Quantitative Trait Loci Influencing Endosperm Proteins and End-use Quality Traits of Hard Red Spring Wheat Breeding Lines

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Abstract: Wheat bread-making quality is influenced by a complex group of traits including dough visco-elastic characteristics. In this study, quantitative trait locus/loci (QTL) mapping and analysis were conducted for endosperm polymeric proteins together with dough mixing strength and bread-making properties in a population of 139 (MN98550 × MN99394) recombinant inbred lines that was evaluated at three environments in 2006. Eleven chromosome regions were associated with endosperm polymeric proteins, explaining 4.2–31.8% of the phenotypic variation. Most of these polymeric proteins QTL coincided with several QTL for dough-mixing strength and bread-making properties. Major QTL clusters were associated with the low-molecular weight glutenin gene Glu-A3, the two high-molecular weight glutenin genes Glu-B1 and Glu-D1, and two regions on chromosome 6D. Alleles at these QTL clusters have previously been proven useful for wheat quality except one of the 6D QTL clusters.

Keywords: quantitative trait locus; wheat quality

Wheat (*Triticum aestivum* L.) bread-making quality is directly related to gluten protein content. Through the size exclusion HPLC, the wheat gluten proteins or endosperm proteins could be separated based on their molecular size/weight, in an order of large to small proteins (Bietz 1984). Several studies have reported significant associations between the variation in molecular weight (Mw) distribution of endosperm proteins and end-use quality of different classes of wheat.

In breeding programs, because of the time, resources, large quantities of grain required to perform several quality traits analyses, full-scale milling and baking tests are only performed on advanced breeding lines to predict varietal performance. To address this challenge, several research groups have used wheat germplasm covering different market classes to identify quantitative trait loci (QTL) and genes influencing specific end-use quality traits (Campbell *et al.* 2001; McCartney *et al.* 2006; Elangovan *et al.* 2008; Mann *et al.* 2009). In this study, we present QTL influencing major endosperm protein fractions, dough-mixing strength, and bread-making properties in hard red spring wheat breeding lines adapted to the Upper Midwest region of the USA.

MATERIAL AND METHODS

A population of 139 recombinant inbred lines (RILs, $F_{6:8}$) was developed from the MN98550 ×

MN99394 cross (Tsilo *et al*. 2011). Parents, RILs, and three checks were grown in yield plots in a randomized complete block design at three Minnesota locations in 2006. A 700-g composite grain sample per line in each location was used for quality analyses. Grain samples were conditioned to 16.5% moisture content and milled using Quadrumat Senior break and reduction grinding heads (C.W. Brabender Instrument Inc., South Hackensack, NJ). Dough properties were determined using a computer-based 35-g mixograph according to AACC Approved Method (AACC 2000). Bread-baking was performed using 25-g flour samples and a straight dough method according to AACC Approved Method (AACC 2000). The SDS extractable and unextractable protein fractions were separated based on the protocol of Gupta *et al.* (1993). Broad-sense heritability was estimated for all traits evaluated. A genetic linkage map was constructed with 531 SSR and DArT marker loci that spanned a distance of 2505 cM over the whole genome of wheat. QTL analysis was performed using composite–interval mapping in WinQTL Cartographer. A QTL was declared at the LOD score ≥ 2.5.

RESULTS AND DISCUSSIONS

Trait distribution for all polymeric proteins, dough mixing and bread making properties showed transgressive segregation (Table 1), suggesting polygenic inheritance. Heritability estimates ranged from 60 to 94% (data not shown).

The QTL results for all traits and their components are summarized in Table 2 and Figure 1. The SE-HPLC absorbance area (AA) and area % (A%) of polymeric proteins represents the quantity of proteins in flour and in protein, respectively. Two major QTL clusters on chromosomes 1B and 1D that influenced many polymeric proteins, dough mixing strength and bread-making properties were mapped at the high-molecular weight (HMW) glutenin *Glu-B1* and *Glu-D1* loci, which is consistent with previous research (Campbell *et al.* 2001

Table 1. Phenotype of polymeric proteins, dough mixing strength and bread-making properties in a recombinant-inbred population evaluated in three environments in 2006

Trait	RIL population (n = 139)				Normality[b]	Parental lines	
	mean	min	max	SD		MN99394	MN98550
Polymeric proteins[a]							
EPP	4615	3757	5441	0.99	0.99^{ns}	4543	4711
UPP	4921	3085	6216	0.97	0.97**	5084	5075
UVHP	951	599	1277	1.00	1.00^{ns}	948	1045
Mixograph parameters							
Midline peak time (MPT) (min)	5.3	2.4	12.9	2.2	0.90***	4.9	5.0
Midline peak value (MPV) (%)	45.0	34.9	55.0	3.7	0.98*	47.0	44.1
Midline peak width (MPW) (%)	17.0	8.6	28.4	3.3	0.95***	19.6	16.0
Midline peak integral (MPI) (% torque × min)	173.7	86.2	365.2	63.8	0.91***	168.6	164.8
Bread-making parameters							
Bake mixing time (BMT) (min)	2.5	1.5	4.2	0.6	0.96***	2.7	2.5
Bake water absorption (BWA) (%)	56.0	52.8	58.7	1.0	0.99^{ns}	55.9	55.9
Bread loaf volume (BLV) (cm^3)	174.3	152.7	195.7	8.0	0.99^{ns}	180.3	173.3

[a]EPP = SDS-extractable polymeric proteins; UPP = SDS-unextractable polymeric proteins; UVHP = SDS-unextractable very high molecular weight polymeric proteins; [b]Shapiro-Wilk normality test was based on the mean of three environments with the null hypothesis stating that the distribution is normal; RIL – recombinant inbred lines; SD – standard deviation
*P < 0.05, **P < 0.01, ***P < 0.001; ^{ns}not significant at P < 0.05

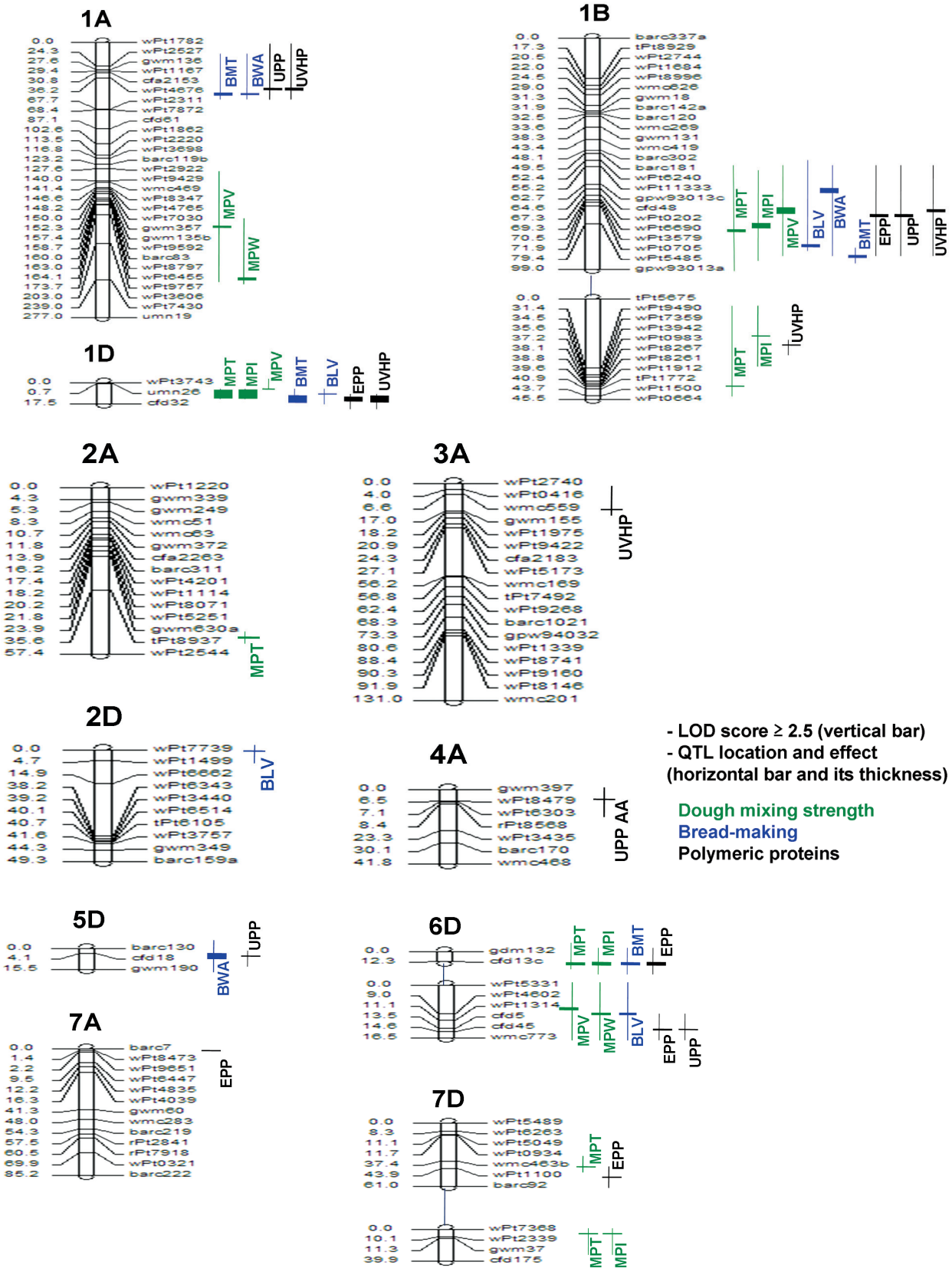

Figure 1. QTL regions for polymeric proteins (EPP, UPP, UVHP), drough-mixing (midline peak time (MPT), midline peak value (MPV), midline peak width (MPW), midline peak integral(MPI)), and bread-making (bake mixing time (BMT), bake water absorbtion (BWA), bread loaf volume (BLV))

Table 2. Summary of individual QTL and their joint-effects on trait values of 139 recombinant inbred lines averaged across three environments

Trait[a]	QTL	QTL information[b]		Multiple regression	
		R^2	add	markers in the model	R^{2c}
EPP (AA)	*QEppaa.mna-1D*	19.4	149	umn26	38.4
	QEppaa.mna-6D.1	19.4	–148	cfd13c	
	QEppaa.mna-6D.2	8.4	–97	cfd5	
	QEppaa.mna-7A	5.8	81	wPt8473	
	QEppaa.mna-7D	6.8	–87	wPt5049	
EPP (A%)	*QEppa.mna-1B*	10.0	–0.35	wPt0202	39.9
	QEppa.mna-1D	29.1	0.60	umn26	
	QEppa.mna-6D	19.2	–0.45	cfd13c	
UPP (AA)	*QUppaa.mna-1A*	12.6	–144	wPt4676	40.7
	QUppaa.mna-1B	18.9	177	wPt0202	
	QUppaa.mna-4A	7.4	–111	wPt8479	
	QUppaa.mna-5D	5.1	93	cfd18	
	QUppaa.mna-6D	5.6	–96	wPt1314	
UPP (A%)	*QUppa.mna-1A*	18.0	–1.09	wPt4676	44.0
	QUppa.mna-1B	10.5	0.85	wPt0202	
UVHP (AA)	*QUvhpaa.mna-1A*	5.1	–27	wPt4676	54.5
	QUvhpaa.mna-1B	12.5	42	wPt0202	
	QUvhpaa.mna-1D	31.8	–68	umn26	
	QUvhpaa.mna-3A	4.2	–24	wPt0416	
UVHP (A%)	*QUvhpa.mna-1A*	22.0	–0.25	wPt4676	72.4
	QUvhpa.mna-1B.1	9.9	0.17	wPt3579	
	QUvhpa.mna-1B.2	5.2	0.12	wPt8267	
	QUvhpa.mna-1D	30.8	–0.30	umn26	
MPT	*QMpt.mna-1B.1*	6.7	0.81	wPt6690	70.9
	QMpt.mna-1B.2	7.1	0.61	wPt1500	
	Glu-1D	48.6	–1.58	umn26	
	QMpt.mna-6D	8.1	0.66	cfd13c	
	QMpt.mna-7D.1	4.8	0.49	wPt0934	
	QMpt.mna-7D.2	6.0	–0.56	wPt7368	
MPI	*QMpi.mna-1B.1*	14.5	25.1	wPt6690	68.9
	QMpi.mna-1B.2	5.5	15.4	wPt0983	
	Glu-1D	50.5	–46.0	umn26	
	QMpi.mna-6D	8.8	19.6	cfd13c	
	QMpi.mna-7D	4.7	–14.1	wPt7368	
MPV	*QMpv.mna-1A*	9.1	1.15	gwm357	55.2
	QMpv.mna-1B	28.8	2.00	cfd48	
	Glu-1D	5.7	–0.86	umn26	
	QMpv.mna-6D	11.3	–1.27	wPt1314	

Table 2 to be continued

Trait[a]	QTL	QTL information[b]		Multiple regression	
		R^2	add	markers in the model	R^{2c}
MPW	*QMpw.mna-1A*	16.5	1.39	wPt6455	54.4
	QMpw.mna-1B	32.8	1.89	cfd48	
	QMpw.mna-6D	7.9	–0.95	cfd5	
BMT	*QBmt.mna-1A*	15.7	–0.26	cfa2153	53.0
	QBmt.mna-1B	9.9	0.20	gpw93013a	
	Glu-1D	38.5	–0.38	umn26	
	QBmt.mna-6D	8.7	0.22	cfd13c	
BLV	*QBlv.mna-1B*	15.1	3.14	wPt0705	40.4
	QBlv.mna-2D	11.6	–2.76	wPt7739	
	QBlv.mna-6D	10.9	–2.71	cfd5	
BWA	*QBwa.mna-1A*	14.6	0.38	wPt4676	26.9
	QBwa.mna-1B	23.1	0.48	gpw93013c	
	QBwa.mna-5D	9.6	0.31	cfd18	

[a]Traits were defined in Table 1

[b]For each QTL, the percent phenotypic variation ($R^2 \times 100$) explained by each QTL are provided along with the additive (add) allele effects for the loci. Positive additive allele effects indicate that the QTL for higher value were contributed by MN99394, and negative indicates that the QTL alleles were contributed by MN98550

[c]The percent phenotypic variation ($R^2 \times 100$) explained by joint effects of all QTL in the multiple regression model

McCartney *et al.* 2006; Elangovan *et al.* 2008; Mann *et al.* 2009). The low-molecular weight *Glu-A3* locus on 1A influenced SDS-unextractable polymeric proteins (UPP and UVHP), bake water absorption and bake mixing time. We detected two QTL clusters on 6D, one cluster near the storage protein locus on the short arm of chromosome 6D as previously reported by Nelson *et al.* (2006). The other QTL cluster was located on the long arm of 6D. This novel QTL cluster on 6DL mapped near the markers *Xcfd5*, *Xcfd45* and *Xwmc773* and influenced dough mixing properties, bread loaf volume and polymeric proteins.

Other QTL influencing quality traits including polymeric proteins were detected on chromosomes 1A, 1B, 2A, 2D, 3A, 4A, 5D, 7A, and 7D. For example, another novel QTL for bread loaf volume was on 2D (R^2 = 11.6%). Elangovan *et al.* (2008) conducted intensive bread loaf volume QTL study in six environments and identified QTL on 1B, 1D, 2A, 3A, 5B, 5D, 6B, and 6D. The 6D QTL cluster identified by Elangovan *et al.* (2008) was the storage protein locus on the short arm. Most of the QTL for polymeric proteins also co-localized with QTL for quality traits and was consistent with the strong correlations between polymeric proteins (EPP and UVHP) and dough and bread-baking properties (Tsilo *et al*. 2010).

The multiple regression analysis of all the QTL for dough-mixing properties showed that the QTL explained 54.4–70.9% of the phenotypic variation (Table 2). All QTL in the multiple regression model explained 26.9–53.0% of the phenotypic variation in bread-making properties and 38.4–72.4% of the phenotypic variation in polymeric proteins (Table 2). DNA markers linked to these QTL may be useful in increasing the frequency of desirable alleles during early generations of breeding.

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