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ORIGINAL PAPER

Genetic control of wheat quality: interactions between chromosomal regions determining protein content and composition, dough rheology, and sponge and dough baking properties

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Abstract While the genetic control of wheat processing characteristics such as dough rheology is well understood, limited information is available concerning the genetic control of baking parameters, particularly sponge and dough (S&D) baking. In this study, a quantitative trait loci (QTL) analysis was performed using a population of doubled haploid lines derived from a cross between Australian cultivars Kukri × Janz grown at sites across different Australian wheat production zones (Queensland in 2001 and 2002 and Southern and Northern New South Wales in 2003) in order to examine the genetic control of protein content, protein expression, dough rheology and sponge and dough baking performance. The study highlighted the inconsistent genetic control of protein content across the test sites, with only two loci (3A and 7A) showing QTL at three of the five sites. Dough rheology QTL were highly consistent across the 5 sites, with major effects associated with the *Glu-B1* and *Glu-D1* loci. The *Glu-D1* 5 + 10 allele had consistent effects on S&D properties across sites; however, there was no evidence for a positive effect of the high dough strength *Glu-B1-al* allele at *Glu-B1*. A second locus on 5D had positive effects on S&D baking at three of five sites. This study demonstrated that dough rheology measurements were poor predictors of S&D quality. In the absence of robust predictive tests, high heritability values for S&D demonstrate that direct selection is the current best option for achieving genetic gain in this product category.

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Introduction

The genetic improvement of wheat has focused on three broad areas, enhancement of yield, overcoming biotic and abiotic stresses, and improvement of processing and end use quality. In continuing to make progress in breeding wheat for food uses, breeders need access to tools that accurately predict processing and end use performance. These tools include both information concerning the genes that need to be combined to achieve a particular quality grade and the tools to rapidly measure the potential of individual lines to achieve the required specifications. The remarkable range of end products in which wheat is used (e.g., breads, noodles, cookies, breakfast cereals) creates a

broad spectrum of performance specifications with differences in genetic determinants.

The genetic analysis of wheat quality has been dominated by the investigation of the relationships between seed storage protein alleles and processing characteristics (for reviews see Bushuk 1998; Shewry et al. 1999; Gras et al. 2001; Goesaert et al. 2005). In bread wheat, genes controlling high molecular weight glutenin subunits (HMW-GS) are encoded by the *Glu-1* loci, *Glu-A1*, *Glu-B1* and *Glu-D1*, located on the long arms of the chromosomes of homoeologous group 1 (Payne 1987). The *Glu-B1* and *Glu-D1* typically each encode two subunits, named as x-type and y-type, whereas alleles so far found at *Glu-A1* in bread wheat cultivars encode either one x-type or neither subunit (Payne and Lawrence 1983; McIntosh et al. 1995). The low molecular weight glutenin subunits (LMW-GS) are controlled by genes at the *Glu-A3*, *Glu-B3* and *Glu-D3* loci on the short arms of chromosome 1AS, 1BS and 1DS, respectively. Genes coding for gliadin proteins are located on the short arms of group 1 and 6 chromosomes (Gao et al. 2007). While defining the glutenin and gliadin alleles provides important clues to the potential quality of a wheat variety, recent research has also identified the importance of understanding the relative expression levels of particular alleles and classes of seed storage proteins in determining quality. For example, the ratio of high- to LMW-GS (Huebner and Bietz 1984; Sutton 1991; Lafiandra et al. 1993) and the size distribution of the polymeric gluten proteins are both factors considered to be important in determining the end product performance of a given genotype grown under specific environmental conditions (MacRitchie 1992; Weegels et al. 1996; Sivri et al. 2004; Don et al. 2005). Control of grain hardness by defined alleles of the puroindoline genes, *Pina* and *Pinb*, has been extensively documented (Morris 2002).

Large scale rheological tests have been widely used as predictors of wheat processing and end product quality. Most commonly used dough rheology instruments are the Farinograph (Brabender 1932), Mixograph (Swanson and Working 1933; Rath et al. 1990; Shelke and Walker 1990), Extensograph (Campbell et al. 1987; Oliver and Allen 1992; Panozzo and Eagles 2000) and Alveograph (Faridi et al. 1987; Trethowan et al. 2001; Angioloni and Rosa 2007; De Vita et al. 2007). Small scale Mixographs, Farinographs and Extensographs have been developed and found to be highly related to traditional tests (Gras et al. 1990; Gras and Békés 1996; Kieffer et al. 1981a, b; Mann et al. 2003, 2005, 2007; Rath et al. 1990, 1994; Smewing 1995).

A wide range of baking methods are used in different markets for the production of differing styles of breads (Sluimer 2005). This study focuses on the sponge and dough method which includes a long fermentation process to develop more intense flavor components (Cauvain 2003). While a number of studies have examined the genetic control of dough properties (Cornish et al. 2001; Crepieux et al. 2005; Huang et al. 2006; Ma et al. 2005; Nelson et al. 2006; Perretant et al. 2000; Zanetti et al. 2001), only a small number of studies have examined the genetic control of end product quality using QTL studies. These include studies of French bread making (Groos et al. 2007), rapid dough breadmaking (Kuchel et al. 2006) Chorleywood bread process and no time mix baking procedure (Law et al. 2005), long-fermentation baking test using AACC method 10-10 (McCartney et al. 2006) and baking using an AACC method 10-10B straight dough method (Rousset et al. 2001). It is interesting to note that for the one parameter consistently measured across all studies, loaf volume, there is considerable variation in the nature of the QTL identified and it is important to note that some QTL, such as those on 2A (Kuchel et al. 2006), 3A (Law et al. 2005; Kuchel et al. 2006; Groos et al. 2007) and 5B, 7A and 7B (Groos et al. 2007) do not map to glutenin loci.

The primary objectives of this work were to (a) investigate linkages between regions of the wheat genome and S&D baking, (b) compare those regions of the genome controlling S&D baking traits, (c) examine the utility of dough rheology tests as predictors of baking outcomes, (d) investigate the impact of environment on the genetic control of baking quality. A doubled haploid wheat population Kukri × Janz (Kammholtz et al. 2001), comprising 160 lines was field trialed at four locations prior to grain composition, milling, rheological testing, baking and QTL analysis.

Materials and methods

Table 1 provides a description of the protein expression, dough rheology and sponge and dough baking parameters used in this study and the abbreviations used to refer to each method.

Germplasm

Kukri × Janz doubled haploid (DH) lines were produced using a wheat × maize (*Zea mays* L) production technique from F1 wheat (Kammholz et al. 2001). Kukri has unique high dough strength, while Janz is considered to have genes for wide adaptation and high yield in Australia. This cross is therefore of interest to study the genetic basis of dough rheology, in particular dough strength and extensibility. The same doubled haploid populations were used in each of the field trials. Table 2 gives the allelic composition of Kukri and Janz for glutenin, puroindoline and reduced height loci.

Table 1 Traits and their heritabilies

Abbreviation	Description	Narrabri		Hillston	
		H	CV	H	CV
Protein expression					
GPC	Grain protein content determined by NIR	0.69	3.5	NA	NA
Ax	HMWGS Glu1Ax protein content (mg/g flour), as determined by reverse phase HPLC	0.74	6.6	0.5	11.3
Bx	HMWGS Glu1Bx protein content (mg/g flour), as determined by reverse phase HPLC	0.88	10.7	0.87	11.8
Dx	HMWGS Glu1Dx protein content (mg/g flour), as determined by reverse phase HPLC	0.64	8.7	0.36	12.6
Dy	HMWGS Glu1Dy protein content (mg/g flour), as determined by reverse phase HPLC	0.48	11.7	0.12	14.3
LMW	Total LMWGS protein content (mg/g flour), as determined by reverse phase HPLC	0.53	3.7	NA	NA
ABG	Total $\alpha\beta$-gliadin protein content (mg/g flour), as determined by reverse phase HPLC	0.72	6.6	0.07	16.2
GG	Total γ-gliadin protein content (mg/g flour), as determined by reverse phase HPLC	0.68	5.3	0.2	14
OG	Total ω-gliadin protein content (mg/g flour), as determined by reverse phase HPLC	0.45	9.2	NA	NA
UPP	Unextractable polymeric protein content (mg protein/g flour) as determined by size exclusion HPLC	0.63	3.2	0.65	3.6
Dough rheology					
WA	Water absorption determined by 4 g Z-arm mixer	0.82	1.5	0.66	2.2
TPDD	Time to peak dough development determined by 4 g Z-arm mixer	0.8	17.4	0.65	27.5
PR	Peak Resistance determined by 4 g Z-arm mixer	0.48	3	0.53	2.8
STAB	Dough Stability determined by 4 g Z-arm mixer	0.77	20.5	0.54	29.1
MT	Mixing time determined by 10 g mixograph. The time from first addition of water to that point in maximum consistency range immediately before first indication of weakening	0.79	6.9	0.86	10.1
MBW	Maximum band width determined by 10 g Mixograph. The maximum bandwidth during the mixing (arbitrary units)	0.81	5	0.8	2.8
RBD	Resistance break down determined by 10 g Mixograph. The slope of decline of resistance after the peak, measured in the percentage of PR	0.56	20	0.43	14.1
Rmax	Maximum resistance determined by modified Kieffer micro-extension testing. Maximum resistance on the extension curve	0.72	15.6	0.81	15.5
Height	Maximum height of the Extensograph curve	ND	ND	ND	ND
Ext_{Rmax}	Extension to maximum resistance determined by Kieffer micro-extension testing	0.73	9.0	0.75	7.9
Length	Maximum length of the dough piece at rupture in an Extensograph test	ND	ND	ND	ND
Sponge and dough baking					
SDV	Sponge and dough loaf volume	0.57	3.1	0.57	3.3
SDOS	Sponge and dough oven spring	0.64	27	0.67	33.6
SDSA	Sponge and dough slice area determined by C-Cell	0.60	3.3	0.56	3.3
SDCN	Sponge and dough cell number determined by C-Cell	0.64	6.9	0.67	6.4
SDACA	Sponge and dough average cell area determined by C-Cell	0.55	1.2	0.58	1.1
SDCWT	Sponge and dough cell wall thickness determined by C-Cell	0.52	2.2	0.61	2.2
SDCF	Sponge and dough crumb firmness determined by texture analyser	0.47	10.6	0.60	7.8
SDML	Sponge and dough crumb Minolta L value	0.28	1.1	0.03	1.2
SDMA	Sponge and dough crumb Minolta A value	0.77	8.2	0.83	7.7
SDMB	Sponge and dough crumb Minolta B value	0.73	5.0	0.87	3.4

NA indicates no genetic variation for the trait, ND not determined at the Narrabri or Hillston sites

Field trials

Field trials were conducted at four sites, Narrabri (northern New South Wales, in 2003), Hillston (southern New South Wales, in 2003), Biloela (central western Queensland, in 2001 and 2002) and Lundavra (southern Queensland, 2002). The Narrabri and Hillston trials included 160 DH lines in a field trial design utilizing 220 plots arranged in a rectangular array of 10 rows by 22 columns (ranges).

NIR

The protein and moisture content of the grain and flour samples from the Narrabri and Hillston sites were

Table 2 Glutenin and Puroindoline alleles for Kukri and Janz

	High molecular weight glutenin alleles			Low molecular weight glutenin alleles			Hardness locus alleles		Reduced height loci
	Glu-A1	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*	*Pina*	*Pinb*	*rht*
Kukri	*a (Ax2*)*	*al (Bx7 + By8*)*	*d* (Dx5 + Dy10)	*d*	*h*	*b*	*Pina-D1b*	*Pinb-D1a*	*Rht2*
Janz	*a (Ax2*)*	*b (Bx7 + By8)*	*a* (Dx2 + Dy12)	*b*	*b*	*b*	*Pina-D1a*	*Pinb-D1b*	*Rht1*

determined by infrared reflectance (NIR) according to AACC method 39-11 (AACC 1999). Grain protein and hardness for samples from the Biloela and Lundavra sites were determined on whole grain samples using a NIR Systems 6500 instrument calibrated against a reference method for protein, RACI method 02-03 (RACI 2003a) and hardness, AACC method 55-30 (AACC 2000c).

Single kernel grain classification

Grain weight, diameter and hardness were measured using a Single Kernel Classification System (SKCS 4100) instrument according to the manufacturer's instructions (Perten Instruments, Springfield IL).

Milling

Grain samples from 216 of the field plots from the Narrabri and Hillston sites were milled at BRI Research using a laboratory scale Buhler mill (MLU-202) according to an optimized BRI Research method which was based on the AACC method 26-21A (AACC 2000a). Grain samples from the Biloela and Lundavra sites were conditioned to 15% moisture content prior to milling through a Buhler, MLU-202, pneumatic mill by AACC Method 26-21A (AACC 2000a) at the Queensland Department of Primary Industries and Fisheries (QDPI&F).

Protein expression analysis

Reversed-phase high performance liquid chromatography (RP-HPLC)

For the analysis of gliadins and glutenins by RP-HPLC, the following procedure was used: Gliadins were extracted from 50 mg flour with 1 ml 70% (v/v) ethanol, vortexed for 1 h at room temperature and centrifuged at 17,000×*g* for 15 min. The supernatant was used for gliadin analysis. Pellets remaining after gliadin extraction were further extracted twice with 1 ml 50% (v/v) propan-1-ol for 30 min at room temperature in 1.5 ml Eppendorf tubes with shaking, and centrifuged at 17,000×*g* for 15 min. The pellets were resuspended in 1 ml 50% (v/v) propan-1-ol/ 0.1 M Tris, pH 6.6/2 M urea/1% (w/v) DTT at 60°C. After 1 h reduction, samples were centrifuged for 15 min, the glutenin containing supernatants were removed and placed in fresh Eppendorf tubes, and alkylated with 4-vinylpyridine (10 µl) at 60°C for 15 min.

RP-HPLC analyses were carried out by injecting 20 µl samples into a Waters HPLC system using a Vydac column (C18, 300 Å pore size, 10 µm particle size, 250 × 4.6 mm ID) at 70°C with a solvent flow rate of 1 ml/min. Elution was achieved using a gradient system formed from two solvents: A, Millipore water containing 0.07% (v/v) of TFA and B, acetonitrile containing 0.05% (v/v) TFA. Separate elution profiles were used to resolve gliadins and polymeric proteins. For gliadins, following a 1 min isocratic elution period when a mobile phase comprising 75% A and 25% B was pumped, a linear gradient from 25% B to 50% B was applied over a 55 min period. Polymeric proteins were resolved with a linear gradient extending from 24% B to 48% B over a 55 min period. Eluents were monitored at 214 nm.

Size exclusion high performance liquid chromatography (SE-HPLC)

SE-HPLC was performed to assess the proportion of the main classes of storage proteins (glutenins, gliadins, albumins/globulins). Total proteins were extracted according to Batey et al. (1991). Extracts (20 µl) were injected into a Phenomenex Biosep™ Sec-4000 column (Phenomenex, Torrance, CA, USA) to run for 35 min with an eluent containing 50% (water + 0.05% TFA) and 50% (acetonitrile + 0.05% TFA) using a Waters HPLC system (Waters Corporation, Milford, MA, USA) comprising two model 510 pumps, a 712 WISP automatic sampler, a model 481 UV–visible detector at 214 nm and using Millennium Chromatography Manager Software Version 2.15 for integration purposes.

The total polymeric protein was separated into extractable and unextractable (residue) fractions based on its extractability in 0.5% (w/v) SDS- 0.05 M Na phosphate buffer (pH 6.9) (without sonication). To solubilize the remaining protein (unextractable protein), the residue was sonicated for 30 s. Both extracts were filtered through 0.45 µm PVDF filters before chromatography on a Phenomenex BIOSEP-SEC 4000 column (Phenomenex, Torrance, CA, USA)

connected to a Beckman System Gold high performance liquid chromatograph using the 0.5% (w/v) SDS- 0.05 M Na phosphate buffer (pH 6.9) as mobile phase.

Dough rheology testing

(1) *Small scale rheology testing for samples from the Narrabri and Hillston sites Z-arm mixer*; Water absorption and z-arm mixing characteristics of 216 samples were carried out according to the milling design. Optimum water absorption values of the DH population lines were determined with a prototype Micro Z-arm Mixer (Bekes et al. 2002) which uses 4 g of test flour per mix. Constant angular velocity (with shaft speeds for the fast and slow blades of 96 and 64 rpm, respectively) was used during all mixes. Torque outputs were calibrated to give Brabender Unit equivalent units. Mixing was carried out in triplicate, each for 20 min. Before adding water to the flour, the baseline was automatically recorded (30 s) by mixing only the solid components. The water addition was carried out in one step using an automatic water pump. The following parameters were determined from the individual mixing experiments by taking the averages: WA%- water absorption determined at 500 BU dough consistency: TPDD (time to peak dough development); the interval, from first addition of water to the point in maximum consistency range immediately before first indication of weakening (AACC method 54-21, AACC 2000b): stability (STAB); the time difference between the points where the top of the curve first intersects 500 BU and where it leaves 500 BU line: breakdown (BD); the difference in BU from top of the curve at peak to top of the curve 5 min after peak (AACC method 54-21, AACC 2000b).
(2) *Mixograph*; Small scale Mixograph (10 g) characterization of 216 flour samples was carried out according to a method based on AACC method 54-40 (AACC 2000b). Samples with variable water absorption corresponding to flour protein were mixed in a 10 g CSIRO prototype Mixograph (Gras et al. 1996) keeping the total dough mass constant. For each of the flour samples, the following parameters were recorded: MT, mixing time (sec); PR, Mixograph peak resistance (arbitrary units, AU); BWPR, band width at peak resistance (arbitrary units, AU); RBD, resistance breakdown (%); BWBD, bandwidth breakdown (%); TMBW, time to maximum bandwidth (s); and MBW, maximum bandwidth (arbitrary units, AU) (Gras et al. 2001).
(3) *Uniaxial Extension Testing*; Doughs with a final mass of 17.5 g were mixed to peak dough development in a 10 g prototype Mixograph for extension testing on the TA.XT2i texture analyzer using a Kieffer rig (Stable Micro Systems, Godalming, UK). Extension at 1 cm/s was carried out in five replicates on a TA.XT2i texture analyzer with a modified geometry Kieffer dough and gluten extensibility rig (Mann et al. 2003). Dough samples for extension testing (~1.0 g/test) were molded with a Kieffer molder, and rested at 30°C and 90% RH for 45 min before extension testing. The Rmax and Ext_{Rmax} were determined from the data with the help of Exceed Expert software (Smewing 1995; Mann 2002). Flour water absorption and dough development time for samples from the Biloela and Lundavra sites were determined using the 50 g Farinograph (Brabender Duisburg, Germany) as outlined in RACI method 06-02 (RACI 2003c). Maximum extensibility and maximum resistance at 45 min were determined from two dough pieces of 150 g each from one mix as outlined in RACI method 06-01 (RACI 2003b) using the Farinograph–Resistograph and Extensograph (Brabender Duisburg, Germany).

Sponge and dough (S&D) baking

Sponge and dough baking was conducted for samples from the Narrabri and Hillston sites by BRI Research, and for samples from the Biloela and Lundavra sites, by QDPI&F. The sponge and dough baking methodology used in these laboratories had previously been co-developed and cross-calibrated (Lever et al. 2005). The BRI Research sponge and dough baking involves a two-step process. In the first step, the sponge is made by mixing part of the total flour with water, yeast and yeast food. The sponge is allowed to ferment for 4 h. In the second step, the sponge is incorporated with the rest of the flour, water and other ingredients to make dough. The sponge stage of the process was made with 200 g of flour and was given 4 h fermentation. The dough was prepared by mixing the remaining 100 g of flour and other ingredients with the fermented sponge. Both the sponge and the doughs were mixed in a 300 g-Farinograph bowl. Mixing time was controlled using Easymix software (BRI Research) to indicate when the dough had reached optimum dough development. The water addition rate was adjusted based on the micro Z-arm (CSIRO) water absorption and was adjusted, if required, during the sponge mixing stage, using Easymix peak height, supplemented by baker's assessment. Each dough sample produced two high-top pup loaves and one square pup loaf. Loaf volume measurements were made on two high top loaves using rape seed displacement on the day of baking. Oven spring was measured from the top of the tin

to the point at which the upper crust surface changes from being rough to smooth. The measurement is made on both sides of the loaf and the results added together. Crumb structure and slice parameters (parameters SDSA, SDCN, SDACA, SDCWT, see Table 1) were measured on the day after using a C-Cell digital image analysis instrument using the manufacturer's analysis software and protocol (Calibre Instruments UK). Crust and crumb color (Minolta Chroma Meter CR 300) were measured on a square sandwich style loaf as was crumb texture (Stable Microsystems, TA XT 2 fitted with a 20 mm diameter probe).

Sponge and dough (S&D) baking for the Biloela and Lundavra sites: Sponge and dough baking quality was measured using a modified formula and procedures developed and described in Lever et al. (2005). The formula modification involved using a bread improver blend based on 0.5 g calcium hydrogen orthophosphate, 0.12 g ammonium sulfate and 1.0 g malt flour.

Statistics and QTL analysis

A genetic linkage map consisting of 246 segregating loci has been constructed for the Kukri x Janz doubled haploid population using 239 microsatellites and 7 allele specific DNA markers analyzed by Syngenta Toulouse (France) and CSIRO Plant Industry (Australia) laboratories. The map was analyzed in the statistical software package R (R Development Core Team, 2006) using the R/qtl library (Broman et al. 2003) resulting a map based on 246 markers spread over 21 linkage groups and scored over 172 genetic lines. The phenotypic data for each trait/site combination was analyzed separately using techniques described in Cullis et al. (2003) to account for field and laboratory variation. QTL analysis was undertaken using the whole of genome regression approach of Verbyla et al. (2007). Chromosomes containing clusters of QTL are shown in Fig. 1. QTL are considered to be members of such regions if their peak interval falls within a region 20 cM proximal, and 20 cM distal, of the modal interval location of that group of QTL.

Multivariate mixed model analysis of traits

A multivariate mixed model analysis of four traits (Protein, Length, Height and SDV) was conducted for three sites analyzed by the same laboratory (QDPI&F) in order to estimate the genetic correlations between traits, while simultaneously accommodating non-genetic variation. The analysis assumes linearity of relationships between traits and in order to achieve this it was necessary to transform the data for each trait to a logarithmic scale (Mann et al. 2005, 2007). As a precursor to the multivariate analysis, each trait was analyzed separately using the techniques described in Cullis et al. (2003). The base-line mixed model for each trait comprised sources of variation for DH lines (that is, genetic variation) and non-genetic variation partitioned according to the experimental designs. The latter comprised variation between field plots, between milling days, between milling samples within milling days (that is, residual milling variation), between blocks of extension testing days, between extension testing days within blocks and residual variation. The base-line analyses were then extended to include modeling of trend, both in the field and the laboratory. All statistical analyses were conducted using the ASReml package (Gilmour et al. 2002). Details of the experimental designs used at the field trial, milling, rheology and baking stages are given in the supplementary data.

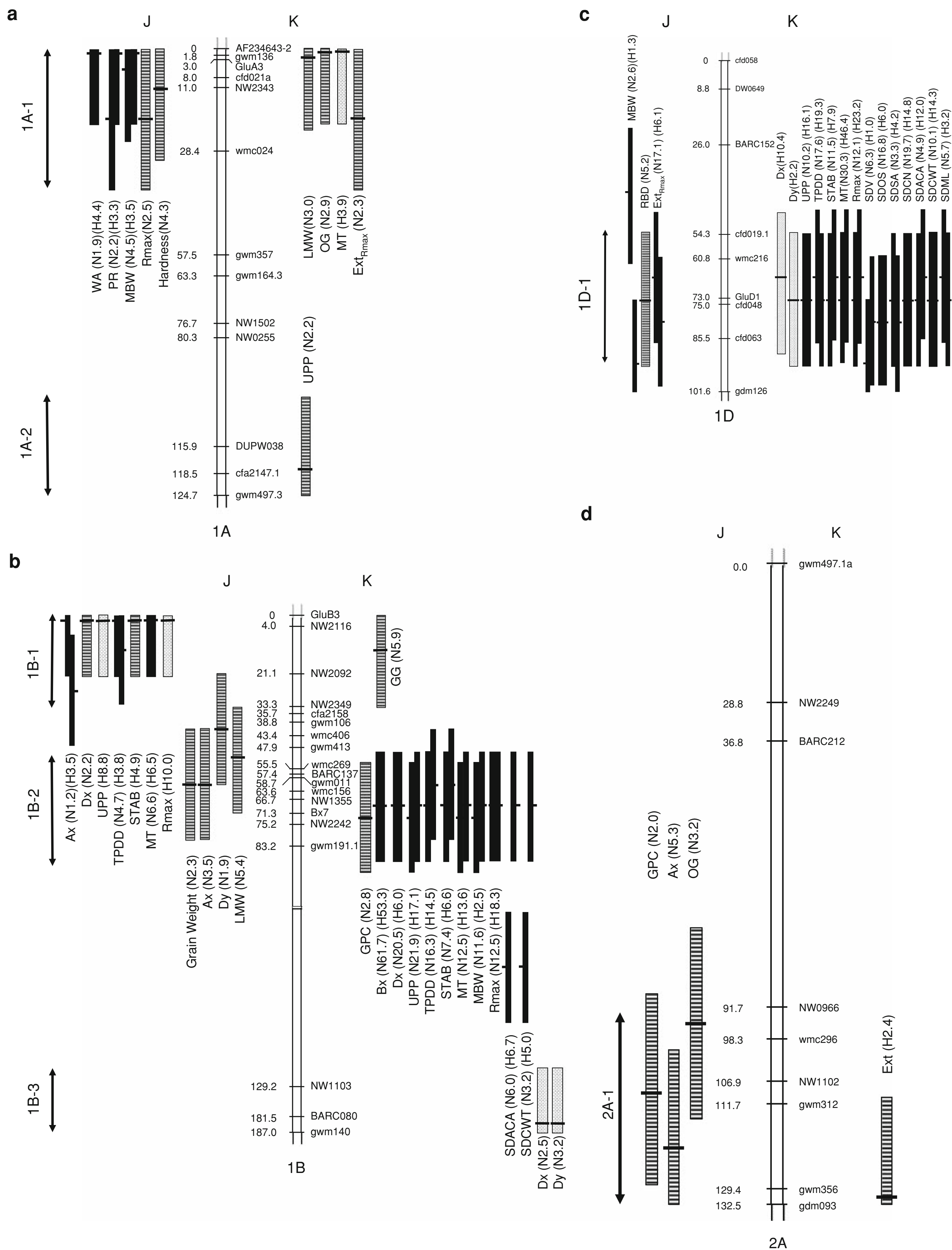
Fig. 1 Maps of chromosomes containing QTL found from analysis of ▶ the Kuri × Janz population grown at the Narrabri and Hillston sites. QTL where the effect is donated by Janz are shown on the left of the chromosome genetic map, and QTL donated by Kukri are shown on the right of the map. QTL expressed at both Narrabri and Hillston are shown by filled bars, QTL expressed only at Narrabri are shown by *bars* filled by horizontal stripes, and QTL expressed only at Hillston are shown by *bars* with shaded fill. *Bars* indicating QTL extend for 20 cM from the mid point of the interval centring the QTL (indicated by a *horizontal bar*). Map distances are in cM. **a** Chromosomes 1A. **b** Chromosome 1B. **c** Chromosome 1D. **d** Chromosome 2A. **e** Chromosome 2B. **f** Chromosome 3A (g) chromosome 4B. **h** Chromosome 4D. **i** Chromosome 5A. **j** Chromosome 5B. **k** Chromosome 7A

Results

The same Kukri × Janz doubled haploid population was phenotyped between 2001 and 2003. Groups at Plant Industry and BRI Research Ltd analyzed the population grown at two sites, Narrabri and Hillston, in 2003, while QDPI&F phenotyped the population grown at Biloela (2001, 2002) and Lundavra (2002). This manuscript reports the joint analysis and interpretation of the results of the two research programs, with both sets of phenotyping being analyzed using the one genetic map and QTL analysis procedures. Four parameters were measured across all of these sites; grain protein content (GPC), Rmax, extensibility, and sponge and dough loaf volume. A summary of the data collected from samples grown at these sites is given in Table 3. The strength of this approach is that it provides a means to identify QTL that are common across sites and years, and common across independent phenotyping programs.

Grain physical parameters

Kukri and Janz differ in puroindoline alleles, with Kukri containing the *Pina-D1b* and *Pinb-D1a* alleles, while Janz contains the *Pina-D1a* and *Pinb-D1b* alleles. QTL for grain hardness were found on chromosome 5D at a position consistent with the *Ha* locus at both the Narrabri (LOD



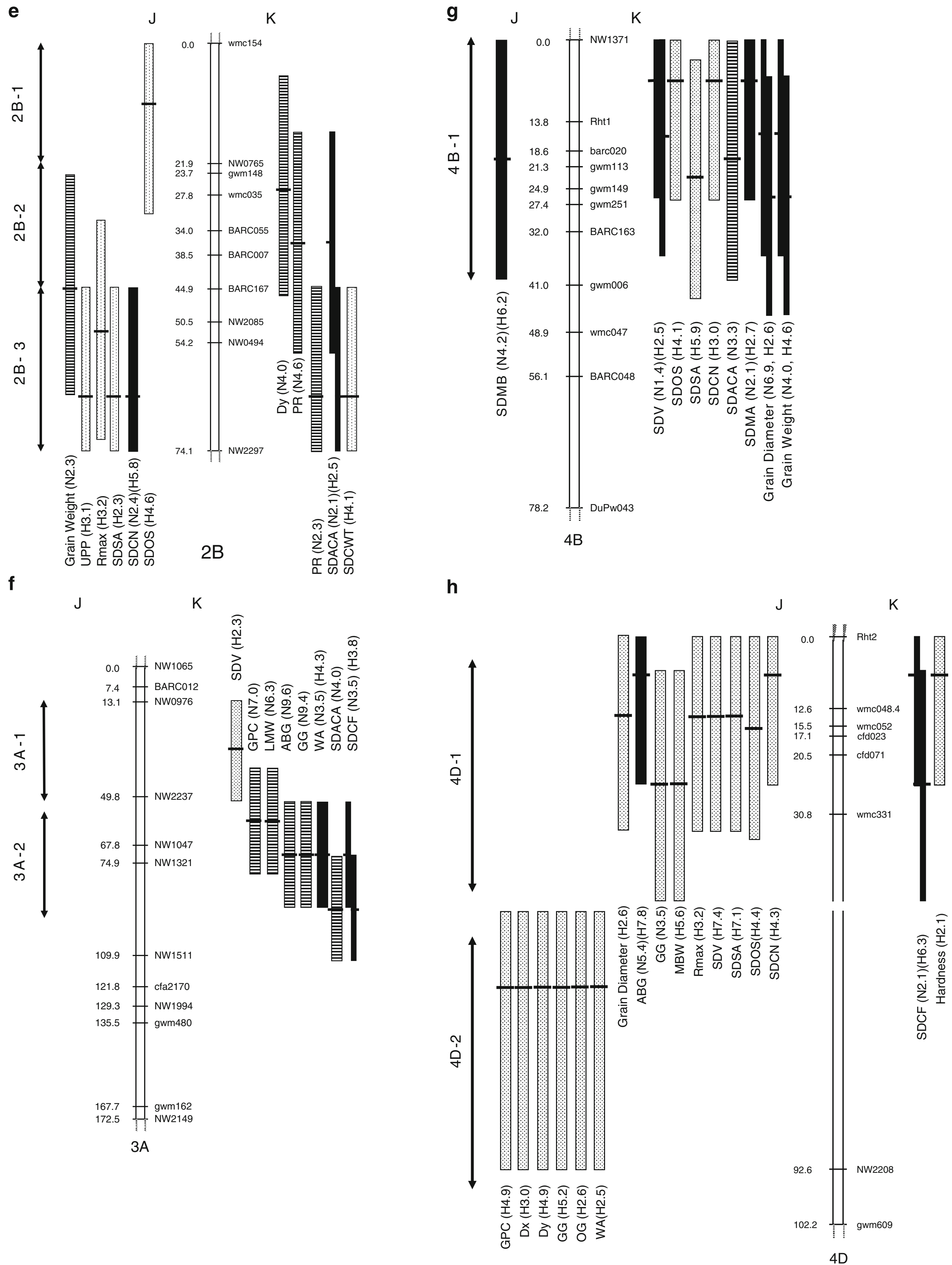


Fig. 1 continued

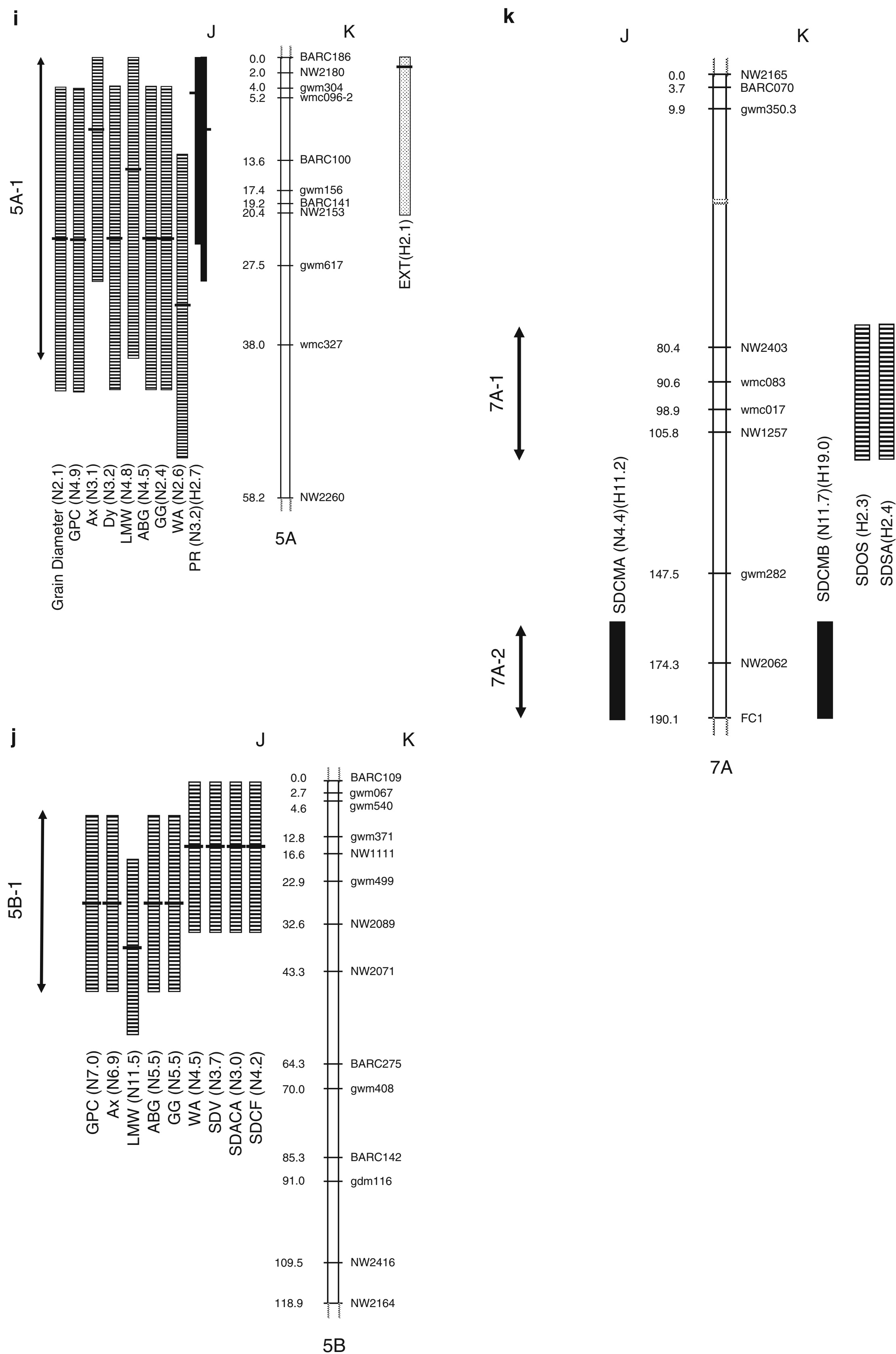


Fig. 1 continued

Table 3 Summary of data from 5 trial sites

Trait[a]	Site	Method[b]	Units	Test plot (Mean ± SD	Test plot minimum	Test plot maximum	Kukri[c] (Mean ± SD)	Janz (Mean ± SD)
GPC	N03	NIR	%	12.9 ± 0.6	11.5	14.4	12.8 ± 0.6	12.6 ± 0.4
	H03	NIR	%	10.5 ± 1.1	8.1	14.1	10.9 ± 1.0	9.6 ± 1
	B01	NIR	%	13.8 ± 0.4	12.8	14.8	13.6 ± 0.6	12.8 ± 0.2
	B02	NIR	%	12.3 ± 0.6	10.9	13.9	12.3 ± 0.4	11.9 ± 0.4
	L02	NIR	%	14.6 ± 0.6	13.1	16.2	15.1 ± 0.7	14.1 ± 0.6
Maximum	N03	Kieffer	N	0.25 ± 0.06	0.12	0.45	0.27 ± 0.05	0.2 ± 0.03
Resistance	H03	Kieffer	N	0.19 ± 0.05	0.09	0.38	0.21 ± 0.02	0.16 ± 0.02
	B01	Extensograph	BU	427 ± 68	265.6	613.8	484 ± 51	336 ± 47
	B02	Extensograph	BU	597 ± 100	288.8	805.0	655.6 ± 92	482.3 ± 64
	L02	Extensograph	BU	588 ± 110	285.0	780.0	678 ± 151	604 ± 54
Extensibility	N03	Kieffer	mm	116.0 ± 15.6	66.1	152.5	119 ± 9.4	121 ± 12.4
	H03	Kieffer	mm	99 ± 11.7	68.9	131.9	102 ± 6	95 ± 5
	B01	Extensograph	cm	22.8 ± 1.5	18.9	26.4	22.3 ± 1.8	22.1 ± 1.0
	B02	Extensograph	cm	18.0 ± 1.7	13.9	24.1	17.8 ± 1.6	17.7 ± 0.77
	L02	Extensograph	cm	22.4 ± 2.2	17.0	26.5	23.7 ± 2	22.6 ± 0.9
SDV	N03	BRI	cm	826 ± 27	764.4	913.8	848 ± 30	809 ± 30
	H03	BRI	cm	749 ± 28	662.5	824.4	739 ± 23	732 ± 20
	B01	QDPI&F	cm	809 ± 30	723.7	883.8	841 ± 30	801 ± 31
	B02	QDPI&F	cm	752 ± 45	647.3	865.8	694 ± 32	809 ± 25
	L02	QDPI&F	cm	768 ± 49	610.0	898.0	784 ± 40	771 ± 27.7

NO3 Narrabri 2003, *H03* Hillston 2003, *B01* Biloela 2001, *B02* Biloela 2002, *L02* Lundavra 2002, *SD* standard deviation, *N* Newtons, *BU* Brabender units, *BRI* sponge and dough baking method used by BRI Ltd, *QDPI&F* sponge and dough baking method used by QDPI&F

[a] Trait abbreviations given in Table 1

[b] Methods outlined in Table 1

[c] Means and standard deviations for Kukri and Janz parental lines calculated from a minimum of 10 replicated plots at each site

10.3) and Hillston (LOD 2.7) with the *Pina-D1b* and *Pinb-D1a* allele conferring a harder grain phenotype. These results are consistent with previous studies of the impact of these alleles (Cane et al. 2004). Additional QTL for grain hardness were also found on 1A (LOD 4.3) and 4D (LOD 2.1) at Narrabri (Fig. 1a). The 4D hardness QTL is in a position consistent with the location of the Kukri-derived *Rht*-2 dwarfing gene (Fig. 1h). Grain weight and diameter were influenced by loci on 4B and 4D consistent with the location of the *Rht*-1 and *Rht*-2 genes, respectively (Fig. 1g, h) where the influence of the *Rht* genes results in reduced seed weight and diameter. A summary of QTL obtained for grain hardness, grain weight and grain diameter is given in supplementary data Table 1.

Quantitative analysis of flour protein content and protein expression

Grain protein content

Average protein content for the sites ranged from 10.5% at Hillston 2003 to 14.6% at Lundavra in 2002 (Table 3). Across all sites except Hillston, the heritability of protein content was high (ranging from 0.69 to 0.93) and CVs were low (ranging from 1.5 to 3.5%) (Table 4). Across the five sites, two QTL, on chromosomes 3A (Fig. 1f) and 7A (Fig. 1k), were present at three of the five sites, albeit with low LOD scores for the QTL on 7A (Table 4). QTL on two regions of chromosome 1B (Fig. 1b) and 5A (Fig. 1i) were observed at two sites (Table 4).

Protein expression levels

Heritability, CV and QTL data for all protein alleles and classes are given in supplementary data Table 2. Analysis of protein expression by quantitative HPLC analysis was conducted for two sites only, Narrabri 2003 and Hillston 2003. The heritabilities of the various classes of seed storage proteins differed, as did the heritabilities for some classes between sites. For example, *Glu-B1x* expression is a highly heritable trait at both sites, whereas Ax, Dx, Dy, gliadin, $\alpha\beta$ gliadin, γ gliadin and low molecular weight glutenin expression were significantly less heritable at Hillston 2003 than Narrabri 2003.

Table 4 QTL obtained at all sites for GPC, uniaxial extension parameters and sponge and dough loaf volume

Chromosome				1A	1B	1B	1B	1D	2B	3A	4A	4D	5A	5B	5D	7A	7B	7B
Marker interval				(5–6)	(2)	(12–15)	(17–18)	(6–7)	(4–9)	(4–6)	(9–10)	(2–5)	(9–10)	(5–8)	(5–8)	(6–7)	(2–5)	(8–9)
Trait[a]	Site	H	CV[b]															
GPC	N03	0.69	3.5			K2.8				K7.0			J4.9	J7.0				
	H03	NA	NA															
	B01	0.93	1.5							K2.6						J1.3		
	B02	0.82	2.5				K2.3									J2.9		
	L02	0.84	2.6			K5.3	K3.8			K11.2			J2.4			J2.1		
Rmax[a]	N03	0.72	15.6	J2.4		K12.5		K12.1										
	H03	0.81	15.5		J10.0	K18.3		K23.2	J3.2			J3.2						
	B01	0.95	6.3		J4.7	K23.6		K56.4	J4.1									K2.4
	B02	0.88	7.7		J1.5	K13.6		K26.0	J6.2		J2.4	J3.8						
	L02	0.80	11.5			K10.6		K18.9			J2.3							K2.7
Ext[b]	N03	0.73	9.0	K2.3				J17.1									K2.6	
	H03	0.75	7.9					J6.1									K2.1	
	B01	0.87	4.4	J4.6		K14.4		J2.5										
	B02	0.86	4.7				K3.7	J8.6				J6.5						
	L02	0.75	7.7			K 2.6		J8.1										
SDV	N03	0.57	3.1					K6.3						J3.7				
	H03	0.57	3.3					K1.0		K2.3		J7.4						
	B01	0.81	2.5					K10.6				J5.9			K4.0		J2.1	
	B02	0.83	16.2			J5.4		J9.0						J4.7	K5.3			
	L02	0.78	18.9			J8.1									K2.2			

QTL data are LOD scores. The donor of the numerically larger effect is indicated by K (Kukri) or J (Janz)

NO3 Narrabri 2003, *H03* Hillston 2003, *B01* Biloela 2001, *B02* Biloela 2002, *L02* Lundavra 2002, *H* heritability

[a] Trait abbreviations and methodologies are described in Table 1. Maximum resistance determined as Rmax for samples from the Narrabri and Hillston sites, and Extensograph Height for samples from the Biloela and Lundavra sites· Extensibility (Ext) determined as Ext_{Rmax} for samples from the Narrabri and Hillston sites, and Extensograph Length for samples from the Biloela and Lundavra sites

[b] *CV* coefficient of variation. QTL at regions only represented at one site and for one trait are not included in the table: GPC, N03, 2A (7), J 2.0; GPC, B01, 7D (3), J 2.4; GPC, L02, 3B (3), J 3.2; GPC, L02, 3B (6), K 2.4; GPC, L02, 4B (3), K 7.4; GPC, H03, 4D; (7) J 4.9; GPC, L02, 7A (3), J 3.1; Height, B02, 3B (3), J 3.4; Height, B02, 3D (10), K 2.8; Height, B01, 3D (16), K 4.8; Height, L02, 5B (10) J 5.8; Ext_{Rmax}, H03, 2A (9), K 2.4; Ext_{Rmax}, H03, 5A (2), K 2.1; Length, L02, 4A (6), K 2.5; Length, B01, 4D (8–9), J 3.9; Length, B01, 5D (3), J 2.0; SDV, B02, 2D (2) J 2.2; SDV, H03, 4B (3) K 2.5; SDV L02, 5B (11) K 4.0; SDV, H03, 5D, (11), K 2.8; SDV, B02, 5D (2) J 5.5; SDV, B01, 7A (2), J 3.9

High Molecular Weight Glutenins: The Kukri × Janz population segregates for x and y type high molecular weight glutenins at the *Glu-B1* locus (*Glu-B1-al*, Kukri; *Glu-B1-b* allele, Janz, Fig. 1b) and *Glu-D1* locus (5 + 10, Kukri; 2 + 12 Janz, Fig. 1c). At the *Glu-A1* locus both parents have the identical Ax1 allele, while the Ay allele is not expressed from this locus. The "x" subunits of the *Glu-A1*, *Glu-B1* and *Glu-D1* loci show differing patterns of control of protein expression.

The expression of *Glu-B1-al* at the protein level gives very strong QTL (Narrabri LOD 61.7; Hillston LOD, 53.3) indicating that *cis* acting factors associated with the gene are the primary controlling elements of *Glu-B1-al* protein expression. The control of expression of high molecular weight glutenin "x" subunits from the *Glu-A1* and *Glu-D1* loci is more complex. While expression of the Dx subunit at the protein level shows a strong QTL at the *Glu-D1* locus from Hillston (LOD 10.4), the expression of *Glu-D1* at the protein level is also controlled by a QTL consistent with the location of the *Glu-B1* locus at both Narrabri and Hillston (Narrabri LOD 20.5; Hillston LOD 6.0). The expression of the Ax protein is complex with a range of different loci influencing expression at each site; Narrabri, 2A; 5A; 5B; and 7D and Hillston; 1B; and 6A. Control of the expression of the Dy subunits at the protein level is independent of the Dx subunit despite their being encoded at the same locus.

Low Molecular Weight Glutenins and Gliadins; Consistent with the absence of heritability for LMW protein expression levels at Hillston, QTL for LMW glutenin subunit expression were observed at Narrabri but not Hillston. At Narrabri, four of the five QTL found for this trait were at the same loci as control grain protein content at the Narrabri site, on chromosomes 1B, 3A, 5A and 5B (Fig. 1j) while a QTL on 1A is independent of protein content and located at a position consistent with the location of the *Glu-A3* locus containing the LMW genes (Fig. 1a).

The expression of the $\alpha\beta$ gliadin proteins showed a consistent QTL on 4D at Narrabri and Hillston (Fig. 1h). At the Narrabri site, loci on 3A, 5A and 5B are all loci associated with increased protein content, while a QTL on 6A is present in a region of the chromosome containing the *Gli-2* locus, the location of the $\alpha\beta$-gliadin genes (Gao et al. 2007). The expression of the γ gliadin family at the protein level shows QTL in different regions of chromosome 4D at the two sites, with QTL in region 4D-1 at Narrabri and 4D-2 at Hillston (Fig. 1h) although it should be noted that there is a lack of marker density in the central region of chromosome 4D and this anomaly may be resolved if a higher marker density map were available for this chromosome. QTL for γ gliadin were also found at the protein content loci on 3A, 5A and 5B. At Narrabri, there is a weak QTL on 6A and a QTL associated with the *Glu-B3* locus on 1B (Fig. 1b). In contrast, the expression of the ω gliadin family at the protein level is under quite different genetic control to other members of the gliadin family, with QTL on 1A (Fig. 1a) and 2A (Fig. 1d) at Narrabri and 3B and 4D (Fig. 1h) at Hillston.

Unextractable Polymeric Protein (UPP); UPP is a parameter determined by measuring the percentage of the polymeric proteins in a dough that are unable to be extracted in an SDS-buffer solution following sonication, and has been suggested to be a predictor of dough strength (Gras et al. 2001). In this analysis, strong QTL for UPP were associated at both the Narrabri and Hillston sites with the *Glu-B1* and *Glu-D1* loci, indicating a strong degree of control of this parameter by HMW-GS. At Hillston, there is also a significant QTL on 1B consistent with the *Glu-B3* locus (Fig. 1b) and a non-glutenin associated QTL on 2B (Fig. 1e).

Dough rheology traits

Analysis of Z-arm mixer (4 g) and Mixograph (10 g) traits was conducted for two sites, Narrabri and Hillston 2003. Heritability, CV and QTL data are given in supplementary data Table 3.

Z-arm mixer water absorption

QTL for water absorption at both sites were associated with either glutenin loci or increased protein content. At both sites, QTL were found on 3A (protein content locus, Fig. 1f) and on 1A at a location consistent with *Glu-A3* locus (Fig. 1a). Additional QTL were found at the Narrabri site on 5A (Fig. 1i) and 5B (Fig. 1j) in positions co-located with QTL for protein content.

Z-arm mixing traits

A number of dough rheology traits are measured by the Z-arm mixer including; time to peak dough development (TPDD), peak resistance (PR) and stability (STAB). TPDD and STAB are both considered to be measures of dough strength (Mann et al. 2007).

TPDD showed very consistent QTL between sites with QTL associated with the *Glu-B3, Glu-B1* and *Glu-D1*. One additional QTL was present on 7D at Narrabri. STAB showed a near identical set of QTL suggesting that these traits are highly correlated. Two QTL for PR were seen across the two sites, QTL on 1A consistent with the *Glu-A3* locus and 5A consistent with a locus controlling protein content at the Narrabri site. At Narrabri, additional QTL were present on 2B and 7B.

Mixograph

Three Mixograph traits were measured at both sites, MT, MBW and RBD (see Table 1). MT showed a very similar set of major QTL to those found for TPDD and STAB, with QTL on 1B consistent with the *Glu-B3* and *Glu-B1* loci, and with a QTL on 1D consistent with *Glu-D1*. QTL for MBW were present at both sites on 1A consistent with the *Glu-A3* locus. QTL at Hillston were also found on 1D, 4D, 5D and 7B. The RBD trait is considered to be the Mixograph equivalent to the stability trait measured by Farinograph, however, tended to show lower heritability values with high CVs along with inconsistent QTL between sites suggesting that this may be a less useful trait for breeding and selection purposes.

Uniaxial extension

Different experimental methods were used for the measurement of extensibility parameters at the Narrabri and Hillston sites compared to the Biloela and Lundavra sites. The Narrabri and Hillston sites were analyzed using a small scale modified Kieffer method, while the Biloela/Lundavra sites were measured using a large scale Extensograph method. In order to avoid confusion between the parameters, and to be consistent with previous publications (Mann et al. 2005, 2007), the terms "height" and "length" are used to denote maximum resistance and extensibility parameters determined using the large scale Extensograph, while Rmax and Ext_{Rmax} denote the equivalent parameters determined using the modified Kieffer method. In this study, each parameter determined by each method showed high heritabilities and acceptable CVs of measurement (Table 4). Two QTL for Rmax/height were found across all five sites, on 1B and 1D corresponding to the *Glu-B1* and *Glu-D1* loci (Table 4; Fig. 1b). For both QTL, high R_{max}/height was associated with the high dough strength allele, *Glu-B1-al* and 5 + 10 on *Glu-B1* and *Glu-D1*, respectively. Two other QTL were common across three sites, a QTL on chromosome 1B at a position consistent with the location of the *Glu-B3* locus (with the "b" allele from Janz associated with increased R_{max}/height, Fig. 1b), and a QTL on 2B (Table 4; Fig. 1e). A QTL for Ext_{Rmax}/Length was found across all sites, on 1D and associated with the low strength 2 + 12 allele at *Glu-D1* Fig. 1c). Two other regions of the genome yielded QTL at two sites, 1B and 7B, while 1A gave QTL which differed in the donor of extensibility at the two sites (Table 4; Fig. 1a).

Sponge and dough baking

Sponge and dough baking was carried out for both the Narrabri and Hillston sites at BRI Research using a small scale baking method previously benchmarked against samples of known baking performance. Both high top and sandwich loaves were baked with loaf volume and crumb structure traits determined on high top loaves; crumb softness was measured on sandwich style loaves using a compression method (TAXT2). Slice area and crumb structure traits were measured using a C-Cell image analysis system, while color parameters were measured using a Minolta Chroma Meter CR 300. C-Cell data are summarized in supplementary data Table 4 and Minolta color data are given in supplementary data Table 5. Samples from the Biloela and Lundavra sites were analyzed by QDPI&F using the sponge and dough baking method of Lever et al. (2005) yielding loaf volume data (Table 4).

Loaf dimensions

Sponge and dough loaf volume was determined for five sites. Heritabilities ranged from 0.57 to 0.83 (Table 4). The CVs for baking fell into two groups, low CVs for the Narrabri, Hillston and Biloela 2001 sites, and high CVs for the Biloela 2002 and Lundavra 2002 sites. QTL were found at three of the five sites in two regions of the genome, 1D and 5D (Table 4). It should be noted, however, that for the region on 1D containing the *Glu-D1* locus, a QTL was also found at one site where the low dough strength 2 + 12 allele was associated with increased loaf volume rather than the high dough strength 5 + 10 allele. Two other QTL were found at two sites, one associated with the *Glu-B1-b* allele at the *Glu-B1* locus, and a second on chromosome 4D (Table 4). The absence of QTL associated with the high dough strength *Glu-B1-al* allele on *Glu-B1* is noteworthy, in fact, the evidence from two sites suggests that increased loaf volume is associated with the lower dough strength *Glu-B1-b* allele. For the Narrabri and Hillston sites, two additional measures of loaf dimensions were used, sponge and dough oven spring (SDOS), and sponge and dough slice area (SDSA). SDOS showed a strong and consistent QTL at both sites on 1D associated with the 5 + 10 allele at *Glu-D1* (Fig. 1c). At Hillston, additional QTL were found on 2B (Fig. 1e), 4B (Fig. 1g), 4D (Fig. 1h) and 7A (Fig. 1k). SDSA represents an additional objective method of measuring loaf dimensions and gives QTL consistent with those obtained for loaf volume.

Crumb parameters

The three crumb parameters measured on sponge and dough breads using C-Cell, cell number (SDCN), average cell area (SDACA), cell wall thickness (SDCWT) all show consistent heritabilities in the 0.55–0.67 range with CVs ranging from 1.1 to 6.9 (Table 1). At both sites, a QTL on 1D consistent with the 5 + 10 allele of *Glu-D1*

Table 5 Estimated genetic correlation matrix of traits for the DH effects for traits from the Biloela and Lundavra sites

	B01 length	B01 height	B01 protein	B01 SDV[a]	B02 length	B02 height	B02 protein	B02 SDV	L02 length	L02 height	L02 protein	L02 SDV
B01 length	1	0.12	0.53	0.29	0.70	0.25	0.51	0.12	0.61	0.14	0.50	0.03
B01 height	0.12	1	0.14	0.26	−0.13	0.86	0.18	−0.48	−0.31	0.86	0.23	−0.46
B01 protein	0.53	0.14	1	0.45	0.35	0.20	0.75	0.12	0.18	0.04	0.62	0.05
B01 SDV	0.29	0.26	0.45	1	0.15	0.27	0.41	−0.04	0.03	0.19	0.36	−0.06
B02 Length	0.70	−0.13	0.35	0.15	1	0.04	0.34	0.23	0.77	−0.05	0.36	0.13
B02 Height	0.25	0.86	0.2	0.27	0.04	1	0.23	−0.39	−0.12	0.78	0.28	−0.39
B02 Protein	0.51	0.18	0.75	0.41	0.34	0.23	1	0.08	0.19	0.09	0.57	0.02
B02 SDV	0.12	−0.48	0.12	−0.04	0.23	−0.39	0.08	1	0.29	−0.44	0.04	0.26
L02 length	0.61	−0.31	0.18	0.03	0.77	−0.12	0.19	0.29	1	−0.19	0.23	0.19
L02 height	0.14	0.86	0.04	0.19	−0.05	0.78	0.09	−0.44	−0.19	1	0.17	−0.42
L02 Protein	0.50	0.23	0.62	0.36	0.36	0.28	0.57	0.04	0.23	0.17	1	−0.02
L02 SDV	0.03	−0.46	0.05	−0.06	0.13	−0.39	0.02	0.26	0.19	−0.42	−0.02	1

The parameters length, height, protein and SDV are defined in Table 1

NO3 Narrabri 2003, *H03* Hillston 2003, *B01* Biloela 2001, *B02* Biloela 2002, *L02* Lundavra 2002

(Fig. 1c) is associated with high cell number. A second QTL consistent across sites was seen on 2B (Fig. 1e). At Hillston, QTL were also found on 4B (Fig. 1g) and 4D (Fig. 1h).

SDACA showed strong QTL at both sites on chromosome 1B. These QTL were aligned to adjacent regions of the chromosome at the two sites with the QTL at Narrabri distal to the 1B-2 region, while the Hillston QTL is closely aligned to the location of *Glu-B1* (Fig. 1b). At the *Glu-D1* loci, SDACA was associated with *Glu-D1* at both sites. A QTL at each site was observed on chromosome 2B although the data is inconclusive as to whether the QTL at each site are located in the same region of 2B (Fig. 1e). At Narrabri, there are additional QTL on 3A (Fig. 1f) and 5B (Fig. 1j) in positions overlapping with GPC QTL on these chromosomes. SDCWT shows near identical QTL to SDCN suggesting that these are highly correlated traits.

Crumb firmness

A sandwich loaf was prepared from each sample in order to test for crumb firmness measured using a texture analyzer. A softer crumb texture is generally preferred by consumers. Crumb firmness showed a consistent trend in that regions of the genome associated with increased protein content at each site tended to be associated with increased softness (i.e., reduced firmness). For example, consistent QTL were found at both sites on chromosome 3A (Fig. 1f), and at 4D (Fig. 1h) at Hillston and 5B (Fig. 1j) at Narrabri, in regions associated with increase protein content. QTL not associated with protein content were also found at Hillston on 4D (Fig. 1h) and 6A.

Loaf color parameters

Both crumb and crust color measurements were made using a Minolta color meter. Crust heritability values for the L, A and B parameters had very low heritabilities and will not be considered further. For the crumb color measurement, L values (associated with the brightness of the material tested) had low heritabilities at both sites, while those for the A and B parameters were high at both sites. A single QTL for crumb L value was seen at both sites, associated with the *Glu-D1* locus (Fig. 1c), suggesting that the smaller cell areas and cell wall thicknesses noted above caused greater brightness values from the crumb surface. Consistent QTL were found for the A and B values across both sites, with loci on 4B (Fig. 1g) and 7A (Fig. 1k), respectively, although the polarity of the A and B QTL was reversed with Janz donating a high “B” yellow color QTL on 4B (low red “A” color, Fig. 1g) and Kukri donating a high “B” yellow color (low red A color) on 7A (Fig. 1k). A consistent QTL were also found on 3B for B value.

Correlation analysis

Correlations between the genetic effects for traits at the same site, and across sites, were examined by multivariate analysis. The results are shown in Table 5. Between site correlations for the traits Extensograph Length and Height, and protein content parameters were high (Length B01: B02 0.7, B01: L02 0.61, B02:L02 0.77; Height B01:B02 0.86, B01:L02 0.86, B02:L02 0.78; Protein B01: B02 0.75, B01: L02 0.62, B02:L02 0.57); however, correlations between loaf volume across sites were low (loaf volume

B01:B02 −0.04, B01:L02 −0.06, B02:L02 0.26). Correlations between traits for each site were also examined. Correlations between Extensograph length and protein content were seen at each site (B01 0.53, B02 0.34, L02 0.23) and consistent weak correlations between extensibility and loaf volume were also seen (B01 0.29; B02 0.23, L02 0.19). Correlations between protein content and loaf volume were inconsistent with Biloela 2001 showing a correlation of 0.45 but neither Biloela 2002 (0.08) or Lundavra 2002 showed any correlation (−0.02). Extensograph height also showed an inconsistent correlation to loaf volume with Biloela 2001 giving a correlation of 0.26 yet Biloela 2002 and Lundavra 2002 showed strongly negative correlations of −0.39 and −0.42, respectively. Correlations between Extensograph length and height were weak and inconsistent (B01, 0.12; B02, 0.04; L02, −0.19). The multivariate analysis provides an estimate of the genetic variance matrix between traits. This was used to determine the following proportion of variance explained by height, length and GPC for loaf volume; B01 27.6, B02 26.9 and L02 20.8%.

Discussion

Enhancing wheat "quality" is an inherently complex breeding objective, involving the combination of component phenotypes (hardness, flour color, baking quality, etc.) underpinned by diverse metabolic pathways, including genes controlling grain architecture, and the synthesis of protein, starch, non-starch polysaccharides, lipids and color pigments. This research has used a genetic mapping approach to investigate the roles of all regions of the genome, not only the roles of known protein loci and protein alleles, in order to identify non-glutenin loci that comprise gene expression networks influencing baking outcomes. The response of these loci to differing production environments has been probed.

The specific focus of this study was to identify regions of the wheat genome controlling the quality of bread made from a specific breadmaking style, sponge and dough baking. S&D baking is a two-stage process differentiated from other breadmaking styles; in the first phase, ingredients are combined and then allowed to undergo an extensive fermentation, followed by a second phase involving further ingredient addition and mixing prior to proofing, molding and baking steps. It is thought that the fermentation process places additional strain on the dough requiring flours with greater protein content and dough strength be used for S&D relative to rapid dough or short fermentation baking processes (Lever et al. 2005). For three sites, Biloela 2001, Biloela 2002 and Lundavra 2002, loaf volume was the sole measure of S&D baking performance, whereas a more comprehensive assessment of baking performance was conducted for the Narrabri 2003 and Hillston 2003 sites using a C-Cell imaging system to measure a range of loaf dimensions and internal structure parameters. In addition, crumb texture was measured using a compression test using a Texture Analyzer.

Consistent QTL for loaf volume were not obtained across all sites, despite high heritability values and low CVs of measurement. Differences in methodologies between the two laboratories (QDPI&F for Biloela 2001, Biloela 2002 and Lundavra 2002; BRI Ltd Narrabri 2003, Hillston 2003) may have contributed to this outcome although some QTL, e.g., 1D, 4D and 5B, were obtained from both laboratories. Two sets of QTL were found at three of the 5 sites. First, a QTL associated with the *Glu-D1* locus with the 5 + 10 allele from Kukri associated with increased baking volume was found at three sites, although it should be noted that a QTL with inverse effect (i.e., the 2 + 12 allele associated with increased volume) was found at one site. A second QTL on 5D, not associated with the hardness locus, was also found. There is no known candidate gene at this location. While the impact of the 5 + 10 allele at *Glu-D1* on loaf volume was not consistent across sites, the contribution of this allele was evident in the C-Cell measurements of loaf dimensions and crumb structure from the Narrabri and Hillston sites. The 5 + 10 allele contributes to slice area (an alternative measure of loaf dimensions) and to the number of cells and their thickness, when 5 + 10 was present. This crumb structure is consistent with performance targets for this breadmaking category. Crumb yellowness is controlled by the same loci controlling flour color, indicating there are no additional genetic factors influencing baking outcomes for crumb color. Loaf firmness is an important quality parameter for sponge and dough baking with a softer texture associated with higher quality breads. QTL for softness were associated with regions of the genome shown to increase protein content, 3A at both Narrabri and Hillston sites, 5B at Narrabri and 4D at Hillston.

QTL affecting the rheological parameters of doughs do not necessarily translate into effects on baking outcomes. There is no support for the use of the *Glu-B1-al* allele from Kukri at *Glu-B1* as a means of increasing S&D baking performance. While this allele provides a considerable increase in dough strength and an increase in extensibility (at two sites), these contributions to the rheology of the dough were not translated into positive impacts on loaf volume or key structural or textural parameters. The use of this allele may be a negative for bakers through increasing the energy and time requirement for mixing, without increasing baking performance. One possible explanation for the difference in impact of the 5 + 10 high strength allele and the *Glu-B1–al* allele is that the 5 + 10 allele contains an additional cysteine

residue which is thought to involve this allele in a more highly cross linked gluten structure (Anderson and Green 1989), whereas the *Glu-B1-al* allele may contribute greater strength through additional mass of a less cross linked structure (Butow et al. 2003). Under the extended fermentation period involved in S&D breadmaking, gluten networks involving the *Glu-B1-al* allele may not remain fully intact, exerting less influence the product of the final baked product.

Flour water absorption is an important industry trait. At both Narrabri and Hillston sites, consistent QTL on chromosomes 1A and 3A were found. The impact of protein content on water absorption confirmed by the presence of QTL on 3A, 5A and 5B (at Narrabri) and 4D (Hillston) all co-locating with GPC QTL. Water absorption has been considered to be a function of starch damage, non-starch polysaccharide content and protein content. It is interesting to note that the only QTL found in this study were associated with protein content despite grain hardness segregating in the population.

QTL for dough rheology were principally associated with the glutenin loci and were highly consistent across sites. The dough strength related parameters TPDD, MT and Rmax/Height were associated with the *Glu-B3* "b" low molecular weight glutenin donated by Janz, and the *Glu-B1-al* ("over-expression-") allele and *Glu-D1-d* (5 + 10) alleles donated by Kukri. A strong QTL for extensibility was found at all sites associated with *Glu-D1*, with increased extensibility associated with the 2 + 12 allele. At three of the five sites, a QTL for extensibility was also found associated with the *Glu-B1-al* allele. Interestingly, this QTL was only found for the Extensograph methodology and not the modified Kieffer method, consistent with a previous study showing that the Extensograph and Kieffer derived extension traits had differences in genetic control (Mann et al. 2007). Studies of rheological properties across sites demonstrated that as individual parameters they were very weak predictors of baking outcomes. Approaches using techniques such as multivariate statistical analysis have been shown to be able to identify combinations of parameters from grain composition and rheology data that can generate useful predictive models for baking outcomes (Dobraszczyk and Morgenstern 2003).

Environment has a major impact on not only total protein content but also on the relative expression of protein classes. Only two QTL for protein content were observed across three of the five sites. Of the QTL observed, a QTL on chromosome 3A observed at three sites is in a location consistent with other studies of the genetic control of protein content in wheat (Groos et al. 2004). The expression of classes of seed storage proteins was investigated by HPLC for samples from two sites, Narrabri 2003 and Hillston 2003. At both sites, loci that influence protein content also influence the relative expression of groups of seed storage proteins. This observation demonstrates why protein content per se can be a misleading indicator of wheat quality—changes in protein composition in response to changes in gene expression patterns in different environments are likely to have more profound effects on dough rheology and baking outcomes than changes driven by protein content alone.

The integrated nature of the experiment conducted, in which QTL for grain physical characteristics, protein composition, dough rheology and baking were studied concomitantly, allowed the identification of effects on the expression of regions of the genome in response to environment. Some regions of the genome showed consistent influences on a wide range of parameters across both the Narrabri and Hillston sites, including Regions 1 and 2 on 1B (Fig. 1b), Region 1 on 1D (Fig. 1c), Region 1 on 4B (Fig. 1g), and Region 2 on 7A (Fig. 1k). However, other regions contained loci that were differentially expressed at the two sites and affected a wide range of parameters in parallel For example, regions predominantly expressed at Narrabri include Region 1 on 1A (Fig. 1a), Region 2 on 3A (Fig. 1f), Region 1 on 5A (Region 1i), Region 1 on 5B (Fig. 1j) and Region 1 on 7A (Fig. 1k). Effects of Regions 1 and 2 on 4D were predominantly observed at Hillston (Fig. 1h).

This study provides further evidence for the contention that wheat quality is a consequence of a network of interacting genes. For example, QTL for the expression of *Glu-D1* subunit 5 were also found at the *Glu-D1* locus but also associated with the *Glu-B1* locus, such that the presence of the high expression *Glu-B1-al* allele is associated with higher levels of subunit 5 from the *Glu-D1* locus being present in the flour. This provides support for the findings of Ravel et al. (2006) who have identified a region of the genome tightly linked to *Glu-B1* as carrying a gene responsible for high molecular weight glutenin over-expression. An alternative mechanism is that the presence of higher levels of the Bx7 interacts with subunit 5 to stabilize a complex, preventing degradition of the subunit.

Utilizing a genetic mapping approach allowed the investigation of the importance of regions outside the glutenin loci. Regions of the genome containing the dwarfing genes *Rht-B1b* (*Rht*-1) and *Rht-D1b* (*Rht*-2) had negative effects on wheat quality parameters. The *Rht-1* allele donated by Janz had negative impacts at both the Narrabri and Hillston sites on grain weight, grain diameter and S&D volume. The *Rht-2* allele donated by Kukri had negative effects at the Hillston site on Rmax and a range of S&D parameters including crumb firmness, cell number, slice area and S&D loaf volume. The avoidance of the *Rht*-1 and *Rht*-2 dwarfing genes in breeding for S&D wheats is therefore indicated. A range of other loci with beneficial

effects on quality were identified for which there are currently no candidate genes. A locus on 3A associated with protein content, protein expression and baking properties has been identified and may represent the same locus as identified previously by Law et al. (2005), Groos et al. (2004, 2007) and Kuchel et al. (2006). Regions on 5A and 5B influencing protein content and protein expression are in positions consistent with loci identified in previous studies (Arbelbide and Bernardo 2006; Blanco et al. 2002; Gonzalez-Hernandez et al. 2004; Groos et al. 2004; Groos et al. 2007; Harjit-Singh et al. 2001). The consistent nature of these effects across studies and their importance in influencing quality outcomes makes these loci attractive targets for map based cloning to identify the causal genes.

In conclusion, this study has demonstrated that an integrated approach to studying the genetic basis of grain, rheological and baking properties provides a powerful mechanism to identify QTL governing quality traits, but also provides a mechanism for re-evaluating assumptions concerning the relationships between predictive tests and end product quality. This study confirms the key role that HMW glutenin loci play in determining dough rheological parameters. However, the study does not support the contention that protein content, dough strength or extensibility are effective predictors of S&D baking volume or that increases in dough strength necessarily translate into superior baking outcomes. The data suggest that there is an important mechanistic difference in the impact of the *Glu-D1* 5 + 10 allele and the *Glu-B1-al* allele. Enrichment of breeding populations for the *Glu-D1* 5 + 10 allele is suggested by the data as a strategy for enhancing the probability of achieving acceptable S&D baking outcomes, however, as heritability values for S&D baking are high, direct selection for the trait will be an important additional strategy. The observations made on the impact of environment, and the interactions between genes, reinforce the view that wheat quality is achieved as a result of the action of complex interactions between genes controlling the level and composition of grain constituents. Further studies will be required to provide a comprehensive roadmap for the reliable achievement of S&D baking outcomes across a wider genetic base and across diverse environments.

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