Table 2 that the data did not consist of full factorials for every allele combination. In most instances several possible combinations of alleles were missing. This was a consequence of the random nature of the assortment of the alleles into the F_3 and F_4 populations. Therefore, the statistical model used for the analysis was required to take into account the unbalanced nature of some of the designs. SAS is well set up to handle this type of unbalanced ANOVA using it's General Linear Models procedure. A main-effects only model was used for each family, with interactions ignored, but with all main effects included for each population analysis. As a consequence, all means are least-square estimates in the Results tables. These main-effects models were then used to establish contrasts between the means that were consistent with a variety of genetical expression models (dominant/recessive, additive, overdominant). There were generally only two degrees of freedom available for the contrasts, but with six models to be tested. Therefore, the actual *P* values determined will be underestimates, though their relative values will be consistent. Equally the overdominant model will underestimate the level of overdominance because the heterozygosity was established in the F_3 seed, and therefore the F4 bulks will consist of 25% of each allele in the homozygous form and 50% as heterozygotes.

Results

Quality differences among the F_4 populations

There were significant differences among the population means for all quality parameters measured, except hardness (Table 2). Population 7 had the greatest value for hardness, sedimentation and MPV, even though both of its parents did not have obviously higher MPV and sedimentation values (Table 1). Its sedimentation value was significantly higher than populations 5, 6 and 10; its MPV value was significantly higher than populations 5, 9 and 10. This implies that recombination may have had an additive effect for these two parameters, especially for MPV. The relatively low Pelshenke time of population 7 could be the result of inheritance from one of the parents, Karamu, whose Pelshenke time was exceptionally low. Population 9 ranked 1st for MPT, and 2nd for hardness, sedimentation and Pelshenke time, but its whole flour protein content was the lowest (Table 2). This result further confirms that greater protein quantity does not necessarily mean better quality of bread-making. The greatest MPT of population 9 could result from both parents, Otane and Pernel (Table 1), as they were both high for this character. Population 6 had the greatest WFP, also consistent with its pedigree as both of its parents, Morahi and Rongotea, had a relatively high WFP.

The original design of the experiments was to produce completely balanced sets of progeny. However, due to limitations on testing F_3 plants, the F_3 allelic segregation could not be entirely balanced. According to the populations' allelic segregation, appropriate populations were chosen for particular allelic comparisons as indicated in Table 3.

Comparison of HMW-GS alleles n, 1 and 2* on *Glu-A1*

The results in Tables 4a and b represent a comparison of results produced from two pairs of alleles at locus *Glu-A1*. The comparisons were carried out separately in different populations but both pairs had the 2* allele involved.

For the pair-wise comparison of the 2* and null (n) alleles, no significant differences were found between

Table 4a Comparison of effects produced by alleles at the *Glu-A1* locus on measured quality parameters; **alleles 2* and n** (see Materials and methods for calculation of range %)

Quality parameters		Wholemeal flour protein (%)	Hardness	SDS sedimentation (ml)	Pelshenke (min)	Mid-line peak time (min)	Mid-line peak value (%)
Allele	2*	15.2	77.6	66.3	130.4	2.02	68.7
	n	15.6	80.5	60.4	97.3	1.86	65.2
value	2*/n	15.8	89.3	68.1	138.7	2.04	70.2
LSD		1.2	15.6	9.4	29.8	0.34	5.5
Range %		3.9	14.2	11.9	33.9	9.1	7.4
Best fitted models (Better on top)							
Model		No	Over-dominant	Over-dominant	Dominant	No	Over-dominant
Allele		significant	2*/n>n>2*	2*/n>2*>n	2*=2*/n>n	significant	2*/n>2*>n
P value		difference	0.081	0.095	**0.013**	difference	0.072
Model			Over-dominant	Dominant	Over-dominant		Dominant
Allele		2*=n	2*/n>n=2*	2*=2*/n>n	2*/n>2*>n	2*/=n	2*=2*/n>n
P value			0.094	0.097	**0.014**		0.083

Table 4b Comparison of effects produced by alleles at the *Glu-A1* locus on measured quality parameters; **Alleles 2* and 1. Bold** represents a significant genetic model for the parameter as indicated in the table

Quality parameters		Wholemeal flour protein (%)	Hardness	SDS sedimentation (ml)	Pelshenke (min)	Mid-line peak time (min)	Mid-line peak value (%)
Allele	2*	13.1	71.1	63.1	178.9	3.17	52.8
	1	15.4	72.1	63.1	164.2	3.14	61.1
value	2*/1	12.9	76.8	60.8	167.5	3.16	58.4
LSD		1.5	14.8	4.4	28.5	0.93	4.4
Range %		18.1	7.8	3.7	8.6	1.0	14.5
Best fitted models (better on top)							
Model		Over-dominant	No	No	No	No	Dominant
Allele		1>2*>2*/1	significant	significant	significant	significant	1=2*/1>2*
P value		**0.014**	difference	difference	difference	difference	**0.033**
Model		Dominant					Over-dominant
Allele		1>2*=2*/1	2*=1	2*=1	2*=1	2*=1	2*/1>1>2*
P value		**0.016**					**0.041**

families possessing these two alleles for WFP and MPT. However, families containing the 2* allele showed greater values for all the other quality parameters except that the possession of allele n resulted in families with higher hardness. These increases were generally not significant in direct comparisons using the LSD. The genetic models 'over-dominant' or 'dominant' for the 2* allele having a greater value were, however, generally applicable and significant at the 10% level for this pairing. For Pelshenke time, the significance with these models was even greater.

For the-pair wise comparison of F_4 families containing the 2* and 1 alleles, the significant models were also 'over-dominant' and 'dominant.' Families possessing allele 1 had a greater value than those possessing allele 2*, both for WFP and MPV. No significant differences (P=0.1 or below) were found between families different for these two alleles for the other measured quality parameters.

Families containing allele 2* had a significantly longer Pelshenke time than those containing the n allele. Possession of allele 2* also resulted in a longer Pelshenke time than possession of allele 1 (though not significantly). This result supports the belief that allele 2* had a positive effect on the dough-strength parameters. The results also suggested that allele 1 is related to high WFP and MPV.

Comparison of HMW-GS alleles 5+10 and 2+12 on *Glu-D1*

For locus *Glu-D1*, the possession of alleles 5+10 and/or 2+12 caused no significant differences between their progeny for WFP, Hardness, MPT and MPV. However, F_4 families containing allele 5+10 had a significantly higher sedimentation volume and longer Pelshenke times than families containing allele 2+12. Possession of allele

Table 5 Comparison of effects produced by alleles at the *Glu-D1* locus on measured quality parameters; **alleles 5+10 and 2+12. Bold** represents a significant genetic model for the parameter as indicated in the table

Quality parameters		Wholemeal flour protein (%)	Hardness	SDS sedimentation (ml)	Pelshenke (min)	Mid-line peak time (min)	Mid-line peak value (%)
Allele	5+10	13.8	72.4	63.0	194.7	3.59	57.9
	2+12	13.4	73.4	58.5	133.2	2.81	54.0
value	5+10/2+12	14.5	72.4	63.3	170.8	3.41	54.0
LSD		1.5	15.8	4.3	28.1	1.80	13.9
Range %		7.9	1.4	7.8	37.0	23.8	7.1
Best fitted models (better on top)							
Model		No significant difference	No significant difference	Dominant (5+10)= (5+10/2+12)>(2+12)	Additive (5+10)>5+ 12/2+12)>(2+12)	No significant difference	No significant difference
Allele							
P value				0.057	**0.002**		
Model				Additive	Dominant		
Allele		5+10=2+12	5+10=2+12	(5+10)>(5+10/2+12) >(2+12)	(5+10)= (5+10/2+12)>(2+12)	5+10=2+12	5+10=2+12
P value				0.105	**0.006**		

Table 6a Comparison of effects produced by alleles at the *Glu-A3* locus on measured quality parameters; **alleles c and d**. **Bold** presents a significant genetic model for the parameter as in the table

Quality parameters		Wholemeal flour protein (%)	Hardness	SDS Sedimentation (ml)	Pelshenke (min)	Mid-line peak time (min)	Mid-line peak value (%)
Allele	d	16.0	78.4	67.3	167.8	2.52	64.6
	c	14.3	76.8	66.2	164.7	2.97	64.2
value	d/c	14.5	76.4	62.1	154.8	3.09	56.5
LSD		1.0	9.2	4.0	18.1	0.94	6.8
Range %		11.4	2.6	8.0	8.0	19.9	14.7
Best fitted models (better on top)							
Model		Dominant	No	Over-dominant	No	No	Over-dominant
Allele		d>c=d/c	significant	d=c>d/c	significant	significant	d=c>d/c
P value		**0.014**	difference	**0.01**	difference	difference	**0.03**
Model		Additive		Over-dominant			Dominant
Allele		d>d/c>c	d=c	d>c>d/c	d=c	d=c	c>d>d/c
P value		**0.019**		**0.012**			**0.053**

Table 6b Comparison of effects produced by alleles at the *Glu-A3* locus on measured quality parameters; **alleles e and d**. **Bold** presents a significant genetic model for the parameter as in the table

Quality parameters		Wholemeal flour protein (%)	Hardness	SDS sedimentation (ml)	Pelshenke (min)	Mid-line peak time (min)	Mid-line peak value (%)
Allele	d	15.7	72.2	54.3	181.1	2.29	63.2
	e	15.3	71.2	53.4	173.2	2.31	60.0
value	d/e	14.9	62.4	53.6	190.2	2.25	60.9
LSD		0.9	17.9	5.8	28.0	0.21	3.8
Range %		5.2	14.3	1.7	9.4	2.6	5.2
Best fitted models (better on top)							
Model		Over-dominant	No	No	No	No	Additive
Allele		d>e>d/e	significant	significant	significant	significant	d>d/e>e
P value		0.080	difference	difference	difference	difference	0.092
Model							Dominant
Allele			d=e	d=e	d=e	d=e	d>e=d/e
P value							0.093

Table 7a Comparison of effects produced by alleles at the *Glu-B3* locus on measured quality parameters; **alleles b and g**. **Bold** presents a significant genetic model for the parameter as in the table

Quality parameters		Wholemeal flour protein (%)	Hardness	SDS sedimentation (ml)	Pelshenke (min)	Mid-line peak time (min)	Mid-line peak value (%)
Allele	b	14.0	73.7	61.4	167.3	3.39	56.8
value	g	12.7	71.6	62.5	171.1	3.49	54.2
	b/g	14.5	76.4	62.8	174.2	3.64	53.9
LSD		1.7	17.3	5.3	33.8	1.99	14.0
Range %		13.1	6.5	2.2	4.0	7.1	5.3
Best fitted models (better on top)							
Model		Over-dominant	No	No	No	No	No
Allele		b/g>b>g	significant	significant	significant	significant	significant
P value		**0.034**	difference	difference	difference	difference	difference
Model		Dominant					
Allele		b=b/g>g	b=g	b=g	b=g	b=g	b=g
P value		0.060					

Table 7b Comparison of effects produced by alleles at the *Glu-B3* locus on measured quality parameters; **alleles b, Sec-12 and Sec-13**. **Bold** presents a significant genetic model for the parameter as in the table

Quality parameters		Wholemeal flour protein (%)	Hardness	SDS sedimentation (ml)	Pelshenke (min)	Mid-line peak time (min)	Mid-line peak value (%)
Allele	b	16.1	69.7	58.7	195.3	2.44	64.5
value	S12	15.0	82.2	49.2	187.3	2.23	60.7
	S13	15.1	65.1	51.6	161.9	2.17	59.1
	bS13	15.6	69.3	55.3	183.3	2.32	62.3
	S13S12	14.9	56.7	54.0	179.7	2.26	60.1
LSD		1.3	25.9	8.3	40.3	0.30	5.4
Range %		7.8	36.7	17.7	18.4	11.8	8.8
Best fitted models (better on top)							
Model		One allele additive with two equal alleles	No	One allele additive with two equal alleles	One recessive two equal alleles	One dominant two equal recessive	One allele additive with two equal alleles
Allele		b>bS13>S13= S12S13=S12	significant	b>bS13>S13= S12S13=S12	bS13=S12S13= S12=b>S13	b>bS13>S13= S12S13=S12	b>bS13>S13= S12S13=S12
P value		**0.041**	difference	**0.015**	0.069	**0.028**	**0.037**
Model		One dominant two equal recessive		One dominant two equal recessive	One allele additive with two equal alleles	One recessive two equal alleles	One recessive two equal alleles
Allele		b=bS13>S12= S12S13=S13	b=S12=S13	b=bS13>S12= S12S13=S13	b=S12>bS13= S12S13>S13	b>S12=S13= S12S13=bS13	b>S12=S13= S12S13=bS13
P value		0.053		**0.021**	0.082	**0.038**	**0.048**

5+10 also resulted in greater WFP, MPT and MPV, and lower hardness than possession of allele 2+12, but not at a significant level. These results give some support to the general finding that possession of allele 5+10 is better for baking quality than allele 2+12. The 'dominant' and 'additive' models were applicable for both SDS sedimentation volume and Pelshenke time, and both models were highly significant for Pelshenke time.

Comparison of LMW-GS alleles c, d and e on *Glu-A3*

F_4 families possessing allele d had higher values than families possessing either of the alleles c or e for all of the measured quality parameters except MPT. F_4 families possessing allele d had a shorter MPT than F_4 families possessing either allele c or e. The higher WFP of families with allele d, over those with allele c, is significant.

Table 8 Comparison of effects produced by alleles at the *Glu-D3* locus on measured quality parameters; **alleles a and b**. **Bold** presents a significant genetic model for the parameter as in the table

Quality parameters		Wholemeal flour protein (%)	Hardness	SDS sedimentation	Pelshenke (min)	Mid-line peak time (min)	Mid-line peak value (%)
Allele	a	14.6	77.7	64.5	151.3	3.01	58.5
	b	15.6	77.7	66.6	162.7	2.19	68.1
value	a/b	14.6	76.2	64.4	173.2	3.38	58.6
LSD		1.2	10.5	4.6	20.7	1.07	7.8
Range %		6.7	1.9	3.4	13.5	41.6	15.5
Best fitted models (better on top)							
Model		Over-dominant	No	No	Over-dominant	Over-dominant	Over-dominant
Allele		b>a>a/b	significant	significant	a/b>b>a	a/b>a>b	b>a>a/b
P value		0.099	difference	difference	0.064	**0.011**	**0.017**
Model						Over-dominant	Dominant
Allele			a=b	a=b		a/b>a=b	a=a/b>b
P value						**0.023**	**0.028**

In general, possession of allele d was better for wheat quality than possession of either allele c or e. The applicable significant genetic models indicated that allele d may be recessive to allele c.

The results of Table 6 suggest that at the *Glu-A3* locus, possession of allele d is more desirable for improving wheat quality than possession of either allele c or e. The shorter MPT of flour containing allele d means that less work input would be required in mixing times with a genotype containing this allele while still maintaining the higher values for the other measured quality parameters. This result agrees with the conclusion of Gupta et al. (1990a) for Rmax in Australian wheat.

Comparison of LMW-GS alleles b, g, Sec-12 and Sec-13 on *Glu-B3*

In Table 7a, F_4 families containing allele b had greater WFP than those containing allele g. The 'over-dominant' genetic model "b/g >b>g" was significant for this pair of alleles for WFP. The higher WFP of allele b agrees with the conclusions of Gupta (1990a) and Cornish et al. (1993). For all the other parameters, there was no significant difference between alleles b and g.

In Table 7b, F_4 families possessing allele b had higher values for all the measured quality parameters than families possessing either Sec-12 or Sec-13, with the exception of hardness. Sec-12 appeared to be a high hardness recessive allele. However, its influence wasn't significant. In general, possession of allele b was better for increasing flour quality characteristics relative to either allele Sec-12 or Sec-13. Genetic models were significant for WFP, SDS sedimentation, MPT and MPV. In the significant genetic models, possession of allele b mainly resulted in higher quality values and was additive with the other two alleles.

Comparison of LMW-GS alleles a and b on *Glu-D3*

For locus *Glu-D3*, possession of allele b lead to higher WFP, SDS sedimentation, Pelshenke time and MPV values, though not significantly so. The MPT associated with allele b was significantly shorter than that associated with allele a. The negative relationship between MPT and MPV, also indicated in Luo (1999), is generally observed. The higher MPV and short MPT associated with allele b on locus *Glu-D3* could be explained by its higher LMW-GS quantity, which resulted in a higher quantity of aggregated proteins. The consequently higher ratio of "Aggregated Protein" (HMW-GS+LMW-GS)/"Non-Aggregated Protein" (Gliadin) provides more polymeric proteins, which can increase the strength, tenacity and resistance of the dough (i.e. MPV). The higher the amount of aggregated glutenin in the dough, the easier or quicker the gluten forms. MPT is the time of maximum resistance, when the gluten network is at its strongest. Therefore, families containing allele b had a higher MPV and, logically, a shorter MPT than allele a. The 'over-dominant' genetic model seemed applicable to this pair of alleles. In general, possession of allele b was associated with better quality characters than the possession of allele a.

Discussion

When considered among all the F_4 cross populations, significant differences existed for every measured quality parameter in at least one population. Hardness seemed to be the least responsive to changes in the glutenin alleles, with only one weak (P=0.08) overdominant significant model resulting. However, the average percentage range resulting across all populations of F_4 families differing in their glutenin alleles was greater for hardness than for several other characters that showed more sig-

Table 9 Overall summary of the effects of the different alleles on the quality parameters

Quality parameter						Locus	Mean range %
WFP	HAR	SED	PEL	MPT	MPV		
Significant (P<0.1) differences between alleles and their direction							
–[a]	**(–)**[b]	2*>n	2*>n	–	2*>n	A1-HMW	13.4
1>2*	–	–	–	–	1>2*	A1-HMW	9
–	–	5+10>2+12	5+10>2+12	–	–	D1-HMW	14.2
d>c	–	**(–)**	–	–	**(–)**	A3-LMW	10.8
(–)	–	–	–	–	d>e	A3-LMW	6.4
b>g	–	–	–	–	–	B3-LMW	6.4
b>Sec13/ Sec12	–	b>Sec13/ Sec12	b/Sec12> Sec13	b>Sec13/ Sec12	b>Sec13/ Sec12	B3-LMW	16.8
b>a	–	–	b>a	a>b	b>a	D3-LMW	13.8
Number (%) of significant differences for that quality parameter							(P=0.47) ↑
6 (75)	1 (12.5)	4 (50)	4 (50)	2 (25)	6 (75)		
Mean range (%) of values across F_4 populations for that quality parameter							
9.2	10.7	7	16.6	14.6	9.8	←(P=0.36)	

[a] "–" Indicates no significant difference
[b] **"(–)"** Indicates that there was a significant difference (P<0.10) but only for the heterozygote relative to one of the homozygotes

nificant differences. This can be explained by assuming hardness was intrinsically more variable than several (sedimentation volume in particular) of the other characters. The differences in the mean percentage ranges were not significant for either the particular quality test or the loci being investigated. Thus, these values did not indicate where the most important responses to changes in alleles were likely to occur. A statistical contrast between all the loci representing HMW alleles (12.2%) and LMW alleles (10.8%) for percentage ranges also did not show a significant statistical difference. This indicated that, for the alleles tested, the average effects on flour quality parameters were about equal for changes in HMW and LMW alleles. This equality of action between HMW and LMW allele changes is consistent with the results of some authors (e.g. Payne 1987), though others have found HMW glutenins to have a greater effect (e.g. Branlard and Felix 1994). In four instances the percentage ranges associated with a particular allele exceeded 30%. This indicated that there were very strong differences in the quality parameters associated with the possession of a particular allele. In this series of trials it was not possible to measure glutenin quantities for all of the F_4 bulks. Therefore, it is not known whether the changes in quality parameters associated with changes in alleles were a consequence of altered total glutenin quantity or altered quality of the glutenins. MPT had only two significant associations with particular alleles, whereas values of the remaining characters were significantly associated with the possession of a particular glutenin in 50% or 75% of the comparisons.

Where changes in an allele led to a significant increase in a quality measure then, in all but one instance, that allele was associated with a higher value for all significant changes in quality parameters. The sole variation was in the *Glu-D3* alleles where MPT and MPV changed in inverse directions (though as discussed in the Results this may be expected) when changing from possessing allele a to allele b. The best-fit genetic model was also consistent for any allele comparison. Most frequently the best-fitting model was a dominant or overdominant model rather than an additive model. The most-consistent exception was for the *Glu-B3* comparison of b, Sec 12 and Sec13, where an additive model dominated. There must be some concern, however, about the reality of some of the overdominant models in a genetic sense, as 50% of the individual grains should have segregated out in the F_4 to be homozygous, since the heterozygous condition was determined in the F_3. This may indicate that such a mixture of alleles creates an overdominant effect, even if the mixture is attained by mixing different homozygous lines.

For *Glu-A1*, 2* appears to be a better baking quality allele (i.e. it confers better values for the quality parameters measured in the F_4 population tested) than allele n. This is consistent with the findings of Payne (1987); Nieto-Taladriz et al. (1994) and Sontag-Strohm et al. (1996). The data presented here suggest that allele 1 may be even better. Studies on Australian wheat cultivars ranked several of the Glu-3 alleles for their effects on Rmax (Gupta and Shepherd 1988; Gupta et al. 1989b, 1990a, b, 1991, 1994; Metakovsky et al. 1990). Our rankings, though on different characters, are entirely consistent with their results. For example we both found *Glu-A3* d >c and e, *Glu-B3* b >g and *Glu-D3* b>a. From among the loci tested it is possible to select a preferred genotype which would be *Glu-A1*: 1, *Glu-B1*: ?, *Glu-D1*: (5+10), *Glu-A3*: d, *Glu-B3*: b, *Glu-D3*: b. It should be stressed that these selections are only from among the alleles tested. The selections also assume that improve-

ments in the quality parameters tested will result in improvements in bread-making quality. Confirmation of this hypothesis will depend on the values of actual bread-making qualities obtained from bulks of these lines in future generations.

In some instances the most significant models showed strong overdominance effects suggesting that F_1 hybrid wheats may have some advantages in quality (e.g. the 2*/n hybrid at the locus *Glu-A1* was superior for several characters). Conversely, often the overdominance was in the direction of a reduced benefit from the hybrid (e.g. the 2*/1 hybrid at the locus *Glu-A1* was inferior for several characters). In this latter situation early generation testing could overemphasise the negative aspects of a particular cross. Though, as specified earlier, these findings on significant overdominance models must be tempered by the knowledge that many of the F_4 grains tested were not heterozygotes but had segregated to produce an F_4 mix of heterozygotes and both types of homozygotes.

In conclusion, this study has provided evidence that some of the glutenin allelic variations in RILs can significantly improve values for at least some of the quality characters which have been found to be related to bread making. The information given above could therefore be a valuable reference for designing a quality breeding programme for bread-making wheat. We also believe that the more extensive testing of the progeny of these RILs, particularly for actual glutenin quantities as well as allelic composition and for actual bread-making quality rather than just for characters given here, will be of even greater use to future breeding programmes.

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