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Flour proteins linked to quality traits in an Australian doubled haploid wheat population

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Abstract. The Cranbrook/Halberd doubled haploid population has provided a unique opportunity to examine in detail the contributions made by a number of different high molecular weight (HMW) and low molecular weight (LMW) glutenin alleles to the dough properties in a set of homogeneous lines of wheat. A range of different instruments was employed, including Farinograph, Extensograph, Do-Corder, Resistograph, and GRL/EasyMix, to study the dough rheology of the lines from 3 sites over 2 years. Correlation studies showed that 2 basic parameters (dough strength and extensibility) were measured by these different instruments. The results presented are mainly from the Extensograph, which is a major Australian standard for determining release and marketing classification of Australian wheats.

Approaches to investigate the data include bulk segregant analysis, distribution of protein alleles in the population, and multiple linear regression. As expected, the HMW glutenin alleles made a major contribution to dough strength, with a minor, but not insignificant, contribution from the LMW glutenin alleles. From a knowledge of their glutenin alleles, a glutenin strength score (GSS) was devised to allow breeders to rank the dough strength of various lines. The GSS scoring system is based on both HMW and LMW glutenin alleles, adding to a total out of 10. Extensibility, on the other hand, was predominantly influenced by protein levels in the flour and environmental conditions such as site and season. However, the LMW glutenin alleles make a significant genetic contribution to the extensibility, which can be assessed by using a glutenin extensibility score. These two glutenin quality scores currently include only the alleles present in the parents, Cranbrook and Halberd, but this could be expanded to include a wider range of alleles by analysis of the quality data from other doubled haploid populations. These quality scores would then be an extremely useful tool for assessing the potential quality of parental and early generation germplasm in wheat breeding programs, by a knowledge of the allelic composition of their HMW and LMW glutenins.

Additional keywords: glutenin, alleles, quality score, rheological properties, strength, extensibility.

Introduction

It is well known that wheat varieties differ in their bread-making ability and that the endosperm proteins, particularly the glutenins, have a major influence on bread-making quality. Wheat varieties at the same protein level have long ago been shown to differ in their bread-making quality, giving the first indication that protein quality, as well as amount is important for good bread-making quality (Finney and Baremore 1948).

Gliadins have been used very successfully to identify wheat varieties using differences in their acid polyacrylamide gel electrophoresis (APAGE) banding patterns (Bushuk and Zilman 1978). This technique was refined in Australia using gradient acrylamide gels, isoelectric focusing (IEF), and 2-dimensional electrophoresis (du Cros and Wrigley 1979). Associations between the electrophoretic patterns of gliadin proteins and quality characteristics enabled wheat cultivars to be grouped into classes, which related to a combination of grain hardness and dough strength (Wrigley *et al.* 1981, 1982). Correlation studies between gliadin bands and the rheological properties (dough strength and extensibility) of a flour dough found that 7–12 gliadin bands accounted for 37–54% of the variation in French-grown wheats (Branlard

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Table 1. *Glu-1* quality score for HMW glutenin alleles

Glu-1 score	*Glu-A1*	*Glu-B1*	*Glu-D1*
4			5+10 (*d*)
3	1 (*a*), 2* (*b*)	17+18 (*i*), 7+8 (*b*)	
2		7+9 (*c*)	2+12 (*a*), 3+12 (*b*)
1	N (*c*)	7 (*a*), 6+8 (*d*)	4+12 (*c*)

and Dardevet 1985). It was realised that gliadin and glutenins could be used as genetic markers for dough quality in Australian wheats (Metakovsky *et al.* 1990). A nomenclature system using lower case alphabet characters to describe groups of bands that were present as a gliadin allelic block was proposed by Metakovsky (1991).

The nomenclature for the high molecular weight (HMW) glutenin subunits was devised by Payne *et al.* (1984*a*). Later, Payne *et al.* (1987) (Table 1) devised a *Glu-1* quality score for the HMW glutenin subunits, to predict the bread-making quality of British-grown wheats. The *Glu-1* quality score for a particular variety is obtained by summing the scores of the individual *Glu-A1*, *Glu-B1*, and *Glu-D1* alleles to give a total score out of 10.

This *Glu-1* score accounted for 55–67% of the variation in the independently established bread-making quality of British-grown wheats. Adoption of this scoring system by breeders in the UK resulted in a dramatic improvement in the quality of new varieties for bread-making, largely through discarding low *Glu-1* score alleles such as *Glu-A1c*, *Glu-B1a*, *Glu-B1d*, and *Glu-D1c*, and selecting alleles with a high *Glu-1* score, particularly *Glu-D1d*. This had a profound effect worldwide in breeding programs characterising germplasm for *Glu-1* alleles (Morgunov *et al.* 1993) and selecting appropriate *Glu-1* alleles to match the dough strength required for different end products. Bushuk (1998) derived an equation to predict unit loaf volume in Canadian wheats from the HMW glutenin subunit composition. This gives similar weighting to the *Glu-B1 b*, *c*, and *i* alleles in the *Glu-1* quality score (Table 1), and a very similar contribution from *Glu-A1a* and *Glu-A1b* alleles.

Payne *et al.*(1987) considered that the *Glu-1* quality score could be improved by including the contribution of the low molecular weight (LMW) glutenin subunits, which account for 80% by weight of the polymeric glutenin in flour (Payne *et al.* 1984*a*). Using a technique to separate the gliadins from the LMW glutenins, the composition of the LMW glutenin alleles could be analysed (Gupta and Shepherd 1990). This technique was simplified by an initial extraction of the gliadins, to enable routine screening of both HMW and LMW glutenin alleles in breeding programs (Singh *et al.* 1991). There is a very close genetic linkage between the LMW glutenins coded by *Glu-3* and the gliadins coded by the *Gli-1* genes on the short arm of chromosome 1 (Singh and Shepherd 1988). This enables the gliadins, with simpler banding patterns than the LMW glutenins, to be used as indicators of *Glu-3* alleles for screening purposes in breeding programs (Singh *et al.* 1991). It is now thought that the LMW glutenin polymers, rather than the gliadin monomers, are important in the formation of the gluten macropolymer and hence affect the rheological properties of a flour dough (Payne *et al.* 1984*b*). Correlation studies were used to predict the physical dough properties using both *Glu-1* and *Glu-3* alleles (Gupta *et al.* 1991) where a correlation of 0.82 was obtained for maximum dough strength and 0.57 for extensibility for 48 Australian wheat cultivars. These results, however, were only tentative as many other variables could have accounted for differences between varieties, other than the glutenin alleles. Dough strength and extensibility are key parameters influencing end product quality and hence important quality objectives in Australian wheat breeding programs.

The availability of doubled haploid populations derived from crosses between wheat lines (Kammholz *et al.* 2001, this issue) provided an opportunity to determine the detailed relationship between the HMW and LMW glutenin proteins and the flour processing qualities with pure homogeneous lines. Glutenin subunit proteins make a major contribution to dough strength and extensibility (reviewed in Gras *et al.* 2001, this issue) and have been identified in the above doubled haploid populations used for genetic mapping studies (Chalmers *et al.* 2001, this issue). The Cranbrook/Halberd cross contains 12 different glutenin alleles. The majority of other alleles found in Australian germplasm are present in the other doubled haploid populations (Cornish *et al.* 2001, this issue). When all the quality data on these 5 populations are analysed it will be possible to produce a comprehensive quality scoring system using both *Glu-1* and *Glu-3* alleles. This scoring system will need to be validated in the germplasm of breeding programs. Proteins can be used as markers for dough quality.

Materials and methods

Germplasm

The parents and the doubled haploid (DH) lines for the Cranbrook/Halberd population are described in Kammholz *et al.* (2001, this issue). The wheat varieties Cranbrook and Halberd were chosen as parents for a doubled haploid population, due to their contrasting rheological attributes. Cranbrook is a wheat variety that was introduced from CIMMYT, Mexico. It was released in Western Australia in 1984 and has medium to high dough strength and high extensibility. It has been used as a parent for a number of good quality varieties including Cunderdin and Carnamah, released in 1996. Halberd, released in 1969, was the only hard-grained variety in the family of wheats based on the soft wheat Insignia. This variety was well adapted to South Australian soils, due to its tolerance of high soil boron levels. It was grown extensively in both South and Western Australia. Halberd produces flour with low dough strength and low extensibility and this was particularly noticeable at lower protein when the quality fell away dramatically. It was known that these two wheat varieties differed at all 6 glutenin loci and hence would provide an opportunity to study the effects of many alleles and possible interactions between alleles. As the

varieties were of diverse parentage it was anticipated that they would be widely separated genetically and highly polymorphic.

Protein alleles

The different procedures for analysing seed storage proteins have been presented by Cornish *et al.* (2001, this issue). The HMW and LMW glutenin subunits were determined using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The nomenclature used in this paper for the HMW glutenin alleles (*Glu-1*) follows Payne *et al.* (1984*a*) and Gupta and Shepherd (1990) for the LMW glutenin alleles (*Glu-3*). In 1995, a doubled haploid (DH) population was produced from a cross between Halberd and Cranbrook. These two parents differ from each other at each of the 6 glutenin loci, with Halberd being *Glu-1 a*, *e*, *d*; *Glu-3 e*, *c*, *c* and Cranbrook *Glu-1 b*, *i*, *a*; *Glu-3 b*, *d*, *a*. Hence there are $2^6 = 64$ possible combinations of seed protein alleles of the progeny. One hundred and seventy DH lines were produced and 60 different glutenin allele combinations were present. The flour samples were tested for homogeneity using SDS-PAGE to determine their HMW and LMW glutenin alleles. Only the pure lines were included in the subsequent statistical analysis.

Trials

In 1997, the 170 doubled haploid lines of the Cranbrook/Halberd population were planted at Roma, Queensland, in 2 blocks according to maturity group (early or mid-season), to simplify harvesting. Each group contained 2 replicates of each parent, a replicate of each line as well, and 3 control varieties (5 replicates of 2 of these control varieties and 2 replicates of the third control in each of the 2 maturity blocks). Another trial planted at Stow, South Australia, in 1997, consisted of a double plot with 170 DH lines plus parents and 85 double plots from 5 control varieties. The double plots were combined to provide enough seed to mill 2 kg of seed after cleaning. The large number of controls randomised throughout the trial was to enable an estimate to be made of the site environmental variation.

In 1998 a Cranbrook/Halberd trial was planted at Stow, South Australia, with the same entries and trial design as in 1997.

Rheological testing of flour doughs

Two kilograms of grain per line was milled on a laboratory Buhler mill and the resultant flour was analysed for physical dough properties. Various physical dough testing instruments were used to record the rheology of a flour/water dough during mixing. The Farinograph and Extensograph testing was conducted by the SARDI, Grain Quality Laboratory, the fast Farinograph by the NSW Agriculture, Agricultural Research Institute, and the Resistograph and GRL/Easymix by the Queensland Department of Primary Industries, Leslie Research Centre. The recorded curves consist of 4 sections: hydration, development, plateau, and a breakdown section. The hydration section shows a rapid increase in resistance with mixing time, during which the water is incorporated into the flour. The development section is a period of more gradual increase in resistance as the gluten structure develops. This is followed by a plateau section of maximum dough resistance, which may range from flat and broad to peaked. The section of decreasing dough resistance is interpreted as dough breakdown. Different parameters are measured from the curves for various instruments but generally include a time to maximum dough resistance (peak development time), a measure of the breakdown characteristics of the dough, and in some instances a measure of the work energy input to mix the dough to peak development time.

Farinograph method

A Farinograph (Brabender Instruments Co.) is a mixer with 2 Z-shaped mixing blades. These blades rotate in opposite directions at different speeds with a 3:2 ratio and the dynamometer shaft rotates at 60 rpm. This produces a gentle folding mixing action with a low rate energy input into the dough. This mixing action matches the traditional low speed mixers that were once used by commercial bakeries where bulk fermentation was used to assist in the rheological development of the dough. The Extensograph method (Westcott and Ross 1995; RACI, Official Testing Method 06–02) involves using 50 g flour (13.5% mb) and adjusting the water addition so that the mixing curve peaks at the 500 Brabender Unit (BU) viscosity line (1 Nm for the 50-g bowl). Parameters measured with the Farinograph included water absorption (WA), dough development time (DDT_s), stability ($Stab_s$), and breakdown 5 min after the peak (BD_s).

Fast Farinograph method

A Do-Corder is a Brabender Farinograph with a variable speed mixer, which was run at 180 rpm for these tests. The aim was to more closely simulate the high-speed mixers used by commercial bakeries, which use mechanical dough development and short fermentation times to obtain an 'optimum' development time. Faster mixing action is thought to discriminate better between stronger flours, with the energy input to maximum peak height or work input being recorded. This faster mixing action can produce a second mixing peak, where the first is the 'hydration peak' and the second is close to the end of the stability phase and relates to the 'optimum mixing' for bread baking (Frazier *et al.* 1975). Parameters included dough development time (DDT_f), dough stability ($Stab_f$), dough breakdown 5 min after the peak (BD_f), Farinograph Quality Number (FQN) (which is defined as Breakdown at BU 30*10), and work input to peak (WI_f).

Resistograph method

A Resistograph is a variant of the Farinograph with a more intensive high shear and high work input mixing action. The bowl has paddle-like blades with 100 g of flour being used for the test. The physical dough properties were studied using a Resistograph mixing head fitted to a Do-Corder (Brabender OHG, Duisburg, Germany) operating at 63 rpm. Measurements of breaking point (BP) and curve weakening angle (CA) were obtained from the mixing curves (Martin *et al.* 1986). For doughs of medium strength and weak flours there are characteristically 2 peaks. The first peak maximum characterises the water banding and the development of the dough, whereas the second peak measures the optimum mixing for bread baking and is just prior to dough breakdown (Shuey 1975).

GRL/Easymix method

Time to peak (time to peak development, s) and work input to peak (work input to peak dough development, Watt h) were measured on doughs prepared using a full baking formula and mixed in an open bowl on a GRL 200 mixer (Hlynka and Anderson 1955) at 160 rpm. Measurements of time to peak (TTP) and work input (WI) were derived using the Easymix software (BRI Australia Ltd, Sydney). The work input values measured are the total work input on the mixer. Oliver and Allen (1992) suggested that mixing to a specific level of work input rather than mixing dough to a peak as a method of achieving 'optimum' development for a bread baking process. This allows the work input to be used as a measure of dough development, which is independent of mixer type or speed.

Extensograph

A Brabender Extensograph measures the rheological properties of a 2% salt water dough after it has been mixed for 5 min to 500 BU viscosity and allowed to rest in a humidity cabinet at 30°C for 45 min The aim is to simulate conditions used in a long fermentation bread baking process. The curve is a record of the resistance to stretching or maximum dough resistance (Rmax) and the amount the cylindrical shaped dough piece can be stretched (the extensibility) (Ext) before

breaking. This test approximates to a tensile strength test and has become the Australian industry standard technique for assessing dough quality (Westcott and Ross 1995; RACI, Official Testing Method 06–01).

Size exclusion high performance liquid chromatography (*SE-HPLC*)

Three different fractions were separated, based on size, on a Phenomenex BIOSEP-S4000 column (5 µm, 500 Å, 7.8 mm by 300 mm). When analysing total protein extracts (10 mg of wholemeal or endosperm were solubilised in 0.5% SDS-phosphate buffer with the aid of 15 s sonication), 3 areas under the chromatogram were established. The chromatogram in the 10.00–18.00 min period from the initiation of the test mainly consisted of glutenins (peak 1). The second sector consisted of gliadins (peak 2), eluting from 18.00 to 21.50 min. Albumin and globulins (peak 3) were the main components of the remaining sector (21.50–24.00 min). The percentage of unextractable polymeric protein (%UPP) was determined based on a sequential extraction using 0.5% SDS-phosphate buffer without sonication (first step) followed by 30 s sonication on the pellet after being re-suspended in the same buffer (Gupta *et al.* 1993). The areas under the chromatogram for the peak 1 sectors of both extracts were used for the calculation of the unextractable percentage value.

All protein extracts were analysed using a Beckman System Gold HPLC, configured with two 126 Pumps, a 166 Detector, and a 507E Autosampler.

Statistical methods

GENSTAT 5, Release 4.1 for Windows 95, Lawes Agricultural Trust, Rothamsted Experimental Station (1998) was used to perform a multiple linear regression analysis of maximum dough resistance (Rmax) against the various glutenin alleles and an ANOVA table of results produced. This software was also used to produce a correlation matrix of the quality parameters.

Results

Instrument measurements

Strength

The instrumental methods used to measure the rheological properties are classified into 2 main groups. The large-scale instruments such as the Farinograph, Fast Farinograph, Resistograph, Extensograph, and GRL/EasyMix use at least 50 g of flour, whereas the small-scale instruments use 10 g or less of flour. It is apparent when Rmax data from an Extensograph is compared with that from other rheological testing instruments that there is a high correlation (r = 0.69–0.88) between many of the parameters measured by the different instruments (data not shown).

This suggests that these instruments are all producing some measurement of the 'strength' of a flour dough. The Do-Corder parameters, particularly dough development time, DDT_f (r = 0.88), and work input, WI_f (r = 0.86), have been found to be highly correlated with dough strength (Rmax). This suggests that greater use could be made of the DoCorder to predict dough strength. Work input to peak has been found to relate to the baking quality of wheats in a mechanical dough development process (Frazier *et al.* 1975). It is also interesting to note that the derived Farinograph Quality Number (FQN) is very strongly correlated to WI_f (r = 0.84).

Extensibility

In contrast to Rmax, when the Extensograph extensibility data are compared with those from other rheological testing instruments, there is only one parameter that correlates highly to it with large-scale rheological testing instruments (>50 g flour). This is the dough breakdown on a Do-Corder ($BD_{f)}$ (r = –0.500). However, the use of small scale testing instruments, such as the force measurement (r = 0.536) with the Probe Test (Oliver and Allen 1993), and the band width at peak resistance (r = 0.693) with a 2-g Mixograph™, has a higher correlation to extensibility. The high correlation between the Extensograph parameters and the parameters as measured by small scale testing instruments such as the Probe Test and the 2-g Mixograph™ suggests that these two techniques will be extremely useful for screening material in breeding programs (Békés *et al.* 2001, this issue).

It was decided to concentrate the analysis on Extensograph parameters (Rmax and Ext) since the Extensograph is accepted as the standard instrument used by wheat breeding programs throughout Australia to assess the rheological quality, and to classify new wheat varieties. Hence, all results presented relate to the Extensograph, unless otherwise stated.

Analysis of Extensograph measurements

Dough strength

Bulk segregant analysis approach. Distribution of the Rmax quality trait and 'bulk segregant analysis-type' of comparison of the alleles in the extreme low and high ends for each population shows that the allelic combinations are not evenly distributed. The lines with a lower Rmax than Halberd (Fig. 1, marked with arrow H) have a predominance of *Glu-B1e*, *Glu-D1a*, *Glu-A3e*, and *Glu-B3c* as found in the parent Halberd, whereas the lines with a higher Rmax than Cranbrook (Fig. 1, marked with an arrow C) have a predominance of *Glu-B1i*, *Glu-D1d*, *Glu-A3b*, and *Glu-B3d* as found in the parent Cranbrook. This suggests that the glutenin alleles are making a major contribution to Rmax.

Allelic distribution in Cranbrook/Halberd population. If the *Glu-1* Score of Payne *et al.* (1987) is applied to the parents Cranbrook and Halberd, a score of 8 is received for each variety. This, however, does not match the average Rmax (6 entries) of Cranbrook of 398 BU and Halberd of 205 BU from the Roma trial.

The mean Rmax of the 8 HMW and 8 LMW glutenin combinations from Roma rep 1 is shown in Fig. 2. The rankings of glutenin alleles from each gene can be determined and are as follows: *Glu-B1i* >*Glu-B1e*, *Glu-D1d* >*Glu-D1a*, *Glu-A3b* >*Glu-A3e*, *Glu-B3d* >*Glu-B3c*, and *Glu-D3a* >*Glu-D3c*.

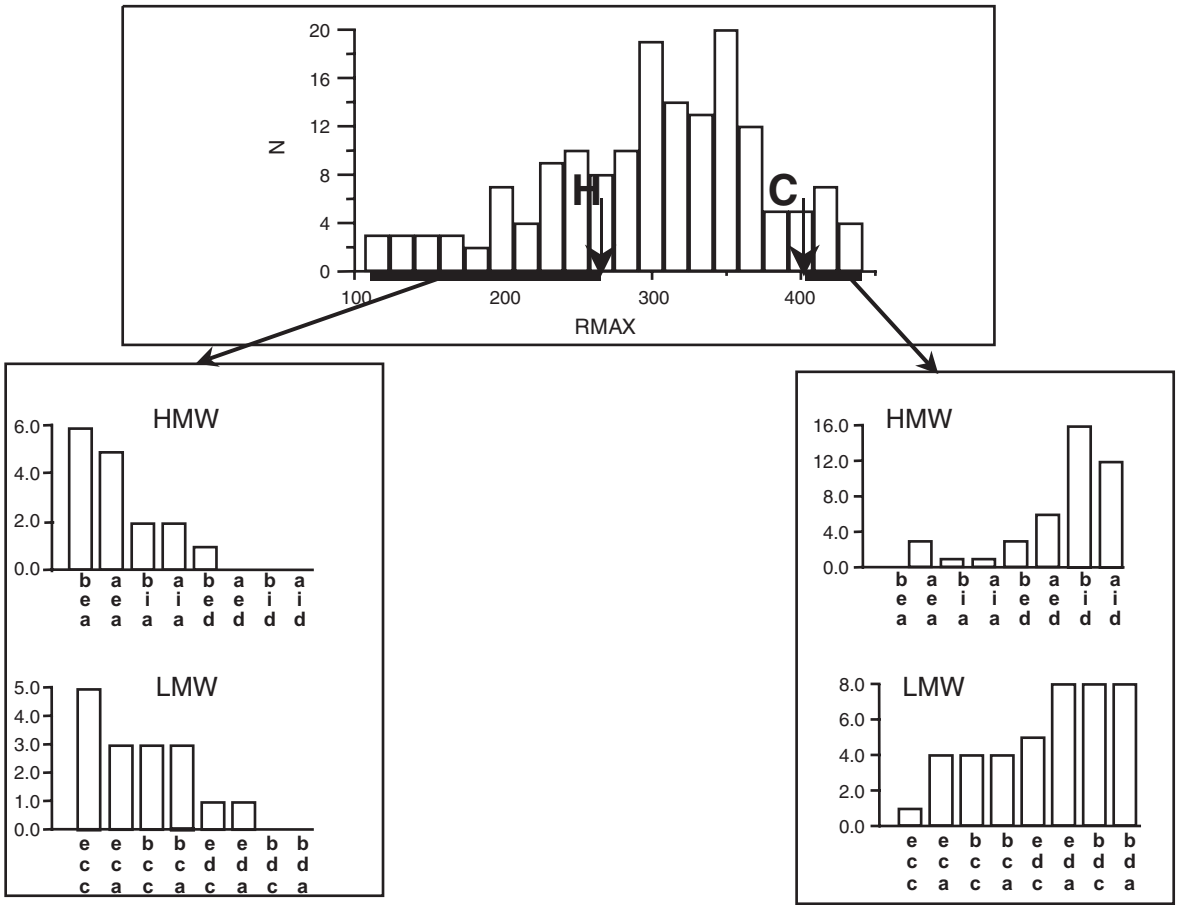

Fig. 1. Distribution of maximum dough resistance and 'bulk segregant analysis-type' of comparison of alleles at the extreme low and high ends of the population.

Multiple linear regression approach. The quality data from the 3 sites over 2 years were analysed by multiple linear regression of the maximum dough resistance (Rmax) against the various glutenin alleles. Samples totalling 679 were included with only one sample with an atypically high Rmax of 715 BU, and a very large standardised residual of 4.00, being excluded as an outlier (Table 2).

This regression accounted for 61% of the variance in Rmax and the standard error of the observation was estimated to be 75 BU. It should be noted that, due to the nature of this test, the standard deviation in measuring Rmax increases with increasing Rmax, although the percentage standard error in Rmax is constant (Table 3). Hence, for lines with lower Rmax (<200 BU), the standard error in predicting Rmax is less than 75 BU, and with higher Rmax (>300 BU) the standard error in predicting Rmax is greater than 75 BU. Logarithmic models were tried but they did not improve the percentage variance accounted for.

In Table 4, the estimate of the regression coefficients for each allele is listed, with the alternative allele being assigned a coefficient value of zero. Although this regression can be used to predict the Rmax for this set of data, it is of little value in predicting absolute values of Rmax as it will shift up or down depending on the environmental effects of site and season. The same rankings and allele weightings were obtained if the 3 sites were treated separately, with only the magnitude of Rmax changing with the site. The site × *Glu* interaction was largest for *Glu-D1d* as this allele makes the major contribution to Rmax. As the rankings and the weightings of the various alleles are constant over different sites, the regression coefficients have been converted to a weighted score out of 10 (Payne *et al.* 1987). This glutenin strength score (GSS) includes contribution from both the *Glu-1* and the *Glu-3* alleles (Table 4) and can be used to predict the relative strength of wheat lines in a breeding trial.

Dough extensibility

Bulk segregant analysis approach. The distribution of the extensibility quality trait and 'bulk segregant analysis-type' of comparison of the alleles in the extreme low and high ends for each population shows that the allelic combinations are not evenly distributed, particularly for the LMW glutenin alleles (Fig. 3). The lines with a lower extensibility than Halberd have a predominance of *Glu-D1d*, *Glu-A3e*,

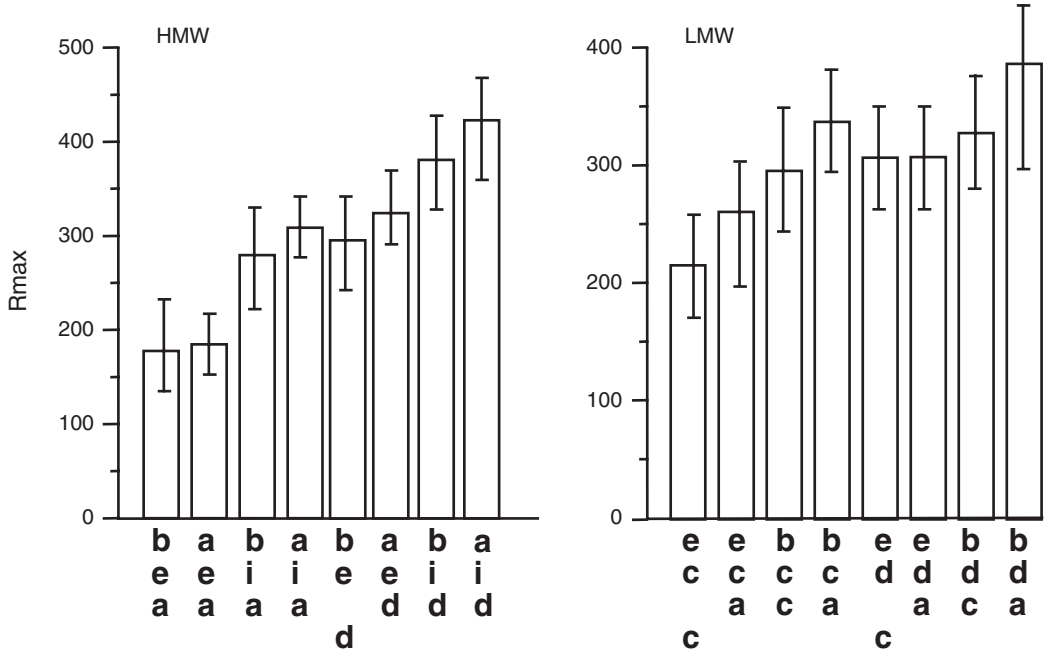

Fig. 2. Maximum dough resistance means (Rmax) for the HMW and LMW glutenin allelic combinations. The standard error of difference (s.e.d.) value at $P = 0.05$ is shown on each of the allelic combinations.

Table 2. Regression analysis of variance for maximum dough resistance

d.f., degrees of freedom; SS, sum of squares; MS, mean squares; VR, variance ratio; *F* pr., probability at this variance ratio

	d.f.	SS	MS	VR	*F* pr.
Regression	6	6 091 531	1 015 255	178.21	<0.001
Residual	671	3 828 413	5697		
Total	678	9 919 944	14 631		

Table 3. Regression estimate of maximum dough resistance

s.e., standard error; *t* (672), *t*-statistic with 672 degrees of freedom; *t* pr., probability at this *t* statistic

	Estimate	s.e.	*t* (672)	*t* pr.
Constant	188.85	7.71	21.65	<0.001
Glu-A1b	–17.44	5.88	–2.97	0.003
Glu-B1i	98.67	5.99	16.46	<0.001
Gli-D1d	142.93	5.97	23.93	<0.001
Glu-A3e	–46.91	5.88	–7.98	<0.001
Glu-B3d	64.95	6.17	10.53	<0.001
Glu-D3c	–39.49	5.9	–6.69	<0.001

Glu-B3c, and *Glu-D3c* as found in the parent Halberd, whereas the lines with a higher extensibility than Cranbrook have a predominance of *Glu-D1a*, *Glu-A3b*, *Glu-B3d*, and *Glu-D3a* as found in the parent Cranbrook. This suggests that particularly the *Glu-3* alleles are making a contribution to extensibility.

Table 4. Predicted glutenin strength score (GSS) for Extensograph maximum dough resistance for each allele

GSS	*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*
3.5			d			
2.5		i				
1.5					d	
1				b		a
0.5	a					
0	b	e	a	e	c	c

Allelic distribution in Cranbrook/Halberd population. The extensibility mean of the 8 HMW and 8 LMW glutenin combinations from Roma rep 1 are shown in Fig. 4. The ranking of glutenin alleles for extensibility is the same as found for Rmax except that *Glu-B1* alleles did not appear to be important.

Multiple linear regression approach. The extensibility or 'stretchability' of a dough is an important quality parameter required for a wide range of end products, including pan bread, flat bread, and biscuits. This parameter has proved to be very difficult to breed for as the genetics of its control are poorly understood. Glutenin alleles at the *Glu-D1* and *Glu-D3* loci influence dough extensibility (Gupta *et al.* 1994). It is known that the extensibility of a flour dough is strongly correlated to the flour protein within a variety ($r = 0.690$ from these data). The LMW glutenin alleles account for 29.0% of the variance with a standard error of observation of 1.46 cm, if the data from the single site Roma

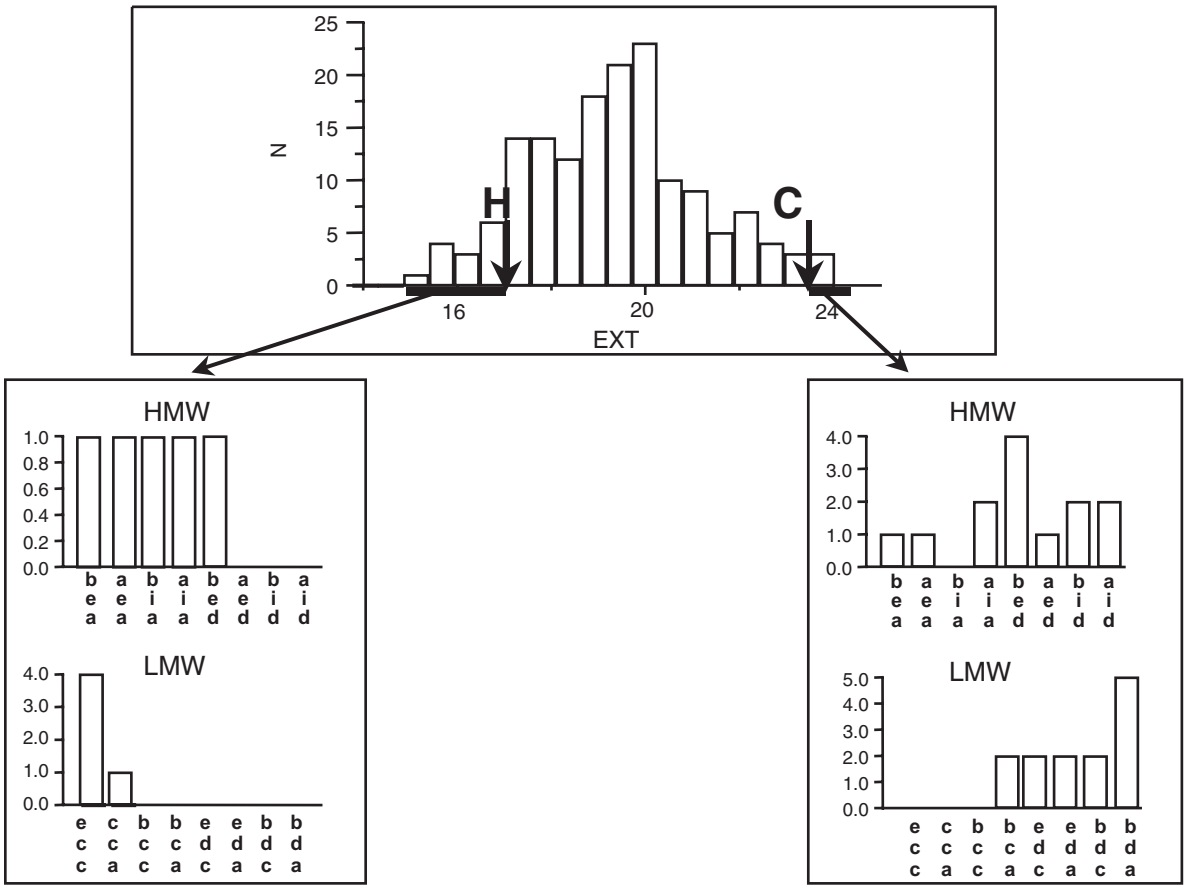

Fig. 3. Distribution of dough extensibility and 'bulk segregant analysis-type' of comparison of alleles at the extreme low and high ends of the population. H, Halberd; C, Cranbrook.

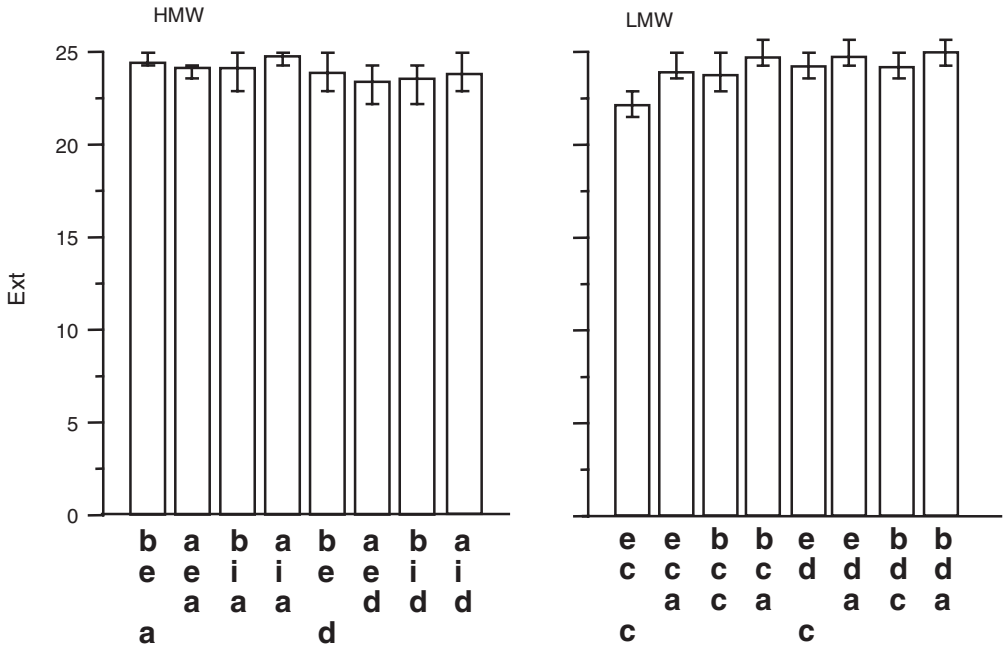

Fig. 4. Dough extensibility means for HMV and LMW allelic combinations. The standard error of difference (s.e.d.) value at $P = 0.05$ probability is shown on each of the allelic combinations.

Table 5. Linear regression models for predicting extensibility

Model	Variance (%)	Standard error (cm)
Glu-3	14.4	2.43
Flour protein (FP)	47.5	1.90
Site	48.4	1.89
FP + Site	54.0	1.78
FP + Site + *Glu-3*	68.7	1.47
FP + Site + *Glu-3*+ *Glu-1*	69.6	1.45

Table 6. Regression analysis of variance for dough extensibility
d.f., degrees of freedom; SS, sum of squares; MS, mean squares; VR, variance ratio; *F* pr., probability at this variance ratio

	d.f.	SS	MS	VR	*F* pr.
Regression	10	3310	330.956	157.61	<0.001
Residual	674	1415	2.100		
Total	684	4725	6.908		

are analysed. When the 3 sites are included the *Glu-3* alleles only account for 14.4% of the variance. Hence a model was used that included both the flour protein and the site as variables in the regression along with the *Glu-3* and *Glu-1* alleles. This resulted in a dramatic improvement in the percentage variance accounted for (70%) and a lower standard error of observation of 1.45 cm (Table 5). Hence, it can be concluded that although all 3 *Glu-3* loci and the *Glu-D1* locus make a significant contribution to the extensibility, the major contribution is not the allelic composition, but the quantity of glutenin protein.

The error variance does not appear to be constant, with lower values of extensibility being more variable than higher ones. This may be an inherent defect of the Extensograph, where it is found that below 100 BU there is possibly chart slippage or the chart gearing mechanism may fail to activate, resulting in a low extensibility measurement or no trace.

Multiple linear regression of extensibility data for all sites with 685 samples was used to perform an ANOVA (Table 6).

In Table 7, the estimates of the regression coefficients for each allele are listed, with the alternative allele being assigned a coefficient value of zero.

In a similar manner to that in which glutenin alleles have been ranked for strength, so a glutenin extensibility score (GES) is proposed for producing a weighted ranking of LMW glutenin alleles (Table 8)

Discussion

Two fundamental properties of a dough (strength and extensibility) can measured by a range of instrumental methods. The magnitude of these two rheological properties of a dough is influenced by the *Glu-1* and *Glu-3* alleles as shown by bulk segregant analysis, a study of the allelic distribution within the Cranbrook/Halberd population, and by multiple linear regression and ANOVA of the population lines.

Table 7. Regression estimate of extensibility
s.e., standard error; *t* (674), *t*-statistic with 674 degrees of freedom; *t* pr., probability at this *t* statistic; Site A, Roma 1997 rep1; Site B, Roma 1997 rep 2; Site C, Stow 1997; Site D, Stow 1998

	Estimate	s.e.	*t* (674)	*t* pr.
Constant	16.050	0.752	21.34	<0.001
Flour protein	0.572	0.049	11.65	<0.001
Site B	0.464	0.156	2.97	0.003
Site C	–1.944	0.208	–9.36	<0.001
Site D	–1.434	0.228	–6.28	<0.001
Glu-A1b	0.072	0.113	0.64	<0.001
Glu-B1i	–0.228	0.114	–1.99	<0.001
Glu-D1d	–0.496	0.115	–4.32	<0.001
Glu-A3e	–0.936	0.113	–8.32	<0.001
Glu-B3d	1.225	0.118	10.38	<0.001
Glu-D3c	–1.134	0.113	–10.07	<0.001

Table 8. Predicted glutenin extensibility score (GES) for Extensograph extensibility for each allele

GES	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*
3			d	a
2.5		b		
2				
1.5	a			
1				
0.5				
0	d	e	c	c

Dough strength

The ranking within the *Glu-1* loci matches that of Payne *et al.* (1987) (Table 1) and the additional *Glu-B1e* allele matches that reported by Bushuk (1998). The magnitude of the various *Glu-1* alleles, however, differs a little from Payne *et al.* (1987) with the *Glu-1* contributing less to the total GSS, to allow for the *Glu-3* contribution. When the rankings of the various alleles were compared with the Australian material of Gupta *et al.* (1991), they indicated either similar rankings (*Glu-B1 i >e*, *Glu-A3 b >e*, and *Glu*-D3 a >*c*) or no data were available (*Gli-B3d v. Glu-B3c*). The major difference to the findings of Gupta *et al.* (1991) was that they found no significant difference between *Glu-D1d* and *Glu-D1a* in Australian material, although this was not the case with the world material. When the GSS (Table 4) is applied to the parents, a score of 6 and 4 is obtained for Cranbrook and Halberd, respectively. This ranks the varieties correctly for Rmax and allows for the possibility of other varieties with different allelic combinations, to obtain higher GSS values, at higher Rmax values than Cranbrook.

Dough extensibility

When the allelic rankings for Rmax (Table 4) and Ext (Table 8) are compared with those of Gupta *et al.*(1991), there is general agreement in the rankings for the *Glu-D1*, *Glu-A3*, and *Glu-D3* loci with no significant difference for the *Glu-A1* and *Glu-B1* locus as found in the Cranbrook/Halberd population. The protein content of a flour is predominantly controlled by the environment rather than the genotype, with the site and year having a major influence. However, genetic gains in improving extensibility can still be made in a breeding program by selecting particular LMW glutenin alleles.

It is interesting to note that the only locus to show contrary ranking for Rmax and Ext is *Glu-D1*, where for Rmax *Glu-D1d* >*Glu-D1a*, whereas the reverse applies for Ext. This means that if one selects the *Glu-D1d* allele rather than *Glu-D1a*, this will result in an average increase in Rmax of 143 BU, but at the expense of a decrease in extensibility of 0.5 cm. Many Australian Prime Hard wheats have been based on the variety 'Cook', which has a medium dough strength and high extensibility. The high extensibility is most probably due to the presence of the (*Glu-D1a*), as well as the particular *Glu–3* alleles that confer high extensibility (*Glu-A3b*, *Glu-B3b*, *Glu-D3b*). This strategy of using the *Glu-D1a* allele extensively in Australian Prime Hard wheats has been quite successful in producing varieties much sought after in the market place.

Breeding strategies

Using the Cranbrook/Halberd doubled haploid population it has been possible to produce a simple scoring system that ranks the *Glu-1* and *Glu-3* alleles for dough strength and extensibility out of 10. The *Glu-1* alleles make the major contribution to dough strength as measured by the GSS. The dough strength is independent of the flour protein level ($r = 0.143$ between Rmax and flour protein). There is a major genetic effect of both the HMW and LMW glutenin alleles on dough strength, which can be selected for by breeders. Extensibility is predominantly controlled by the amount of glutenin, which is determined by the environmental conditions during grain filling and deposition of the endosperm proteins. The *Glu-3* alleles modify the level of extensibility with particular combinations enhancing it. This will allow breeders to select lines with higher extensibility by ensuring that the protein achievement is adequate and the *Glu-3* alleles are optimum for a high GES. The same ranking of alleles is found for dough strength and extensibility, with the exception of alleles at the *Glu-D1* locus. However, the balance between the *Glu-1* and *Glu-3* alleles is different for each parameter, with the *Glu-1* alleles playing the major role in dough strength but the *Glu-3* alleles the major role in extensibility. This means that it will be possible, by judicious selection of both *Glu-1* and *Glu-3*, to obtain a wide range of dough strengths required for optimum processing for different end products, while still achieving a reasonable compromise of medium to high dough extensibility. Additional alleles can be added to the GSS and GES score, when the other doubled haploid populations are analysed for strength and extensibility. This refined scoring system has the potential to result in major improvements in the rheological properties of Australian wheats and hence end-product quality, if implemented by breeding programs throughout Australia.

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