Theor Appl Genet (2005) 111: 410–422
DOI 10.1007/s00122-005-2001-0

ORIGINAL PAPER

W. Ma · R. Appels · F. Bekes · O. Larroque
M. K. Morell · K. R. Gale

Genetic characterisation of dough rheological properties in a wheat doubled haploid population: additive genetic effects and epistatic interactions

Received: 20 June 2004 / Accepted: 10 March 2005 / Published online: 18 June 2005

Abstract Doubled haploid lines ($n = 160$) from a cross between wheat cultivars 'Cranbrook' (high dough extensibility) and 'Halberd' (low dough extensibility) were grown at three Australian locations. The parents differ at all high- and low-molecular-weight glutenin loci. Dough rheological parameters were measured using small-scale testing procedures, and quantitative trait locus (QTL) mapping procedures were carried out using an existing well-saturated genetic linkage map for this cross. Genetic parameters were estimated using three software packages: QTLCartographer, Epistat and Genstat. Results indicated that environmental factors are a major determinant of dough extensibility across the three trial sites, whereas genotypic factors are the major determinants of dough strength. Composite interval mapping analysis across the 21 linkage groups revealed that as expected, the main additive QTLs for dough rheological properties are located at the high- and low-molecular-weight glutenin loci. A new QTL on chromosome 5A for M-extensibility (a mixograph-estimated measure of extensibility) was detected. Analysis of epistatic interactions revealed that there were significant conditional epistatic interactions related with the additive effects of glutenin loci on dough rheological properties. Therefore, the additive genetic effects of glutenins on dough rheological properties are conditional upon the genetic background of the wheat line. The molecular basis of the interactions with the glutenin loci may be via proteins that modify or alter the gluten protein matrix or variations in the expression level of the glutenin genes. Reverse-phase high performance liquid chromatography analysis of the molar number of individual glutenin subunits across the population showed that certain conditional epistases resulted in increased expression of the affected glutenin. The epistatic interactions detected in this study provide a possible explanation of the variable genetic effects of some glutenins on quality attributes in different genetic backgrounds and provide essential information for the accurate prediction of glutenin related variance in marker-assisted wheat breeding.

Keywords Wheat · Dough quality · Additive QTLs · Digenic epistatis · Conditional epistatic interactions · Glutenins

Communicated by A. Charcosset

W. Ma · F. Bekes · O. Larroque · M. K. Morell · K. R. Gale (✉)
CSIRO Plant Industry, GPO Box 1600, ACT 2601, Australia
E-mail: Kevin.Gale@deh.gov.au
Tel.: +61-2-6274 2737

W. Ma · R. Appels
Western Australia Department of Agriculture and CRC for Molecular Plant Breeding, Biological Sciences, Murdoch University, WA, 6152, Australia

Introduction

Wheat dough has unique properties, the most important of which is the viscoelasticity of its gluten. This special property allows the baking of bread, which has been a basic food for man throughout recorded history, and probably for a much longer period; it remains the principal food product made from wheat (Briggle and Curtis 1987). The physical properties (rheological properties) of wheat-flour doughs include their extensibility and resistance to extension, which influence their mixing behaviour very strongly, and are important factors in the wheat varieties' bread-making quality. Dough extensibility is the extent to which dough can be elongated before rupture. Maximum resistance (Rmax) is a measure of the resistance of the dough to extension, or the maximum force required to stretch a piece of dough. These two characteristics of wheat dough (rheological properties) reflect the elastic and film-forming properties of a given dough, which give wheat its unique bread-making specificity (Simmonds 1989; Eliasson and Larsson 1993).

Different types of breads have different requirements for dough extensibility and strength. In most cases, high

dough extensibility and strength are desirable (Pogna et al. 1996). Strong dough will form a cohesive mass that has resistance to extension and can retain stability during mixing (Simmonds 1989). Extensible doughs are able to hold the gas produced during fermentation within evenly distributed discrete cells within their structure. This results in a loaf crumb in which the gas cells are of regular size and even distribution (Simmonds 1989). Such a crumb structure appears light in colour, fine and silky in structure, both highly desirable characteristics of bread. Weak gluten will make the gas cells expand excessively during fermentation, causing their walls to collapse and the cells to coalesce. The resulting bread will have very open texture with a coarse wall structure (Finney et al. 1987; Simmonds 1989). For those reasons, dough extensibility and strength are among the most important quality factors selected during breeding. However, breeding wheat varieties with desirable rheological properties requires significant investment, as the measurement of dough rheological properties is time-consuming and often subject to systematic errors. Furthermore, dough extensibility is a trait with low heritability, making selective breeding more difficult.

The protein matrix (gluten) formed by the wheat storage proteins in dough are major factors determining the dough rheological properties. Wheat storage proteins consist of two major fractions: the gliadins and the glutenins (Nieto-Taladriz et al. 1994). Gliadins are monomeric proteins and can be separated into four groups, alpha-, beta-, gamma- and omega-gliadins, based on their size. Gluten is an aggregate of high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS) held together by disulphide bonds (Payne et al. 1981, 1987; Jackson et al. 1983). There is wide variation between wheat varieties in the composition of gliadins and glutenin subunits (Nieto-Taladriz et al. 1994). There is now a considerable body of evidence showing that wheat dough strength is mainly a function of grain glutenin composition, with minor or modifying effects from gliadins (Wall 1979; Branlard and Dardevet 1985, 1994; Payne et al. 1987; Bekes et al. 2001; Cornish et al. 2001). This knowledge is currently used in breeding wheat varieties with high dough strength (Cornish et al. 2001; Eagles et al. 2001). From a breeder's perspective, it is important to know whether there are genetic factors (genes) other than glutenins and gliadins that affect dough strength. These factors may represent minor proteins in wheat grain that modify gluten functionality (e.g. by affecting the degree of cross-linking and thus size distribution of the polymer), or transcription factors that control glutenin expression level. These factors may be identified as quantitative trait loci (QTLs) that have independent additive effects or loci that show epistatic interaction with the major glutenin loci. Identifying such genetic factors or QTLs for dough rheological properties will provide wheat breeders with new information on gluten quality attributes in different genetic backgrounds and markers for additional sources of genetic variation other than those directly linked to the glutenin structural genes.

Advances in statistical methods for QTL mapping, such as those made by Haley and Knott (1992), Jansen and Stam (1994) and Zeng (1994), facilitate accurate location of additive genetic factors for any given trait. Chase et al. (1997) developed a statistical procedure and corresponding software, Epistat, for analysing digenic epistatic interactions based on estimates of log-likelihood ratio (LLR), which is computed as the log of the ratio of the additive model and the epistatic model.

We report here the identification of QTLs and their conditional digenic epistatic interactions for dough rheological properties based on the analysis of a doubled haploid (DH) population with a well-saturated genetic linkage map and extensive quality trait data from three field trials.

Materials and methods

Genetic materials

A wheat × maize induced DH population, 'Cranbrook' × 'Halberd', consisting of 160 lines was used in this study (Kammholz et al. 2001). Both 'Cranbrook' and 'Halberd' are hard-grained varieties that differ widely in various quality and agronomic traits. All of the six HMW and LMW glutenin loci are polymorphic between the two parental lines (Table 1). 'Cranbrook' has high dough extensibility, whereas 'Halberd' possesses relatively low extensibility; hence the prime purpose of the cross was to study the genetics of dough rheology.

Field trials and quality assessment

Field trials of the DH population were carried out at three sites in Australia: Roseworthy in South Australia (1996), and Roma and Stowe in Queensland (1997). These sites represent three typical wheat production environments in Australia. A Latinised row column design was used with two replications of each line at the Roma and Roseworthy field sites and an un-replicated design at Stowe. Each entry was planted in a plot of 1.9 m × 8 m, using 742 seeds, equal to the commercial seeding rate of 10^6 plants/ha.

Table 1 High- and low-molecular-weight glutenin compositions of two parents

Parent	*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*
'Cranbrook'	*b* (2*)	*i* (17 + 18)	*a* (2 + 12)	*b*	*d*	*a*
'Halberd'	*a* (1)	*e* (20)	*d* (5 + 10)	*e*	*c*	*c*

Dough testing

Dough rheological parameters including extensibility and dough Rmax were measured using a small-scale testing procedure (Bekes et al. 2001). Briefly, 2 g flour samples were mixed in duplicate using a 2-g mixograph (TMCO, Lincoln, Neb., USA) with water adsorption calculated by the American Association of Cereal Chemists method (1998), to peak dough development. The resulting 3.5 g of dough was rested (30°C, >90% relative humidity), moulded into 6-mm diameter strips and tested in duplicate using a micro-extension tester (Bekes et al. 2001) with a 6-mm diameter hook, at an elongation rate of 1 cm/s. Rmax to extension (N) and extension at rupture (cm) were calculated. A predicted extensibility value (M-extensibility, the estimate of extensibility from measurement of protein quantity and mixograph data) was also calculated using mixograph data as specified by Bekes et al. (2001), i.e.

$$\mathrm{M-EXT} = 9.7220 + 0.4593\mathrm{PROT} + 0.0465\mathrm{MBW} - 0.00855\mathrm{BWBD}$$

where M-EXT is M-extensibility, MBW is maximum bandwidth, and BWBD is bandwidth breakdown.

Statistical and QTL analysis

Genstat (version 5-4.1, Lawes Agriculture Trust, 1998) was used to conduct all statistical analyses, including basic statistics, skewness and kurtosis of data distribution, and variance component analysis of the three field trials using residual maximum likelihood method. The mean heritability of each trait was calculated as σ2genotype/(σ2genotype + σ 2 residual/no. reps). Windows QTLCartographer (Basten et al. 1997; Wang et al. 2002) was used to conduct composite interval mapping based on an existing genetic linkage map (Chalmers et al. 2001) with a 1-cM walking speed and the background marker searching model VI (Basten et al. 1997) with a 10-cM window size. Epistat (Chase et al. 1997) was used for genome-wide searching of conditional epistatic interactions. The LLR value 4 ($P=0.005$) was used as threshold of epistasis for each individual trial. The detected interaction was reported if the Monte Carlo simulation-based P-value (by using program Mntecrlo, a companion program of Epistat) was below 0.05 in at least one another trial, and the interaction pattern was consistent among the three field trials.

Reversed-phase high performance liquid chromatography

In order to examine the molecular bases of conditional epistases involving glutenins in this study, reversed-phase high performance liquid chromatography (RP-HPLC) was used to determine HMW-GS/LMW-GS ratio (H/L). The whole procedure was performed as described by Larroque et al. (2000). Millenium 32 chromatography manager software was used for integration. To obtain the H/L value, two sectors were defined based on elution time. The first, corresponding to HMW-GS, comprised the more hydrophilic components (13–28 min), whereas the second corresponded to LMW-GS (28–48 min), which are less hydrophilic.

Results

Statistical analysis of field trial data

A basic descriptive statistics result is listed in Table 2. Notable differences were observed between the three trials. Most quality data measurements had low skewness and kurtosis, indicating normal distribution.

Variance component analysis (Table 3) indicated that Rmax possessed a high genotypic variance component (63.8%) and a low environmental variance component

Table 2 Basic statistical parameters of nine measurements

Trait*	RosMext (cm)	RomMext (cm)	StoMext (cm)	RosExt (cm)	RomExt (cm)	StoExt (cm)	RosRmax (AU)	RomRmax (AU)	StoRmax (AU)
'Cranbrook'	25.10	25.96	24.85	21.53	25.60	25.22	1106.14	1010.31	821.62
'Halberd'	18.24	20.64	19.11	12.36	13.06	14.28	1101.50	1021.73	820.14
Mean	19.31	23.82	20.14	13.03	17.83	17.31	1102.70	1019.99	807.18
Standard error	0.09	0.09	0.16	0.28	0.17	0.17	25.11	28.60	23.09
Median	19.35	23.80	20.20	12.56	17.90	17.42	1066.50	965.00	768.00
Standard deviation	1.14	1.08	2.07	3.46	2.18	2.10	315.57	360.65	291.21
Kurtosis	0.72	−0.02	0.52	0.23	−0.02	−0.35	−1.00	−0.07	−0.10
Skewness	−0.23	−0.21	−0.32	0.48	−0.17	−0.30	0.12	0.53	0.65
Minimum	15.20	20.70	13.80	5.17	11.58	12.24	510.00	344.00	278.00
Maximum	22.20	26.40	25.40	25.00	22.87	21.84	1732.00	2014.00	1662.00

*_RosMext_, _RomMext_ and _StoMext_ Mixograph-estimated measure of extensibility (M-extensibility) measured at Roseworthy, Roma and Stowe, respectively. _RosExt_, _RomExt_ and _StoExt_ Extensibility measured at Roseworthy, Roma and Stowe, respectively. _RosRmax_, _RomRmax_ and _StoRmax_ Maximum resistance (Rmax) measured at Roseworthy, Roma and Stowe, respectively. _AU_ Amylograph units

Table 3 Variance components analysis of the three dough rheological parameters

Source*		E	G	G × E	Reps	e	G/G × E
Ext (cm)	Component	6.411, 61.1%	1.554, 14.8%	0.229, 2.2%	0.045, 0.4%	2.26, 21.5%	6.78
	Standard error	6.42	0.237	0.175	0.074	0.184	
Mext (cm)	Component	5.7466, 73.2%	0.8841, 11.3%	0.2936, 3.7%	0.0001, 0%	0.9203, 11.7%	3.01
	Standard error	5.7538	0.1349	0.0882	0.0048	0.0780	
Rmax (Brabender units)	Component	400, 3.3%	7462, 63.8%	1839, 15.4%	32, 0.3%	2062, 17.2%	4.06
	Standard error	420	952	278	55	172	

**E* Environmental variance, *G* genotype variance, *G* × E genotype × environment variance, *Reps* variance for replications in environments, *e* pooled error.

(3.3%). In contrast, both extensibility and M-extensibility showed a high environmental component (61.1% and 73.2%, respectively) and a low genotypic component (14.8% and 11.3%, respectively). The three quality parameters, Rmax, extensibility and M-extensibility, all had relatively low values for genotype × environment (G × E) interactions, especially for extensibility, which had the highest G/G × E ratio. The variance values between replicates were close to zero.

Heritability analysis (Table 4) revealed that no significant difference was found between the two measurements of extensibility (extensibility and M-extensibility). A significant difference was evident however, between the two environments for both extensibility and M-extensibility. In the Roseworthy environment, the heritability was 82.9% and 83.1% for extensibility and M-extensibility, respectively. At Roma, the heritability became much lower, being 38.3% and 39.1% for the two measurements, respectively. In contrast, heritability estimates for Rmax were consistent at both field trials, with 91.0% and 87.6% at Roma and Roseworthy, respectively. Because the Stowe field trial was unreplicated, no heritability data were calculated for this environment.

Composite interval mapping (additive QTLs)

Major QTLs for the traits under study were located on group 1 chromosomes by conducting composite interval mapping over all 21 chromosomes (linkage groups). Figures 1, 2 and 3 illustrate the position of the significant QTLs. On chromosome 1A (Fig. 1), one significant QTL was located at the *Glu-A3* region, which was significant for eight of the nine trait measurements, including extensibility and M-extensibility at all three trials, and Rmax at the Roseworthy and Roma field trials. Rmax data from the Stowe field trial was found not to be significant. Extensibility data from the Roseworthy and Stowe, M-extensibility from Roma, and Rmax from Roma, had LLR values above 22, whereas the other three data measurements had LLR values below 15. The HMW glutenin region, *Glu-A1*, did not show any significant effects on any of the nine trait measurements. The 'Cranbrook'-type allele (*Glu-A3b*) had a positive association with all data measurements.

Table 4 Heritability estimation of the three parameters

E[a]	Ext (%)	Mext (%)	Rmax (%)
ROS	82.9	83.1	91.0
ROM	38.3	39.1	87.6
Combined	14.6	90.0	92.3

[a] *ROS* Roseworthy, *ROM* Roma; Stow environment heritability data were not calculated as this site was an unreplicated trial.

Two QTLs were identified on chromosome 1B (Fig. 2), with one located at the LMW glutenin (*Glu-B3*) locus and another one located at the HMW glutenin (*Glu-B1*) locus. The first QTL was significant for all of the trait measurements, except for extensibility in the Roma trial. It is worth noting that this QTL had a higher LLR value than the one located on chromosome 1A (*Glu-A3* locus). The LLR value for this QTL was above 30 for six trait measurements, including extensibility at Stowe and Roseworthy, M-extensibility at Roseworthy and Roma, and Rmax at Roseworthy and Roma. The second QTL on chromosome 1B consisted of three high LLR peaks (LLR > 38), which were related to the three Rmax measurements. Extensibility at Roseworthy was the only extensibility-related measurement that had a significant LLR peak at this region; however, the peak location was not located at the *Glu-B1* locus and the LLR value was below 18. Again, the 'Cranbrook'-type alleles of *Glu-B1* (*i*) and *GluB3* (*d*) made a positive contribution to the relevant traits, with the only exception being the putative QTL for extensibility at Roseworthy at the *Glu-B1* locus, which revealed a positive contribution from the 'Halberd'-type allele (*e*).

Chromosome 1D contained a highly significant QTL at the HMW glutenin region (*Glu-D1*) associated with Rmax measurements from all three environments (LLR > 64, Fig. 3). Again, the extensibility trait data from the Roseworthy site was the only extensibility-related measurement that had a significant LLR value associated with this chromosomal region, with a much lower LLR value (< 18). The LMW glutenin region of chromosome 1D (*Glu-D3*) was also found to have significant genetic effects, for extensibility at Roma and Stowe, M-extensibility at Roma and Stowe, and for Rmax at Roma and Roseworthy. The 'Cranbrook'-type allele of *Glu-D3* (*a*) had a positive association with all trait measurements while the 'Halberd'-type allele of *Glu-D1* (*d*) had a positive contribution to Rmax.

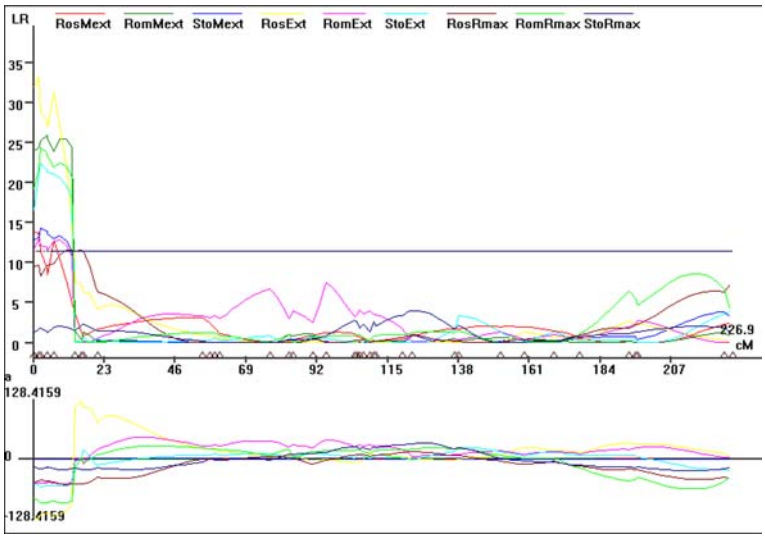

Fig. 1 Composite interval mapping results on chromosome 1A. *RosMext*, *RomMext* and *StoMext* Mixograph-estimated measure of extensibility (M-extensibility) measured at Roseworthy, Roma and Stowe, respectively. *RosExt*, *RomExt* and *StoExt* Extensibility measured at Roseworthy, Roma and Stowe, respectively. *RosRmax*, *RomRmax* and *StoRmax* Maximum resistance (Rmax) measured at Roseworthy, Roma and Stowe, respectively

A significant QTL was also located on chromosome 5A (Fig. 4), representing the only QTL detected outside the glutenin loci in this study. This QTL was significant for M-extensibility at Roma (LLR = 20.8) and Stowe (LLR = 21.0). The peak was at the location of marker PAAG/MCCC4. No significant effects were detected for this QTL on M-extensibility at Roseworthy, or extensibility or Rmax at the three field trial sites.

Epistasis analysis

A genome-wide search of epistatic interactions involving all glutenin loci for the three studied parameters was performed. Because the majority of glutenins have additive genetic effects on dough rheological properties, the detected epistases were mainly conditional epistases (Chase et al. 1997).

Extensibility

A genome-wide search of digenic interactions for extensibility revealed one pair of significantly interacting loci, the LMW glutenin locus (*Glu-B3*, chromosome 1B) and a microsatellite marker, GWM044, located on chromosome 7D. This interaction had LLR values of 4.62 and 6.65, and MonteCarlo-based *P*-values of 0.0037 and 0.0007 for the Roseworthy and Roma field trials, respectively. The interaction was not significant for the Stowe trial (LLR = 0.64). At the Roseworthy site, *Glu-B3* demonstrated a highly significant additive genetic effect on extensibility (*Glu-B3 d* allele over *c* allele, LLR = 12.11, $P < 9.65 \times 10^{-7}$). However, this additive genetic effect was not present for *Glu-B3* when combined with the 'Halberd' background of marker GWM044 (LLR = 1.48, $P > 0.05$). The additive effect of *Glu-B3* on extensibility in the 'Cranbrook' background of marker GWM044 was highly significant, with an LLR value of 12.71

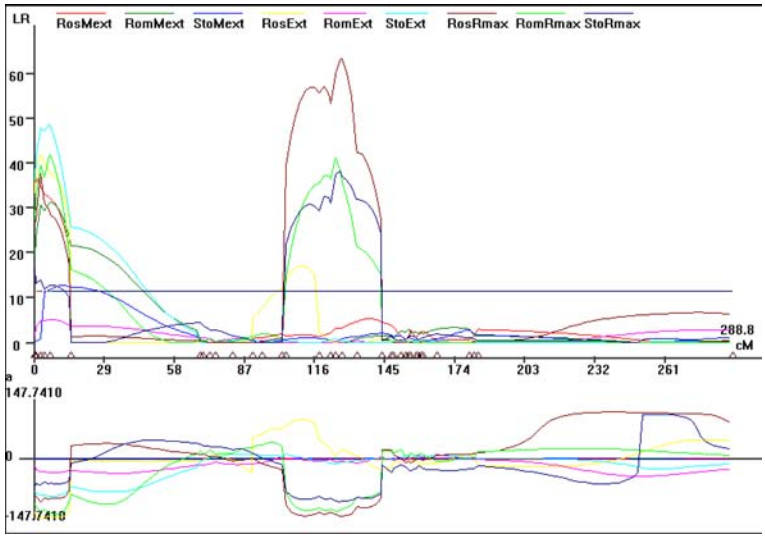

Fig. 2 Composite interval mapping results on chromosome 1B *RosMext*, *RomMext* and *StoMext* Mixograph-estimated measure of extensibility (M-extensibility) measured at Roseworthy, Roma and Stowe, respectively. *RosExt*, *RomExt* and *StoExt* Extensibility measured at Roseworthy, Roma and Stowe, respectively. *RosRmax*, *RomRmax* and *StoRmax* Maximum resistance (Rmax) measured at Roseworthy, Roma and Stowe, respectively

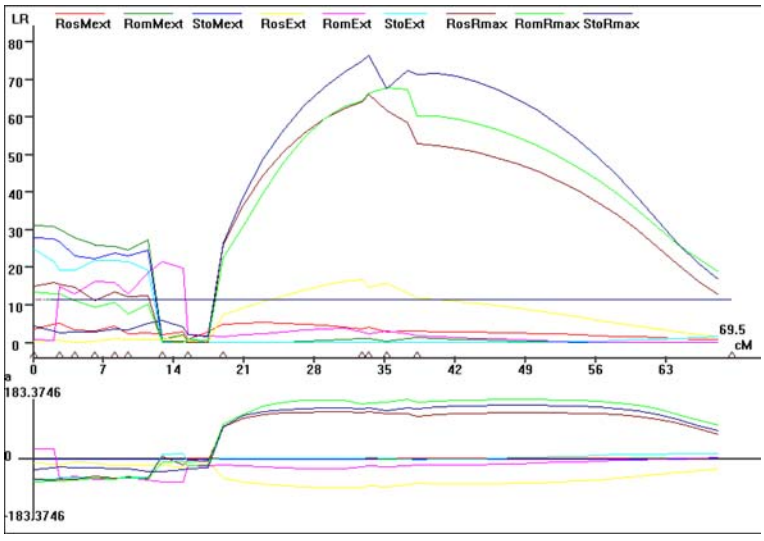

Fig. 3 Composite interval mapping on chromosome 1D *RosMext*, *RomMext* and *StoMext* Mixograph-estimated measure of extensibility (M-extensibility) measured at Roseworthy, Roma and Stowe, respectively. *RosExt*, *RomExt* and *StoExt* Extensibility measured at Roseworthy, Roma and Stowe, respectively. *RosRmax*, *RomRmax* and *StoRmax* Maximum resistance (Rmax) measured at Roseworthy, Roma and Stowe, respectively

($P < 9.65\times10^{-7}$). At the Roma field trial site, *Glu-B3* did not show a significant additive effect on extensibility (Fig. 2). For the Roma site, it was confirmed that *Glu-B3* did not have a significant additive effect in the presence of the 'Halberd' allele of marker GWM044 (LLR = 1.03, $P > 0.05$) but showed a significant additive effect on extensibility in the presence of the 'Cranbrook' allele of this marker (LLR = 4.28, $P < 0.004$). For the Stowe site, *Glu-B3* showed a significant additive effect on extensibility (LLR = 14.28), and this effect was evident in the presence of either the 'Cranbrook' or 'Halberd' alleles of marker GWM044 (LLR = 9.33 and 5.19, respectively).

M-extensibility

A total of seven pairs of conditional epistasis were detected for M-extensibility (Table 5). Among these, *Glu-A3* interacted with four non-glutenin chromosomal regions, including chromosome 2D (single significant marker, CDO366), chromosome 3A (single significant marker, PAGA/MGCG290), chromosome 7A (ten significant markers) and chromosome 7B (single significant marker, PACT/MCAT3). *Glu-B3* interacted with two non-glutenin chromosomal regions: chromosome 6A (three significant markers) and chromosome 7A (two significant markers). Significant interaction was also detected between *Glu-B3* and *Glu-D3*. These epistases were all significant based on MonteCarlo simulation for the Roma and Stowe field trials but were not significant for the Roseworthy field trial.

Whereas the 'Halberd' allele of marker CDO366 was required for *Glu-A3* to express its effect on M-extensibility, the 'Cranbrook'-type alleles of the other three interacting chromosomal regions were required for the expression of the positive effect of *Glu-A3* on M-extensibility. For *Glu-B3* and *Glu-D3*, the 'Halberd'-type alleles of their interacting regions were required for their positive effects on M-extensibility.

Some statistical parameters related to the interaction between *Glu-B3* and *Glu-D3* are listed in Table 6. In detail, the *P*-value for the effect of *Glu-B3* on M-extensibility decreased from 0.23 at Roma and 0.53 at Stowe in the presence of the 'Cranbrook' allele of *Glu-D3* (*a* allele) to 4.1E-6 at Roma and 3.3E-6 at Stow in the presence of the 'Halberd' allele of *Glu-D3* (*c* allele, Table 6). For *Glu-D3*, the significance of the effect on M-extensibility increased from $P = 0.11$ at Roma and

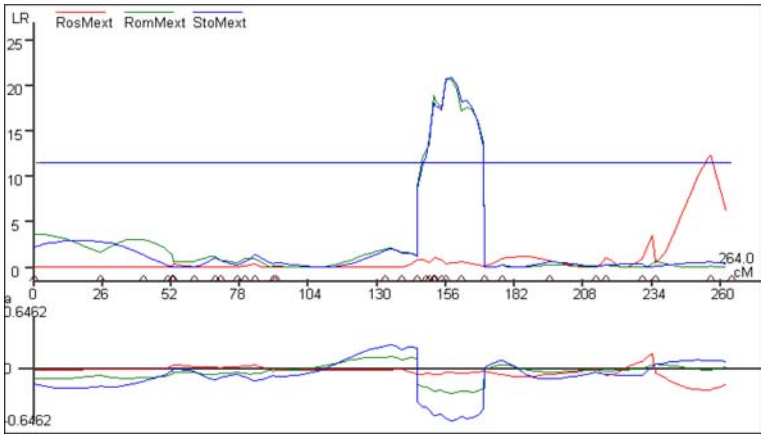

Fig. 4 Composite interval mapping for M-extensibility on chromosomes 5A *RosMext*, *RomMext* and *StoMext* Mixograph-estimated measure of extensibility (M-extensibility) measured at Roseworthy, Roma and Stowe, respectively

Table 5 Epistases and their statistical parameters for M-extensibility

Marker1	Marker2[a]	ROS				ROM				Stow			
		LLR[b]	*P*-value	Marker1 *P*-value	Marker2 *P*-value	LLR	*P*-value	Marker1 *P*-value	Marker2 *P*-value	LLR	*P*-value	Marker1 *P*-value	Marker2 *P*-value
GluA3	CDO366 (2D,h)	0.196	0.7477	0.0163	0.2048	4.573	0.0042	0.0083	0.0489	4.840	0.0033	0.0062	0.0405
	PAGA/MGCG290 (3A,c)	0.575	0.3530	0.0128	0.7987	4.008	0.0084	0.0040	0.4559	4.270	0.0064	0.0024	0.4408
	PAGC/MCCT5 (7A,c)	0.035	0.9146	0.0253	0.7842	4.693	0.0024	0.0031	0.4980	4.501	0.0049	0.0023	0.5424
	PAGC/MCAA8 (7A,c)	0.051	0.8566	0.0293	0.7804	4.431	0.0043	0.0062	0.7055	4.413	0.0043	0.0072	0.6992
	PACG/MGAC450 (7A,c)	0.023	0.8367	0.0134	0.8045	4.028	0.0068	0.0021	0.6441	4.126	0.0048	0.0013	0.6639
	PACC/MCAG8 (7A,c)	0.151	0.6719	0.0068	0.5550	4.054	0.0075	0.0056	0.3151	3.779	0.0062	0.0037	0.3077
	PAGC/MCCT179 (7A,c)	0.047	0.9102	0.0055	0.8980	6.110	0.0007	0.0009	0.8867	5.646	0.0015	0.0015	0.8742
	PACC/MCAC7 (7A,c)	0.092	0.9047	0.0034	0.7824	5.441	0.0016	0.0038	0.4799	5.579	0.0011	0.0042	0.4348
	PAAC/MCCA3 (7A,c)	0.084	0.9580	0.0080	0.6516	4.121	0.0085	0.0014	0.7965	3.991	0.0074	0.0014	0.7641
	PAGC/MCCT6 (7A,c)	0.089	0.7901	0.0267	0.7283	4.628	0.0026	0.0046	0.7879	4.479	0.004	0.0025	0.7692
	PATA/MCTT2 (7A,c)	0.065	0.7421	0.0091	0.9978	4.830	0.0026	0.0014	0.9051	3.847	0.0084	0.0009	0.9575
	PACC/MCCG229 (7A,c)	0.028	0.9300	0.0060	0.7378	4.933	0.0022	0.0031	0.6723	4.634	0.0032	0.0028	0.6665
	PACT/MCAT3 (7B,c)	0.150	0.6496	0.0035	0.0161	3.920	0.0065	0.0009	0.1584	4.334	0.0059	0.0008	0.2306
GluB3	PAGA/MCAG178 (1D,h)	1.339	0.1213	0.0000	0.0127	5.190	0.0015	0.0003	0.0000	4.482	0.0042	0.0007	0
	PAAT/MCCA304 (1D,h)	0.781	0.2432	0.0000	0.0423	6.786	0.0004	0.0000	0.0000	6.381	0.0007	0	0
	GluD3 (1D,h)	1.185	0.1318	0	0.0291	3.870	0.0083	0	0	3.5580	0.009	0	0
	PAGT/MCCC1 (6A,h)	0.377	0.5173	0.0000	0.0060	4.089	0.0062	0.0000	0.3705	3.901	0.0063	0	0.4456
	PAGA/MCAT8 (6A,h)	0.658	0.2837	0.0001	0.0002	4.722	0.0023	0.0000	0.3994	4.293	0.0051	0	0.4415
	PAGC/MCAG4 (6A,h)	0.374	0.4174	0.0000	0.0003	4.229	0.0063	0.0001	0.3512	3.841	0.0074	0	0.4289
	PACG/MGAC255 (7A,h)	0.945	0.1834	0.0000	0.4936	4.963	0.0024	0.0000	0.0419	4.913	0.0027	0.0001	0.0539
	wmc116 (7A,h)	0.567	0.3170	0.0000	0.4272	3.779	0.0082	0.0005	0.1358	4.087	0.0069	0.0016	0.1544
GluD3	PAAT/MCCA285 (1B,h)	0.820	0.2118	0.0475	0.0000	5.556	0.0016	0.0000	0.0000	5.205	0.0037	0	0.0001
	GluB (1B,h)	1.008	0.1698	0.0603	0.0000	4.093	0.0072	0.0000	0.0000	3.738	0.0096	0	0
	PAGG/MCAC174 (1B,h)	0.322	0.4337	0.0921	0.0000	8.161	0.0006	0.0000	0.0000	7.837	0.0006	0	0

[a]Specifies chromosomal location and the advantageous allele. *c* 'Cranbrook'-type allele, *h* 'Halberd'-type allele
[b]*LLR* Log-likelihood ratio

$P = 0.09$ at Stowe in the presence of the 'Cranbrook' allele of *Glu-B3* (*d* allele) to $P = 6.5 \times 10^{-8}$ at Roma and $P = 4.2 \times 10^{-7}$ at Stowe in the presence of the 'Halberd' allele of *Glu-B3* (*c* allele, Table 7). The interaction between *Glu-B3* and *Glu-D3* was not significant at the Roseworthy site (LLR = 1.19, $P > 0.15$).

Dough Rmax

Five glutenin loci (*Glu-B1*, *-D1*, *-A3*, *-B3* and *-D3*) have significant effects on dough Rmax in this study, and four of these, *Glu-B1*, *-D1*, *-B3* and *-D3*, require the presence of a specific allele of other, non-glutenin loci to express their full effects (Table 8). *Glu-B1* interacts with three non-glutenin chromosomal regions, including the telomere region of the long arm of chromosome 1A (two significant markers), and the short and long arms of chromosome 3A (two and eight significant markers respectively). *Glu-B3* interacts with one non-glutenin region near the distal end of chromosome 1D (two significant markers). The *Glu-D1* locus interacts with a chromosome region on the long arm of chromosome 2B (two significant markers).

The most significant epistasis detected in this study was for the trait of dough Rmax, between the HMW

Table 6 Genetic effects of *GluB3* on M-extensibility

Trial	*d-c*		*dc-cc*[a]		*da-ca*[b]	
	a(cm)[c]	*P*-value	*a* (cm)[c]	*P*-value	*a* (cm)[c]	*P*-value
ROS	0.8	$7.0E0\times10^{-6}$	1.1	$3.3\times10^{-}$ E-5^{5}	0.45	0.053
ROM	0.7	$3.5\times10^{-E\text{-}55}$	1.2	$4.1\times10^{-}$ E-6^{6}	0.3	0.23
STO	1.3	$5.1\times10^{-}$ E^{5} −5	1.9	$3.3\times10^{-}$ E-6^{6}	0.4	0.53

[a]*dc-cc GluB3d-GluB3c* at the *GluD3c* ('Halberd'-type allele) background
[b]*da-ca GluB3d-GluB3c* at the *GluD3a* ('Cranbrook'-type allele) background;
[c]Mean difference

glutenin locus *Glu-B1* and a region of the long arm of chromosome 3A (Table 8). The marker pAGT/mCAG2 did not express an independent genetic effect on Rmax ($P > 0.6$ at the three field trials, Table 8). However, the LLR values of the interaction between *Glu-B1* and marker pAGT/mCAG2 for the Roseworthy, Roma and Stowe field trials were 8.51, 4.85 and 12.00, with the Monte Carlo simulation-based *P*-values of 0.0001, 0.0032 and 10^{-8}, respectively. This epistasis demonstrated that the significant effect of *Glu-B1* on dough Rmax was dependent on the presence of the 'Cranbrook'-type allele of its interacting chromosome region, with no significant genetic effect apparent in the presence of the 'Halberd' allele of the interacting region. For example, for the Stowe field trial, the *Glu-B1* locus had a significant genetic effect on Rmax, with an LLR value of 11.07 [*Glu-B1i*−*Glu-B1e* = 913.95−691.72 = 222.23 Amylograph units (AU)]. The LLR value of this genetic effect dropped sharply to 0.05 ($P > 0.3$) in the presence of the 'Halberd' allele of marker pAGT/mCAG2. The mean difference between genotype *Glu-B1e* and *i* decreased from 222.23 AU across the population to 19.81 AU in the presence of the 'Halberd' allele of marker pAGT/mCAG2. In the presence of the 'Cranbrook' allele of this marker, the additive effect of allele *Glu-B1i* over *Glu-B1e* was highly significant, with an LLR value of 15.01. The mean difference between *Glu-B1e* and *i* increased from 140.28 AU in association with the 'Halberd' allele of marker pAGT/mCAG2, to 389.16 AU (*Glu-B1i* mean = 1008.43 AU, *Glu-B1e* mean = 619.27 AU). Similar interaction patterns were revealed at the Roseworthy and Roma field trials. *Glu-B1* expressed an independent additive effect at Roseworthy and Roma (LLR = 20.59 and 16.33, respectively; the mean difference between *Glu-B1i* and *e* genotypes = 330.45 and 332.56 AU for Roseworthy and Roma, respectively). The LLR values of this additive effect increased from 1.94 at Roseworthy and 2.21 at Roma in the presence of the 'Halberd' allele of marker AGT/mCAG2, to 21.17 at Roseworthy and 15.35 at Roma in the presence of the 'Cranbrook' allele of this marker. Concurrently, the mean difference for Rmax increased from 140.28 AU at Roseworthy and 169.81 AU at Roma in the presence of the 'Halberd' allele of marker AGT/mCAG2 to 465.65 AU at Roma and 482.87 AU at Roseworthy in the presence of the 'Cranbrook' allele of this marker.

For Rmax, the two glutenin to glutenin interactions detected in this study were between *Glu-B1* and *Glu-B3*, and *Glu-D1* and *Glu-B3*. Unlike the interaction between *Glu-B3* and *Glu-D3* for M-extensibility, where each glutenin required the inferior allele of the interacting glutenin to express the positive glutenin–glutenin effect, for the two glutenin interactions for dough Rmax, each glutenin required the positive allele of the interacting glutenin to express a positive effect. Table 9 indicates that the 'Cranbrook'-type alleles of *Glu-B1* and *-B3* and the 'Halberd'-type allele of *Glu-D1* are the required alleles for its interacting glutenin to express its enhanced effect. These three alleles also had independent positive effects on dough Rmax. For example, *Glu-D1* showed LLR values of 7.57, 5.93 and 9.17 for the Roseworthy, Roma and Stowe field trials, respectively, in the presence of the 'Halberd' allele of *Glu-B3* (*c* allele, has negative association on dough Rmax). The three corresponding LLR values increased to 10.08, 12.80 and 12.86, respectively, in combination with the 'Cranbrook' allele of *Glu-B3* (*d* allele, has positive effect on dough Rmax).

Table 7 Genetic effects of *GluD3* on M-extensibility

Trial	*a-c*		*ac-cc*		*ad-cd*	
	a (cm)[a]	*P*-value	*a* (cm)[a]	*P*-value	*a* (cm)[a]	*P*-value
ROS	0.3	0.058	0.6	0.011	0	0.65
ROM	0.8	0.0006	1.2	$6.5\times10^{-}$ E-8^{8}	0.3	0.11
STO	1.6	0.0003	2.2	$4.2\times10^{-}$ E-7^{7}	0.8	0.09

[a]*ac-cc GluB3d-GluB3c* at the *GluD3c* ('Halberd'-type allele) background
[b]*ad-cd GluB3d-GluB3c* at the *GluD3a* ('Cranbrook'-type allele) background;
[c]Mean difference

Table 8 Epistases and their statistical parameters for dough Rmax

Marker1	Marker2	ROS				ROM				STO			
		LLR	*P*-value	Marker1 *P*-value	Marker2 *P*-value	LLR	*P*-value	Marker1 *P*-value	Marker2 *P*-value	LLR	*P*-value	Marker1 *P*-value	Marker2 *P*-value
GluB1	PACG/MCCA2 (1A,c)	2.366	0.0431	0.0000	0.0270	5.431	0.0040	0.0000	0.0043	3.642	0.0018	0	0.0517
	PACA/MCAC6 (1A,c)	3.722	0.0099	0.0000	0.0593	5.839	0.0023	0.0000	0.0621	3.885	0.0097	0	0.3513
	PACA/MCAG2 (3A,c)	2.886	0.0093	0.0000	0.3834	0.307	0.4730	0.0000	0.7034	4.666	0.0043	0	0.5194
	CDO460 (3A,c)	4.181	0.0065	0.0000	0.7611	0.374	0.4128	0.0000	0.9493	8.284	0.0003	0	0.681
	PACA/MCCT5 (3A,c)	3.816	0.0084	0.0000	0.4990	5.736	0.0016	0.0000	0.0643	2.276	0.044	0	0.1577
	PAGT/MCAG3 (3A,c)	4.243	0.0056	0.0000	0.3877	6.510	0.0012	0.0000	0.2103	3.759	0.0112	0	0.2149
	PAGT/MCAG2 (3A,c)	8.513	0.0001	0.0000	0.7657	4.851	0.0032	0.0000	0.6008	12.000	0	0	0.6138
	wmc169 (3A,c)	6.705	0.0005	0.0000	0.4548	3.462	0.0132	0.0000	0.9375	10.16	0.0001	0	0.3401
	PAGT/MCAT1 (3A,c)	4.360	0.0043	0.0000	0.3952	3.139	0.0206	0.0000	0.7026	13.939	0	0	0.3277
	PACC/MCCG134 (3A,c)	9.535	0.0001	0.0000	0.6985	2.237	0.0658	0.0000	0.2852	11.292	0.0001	0	0.6963
	PACA/MCGA6 (3A,c)	7.963	0.0004	0.0000	0.9573	2.119	0.0759	0.0000	0.6410	12.165	0	0	0.3401
	PAAC/MCTA7 (3A,c)	8.150	0.0004	0.0000	0.6609	2.187	0.0834	0.0000	0.3586	10.98	0	0	0.724
GluB3	PAAG/MCAG3 (1D,c)	4.332	0.0049	0.0000	0.8571	0.612	0.3083	0.0001	0.8528	1.707	0.0822	0.0498	0.8559
	pACT/mCAT2 (1D,c)	5.451	0.0031	0.0000	0.9867	1.201	0.1839	0.0000	0.8046	2.537	0.0347	0.0091	0.981
GluD1	PACT/MCTG6 (2B,h)	3.947	0.0073	0.0000	0.1213	5.181	0.0028	0.0000	0.7406	2.624	0.0363	0	0.1823
	PAAT/MCAT3 (2B,h)	4.176	0.0078	0.0000	0.0562	3.159	0.0233	0.0000	0.6800	1.69	0.1762	0	0.0966
GluD3	PACA/MCCT2 (3D,h)	0.352	0.4272	0.0604	0.6192	5.972	0.0010	0.0000	0.8040	5.097	0.0036	0	0.7443
	PACG/MCGG161 (7B,c)	2.679	0.0265	0.0486	0.0202	4.249	0.0065	0.0000	0.5113	4.235	0.0051	0	0.5973

RP-HPLC data analysis

Thirty DH lines were selected from grain samples grown at the Roma field trial site for RP-HPLC analysis. Lines were selected based on three highly significant interactions observed between glutenin and non-glutenin loci to preliminarily assess whether the three epistatic interactions resulted from altered expression levels of the glutenin proteins. The three selected interactions were (1) the region on the long arm of chromosome 2B that interacts with *Glu-D1* for Rmax (Table 8), (2) the region on the long arm of chromosome 3A that interacts with *Glu-B1* for Rmax (Table 8) and (3) a region on the long arm of chromosome 7A that interacts with *Glu-A3* for M-extensibility (Table 5). The three representative markers corresponding to these three regions were PAAT/MCAT3, PAGT/MCAG2 and PAGC/MCCT179, respectively. The mean values for H/L of these selected DH lines are listed in Table 10. Results indicated that the mean H/L ratio differed significantly between the 'Cranbrook'-type and 'Halberd'-type DH lines for the loci represented by markers PAAT/MCAT3 ($P = 0.0084$) and PAGC/MCCT179 ($P = 0.0001$). Positive effect for two interactions correlated with differences in H/L ratio, indicating quantitative variation in glutenin expression. No significant difference in H/L was identified between the DH groups for the locus represented by marker PAGT/MCCT179 marker ($P = 0.1626$). This is suggesting that there are various mechanisms under epistasis between glutenin and non-glutenin regions; variation in glutenin expression may be one of the causes of these epistatic interactions.

Discussion

Effects of glutenin allelic composition

In selection for wheat quality, it is important to be able to detect QTLs of small magnitude, because these may play an important role in achieving the ultimate selection response in a breeding program (Kearsey and Pooni 1996). In the current study, we scanned the whole genome for loci affecting dough properties in this cross. The QTLs detected were mainly major QTLs that are located at the glutenin loci. Similar results were obtained in this

Table 9 Glutenin interactions for Rmax

Marker1	Marker2	ROS				ROM				STO			
		LLR	*P*-value	Marker1 *P*-value	Marker2 *P*-value	LLR	*P*-value	Marker1 *P*-value	Marker2 *P*-value	LLR	*P*-value	Marker1 *P*-value	Marker2 *P*-value
GluB1	PAAT/MCCA285 (1B,c)	5.296	0.0150	0.0000	0.0000	4.404	0.0223	0.0000	0.0001	1.447	0.4173	0	0.0317
	GluB3 (1B,c)	4.238	0.0211	0.0000	0.0000	3.391	0.0412	0.0000	0.0000	1.023	0.5766	0	0.0662
	PAGG/MCAC174 (1B,c)	4.162	0.0729	0.0000	0.0005	3.082	0.1386	0.0000	0.0014	0.956	0.4819	0.0003	0.0829
GluB3	PAGG/MCTC5 (1B,c)	4.863	0.0081	0.0000	0.0000	4.106	0.0159	0.0000	0.0000	0.855	0.2585	0.0242	0
	GluB1 (1B,c)	4.238	0.0211	0.0000	0.0000	3.391	0.0412	0.0001	0.0000	1.023	0.2326	0.0213	0
	PACG/MGAC288 (1D,h)	3.534	0.0279	0.0000	0.2245	2.874	0.0477	0.0000	0.1696	4.625	0.0067	0.0227	0.0034
	CDO580 (1D,?)	4.297	0.0161	0.0000	0.2148	2.500	0.0868	0.0000	0.1050	5.397	0.0044	0.0162	0.0206
	PAGG/MCTG7 (1D,h)	2.705	0.0751	0.0003	0.1965	1.637	0.1743	0.0009	0.0696	6.132	0.002	0.1188	0.0005
	CDO1173 (1D,h)	4.185	0.0125	0.0000	0.0633	3.442	0.0241	0.0001	0.0255	6.048	0.0024	0.0314	0.0021
	PAGC/MCCA8 (1D,h)	5.960	0.0027	0.0005	0.0379	3.217	0.0279	0.0020	0.0780	8.102	0.0009	0.0383	0.0116
	PAAT/MCAT8 (1D,h)	3.792	0.0162	0.0000	0.0304	1.764	0.1243	0.0000	0.0980	5.615	0.0023	0.0117	0.0052
	wmc429 (1D,h)	5.350	0.0049	0.0002	0.0519	3.063	0.0400	0.0001	0.0543	6.017	0.0016	0.0261	0.0013
	BCD402 (1D,h)	4.834	0.0105	0.0001	0.0016	4.109	0.0233	0.0005	0.0000	2.870	0.0378	0.0171	0
	GluD1 (1D,h)	4.036	0.0208	0.0000	0.0004	4.161	0.0223	0.0000	0.0000	2.246	0.0578	0.0234	0
	PAAA/MCCC3 (1D,h)	3.908	0.0232	0.0000	0.0003	4.006	0.0208	0.0002	0.0000	1.947	0.076	0.0249	0
GluD1	xKSUD14 (B)(1B,c)	3.462	0.0145	0.0007	0.0000	4.583	0.0095	0.0004	0.0004	3.646	0.0686	0	0.0315
	PAAT/MCCA285 (1B,c)	5.486	0.0029	0.0004	0.0000	4.709	0.0115	0.0000	0.0000	3.17	0.1077	0	0.0334
	GluB3 (1B,c)	4.036	0.0125	0.0001	0.0000	4.161	0.0118	0.0000	0.0000	2.248	0.2336	0	0.0896
	PAGG/MCAC174 (1B,c)	5.506	0.0034	0.0008	0.0000	4.321	0.0121	0.0005	0.0009	3.30	0.1382	0	0.1621

cross by analysis of glutenin allele effects using data obtained by different dough testing methods (Cornish et al. 2001; Gras et al. 2001). We detected one additional significant QTL located on chromosome 5A for M-extensibility. Other genetic factors that have small additive genetic effects on dough rheological properties may be present in this cross but were undetected due to limitations of the experimental design. Spatial errors related with the field trials and/or laboratories sample testing orders have not been considered in this study and this would influence the detection of small QTLs (Echermann et al. 2001). In addition, the population size (160 lines) may limit the detection of minor QTLs (Beavis 1994, 1998; Melchinger 1998).

Data analysis approaches

Two QTL analysis procedures were used in the current study, composite interval mapping (Zeng 1993, 1994; Basten et al. 1997) and epistasis analysis (Chase et al. 1997). A comparison between simple interval mapping (Lander and Botstein 1989) and composite interval mapping in the current study indicated that composite interval mapping has the ability to more clearly define QTL regions relative to simple interval mapping. Composite interval mapping on chromosome 1B led to the identification of two QTLs for dough rheological properties on the chromosome, one corresponding to the *Glu-A1* region and one to the *Glu-B1* region (Fig. 2), while simple interval mapping generated a result that is relatively inconclusive (Fig. 5).

We found Epistat (Chase et al. 1997) to be a versatile software package that allows the user to scan the data set quickly for potential interactions and apply statistical tests to determine the significance of the candidate loci. Epistat permits the analysis of all markers and can detect two kinds of epistatic effects, conditional and coadaptive epistasis. Conditional epistasis indicates that the primary additive effects of a QTL are conditional upon the presence of a particular allele from another locus, which may decrease or increase the primary QTL effect. In contrast, coadaptive epistasis indicates that one locus has no genetic effect when it acts independently; however, in the presence of a specific allele of a second locus, the locus has a genetic effect. Epistat was initially designed for analysis of recombinant inbred populations. Due to the similarity in genetic structure between DH populations and recombinant inbred populations, we have used Epistat in this study to analyse glutenin-related conditional epistatic interactions in a DH population. In addition to the glutenin-related conditional epistases, a complete pairwise search resulted in the identification

Table 10 Associations between three non-glutenin markers and high-molecular-weight glutenin subunit/low-molecular-weight glutenin subunit ratio

Marker	'Cranbrook'-type mean	'Halberd'-type mean	*P*-value
PAAT/MCAT3 (2B)	0.50	0.44	0.0084
PAGT/MCAG2 (3A)	0.43	0.46	0.1626
PAGC/MCCT179 (7A)	0.40	0.49	0.0001

of a large number of coadaptive epistasis for the dough properties studied. However, these co-adaptive epistases were found to have inconsistent interaction patterns across the three different field trial sites used in this study.

Variance components

Variance component analysis indicated that the primary variance component for Rmax was from genotypic factors, whereas extensibility was highly sensitive to environmental variation. It is interesting to note that Rmax and extensibility have similar G/G × E ratios. Nevertheless, the results suggest that whilst Rmax is amenable to genetic manipulation and thus breeding gain, extensibility as a trait is relatively difficult to target by selective breeding. The results of variance component analysis in this study are compatible with an independent study based on a smaller population and two field trials (Ma 2001).

As expected, both LMW and HMW glutenin loci were found to affect dough Rmax, with the HMW glutenin loci having the largest effect. The effects of the HMW glutenin loci on Rmax were consistent across all three environments, whereas the effects of the LMW glutenin loci were subject to variation between environments (see below).

Additive QTLs

Extensibility was mainly affected by the LMW glutenins, especially *Glu-A3* and *-B3* in this population. However, the effects of the LMW-GS on extensibility were not as robust as those of the HMW glutenins on Rmax across the three environments. At Roma, *Glu-A3* had a less significant additive effect on extensibility than at Roseworthy and Stowe. *Glu-B3* had no significant effect on extensibility at Roma; in contrast, there was significant effect observed at Roseworthy and Stowe. This observation is consistent with the observation that the heritability of extensibility for the Roma field trial was significantly lower than that for the Roseworthy trial. The inconsistency of effects of *Glu-3* loci on dough extensibility between environments makes selection for high dough extensibility in wheat a challenging task. The *Glu-D3* region was generally found to have a higher additive effect on M-extensibility than on extensibility, which is in agreement with the results of Bekes et al. (2001).

A key requirement for modern wheat cultivars targeting the high wheat grades, is to produce doughs with a balance between strength and extensibility. Usually, dough strength and extensibility are negatively correlated (Bekes et al. 2001). Interestingly, in this population, the direction of effect of all three LMW glutenin loci were the same for Rmax and extensibility, indicating that with respect to selection based on LMW glutenin alleles, dough strength and extensibility were not mutually exclusive. However, different results were evident for the effects of the HMW-GS. The *Glu-B1* locus was found to have a highly significant effect on Rmax, with no effect on extensibility, whereas the *Glu-D1* locus was shown to have opposite effects on Rmax and extensibility (positive effect on Rmax, negative effect on extensibility). Therefore, according to the current study, attaining a balance between dough strength and exten-

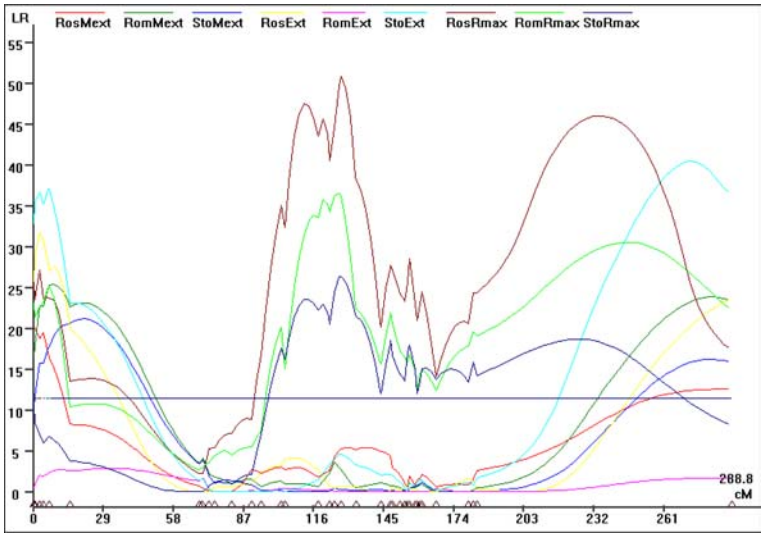

Fig. 5 Simple interval mapping on group 1B *RosMext*, *RomMext* and *StoMext* Mixograph-estimated measure of extensibility (M-extensibility) measured at Roseworthy, Roma and Stowe, respectively. *RosExt*, *RomExt* and *StoExt* Extensibility measured at Roseworthy, Roma and Stowe, respectively. *RosRmax*, *RomRmax* and *StoRmax* Maximum resistance (Rmax) measured at Roseworthy, Roma and Stowe, respectively

sibility by selection for HMW glutenin alleles alone may be difficult.

A new QTL for M-extensibility was identified on chromosome 5A. There is no apparent candidate gene for this QTL. Interestingly, this QTL is not significant for extensibility as measured by small-scale extensograph. Since M-extensibility is calculated from mixograph data (Bekes et al. 2001), this QTL may represent a gene or genes directly related to mixing properties. Further work is required to determine the basis of this QTL.

QTLs controlling flour protein content were also analysed in this DH population (data not shown), and no QTL for Rmax or extensibility was identified linked to flour protein content. This indicated that optimised protein type may play more important role than total protein content in determining dough physical properties.

Epistasis

The conditional digenic epistases reported in this study between glutenin and non-glutenin loci are the first reported. We conclude that important genetic factors, other than the glutenin structural genes, influence dough rheological properties through their effect on glutenin loci. The analysis of three epistatic interactions by RP-HPLC analysis of a sub-population of the lines ($n = 30$) showed that two of these were associated with variation in HMW glutenin expression, as indicated by variation in the HMW-to-LMW glutenin ratio. The most significant epistasis observed (between *Glu-B1* and marker PAGT/MCAG2 for Rmax) does not appear to be related to glutenin expression as judged by RP-HPLC. The molecular basis of this interaction is unknown and indicates that other mechanisms, in addition to effects on gene expression, may cause epistasis. One such mechanism may be the direct interaction between the gene products from the two interacting loci. Further RP-HPLC analysis of the whole DH population is currently underway.

The results of this and other studies emphasise the effects of genetic background on wheat functionality in addition to those of the glutenin loci. It is interesting that the identification of conditional epistatic interactions for glutenins in the current study did not identify additional variation for quality that is unrelated to the glutenin loci, but indicates that breeders may more accurately manipulate glutenin loci and their modifying loci to achieve breeding goals.

The current study suggests that the genetic background influences the expression of the effects of *Glu-A3*, *Glu-B1* and *Glu-D1* on dough properties. The HMW glutenin pair Dx5 + Dy10 (*Glu-D1 d* allele) has been shown to have a beneficial effect on dough strength and has been extensively selected for in breeding high-quality wheats (Varghese et al. 1996). It is thought that the extra cysteine residue of subunit Dx5 is responsible for the effect of this allele on dough quality (Greene et al. 1988; Gupta and MacRitchie 1991, 1994). However, Flavell et al. (1989) suggested that a more regular pattern of β turns in the central repetitive domain of subunit Dy10 may be responsible for the effect of this allele on dough quality. The current study demonstrated that the genetic background affects the expression of the positive effect of *Glu-D1 d* on dough strength. Specifically, the presence of the 'Halberd' allele of the locus defined by marker PAAT/MCAT3 or PACT/MCTG6 on chromosome 2B is required. The 'Halberd'-type alleles of these markers are associated with lower H/L ratio (Table 10). This information has important implications for the utilisation of *Glu-D1 d*-related variation in breeding programs. Similarly, HMW glutenin locus *Glu-B1* required other non-glutenin loci to express its full effect on Rmax in this population. These non-glutenin loci include 'Cranbrook'-type alleles of three different chromosomal regions (Table 8). Among them, the chromosome 3A long arm region (represented by marker PAGT/MCAG2) has the most significant interaction with *Glu-B1*. There is no indication of any relationship between this chromosomal region (marker PAGT/MCAG2) and the expression level of *Glu-B1* protein as indicated by RP-HPLC (Table 10).

One of the observed epistatic interactions resulted in a stable QTL for extensibility. Across the three field trials, *Glu-B3* had significant genetic effect on extensibility in two trials (Roseworthy and Stowe) but no significant effect for the Roma trial. This indicated that G × E interactions were associated with the *Glu-B3* effect on extensibility. However, significant epistasis was detected between marker GWM044 and *Glu-B3* for the Roseworthy and Roma field trials. Consequently, *Glu-B3* had a significant genetic effect on extensibility across the three field trials in association with the 'Cranbrook' allele of marker GWM044.

Glu-B3 also showed interaction with *Glu-D3* for M-extensibility and with *Glu-B1* and *Glu-D1* for Rmax. The epistasis between *Glu-B3* and *Glu-D3* suggested that each of these two loci had similar genetic effects on M-extensibility to the combination of two (Tables 6, 7), whereas the interactions between *GluB3* and *GluB1*, *D1* indicate that *GluB3 d* allele, which has positive effects on both extensibility and strength, significantly enhances both *GluB1*'s and *D1*'s effects on dough strength. These interaction patterns may be used by wheat breeders in order to produce balanced quality wheats.

Summary

Our results from the detailed analysis of the 'Cranbrook' × 'Halberd' population demonstrated that selection based on LMW-GS during breeding may be used to achieve a desirable balance between dough extensibility and Rmax. The positive effect of *Glu-D1d* over *Glu-D1a* on dough strength was conditional upon the presence of a specific non-glutenin locus in this study. The two types of epistatic interactions, those between glutenins and

non-glutenin genes and these between glutenins, were found to be equally important. These results indicated that some glutenins required additional factors to express their additive genetic effects, with one probable factor being the control of glutenin expression. The development and validation of markers based on glutenin epistases should increase the efficiency of selection for elite wheat quality breeding lines.

References

Basten CJ, Weir BS, Zeng ZB (1997) QTL Cartographer: a reference manual and tutorial for QTL mapping. Department of Statistics, North Carolina State University, Raleigh, NC

Beavis WD (1994) The power and deceit of QTL experiments: lessons from comparative QTL studies. In: Proceedings of the 49th annual corn and sorghum industry research conference, Washington DC, pp 250–253

Beavis WD (1998) QTL analysis: power, precision and accuracy. In: Paterson AH (ed) Molecular dissection of complex traits. CRC, Boca Raton, pp 145–162

Bekes F, Gras PW, Anderssen RS, Appels R (2001) Quality traits of wheat determined by small-scale dough testing methods. Aust J Agric Res 52:1325–1338

Branlard G, Dardevet M (1985) Diversity of grain protein and bread wheat quality. II. Correlation between high-molecular-weight subunits of glutenin and flour quality characteristics. J Cereal Sci 3:345–354

Branlard G, Dardevet M (1994) A null *Gli-D1* allele with a positive effect on bread wheat quality. J Cereal Sci 20:235–244

Briggle LW, Curtis BC (1987) Wheat worldwide. Heyne EG (ed) Wheat and wheat improvement. ASA, Madison, pp 1–31

Chalmers KJ, Campbell AW, Kretschmer J, Karakousis A, Henschke PH, Pierens S (2001) Construction of three linkage maps in bread wheat, *Triticum aestivum*. Aust J Agric Res 52(12):1089–1119

Chase K (1997) User's manual and tutorial guide for Epistat. http://www.larklab.4bit.net

Chase K, Adler FR, Lark KG (1997) Epistat: a computer program for identifying and testing interactions between pairs of quantitative trait loci. Theor Appl Genet 94:724–730

Cornish GB, Békés F, Allen HM, Martin DJ (2001) Flour proteins linked to quality traits in an Australian doubled haploid wheat population. Aust J Agric Res 52(12):1339–1348

Eagles HA, Bariana HS, Ogbonnaya FC, Rebetzke GJ, Hollamby GJ, Henry RJ (2001) Implementation of markers in Australian wheat breeding. Aust J Agric Res 52(12):1349–1356

Echermann PJ, Verbyla AP, Cullis BR, Thompson R (2001) The analysis of quantitative traits in wheat mapping populations. Aust J Agric Res 52(12):1195–1206

Eliasson AC, Larsson K (1993) Cereals in breadmaking: a molecular colloidal approach. Dekker, New York

Finney KF, Yamazaki WT, Youngs VL, Rubenthaler GL (1987) Quality of hard, soft, and duram wheats. In: Wheat and wheat improvement, 2nd edn. Agronomy Monograph no. 13. ASA, Madison, WI, pp 677–748

Flavell RB, Goldsbrough AP, Robert LS, Schnick D, Thompson RD (1989) Genetic variation in wheat HMW glutenin subunits and the molecular basis of breadmaking quality. Biotechnology 7:1281–1285

Gras PW, Anderssen RS, Keentok M, Bekes F, Appels R (2001) Gluten protein functionality in wheat flour processing: a review. Aust J Agric Res 52(12):1311–1324

Greene FC, Anderson OD, Yip RE, Halford NG, Malpica-Romero JM, Shewry PR (1988) Proceedings of the 7th international wheat genetics symposium. IPSR, Cambridge, pp 735–740

Gupta RB, MacRitchie (1991) A rapid one-step one-dimensional SDS procedure for analysis of subunit composition of glutenin in wheat. J Cereal Sci 14:105–109

Gupta RB, MacRitchie (1994) Allelic variation at glutenin subunit and gliadin loci, *Glu-1, Glu-3* and *Gli-1* of common wheats. II. Biochemical basis of the allelic effects on dough properties. J Cereal Sci 19:19–29

Haley CS, Knott SA (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69:315–324

Jackson EA, Holt LM, Payne PI (1983) Characterization of high-molecular-weight gliadin and low-molecular-weight glutenin subunits of wheat endosperm by two dimensional electrophoresis and the chromosomal localization of their controlling genes. Theor Appl Genet 66:29–37

Jansen RC, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136:1447–1455

Kammholz SJ, Campbell AW, Sutherland MW, Hollamby GJ, Martin PJ, Eastwood RF (2001) Establishment and characterisation of wheat genetic mapping populations. Aust J Agric Res 52(12):1079–1088

Kearsey MJ, Pooni HS (1996) The genetical analysis of quantitative traits, 1st edn. Chapman & Hall, London

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Larroque, OR, Bekes, F, Wrigley, CW, WG Rathmell (2000) Analysis of gluten proteins in grain and flour blends by RP-HPLC. Proceedings Gluten 2000, Bristol, UK, April 2000, pp 136–139

Ma W (2001) Molecular mapping of wheat quality attributes in a wheat doubled haploid population W21MMT70 × 'Mendos'. PhD Dissertation, University of Southern Queensland, Queensland, Australia

Melchinger AE, Utz HF, Schön CC (1998) Quantitative trait locus (QTL) mapping using different testers and independent population samples in maize reveals low power of QTL detection and large bias in estimates of QTL effects. Genetics 149:383–403

Nieto-Taladriz MT, Perretant MR, Rousset M (1994) Effect of gliadins and HMW and LMW subunits of glutenin on dough properties in the F_6 recombinant inbred lines from a bread wheat cross. Theor Appl Genet 88:81–88

Payne PI, Holt LM, Law CN (1981) Structural and genetical studies on the high molecular weight of wheat gluten. Part I: Allelic variation in subunits amongst varieties of wheat (*Triticum aestivum*). Theor Appl Genet 60:229–239

Payne PI, Seekings JA, Worland AJ, Jarvis MG, Holt LM (1987) Allelic variation of glutenin subunits and gliadins and its effects on bread-making quality in wheat: analysis of F5 progeny from 'Chinese Spring' × 'Chinese Spring' (Hope 1A). J Cereal Sci 6:103–118

Pogna NE, Tusa P, Boggini G (1996) Genetic and biochemical aspects of dough quality in wheat. Adv Food Sci 18(5–6):145–151

Simmonds DH (1989) Inherent quality factors in wheat. Wheat and wheat quality in Australia: 31–61. Australia Wheat Board

Soller M, Brody T, Genizi A (1976) The power of experimental designs for the detection of linkage between marker loci and quantitative loci in crosses between inbred lines. Theor Appl Genet 47:35–39

Varghese JP, Struss D, Kazman ME (1996) Rapid screening of selected European winter wheat varieties and segregating populations for the *Glu-D1d* allele using PCR. Plant Breed 115:451–454

Wall JS (1979) The role of wheat proteins in determining baking quality. In: Laidman DL, Wyn Jones RG (eds) Recent advances in the biochemistry of cereals. Academic, New York, pp 275–311

Wang SC, Basten CJ, Zeng ZB (2002) Windows QTLCartographer. Statistical genetics, North Carolina State University

Zeng ZB(1993) Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. Proc Natl Acad Sci USA 90:10972–10976

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468