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ORIGINAL PAPER

Mapping a major QTL for malt extract of barley from a cross between TX9425 × Naso Nijo

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Abstract

Key message **One major QTL-controlling malt extract was identified on 2H, based on the data from four different environments and a large number of DH lines, determining 48 % of phenotypic variation. This QTL is of a high value for marker-assisted selection.**

Abstract Improving malting quality traits is one of the major breeding objectives for barley breeding programmes. Among different quality traits, malt extract is one of the most important, determining the yield of beer production. The use of molecular markers linked to loci affecting the quality traits can greatly improve selection efficiency. However, the discovery of closely linked markers relies on not only the availability of the loci, but the accuracy of phenotyping. In this experiment, 188 doubled-haploid lines derived from the cross between a Japanese malting barley and a Chinese feed barley were grown in four different environments (two sites × 2 years). Different quality traits were determined and used to map QTL for these traits. Several QTLs were identified for different quality traits. One major QTL-controlling malt extract was identified on 2H and determined 48 % of phenotypic variation with the closest marker of GBM1121. This QTL was consistently expressed in all four environments and is of a high value for marker-assisted selection in malting barley breeding.

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Introduction

The malting quality of barley is an important character in the economics of producing barley for brewing. It represents the comprehensive effects of a number of interacting component traits, which include malt extract, Kolbach index, diastatic power, malt total nitrogen content, malt soluble nitrogen content, free amino nitrogen and malt viscosity (Burger and LaBerge 1985). Most malting quality traits are quantitatively inherited (Peterson and Foster 1973) and generally of low heritability (Sparrow 1970), with many genes involved (Fox et al. 2003). The heritability of malt extract estimated in the F_2 and F_3 with different methods and populations ranged from 8 to 70 % (Foster et al. 1967; Rasmusson and Glass 1965). Relatively higher heritability (50–86 %) was reported for diastatic power (Hockett and Nilan 1985). The environment and the genotype × environment interaction also showed significant effects on malt quality (Hayter and Riggs 1973; Pomeranz et al. 1976; Carreck and Christian 1991; Coles et al. 1991), adding to the difficulties in making effective selection in early generations.

Quantitative traits locus (QTL) analysis provides a powerful tool for dissecting complex traits and for identifying chromosome regions and molecular markers linked to these traits. The use of molecular markers associated with these traits can not only improve selection efficiency by avoiding environmental effects but also make it possible to select these traits at an earlier stage of the breeding programme. Many QTL for malting qualities have been identified in barley germplasm from Australia (Barr et al. 2003; Pallotta et al. 2003; Coventry et al. 2003; Zhang et al. 2012), North America (Hayes et al. 1993; Oziel et al. 1996; Bezant et al. 1997; Mather et al. 1997; Marquez-Cedillo et al. 2000; Elía et al. 2010; Zhou et al. 2012b), and Europe (Chalmers et al. 1993; Thomas et al. 1996; Powell et al. 1997; Elía et al. 2010). QTL-controlling malt quality traits were identified in almost all chromosomes. For example, QTL have been mapped to all seven chromosomes for malt extract (Hayes et al. 1993, 1996; Oziel et al. 1996; Thomas et al. 1996; Bezant et al. 1997; Mather et al. 1997; Marquez-Cedillo et al. 2000; Hoffman and Dahleen 2002; Barr et al. 2003; Cakir et al. 2003; Pallotta et al. 2003; Coventry et al. 2003; Emebiri et al. 2004; Han et al. 2004; Gao et al. 2004; Li et al. 2005; Von Korff et al. 2008; Laidò et al. 2009; Schmalenbach and Pillen 2009; Elía et al. 2010; Panozzo et al. 2007; Zhou et al. 2012b), diastatic power (Hayes et al. 1993, 1996; Thomas et al. 1996; Oziel et al. 1996; Mather et al. 1997; Powell et al. 1997; Marquez-Cedillo et al. 2000; Hoffman and Dahleen 2002; Barr et al. 2003; Cakir et al. 2003; Coventry et al. 2003; Pallotta et al. 2003; Emebiri et al. 2004; Han et al. 2004; Gao et al. 2004; Panozzo et al. 2007; Elía et al. 2010; Zhou et al. 2012b), wort viscosity (Mather et al. 1997; Emebiri et al. 2004; Panozzo et al. 2007; Von Korff et al. 2008; Laidò et al. 2009; Schmalenbach and Pillen 2009; Zhou et al. 2012b) and Kolbach index (Oziel et al. 1996; Marquez-Cedillo et al. 2000; Hoffman and Dahleen 2002; Schmalenbach and Pillen 2009; Elía et al. 2010; Zhou et al. 2012b).

Accurate phenotyping is the major barrier to locate QTL-controlling quantitative traits (Zhou 2011). The evaluation of malting quality is often affected by many environmental factors, which include soil properties, temperature, water and fertiliser supply. These factors cause great variability and low heritability, which may lead to different QTL for quality traits, especially those determining a relatively lower percentage of phenotypic variation. In previous studies, QTL for malt quality was detected in different genomic regions due to the effect of the different genetic backgrounds of crosses used, and/or genotype × environment interactions of the QTL. For examples, QTL on 2H for malt extract with Morex contributing the higher extract was identified in different positions from different populations (Oziel et al. 1996; Elía et al. 2010). Even using a same population (Blenheim × E224/3), different QTL for malt extract on 2H was identified in two different reports with not only a different number of QTL being identified but also different positions where the QTL were located (Thomas et al. 1996; Powell et al. 1997). Therefore, the identification of QTL for quality traits should be based on the results from multi-environments.

A Chinese land race, TX9425, has been used as a donor for favourable alleles for various traits, e.g., waterlogging tolerance (Pang et al. 2004; Zhou et al. 2007; Xu et al. 2012); salinity tolerance (Xu et al. 2012; Zhou et al. 2012a), disease resistance (Li et al. 2009; Li and Zhou 2011) and dwarf genes (Wang et al. 2010). However, this variety also showed some unfavourable traits, for example, thicker husk, short spike length and high grain density (Chen et al. 2012), which may lead to low malting quality. It is important to discover the relationships between the favourable and unfavourable traits to provide a useful strategy to take advantage of the favourable traits from this landrace variety. For example, when using this germplasm to improve stress tolerance of a malting variety, selections should be made against the unfavourable malting quality traits from this landrace. In this study, 188 doubled-haploid (DH) lines derived from the cross between a Japanese malting barley and a Chinese feed barley were grown in four different environments (two sites × 2 years). Most of the major quality traits were tested and a high-density genetic map (551 DArT and 75 SSR markers) was used to map these traits.

Materials and methods

Plant materials and field experiments

A total of 188 DH lines were produced from the F_1 of the barley cross between TX9425 and Naso Nijo by the anther culture method. TX9425 is a Chinese two-rowed feed variety, which showed shorter plant height and good tolerance to various stresses (Wang et al. 2010, 2014). In contrast, Naso Nijo is a Japanese two-rowed malting barley with good agronomic traits but less tolerant to various stresses. Both parental varieties and all DH lines were provided by the Tasmanian Institute of Agriculture, The University of Tasmania. All the DH lines and parents were grown in Hangzhou (HZ, 30.25°N, 120.17°E), Zhejiang Province and Yancheng, Jiangsu Province (YC, 33.38°N, 120.12°E) in two successive growing seasons, 2006–2007 (06) and 2007–2008 (07). HZ had slightly high temperatures and much higher rainfall than YC during the grain filling period. One hundred and fifty vigorous seeds of each line or variety were sown in 2-m-long rows with a 0.25-m row spacing. All experiments were arranged in a randomized complete block design with three replications. The soil type

was silt-loam with medium fertility. All plots were supplied with only 150 kg/ha N, 600 kg/ha $Ca(H_2PO4)_2 \cdot H_2O$ and 150 kg/ha KCl as base fertilizer before sowing. No further fertilizer was applied to the plots. Fungicides were not required as no severe diseases were observed. Hand weeding was undertaken when needed. On maturity, grains of each line or variety were harvested for further analysis.

Micro-malting and malting quality analysis

The barley grains were screened through a 2.2 mm sieve, with the grains retained on the sieve being used for micro-malting. Grain samples (200 g of each line) were micro-malted in an automatic micro-malting system (Joe White Micro-malting Systems, Australia) using the following protocol: 5 h steep, 8 h air-rest, 4 h steep, 9 h air-rest, 3 h steep and 1 h air-rest, all at 15 °C. Germination lasted 4 days at 15 °C, and the kilning scheme was: 2 h at 50 °C, 4 h at 55 °C, 6 h at 60 °C, 4 h at 65 °C, 1 h at 70 °C, 1 h at 75 °C, 1 h at 80 °C, and 2 h at 82 °C. The malt quality traits, malt extract (ME), malt total nitrogen content (TN), malt soluble nitrogen content (SN), Kolbach index (KI), free amino nitrogen (AN), viscosity (VI) and diastatic power (DP), were determined according to the official methods from the European Brewery Convention (1998).

Statistical analysis

A genetic linkage map produced from the TX9425/Naso Nijo DH population using 551 DArT and 75 microsatellite (SSR) markers (Xu et al. 2012; Wang et al. 2014) was used for QTL analysis. The QTL analyses were based on the mean of the three replications from each location and year. The software package MapQTL6.0 (Van Ooijen and Kyazma 2009) was used to detect QTL which were first analysed by interval mapping (IM). The closest marker at each putative QTL identified using IM was selected as a cofactor, and the selected markers were used as genetic background controls in the approximate multiple QTL model (MQM). A logarithm of the odds (LODs) threshold values applied to declare the presence of a QTL were estimated by performing the genome wide permutation tests using at least 1000 permutations of the original data set for each trait, resulting in a 95 % LOD threshold around 3.0. The percentage of variance explained by each QTL (R^2) was obtained by using restricted MQM mapping. Graphical representation of linkage groups and QTL was carried out using MapChart 2.2 (Voorrips 2002).

Examination of ME QTL region for potential genes

In order to identify potential genes underlying ME QTL, we took the closest marker GBM1121 to localise it on the POPseq genetic map of Morex × Barke (Mascher et al. 2013). All barley gene models have been assigned on the map. Thus, the information on the map can be used to identify genes at a certain location. Barley population sequencing data were downloaded from ftp://ftpmips.helmholtz-muenchen.de/plants/barley/public_data/popseq_IPK/ (Mascher et al. 2013). The GBM1121 marker primer sequences (ACCACCCCCATCCATCAG/GACTGCACCT TGTAGCCGAT) were used to blast barley databases on http://webblast.ipk-gatersleben.de/barley/. A WGS Morex_contig_44922 was identified containing the marker sequence. The morex_contig_44922 was located at 14.38 cM on 2H. After the identification of the GBM1121 marker location, we searched a 10 cM region with 5 cM up and 5 cM down from 14.38 cM for ME candidate genes. The annotated genes between 9.38 and 19.38 cM were fetched (Supplemental Table S1) and examined for potential genes contributing to ME difference between barley lines.

Results

Malting quality for parents and DH lines

Mean values of different quality traits for the parents and the DH population in each environment are shown in Table 1. Naso Nijo showed higher values for ME, SN, AN, KI and DP in all environments, while TX9425 had higher VI. Transgressive segregation was found with some DH lines showing higher or lower values than both parents (Table 1). Effects of genotypes, locations and years were highly significant for all the traits (Table 2). For example, the two parents, TX9425 and Naso Nijo, and DH lines showed generally higher ME, lower DP and TN in HZ06 and HZ07 trials than in YC06 and YC07 trials. Interactions between genotypes, locations and years were also significant for most of the traits. Relatively low heritabilities were found for all quality traits with the broad-sense heritabilities (h_B^2) ranging from 0.09 for VI to 0.38 for AN (Table 1). As expected, many of malting quality traits correlated with each other (Table 3). ME showed significant positive correlation with AN, KI and DP, and significant negative correlation with TN and VI.

QTL analysis for quality traits

QTL for malt extract

Based on average values from all different environments, two significant QTL were found to be associated with ME (Fig. 1; Table 4). A major QTL, *QMe.NaTx-2H*, was identified on 2H with the closest marker of GBM1121. This QTL

Table 1 Mean, range and h_B^2 of malt quality parameters tested in different environments

Trait	Environment	TX	NN	DH		h_B^2
				Mean ± SD	Range	
ME (%)	HZ06	77.95	82.99	81.0 ± 1.44	75.2–84.2	0.38
	HZ07	76.95	81.11	79.81 ± 1.24	75.9–82.29	
	YC06	76.22	79.57	79.6 ± 1.73	74.16–84.19	
	YC07	76.93	79.37	78.79 ± 1.28	75.4–81.98	
TN (%)	HZ06	1.85	1.67	1.81 ± 0.14	1.55–2.15	0.12
	HZ07	1.89	1.82	1.88 ± 0.1	1.55–2.15	
	YC06	2.16	2.0	1.89 ± 0.19	1.42–2.38	
	YC07	2.14	2.17	2.21 ± 0.13	1.85–2.53	
SN (%)	HZ06	0.73	0.82	0.84 ± 0.08	0.64–1.15	0.34
	HZ07	0.63	0.71	0.73 ± 0.06	0.58–0.92	
	YC06	0.69	0.82	0.75 ± 0.07	0.57–1.07	
	YC07	0.71	0.8	0.8 ± 0.07	0.65–1.03	
VI (cP)	HZ06	1.09	0.93	0.95 ± 0.04	0.88–1.16	0.09
	HZ07	1.03	0.91	0.94 ± 0.04	0.65–1.05	
	YC06	1.09	0.91	0.96 ± 0.05	0.87–1.10	
	YC07	1.01	0.93	0.95 ± 0.03	0.91–1.10	
AN (mg/L)	HZ06	149.17	186.68	187.46 ± 24.05	126.29–260.0	0.38
	HZ07	116.75	148.4	151.29 ± 20.34	108.22–213.19	
	YC06	140.41	186.08	163.62 ± 20.28	112.29–247.13	
	YC07	145.59	167.22	165.95 ± 17.29	129.51–230.82	
KI (%)	HZ06	39.74	48.89	46.74 ± 3.67	35.66–55.63	0.25
	HZ07	33.95	38.76	39.03 ± 3.48	31.23–53.25	
	YC06	31.89	40.6	4011 ± 3.72	29.39–49.77	
	YC07	37.77	42.24	41.55 ± 3.27	34.07–51.51	
DP (°WK)	HZ06	283.03	332.68	316.53 ± 52.86	150.45–466.27	0.28
	HZ07	321.89	413.56	384.05 ± 46.08	253.15–509.4	
	YC06	336.65	428.38	364.06 ± 48.56	240.41–476.63	
	YC07	344.82	436.62	409.08 ± 43.16	284.9–523.7	

SD means standard deviation; h_B^2: broad-sense heritability calculated as the ratio of the genetic variation divided by phenotypic variation (genetic and environmental variation). *ME* malt extract, *TN* malt total nitrogen content, *SN* malt soluble nitrogen content, *VI* viscosity, *AN* free amino nitrogen, *KI* Kolbach index and *DP* diastatic power

Table 2 The mean squares and their significance for malt quality traits in DH population lines based on an ANOVA

Source of variation	ME	TN	SN	VI	AN	KI	DP
Block	21.47**	31.86**	48.51**	32.36**	89.63**	27.84**	42.26**
Genotype (*G*)	9.8**	5.43**	9.26**	1.80**	10.66**	5.53**	7.48**
Location (*L*)	546.58**	1525.05**	18.08**	12.78**	38.48**	151.66**	413.39**
Year (*Y*)	382.22**	1413.82**	159.08**	11.87**	564.87**	353.31**	994.79**
$Y \times L$	13.15**	589.31**	1137.77**	0.88	709.65**	753.41**	39.76**
$G \times Y$	2.46**	2.91**	2.47**	0.55	2.13**	1.21	2.42**
$G \times L$	1.91**	4.13**	2.18**	0.57	1.76**	1.25	2.67**
$G \times L \times Y$	2.30**	4.11**	3.66**	0.69	3.09**	1.60**	2.71**

ME malt extract, *TN* malt total nitrogen content, *SN* malt soluble nitrogen content, *VI* viscosity, *AN* free amino nitrogen, *KI* Kolbach index and *DP* diastatic power

** Significant at the 1 % level

explained 48.4 % of the phenotypic variance with a LOD value of 30.1 (Fig. 2; Table 4). A minor *QMe.NaTx-1H* was also found on 1H with nearest marker being bPb-8884, explaining 5 % of the phenotypic variation. Environments showed significant effects on ME. As shown in Table S2, *QMe.NaTx-1H* was not identified in YC06 and HZ07. In

Table 3 Correlation coefficients between agronomic traits in DH population lines from Naso Nijo × TX9425 (calculated from the average value of three locations)

Correlation coefficients	ME	TN	SN	VI	AN	KI
TN	−0.23**					
SN	0.18	0.34**				
VI	−0.45**	0.42**	−0.43**			
AN	0.24**	0.16	0.93**	−0.55**		
KI	0.33**	−0.34**	0.76**	−0.71**	0.82**	
DP	0.35**	0.1	0.44**	−0.44**	0.45**	0.38**

ME malt extract, *TN* malt total nitrogen content, *SN* malt soluble nitrogen content, *VI* viscosity, *AN* free amino nitrogen, *KI* Kolbach index and *DP* diastatic power

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

contrast, *QMe.NaTx-2H* was identified in all environments. The alleles for higher malt extract of both QTL were from Naso Nijo. Some more QTL were also found from different environments. These included one on 7H from the HZ06 trial and one on 4H from the HZ07 trial (Table S2) with Naso Nijo alleles also contributing the higher ME.

QTL for viscosity

Four QTLs (*QVi.NaTx-1H*, *QVi.NaTx-3H*, *QVi.NaTx-5H* and *QVi.NaTx-7H*) were detected for VI based on the average values from all environments (Table 4). These QTL explained 6.7–17.8 % of the phenotypic variation. The total phenotypic variation explained by these four QTL was about 46 %. However, all the QTL showed significant interactions with environments with none being detected in all four environments. All four QTL were identified in YC07 but only one QTL (*QVi.NaTx-3H*) was detected in the HZ06 trial (Table S2). Surprisingly, TX9425 alleles for two of the QTL contributed low VI, indicating the possibility of combining favourable alleles from both parents to produce varieties with even lower VI.

QTL for diastatic power

Four QTL were identified for DP (Table 4). *QDp.NaTx-1H* explained 4.4 % of the phenotypic variance, with bPb-0081 being the closest marker. *QDp.NaTx-2H* was located on 2H with the nearest marker being bPb-1196, explaining 8.9 % of the phenotypic variation. *QDp.NaTx-7H* was located on 7H with the closest marker being HVWAXYG, explaining 13 % of the phenotypic variation. *QDp.NaTx-5H* was found on 5H with the closest marker being GBM1039. This QTL explained 20.9 % of the phenotypic variance with a LOD value of 13.75 (Table 4). Among these QTLs, *QDp.NaTx-1H* was only significant in the YC07 trial, *QDp.NaTx-2H* was significant in the HZ06 trial and HZ07 trial, and *QDp.NaTx-7H* was significant in the YC06 trial and HZ07 trial, while *QDp.NaTx-5H* was significant in three environments. Naso Nijo alleles increased DP in most QTL (Table 4).

QTL for Kolbach index

Two QTL were found to be associated with KI (Fig. 1; Table 4). *QKi.NaTx-5H* was located on 5H with the nearest marker being bPb-7444, explaining 7.4 % of the phenotypic variation. *QKi.NaTx-7H* was found on 7H with the closest marker being bPb-4725. This QTL explained 15.4 % of the phenotypic variance with a LOD value of 7.01. Naso Nijo alleles contributed higher KI in both QTL (Table 4).

QTL for nitrogen attributes

Based on the average values from all four environments, two QTL were found to be associated with TN. The first QTL was located at the position of around 66 cM of 1H, explaining 14.7 % of the phenotypic variation. The second QTL was located at 12 cM on 2H, explaining 12.8 % of the phenotypic variation (Table 4; Fig. 1). Environments showed significant effects on QTL for TN with almost no QTL being detected in more than two environments (Table S2). The QTL identified for SN also varied between environments. No QTL were identified for SN from the HZ06 and YC07 trials (Table S2). Two QTL were found for AN. Among them, the QTL on 7H was located on the same position to that for SN (Fig. 1; Table 4). Again some different QTL were detected from different environments (Table S2).

Searching for possible candidate genes underlying ME QTL

The identification of the most important QTL for ME on 2H (*QMe.NaTx-2H*) encouraged us to further investigate for candidate genes underlying the QTL. We had examined all annotated genes near the marker GBM1121 on 2H. One hundred and seventy-three high confident genes

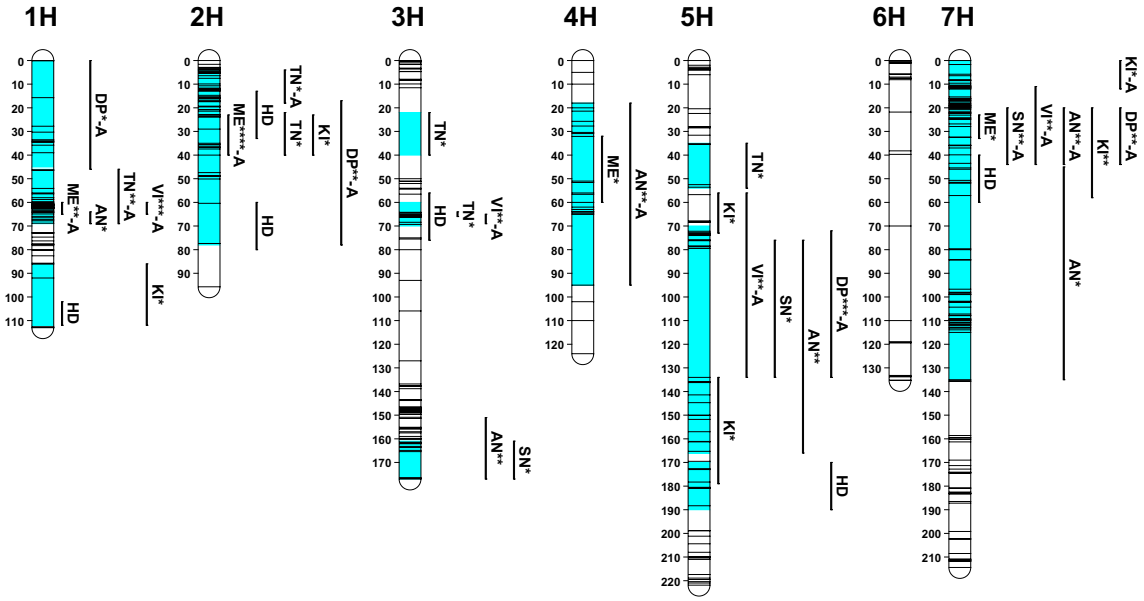

Fig. 1 QTL for malting quality traits. The number of '*' indicating the number of environments that QTL was detected and '-A' indicating that the QTL was detected based on the average values of all environments. QTL for heading dates (HD) were also presented. *ME* malt extract, *TN* malt total nitrogen content, *SN* malt soluble nitrogen content, *KI* Kolbach index, *AN* free amino nitrogen, *VI* viscosity and *DP* diastatic power

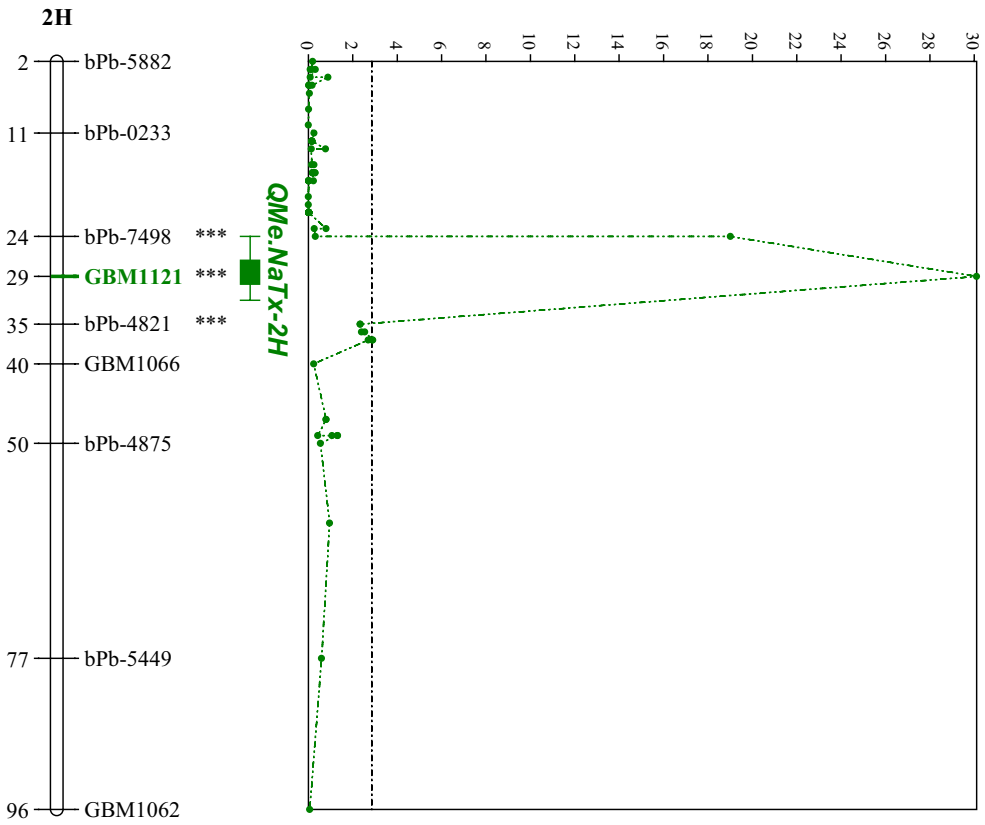

Fig. 2 The major QTL for malt extract (ME) on 2H from MQM mapping results. Only a few selected markers were presented

were found in a 10 cM region underlying the QTL. A gene (MLOC_60943.2) coding for a cell wall hydrolytic enzyme endo-1,4-xylanase A was identified (Supplemental Table S1). This gene is at the same position as the marker GBM1121, both of which were at 14.38 cM according to PopSeq map (Mascher et al. 2013).

Table 4 QTLs for malt quality parameters in the DH population of Naso Nijo × TX9425 (Average value)

Trait	Linkage group	QTL name	2_LOD interval	Nearest marker	Position (cM)	LOD	R^2 (%)	Source of positive effect
ME	1H	QMe.NaTx-1H	54.117–59.735	bPb-8884	55.972	4.35	5	NN
	2H	QMe.NaTx-2H	23.927–35	GBM1121	29	30.1	48.4	NN
TN	1H	QTn.NaTx-1H	64.937–67.334	0501C	65.922	7.13	14.7	NN
	2H	QTn.NaTx-2H	7.534–17.046	bPb-5153	12.586	5.43	12.4	NN
SN	7H	QSn.NaTx-7H	20.79–43.473	HVWAXYG	26.8	5.6	12.8	NN
VI	1H	QVi.NaTx-1H	61.053–61.384	bPb-8509	61.153	10.24	17.8	TX
	3H	QVi.NaTx-3H	65.475–68.435	Bmac0209	65.653	5.04	7.7	TX
	5H	QVi.NaTx-5H	72.247–134	HVACL1	76.122	4.51	6.7	NN
	7H	QVi.NaTx-7H	20.79–27.876	bPb-5902	23.104	7.9	14.5	TX
AN	4H	QAn.NaTx-4H	51.615–95	bPb-0365	56	3.75	7.5	NN
	7H	QAn.NaTx-7H	20.79–43.473	HVWAXYG	26.8	5.59	11.5	NN
KI	5H	QKi.NaTx-5H	56.707–72.244	bPb-7444	67.992	4.12	7.4	NN
	7H	QKi.NaTx-7H	10.695–11.591	bPb-4725	11.334	7.01	15.4	NN
DP	1H	QDp.NaTx-1H	0–46.328	bPb-0081	0.013	3.33	4.4	NN
	2H	QDp.NaTx-2H	17.046–40	bPb-1196	36.2	6.39	8.9	NN
	5H	QDp.NaTx-5H	76.229–134	GBM1039	78.4	13.75	20.9	NN
	7H	QDp.NaTx-7H	23.104–32.38	HVWAXYG	26.8	9.1	13	NN

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 and Kyazma 2009)

ME malt extract, *TN* malt total nitrogen content, *SN* malt soluble nitrogen content, *VI* viscosity, *AN* free amino nitrogen, *KI* Kolbach index and *DP* diastatic power

Discussion

Many Chinese germplasm have shown a wide range of stress tolerances such as salinity (Chen et al. 2007) and waterlogging tolerance (Zhou et al. 2007) but relatively poor malting quality. To effectively use these germplasm in improving malting barley for stress tolerance, it is important to have an effective tool to select against poor quality since most malting quality traits are quantitatively inherited (Peterson and Foster 1973) and generally of low heritability (Sparrow 1970). The environment also showed significant effects on malt quality (Coles et al. 1991). In the current experiment, even though the growth conditions in both sites were quite similar with only slightly higher temperatures and higher rainfall in HZ, very significant effects of environment on malting quality were observed (Table 2). For example, the average ME of all DH lines ranged from 81.0 (HZ06) to 78.8 (YC07) (Table 1). Significant interactions were also found between sites/years and genotypes (Table 2). Thus, the data from multi-sites or years should be used to increase the accuracy of quality assessment.

ME is one of the most important quality traits for barley, determining the yield of beer production. However, ME is a very complex quantitative trait and can be easily affected by environmental factors. Direct selection on ME has been shown to be less effective. Researchers have been trying to identify molecular markers linked to the trait to assist the selection. Many QTL conferring ME have been reported on all seven chromosomes (Hayes et al. 1993, 1996; Oziel et al. 1996; Thomas et al. 1996; Bezant et al. 1997; Mather et al. 1997; Marquez-Cedillo et al. 2000; Hoffman and Dahleen 2002; Barr et al. 2003; Cakir et al. 2003; Pallotta et al. 2003; Emebiri et al. 2004; Han et al. 2004; Gao et al. 2004; Li et al. 2005; Von Korff et al. 2008; Laidò et al. 2009; Schmalenbach and Pillen 2009; Elía et al. 2010; Panozzo et al. 2007; Zhou et al. 2012b). However, most of the QTL determined only a small amount of phenotypic variation. For example, Hayes et al. (1993) (Pillen et al. 2003) found that ME was controlled by 33 QTL, which makes it hard for plant breeders to use molecular markers to select for this trait. To be effective for MAS, the QTL should have sufficient allelic effect. In the present study, two QTLs (*QMe.NaTx-1H* and *QMe.NaTx-2H*) were identified for ME on 1H and 2H, respectively. The QTL (*QMe.NaTx-1H*) was located on similar positions to previously reported (Hayes et al. 1993; Panozzo et al. 2007; Elía et al. 2010) (Table S3) (Kjar et al. 1995; Hori et al. 2003; Baghizadeh et al. 2007). This QTL only determined 5 % of the phenotypic variation and showed significant interaction

with environments. It was only detected in two of the four environments. The most important QTL for ME on 2H (*QMe.NaTx-2H*) was consistently expressed in all of the environments and determined 48 % of phenotypic variation (Fig. 2; Table 4). Several QTLs with relatively greater effects on ME were reported on 2H. When compared to their positions with the current QTL by using different consensus maps (Wenzl et al. 2006; Varshney et al. 2007; Alsop et al. 2011), most of them are on a different position from the QTL identified in this study (Thomas et al. 1996; Hayes et al. 1996; Bezant et al. 1997; Marquez-Cedillo et al. 2000; Pallotta et al. 2003; Coventry et al. 2003; Emebiri et al. 2004; Panozzo et al. 2007; Elía et al. 2010). A few QTL on 2H, which are located on a similar position, determined a relatively smaller amount of phenotypic variation (Hayes et al. 1993; Oziel et al. 1996; Hoffman and Dahleen 2002; Zhou et al. 2012b) (Table S3).

Since the position of this QTL (*QMe.NaTx-2H*) was on a similar position where a QTL was found for heading date (Wang et al. 2010, 2014) (Fig. 1), further analysis was conducted with MapQTL6.0 using heading date data from this population (Wang et al. 2014) as a covariate. Results confirmed that this QTL was not affected by heading date with the LOD value and R^2 for 2H ME QTL changed from 30.1 and 48.4 to 30.8 and 48.5, respectively, after using heading date data as a covariate. The percentage of husks showed significant effect on ME (Fox et al. 2006) and a preliminary study showed that a QTL for hull thickness was found on the short arm of 2H (Collins et al. 1999) with the allele that increased ME showed decreasing effect on hull thickness. To confirm whether the alleles controlling ME and hull thickness are the same, a few selected DH lines with highest or lowest ME were tested for the husk content. Results showed that high ME was not necessarily linked with low husk content. For example, a DH line (No. 45181) from the YC07 trial had both high ME (80.0 %) and high hull content (11.3 %) and another DH line (No. 45006) from the same trial showed both low ME (75.9 %) and low hull content (9.7 %). However, on the other hand, barley grain husk and endosperm cell wall degradations might be important in the contribution to ME or to ME difference between barley lines. It was estimated that hydrolysis of cell wall components contributed 18.5 % soluble sugar to endospermic sugar pool (Morrall and Briggs 1978). Heteroxylan was a major component of barley cell wall consisting of 71 % (w/w) and 20 % (w/w) in aleurone layer and starchy endosperm cell walls, respectively (Fincher 2010). The gene (MLOC_60943.2) located at the same position as the marker GBM1121 coded for an important enzyme endo-1,4-beta-xylanase. This enzyme hydrolysed the backbone of heteroxylan and released soluble polysaccharides (Fincher 2010). Endo-1,4-xylanase A might have dual roles. Firstly, it degraded cell walls and facilitated the release of starch hydrolytic enzymes from aleurone and scutellar epithelial layers to starch endosperm (Fincher 2010; McFadden et al. 1988). Secondarily, the hydrolytic products of heteroxylans by endo-1,4-xylanase were soluble and were able to contribute to the ME difference between barley lines.

DP is another complex and important quality trait, which is the collective activity of starch degrading enzymes in malt. QTL-controlling diastatic power was also identified in every chromosome (Hayes et al. 1993, 1996; Thomas et al. 1996; Oziel et al. 1996; Mather et al. 1997; Marquez-Cedillo et al. 2000; Hoffman and Dahleen 2002; Barr et al. 2003; Cakir et al. 2003; Coventry et al. 2003; Pallotta et al. 2003; Emebiri et al. 2004; Han et al. 2004; Gao et al. 2004; Panozzo et al. 2007; Elía et al. 2010; Zhou et al. 2012b). In this experiment, four QTLs were identified for DP, with most of them being in similar positions to those previously reported. The major QTL (*QDp.NaTx-5H*) determined more than 20 % of the phenotypic variation. Further studies are needed to prove the differences between this QTL and a previously reported major QTL (Oziel et al. 1996; Hoffman and Dahleen 2002) (Table S3). The QTL on 7H (*QDp.NaTx-7H*) determined not only 13 % of the phenotypic variation of DP, but also 12.8 % of SN and 11.5 % of AN. The QTL for VI and KI on 7H were also located on a similar position to *QDp.NaTx-7H*, so it is not surprising that these traits are closely related (Table 3).

In conclusion, a total of 17 QTLs were identified for seven malting quality traits, explaining 4.4–48.4 % of the phenotypic variation. The most significant QTL was identified on 2H for ME, which seems to be different from previously reported. This QTL determined a large proportion of the phenotypic variation with little effect from the environment and should be very effective when used in a breeding programme.

Author contribution statement J.W., M.Z. constructed the population and molecular map; J.W. designed the experiments; J.W., J.Y., J.Z., Q.J., W.H., Y.S. performed the experiments; Q.Z. and C.L. performed genomic analysis for potential genes; J.M., M.Z. analysed the data and wrote the paper.

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