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The genetic control of milling yield, dough rheology and baking quality of wheat

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Abstract Improving the end-use quality of wheat is a key target for many breeding programmes. With the exception of the relationship between glutenin alleles and some dough rheological characters, knowledge concerning the genetic control of wheat quality traits is somewhat limited. A doubled haploid population produced from a cross between two Australian cultivars 'Trident' and 'Molineux' has been used to construct a linkage map based largely on microsatellite molecular makers. 'Molineux' is superior to 'Trident' for a number of milling, dough rheology and baking quality characteristics, although by international standards 'Trident' would still be regarded as possessing moderately good end-use quality. This population was therefore deemed useful for investigation of wheat end-use quality. A number of significant QTL identified for dough rheological traits mapped to HMW and LMW glutenin loci on chromosomes 1A and 1B. However, QTL associated with dough strength and loaf volume were also identified on chromosome 2A and a significant QTL associated with loaf volume and crumb quality was identified on chromosome 3A. A QTL for flour protein content and milling yield was identified on chromosome 6A and a QTL associated with flour colour reported previously on chromosome 7B was confirmed in this population. The detection of loci affecting dough strength, loaf volume and flour protein content may provide fresh opportunities for the application of marker-assisted selection to improve bread-making quality.

Abbreviations HMW: High molecular weight · LMW: Low molecular weight · MAS: Marker-assisted selection · QTL: Quantitative trait locus

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Introduction

Baking quality is a major target for wheat improvement. Since the value of cross hybridisation and selection was first recognised, varieties have been selected to perform better in mills and processing facilities. Due to the constraints of population size and sample size, full-scale mill and bakery testing is not performed routinely. In an effort to reduce the resources required to improve wheat end-use quality, breeding programmes utilise small-scale testing equipment to predict varietal performance. A number of characteristics are measured to predict baking quality. These may include traits such as particle size index (a grain hardness measure), flour protein content, water absorption, flour paste viscosity, dough resistance and dough extensibility. However, these small-scale assessment methods can still be expensive, laborious and require quantities of grain in excess of that available at the early stages of a breeding programme. For similar reasons, these assays are also unable to be used to select individual plants within populations. In addition, environmental variation, originating from both the field and laboratory, may reduce the heritability of phenotypic quality selection. With the advent of molecular

marker-assisted selection (MAS), opportunity exists to replace some of the current phenotypic based selection activities with genotypic selection.

A number of authors have reported success in teasing apart the complex genetic basis of wheat quality. Payne et al. (1987) evaluated some of the alleles at the high molecular weight (HMW) glutenin loci (*Glu-A1*, *Glu-B1* and *Glu-D1*) and scored their importance to wheat quality. Later, the combined effects of alleles at both the HMW and low molecular weight (LMW) glutenin loci on dough strength were investigated (Gupta et al. 1989; Nieto-Taladriz et al. 1994; Eagles et al. 2002b). In the study of Eagles et al. (2002b) almost half the phenotypic variation in dough resistance and extensibility was explained by the main and two-way interaction effects of the six glutenin loci. Other genes involved in the control of grain hardness (*Pina-D1* and *Pinb-D1*) have been identified (Giroux and Morris 1997) and their effects on flour and dough performance characterised (Cane et al. 2004). In addition, alleles at the granule-bound starch synthase (GBSS) loci have been shown to be associated with the amylose fraction of the grain starch and consequently noodle texture (Nakamura et al. 1993). Beyond these well-characterised loci and QTL affecting grain protein content (Groos et al. 2003; Turner et al. 2004; Prasad et al. 1999, 2003), few studies have reported additional genetic loci that influence dough rheology and baking quality (Perretant et al. 2000; Groos et al. 2004; Law et al. 2005).

A doubled haploid population (T/M DH) was produced from a cross between the South Australian cultivars 'Trident' and 'Molineux' (Ranjbar 1997) to study the inheritance of various agronomic and disease resistance traits. Recently, a linkage map has been completed using this population (K.J. Williams, K.J. Willsmore, S. Olson, M. Matic and H. Kuchel, submitted) making detailed genetic analysis possible. 'Trident' and 'Molineux' vary markedly for their end-use quality. More particularly, 'Molineux' has excellent dough rheology characteristics possessing high dough resistance and extensibility. In comparison, 'Trident' has only moderate dough resistance and relatively low extensibility, making this population a good candidate for genetic investigation.

Materials and methods

Genetic resources

A doubled haploid population (T/M DH) consisting of 182 individuals produced from a cross between 'Trident' (VPM1/5*Cook//4*Spear, released in 1993 by the University of Adelaide) and 'Molineux' (Pitic 62/Festiguay//Warigal, released in 1988 by the University of Adelaide) was used as the basis for this study (Ranjbar 1997). A genetic linkage map was produced using 260 microsattelite, sequence-tagged site and protein markers (Williams et al., submitted). 'Trident' and 'Molineux' differed for HMW and LMW glutenin loci (*Glu-B1*, *Glu-A3*, *Glu-B3*) and consequently these were included in the map (Ranjbar 1997).

Samples for quality analysis

Grain for quality analysis was harvested from field experiments conducted in 1996 and 2003. In 1996, the trial was located at Roseworthy Campus, University of Adelaide, South Australia, while in 2003 samples were formed from composites of grain from two sites, Roseworthy and Kapunda, South Australia. These two sites are located within 30 km of each other and were grown under normal growing conditions. Grain yields averaged 2,784 and 2,307 kg ha^{-1} at Roseworthy and Kapunda, respectively, whilst average protein levels of check varieties grown at both locations were within 2%. DH lines were grown in plots constituting six rows and were 1.3 m wide and 3.2 m long. Two replicates of each line were arranged in a randomised rectangular array 12 plots deep. In 1996, 110 of the 182 DH lines were included in the field experiment and were therefore available for quality testing, while in 2003 all 182 DH lines were grown. The grain used for analysis was that retained above a 2 mm sieve. All grains appeared physically sound with no signs of sprouting. Falling number tests on grain samples taken from pre-harvest sprouting susceptible control varieties at the same sites confirmed the grains were sound and able to be used for quality analysis.

Quality analyses

Grain hardness was measured by NIR (Bran & Luebbe, Germany), utilising an NIR calibration previously produced for particle-size index (PSI) (Symes 1965). Samples were milled on a Buhler mill after conditioning to a predetermined moisture basis [moisture (%) = 20.1 − PSI(× 0.25]. Flour yield (FY) was assessed as the percentage of total grain weight accounted for by the combination of all flour fractions. Flour protein (FP) was determined using NIR (Bran & Luebbe) calibrated against Dumas nitrogen content. A Minolta colour meter (Minolta, Japan) was used to determine the colour (Min-a and Min-b*) and brightness of the flour (Min-L). Water absorption (WA), dough stability (Stab) and dough development time (DT) were measured using a farinograph (Brabender, Germany) according to AACC method 54-21 (American Association of Cereal Chemists 1987). The dough performance characteristics, dough resistance (R_{max}) and dough extensibility (Ext), were determined using an extensograph (Brabender) according to AACC method 54-10 while flour paste viscosity (RVA) was measured using a rapid visco analyser (Newport Scientific, Australia), following the protocol provided in the operating manual. In 2003, flour samples were used for further end-use quality

characterisation. Loaves of bread were baked from 100 g of flour and the loaf volume (Vol) and crumb score (Sc) assessed. Flour, yeast (3 g), sodium chloride (2 g), malt flour (0.5 g), ammonium dihydrogen orthophosphate (0.1 g), potassium chromate (0.1 mg) and water (volume determined from farinograph WA) were mixed in a Finney pin mixer (National Manufacturing Company, USA) to form dough. Dough was fermented in an air-tight container for 2 h 50 min before moulding, panning and final proving for 55 min at 30°C (85–90% relative humidity). Loaves were baked for 25 min at 220°C and loaf volume and crumb quality scores assessed.

Statistical design and analysis

Quality trait data from 1996 were produced on a single sample for each line and consequently the raw data were used directly for QTL analysis. In 2003, however, a single DH line composite was formed using equal proportions of grain from Roseworthy and Kapunda. In order to account for laboratory-sourced error (Smith et al. 2001), a partially replicated and completely randomised design (one-third of lines replicated twice) was produced from the entry list. For the lines replicated, the line composite was split into two lab samples. Due to processing constraints (labour), milling and farinograph days were confounded, while the samples tested on each of the extensograph processing days were formed from the sum of two consecutive milling days. Samples were re-randomised such that different within-day randomisations were used for milling, farinograph and extensograph assays. Best linear unbiased predictors (BLUPs) for each of the quality characters measured on the 2003 samples were determined using the REML directive within GENSTAT 6 (Payne et al. 2002). Any significant processing day or within-day effects were fitted as random and fixed effects, respectively. The VFUNCTION procedure of GENSTAT was used to calculate broad sense heritabilities (Nyquist 1991). Given that significant correlations are known to exist between quality traits (Eagles et al. 2002a), the effects of FP, PSI and FY on the remaining traits were assessed for each of the two data sets. Where a significant relationship was established an 'adjusted' value was calculated and used for mapping (Eagles et al. 2002b). Likewise, FP is often heavily dependent on grain yield. Consequently, the relationship between FP and grain yield was determined (using the average grain yield from Roseworthy and Kapunda) for the 2003 data and in turn this was used to produce an adjusted FP figure independent of grain yield. QTL mapping was undertaken using both the adjusted and unadjusted data. For the 2003 data set, adjustments were made during the REML analysis described above, fitting FY, FP and PSI where significant ($P < 0.05$) as fixed covariates. In the case of FP, the mean grain yield achieved by each of the lines at Roseworthy and Kapunda was used as the fixed covariate. For the unreplicated 1996 data, the effects of FY, FP and PSI were calculated using general linear regression for each of the traits. These estimates were then used to adjust the raw data.

The location of chromosomal regions (QTL) significantly associated with each of the quality traits was initially detected by single marker regression analysis and later examined using the interval mapping (Haley and Knott 1992) approach provided by MAP MANAGER QTX (Manly and Olson 1999). A QTL with an LOD between two and three was considered suggestive whilst a QTL with an LOD greater than three was considered significant.

Results

Data summary

For many of the traits assessed, the correlation between results achieved in 1996 and those collected in 2003 was relatively poor indicating strong environmental effects. Consequently, the two data sets were treated separately for further analyses, with the larger semi-replicated 2003 data set used for primary investigations and the smaller un-replicated 1996 data set used for validation of QTL stability. In 2003, where significance could be assessed, the T/M DH population showed significant ($P < 0.001$) variation for each of the traits measured. As expected, the correlation between the traits within a year was at times high (Table 1). Most notable were the high correlations observed between PSI, FP, FY and many of the other traits assessed. Consequently, the adjusted values (see Materials and methods) for both the 1996 and 2003 data were often used for QTL analysis to ensure chromosomal regions conferring effects independent of PSI, FP and FY were identified (the suffix 'adj' is used to denote the application of adjusted figures).

For many traits, the performance of the parents 'Trident' and 'Molineux' was well within the bounds of the population providing evidence of transgressive segregation (Table 2). For most traits 'Molineux' was superior to 'Trident' except for WA and RVA where 'Trident' was superior. While both 'Trident' and 'Molineux' would be classed as hard grain textured varieties and both possess the same alleles at the hardness loci (*Pina-D1a* and *Pinb-D1b*) (H. Kuchel, unpublished data), there was a significant difference ($P < 0.05$) observed between them and within the population for PSI. Likewise, both 'Trident' and 'Molineux' carry the same *GBSS* alleles (H. Kuchel, unpublished data) and yet they showed significantly ($P < 0.001$) different flour paste viscosity characteristics (RVA). Most striking, however, was the large difference between 'Trident' and 'Molineux' observed for the dough rheology trait R_{max}. Transgressive segregation did not appear to exist for Ext where 'Trident' and 'Molineux' did not significantly ($P < 0.05$) differ from the low and high extremes of the population, respectively.

Table 1 Statistically significant (normal font, $P < 0.05$; italic, $P < 0.01$; bold-italic, $P < 0.001$) observed correlation coefficients for the 2003 unadjusted means are displayed in the lower triangle and for the 2003 adjusted means in the upper

	PSI	FY	Min-L	Min-a	Min-b*	FP	WA	DT	Stab	R_{max}	Ext	RVA	Vol	Sc
PSI	**0.50**													
FY	*0.24*	**0.88**												
Min-L	*−0.37*	−0.19	**0.81**	0.18	*−0.60*							*0.23*	0.19	*0.23*
Min-a	0.15	*0.21*		**0.96**	*−0.87*									
Min-b*			*−0.51*	*−0.79*	**0.98**									
FP	***0.24***	*−0.20*	*−0.57*	*0.20*	*0.18*	**0.92**								
WA		−0.16	*−0.33*		0.15	0.64	**0.77**	*−0.36*	*−0.57*	*−0.60*	*−0.36*			
DT	−0.15	*−0.41*				*0.39*		**0.53**	*0.70*	*0.70*	*0.22*		0.18	*0.24*
Stab	*−0.25*	*−0.38*	0.19				*−0.29*	*0.70*	**0.87**	*0.81*	0.18		0.15	*0.20*
R_{max}	*−0.24*	*−0.23*	*0.23*			−0.19	*−0.51*	*0.57*	*0.79*	**0.87**	0.16		0.16	*0.21*
Ext			*−0.16*			*0.44*		*0.34*	*0.20*		**0.41**		*0.29*	*0.29*
RVA		−0.18	*0.18*									**0.80**		
Vol		*−0.27*				*0.25*	0.17	*0.33*	*0.25*		*0.35*		**0.39**	*0.91*
Sc		*−0.30*	*0.18*					*0.34*	*0.30*	*0.22*	*0.27*		*0.91*	**0.47**

Broad sense heritabilities for the adjusted quality traits are presented on the diagonal (bold values)

Table 2 A summary of the unadjusted BLUPs calculated from the quality data collected on the T/M DH population in 2003

Trait	Description	'Trident'	'Molineux'	Average	Range (min–max)
PSI	Grain hardness	18.8	22.1	20.7	15.9–23.7
FY (%)	Flour extraction	75.7	77.3	76.5	72.2–79.8
Min-L	Flour brightness	91.7	91.54	91.63	90.87–92.86
Min-a	Flour colour (red-green)	−0.96	−0.33	−0.63	−1.06 to −0.25
Min-b*	Flour colour (yellow)	12.2	9.6	10.8	8.2–13.5
FP (%)	Flour protein	12.8	13.2	12.9	10.5–14.9
WA (%)	Water absorption	63.4	61.1	62.2	59.0–65.8
DT (min)	Dough development time	6.1	7.0	6.4	4.9–8.3
Stab (cm)	Dough stability	8.1	9.2	6.8	3.3–10.2
R_{max} (BU)	Max dough resistance	285.9	356	315.5	154.6–527.6
Ext (cm)	Dough extensibility	20.1	23.9	21.2	19.3–24.0
RVA (rvu)	Flour paste viscosity	163.2	131.1	157.5	118.1–189.7
Vol	Loaf volume	628	667	647	598–688
Sc	Crumb score	35	39	38	31–42

QTL analysis: flour protein and milling yield

Significant QTL associated with unadjusted FP were detected on chromosomes 1B, 6A (designated *QFpc.agt-6A*) and 6D in 1996 and 2003, on 7A in 2003 and 7D in 1996 (Table 3). Significant QTL associated with FP adjusted for grain yield in 2003 were identified on chromosome 6A, coincident with *QFpc.agt-6A*, and on 2D, not identified using unadjusted data. Interestingly QTL on this chromosome were also associated with WA in both 1996 and 2003. When adjusted for FP, the association of QTL identified for FP with WA reduced in significance whilst a smaller QTL on 2DL approximately 40 cM from the FP QTL was detected for both the 1996 and 2003 data sets. An association at *QFpc.agt-6A* was detected with FYadj (LOD 5.1) in 2003. The 'Trident' allele at the 6A locus was associated with higher FY. *QFpc.agt-6A* was also associated with WA, PSI and Ext; however, no effect on these traits could be demonstrated when adjusted for the effects of FP.

QTL analysis: flour colour and brightness

The flour colour traits, Min-L, Min-a and Min-b*, were shown to be significantly ($P < 0.01$) correlated with flour protein and therefore the FP adjusted values were used for QTL mapping. A major QTL was identified on chromosome 7B that explained 61 and 48% of the phenotypic variation in Min-b*adj in 1996 (LOD 19.7) and 2003 (LOD 23.3), respectively. This same chromosome region had a significant effect on Min-Ladj and Min-aadj in both 1996 (LOD 2.2 and LOD 13.2) and 2003 (LOD 6.5 and LOD 20.8). The 'Molineux' allele was associated with high Min-Ladj and Min-aadj values. In 2003 an additional QTL associated with Min-aadj was identified very close to, but proximal of, the *Glu-B3* locus on chromosome 1BS. However, this QTL did not have any effect on flour colour in 1996 or appear to be coincident with the FP QTL previously identified on chromosome 1B. Likewise, a significant QTL was detected for Min-Ladj (2003 data) on chromosome 1AS coincidental with the *Glu-A3* locus.

Table 3 A summary of QTL identified in the T/M DH population for wheat end-use quality traits for the years 1996 and 2003

Trait	Chr	Closest marker(s)	2003			1996		
			LOD	%	Add	LOD	%	Add
FP	1B	*Xgwm011–Xbarc181*	3.1	7	−0.22	2.2	9	−0.30
	6A	*Xbarc171–Xbarc113*	2.8	7	0.22	2	9	0.29
	6D	*Xgdm141–Xgwm325*	3.4	8	0.28	2.8	13	0.38
	7A	*Xwmc65*	2.5	6	0.21			
	7D	*Xcfd31–Xgwm44*				2.9	13	−0.53
FPadj[a]	2D	*Xgwm484–Xwmc144*	2.7	7	−0.20			
	6A	*Xbarc171*	2.7	7	0.19			
FYadj[b]	1A	*Xwmc312*	2.2	5	0.22			
	2A	*Xgwm558*				3.3	15	0.65
	6A	*Xbarc107–Xbarc171*	5.1	12	−0.36	1.9	9	−0.55
Min-Ladj	1A	*Xpsp2999*	6.3	10	0.06			
	7B	*Xgwm273–Xgwm146*	6.5	22	0.09	2.2	15	0.12
Min-aadj	1B	*Xpsp3000–Xgwm264*	2.5	6	0.05			
	7B	*Xgwm273–Xgwm146*	20.8	44	0.11	13.2	62	0.18
Min-badj[b]	7B	*Xgwm273–Xgwm146*	23.3	48	−0.65	19.7	77	−0.95
WAadj	1A	*Xpsp2999*	3.6	10	−0.29	2.9	13	−0.58
	1B	*Glu-B3*	3.4	8	−0.25			
	2A	*Xgwm558*	3.4	8	−0.26			
	2D	*Xgwm484–Xwmc144*	3.5	8	−0.28			
	2D	*Xgwm539–Xcfd44*	4.7	11	−0.44	2	9	−0.61
DTadj	1B	*Glu-B3*	5.2	12	0.15			
Stabadj	1A	*Xpsp2999*	5.7	15	0.61			
	1B	*Glu-B3*	9.3	20	0.72			
	2A	*Xbarc15*	3	7	0.43			
R_{max}adj	1A	*Xpsp2999*	5.3	14	22.9	1.9	10	21.0
	1B	*Glu-B3*	10.5	23	30.1	2	8	18.9
	2A	*Xbarc15*	4.9	12	21.6	1.5	7	17.5
Extadj	1A	*Xpsp2999*	8.8	20	0.26	3.2	14	0.39
	1B	*Glu-B1*				2.1	10	−0.32
	3D	*Xgwm191*	3.1	8	0.18			
RVAadj	2B	*Xbarc18*	3.8	10	−4.4			
	7A	*Xwmc65*	3.5	9	−4.2			
	7D	*Xbarc58*	2.9	7	−4.10			
Voladj[a]	2A	*Xbarc15*	2	5	2.8			
	3A	*Xgwm666*	3.7	11	5.1			
Scadj[a]	2A	*Xbarc15*	1.9	5	0.34			
	3A	*Xgwm666*	4.3	13	0.72			

The LOD and percent variation accounted for by each of the QTL are provided along with the additive (add) allele effects for the loci. A superior expression of the trait is indicated by a positive effect if the allele is carried by 'Molineux' and negative if carried by 'Trident'

[a]This trait was not available for the 1996 data set and hence QTL stability could not be determined

[b]The unadjusted data of 1996 were used as neither FP nor FY nor PSI was significantly related to the trait's expression

QTL analysis: dough rheology and baking performance

The glutenin loci segregating in this population were shown to confer major effects on dough rheology. 'Molineux' possesses the alleles *Glu-B1b* (7 + 8), *Glu-A3c* and *Glu-B3b* while 'Trident' carries *Glu-B1c* (7 + 9), *Glu-A3e* and *Glu-B3h*. For both the 2003 and 1996 data sets a significant QTL was detected at the *Glu-A3* locus that was associated with R_{max}adj (LOD 5.3 and LOD 1.9), Extadj (LOD 8.8 and LOD 3.2) and WAadj (LOD 3.6 and LOD 2.9) whilst in 2003 it was also associated with Stabadj (LOD 5.7). At the other LMW glutenin locus segregating in this population, *Glu-B3*, a significant association was identified with R_{max}adj in both 2003 and 1996 (LOD 10.5 and LOD 2) but was not related to Extadj in either year. This QTL on chromosome 1B was also found to be associated with some of the other dough and flour quality traits in 2003, namely, WAadj (LOD 3.4), DTadj (LOD 5.2) and Stabadj (LOD 9.3). In contrast, no significant relationships were found between the *Glu-B1* locus and any of the quality traits assessed. 'Molineux' carried the favourable allele at both the *Glu-A3* and *Glu-B3* loci for each of the traits assessed, except WAadj. Interestingly, none of the glutenin loci were shown to be associated with the bread-baking traits, loaf volume and crumb score. However, a novel QTL on chromosome 2A (*QRmx.agt-2A*) was found to be associated with R_{max}adj (LOD 4.9), WAadj (LOD 3.4) and Stabadj (LOD 3). While not significant, this region on 2A may also be involved in the control of Voladj (LOD 2) and Scadj (LOD 1.9) (Fig. 1). For all but WAadj, 'Molineux' carried the favourable allele at *QRmx.agt-2A*. A second final QTL associated with the baking tests Voladj and Scadj was identified on chromosome 3A (*QBVol.agt-3A*) (LOD 3.7 and LOD 4.3) close to, but distal of, *Xgwm666*. This region was not

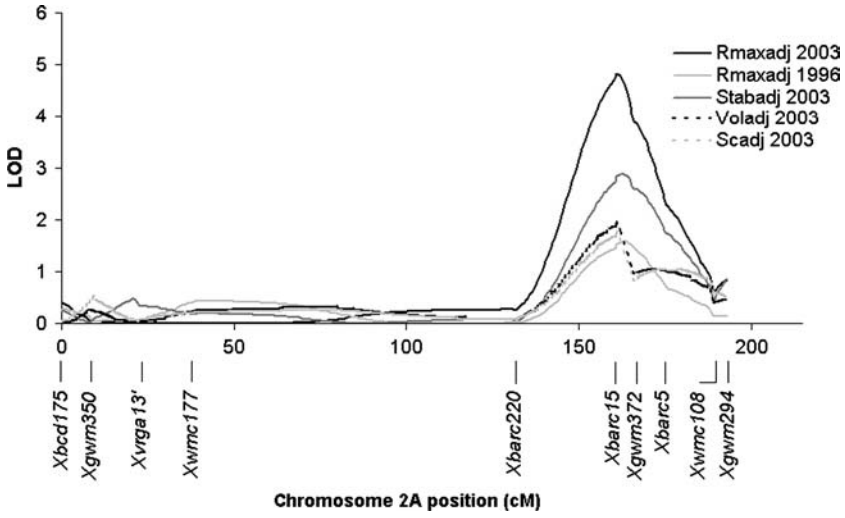

Fig. 1 Association between chromosome 2A and dough rheological and baking quality traits

associated with any other traits in either year. Again, the positive allele was carried by 'Molineux'.

Flour paste viscosity (RVA) in 2003 showed significant association with three QTL in 2003. However, no significant QTL for RVA were identified from the 1996 data set. QTL on chromosomes 2B, 7A and 7D each accounted for between 7 and 10% of the phenotypic variation in RVAadj in 2003. Interestingly, neither the chromosome 2B nor 7D QTL was found to be associated with any of the other traits measured, whilst the QTL on 7A showed some suggestive linkage with the expression of FP content.

Marker-assisted selection of QTL for improved dough rheology

In order to determine the potential of MAS for wheat quality in this cross, the genotypic effects for the marker loci closest to the QTL for WA, R_{max}, Ext, Vol and Sc were determined (Table 4). When considered in a general linear model, the additive effect of the markers closest to the QTL located on chromosomes 1A, 1B and 2A explained 36.7% of the phenotypic variation in R_{max}adj in 2003. Consequently, based on a broad sense heritability of 0.87 one can calculate that approximately 42.2% of the genetic variation for R_{max}adj in this cross could be explained and, therefore manipulated, by markers linked to these QTL. No significant interactions between the QTL were observed. Although FP was shown to have the largest effect on Ext, the single QTL on chromosome 1A, identified using the 2003 data set explained 20.5% of the phenotypic variation in Extadj or 50% of the genetic variation. For Voladj, markers linked to the two QTL identified on chromosomes 2A and 3A did not show any interaction effect, but their main effects accounted for 11.3% of the phenotypic variation. Given the low heritability for Voladj, this equates to 29% of the genetic variation. Meanwhile, the same two QTL explained 13% of the phenotypic variation of Sc or 27.7% of that deemed to be of genetic origin. As was presented in Table 3, many of the loci controlling dough rheology were also associated with WAadj. Linear modelling showed that markers linked to the two LMW glutenin loci and the QTL on chromosomes 2A and 2D accounted for 23.5% of the phenotypic variation in WAadj, which can be calculated to 30.5% of the genetic component of WAadj.

Table 4 Genotypic effects associated with molecular markers linked to the major QTL for some of the 2003 dough rheology and bread-baking data

Marker	WAadj (%)	R_{max}adj (BU)	Extadj (cm)	Voladj	Scadj
Xpsp2999-1A	−0.55 ± 0.13	42.7 ± 7.6	0.53 ± 0.08		
Glu-B3	−0.38 ± 0.13	49.9 ± 7.6			
Xbarc15-2A	−0.38 ± 0.13	34.4 ± 7.6		5.2 ± 1.9	0.69 ± 0.24
Xgwm539-2D	−0.46 ± 0.13				
Xgwm666-3A				6.9 ± 1.9	0.93 ± 0.24
Variance explained (%)[a]	30.5	42.2	50	29	27.7

An increase in the magnitude of the trait is indicated by a positive effect if the allele is carried by 'Molineux' and negative if carried by 'Trident'

[a]The percentage of genotypic variance explained by the additive marker effects

Discussion

QTL associated with flour protein content

The strong relationship between grain protein content and the grain yield of wheat cultivars (Lawlor 2002) can substantially hinder the rate of genetic gain for superior protein content. Consequently, in order to identify molecular markers that may be used to manipulate the protein content, QTL that are involved in the control of protein content independent of the effects of grain yield must be identified. A significant outcome of this study was the identification of a QTL, *QFpc.agt-6A*, associated with flour protein independent of grain yield. Turner et al. (personal communication, 2006) also found a QTL-associated grain protein close to *Xgwm334*, placing it around 50 cM away from the QTL located in this study. *QFpc.agt-6A* was identified in both 2003 and 1996 making it a possible candidate for the improvement of protein content in wheat through MAS. While genetic gain may be achieved for grain protein through selection for *QFpc.agt-6A* it should be noted that this locus was also found to be associated with flour yield. The alleles carried by 'Molineux' could be linked in repulsion or alternatively this relationship may be the result of an undesirable pleiotropic effect.

QTL associated with flour colour

QTL associated with flour colour (Minolta b*) have been identified previously on chromosome 7A (Parker et al. 1998) and used in breeding programmes to improve the flour quality of wheat cultivars by MAS (Eagles et al. 2001). Association with flour colour has also been reported for the homoeologous position on chromosome 7B (Ma et al. 1999; Mares and Campbell 2001). Here we present a QTL coincident with the QTL detected previously on chromosome 7B. The marker linked to Min-b* (*Xgwm344*) identified in this study could be used in this population to efficiently manipulate flour colour to suit the breeder's end-use target.

The effects of glutenin loci on dough rheology

Previous authors have shown that the glutenin loci on chromosomes 1A, 1B and 1D control much of the variation in dough rheology traits. Perretant et al. (2000) and Groos et al. (2004) both identified QTL associated with dough strength and tenacity located at various glutenin loci. Further, the effects of the *Glu-A3* and *Glu-B3* loci on dough extensibility have been clearly demonstrated (Appels et al. 2001). However, given that there are multiple alleles at each of the glutenin loci, the significance of these genes on dough rheology in any given cross will be largely determined by which alleles are carried by the parents. The *Glu-B1* locus was shown to have a small but significant effect on Ext in 1996 only. This is not surprising, as the difference between the effects of the *Glu-B1b* and *Glu-B1c* alleles on both R_{max} and Ext have been shown to be statistically insignificant (Eagles et al. 2002b). Eagles et al. (2002b) showed that lines possessing the *Glu-A3e* allele produced dough with lower extensibility and R_{max} than lines possessing the *Glu-A3c* allele, although in their work using unbalanced data sets the effects were not statistically significant. However, this study confirmed that the *Glu-A3e* allele is undesirable and should be avoided by wheat breeders. In terms of dough properties the glutenin allele complement possessed by 'Molineux' is the most desirable allelic combination that could be achieved from a cross between 'Trident' and 'Molineux'.

QTL for baking performance

In this study, no association could be demonstrated between the glutenin loci and loaf volume. This conflicts with a previous study (Rousset et al. 2001) that reported a significant relationship between the *Glu-B3* locus and loaf volume. However, the alleles carried by the two parents used in that study differed (*Glu-B3e* and *Glu-B3a*; parental alleles determined from McIntosh et al. 2003) from those segregating in the T/M DH population. This allelic difference at *Glu-B3* may have resulted in greater variation in dough rheology properties and potentially bread-making qualities. In a study of just 26 Canadian-grown varieties, Ng and Bushuk (1988) also reported an association between glutenin subunits and loaf volume. However, in support of the findings presented here, Skerritt et al. (2003) showed a relationship between glutenin alleles and dough rheology but reported a lack of association between the glutenin alleles and bread-making quality. Likewise, Hamer et al. (1992) questioned the accuracy of using HMW glutenin subunits to predict baking quality. In this study we identified two QTL associated with baking quality, *QRmx.agt-2A* and *QBVol.agt-3A*. These QTL on chromosomes 2A and 3A may account for some of the genetic variation that could not be explained by the HMW and LMW glutenin loci. It seems likely that the possible effect of *QRmx.agt-2A* on loaf volume and crumb structure is mediated by raised levels of dough resistance. A relationship between chromosome 2A and R_{max} was also reported by Ma et al. (1999). However, this does not explain why this locus was related to both R_{max} and Vol but the glutenins were found to be related to R_{max} and not Vol. Similarly, the *QBVol.agt-3A* allele inherited from 'Molineux' was associated with an increase in the loaf volume and crumb score without any changes in the dough rheology traits. Law et al. (2005) also mapped a QTL associated with loaf volume and crumb score to chromosome 3A of wheat. They proposed that for the population being studied, a single gene (*Lvl 1*) was responsible for the variation observed. The exact position of *Lvl 1* could not be determined, but

was predicted to be distal of *Xgwm720*. In this study, *QBVol.agt-3A* was located close to, but distal of, *Xgwm666*, placing it in a similar position (Somers et al. 2004) to *Lvl 1*. Consequently, this report confirms in an unrelated cross the role of gene(s) on chromosome 3A in the control of loaf volume and crumb score. This may provide confidence in the use of molecular markers linked to *Lvl 1* for the improvement of baking quality. In addition, the possibility of altering the baking quality of wheat without detrimentally affecting other end uses or dough rheology specifications is highly attractive.

Implications for improvement of water absorption

Although 'Molineux' did not represent the lowest extreme in WA observed in the T/M DH population, no QTL were identified where the 'Molineux' allele was associated with superior levels of WA. In fact the undesirable and most likely pleitropic relationship between WA and dough strength provides a quandary for wheat breeders. With improvements in WA and dough strength juxtaposed, it may be difficult to meet the apparently competing desires of end users. Consequently, it would be useful to identify new loci or alleles that act on WA and baking quality independently of dough rheology, allowing improvement in both. In this study the *QBVol.agt-3A* locus has been shown to be related to baking performance independent of WA.

Conclusions

Phenotypic selection for improved baking quality is not a feasible prospect for the early stages of a wheat breeding programme. Even later in the breeding process, dough rheology is used as a predictor of baking quality, with the baking tests themselves often left until the final stages of cultivar development. Consequently, direct genetic selection for improved end-use quality of wheat using MAS is desirable. HMW and LMW glutenin alleles are already being used by breeding programmes throughout the world to select for improved dough rheological characters. However, the relationship between these loci and final baking quality is less certain. The detection and confirmation of loci associated with baking quality presented here may provide greater opportunities for wheat breeders to improve end-use quality through MAS. MAS for wheat quality may be used as a replacement for early generation phenotypic selection or used in segregating populations to improve the economic efficiency and genetic gain of breeding programmes (Kuchel et al. 2005). In either case, the large proportion of genetic variation that can be manipulated using molecular makers linked to glutenin genes and other QTL should offer confidence in direct genetic selection and a major advantage over other phenotypic tests.

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