

Molecular detection of QTL for agronomic and quality traits in a doubled haploid barley population

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Abstract

In order to efficiently use new dwarf germplasm Huaai 11 for breeding program, it is important to identify the QTL of agronomic and quality traits. In the present study, QTL analysis was performed for ten agronomic and one quality traits using a DH population of a 122 lines derived from the cross of Huaai 11 × Huadamai 6. Composite interval mapping procedures detected a total of 17 QTL which were mapped onto five chromosomes. Six QTL, Qhd2-12, Qmsl2-15 and Qmsl7-7, Qgwp7-7, Qgws7-7 and Qtgw7-10 were detected in all years. Ten QTL were found on chromosome 7H. The QTL on chromosome 7H has effect on grain weight per plant, grain protein content, and main spike length, which accounted for 35.11%, 45.74% and 54.88% of phenotypic variation, respectively. Positive transgressive segregation was found for all traits. Some of QTL identified in this study could be targeted for an efficient transfer into new cultivars by applying MAS.

Keywords: agronomic traits; quality trait; QTL; MAS; *Hordeum vulgare*.

Abbreviations: QTL - quantitative trait locus; DH - double haploid; MAS - marker assisted selection; HD - Heading date; SP - spike numbers per plant; MSL - main spike length; SMS - spikelet number of main spike; SLP - spikelet number per plant; GP - grain number per plant; GS - grain number per spike; GWP - grain weight per plant; GWS - grain weight per spike; TGW - 1000 grain weight; NT - number of tillers; GPC - grain protein content.

Introduction

In barley breeding programs, yield and quality are the most important traits to be considered. Yield is determined by many agronomic traits. Most of these traits are controlled by QTL. The application of molecular markers linked with QTL offers a tool for the accelerated improvement of quantitative traits in breeding (Thomas et al., 1995). The construction of genetic maps of the barley genome made it possible to map the regions controlling the expression of quantitative traits (Graner et al., 1991; Heun et al., 1991; Kleinhofs et al., 1993). Linkage maps of different barley populations have been used for mapping QTL controlling agronomic traits (Teulat et al., 2001; Pillen et al., 2003; Li et al., 2005; Sameri et al., 2006; Schmalenbach et al., 2009; Wang et al., 2010). The use of molecular markers associated with these traits can greatly improve selection efficiency. Yield is one of the most important breeding objectives. Agronomic traits, such as heading date, spike number per plant, main spike length, spikelet number of main spike, spikelet number per plant, grain number per plant, grain number per spike, grain weight per plant, grain weight per spike, 1000 grain weight, and number of tillers, could directly or indirectly affect the yield. Among agronomic traits, heading date is one of the important quantitative traits. Heading date is determined by three main factors: vernalization response, photoperiodic response, and earliness *per se* genes in barley (Takahashi & Yasuda, 1971; Roberts et al., 1988; Gallagher et al., 1991). Vernalization response loci, *Vrn-H1*, *Vrn-H2* and *Vrn-H3*, are located on chromosome 5HL, 4HL and 7HS, respectively (Takahashi &

Yasuda, 1971; Laurie et al., 1995) and their candidate genes and functions have been reported (Dubcovsky et al., 2005; Fu et al., 2005; von Zitzewitz et al., 2005; Yan et al., 2006; Casao et al., 2010). Photoperiod response loci, *Ppd-H1* and *Ppd-H2*, are located on chromosome 2HS and 1HL, respectively (Laurie et al., 1994; Laurie et al., 1995; Karsai et al., 1997). Turner et al. (2005) cloned the candidate gene for the long-day responsive *Ppd-H1* locus, and Faure et al. (2007) studied the functions of *Ppd-H2* (Casao et al., 2010). The "earliness *per se*" genes including recessive alleles and their earlier synonyms: *eam7*, *eam8*, *eam9* and *eam10*, are located on chromosome 6HS, 1HL, 4HL, and 3HL, respectively (Takahashi & Yasuda, 1971; Gallagher et al., 1991), and the 'eps' loci identified through QTL analyses are located on chromosome 2H, 3H, 4H, 5H, 6H and 7H (Laurie et al., 1995). Heading date is controlled by a large number of QTL (Hayes et al., 1993; Marquez-Cedillo et al., 2001; Pillen et al., 2003; Sameri & Komatsuda, 2004; von Korff et al., 2006; Wang et al., 2010). QTL underlying many important agronomic traits have been identified and mapped. For example, QTL for spike number per plant were mapped on chromosome 3HL and 5HL (Sameri et al., 2006); for spike length on chromosome 1H, 2H, 3H, 4H, 5H and 7H (Hori et al., 2003; Li et al., 2005; Sameri et al., 2006; Baghizadeh et al., 2007; Wang et al., 2010); for spikelet number per spike on chromosome 1H, 2H, 5H, 7H (Li et al., 2005; Baghizadeh et al., 2007); QTL for grain number per spike on chromosome 1H (Pillen et al., 2003), 2H (Li et al., 2005;

Table 1. Means, range and SD of traits investigated for the Huaai 1/Huadamai6 DH lines and parental lines.

Traits	Parental lines		DH lines			Heritability %
	Huaai 1	Huadamai6	Mean	SD	Range	
SP	8.63	11.71	8.58	1.52	5.21-13.58	90.85
MSL	4.93	10.00	6.59	1.70	4.05-10.51	87.77
SMS	56.75	34.04	55.38	19.16	20.83-85.75	93.58
HD	125.67	133.83	126.52	7.03	107.67-139.83	66.15
SLP	457.75	363.83	426.90	132.38	184.08-709.00	81.71
GP	296.00	309.17	277.72	73.66	131.00-430.29	58.31
GS	33.72	26.58	33.03	10.19	13.99-51.50	60.17
GWP	8.23	13.99	8.92	2.33	4.17-14.46	64.04
GWS	0.94	1.21	1.04	0.28	0.46-1.91	63.02
TGW	26.60	44.37	31.70	7.58	19.39-52.08	58.15
NT	10.79	13.79	11.12	1.47	7.13-15.46	79.53
GPC	14.90	12.95	13.89	0.81	12.65-15.94	76.03

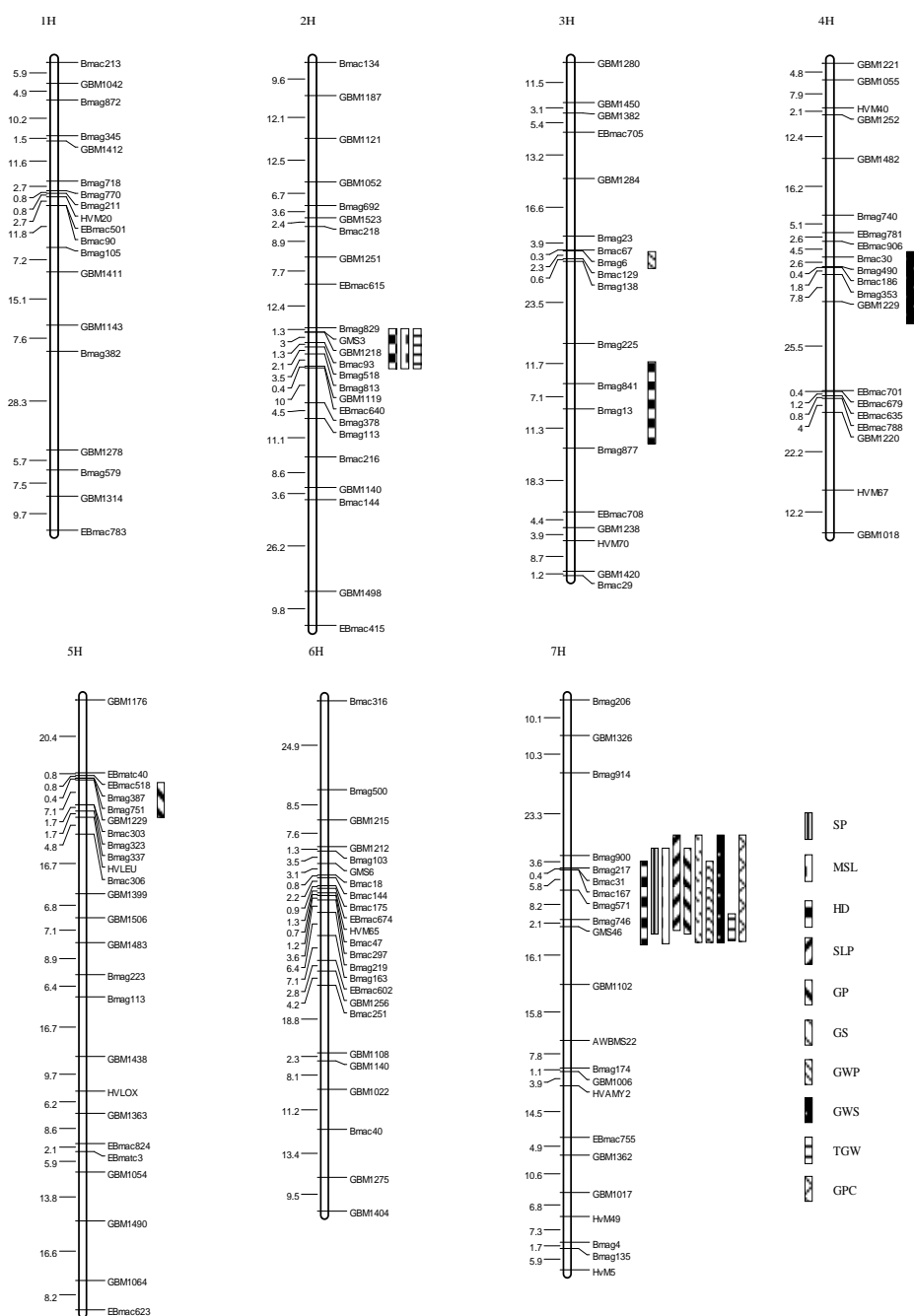


Fig 1. Map locations of 17 agronomical and quality traits QTL for Huaai 11 × Huadamai 6 DH population.

Baghizadeh et al., 2007; Wang et al., 2010), 3H and 4H (Teulat et al., 2001), and for number of tillers on chromosome 3H, 4H, 6H (Teulat et al., 2001). QTL conferring grain weight per plant (yield) and 1000 grain weight were detected on all seven chromosomes (Backes et al., 1995; Teulat et al., 2001; Hori et al., 2003; Pillen et al., 2003; Li et al., 2005; von Korff et al., 2006; Baghizadeh et al., 2007; Wang et al., 2010). Three traits, spikelet number per plant, grain number per plant and grain weight per spike are important agronomic traits, but the QTL controlling these traits had so far not been reported. Protein content is one of the most important quality breeding objectives. QTL for grain protein content were identified on 1H, 2H, 4H, 5H and 7H (Marquez-Cedillo et al., 2000; See et al., 2002; Emebiri et al., 2003; Pillen et al., 2003; Emebiri et al., 2005; Li et al., 2005). Huaai 11 is a new source of dwarf discovered in our research group for broadening the genetic base of dwarfism. This dwarf trait was controlled by a recessive dwarfing gene *btwd1*, and mapped onto the long arm of chromosome 7H (Ren et al., 2010). Many QTL were detected for some above-mentioned agronomic traits using conventional barley linkage mapping based on experimental populations derived from a bi-parental cross. However, the effects of these QTL often turn out to be unique to a specific genetic background, which has limited their application in breeding programs involving Huaai 11. In order to efficiently use this new germplasm for barley breeding program, it is important to characterize the QTL controlling agronomic traits in this line. The objective of the study was to detect QTL for agronomic and quality traits in the DH population derived from Huaai 11 × Huadamai 6. This information could be useful for developing new varieties for plant breeders using MAS.

Results

Phenotypic variation

Mean values of traits for the parent Huaai 11, Huadamai 6, and the DH lines are shown in Table 1. Large differences between the two parents were observed for all agronomic traits and one quality trait. Huadamai 6 showed higher values than Huaai 11 for Heading date (HD), spike number per plant (SP), main spike length (MSL), grain number per plant (GP), grain weight per plant (GWP), grain weight per spike (GWS), 1000 grain weight (TGW) and number of tillers (NT) over all years. Huaai 11 had higher spikelet number of main spike (SMS), spikelet number per plant (SLP), grain number per spike (GS) and grain protein content (GPC) than Huadamai 6. DH lines showed significant differences for all traits measured in this experiment (Table 1). The wide range of variation of the investigated traits (Table 1) and the normal distributions of phenotype (data not shown) indicated transgressive segregations, suggesting polygenic inheritance of the traits. Heritability estimates ranged from 58.15%-93.58% (Table 1), indicating that it was possible to detect QTL for these traits by using a suitable linkage map. Analysis of variance of all traits also showed significant effects of year and genotype except spikelet number of main spike, no significant difference effects of interaction between genotype and year in addition to grain weight per plant (GWP), grain weight per spike (GWS) and grain protein content (GPC) (Table 2). As expected, many of the traits correlated with each other (Table 3). There were significant positive correlation between the most of agronomic traits, but the quality trait grain protein content and other agronomic traits were significant negatively correlated.

Identification of QTL associated with different traits

The 536 microsatellite markers were evaluated for polymorphism between the two parents Huaai 11 and Huadamai 6. A total of 166 microsatellite markers were polymorphic. Genotype data for 7 microsatellite markers were incomplete, and not included in the analysis. Linkage analysis found that 6 microsatellite loci unlinked, 153 microsatellite markers were mapped onto seven barley chromosomes (Fig 1). The genetic map spanned 1051.8 cM with an average marker distance of 6.9 cM. Of the eleven agronomic traits, genotype of spikelet number of main spike (SMS) was no significant difference, so this trait was excluded for QTL analysis. QTL analyses for all agronomic traits and quality trait. A total of 17 QTL were mapped onto five chromosomes based on data from all years for nine agronomic traits and one quality trait. The LOD value for each QTL was given in Table 4. The QTL were mapped on chromosome 2H, 3H, 4H, 5H and 7H. Among them, ten QTL were found on the chromosome 7H and six QTL were detected in all years (Table 4; Fig 1).

Heading date

Three QTL, distributed on three chromosomes, were significantly associated with heading date. Qhd2-12 on chromosome 2H was detected in all three years and accounted for 10.38% to 22.93% of the phenotypic variation with the effect on shortening heading date. The Qhd3-13 on chromosome 3H was detected in both years 2009 and 2010, and accounted for 8.35% to 21.29% of the phenotypic variation with the effect on delaying heading date. The Qhd7-5 on chromosome 7H was detected in 2009 and accounted for 11.58% of the phenotypic variation with the effect on shortening heading date (Table 4; Fig 1).

Main spike length

Two significant QTL have effect on main spike length in all three years. The QTL, Qms12-15 on chromosomes 2H accounted for 5.22% to 8.20% of the phenotypic variation with the effect on decreasing main spike length. The QTL, Qms17-7 showed major effect in controlling this trait, and accounted for 47.08% to 54.88% of the phenotypic variation with the effect on decreasing main spike length (Table 4; Fig 1).

Spike number per plant and spikelet numbers per plant

One QTL, Qsp7-7 on chromosome 7H associated with spike number per plant, was detected in 2009 and accounted for 15.36% of the phenotypic variation with the effect on decreasing spike number per plant. No significant QTL was identified in 2008 and 2010 years for spike number per plant (Table 4; Fig 1). One QTL, Qslp7-7 significantly influenced spikelet number per plant were detected in 2009. This QTL was mapped onto chromosomes 7H and explained for 9.30% of the phenotypic variance with the effect on decreasing spikelet number per plant. No significant QTL was identified in 2010 for spikelet number per plant (Table 4; Fig 1). In general, an increase in numbers of tillers can result in increased spike number per plant. Spike number per plant and number of tillers shows a significant positive correlation (Table 3), QTL was only detected for spike number per plant, but not QTL was detected for numbers of tillers.

Table 2. Analysis of variance on agronomic and quality traits in DH lines from Huaai 11 × Huadamai 6.

Source of variation	SP	MSL	SMS	HD	SLP	GP	GS	GWP	GWS	TGW	NT	GPC
Year	28.18**	49.07**	7.99**	143.13**	47.37**	349.37**	223.40**	1111.19**	637.85**	95.13**	75.62**	187.50**
Genotype	9.60**	690.43**	2.59	9.18**	16.36**	34.97**	8.41**	215.43**	84.99**	46.83**	5.13*	156.40**
Year× Genotype	3.74	0.729	0.26	1.829	2.09	1.41	0.056	14.92**	11.67**	3.47	0.368	8.19**

* Significant at the 5% level, ** Significant at the 1% level.

Table 3. Correlation coefficients between agronomic traits in DH lines from Huaai 11 × Huadamai 6.

	MSL	SMS	HD	SLP	GP	GS	GWP	GWS	TGW	NT	GPC
SP	0.346**	-0.455**	0.347**	0.009	0.137*	-0.215**	0.328**	-0.12	0.522**	0.724**	-0.169**
MSL		0.028	0.440**	0.234**	0.417**	0.296**	0.590**	0.491**	0.529**	0.352**	-0.545**
SMS			0.030	0.867**	0.624**	0.759**	0.295**	0.490**	-0.470**	-0.170**	-0.408**
HD				0.268**	0.546**	0.429**	0.621**	0.523**	0.435**	0.376**	-0.358**
SLP					0.816**	0.767**	0.541**	0.574**	-0.242**	0.193**	-0.552**
GP						0.925**	0.865**	0.869**	0.139*	0.368**	-0.668**
GS							0.739**	0.884**	-0.014	0.095	-0.613**
GWP								0.925**	0.592**	0.496**	-0.627**
GWS									0.422**	0.238**	-0.633**
TGW										0.418**	-0.231**
NT											-0.182**

* Significant at the 5% level, ** Significant at the 1% level

Table 4. QTL, their locations and effects for agronomic and quality traits of the Huaai 11/Huadamaï6 DH lines.

Traits	Years	QTL	Marker	chromosome	LOD	Position	Range (cM)	Heritability (%)	Additive effect
HD	2008	Qhd2-12	GBM1218	2H	3.69	77.2	75.9-87.1	10.38	-2.35
		Qhd2-12	GBM1218	2H	9.04	77.9	75.9-87.1	22.93	-3.73
	2009	Qhd3-13	Bmag13	3H	3.77	97.6	89.4-113.4	8.35	2.29
		Qhd7-5	Bmag217	7H	4.85	46.1	45.3-68.6	11.58	-2.74
		Qhd2-12	GBM1218	2H	7.55	78.2	75.9-87.1	18.61	-3.40
		Qhd3-13	Bmag13	3H	7.72	99.6	85.4-115.6	21.29	3.70
2010	Qhd2-12	GBM1218	2H	7.55	78.2	75.9-87.1	18.61	-3.40	
	Qhd3-13	Bmag13	3H	7.72	99.6	85.4-115.6	21.29	3.70	
SP	2009	Qsp7-7	Bmac167	7H	5.66	47.7	38.4-64.6	15.36	-0.70
MSL	2008	Qmsl2-15	Bmag813	2H	6.47	83.5	77.2-85.6	7.61	-0.50
		Qmsl7-7	Bmac167	7H	26.55	47.7	47.3-49.5	47.08	-1.31
	2009	Qmsl2-15	Bmag813	2H	8.76	83.5	75.9-87.1	8.20	-0.48
		Qmsl7-7	Bmac167	7H	31.76	47.7	38.4-68.6	48.66	-1.22
	2010	Qmsl2-15	Bmag813	2H	5.10	83.5	75.9-87.1	5.22	-0.43
		Qmsl7-7	Bmac167	7H	31.05	47.7	40.4-65.6	54.88	-1.45
SLP	2009	Qslp7-7	Bmac167	7H	3.08	47.7	36.4-64.6	9.30	-42.80
GP	2009	Qgp5-8	Bmag323	5H	3.50	30.2	22.4-31.9	8.07	-21.80
		Qgp7-7	Bmac167	7H	9.85	47.7	38.4-64.6	26.00	-36.96
GS	2009	Qgs7-7	Bmac167	7H	4.38	47.7	36.4-68.6	12.25	-2.71
GWP	2009	Qgwp3-9	Bmac129	3H	3.73	56.3	54.0-56.9	6.20	0.50
		Qgwp7-7	Bmac167	7H	15.80	47.7	41.7-68.6	33.51	-1.16
	2010	Qgwp7-7	Bmac167	7H	15.76	48.5	42.6-64.6	35.11	-2.00
GWS	2009	Qgws4-12	Bmag353	4H	3.80	63.4	53.6-73.2	10.40	0.06
		Qgws7-7	Bmac167	7H	13.03	47.7	38.4-68.6	14.79	-0.08
	2010	Qgws7-7	Bmac167	7H	8.76	48.5	36.4-66.4	23.50	-0.22
TGW	2009	Qtgw7-10	GMS46	7H	3.25	64.6	63.8-68.6	11.75	-2.21
		Qtgw2-14	Bmag518	2H	3.64	81.5	75.9-85.6	9.05	-2.81
	2010	Qtgw7-10	GMS46	7H	4.91	64.6	63.8-68.6	16.39	-3.74
GPC	2009	Qgpc7-7	Bmac167	7H	22.32	47.7	36.4-68.6	45.74	0.78
	2010	Qgpc7-7	Bmac167	7H	7.17	49.5	38.4-64.6	21.69	0.40

Grain number per plant and grain number per spike

Two QTL were detected in 2009 for grain number per plant, Qgp5-8 and Qgp7-7. These two QTL explained for 8.07% and 26.00% of the phenotypic variance, respectively. The Qgp5-8, located on chromosome 5H, and Qgp7-7, located on chromosome 7H, both have the effect on decreasing grain number per plant (Table 4; Fig 1). One QTL, Qgs7-7, was identified for grain number per spike, and located on chromosome 7H in 2009. This QTL showed effect on decreasing grain number per spike and explained for 12.25% of the phenotypic variance. No significant QTL for grain number per plant and grain number per spike were identified in 2010 (Table 4; Fig 1).

Grain weight per plant and grain weight per spike

Two QTL were detected for grain weight per plant. The Qgwp7-7, located on chromosome 7H, was detected in two years, and had the largest effect on decreasing grain weight per plant, and explained 33.51% and 35.11% of the phenotypic variance in 2009 and 2010, respectively. The Qgwp3-9 was located on chromosome 3H with an effect on increasing grain weight per plant. It was only detected in 2009 and explained 6.20% of the phenotypic variance (Table 4; Fig 1). Two QTL were identified for grain weight per spike. The Qgws7-7, located on chromosome 7H, was detected in two years, and had the effect on decreasing grain weight per spike, and explained 14.79% and 23.50% of the phenotypic variance in 2009 and 2010, respectively. The Qgws4-12 on chromosome 4H was only detected in 2009, and accounted for 10.40% of the phenotypic variation with the effect on increasing grain weight per spike (Table 4; Fig 1).

1000 grain weight

Two QTL were identified for 1000 grain weight. The Qtgw7-10, located on chromosome 7H, was detected in two years, and had the effect on decreasing 1000 grain weight, and explained 11.75% and 16.39% of the phenotypic variance in 2009 and 2010, respectively. The Qtgw2-14 on chromosome 2H was only detected in 2010, and explained 9.05% of the phenotypic variance with effect on decreasing 1000 grain weight (Table 4; Fig 1).

Grain protein content

One QTL, Qgpc7-7 on chromosome 7H, was identified for grain protein content in two years, with effect on increasing grain protein content, and explained 45.74% and 21.69% of the phenotypic variance in 2009 and 2010, respectively (Table 4; Fig 1).

Discussion

Many agronomic and quality traits are controlled by quantitative trait loci (QTL). QTL analysis is a useful approach to discover and dissect complex traits and to identify favorable alleles in diverse germplasm (Paterson et al., 1988). In the present study, we detected 17 QTL for ten agronomic traits and one quality trait using a DH population derived from a cross between a dwarfing barley cultivar Huaai 11 and a common feed barley cultivar Huadamaï 6 in combination with composite interval mapping (CIM).

QTL for agronomic traits

Three QTL were significantly associated with heading date. The Qhd2-12 was detected in all three years and the Qhd3-13 was detected in two years, another QTL, Qhd7-5 was only detected in one year with minor effect. QTL for heading date were found on chromosome 1H, 2H, 3H, 4H, 5H 6H and 7H (Backes et al., 1995; Laurie et al., 1995; Marquez-Cedillo et al., 2001; Teulat et al., 2001; Pillen et al., 2003; Li et al., 2005; von Korff et al., 2006; Wang et al., 2010). Qhd2-12 was located on the centromeric region of chromosome 2H, which is coincident with the *earliness per se* locus *Eam6* (or *eps2S*) (Laurie et al., 1995; Franckowiak & Konishi, 2002; Horsley et al., 2006). Teulat et al. (2001) and von Korff et al. (2006) reported that QTL for heading date on chromosome 3H was linked with the marker *Bmag13* and *HVM62*, since the *Bmag13* was located between the interval of *Bmag225-Ebmac708*, suggesting that Qhd3-13 is the same as to the previously reported QTL on chromosome 3H for heading date. QTL conferring heading date were reported on 7HS (Backes et al., 1995; Li et al., 2005; von Korff et al., 2006) and 7HL (Teulat et al., 2001; Pillen et al., 2003; von Korff et al., 2006). Qhd7-5 is close to the centromere of chromosome 7H, and different from those QTL on chromosome 7H reported previously. The Qhd7-5 is a newly identified QTL for heading date. One QTL Qsp7-7 for spike numbers per plant and one QTL Qslp7-7 for spikelet number per plant were identified in 2009. No significant QTL was identified in year 2008 and 2010 for spike number per plant and spikelet number per plant. It may be due to two traits influenced by environment. QTL for spike number per plant were on 3H, 4H, 5H and 6H (Teulat et al., 2001; Sameri et al., 2006). The Qsp7-7 on chromosomes 7H for spike numbers per plant is a newly identified QTL in this study. QTL for spikelet number per spike were reported on 2H, 5H and 7H (Li et al., 2005), but the QTL for controlling spikelet number per plant are rarely reported in barley. Two QTL for main spike length, Qmsl2-15 and Qmsl7-7, were detected in all three years, indicating that these two QTL are not affected by the environments. QTL conferring spike length was reported on chromosomes 1H, 2H, 3H, 4H, 5H and 7H (Hori et al., 2003; Li et al., 2005; Sameri et al., 2006; Baghizadeh et al., 2007; Wang et al., 2010). Examining the 2H and 7H linkage maps of Varshney et al. (2007) and GrainGenes2.0 (<http://wheat.pw.gov>) revealed that Qmsl2-15 is close to the centromere of the 2HL. It is different from those QTL on chromosomes 2H reported previously (Hori et al., 2003; Li et al., 2005; Sameri et al., 2006; Wang et al., 2010). Qmsl7-7 is likely the same as to Qel7.1 on chromosome 7H reported previously (Li et al., 2005), and different from the QTL on chromosomes 7H reported by Sameri et al. (2006). Two QTL Qgp5-8 and Qgp7-7 for grain number per plant and one QTL Qgs7-7 for grain number per spike, was identified. QTL for grain number per spike are reported to be located on chromosomes 1H (Pillen et al., 2003), 2H (Li et al., 2005; Baghizadeh et al., 2007; Wang et al., 2010), 3H and 4H (Teulat et al., 2001). Schmalenbach et al. (2009) detected eight QTL for grains per ear on chromosomes 1H, 2H, 3H, 4H and 7H in wild barley introgression lines. In our study, we detected Qgs7-7 that is different from the QTL on chromosomes 7H reported by Schmalenbach et al. (2009). QTL for controlling grain number per plant are rarely reported in barley. The Qgp5-8 and Qgp7-7 are both new QTL for grain number per plant, and the Qgs7-7 is a new QTL for grain number per spike. Two QTL on chromosome 3H and 7H were detected for grain weight per plant. QTL conferring grain yield was reported to be located on all seven

chromosomes (Marquez-Cedillo et al., 2001; Teulat et al., 2001; Pillen et al., 2003; Li et al., 2005; von Korff et al., 2006; Schmalenbach et al., 2009). Examining the 3H linkage maps of Varshney et al. (2007) suggested that Qgwp3-9 is close to the centromere of the 3H, and different from those previously reported QTL on chromosomes 3HL (Marquez-Cedillo et al., 2001; Pillen et al., 2003; Li et al., 2005; von Korff et al., 2006) and on chromosomes 3HS (von Korff et al., 2006). The Qgwp7-7 is close to the centromere of the 7H, and different from those reported QTL on 7HL (Teulat et al., 2001; Pillen et al., 2003; von Korff et al., 2006; Schmalenbach et al., 2009) and 7HS (von Korff et al., 2006; Schmalenbach et al., 2009). Two QTL, Qgws4-12 and Qgws7-7 for grain weight per spike were identified. The Qgws7-7 was identified in both two years, indicating this QTL was less affected by the environments. QTL for controlling grain weight per spike are rarely reported in barley. Two QTL, Qtgw2-14 and Qtgw7-10 were identified for 1000 grain weight. The Qtgw7-10 located on chromosomes 7H was detected in two years, suggesting that this QTL is also less affected by environment. The Qtgw2-14 on chromosome 2H was only identified in one year. QTL conferring 1000 grain weight was reported to be located on all seven chromosomes (Kicherer et al., 2000; Teulat et al., 2001; Hori et al., 2003; Pillen et al., 2003; Li et al., 2005; von Korff et al., 2006; Schmalenbach et al., 2009; Wang et al., 2010). Pillen et al. (2003) reported GMS3, Bmag353, Bmac186, GBM1102 and Bmag120 associated with QTL for 1000 grain weight. The Qtgw2-14 is on a similar location with a QTL on chromosome 2H reported by Pillen et al. (2003) and Schmalenbach et al. (2009). The Qtgw7-10 is close to the QTL on chromosome 7H reported by Pillen et al. (2003) and von Korff et al. (2006).

QTL for quality traits

In barley, high grain protein percentage might be desirable in feed barley cultivar, but low grain protein concentration is desirable for malt and beer production. One QTL was identified for grain protein content on chromosome 7H. QTL conferring grain protein content was reported on chromosomes 1H, 2H, 4H, 5H and 7H (Marquez-Cedillo et al., 2000; See et al., 2002; Emebiri et al., 2003; Pillen et al., 2003; Emebiri et al., 2005; Li et al., 2005). The Qgpc7-7 was on a similar position to a QTL identified for grain protein content by Li et al. (2005) and Emebiri et al. (2005), and different from those reported by Marquez-Cedillo et al. (2000). The protein content is an important indicator of quality traits in breeding. Pillen et al. (2003) reported grain protein content with negative correlations with yield, thousand-grain weight, ears per square meter, harvest index and kernels per ear. In our study, grain protein content showed a significant negative correlation with all agronomic traits (Table 3). Therefore, it is equally important to select good agronomic traits and quality traits in barley breeding. The plant architecture of newly discovered dwarfing germplasm Huaai 11 consisted of desirable agronomic traits such as shortened stature and early maturity. In this study, QTL were mapped in a DH population derived from a cross between Huaai 11 × Huadamai 6. Twelve traits were measured in DH population. A total of 17 QTL were identified and 11 of them were first reported in this study. Phenotypic variance explained by these QTL varied from 5.22% to 54.88%. The identified QTL may be useful tools for marker-assisted selection in barley breeding programs and further genetic analyses.

Materials and methods

Plant materials and field experiments

The genetic material used in this study was a population of 122 DH lines derived from a cross between dwarfing barley cultivar Huaai 11 and a common feed barley cultivar Huadamai 6 using anther culture. The DH population lines and two parents were planted on the Experimental Farm of Huazhong Agricultural University, Wuhan, China. The field trials were conducted using a randomized complete block design with three replications in 2007-2008, 2008-2009 and 2009-2010, respectively. Each of the DH and parental lines were grown in three rows in a plot of 0.6×1.5 m². The length of line is 1.5 m, spacing of the lines is 0.2 m and spacing of the plants is 0.1 m. In 2007-2008, agronomic traits (HD, SP, MSL, SMS) and quality traits (GPC) were measured. In 2008-2009 and 2009-2010, agronomic traits (HD, SP, MSL, SMS, SLP, GP, GS, GWP, GWS, TGW, NT) and quality traits (GPC) were measured. Heading date (HD) was recorded as “the number of days from sowing to 50% of the heads had emerged from the flag leaf sheath in a plot”; spike number per plant (SP) was measured as “over five grains of the spike in one plant”; main spike length (MSL) was measured as “the length from the base of main spike to the tip of main spike (excluding awns)”; spikelet number of main spike (SMS) was measured as “all spikelet number of main spike”; spikelet number per plant (SLP) was measured as “all spikelet number of the spike of one plant”; grain number per plant (GP) was measured as “the number of all grains in one plant”; grain number per spike (GS) was measured as “the average grain number of each spike”; grain weight per plant (GWP) was measured as “the weight of all grains in one plant”; grain weight per spike (GWS) was measured as “the average grain weight of each spike”, 1000 grain weight (TGW) was measured as “grain weight per plant divided by grain number per plant multiplied by 1000”; number of tillers (NT) was measured as “number of tillers of the whole plant”; and grain protein content (GPC) was measured using near-infrared grain analyzer. The mean of the twelve plants (in three replicates, four plants from each replicate were measured) was calculated for each traits.

SSR genotyping and statistical analysis

The leaves from each DH lines and parents were collected and frozen for DNA extraction according to Stein et al. (2001). Five hundred and thirty-six SSR markers that have been published in Ramsay et al. (2000) and Varshney et al. (2007) distributed on all seven barley chromosomes were used to screen polymorphism between the two parental lines. A set of 153 polymorphic SSR markers were used to genotype the 122 individuals from the DH population. Linkage map was constructed using the software MAPMAKER 3.0 (Lander et al., 1987). The genetic distance (centimorgan, cM) was derived using Kosambi function (Liu & Meng, 2003). The most likely location of QTL and their genetic effects were initially detected by composite interval mapping using QTL Cartographer version 2.5 (Wang et al., 2007). A series of 1,000 permutations were run to determine the experimental wise significance level at $P = 0.05$ of logarithm of the odds ratio ($\text{LOD} \geq 3$) for the trait (Churchill & Doerge, 1994). Composite interval mapping (CIM) was employed to identify QTL and estimate their effects using Model 6 of the Zmapqtl program module. Cofactors were chosen using the forward-backward method of stepwise regression. The genome was scanned at 1-cM intervals and

the window size was set at 10 cM. The percentage of phenotypic variance explained by a specific QTL value was taken as the peak QTL position as determined by QTL Cartographer 2.5. For the measurements and comparisons of variability among the agronomical and quality traits, we calculated the standard deviation (SD). Analysis of variance (ANOVA) was carried out with SAS software.

Conclusion

QTL were mapped in a DH population derived from a cross between Huaai 11 \times Huadamai 6. Twelve traits were measured in DH population in three years. A total of 17 significant QTL were identified for 10 traits and 11 of them were first reported in this study. Some of QTL identified may be useful tools for MAS in barley breeding programs and further genetic analyses.

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