Genetica (2010) 138:1191–1200
DOI 10.1007/s10709-010-9511-7

Mapping of quantitative trait loci controlling barley flour pasting properties

Junmei Wang · Jianming Yang · David McNeil · Meixue Zhou

Received: 9 April 2010 / Accepted: 21 October 2010 / Published online: 10 November 2010

Abstract Pasting properties are important characteristics of barley starch from a processing standpoint. A shorter time to peak viscosity and lower pasting temperature are favorable to both malting and food processing. This study was conducted to identify quantitative trait loci (QTLs) determining pasting properties of barley flour using a doubled haploid population of 177 lines from the cross between six-rowed Yerong and two-rowed Franklin. Yerong is a feed barley with a longer time to peak viscosity and a higher pasting temperature than the other parent Franklin which is a malting barley. Field trials were conducted in three different sites/years. Seven different parameters representing the pasting properties were measured using a Rapid Visco-analyser (RVA). DH lines showed significant differences in all seven parameters in most of the sites/years. For example, the pasting temperature of different DH lines ranged from 73.8 to 89.5 in 2006/2007 MTP field trial. Twenty one QTLs were associated with flour pasting properties. These QTLs were distributed on 11 chromosome regions. Genetic variance explained by these QTLs varies from 4.4 to 15.2%. The most important QTLs controlling the time to peak viscosity and pasting temperature were located on 1H, 2H, 3H and 7H. Results showed that some of the pasting properties can be effectively selected by the combination of several molecular markers.

J. Wang · D. McNeil · M. Zhou (✉)
Tasmanian Institute of Agricultural Research, University of Tasmania, P.O. Box 46, Kings Meadows, TAS 7249, Australia
e-mail: mzhou@utas.edu.au

J. Wang · J. Yang
Institute of Crop and Nuclear Technology Utilization, Zhejiang Academy of Agricultural Sciences, 310021 Hangzhou, China

Keywords Barley (*Hordeum vulgare* L.) · Quantitative trait loci (QTLs) · Pasting property · Rapid Visco-analyser (RVA)

Introduction

Barley is an important primary source in both brewing and feed industries. It also remains an important food crop today in some countries (Baik and Ullrich 2008). Starch is the principal constituent of the total reserve carbohydrate and accounts for over 60% by weight of barley grain (Back-Knudsen et al. 1987). Its content and properties are crucial in determining malting, feed and food quality (MacGregor and Fincher 1993; Jadhav et al. 1998). During the brewing process, degradation products from starch are central in providing substrates for the fermentative phase, and the fermentative sugars contribute to malt extract production (Evans et al. 2009). Therefore, the physico-chemical properties of starch have a significant impact on the resultant malt quality and brewhouse performance (Dunn et al. 1996). Food products containing or prepared from barley grain or flour with different amylose contents exhibit wide variation in processing properties and product quality (Baik and Ullrich 2008). Starches of low amylose content exhibit lower pasting temperature, and greater hot-paste viscosity, swelling power, granule fragility and freeze—thaw stability than do starches with higher amylose contents (Zheng and Sosulski 1998). Lower pasting temperatures of starch would be expected to be an advantage during processing as sufficient gelatinization would occur during the short processing times used commercially (Zhou et al. 1998). Gelatinization is also essential for complete enzymic hydrolysis of starch during mashing (MacGregor and Fincher 1993).

The functional properties of starch are affected strongly by genetic factors (Oliveira et al. 1994; Dunn et al. 1996; Stuart et al. 1997; Tester 1997). Oliveira et al. (1994) reported high heritability of starch granule traits based on genotype means in a study including 14 genotypes but the heritability was low in the parent-offspring heritability evaluation. Environmental factors also have significant impacts on the starch properties of barley. In general, high growth temperatures facilitate amylopectin crystallization and increase gelatinization temperatures, delay the onset and depress the extent of swelling of granules when heated in water (Tester 1997; Kiseleva et al. 2003; Anker-Nilssen et al. 2006).

Much of the published data on starch properties have been obtained on isolated starch because of the problems of separating the effects due to starch, glucans and other components (Zhou et al. 1998). However, starch isolation requires a difficult and time-consuming procedure even when employed on a laboratory scale. The use of the whole meal flour in predicting starch properties would be more practical in a breeding program. Our previous studies showed that pasting properties of barley flour were closely related to malting quality (Zhou and Mendham 2005) and the flour pasting temperature, which showed significant correlation with malt extract, was mainly controlled by additive effects (Zhou et al. 2008). Flour pasting properties can be affected by various chemical components in the flour such as lipids (Zhou et al. 1999), protein and beta-glucan (Zhou et al. 2000). The content in the grain of these components are easily affected by environment and agricultural practices (Lalic et al. 2007). Thus, to improve the effectiveness of selecting barley pasting properties, it is necessary to use molecular marker assisted selection. However, there are few reports on the QTLs controlling pasting properties of barley starch. Our preliminary studies showed several significant QTLs associated with barley flour pasting properties (Zhou et al. 2008). As a follow-on study, the pasting properties measured from a DH population growing in three different sites/years were used for identifying QTLs controlling barley flour pasting properties.

Materials and methods

Plant material

A population of 177 doubled haploid (DH) lines was produced from F_1 plants of the barley cross between Yerong (an Australian six-rowed feed barley) and Franklin (an Australian two-rowed malting barley) by the anther culture method. Both parents are spring type with Yerong being about 10 days earlier than Franklin.

Field experiment

The DH lines and parents were grown at Mt Pleasant Laboratories (MTP), Tasmania, in the 2006/2007 and 2007/2008 growing seasons and Forthside Vegetable Research Station (FVRS), Tasmania, in the 2007/2008 growing season. Field trials at MTP were sown in early winter (June) and FVRS trial was sown in late winter (August). Both 2006/2007 and 2007/2008 MTP trials were relatively dry due to lower than normal rainfall during the growing season, with total rainfall between October and December being 57 mm in 2006/2007 and 132 mm in 2007/2008. These rainfalls were much less than the average rainfall (162 mm). In contrast, the rainfall in FVRS reached 205 mm during these 3 months. However, this was still less than the average (225 mm). Each line was grown in a 2 m row plot with 0.4 m between rows. All agronomic practices, including fertilization, weed and disease control, were in accordance with local practice. All experiments were arranged as a randomized complete block design with two replications.

The measurements of pasting properties

The whole barley grains were ground on a Cyclotech 1903 (Tecator, Sweden) Sample Mill fitted with a 1 mm screen. Ground flour (4.0 g) was made into a slurry with 0.1 M silver nitrate solution (25 ml). The pasting properties of the slurry were determined with a Rapid Visco-analyser (RVA-4D, Newport Scientific, Sydney, NSW, Australia) using the test profile with a stirring speed of 960 rpm for 10 s and 160 rpm for the remainder of the test and with the temperature programmed to rise from 50 to 95°C in 3.7 min, to hold for 2.5 min, to cool to 50°C in 3.8 min, and to hold for 2 min. RVA measurements are reported in Rapid Visco-analyser Units (RVU) and minutes. An RVU is approximately equal to 10 cP. The RVA measurements were as follows (Zhou and Mendham 2005): Peak Viscosity (PV)—highest viscosity during heating; Time to Peak Viscosity (TTPV); Trough (TR)—lowest viscosity after cooling started; Breakdown (BD)—peak viscosity minus trough; Final Viscosity (FV)—maximum viscosity after the temperature had returned to 50°C; Setback (SB)—final viscosity minus trough; Pasting Temperature (PT)—temperature when the rate of increase in viscosity reaches 11.5 RVU in 0.2 min, effectively the beginning of the rapid increase towards PV.

QTL analysis

A genetic linkage map was constructed using 496 DArT (Diversity Arrays Technology) markers and 28 microsatellite markers (Wenzl et al. 2006; Li et al. 2008). Using the software package MapQTL5.0 (van Ooijen and Kyazma 2004), QTLs were first analyzed by interval mapping (IM).

The closest marker at each putative QTL identified using interval mapping was selected as a cofactor and the selected markers were used as genetic background controls in the approximate multiple QTL model (MQM) of MapQTL5.0. Logarithm of the odds (LOD) threshold values applied to declare the presence of a QTL were estimated by performing the genome wide permutation tests implemented in MapQTL version 5.0 using at least 1,000 permutations of the original data set for each trait, resulting in a 95% LOD threshold of 2.9. A different QTL mapping software—QTL Network (Yang et al. 2005) was used for the analysis on the possible interactions within QTLs and between QTL and environment. Permutation tests were carried out using 1,000 iterations at 1-cM intervals. A minimum separation of 10 cM ('filtration window') was used to define individual adjacent QTLs. The QTL Network calculates a P value for significance and in the present study a threshold of $P < 0.05$ was used to declare a significant QTL. For the measurements and comparisons of variability among the traits, the standard deviation (SD) was calculated using the software developed by Tang and Feng (2007).

Definition of QTL names

All the DH lines harvested from different environments were measured for their flour pasting properties and used to identify QTLs controlling different RVA measurements. Names for the tentative QTLs identified from different environments were given by a 2–4 letter acronym for the trait followed by a number identifying the environment (1 = 2006/2007 MTP; 2 = 2007/2008 MTP and 3 = 2007/2008 FVRS), a ".", and a serial number. Final QTL names, identified from the average values of three experiments, were given by a "Q", followed by a 2–4 letter acronym for the trait, a ".", a four letter string of the first two letters of the parents, a "–", and the "H" chromosome number.

Results

Phenotypic variation between two parents and among the DH lines

The mean values of pasting properties are shown in Table 1. The parents of the DH population differ significantly in pasting properties. Yerong had longer TTPV, higher PT, lower PV and BD over all environments. For TR, FV and SB, Franklin showed significantly higher values than Yerong at MTP in 2006/2007 but no significant differences between the two parent varieties were found from the trials at MTP in 2007/2008 and at FVRS in 2007/2008. There was great variation among DH lines for all the RVA measurements. Environments showed significant effects on pasting properties with DH lines grown at MTP in 2006/2007 having lower PV, TR, BD, FV, and higher PT than those grown at FVRS (Table 1).

Table 1 Mean and standard deviation for pasting properties of Yerong, Franklin and the DH population lines of their cross

Traits	PV	TR	BD	FV	SB	TTPV	PT
MTP 2006/2007							
Yerong	307	179	129	303	124	5.87	88.1
Franklin	423	204	219	345	140	5.67	79.1
DH lines-minimum	265	144	97	281	123	5.47	73.8
DH lines-maximum	458	240	233	416	185	6.07	89.5
DH lines-mean	366	191	176	341	151	5.74	83.7
DH lines-SD*	32.67	19.32	20.35	26.50	11.16	0.12	2.91
MTP 2007/2008							
Yerong	393	220	172	364	144	5.93	85.8
Franklin	410	210	200	354	143	5.78	80.9
DH lines-minimum	304	170	124	312	125	5.53	75.0
DH lines-maximum	487	262	244	426	176	6.13	89.6
DH lines-mean	412	217	195	364	147	5.83	82.7
DH lines-SD	27.60	16.83	19.77	22.03	8.56	0.11	2.50
FVRS 2007/2008							
Yerong	406	229	177	369	140	5.58	83.5
Franklin	431	231	200	371	140	5.52	81.9
DH lines-minimum	358	178	140	316	111	5.37	77.9
DH lines-maximum	495	292	235	449	172	5.90	87.2
DH lines-mean	434	233	201	377	145	5.59	82.1
DH lines-SD	23.67	18.60	18.13	22.89	8.75	0.11	1.65

**SD* standard deviation, *PV* peak viscosity, *TR* trough, *BD* breakdown, *FV* final viscosity, *SB* setback, *TTPV* time to peak viscosity, *PT* pasting temperature, *MTP* Mt Pleasant Laboratories, *FVRS* Forthside Vegetable Research Station, *2006/2007* 2006/2007 growing season, *2007/2008* 2007/2008 growing season

Identification of QTLs associated with pasting properties

Peak viscosity (PV)

Two major QTLs controlling PV were found on 1H *(QPv.YeFr-1H)* and 4H *(QPv.YeFr-4H)*, respectively, explaining more than a total of 25% of the phenotypic variation (Table 2; Fig. 1). The Yerong allele increased PV on 1H but decreased PV on 4H. The QTL on 1H *(QPv.YeFr-1H)* was found for both the 2007/2008 MTP *(PV2.2)* and 2007/2008 FVRS *(PV3.1)* trials while the one on 4H *(QPv.YeFr-4H)* was only identified in the 2007/2008 MTP *(PV2.3)* trial. Two more QTLs (*PV2.1* and *PV2.4*) controlling PV were found on Chromosomes 1H and 5H at MTP in 2007/2008. No QTL was identified from the 2006/2007 MTP trial (Table 3).

Trough (TR)

Two significant QTLs associated with TR were identified. One QTL (*QTr.YeFr-2H*) was located on chromosome 2H with the nearest marker being bPb-3653 (105.0 cM), explaining 15.2% of the genetic variation. The other QTL (*QTr.YeFr-7H*) was located on chromosome 7H with the nearest marker being bPb-7263 (150.8 cM) and explaining 9.3% of the genetic variation (Table 2; Fig. 1). For these two QTLs, the Yerong alleles showed increasing effect on 2H but decreasing effect on 7H. The QTL on 2H (*QTr.YeFr-2H*) was found at all three environments (*TR1.1*, *TR2.1* and *TR3.2*) while the QTL on 7H was only found at MTP in 2007/2008 (*TR2.2*). From the 2007/2008 FVRS trial, one extra QTL (*T3.1)* was found on chromosome 2H (Table 3).

Breakdown (BD)

Four QTLs (*QBd.YeFr-1H*, *QBd.YeFr-2H, QBd.YeFr-3H* and *QBd.YeFr-4H*) were detected on chromosomes 1H, 2H, 3H and 4H for BD. These four QTLs explained a total of 41% of the genetic variation (Table 2; Fig. 1). The Yerong alleles showed increasing effect only on 3H. Three of the four QTLs were identified at MTP in 2006/2007 (*BD1.1, BD1.1,* and *BD1.3*). Fewer QTLs were found from the other two trials (Table 3).

Table 2 QTLs for pasting properties detected in the DH population of Yerong × Franklin (Average value)

Trait	Linkage group	QTL name	2_LOD interval	Nearest marker	Position (cM)	LOD	R^2 (%)	Additive effect
PV	1H	QPv.YeFr-1H	93–113	HVHVA1	102.0	6.62	14.4	8.8
	4H	QPv.YeFr-4H	45–65	bPb-0130	58.2	5.46	11.8	−7.9
TR	2H	QTr.YeFr-2H	91–113	bPb-3653	105.0	6.90	15.2	6.0
	7H	QTr.YeFr-7H	116–156	bPb-7623	150.8	4.37	9.3	−4.7
BD	1H	QBd.YeFr-1H	93–113	bPb-1078	103.2	7.47	13.0	−6.0
	2H	QBd.YeFr-2H	85–108	bPb-4577	105.5	5.17	8.7	−4.8
	3H	QBd.YeFr-3H	46–61	Bmag0006	54.1	4.99	8.4	4.7
	4H	QBd.YeFr-4H	31–63	bPpb-0130	58.2	6.28	10.7	−5.4
FV	2H	QFv.YeFr-2H	5–16	Bmac0134	7.6	4.92	12.0	6.9
SB	1H	QSb.YeFr-1H	75–116	bPb-3984	103.5	3.56	6.7	2.0
	2H	QSb.YeFr-2H	5–49	Bmac0134	7.6	6.63	13.1	2.8
	3H	QSb.YeFr-3H	115–132	bPb-4830	123.2	5.75	11.2	−2.6
TTPV	1H	QTpv.YeFr-1H	48–66	bPb-4590*	56.7	8.86	14.0	0.033
	2H	QTpv.YeFr-2H	19–41	bPb-8750*	31.9	8.80	14.0	−0.033
	3H	QTpv.YeFr-3H	43–76	bPb-2394	54.2	4.38	6.7	0.022
	6H	QTpv.YeFr-6H	34–58	bPb-0857*	51.2	4.64	7.1	0.023
	7H	QTpv.YeFr-7H	60–79	bPb-3157*	70.9	3.02	4.4	0.019
PT	1H	QPt.YeFr-1H	92–151	HVHVA1	102.0	3.62	5.8	−0.47
	3H	QPt.YeFr-3H.1	0–28	bPb-1264	0.7	4.71	9.1	0.58
	3H	QPt.YeFr-3H.2	40–58	bPb-2394	54.2	6.46	12.9	0.66
	7H	QPt.YeFr-7H	67–94	bPb-9825*	85.8	5.15	8.5	0.58

The position is that of the nearest marker; R^2 means percentage genetic variance explained by the nearest marker; Two LOD support intervals were used to indicate the 95% confidence intervals (van Ooijen 1992)

* Markers originated from Franklin. *PV* peak viscosity, *TR* trough, *BD* breakdown, *FV* final viscosity, *SB* setback, *TTPV* time to peak viscosity, *PT* pasting temperature

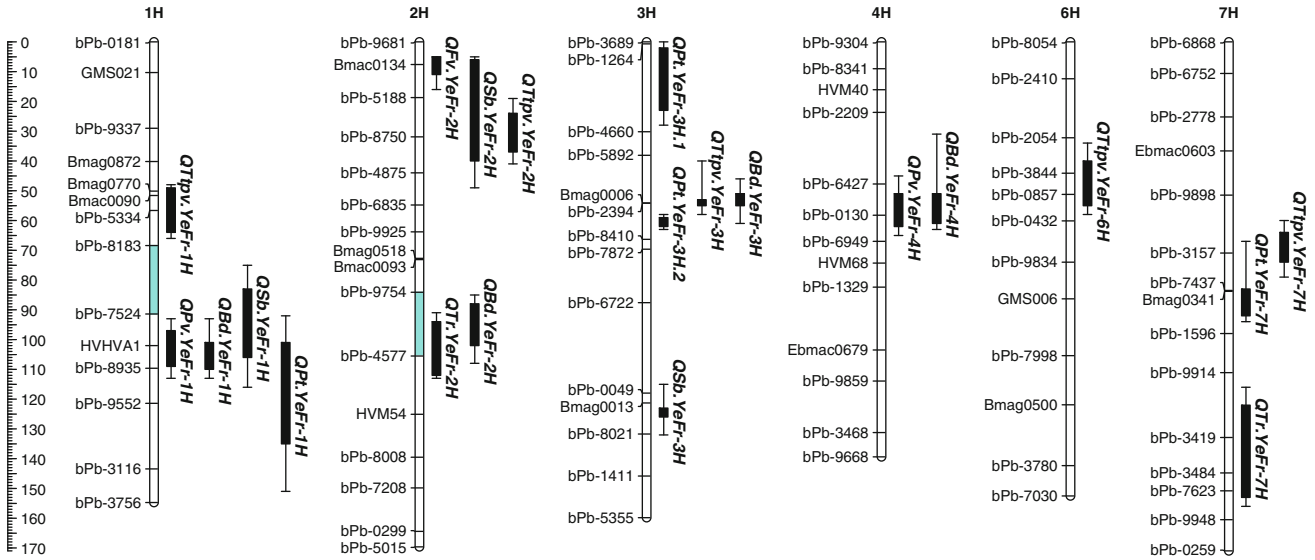

Fig. 1 Quantitative trait loci (QTLs) identified for pasting properties in the DH population from Yerong × Franklin. Each QTL name was given by a "Q", followed by a 2–4 letter acronym for the trait, a ".", a four letter string of the first two letters of the parents, a "–", and the "H" chromosome number. Only SSR markers and a few DArT markers are shown in the map. The units in the rule are cM. For detailed map, please refer to Li et al. (2008)

Final viscosity (FV)

Based on the average value from three different environments, only one significant QTL associated with FV was identified. This QTL (*QFv.YeFr-2H)* was located on chromosome 2H and only explained 12.0% of the genetic variation (Table 2; Fig. 1) with the Yerong allele increasing FV. QTLs identified from different environments were not consistent. From the MTP trial in 2007/2008, one QTL (*FV2.1*) was located on chromosome 7H with the position of 132.9 cM, and explained 12.4% of the genetic variation. One QTL (*FV3.1*) controlling this trait was found on chromosome 2H at FVRS in 2007/2008, explaining 12.5% of the genetic variation. No significant QTL was identified for FV at MTP in 2006/2007 (Table 3).

Setback (SB)

Three QTLs (*QSb.YeFr-1H, QSb.YeFr-2H* and *QSb.YeFr-3H*) were found to be associated with SB based on the average value of the three trials. *QSb.YeFr-1H* was located on 1H with the nearest marker being bPb-3984. *QSb.YeFr-2H* was located on 2H with the nearest marker being Bmac0134 and *QSb.YeFr-3H* was located on 3H with the nearest marker being bPb-4830. These three QTLs accounted for a total of 31% of the variation (Table 2; Fig. 1). The Yerong alleles increased SB in two (1H and 2H) of three QTLs. Some different QTLs were found when the analysis was based on individual trials. At MTP in 2006/2007, the two QTLs (*SB1.1* and *SB1.2*) were located on chromosomes 2H and 4H, and explained 12.1% and 6.7% of genetic variation, respectively. At MTP in 2007/2008, one QTL (*SB2.1*) was located on chromosome 2H with the position of 7.6 cM, explaining 7.9% of the genetic variation and the other QTL *SB2.2* was located on chromosome 3H with the position of 123.2 cM for the nearest marker, explaining 13.3% of the genetic variation. At FVRS in 2007/2008, the two QTLs (*SB3.1* and *SB3.2*) were located on chromosome 2H with the position of 31.9 cM for the nearest marker and on 3H with the position of 123.2 cM for the nearest marker (Table 3).

Time to peak viscosity (TTPV)

Based on the average value across the trials, five significant QTLs (*QTtpv.YeFr-1H, QTtpv.YeFr-2H, QTtpv.YeFr-3H, QTtpv.YeFr-6H* and *QTtpv.YeFr-7H*) were found for TTPV, explaining a total of nearly 50% of the variation (Table 2; Fig. 1). For *QTtpv.YeFr-1H, QTtpv.YeFr-6H* and *QTtpv.YeFr-7H,* the Franklin alleles increased TTPV while for the other two QTLs, the Yerong alleles increased TTPV. A slightly different set of QTLs were identified from individual environments. Three QTLs (*TTPV1.1*, *TTPV1.2* and *TTPV1.3*) were found for TTPV at MTP in 2006/2007 with only TTPV1.3 being at the same position as *QTtpv.YeFr-7H*. Among the two QTLs (*TTPV2.1* and *TTPV2.2*) detected from MTP in 2007/2008, *TTPV2.1* was at the same position as *QTtpv.YeFr-1H*. Four significant QTLs (*TTPV3.1*, *TTPV3.2*, *TTPV3.3* and *TTPV3.4*) were also identified for TTPV at FVRS in 2007/2008. All of the four QTLs were at same or similar positions to those identified from the average values (Table 3).

Table 3 QTLs for pasting properties detected in the DH population of Yerong × Franklin

Trait	Location	Linkage group	QTL name	2_LOD interval	Nearest marker	Position (cM)	LOD	R^2 (%)	Additive effect
PV	MTP 2006/2007	–	–	–	–	–	–	–	–
	MTP 2007/2008	1H	*PV2.1*	20–63	bPb-1604	44.8	3.4	7.1	*7.4*
		1H	*PV2.2*	86–113	HVHVA1	102.0	4.18	8.6	8.3
		4H	*PV2.3*	54–63	bPb-0130	58.2	6.90	13.2	−10.2
		5H	*PV2.4*	50–91	bPb-8101	58.8	3.15	5.7	6.7
	FVRS 2007/2008	1H	*PV3.1*	87–112	bPb-9108	105.0	3.70	9.2	−7.9
TR	MTP 2006/2007	2H	*T1.1*	88–108	bPb-3653	105.0	5.21	12.8	7.1
	MTP 2007/2008	2H	*T2.1*	95–122	bPb-9258	119.6	3.66	7.8	4.7
		7H	*T2.2*	121–144	bPb-3419	132.9	7.36	16.5	−6.9
	FVRS 2007/2008	2H	*T3.1*	0–17	Bmac0134	7.6	4.28	9.8	5.8
		2H	*T3.2*	87–114	bPb-4577	105.5	4.32	9.9	5.9
BD	MTP 2006/2007	1H	*BD1.1*	91–117	bPb-5198	128.1	5.59	10.8	−6.65
		3H	*BD1.2*	44–67	Bmag0006	54.1	2.93	5.5	4.8
		4H	*BD1.3*	41–65	bPb-0130	58.2	5.70	11.0	−6.9
	MTP 2007/2008	2H	*BD2.1*	81–100	bPb-4040	82.8	9.41	20.6	−9.2
		4H	*BD2.2*	47–64	bPb-1762	54.3	3.19	6.1	−5.0
	FVRS 2007/2008	1H	*BD3.1*	87–114	bPb-3473	122.3	2.98	6.6	−5.2
		2H	*BD3.2*	7–38	bPb-3186	18.1	5.46	10.1	6.4
		7H	*BD3.3*	36–64	bPb-9898	51.5	3.1	6.1	5.0
FV	MTP 2006/2007	–	–	–	–	–	–	–	–
	MTP 2007/2008	7H	*FV2.1*	119–147	bPb-3419	132.9	5.10	12.4	−7.8
	FVRS 2007/2008	2H	*FV3.1*	5–12	Bmac0134	7.6	5.86	12.5	8.1
SB	MTP 2006/2007	2H	*SB1.1*	9–71	bPb-8750	31.9	5.41	12.1	−3.9
		4H	*SB1.2*	34–70	bPb-1762	54.3	3.07	6.7	−3.0
	MTP 2007/2008	2H	*SB2.1*	0–43	Bmac0134	7.6	3.64	7.9	2.4
		3H	*SB2.2*	104–133	bPb-4830	123.2	5.95	13.3	−3.1
	FVRS 2007/2008	2H	*SB3.1*	6–47	bPb-8750	31.9	4.72	10.7	−2.8
		3H	*SB3.2*	108–132	bPb-4830	123.2	3.93	8.8	−2.6
TTPV	MTP 2006/2007	2H	*TTPV1.1*	0–34	bPb-8959	0.1	3.60	8.2	−0.033
		2H	*TTPV1.2*	91–110	bPb-4577	105.5	8.36	17.9	0.049
		7H	*TTPV1.3*	52–75	bPb-4541	68.5	5.66	10.9	0.038
	MTP 2007/2008	1H	*TTPV2.1*	49–79	bPb-5334	56.5	4.76	10.2	0.035
		2H	*TTPV2.2*	5–29	Bmac0134	7.6	7.12	14.8	0.042
	FVRS 2007/2008	1H	*TTPV3.1*	49–64	bPb-5292	50.6	10.05	17.7	0.048
		2H	*TTPV3.2*	28–41	bPb-8750	31.9	10.89	20.6	−0.051
		3H	*TTPV3.3*	46–57	bPb-2394	54.2	8.42	12.1	0.040
		6H	*TTPV3.4*	29–56	bPb-2062	51.1	4.31	7.0	0.030
PT	MTP 2006/2007	3H	*PT1.1*	0–26	bPb-1264	0.7	3.52	8.8	0.89
		3H	*PT1.2*	41–63	Bmag0006	54.1	3.25	8.2	−0.85
		7H	*PT1.3*	83–94	bPb-9825	85.8	8.27	19.5	1.35
	MTP 2007/2008	3H	*PT2.1*	0–22	bPb-3689	0.0	3.36	8.4	0.73
		3H	*PT1.2*	35–68	Bmag0006	54.1	3.33	8.3	−0.72
		7H	*PT2.3*	65–94	bPb-7915	84.2	6.32	15.2	1.00
	FVRS 2007/2008	2H	*PT3.1*	24–70	bPb-2501	45.5	6.30	15.1	−0.71
		3H	*PT3.2*	53–75	bPb-2394	54.2	3.81	9.9	0.57

The position is that of the nearest marker; R^2 means percentage genetic variance explained by the nearest marker; *MTP* Mt Pleasant Laboratories, *FVRS* Forthside Vegetable Research Station, *2006/2007*, 2006/2007 growing season; *2007/2008*, 2007/2008 growing season. Two LOD support intervals were used to indicate the 95% confidence intervals (van Ooijen 1992). *PV* peak viscosity, *TR* trough, *BD* breakdown, *FV* final viscosity, *SB* setback, *TTPV* time to peak viscosity, *PT* pasting temperature

Pasting temperature (PT)

Four QTLs (*QPt.YeFr-1H, QPt.YeFr-3H.1*, *QPt.YeFr-3H.2* and *QPt.YeFr-7H*) were identified for PT. These QTLs were located on 1H, 3H and 7H. The Franklin alleles increased PT in two QTLs (*QPt.YeFr-1H* and *QPt.YeFr-7H*) and decreased PT for the other two QTLs. The total genetic variation explained by all four QTLs was 36% (Table 2; Fig. 1). QTLs on 3H and 7H were found at MTP in 2006/2007 (*PT1.1*, *PT1.2* and *PT1.3*) and at MTP in 2007/2008 (*PT2.1, PT2.2* and *PT2.3*) but only *QPt.YeFr-3H.2* was identified at FVRS in 2007/2008 (*PT3.2)* and one different QTL was found on 2H (*PT3.1*) (Table 3).

Discussion

Flour pasting properties and malting and processing quality

Hulled barley grains contain about 13% hull of the grain (by weight) on average. The hull is removable but only with difficulty (Evers et al. 1999). The husk removal process is a complicated process and not practical in the measurement of flour pasting properties since uneven damage could happen to different grains. Moreover, the whole barley grain was generally used for stock feed and in brewery. While using the whole grains, the flour pasting properties can be significantly affected by starch concentration (higher husk content will lead to lower starch content) and composition (Zheng and Sosulski 1998) as well as protein, lipid and beta-glucan content (Zhou et al. 1999, 2000). Our previous studies showed that the whole grain flour pasting properties were closely related to malt extract (Zhou and Mendham 2005) with low pasting temperatures being related to high malt extract. Glennie-Holmes (1995) also found that good malting quality was associated with low time to peak viscosity and final viscosity, but not necessarily with low peak viscosity or peak area. Stuart et al. (1997) reported that malt extract was closely and negatively correlated with the pasting temperature, as well as trough, peak viscosity and peak time. They also suggested that the highly significant relationship between PT and malt extract might be due to PT being an indicator of the relative ease of starch solubilisation and hydrolysis. Therefore, starch with lower PT might be more accessible in malted barley and more importantly, the granules might swell more easily under mashing conditions and were thus more susceptible to enzyme hydrolysis. Lower pasting temperatures of starch are also favorable for processing as sufficient gelatinization would occur during the short processing times used commercially (Zhou et al. 1998). Among the seven traits reported on here, TTPV and PT are the most important as both shorter TTPV and lower PT are beneficial to food processing (Zhou et al. 1998; Zheng and Sosulski 1998) and malting (Zhou and Mendham 2005; MacGregor and Fincher 1993).

Environmental effects on flour pasting properties

It is not surprising that selecting for flour pasting properties could be affected by environmental factors since most of the flour components are affected by environments (Garcia 1985; Narasimhalu et al. 1994; Tester 1997). In this experiment, flour pasting properties showed great variations among different environments. For example, most of the RVA measurements had greater variation among DH lines from the 2006/2007 MTP trials and the flours of the DH lines from the 2006/2007 MTP trial showed significantly lower PV and higher PT. Both the 2006/2007 MTP and 2007/2008 MTP trials experienced drier conditions during the grain filling stage that lead to relatively lower 1,000 grain weights (38.2 and 39.3 g for Franklin and Yerong, respectively, from the 2007/2008 MTP trial) than those found for the 2007/2008 FVRS trial (47.7 and 47.8 g for Franklin and Yerong, respectively). Grain protein content was also found to be higher in both the 2006/2007 MTP (10.0–19.3%) and 2007/2008 MPT (9.2–16.2%) trials than that found in the 2006/2007 FVRS trial (7.4–14.1%). High protein content is generally associated with low starch content. In the current experiments, protein content (data not shown) showed significant, negative correlations with PV ($r = -0.56$), TR ($r = -0.56$), BD ($r = -0.40$) and FV ($r = -0.40$) but positive correlation with SB ($r = 0.19$), TTPV ($r = 0.35$) and PT ($r = 0.40$). The dry conditions encountered by the MTP trials showed greater effects on flour pasting properties, especially TTPV and PT of late maturing lines. For example, the correlation between days to flowering and the differences between the 2007/2008 FVRS and the 2006/2007 MTP is 0.32 for TTPV and 0.25 for PT, both very significant.

QTLs associated with flour pasting properties

QTL analysis on barley grain qualities not only provides a better understanding of the genetic factors influencing these traits but also helps to find valuable markers for molecular marker-assisted selection (MAS). Up to now, most of the studies have been concentrating on QTLs associated with malting qualities such as extract (Hayes et al. 1993; Thomas et al. 1996; Mather et al. 1997; Marquez-Cedillo et al. 2000; Emebiri et al. 2004; Gao et al. 2004; Panozzo et al. 2007), diastatic power (Mather et al. 1997; Han et al. 1997; Marquez-Cedillo et al. 2000; Emebiri et al. 2004; Panozzo et al. 2007), protein content (Marquez-Cedillo et al. 2000; Emebiri et al. 2004;

Panozzo et al. 2007) and beta-glucan content (Han et al. 1995; Mather et al. 1997; Gao et al. 2004; Emebiri et al. 2004). The QTLs identified for malting quality cover nearly all the different chromosomes. Borém et al. (1999) reported some QTLs affecting the starch granule traits, which were located on chromosome 2 (2H), chromosome 4 (4H) and chromosome 7 (5H). There is no report on QTLs controlling pasting properties of barley flour. In this study, a total of 21 significant QTLs were found for different RVA measurements. These QTLs were not evenly distributed on the seven chromosomes. More QTLs were identified on 1H, 2H and 3H while no QTL were found on 5H (Fig. 1). It is not surprising that row type (two or six rowed) may have significant effects on pasting properties. On average, six rowed lines had higher TR ($r = 0.39$) and FV ($r = 0.30$), and lower BD ($r = -0.34$) but there was no obvious correlation between row type and PV ($r = 0.04$), SB ($r = -0.11$), TTPV ($r = -0.06$) and PT ($r = -0.14$). As can be seen from Table 2 and Fig. 1, some QTLs for TR and BD were in the region where the QTL for row type gene (Vrs1) was located (84-105 cM on 2H in this population).

Significant correlations existed among different RVA parameters. In this experiment, PV was positively correlated with TR ($r = 0.76$), BD ($r = 0.73$) and FV ($r = 0.69$) but negatively correlated with PT ($r = -0.64$). Thus it is not surprising that twenty one QTLs associated with flour pasting properties were distributed on only 11 chromosome regions with some QTLs being associated with different parameters. A good example is that *QPt.YeFr-1H, QSb.YeFr.1H, QPv.YeFr-1H and QBd.YeFr.1H* were all in the same region. The marker bPb-2394 on chromosome 3H was associated with TTPV and PT. This marker can be used to select for short TTPV and low PT, which are all favorable to high malt extract (Zhou and Mendham 2005). Similarly, the marker HVHVA1 on chromosome 1H is associated with PV and PT. The selection of the combination of several molecular markers can be effective for some traits. Five QTLs were associated with TTPV (time to peak viscosity) which explained nearly 50% of the genetic variation of this trait. The favorable alleles (towards shorter TTPV) were from both parents (Table 2) thus the selection would allow the combination of the favorable alleles of both parents. Figure 2 shows the phenotypic difference in TTPV with and without selecting for these markers. DH lines with combined favorable alleles from both parents showed much shorter TTPV than both parents while the TTPV of those lines with no favorable alleles was longer than both parents. PT was found to be highly correlated with malt extract (Zhou and Mendham 2005) and is also very important in food processing (Zhou et al. 1998). In this study, QTLs for PT were found on chromosomes 1H (102.0 cM), 3H (0.7 cM and 54.2 cM) and 7H (85.8 cM). Among them, the QTL on

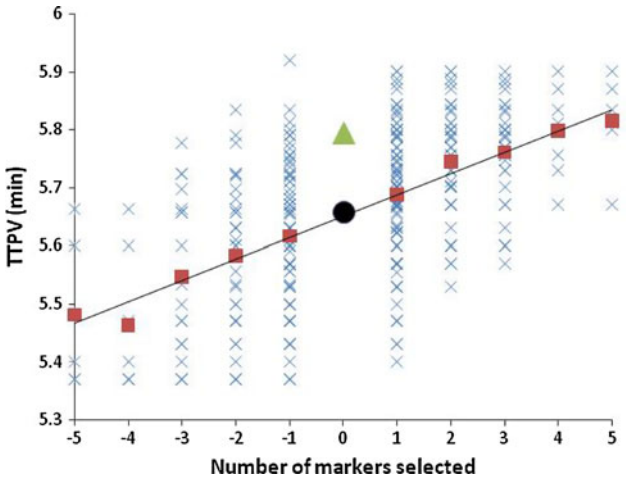

Fig. 2 Effectiveness of selecting TTPV using molecular markers. −5 ∼ −1: different number of favorable markers; 1 ∼ 5: number of unfavorable markers; −5 and 5: all the markers shown in Table 2; −4 and 4: markers from 1H, 2H, 3H and 6H; −3 and 3: markers from 1H, 2H and 3H; −2 and 2: markers from 1H and 2H; −1 and 1: marker from 1H only; 0: without marker selection; *filled triangle* TTPV of Yerong; *filled circle* TTPV of Franklin; *multi symbol* TTPV of DH lines; *filled square* average TTPV of different marker selections

chromosome 1H was at a similar position to the one QTL for malt extract reported by Panozzo et al. (2007), and the QTL on chromosome 7H was at a similar position to the one QTL for malt extract reported by Han et al. (2004), by locating QTLs on the consensus map (Varshney et al. 2007). Some of the QTLs for other traits related to malting quality were also located at similar positions of QTLs for malt extract or diastatic power reported earlier. For example, the QTL on chromosome 1H (102 cM) for PV was at a similar position to the one QTL for malt extract reported by Marquez-Cedillo et al. (2000).

Environmental effects on QTLs associated with flour pasting properties

The identification of QTLs for pasting properties was greatly affected by growth conditions. For example, QTLs for PT from MTP in both growing seasons were very similar (located on 3H and 7H) whereas those from FVRS in 2007/2008 were totally different (located on 1H and 2H). As mentioned above, growth conditions at FVRS were very different from those at MTP. FVRS always produces plump grains for both early and late varieties and protein contents are generally lower and have less variation. Great variations were found in maturity among DH lines with the earliest ones being 20 days earlier than the latest ones., The dry season at MTP caused problems in grain filling especially for late maturity lines. Thus maturity may have some effects on the QTLs identified for pasting properties. However, no QTL for pasting properties on 2H and 7H

were at the same position as the QTL for ear emergency on 2H (46–73 cM) and 7H (36–48 cM). Further analysis on the possible interactions within QTLs and between QTL and environment, using a different QTL mapping software—QTL Network (Yang et al. 2005)—revealed that most of the QTLs had only additive effect with only *QBd.YeFr-2H, QTtpv.YeFr-1H, QTtpv.YeFr-3H, QTtpv.YeFr-7H* and *QPT.YeFr-7H* showing some additive × environment interaction. Epistatic effects were found between *QBd.YeFr-2H* and *QBd.YeFr-3H*, *QPt.YeFr-1H* and *QPT.YeFr-3H.2*, and *QPT.YeFr-3H.2* and *QPT.YeFr-7H*. Environmental effects on QTLs associated with malting quality have also been reported by other researchers. Hayes and Iyamabo (1994) reported that different environments often located a QTL into different sized intervals, and a number of minor QTLs were detected in limited environmental situations. Gao et al. (2004) also found that, in different experiments, QTLs were mapped to different chromosomal locations or to similar chromosomal locations with different interval sizes, or that under certain environmental conditions a QTL could even be missing. These results indicate the complexity of pasting properties and the importance of evaluating these traits in multiple environments.

In conclusion, many QTLs were identified for different barley flour pasting properties. As DArT markers can easily be sequenced and barley consensus maps (Wenzl et al. 2006) have been constructed to link DArT markers with many SSR and RFLP markers, the markers identified in this study can be effectively used in breeding programs for improving pasting properties of barley flour, particularly processing and malting quality related traits, TTPV (QTLs determined nearly 50% of the genetic variation) and PT.

Acknowledgments This work was supported by Grains Research and Development Corporation of Australia (UT8) grant to M.Z. and National Natural Science Foundation of China (30700485) and Zhejiang Natural Science Foundation (Y306641) grant to J.W.

References

Anker-Nilssen K, Færgestad EM, Sahlstrøm S, Uhlen AK (2006) Interaction between barley cultivars and growth temperature on starch degradation properties measured in vitro. Anim Feed Sci Technol 130:3–22

Back-Knudsen KE, Aman P, Eggum B (1987) Nutritive value of Danish-grown barley varieties. I. Carbohydrates and other major constituents. J Cereal Sci 6:173–186

Baik BK, Ullrich S (2008) Barley for food: characteristics, improvement, and renewed interest. J Cereal Sci 48:232–242

Borém A, Mather DE, Rasmusson DC, Fulcher RG, Hayes PM (1999) Mapping quantitative trait loci for starch granule traits in barley. J Cereal Sci 29:153–160

Dunn CA, Bonnici MJ, Logue SJ, Long NR, Allan GR, Stuart IM (1996) An assessment of the physical and chemical properties of barley starch to predict malt quality. In: Proceedings of 24th Institute of Brewing Convention (Asia Pacific Region), Singapore, pp 120–128

Emebiri LC, Moody DB, Panozzo JF, Read BJ (2004) Mapping of QTL for malting quality attributes in barley based on a cross of parents with low grain protein concentration. Field Crops Res 87:195–205

Evans DE, Li C, Eglinton JK (2009) Theproperties and genetics of barley malt starch degrading enzymes. In: Li C, Zhang G (eds) Genetics and improvement of barley malt quality. Springer, Berlin, pp 143–189

Evers AD, Blakeney AB, O'Brien L (1999) Cereal structure and composition. Aust J Agric Res 50:629–650

Gao W, Clancy JA, Han F, Jones BL, Budde A, Wesenberg DM, Kleinhofs A, Ullrich SE (2004) Fine mapping of a malting-quality QTL complex near the chromosome 4HS telomere in barley. Theor Appl Genet 109:750–760

Garcia LF (1985) Relationships between vegetative growth, grain yield and grain protein content in six winter barleys. Can J Plant Sci 65:523–532

Glennie-Holmes M (1995) Studies on barley and malt with the rapid viscoanalyser: [II] The effect of modification viscograms. J Inst Brew 101:19–28

Han F, Ullrich SE, Chirat S, Menteur S, Jesrin L, Sarrafi A, Hayes PM, Jones BL, Blake TK, Wesenberg DM, Kleinhofs A, Lilian A (1995) Mapping of β-glucan content and β-glucanase activity loci in barley grain and malt. Theor Appl Genet 91:921–927

Han F, Ullrich SE, Kleinhofs A, Jones BL, Hayes PM, Wesenberg DM (1997) Fine structure mapping of the barley chromosome-1 centromere region containing malting-quality QTLs. Theor Appl Genet 95:903–910

Han F, Clancy JA, Jones BL, Wesenberg DM, Kleinhofs A, Ullrich SE (2004) Dissection of a malting quality QTL region on chromosome 1 (7H) of barley. Mol Breed 14:339–347

Hayes PM, Iyamabo O (1994) Summary of QTL effects in the 'Steptoe' × 'Morex' population. Barley Genet Newsl 23:98–143

Hayes PM, Liu BH, Knapp SJ, Chen F, Jones B, Blake T, Franckowiak J, Rasmusson D, Sorrells M, Ullrich SE, Wesenberg D, Kleinhofs A (1993) Quantitative trait locus effects and environmental interaction in a sample of North American barley germplasm. Theor Appl Genet 87:392–401

Jadhav SJ, Lutz SE, Ghorpade VM, Salunkhe DK (1998) Barley: chemistry and value-added processing. Critical Rev Food Sci Nutri 38:123–171

Kiseleva VI, Tester RF, Wasserman LA, Krivandin AV, Popov AA, Yuryev VP (2003) Influence of growth temperature on the structure and thermodynamic parameters of barley starches. Carbohydr Polym 51:407–415

Lalic A, Kovacevic J, Simic G, Drezner G, Guberac V (2007) Environmental effects on grain yield and malting quality parameters of winter barley. Cereal Res Commun 35:709–712

Li HB, Vaillancourt R, Mendham NJ, Zhou MX (2008) Comparative mapping of quantitative trait loci associated with waterlogging tolerance in barley (*Hordeum vulgare* L.). BMC Genomics 9:401

MacGregor AW, Fincher GB (1993) Carbohydrates of the barley grain. In: MacGregor AW, Bhatty RS (eds) Barley: chemistry and technology. American Association of Cereal Chemists, St. Paul, p 73

Marquez-Cedillo LA, Hayes PM, Jones BL, Kleinhofs A, Legge WG, Rossnagel BG, Sato K, Ullrich E, Wesenberg DM, North American Barley Genome Mapping Project (2000) QTL analysis of malting quality in barley based on the doubled-haploid progeny of two elite North American varieties representing different germplasm groups. Theor Appl Genet 101:173–184

Mather DE, Tinker NA, LaBerge DE, Edney M, Jones BL, Rossnagel GG, Legge WG, Briggs KG, Irvine RB, Kasha KJ (1997) Regions of the genome that affects grain and malt quality in a North American two-row barley cross. Crop Sci 37:544–554

Narasimhalu P, Kong D, Choo TD, Ferguson T, Therrien MC, Ho KM, May KW, Jui P (1994) Effects of environment and cultivar on total mixed-linkage β-glucan content in eastern and western Canadian barleys (*Hordeum vulgare* L.). Can J Plant Sci 75:371–376

Oliveira AB, Rasmusson DC, Fulcher RG (1994) Genetic aspects of starch granule traits in barley. Crop Sci 34:1176–1180

Panozzo JF, Eckermann PJ, Mather DE, Moody DB, Black CK, Collins HM, Barr AR, Lim P, Cullis BR (2007) QTL analysis of malting quality traits in two barley populations. Aust J Agr Res 58:858–866

Stuart IM, Dunn CA, Allan GR (1997) The prediction of malt quality using Rapid Viscoanalyser. In: Proceedings of the sixth institute of brewing (Central and Southern African Section) convention, Durban, pp 170–174

Tang QY, Feng MG (2007) DPS Data processing system: experimental design, statistical analysis, and data mining. Science Press, Beijing

Tester RF (1997) Influence of growth conditions on barley starch properties. Int J Biol Macromol 21:37–45

Thomas WTB, Powell W, Swanston JS, Ellis RP, Chalmers KJ, Barua UM, Jack P, Lea V, Forster BP, Waugh R, Smith DB (1996) Quantitative trait loci for germination and malting quality characters in a spring barley cross. Crop Sci 36:265–273

van Ooijen JW (1992) Accuracy of mapping quantitative trait loci in autogamous species. Theor Appl Genet 84:803–811

van Ooijen JW, Kyazma BV (2004) MapQTL® 5, software for the mapping of quantitative trait loci in experimental populations. Wageningen, The Netherlands

Varshney RK, Marcel TC, Ramsay L, Russell J, Röder MS, Stein N, Waugh R, Langridge P, Nike RE, Graner A (2007) A high density barley microsatellite consensus map with 775 SSR loci. Theor Appl Genet 114:1091–1103

Wenzl P, Li HB, Carling J, Zhou MX, Raman H, Paul E, Hearnden P, Maier C, Xia L, Caig V, Ovesná J, Cakir M, Poulsen D, Wang JP, Raman R, Smith K, Muehlbauer G, Chalmers K, Kleinhofs A, Huttner E, Kilian A (2006) A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. BMC Genomics 7:206–228

Yang J, Hu CC, Ye XZ, Zhu J (2005) QTL network—2.0. Institute of Bioinformatics, Zhejiang University, Hangzhou

Zheng GH, Sosulski FW (1998) Determination of water separation from cooked starch and flour pastes after refrigeration and freeze-thaw. J Food Sci 63:134–139

Zhou MX, Mendham NJ (2005) Predicting barley malt extract with a Rapid Viscoanalyser. J Cereal Sci 41:31–36

Zhou MX, Robards K, Glennie-Holmes M, Helliwell S (1998) Structure and pasting properties of oat starch. Cereal Chem 75:273–281

Zhou MX, Robards K, Glennie-Holmes M, Helliwell S (1999) Effects of oat lipids on groat meal pasting properties. J Sci Food Agric 79:585–592

Zhou MX, Robards K, Glennie-Holmes M, Helliwell S (2000) Effects of enzyme treatment and processing on pasting and thermal properties of oats. J Sci Food Agric 80:1486–1494

Zhou MX, Li HB, Chen ZH, Mendham NJ (2008) Combining ability of barley flour pasting properties. J Cereal Sci 48:789–793