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ORIGINAL PAPER

QTL mapping for quantities of protein fractions in bread wheat (*Triticum aestivum* L.)

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Abstract One of the key targets of breeding programs in bread wheat is to improve the end-use quality. The relationships between quantities of protein fractions and dough rheological characters have been well established, but there is little information on the genetic control of quantities of protein fractions. Two hundred and forty F_6 recombinant inbred lines derived from a cross between two Chinese wheat cultivars, PH82-2 and Neixiang 188, were sown at Jiaozuo in Henan province in the 2005–2006 and 2006–2007 cropping seasons, and inclusive composite interval mapping was used to dissect main effect quantitative trait loci (M-QTLs) and digenic epistatic QTLs (E-QTLs) for quantities of protein fractions. A total of 55 M-QTLs and 77 pairs of E-QTLs affecting the quantities of protein fractions including GLU-A1 (QGA1), GLU-B1 (QGB1), GLU-D1 (QGD1), HMW-GS (QHMW), GLU-A3 (QGA3), GLU-B3 (QGB3), LMW-GS (QLMW), glutenin (QGLU) and the ratio of the quantity of glutenin to those of gliadin were identified, with M-QTLs contributing 39.3–95.6% of the phenotypic variance explained (PVE), and E-QTLs accounting for 1.4–33.5% of the PVE. Among the M-QTLs, 33 were consistent in two seasons and in the mean value of two seasons with similar effects in both magnitude and direction, including major genes on HMW and LMW glutenin loci linked to *Sec1* and *Glu-B1c*, *Glu-D1d*, *Glu-A3a*, and grain hardness locus *Ha*, indicating that these genes were the most important determinants of gluten strength, and they might have significant effects on dough properties not only through effects on allelic composition, but also by influencing quantities of protein fractions. The effects of E-QTLs were more influenced by environments, compared with those of M-QTLs, with only two pairs of E-QTLs consistent in two seasons and in the mean value of two seasons. The M-QTLs were detected in 12 marker intervals, all of which involved E-QTLs on quantities of protein fractions, whereas only 40 of 77 pairs of E-QTLs involved intervals in which M-QTLs were detected. The results indicated that besides main effects, epistatic effects were also important factors in determining quantities of protein fractions in wheat.

Abbreviations

HMW-GS	High-molecular-weight glutenin subunit
LMW-GS	Low-molecular-weight glutenin subunit
QGA1	Quantity of GLU-A1
QGB1	Quantity of GLU-B1
QGD1	Quantity of GLU-D1
QHMW	Quantity of HMW-GS
QGA3	Quantity of GLU-A3
QGB3	Quantity of GLU-B3
QLMW	Quantity of LMW-GS
QGLU	Total quantity of glutenin
QGLUGLI	Ratio of quantity of glutenin to those of gliadin
QPRO	Protein content

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QTLs	Quantitative trait loci
ICIM	Inclusive composite interval mapping
M-QTL	Main-effect QTL
E-QTL	Digenic epistatic QTL
MAS	Marker-assisted selection
PVE	Phenotypic variance explained

Introduction

Quality improvement is a major priority in wheat (*Triticum aestivum*) breeding. The dough properties reflected by Mixograph, Farinograph and Extensograph parameters have been reported to be influenced by properties of glutenin and gliadin storage proteins. It is generally agreed that dough strength is partly determined by particular variants of high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunit (LMW-GS) (Payne and Corfield 1979; Gupta and MacRitchie 1994), with minor or modifying effects from gliadins (Payne et al. 1984; Békés et al. 2001). Both HMW-GS and LMW-GS are important in determining dough properties, and the total effect of the HMW-GS is larger than that of LMW-GS (Payne et al. 1984; Gupta and MacRitchie 1994; He et al. 2005). Recent research has also indicated the importance of the relative expression levels of particular alleles and storage proteins in quality determination (Weegels et al. 1996; Larroque et al. 2001; Nelson et al. 2006; Zhang et al. 2007a, 2007b; Zhang et al. 2009a). The allelic composition of storage proteins (Payne et al. 1984; Gupta and MacRitchie 1994; He et al. 2005), the quantity of protein fractions (Zhang et al. 2007b; Weegels et al. 1996; Nelson et al. 2006) and the ratio of the quantity of glutenin to the quantity of gliadin (Zhang et al. 2007a, 2007b; Zhang et al. 2009a) are all important determinants of quality.

Changes in relative proportions of storage proteins mainly resulted from genetic effects, but significant effects of environment and genotype by environment interaction have also been found (Triboi et al. 2000; Charmet et al. 2005). The numbers, locations, effects, and interactions of loci affecting the quantity of storage proteins can be determined by QTL analysis (Amiour et al. 2003). There are few reports of QTLs controlling the quantity of glutenin fractions (Guillaumie et al. 2004; Ravel et al. 2006b). Charmet et al. (2005) found several QTLs associated with protein content, with one major QTL explaining 70.0% of the total variation in the quantity of x type fractions of *Glu-B1*. The quantity of glutenin subunits, especially $Bx7^{OE}$ resulted from a duplication of the *Glu-B1-1a* gene (D'Ovidio et al. 1997; Lukow et al. 2002), has a significant effect on dough strength (Kolster et al. 1992).

Although epistasis is thought to contribute substantially to the control and evolution of quantitative traits (Doebley et al. 1995; Yi et al. 2005; Li et al. 2008; Zhang et al. 2008), there are no reports on detection of epistatic QTLs for quantities of protein fractions. The present study was designed to investigate the genetic basis for variation in the quantity of glutenin, the quantity of seven glutenin fractions (GLU-A1, GLU-B1, GLU-D1, HMW-GS, GLU-A3, GLU-B3, LMW-GS) and the ratio of the quantity of glutenin to the quantity of gliadin using 240 recombinant inbred lines (RILs) derived from a cross between PH82-2 and Neixiang 188, with inclusive composite interval mapping (ICIM) which efficiently identifies additive and digenic epistatic QTLs (Li et al. 2007, 2008; Zhang et al. 2008). The information obtained was expected to benefit the development of breeding strategies for improving protein quality.

Materials and methods

Plant materials

The plant materials and experimental design were detailed in Zhang et al. (2009a). PH82-2 and Neixiang 188 were two leading cultivars in the Yellow and Huai Valley wheat region, the most important Chinese wheat production area, from the early 1990s to 2003. They differ from each other in gluten strength and at most of HMW and LMW glutenin subunit loci. PH82-2 has hard grain and good quality for the production of Chinese steamed bread and dry white noodles (He et al. 2004), with glutenin alleles *Glu-A1a*, *Glu-B1h*, *Glu-D1a*, *Glu-A3d* and *Glu-B3d*, respectively. Neixiang 188 has soft grain, but sticky dough and inferior quality for the production of Chinese steamed bread and dry white noodles, with glutenin alleles *Glu-A1a*, *Glu-B1c*, *Glu-D1d*, *Glu-A3a* and *Glu-B3j*, respectively. These two cultivars were crossed in 1998, and 240 F_2-derived F_6 RILs were obtained, among which more than half (58.3%) of the lines possessed the *Glu-B1h* and more than half (64.3%) of the lines carried the *Glu-B3d* allele, suggesting overrepresentation of the PH82-2 alleles at these two loci in the population (Zhang et al. 2009a). The 240 RILs were sown in a latinized alpha lattice design with three partial replications at Anyang and Jiaozuo in Henan Province, and Taian in Shandong in the 2005–2006 and 2006–2007 cropping seasons. Grain samples from both seasons at Jiaozuo were analyzed. Grain hardness was tested on 300-kernel samples using a Perten 4100 Single Kernel Characterization System (SKCS, Perten Instruments North America Inc., Reno, NV). Hard, medium hard, and soft samples were tempered to 16, 15.5, and 14% moisture contents, respectively, and milled into flour using a

Brabender Quadrumat Junior mill according to AACC approved method 26-21A (AACC 2000) for quality testing with an extraction rate around 65%. Flour protein content (14% moisture basis) was determined with a Foss-Tecator 1241 near infrared transmittance (NIT) analyzer (Foss, Högänas, Sweden). Flour moisture content and Falling Number were determined according to AACC approved methods 44-15A and 56-81B, respectively. Falling Number tests on grains confirmed that all samples were free from sprouting.

RP-HPLC and SE-HPLC

The procedures for glutenin protein extraction and RP-HPLC analysis on glutenin protein fractions including GLU-A1, GLU-B1, GLU-D1, HMW-GS, GLU-A3, GLU-B3 and LMW-GS, and total glutenin were conducted following the method of Larroque et al. (2001) and Zhang et al. (2007a). Extraction of polymeric protein and separation by SE-HPLC was carried out for the ratio of quantity of glutenin to gliadin as described by Larroque et al. (2000). Millenium^{32} Chromatography Manager software was used to determine the absorption peak integrations of all protein fractions.

Statistical analyses

All data were analyzed by fitting an appropriate spatial model with rows and columns in each trial (Gilmour et al. 1997; Zhang et al. 2009a). The best linear unbiased predictions from the best-fit model for all traits were used for subsequent analysis. Mean, standard deviation and range were determined using SAS PROC MEANS (SAS Institute 2000). Variance and covariance components were estimated following Holland et al. (2003). Genotypic correlations and response standard errors were estimated following Becker (1984). Broad-sense heritability on across-year genotype mean bases and standard errors were calculated following Holland et al. (2003).

Marker analysis and construction of the genetic map

Genomic DNA was extracted from young leaves including the two parents and RILs using the CTAB method (Sambrook et al. 1989). A total of 2033 SSR markers (Röder et al. 1998; Pestsova et al. 2000; Song et al. 2002; http://www.wheat.pw.usda.gov) were assayed on the two parents to identify polymorphisms. One hundred and eighty-eight polymorphic SSR markers, a rye secalin marker *Sec1* for identifying the 1RS chromosome arm (Singh et al. 1990), an STS marker *YP7A* for a phytoene synthase gene (He et al. 2008) and four glutenin allele markers (*Glu-B1c* and *Glu-D1d*, *Glu-A3a* and *Glu-B3j*) were used to genotype the population and to construct a genetic map for subsequent QTL analysis. The genetic map was constructed with MapManager QTXb20 (Chmielewicz and Manly 2002). A significance level with $P = 1e{-}5$ to avoid Type I error was applied in declaring linkage of markers. Orientations of unlinked groups were based on microsatellite consensus maps (Shi et al. 2003; Somers et al. 2004). The Kosambi mapping function (Kosambi 1944) was used to transform recombination frequencies into map distances. The linkage map covered 1,682.1 cM with an average marker interval of 8.67 cM on 19 wheat chromosomes. No polymorphic markers were identified on chromosomes 3D and 6A (Zhang et al. 2009b).

QTL analysis

QTL analysis was performed using the modified algorithm ICIM (Li et al. 2007, 2008; Zhang et al. 2008), implemented in the integrated software QTL IciMapping (http://www.isbreeding.net). In the first step of ICIM, probability values for entering variables (PIN) of 0.01 and probability values for removing variables (POUT) of 0.02, were used to select significant markers for additive QTL (Li et al. 2007). For additive by additive epistatic QTL, stricter probabilities were used due to the large numbers of marker interactions in the linear model (Li et al. 2008). In the second step a threshold LOD of 3.0 was used to declare significant additive main effect QTLs (M-QTLs) and digenic epistatic QTLs (E-QTLs). In ICIM, marker selection was conducted only once through stepwise regression by considering all marker information simultaneously, and the phenotypic values were then adjusted by all markers retained in the regression equation except for the two markers flanking the current mapping interval. The adjusted phenotypic values were finally used in interval mapping (IM) until the testing position moved into a new interval, completely guaranteeing both QTL effect at the current testing interval and regression coefficients of the background markers. Phenotypic variance explained (PVE) by each M-QTL was estimated according to the formula $PVE = a^2/S^2 \times 100$, where a is the additive variance of the QTL, and S^2 is the phenotypic variance.

Results

Phenotypic analysis of quantities of protein fractions

Analysis of variance indicated that both lines and years had highly significant effects on all traits except for quantity of GLU-A3 (QGA3), which was not significantly affected by year (Table 1). Consequently, the dataset was separated by year for further analysis, although lines contributed larger

Table 1 Sums of squares and broad-sense heritabilities for quantities of protein fractions in PH82-2/Neixiang 188 RILs across two seasons

Source	df	QGA1	QGB1	QGD1	QHMW	QGA3	QGB3	QLMW	QGLU	QGLUGLI	QPRO
Lines (L)	167	24.02**	172**	85**	666**	397.75**	1253**	2995**	5150**	2.79**	248**
Years (Y)	1	0.19**	12**	35**	8**	0.02	6**	67**	27**	0.05**	7**
L × Y[a]	167	3.79	21	10	52	5.30	81	205	412	0.08	26
H_b^2		0.84 ± 0.02	0.88 ± 0.02	0.89 ± 0.02	0.92 ± 0.01	0.99 ± 0.01	0.94 ± 0.01	0.93 ± 0.01	0.92 ± 0.01	0.97 ± 0.01	0.90 ± 0.02

QGA1 quantity of GLU-A1, *QGB1* quantity of GLU-B1, *QGD1* quantity of GLU-D1, *QHMW* quantity of HMW-GS, *QGA3* quantity of GLU-A3, *QGB3* quantity of GLU-B3, *QLMW* quantity of LMW-GS, *QGLU* total quantity of glutenin, *QGLUGLI* ratio of quantity of glutenin to quantity of gliadin, *QPRO* protein content

[a] Including error

** Significant at $P = 0.01$

effects than years and line by year interaction on all traits. All the traits had high broad-sense heritability, ranging from 0.84 to 0.99.

A wide range of variation was observed for all traits between the two parents and among the RILs in both years (Table 2). The mean value of the quantity of GLU-A1 (QGA1) for the RILs was biased towards the low value parent PH82-2 with normal distributions in both seasons. The mean values of the quantities of GLU-B1 (QGB1), GLU-D1 (QGD1) and HMW-GS (QHMW) for the RILs were slightly biased towards the high value parent Neixiang 188 with normal distributions. The mean values of QGA3, the quantities of GLU-B3 (QGB3) and LMW-GS (QLMW), the ratio of quantity of glutenin to gliadin (QGLUGLI) and protein content (QPRO) for the RILs were slightly biased towards the high value parent PH82-2 with normal distributions, whereas the mean value of the total quantity of glutenin (QGLU) for the RILs was higher than that of high value parent PH82-2 with normal distributions. The range of variation observed for all traits were much greater among the RILs than between the two parents. The wide ranges of variation and the normal phenotypic distributions indicated transgressive segregation and polygenic inheritance of the traits.

Genotypic correlations among quantities of protein fractions

There were highly significant positive correlations among QGA1, QGB1, QGD1, QHMW and QGLU, among QGB3, QLMW and QGLU, and between QGLUGLI and QPRO (Table 3). Low to medium positive (but significant) correlations were observed between QGA1, QGB1, QGD1, QHMW and QGA3, QGB3, QLMW, QPRO, between QGA3 and QGB3, QLMW, and between QGA3, QGB3, QLMW, QGLU and QGLUGLI, QPRO, whereas low to medium negative (but significant) correlations were observed between QGA1, QGB1, QGD1, QHMW and QGLUGLI.

M-QTLs for quantities of protein fractions

Fifty-five M-QTLs for all quantities of protein fractions were identified and mapped in 12 marker intervals (Table 4; Fig. 1). Six M-QTLs for QGA1 mapped to chromosomes 1B (two M-QTLs), 1D, 3A, 3B and 5D, among which *QGlu-a1.caas-1B1* linked to *Sec1* and *QGlu-a1.caas-1D* linked to *Glu-D1d* accounted for 5.6–13.9% of the PVE in two seasons and in the mean value of two seasons with positive effects from PH82-2. *QGlu-a1.caas-3B* flanked by marker interval *Xwmc3-Xbarc68.1* and *QGlu-a1.caas-5D* linked to *Ha* accounted for 4.1–20.2% of the PVE in two seasons and in the mean value of two

Table 2 Means and ranges of quantities of protein fractions in PH82-2/Neixiang 188 RILs and parents in two seasons

Trait	2005–2006				2006–2007			
	Parent		RIL		Parent		RIL	
	PH82-2	Neixiang 188	Mean ± SD	Range	PH82-2	Neixiang 188	Mean ± SD	Range
QGA1[a]	1.8	2.2	1.8 ± 0.3	1.1–2.8	1.7	2.1	1.8 ± 0.3	1.2–2.8
QGB1	3.8	4.7	4.5 ± 0.8	2.5–6.8	3.4	4.7	4.2 ± 0.7	2.7–6.4
QGD1	2.9	3.4	3.2 ± 0.5	2.0–4.6	3.3	4.4	3.8 ± 0.6	2.4–5.4
QHMW	8.4	10.3	9.5 ± 1.5	5.6–14.2	8.4	11.2	9.8 ± 1.4	6.5–14.3
QGA3	2.7	0.6	1.8 ± 1.1	0.4–4.0	2.3	0.5	1.8 ± 1.1	0.4–4.1
QGB3	14.4	12.1	14.1 ± 2.1	9.4–19.6	13.8	11.7	13.9 ± 1.9	9.5–18.8
QLMW	25.5	21.9	24.7 ± 3.1	16.5–33.8	24.1	20.6	23.8 ± 2.9	16.6–33.5
QGLU	33.9	32.2	34.2 ± 4.3	22.6–45.9	32.5	31.8	33.7 ± 3.9	24.4–46.4
QGLUGLI	0.85	0.76	0.83 ± 0.09	0.64–1.01	0.86	0.71	0.81 ± 0.09	0.58–0.99
QPRO	13.9	10.9	12.6 ± 1.0	10.2–15.0	13.9	11.6	12.9 ± 0.8	10.7–14.9

[a] The quantities of protein fractions are in 10^6 absorbance units of HPLC corresponding to 1 mg of flour, abbreviated as AU

Table 3 Genotypic correlations among quantities of different protein fractions in PH82-2/Neixiang 188 RILs in the mean value of two seasons

Variable	QGB1	QGD1	QHMW	QGA3	QGB3	QLMW	QGLU	QGLUGLI	QPRO
QGA1	0.98 ± 0.02	0.91 ± 0.02	0.99 ± 0.01	0.17 ± 0.08	0.35 ± 0.08	0.52 ± 0.07	0.75 ± 0.04	−0.39 ± 0.07	0.49 ± 0.07
QGB1		0.86 ± 0.03	0.98 ± 0.01	0.22 ± 0.08	0.19 ± 0.08	0.40 ± 0.07	0.66 ± 0.05	−0.50 ± 0.06	0.35 ± 0.08
QGD1			0.94 ± 0.01	0.28 ± 0.08	0.44 ± 0.07	0.59 ± 0.05	0.81 ± 0.03	−0.31 ± 0.08	0.41 ± 0.07
QHMW				0.24 ± 0.08	0.36 ± 0.06	0.51 ± 0.06	0.75 ± 0.04	−0.43 ± 0.07	0.40 ± 0.07
QGA3					0.20 ± 0.08	0.53 ± 0.06	0.49 ± 0.06	0.19 ± 0.08	0.22 ± 0.08
QGB3						0.91 ± 0.01	0.81 ± 0.03	0.57 ± 0.06	0.47 ± 0.07
QLMW							0.95 ± 0.01	0.43 ± 0.07	0.47 ± 0.07
QGLU								0.18 ± 0.08	0.51 ± 0.06
QGLUGLI									0.76 ± 0.04

$r_{240,0.01} = 0.16$, $r_{240,0.001} = 0.20$

seasons with positive effects from Neixiang 188. Two additional M-QTLs on chromosome 1B in 2005–2006 and in the mean value of two seasons, and on chromosome 3A in 2006–2007 contributed 5.0–23.4% of the PVE. No QTL for QGA1 mapped on chromosome 1A as both parents possessed *Glu-A1a*. Seven M-QTLs associated with QGB1 were located on chromosomes 1A, 1B (two M-QTLs), 1D, 3A, 4A and 5D, among which *QGlu-b1.caas-1B1*, *QGlu-b1.caas-1B2* linked to *Glu-B1c*, *QGlu-b1.caas-1D* and *QGlu-b1.caas-3A* in marker interval *Xwmc664-Xwmc21* accounted for 3.7–24.9% of the PVE in two seasons and in the mean value of two seasons with positive effects from PH82-2. *QGlu-b1.caas-5D* accounted for more than 11.3% of the PVE in two seasons and in the mean value of two seasons with positive effects from Neixiang 188. Two additional M-QTLs on chromosomes 1A and 4A contributed 5.1 and 4.7% of the PVE in 2005–2006. Six M-QTLs associated with QGD1 were detected on chromosomes 1B, 1D, 2B, 3A, 5D and 7A, among which *QGlu-d1.caas-1B* linked to *Sec1* and *QGlu-d1.cass-1D* accounted for 10.7–33.3% of the PVE in two seasons and in the mean value of two seasons with positive effects from PH82-2. *QGlu-d1.caas-5D* linked to *Ha* accounted for 9.7–18.0% of the PVE in two seasons and in the mean value of two seasons with positive effects from Neixiang 188. Three additional M-QTLs on chromosome 2B in 2006–2007 and in the mean value of two seasons, and on chromosomes 3A and 7A in 2006–2007 contributed 3.1–12.6% of the PVE. Six M-QTLs associated with QHMW were detected on chromosomes 1A, 1B, 1D, 2B, 3A and 5D, among which *QHmw-gs.caas-1B* linked to *Sec1* and *QHmw-gs.caas-1D* accounted for 11.7–18.5% of the PVE in two seasons and in the mean value of two seasons with positive effects from PH82-2. *QHmw-gs.caas-5D* accounted for 10.1–21.2% of the PVE in two seasons and in the mean value of two seasons with positive effects from Neixiang 188. Three additional M-QTLs on chromosome 1A in 2005-2006 and in the mean value of two seasons, on chromosome 2B in 2006-07, and on chromosome 3A in 2006–2007 and in the mean value of two seasons contributed 3.4–4.5% of the PVE.

Table 4 M-QTLs for quantities of protein fractions detected in PH82-2/Neixiang 188 RILs in two seasons and in the mean value of two seasons

Trait	QTL[a]	Marker interval	2005–2006			2006–2007			Mean value		
			A[b]	LOD	PVE (%)	A	LOD	PVE(%)	A	LOD	PVE(%)
QGA1	*QGlu-a1.caas-1B1*	*HVM23-Sec1*	−0.1	7.9	11.0	−0.1	8.1	13.9	−0.1	5.3	11.5
	QGlu-a1.caas-1B2	*Xbarc61- Glu-B1c*	−0.2	15.2	23.4				−0.1	4.4	6.1
	QGlu-a1.caas-1D	*Xcfd48.1- Glu-D1d*	−0.1	7.7	10.8	−0.1	3.5	5.6	−0.1	7.1	10.0
	QGlu-a1.caas-3A	*Xwmc664-Xwmc21*				−0.1	3.2	5.0			
	QGlu-a1.caas-3B	*Xwmc3-Xbarc68.1*	0.1	3.0	4.6	0.1	3.1	4.8	0.1	3.1	4.1
	QGlu-a1.caas-5D	*Xcfd18-Ha*	0.1	12.0	20.2	0.1	5.6	10.0	0.1	11.4	19.9
QGB1	*QGlu-b1.caas-1A*	*DuPw38-Xcfa2147*	0.2	4.0	5.1						
	QGlu-b1.caas-1B1	*HVM23-Sec1*	−0.3	9.8	13.8	−0.2	9.4	11.4	−0.3	11.0	13.2
	QGlu-b1.caas-1B2	*Xbarc61- Glu-B1c*	−0.2	7.6	10.4	−0.4	21.0	24.9	−0.3	12.4	15.0
	QGlu-b1.caas-1D	*Xcfd48.1- Glu-D1d*	−0.2	4.5	5.7	−0.2	6.1	5.8	−0.2	6.9	7.6
	QGlu-b1.caas-3A	*Xwmc664-Xwmc21*	−0.1	3.0	3.8	−0.2	5.2	4.9	−0.1	3.5	3.7
	QGlu-b1.caas-4A	*DuPw202-Xgwm160*	0.2	3.6	4.7						
	QGlu-b1.caas-5D	*Xcfd18-Ha*	0.3	11.5	18.7	0.2	8.8	11.3	0.3	11.7	15.6
QGD1	*QGlu-d1.caas-1B*	*HVM23-Sec1*	−0.2	6.9	10.7	−0.2	11.7	12.5	−0.2	15.5	18.2
	QGlu-d1.caas-1D	*Xcfd48.1- Glu-D1d*	−0.3	17.6	31.0	−0.3	22.5	28.1	−0.3	25.1	33.3
	QGlu-d1.caas-2B	*Xbarc160-Xwmc474.1*				−0.1	3.3	3.1	−0.1	3.2	3.1
	QGlu-d1.caas-3A	*Xwmc664-Xwmc21*				−0.2	6.6	6.7			
	QGlu-d1.caas-5D	*Xcfd18-Ha*	0.2	9.7	18.0	0.1	7.3	11.0	0.2	8.7	9.7
	QGlu-d1.caas-7A	*Xbarc121-Xbarc49*				−0.2	11.8	12.6			
QHMW	*QHmw-gs.caas-1A*	*DuPw38-Xcfa2147*	0.3	3.4	4.5				0.3	3.0	3.4
	QHmw-gs.caas-1B	*HVM23-Sec1*	−0.5	8.2	11.7	−0.6	12.7	13.4	−0.6	11.5	14.5
	QHmw-gs.caas-1D	*Xcfd48.1- Glu-D1d*	−0.6	10.9	15.8	−0.6	16.4	18.4	−0.6	14.4	18.5
	QHmw-gs.caas-2B	*Xbarc200-Xgwm257*				−0.3	3.5	3.9			
	QHmw-gs.caas-3A	*Xwmc664-Xwmc21*				−0.3	4.0	3.9	−0.3	3.1	3.4
	QHmw-gs.caas-5D	*Xcfd18-Ha*	0.7	12.5	21.2	0.5	9.4	10.1	0.6	12.1	17.1
QGA3	*QGlu-a3.caas-1A*	*Xcfa2153-Glu-A3a*	1.0	60.2	75.5	0.9	58.1	74.9	0.9	61.4	74.9
	QGlu-a3.caas-1B	*Glu-B1c -Xcfd48.3*				−0.2	3.7	2.0	−0.1	3.9	1.8
	QGlu-a3.caas-1D	*Xcfd48.1- Glu-D1d*	−0.2	4.4	2.3				−0.2	4.7	2.2
	QGlu-a3.caas-3B	*Xwmc3-Xbarc68.1*	0.2	3.6	2.4	0.2	4.5	3.1	0.2	4.3	2.7
QGB3	*QGlu-b3.caas-1B1*	*HVM23-Sec1*	1.5	26.7	47.1	1.4	38.2	50.0	1.4	34.0	50.5
	QGlu-b3.caas-1B2	*Xbarc61- Glu-B1c*	−0.5	3.9	4.9	−0.5	8.5	7.1	−0.5	6.2	6.1
	QGlu-b3.caas-1D	*Xcfd48.1- Glu-D1d*	−0.9	12.5	17.9	−1.0	24.0	25.6	−0.9	18.8	21.8
	QGlu-b3.caas-3A	*Xwmc21-Xwmc505.2*				−0.3	3.9	3.1			
	QGlu-b3.caa-5D	*Xcfd18-Ha*	0.8	9.2	14.8	0.6	10.6	10.2	0.7	10.7	12.7
QLMW	*QLmw-gs.caas-1A*	*Xcfa2153-Glu-A3a*	0.7	8.2	11.2	1.1	10.8	12.9	0.9	7.4	9.3
	QLmw-gs.caas-1B1	*HVM23-Sec1*	1.5	12.4	19.1	1.4	16.2	21.2	1.4	15.2	21.3
	QLmw-gs.caas-1B2	*Xbarc61- Glu-B1c*	−0.8	4.5	6.4	−0.8	6.7	7.7	−0.8	5.9	7.2
	QLmw-gs.caas-1D	*Xcfd48.1- Glu-D1d*	−1.5	14.0	21.9	−1.5	19.7	27.1	−1.4	16.0	22.3
	QLmw-gs.caas-3A	*Xwmc21-Xwmc505.2*				−0.7	4.1	4.8			
	QLmw-gs.caas-5D	*Xcfd18-Ha*	1.5	11.9	21.4	1.1	9.5	13.0	1.2	10.4	15.4
QGLU	*QGlutenin.caas-1A1*	*DuPw38-Xcfa2147*	1.0	3.0	4.9						
	QGlutenin.caas-1A2	*Xcfa2153-Glu-A3a*				1.1	6.0	7.9	0.9	3.3	4.9
	QGlutenin.caas-1B1	*HVM23-Sec1*				0.9	3.4	4.4			
	QGlutenin.caas-1B2	*Xbarc61- Glu-B1c*				−1.2	7.2	9.6			
	QGlutenin.caas-1D	*Xcfd48.1- Glu-D1d*	−2.1	13.0	24.9	−2.0	17.2	26.6	−1.9	13.1	22.4
	QGlutenin.caas-3A	*Xwmc664-Xwmc21*				−1.1	5.8	7.8			
	QGlutenin.caas-4A	*DuPw202-Xgwm160*	1.0	3.0	5.1						
	QGlutenin.caas-5D	*Xcfd18-Ha*	2.2	12.2	26.2	1.5	10.0	15.2	1.7	10.2	19.2

Table 4 continued

Trait	QTL[a]	Marker interval	2005–2006			2006–2007			Mean value		
			A[b]	LOD	PVE (%)	A	LOD	PVE(%)	A	LOD	PVE(%)
QGLUGLI	*QGlugli.caas-1A*	*Xcfa2153-Glu-A3a*	0.2	7.6	3.8	0.2	10.8	4.9	0.2	8.9	3.9
	QGlugli.caas-1B	*HVM23-Sec1*	0.8	62.7	74.7	0.9	75.1	73.6	0.8	66.5	73.2
	QGlugli.caas-3A	*Xwmc664-Xwmc21*				0.1	4.8	2.3	0.1	4.2	2.1
QPRO	*QProtein.caas-3A*	*Xwmc664-Xwmc21*	−0.3	6.3	9.0	−0.3	6.8	10.1	−0.3	5.8	9.4
	QProtein.caas-3B	*Xwmc3-Xbarc68.1*	0.2	3.6	4.5	0.2	3.2	4.2	0.2	3.0	4.3
	QProtein.caas-4A	*DuPw202-Xgwm160*				0.2	3.3	4.5			
	QProtein.caas-5D	*Xcfd18-Ha*	0.5	13.2	30.8	0.4	10.9	19.1	0.4	12.6	25.6

[a] Nomenclature for QTL: *Q* followed by a trait designator, a hyphen (-) and chromosome on which the QTL located

[b] Positive additive effects are associated with increased effects from Neixiang 188 alleles, and negative additive effects are associated with increased effects from PH82-2 alleles

An M-QTL for QGA3 coincidental with *Glu-A3a* accounted for 74.9–75.5% of the PVE in two seasons and in the mean value of two seasons with positive effects coming from Neixiang 188. *QGlu-a3.caas-3B* accounted for 2.4–3.1% of the PVE in two seasons and in the mean value of two seasons with positive effects from Neixiang 188. Two additional M-QTLs on chromosome 1D in 2005–2006 and in the mean value of two seasons, and on chromosome 1B in 2006–2007 and in the mean value of two seasons contributed 1.8–2.3% of the PVE. Five M-QTLs for QGB3 were located on chromosomes 1B (two M-QTLs), 1D, 3A and 5D, among which *QGlu-b3.caas-1B1* and *QGlu-b3.caas-5D* accounted for 10.2–50.5% of the PVE in two seasons and in the mean value of two seasons with positive effects from Neixiang 188. *QGlu-b3.caas-1B2* and *QGlu-b3.caas-1D* accounted for 4.9–25.6% of the PVE in two seasons and in the mean value of two seasons with positive effects from PH82-2. An additional M-QTL on chromosome 3A in 2006–2007 contributed 3.1% of the PVE. Six M-QTLs associated with QLMW were mapped on chromosomes 1A, 1B (two M-QTLs), 1D, 3A and 5D, among which *QLmw-gs.caas-1A* linked to *Glu-A3a*, *QLmw-gs.caas-1B1*, and *QLmw-gs.caas-5D* accounted for 9.3–21.4% of the PVE in two seasons and in the mean value of two seasons with positive effects from Neixiang 188. *QLmw-gs.caas-1B2* and *QLmw-gs.caas-1D* accounted for 6.4–27.1% of the PVE in two seasons and in the mean value of two seasons with positive effects from PH82-2. An additional M-QTL on chromosome 3A in 2006–2007 contributed 4.8% of the PVE.

Eight M-QTLs associated with QGLU were mapped on chromosomes 1A (two M-QTLs), 1B (two M-QTLs), 1D, 3A, 4A and 5D, among which *QGlutenin.caas-5D* accounted for 15.2–26.2% of the PVE in two seasons and in the mean value of two seasons with positive effects from Neixiang 188. *QGlutenin.caas-1D* accounted for 22.4–26.6% of the PVE in two seasons and in the mean value of two seasons with positive effects from PH82-2. Six additional M-QTLs on chromosomes 1A and 4A in 2005–2006, on chromosomes 1B (two M-QTLs) and 3A in 2006–2007, and on chromosome 1A in 2006–2007 and in the mean value of two seasons, contributed 4.4–9.6% of the PVE. Three M-QTLs associated with QGLUGLI were mapped on chromosomes 1A, 1B and 3A, among which *QGlugli.caas-1B* linked to *Sec1* accounted for 73.2–74.7% of the PVE in two seasons and in the mean value of two seasons with positive effects from Neixiang 188. *QGlugli.caas-1A* accounted for 3.8–4.9% of the PVE in two seasons and in the mean value of two seasons with positive effect from Neixiang 188. An additional M-QTL on chromosome 3A contributed 2.3 and 2.1% of the PVE in 2006–2007 and in the mean value of two seasons.

Four M-QTLs associated with QPRO were mapped on chromosomes 3A, 3B, 4A and 5D, among which *QProtein.caas-5D* and *QProtein.caas-3B* accounted for 19.1–30.8 and 4.2–4.5% of the PVE in two seasons and in the mean value of two seasons with positive effects from Neixiang 188. *QProtein.caas-3A* accounted for 9.0–10.1% of the PVE in two seasons and in the mean value of two seasons with positive effects from PH82-2. An additional M-QTL on chromosome 4A in 2006–2007 contributed 4.5% of the PVE.

E-QTLs for the quantities of protein fractions

A genome-wide search for E-QTLs among all the traits was performed, with a total of 77 pairs of E-QTLs in 37 marker intervals being identified among the RILs (Table 5). One, five, thirteen, nine, twelve, five, seven, twelve, six and seven pairs of E-QTLs were detected for QGA1, QGB1, QGD1, QHMW, QGA3, QGB3, QLMW, QGLU, QGLUGLI and QPRO, respectively, with the estimated total contributions of 3.8–21.7% in 2005–2006, 1.4–33.5% in 2006–2007 and 1.8–7.8% in the mean value of two

seasons. All 12 marker intervals in which M-QTLs were detected were involved in epistatic effects (Tables 4, 5). Forty pairs of E-QTLs were involved in interactions between two M-QTL-containing marker intervals or between an M-QTL-containing marker interval and some other marker interval unrelated with M-QTLs, but 37 pairs of E-QTLs were involved in interactions between two marker intervals in which no M-QTLs had been detected. Most E-QTLs were detected in only one season and only two pairs of E-QTLs were detected in both seasons and in the mean value of two seasons.

Discussion

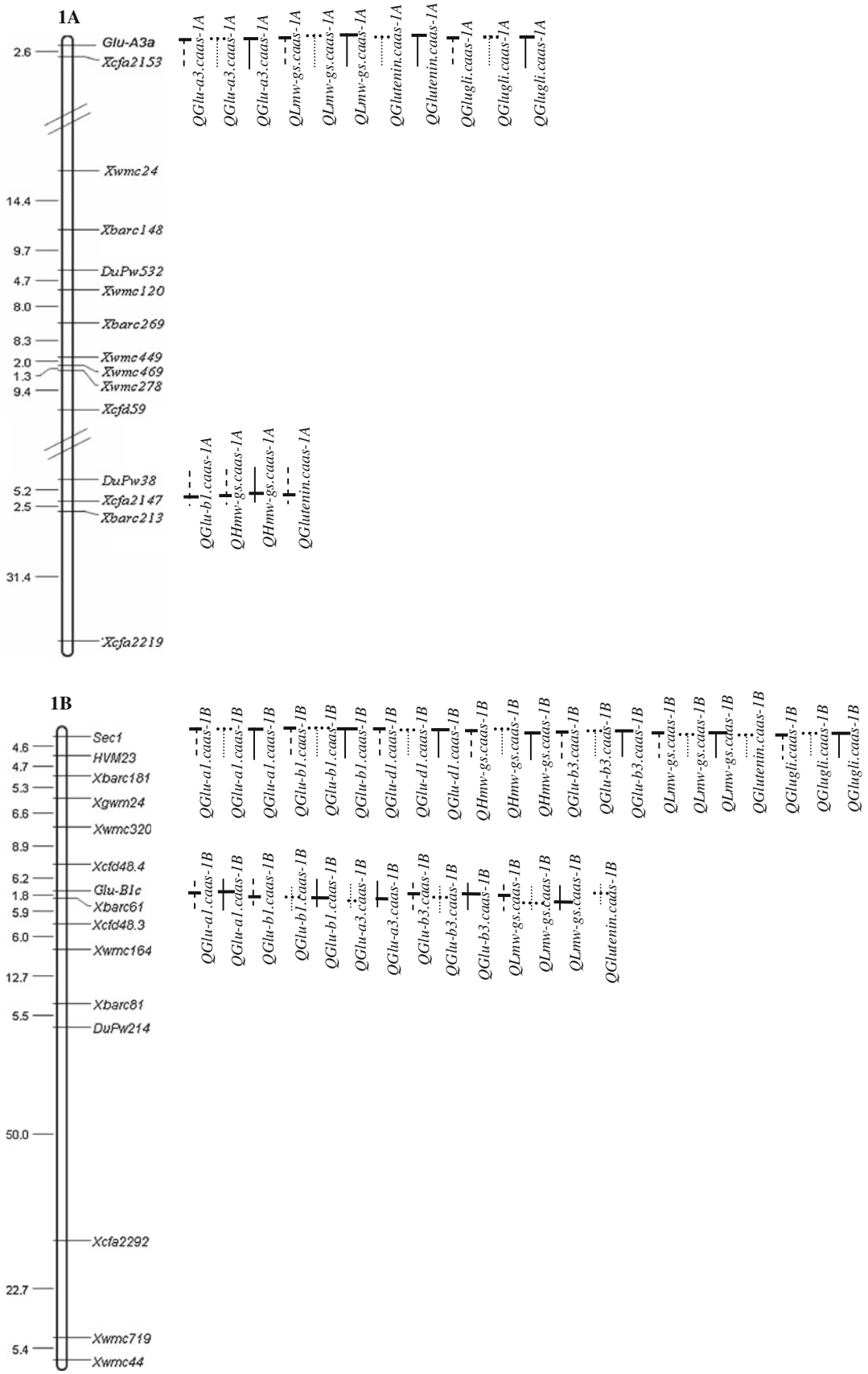
Relationship between QTLs for quantities of protein fractions and major gene loci for wheat quality

Using the current RIL population, Zhang et al. (2009a) indicated that for mixograph parameters, (1) the major loci for HMW- and LMW-GS were associated with the variation, with *Glu-D1* and *Glu-B3* playing the most important roles, (2) additive effects of HMW- and LMW-GS contributed to most of the variation, (3) epistatic effects were also important and could negate at least part of the additive effects of individual loci, and (4) quantities of glutenin protein fractions, especially the total quantity of glutenin, LMW-GS and GLU-B3, showed highly significant correlations with most of the mixograph parameters, and the ratio of quantity of glutenin to quantity of gliadin is important to wheat quality. Therefore, it was necessary to determine the precise effects of QTLs for quantities of protein fractions, and to identify their potential molecular markers for marker-assisted selection (MAS) in breeding. Kolster et al. (1992) suggested that glutenin loci share a common regulatory mechanism. Approaches to the identification of regulators have included genetic linkage analysis using wheat populations. Guillaumie et al. (2004) mapped the SPA (storage protein activator) factor to the same chromosome arm as the HMW glutenin structural genes located on. Ravel et al. (2006a) mapped the DNA binding with one finger (Dof) wheat prolamin-box binding factor to the long arm of wheat chromosome 5. As pointed out by Bernacchi et al. (1998), one way to verify a QTL is to investigate the stability of its effect across environments. A total of 55 M-QTLs for quantities of protein fractions were detected in the present study. Among these, 33 were consistent in two seasons and in the mean value of two seasons, including four for QGA1, five for QGB1, three for QGD1, three for QHMW, two for QGA3, four for QGB3, five for QLMW, two for QGLU, two for QGLUGLI and three for QPRO, with most of these accounting for more than 10% of the PVE for the corresponding traits. *QGlu-a3-caas-1A*, which is closely linked with *Glu-A3a* accounted for more than 74.9% of the PVE for QGA3. This is in agreement with Ma et al. (2005), Ravel et al. (2006b) and Storlie et al. (2009), who indicated that some or all of the glutenin alleles shared regulatory loci for the expression of HMW glutenins on several chromosome arms including 1A, 1B, 1D and 5D.

Fig. 1 Fifty-five M-QTLs for quantities of protein fractions located on the genetic map of PH82-2/Neixiang 188. Mapped markers are indicated on the *right* and their corresponding genetic distances (cM) are indicated on the *left*. The positions of maximum LOD values for QTLs are shown by *horizontal lines*. Gaps in the genetic map are indicated with *hatched lines*. The reduced recombination frequencies and lower map distances on chromosome 1B might be due to reduced pairing between the rye arms in PH82-2/Neixiang 188 RILs. *Sec1* should be on the short arm, whereas all the other markers were presumably on the long arm of chromosome 1B ▶

Some loci detected in this study co-mapped to loci for wheat quality parameters detected in previous studies. For example, using the same population, Zhang et al. (2009b) reported that QTL on chromosome 1D linked to *Glu-D1d* had large effects on Zeleny sedimentation volume, Mixograph peak time (MPT), peak width (MPW) and width at 8 min (MTxW), accounting for 16.2–43.1% of the PVE; QTL linked to *Sec1* on chromosome 1BL.1RS showed large negative effects on Zeleny sedimentation volume, MPT, MPW, MTxW and noodle springiness, accounting for 8.1–42.2% of the PVE; QTL linked to *Ha* on chromosome 5DS showed large effects on flour protein content, MPT, MPW, RVA peak viscosity and final viscosity, and noodle springiness, accounting for 9.4–27.1% of the PVE; the grain hardness gene effects on dough strength and some mixing traits also echoed that of Nelson et al. (2006); and QTLs for dough strength co-located to various glutenin loci (Perretant et al. 2000).

The significant effects of *Glu-D1d*, *Sec1* and *Ha* on protein quality are clearly documented. He et al. (2005), Nieto-Taladriz et al. (1994) and Zhang et al. (2007a) indicated that *Glu-D1d* was significantly and positively correlated with high protein quality, whereas *Glu-B3j* (*Sec1*) showed a strong negative effect on all protein quality traits. Grain hardness is a strong predictor of dough handling and loaf texture characteristics (Symes 1965; Perretant et al. 2000). Some association of quantities of protein fractions as the genetic basis of dough properties with *Ha*, *Glu-D1d* and *Sec1* appeared in this study, but it remains unclear whether the responsible loci are the genes themselves. In a separate study, we found that lines with *Ha* and *Glu-D1d* had significantly higher quantities of glutenin, LMW-GS, *Glu-B3* and HMW-GS than those without the gene, whereas *Sec1* significantly decreased quantities of LMW-GS, and thus lowered quantity of glutenin (data not shown). It seems that the HMW-GS allele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QHmw-gs.caas-1A
QGlutenin.caas-1A
1B
Sec1
HVM23
Xbarc181
Xgwm24
Xwmc320
Xcfd48.4
Glu-B1c
Xbarc61
Xcfd48.3
Xwmc164
Xbarc81
DuPw214
Xcfa2292
Xwmc719
Xwmc44
4.6
4.7
5.3
6.6
8.9
6.2
1.8
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5.4
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QGlu-b1.caas-1B
QGlu-b1.caas-1B
QGlu-b1.caas-1B
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QGlu-a3.caas-1B
QGlu-b3.caas-1B
QGlu-b3.caas-1B
QGlu-b3.caas-1B
QLmw-gs.caas-1B
QLmw-gs.caas-1B
QLmw-gs.caas-1B
QGlutenin.caas-1B
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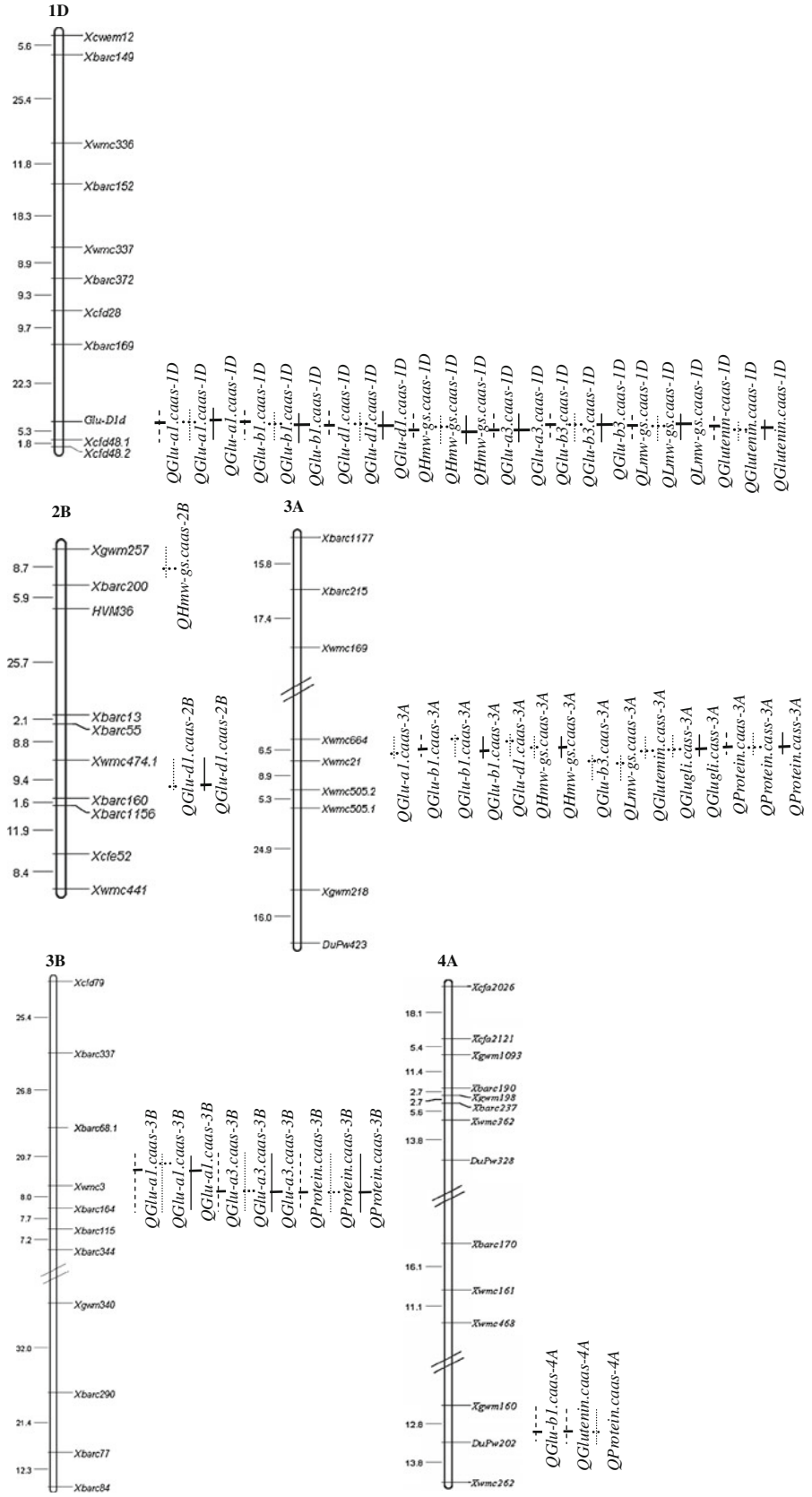

Fig. 1 continued

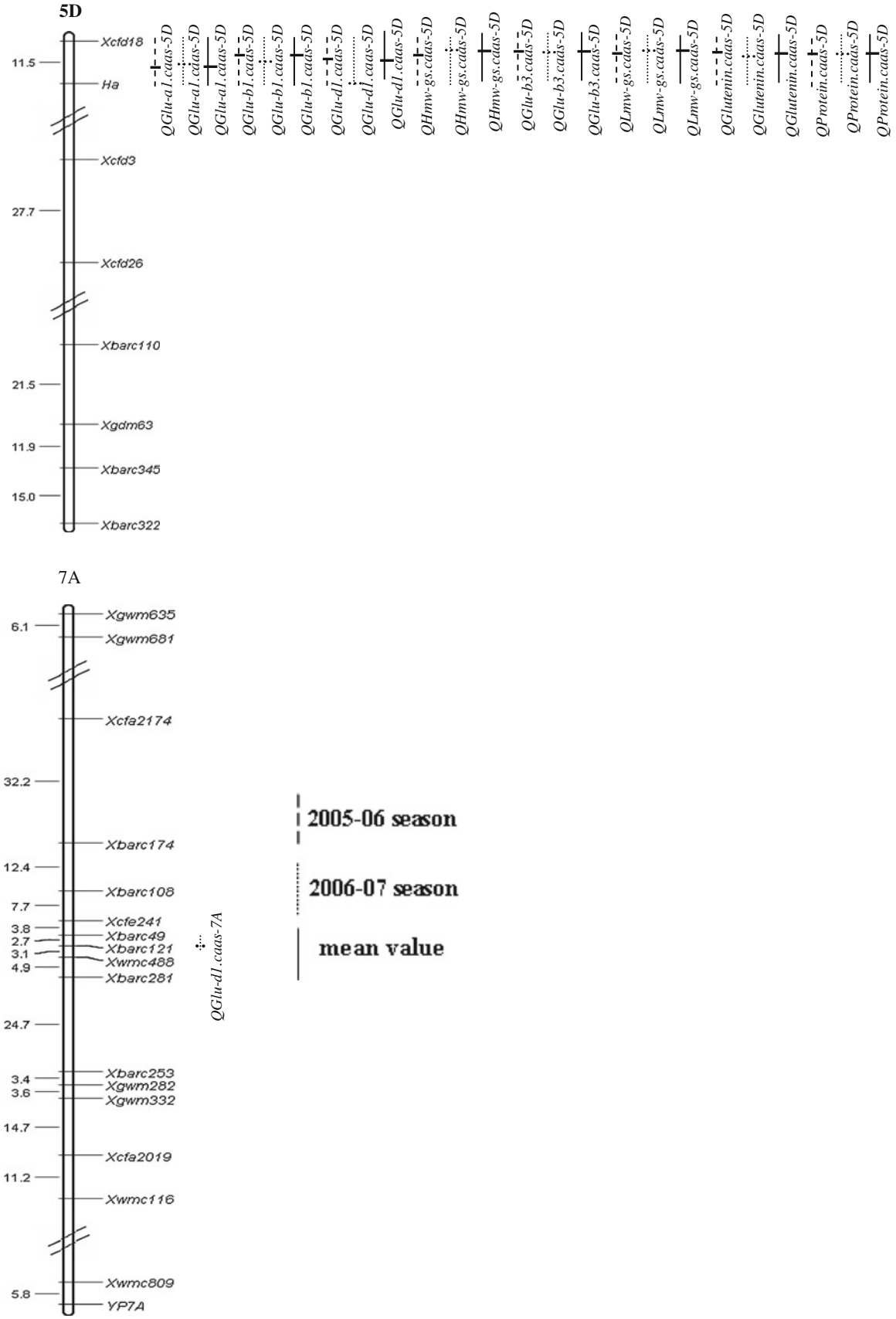

Fig. 1 continued

Table 5 E-QTLs for quantities of protein fractions detected in PH82-2/Neixiang 188 RILs in two seasons and in the mean value of two seasons

Trait	QTL$_1$[a]	Marker interval	QTL$_2$[a]	Marker interval	2005–2006			2006–2007			Mean value		
					AA[b]	LOD	PVE (%)	AA[b]	LOD	PVE (%)	AA[b]	LOD	PVE (%)
QGA1	*QGlu-a1.caas-7A*	*Xgwm332-Xcfa2019*	*QGlu-a1.caas-1D*	*Xbarc372-Xcfd28*	−0.8	5.0	3.8	−0.8	5.5	4.6	−1.0	5.4	5.5
QGB1	*QGlu-b1.caas-2B*	***Xbarc160-Xwmc474.1***[c]	*QGlu-b1.caas-3B*	***Xwmc3-Xbarc68.1***	−0.1	3.3	3.3						
	QGlu-b1.caas-2B	***Xbarc160-Xwmc474.1***	*QGlu-b1.caas-5B*	*Xwmc28-Xbarc142*				0.1	3.1	5.8			
	QGlu-b1.caas-5D	***Xcfd18-Ha***	*QGlu-b1.caas-5B*	*Xwmc28-Xbarc142*	−0.1	3.8	3.3				−0.1	3.7	3.6
	QGlu-b1.caas -7A	*Xgwm332-Xcfa2019*	*QGlu-b1.caas-3A*	*Xbarc215-Xbarc1177*	0.1	5.9	6.5						
	QGlu-b1.caas-7A	***Xbarc121-Xbarc49***	*QGlu-b1.caas-1B*	*Xcfa2292-Xwmc719*	0.1	4.1	3.1						
QGD1	*QGlu-d1.caas-1A*	***DuPw38-Xcfa2147***	*QGlu-d1.caas-7B*	*DuPw398-Xwmc311*	0.2	3.6	3.2						
	QGlu-d1.caas-1B	*Xcfa2292-Xwmc719*	*QGlu-d1.caas-6D*	*Xcfd76-Xbarc204*				−0.1	11.5	3.3			
	QGlu-d1.caas-1B	***HVM23-Sec1***	*QGlu-d1.caas-7B*	*DuPw398-Xwmc311*				0.1	5.1	1.2			
	QGlu-d1.caas-1D	***Xcfd48.1- Glu-D1d***	*QGlu-d1.caas-1B*	***Glu-B1c -Xbarc61***	−0.2	5.4	5.1						
	QGlu-d1.caas-1D	***Xcfd48.1- Glu-D1d***	*QGlu-d1.caas-3A*	*Xbarc215-Xbarc1177*				−0.1	4.0	1.1			
	QGlu-d1.caas-1D	*Xcwem12-Xbarc149*	*QGlu-d1.caas-5B*	*Xwmc28-Xbarc142*	−0.1	3.0	2.5	−0.1	4.4	1.3			
	QGlu-d1.caas-2A	*PPO18-Xwmc198*	*QGlu-d1.caas-7B*	*DuPw398-Xwmc311*				−0.1	7.4	1.9			
	QGlu-d1.caas-2A	*Xwmc522-Xwmc296*	*QGlu-d1.caas-3A*	*Xwmc21-Xwmc505.2*				−0.2	18.1	5.6	−0.1	7.9	3.4
	QGlu-d1.caas-2A	*Xwmc522-Xwmc296*	*QGlu-d1.caas-7A*	***Xbarc121-Xbarc49***				0.1	7.6	1.9			
	QGlu-d1.caas-2B	***Xbarc200-Xgwm257***	*QGlu-d1.caas-2B*	***Xbarc160-Xwmc474.1***	−0.2	3.2	3.2						
	QGlu-d1.caas-2B	***Xbarc200-Xgwm257***	*QGlu-d1.caas-4A*	*DuPw202-Xgwm160*				−0.1	4.0	1.0			
	QGlu-d1.caas-2B	***Xbarc200-Xgwm257***	*QGlu-d1.caas-5A*	*Xgwm443-Xcwem44*				−0.1	5.6	1.6			
	QGlu-d1.caas-5A	*Xbarc303-Xgdm68*	*QGlu-d1.caas-3B*	*Xbarc77-Xbarc290*				0.1	9.9	2.6			
QHMW	*QHmw-gs.caas-1B*	***Glu-B1c -Xbarc61***	*QHmw-gs.caas-4A*	*DuPw328-Xbarc170*	0.1	7.8	5.9						
	QHmw-gs.caas-1D	*Xbarc372-Xcfd28*	*QHmw-gs.caas-7B*	*DuPw450-Xgwm400*				0.1	3.6	2.8			
	QHmw-gs.caas-1D	*Xbarc372-Xcfd28*	*QHmw-gs.caas-2D*	*DuPw347-Xcfd56*				−0.1	6.9	5.9	−0.1	3.9	3.8
	QHmw-gs.caas-1D	*Xcwem12-Xbarc149*	*QHmw-gs.caas-5B*	*Xwmc28-Xbarc142*	−0.1	4.9	4.0						
	QHmw-gs.caas-2B	***Xbarc200-Xgwm257***	*QHmw-gs.caas-7B*	*DuPw450-Xgwm400*	−0.1	3.5	2.7	−0.1	3.8	3.1			
	QHmw-gs.caas-7A	*Xgwm681-Xgwm635*	*QHmw-gs.caas-4A*	*Xbarc190-Xgwm1093*	−0.1	3.1	2.2						
	QHmw-gs.caas-7A	*Xgwm681-Xgwm635*	*QHmw-gs.caas-5A*	*Xbarc303-Xgdm68*	0.1	3.3	2.2						
	QHmw-gs.caas-5D	*Xbarc345-Xgdm63*	*QHmw-gs.caas-6B*	*Xwmc494-Xgdm113*	0.1	3.2	2.2						
	QHmw-gs.caas-7A	***Xbarc121-Xbarc49***	*QHmw-gs.caas-4A*	*DuPw328-Xbarc170*	−0.1	3.9	2.5						

Table 5 continued

Trait	QTL_1^a	Marker interval	QTL_2^a	Marker interval	2005–2006			2006–2007			Mean value		
					AA^b	LOD	PVE (%)	AA^b	LOD	PVE (%)	AA^b	LOD	PVE (%)
QGA3	*QGlu-a3.caas-1B*	***Glu-B1c -Xbarc61***	*QGlu-a3.caas-7A*	***Xbarc121-Xbarc49***	−0.2	4.3	2.6						
	QGlu-a3.caas-1D	*Xcwem12-Xbarc149*	*QGlu-a3.caas-5B*	*Xwmc28-Xbarc142*				−0.3	4.5	3.5			
	QGlu-a3.caas-2A	*PPO18-Xwmc198*	*QGlu-a3.caas-6B*	*DuPw655-Xbarc79*	−0.4	8.4	5.4				−0.2	3.0	2.4
	QGlu-a3.caas-2A	*PPO18-Xwmc198*	*QGlu-a3.caas-7B*	*DuPw398-Xwmc311*				−0.3	6.3	4.0			
	QGlu-a3.caas-2A	*Xwmc522-Xwmc296*	*QGlu-a3.caas-3B*	***Xwmc3-Xbarc68.1***	0.2	4.1	3.6	0.2	3.1	1.7			
	QGlu-a3.caas-2B	***Xbarc200-Xgwm257***	*QGlu-a3.caas-2D*	*DuPw347-Xcfd56*	−0.2	3.7	2.2						
	QGlu-a3.caas-2B	***Xbarc200-Xgwm257***	*QGlu-a3.caas-7B*	*DuPw450-Xgwm400*				−0.2	3.7	2.3			
	QGlu-a3.caas-2B	***Xbarc160-Xwmc474.1***	*QGlu-a3.caas-7A*	***Xbarc121-Xbarc49***				0.2	3.6	2.3			
	QGlu-a3.caas-4A	*Xbarc190-Xgwm1093*	*QGlu-a3.caas-1A*	***DuPw38-Xcfa2147***	−0.3	5.3	3.9						
	QGlu-a3.caas-4A	*Xbarc190-Xgwm1093*	*QGlu-a3.caas-1A*	***Xcfa2153-Glu-A3a***				0.2	3.4	2.0			
	QGlu-a3.caas-1D	*Xbarc372-Xcfd28*	*QGlu-a3.caas-7A*	*Xgwm332-Xcfa2019*				−0.2	3.3	2.7			
	QGlu-a3.caas-4A	***DuPw202-Xgwm160***	*QGlu-a3.caas-7B*	*DuPw450-Xgwm400*	−0.2	3.5	2.0						
QGB3	*QGlu-b3.caas-1A*	*Xwmc449-Xwmc469*	*QGlu-b3.caas-1B*	*Xcfa2292-Xwmc719*	−0.2	7.4	2.5						
	QGlu-b3.caas-1B	***HVM23-Sec1***	*QGlu-b3.caas-5D*	*Xbarc345-Xgdm63*	−0.2	4.9	1.6	−0.1	3.1	0.8			
	QGlu-b3.caas-2A	*Xwmc170-Xcfe53*	*QGlu-b3.caas-2A*	*Xwmc522-Xwmc296*	−0.2	7.2	2.5	−0.1	4.5	1.5	−0.2	4.9	1.8
	QGlu-b3.caas-4A	***DuPw202-Xgwm160***	*QGlu-b3.caas-6D*	*Xcfd76-Xbarc204*	−0.2	5.6	2.2	−0.1	4.4	1.4			
	QGlu-b3.caas-5B	*Xwmc28-Xbarc142*	*QGlu-b3.caas-3A*	*Xbarc215-Xbarc1177*				0.1	4.3	1.6			
QLMW	*QLmw-gs.caas-1A*	***DuPw38-Xcfa2147***	*QLmw-gs.caas-5D*	***Xcfd18-Ha***				−0.3	6.8	1.6	−0.3	5.1	2.4
	QLmw-gs.caas-1B	***Glu-B1c -Xbarc61***	*QLmw-gs.caas-3B*	***Xwmc3-Xbarc68.1***	0.5	5.3	4.7				0.3	3.8	1.8
	QLmw-gs.caas-1D	***Xcfd48.1- Glu-D1d***	*QLmw-gs.caas-4A*	*DuPw328-Xbarc170*	0.3	3.1	1.8	0.3	4.7	2.0			
	QLmw-gs.caas-2B	***Xbarc200-Xgwm257***	*QLmw-gs.caas-2D*	*DuPw347-Xcfd56*				−0.2	3.4	1.5			
	QLmw-gs.caas-2B	***Xbarc160-Xwmc474.1***	*QLmw-gs.caas-6B*	*Xwmc494-Xgdm113*	−0.4	4.4	3.2						
	QLmw-gs.caas-5B	*Xwmc28-Xbarc142*	*QLmw-gs.caas-7B*	*DuPw450-Xgwm400*	−0.4	3.6	2.7						
	QLmw-gs.caas-7B	*DuPw398-Xwmc311*	*QLmw-gs.caas-4D*	*Xwmc285-Xbarc1118*				−0.4	9.0	4.5			

Table 5 continued

Trait	QTL_1[a]	Marker interval	QTL_2[a]	Marker interval	2005–2006			2006–2007			Mean value		
					AA[b]	LOD	PVE (%)	AA[b]	LOD	PVE (%)	AA[b]	LOD	PVE (%)
QGLU	*QGlutenin.caas-1A*	*Xwmc449-Xwmc469*	*QGlutenin.caas-1B*	*Xcfa2292-Xwmc719*				0.5	13.5	2.4			
	QGlutenin.caas-1A	*Xwmc449-Xwmc469*	*QGlutenin.caas-7B*	*DuPw398-Xwmc311*				0.6	20.7	4.1			
	QGlutenin.caas-1A	*Xwmc449-Xwmc469*	*QGlutenin.caas-5D*	***Xcfd18-Ha***				0.5	13.9	2.5			
	QGlutenin.caas-1B	***HVM23-Sec1***	*QGlutenin.caas-2B*	***Xbarc160-Xwmc474.1***				0.3	3.6	1.7			
	QGlutenin.caas-1D	*Xbarc372-Xcfd28*	*QGlutenin.caas-3B*	*Xbarc77-Xbarc290*				−0.5	16.2	3.1			
	QGlutenin.caas-1D	*Xcwem12-Xbarc149*	*QGlutenin.caas-5B*	*Xwmc28-Xbarc142*				−0.6	16.7	3.3			
	QGlutenin.caas-2A	*Xwmc522-Xwmc296*	*QGlutenin.caas-3B*	***Xwmc3-Xbarc68.1***				0.8	30.0	6.8			
	QGlutenin.caas-2B	***Xbarc200-Xgwm257***	*QGlutenin.caas-3B*	***Xwmc3-Xbarc68.1***				−0.6	18.3	3.6			
	QGlutenin.caas-2B	***Xbarc200-Xgwm257***	*QGlutenin.caas-3A*	*Xwmc21-Xwmc505.2*				−0.6	15.9	3.5			
	QGlutenin.caas-1D	*Xbarc372-Xcfd28*	*QGlutenin.caas-4B*	*Xwmc47-Xbarc163*				0.5	13.8	2.5			
	QGlutenin.caas-6B	*Xwmc494-Xgdm113*	*QGlutenin.caas-3B*	*Xbarc77-Xbarc290*	−0.8	3.7	5.4				−0.7	5.4	5.7
	QGlutenin.caas-6B	*Xwmc494-Xgdm113*	*QGlutenin.caas-5D*	***Xcfd18-Ha***	−0.7	3.0	3.6				−0.4	3.5	2.1
QGLUGLI	*QGlugli.caas-1A*	*Xwmc449-Xwmc469*	*QGlugli.caas-1D*	*Xcwem12-Xbarc149*				−0.1	3.2	1.4			
	QGlugli.caas-1A	*Xwmc449-Xwmc469*	*QGlugli.caas-2A*	*Xwmc522-Xwmc296*	0.1	3.2	1.4						
	QGlugli.caas-1B	***HVM23-Sec1***	*QGlugli.caas-2B*	***Xbarc200-Xgwm257***	0.1	3.6	1.7						
	QGlugli.caas-4A	*DuPw202-Xgwm160*	*QGlugli.caas-7B*	*DuPw398-Xwmc311*	−0.1	8.2	2.1				−0.1	4.6	1.3
	QGlugli.caas-7A	*Xgwm332-Xcfa2019*	*QGlugli.caas-7A*	***Xbarc121-Xbarc49***	−0.1	3.1	1.6				−0.1	4.1	1.0
	QGlugli.caas-7A	*Xgwm332-Xcfa2019*	*QGlugli.caas-7B*	*DuPw450-Xgwm400*	−0.1	3.6	1.3						
QPRO	*QProtein.caas-1B*	***HVM23-Sec1***	*QProtein.caas-3B*	*Xbarc77-Xbarc290*				0.2	5.2	4.3			
	QProtein.caas-1D	*Xbarc372-Xcfd28*	*QProtein.caas-2A*	*PPO18-Xwmc198*				0.2	5.1	3.6	0.2	3.3	6.4
	QProtein.caas-1D	*Xbarc372-Xcfd28*	*QProtein.caas-5B*	*Xwmc28-Xbarc142*	−0.2	5.3	6.4						
	QProtein.caas-3A	*Xwmc21-Xwmc505.2*	*QProtein.caas-4A*	*DuPw202-Xgwm160*	−0.2	3.5	4.5						
	QProtein.caas-5D	*Xbarc345-Xgdm63*	*QProtein.caas-5D*	***Xcfd18-Ha***				−0.1	3.1	2.0			
	QProtein.caas-7A	*Xgwm681-Xgwm635*	*QProtein.caas-3B*	*Xbarc77-Xbarc290*	−0.2	3.5	3.6						
	QProtein.caas-7A	*Xgwm681-Xgwm635*	*QProtein.caas-6D*	*Xcfd76-Xbarc204*	0.2	5.2	5.6						

[a] Nomenclature for QTL: see footnote to Table 4

[b] The effect of the pair of the epistatic QTLs

[c] Bold letters indicate an M-QTL

Glu-D1d, *Sec1* and grain hardness gene *Ha*, had significant effects on dough properties, not only through effects on allelic composition, but also by influencing quantities of protein fractions. Therefore, the effects of *Glu-D1d*, *Sec1* and *Ha* on protein quality, which have already been exploited by breeding programs worldwide in selection for improved dough rheological characters, were reconfirmed in this study by QTL mapping. The detection and confirmation of loci associated with quantities of protein fractions presented here offer great confidence to wheat breeders for improving end-use quality through MAS.

Besides the major QTLs found on chromosomes 1A, 1B, 1D and 5D, some minor QTLs were also found in two seasons and in the mean value of two seasons, including *QGlu-b1-caas-3A* and *QProtein.caas-3A* linked to *Xwmc21* on chromosome 3A, and *QGlu-a1.caas-3B* and *QGlu-a3.caas-3B* linked to *Xwmc3* on chromosome 3B. QTLs on chromosome 3A have also been found for flour protein content (Mann et al. 2009; Zhang et al. 2009b) and loaf volume (Kuchel et al. 2006). Huang et al. (2006) found a QTL for MPT on chromosome 3B located in a similar position to the present genes according to the composite wheat map (http://wheat.pw.usda.gov/ggpages/SSRclub/GeneticPhysical/), as well as *QZs.caas-3B* and *QMpw.caas-3B* in Zhang et al. (2009b). Therefore, markers *Xwmc3* and *Xwmc21*, closely linked to these QTLs, have practical implications for breeding as they are potential candidates for improving protein quality in wheat through MAS. These QTLs may account for some of the genetic variation in protein quality that cannot be explained by major genes such as *Glu-D1d*, *Sec1* and *Ha*.

Relationship between M-QTLs and E-QTLs

An important outcome of the present investigation is the characterization of the relative importance of M-QTLs and E-QTLs, and their environmental variation in controlling the expression of quantities of glutenin protein and its fractions. It showed that M-QTLs were the most important determinants of each trait, accounting for 39.3–95.6% of the PVE. Moreover, the effects of individual M-QTL were also highly variable, contributing from as little as 2.0% (a minor gene) to as much as 75.5% of the PVE (a major gene). The total effects of E-QTLs were much smaller than those of the M–QTLs, but varied considerably among traits, ranging from 3.8 to 33.5% of the PVE. Although M-QTLs were frequently the most important determinants of the quality traits, interactions between M-QTLs or even interactions between QTLs that did not have detectable main effects may have had sizable effects on quality traits. Among the 77 pairs of E-QTLs, 10 were detected between two M-QTLs, 30 were detected between an M-QTL and a modifying factor, and the other 37 involved two loci that were not detected as M-QTLs. The 55 M-QTLs were detected in 12 marker intervals, all of which were involved in digenic epistatic interactions on quantities of protein fractions, suggesting that wheat quality is a consequence of a network of interacting genes, and if epistasis was ignored in QTL mapping, many factors involved only in E-QTLs would not be detected and the M-QTLs detected would be confounded with epistatic effects, as predicted by Doebley et al. (1995).

Of the 55 M-QTLs, 33 were consistent (similar in both the direction and magnitude of their effects) in two seasons and in the mean value of two seasons, whereas only 2 of the 77 pairs of E-QTLs were consistent in two seasons and in the mean value of two seasons, suggesting that E-QTLs were more influenced by environmental effects than M-QTLs. Moreover, 14 M-QTLs found in one of the seasons were not detected in the mean value of two seasons, and 58 pairs of E-QTLs found in one of the seasons were not detected in the mean value of two seasons. Breeders must acknowledge such complexity and test the effects of individual QTL in specific genetic backgrounds to ensure the predicted phenotypes of the genes of interest. These QTLs, E-QTLs, M-QTL by environment interactions and E-QTL by environment interactions detected here provide evidence for interaction between QTLs and 'background' or modifying loci, and indicate that interactions between QTLs, E-QTLs and environment might be the prevalent situation in quantitative trait inheritance, in agreement with Doebley et al. (1995). Thus, identification and characterization of QTLs remain a largely unexploited but very important area of research for genetic improvement of wheat gluten strength.

The genetic basis of trait correlations

Genotypic correlations between traits are assumed to be contributed by either gene linkages or pleiotropic effects (Xu 1997). Twelve chromosomal regions for quantities of protein fractions were identified, with six QTL clusters in both seasons for different traits, as shown in Fig. 1. Among the clusters, one (*QGlu-a1-caas-1B* for QGA1, *QGlu-b1-caas-1B* for QGB1, *QGlu-d1-caas-1B* for QGD1, *QHmw-gs-caas-1B* for QHMW with positive effects from PH82-2 and *QGlugli.caas-1B* for QGLUGLI with positive effect from Neixiang 188) mapped on chromosome 1B closely linked to *Sec1*. Another (*QGlu-a1-caas-1D* for QGA1, *QGlu-b1-caas-1D* for QGB1, *QGlu-d1-caas-1D* for QGD1 and *QHmw-gs-caas-1D* for QHMW with positive effects from PH82-2) mapped on chromosome 1D closely linked to *Glu-D1d*. These are consistent with the strong positive correlations among QGA1, QGB1, QGD1 and QHMW, and the negative correlations of QGLUGLI with QGA1, QGB1, QGD1 and QHMW. QTLs for QGLU, QLMW and

QGB3 were also detected near *Glu-D1d*, in agreement with the positive correlations among QGLU, QLMW and QGB3. However, the exotic introgression of QTLs linked to *Sec1* was associated with reductions in QGA1, QGB1, QGD1 and QHMW, and with increases in QGB3 and QLMW. Therefore, it is likely that the counteracting effects of *Sec1* reduce the correlations between the traits.

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