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ORIGINAL PAPER

A genome-wide association study of malting quality across eight U.S. barley breeding programs

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Abstract

Key message **We report malt quality QTLs relevant to breeding with greater precision than previous mapping studies. The distribution of favorable alleles suggests strategies for marker-assisted breeding and germplasm exchange.**

Abstract This study leverages the breeding data of 1,862 barley breeding lines evaluated in 97 field trials for genome-wide association study of malting quality traits in barley. The mapping panel consisted of six-row and two-row advanced breeding lines from eight breeding populations established at six public breeding programs across the United States. A total of 4,976 grain samples were subjected to micro-malting analysis and mapping of nine quality traits was conducted with 3,072 SNP markers distributed throughout the genome. Association mapping was performed for individual breeding populations and for combined six-row and two-row populations. Only 16 % of the QTL we report here had been detected in prior bi-parental mapping studies. Comparison of the analyses of the combined two-row and six-row panels identified only two QTL regions that were common to both. In total, 108 and 107 significant marker-trait associations were identified in all six-row and all two-row breeding programs, respectively. A total of 102 and 65 marker-trait associations were specific to individual six-row and two-row breeding programs, respectively indicating that most marker-trait associations were breeding population specific. Combining datasets from different breeding program resulted in both the loss of some QTL that were apparent in the analyses of individual programs and the discovery of new QTL not identified in individual programs. This suggests that simply increasing sample size by pooling samples with different breeding history does not necessarily increase the power to detect associations. The genetic architecture of malting

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quality and the distribution of favorable alleles suggest strategies for marker-assisted selection and germplasm exchange.

Introduction

Malting quality in barley (*Hordeum vulgare* L.) is determined by a complex suite of traits (Fox et al. 2003). For some of these traits, there is consensus among the end-users for a target value while for other traits the specific target will depend on the market class (two-row or six-row), beer style, and brewing process. In the U.S., brewers who are members of the American Malting Barley Association (AMBA; http://ambainc.org) have defined acceptable ranges and desired target values for 18 barley and malting quality traits. Barley producers that grow industry-endorsed cultivars that meet these standards will generally receive a premium price that is higher than that for feed barley. Developing malting barley cultivars through breeding requires evaluation of large numbers of lines for these malting quality traits. Measuring some of the more important quality traits involves malting individual grain samples and subjecting them to the first stages of brewing to make wort. While this process has been scaled down to accommodate research samples, it is laborious and expensive and, thus, a limiting step for breeders desiring to improve malt quality (Schmitt and Budde 2010). From such analyses, selection can be imposed for multiple traits including but not limited to kernel weight, kernel plumpness, malt extract, barley protein, wort protein, soluble/total ratio (Kolbach index), diastatic power, α-amylase, and β-glucan (Clancy and Ullrich 1988). Characterizing the genetic architecture of these quality traits would facilitate marker-assisted breeding methods, which could both increase the speed and decrease the cost of breeding malting barley cultivars.

Several bi-parental mapping studies have been conducted to identify quantitative trait loci (QTL) for malting quality traits. Within North American breeding germplasm, these studies have used the six-row cultivar Morex and two-row cultivar Harrington, which were the industry standards for malting quality in the 1980's and 90's (Rasmusson and Wilcoxon 1979; Harvey and Rossnagel 1984). To generate ample molecular markers for mapping and sufficient phenotypic variation for quality traits, these parents were used in wide crosses. A two-row (Harrington) by six-row (Morex) population was generated by Marquez-Cedillo et al. (2000). Morex was also crossed with a feed barley parent to generate the Steptoe × Morex population (Kleinhofs et al. 1993; Hayes et al. 1993) and with Dicktoo to generate the winter by spring habit cross of Dicktoo × Morex (Oziel et al. 1996). QTL studies conducted with these mapping populations have identified 70 QTL associated with a suite of malting quality traits (Fig. 1). Several QTL of note that have received additional study are located on the short arm of chromosome 7H and are associated with malt extract, α-amylase, and diastatic power (Han et al. 1997, 2004). Another QTL region associated with malt extract, diastatic power, β-glucan, and α-amylase initially identified in the Steptoe × Morex population was fine-mapped on the short arm of chromosome 4H near the telomere (Gao et al. 2004).

Despite the wealth of genetic information accumulating for malting quality in barley and plant traits in general, translation to crop improvement has been limited (Bernardo 2008). This barrier to application of markers in breeding is due, in part, to population specificity of QTL and QTL by environment interactions (Podlich et al. 2004; Holland 2007; Sneller et al. 2009). One particular challenge in breeding is that QTL identified in wide-cross mapping populations may not be segregating in breeding program populations. This is the case for the two malting quality QTL on 4H and 7H described above within the breeding program at the University of Minnesota (Condon et al. 2008). To generate QTL information on malting quality that will be more useful in breeding applications, it would be desirable to study the genetic architecture of quality in elite breeding germplasm. Association mapping can be used to identify QTL using mapping panels that are designed for a specific context and that may be segregating for more than two alleles at a locus (Jannink et al. 2001). Panels designed from locally adapted breeding material tested over several years and environments can be used to identify QTL that are more relevant to breeding and effective in the target breeding environment (Pozniak et al. 2012).

The success and the power of genome-wide association studies (GWAS) to detect QTL depends, in part, on sufficient polymorphic markers distributed across the genome to ensure adequate linkage disequilibrium (LD) between markers and QTL (Arbelbide et al. 2006). Powerful genotyping technologies are now available to provide the desired level of marker density at increasingly affordable costs. Currently, single nucleotide polymorphism (SNP) Oligo Pool Assay (OPA) and iSelect genotyping in barley provide over 7,000 mapped SNP loci, which can be used in a high-throughput genotyping format (Close et al. 2009; Comadran et al. 2012). In barley, this SNP platform has been used with elite breeding panels to conduct association mapping for several disease resistance traits (Massman et al. 2011; Zhou and Steffenson 2013) and food quality traits (Mohammadi et al. 2014).

To effectively leverage current marker technologies to conduct GWAS, researchers have utilized historic data from long-term breeding trials thereby substantially reducing the cost of genetic studies (Podlich et al. 2004; Sneller et al. 2009; Crossa et al. 2007). Mapping with such unbalanced

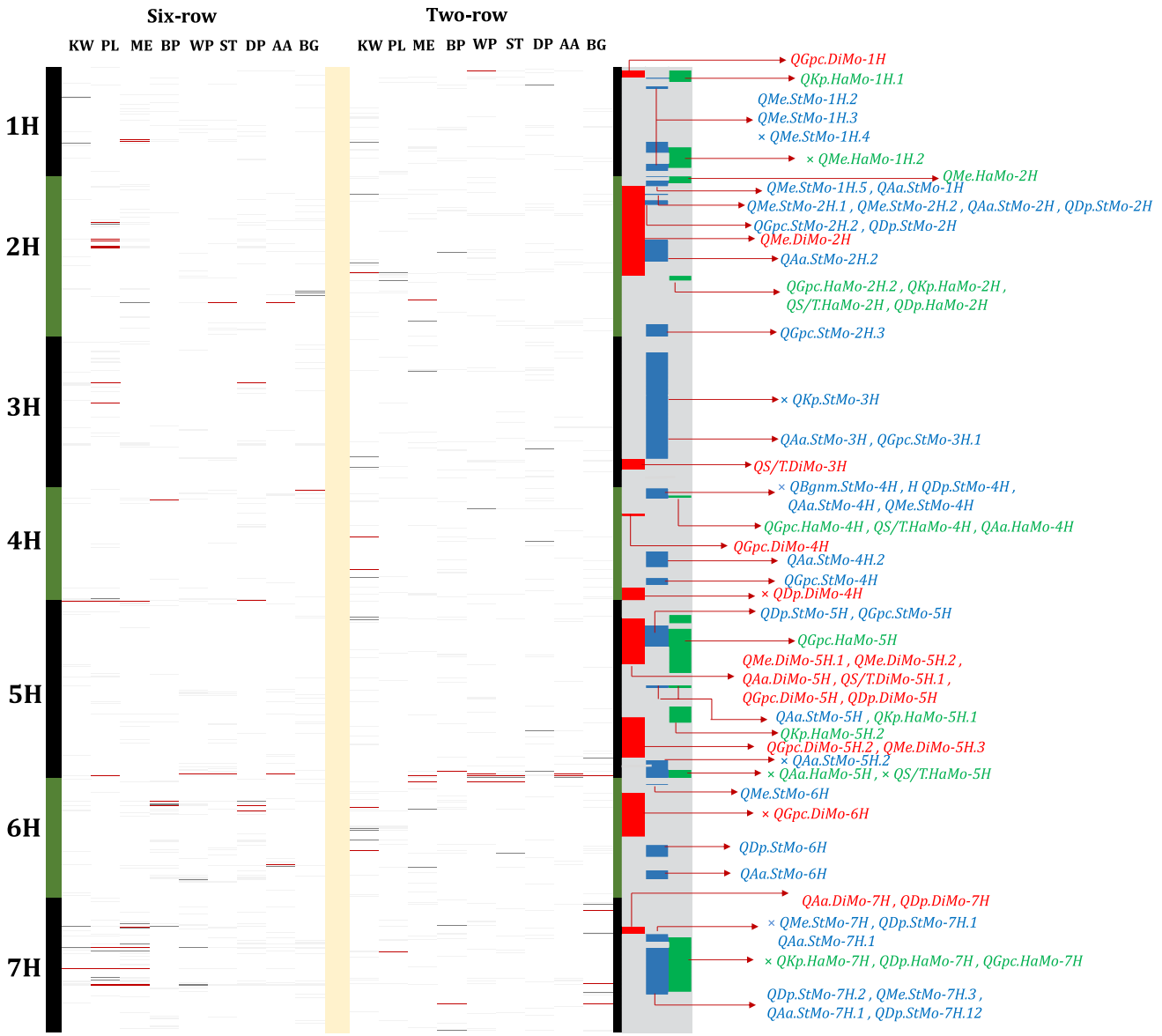

Fig. 1 Heatmap of genome-wide association signals detected in the six-row panel (AB6 + MN6 + ND6) and the two-row panel (AB2 + MT2 + ND2 + WA2) for nine traits. Signals with $1.3 \leq -\log P \leq 3$ are shown in *light gray*; $3 \leq -\log P \leq 4$ in *dark gray*; $-\log P \geq 4$ in *dark red*. Traits include kernel weight (KW), kernel plumpness (PL), malt extract (ME), barley protein (BP), wort protein (WP), soluble/total ratio (ST), diastatic power (DP), α-amylase (AA), and β-glucan (BG). The three color-coded columns on the right depict the coordinates of previously identified QTLs in bi-parental mapping populations Diktoo × Morex 'DiMo' (*red*), Steptoe × Morex 'StMo'(*blue*), and Harrington × Morex 'HaMo' (*green*). The QTLs marked by '×' are those which were detected in this analysis. Values used to generate the heatmap are given in Table S5 (color figure online)

datasets requires methods to estimate breeding line values that account for experimental design effects such as year, location, and trial. These estimates can be acquired by extracting trial effects in a mixed-model and subsequently using trial effects to adjust phenotypic values in a two-step process (Stich et al. 2008). Alternatively, mixed-models can be used to extract the best unbiased linear estimates (BLUEs) for entries included in the association panel in one step (Pozniak et al. 2012). In addition to adjusting for trial effects to obtain accurate genetic estimates of breeding lines, it is also necessary to account for population structure existing in association panels (Zhao et al. 2007). Population structure is defined by differential relatedness among subgroups and is mainly caused by breeding history. Relatedness among individuals that is not accounted for in models used to detect QTL can result in false positive marker-trait associations (Pritchard et al. 2000). In barley, major subpopulation structure has been associated with

the market classes of six-row and two-row, growth habit classes of winter and spring, geographic origin, and breeding program (Malysheva-Otto et al. 2006; Hamblin et al. 2010; Zhou et al. 2012).

An important goal of the U.S. Barley Coordinated Agricultural Project (Barley CAP http://www.barleycap.org) was to assemble large sets of marker and trait data that could be used to conduct GWAS in elite breeding germplasm. In this study, malting quality data from standard breeding trials conducted over a 4-year period from eight U.S. barley breeding programs and 3,072 SNP markers were used in association mapping. Analyses conducted within and across breeding programs reveal the genetic architecture of these traits and segregation of favorable alleles within elite breeding germplasm. This insight is used to suggest strategies for germplasm exchange among breeding programs and marker-based selection to improve malting quality.

Materials and methods

Germplasm, association mapping panels, and malting quality traits

To conduct association mapping, we assembled malting quality data from eight barley breeding programs in the United States that participated in the Barley CAP. The germplasm included both six-row(spring and winter growth habit) and two-row spring barley breeding lines selfed to at least the F_4 generation that were representative of breeding germplasm in the eight breeding programs from 2006 to 2009. Each breeding program contributed 96 entries in each of the 4 years of the project. The six-row spring breeding lines were contributed by USDA at Aberdeen, ID (AB6), University of Minnesota (MN6), North Dakota State University (ND6), and the six-row winter breeding lines were contributed by Oregon State University (OR6), and the two-row spring breeding lines were contributed by USDA at Aberdeen, ID (AB2), Montana State University (MT2), North Dakota State University (ND2), and Washington State University (WA2). The lines that were evaluated for malting quality were chosen as part of the routine breeding procedures for each program and in some cases included all 96 lines, while in other cases included only a subset of the entries. Some lines designated as food barley were not included in the analysis because food barley lines often have high levels of β-glucan and are hulless, which is undesirable in malting barley. Lines designated as feed barley were retained in the analyses because the distinction between malt and feed is not always clear. Because most of the breeding programs only submitted lines for malt analysis that would be under consideration for release as malting varieties, we reasoned that truly non-malting types should be excluded. Initially, association mapping was conducted on each individual breeding population. Subsequently, three six-row spring (AB6, MN6, and ND6) and four two-row spring (AB2, MT2, ND2, and WA2) breeding populations were combined to make joint six-row (6R) and joint two-row (2R) association mapping panels, respectively. Analysis of the OR6 program has been previously reported (Gutiérrez et al. 2011). Each breeding program generated their micro-malting datasets by shipping a bulked sample of the replicates of each entry in each of their breeding trials to the USDA–ARS Cereal Crops Research Unit located in Madison, WI, where micro-malting assessments were conducted following American Society of Brewing Chemists protocols (ASBC 1992). Full description of methods used for malt quality measurements is detailed in Budde et al. (2010). Each sample was analysed for nine traits including kernel weight (mg), kernel plumpness (%), malt extract (%), barley protein (%), wort protein (%), soluble/total protein ratio, diastatic power (°ASBC), α-amylase (20°DU), and β-glucan (ppm).

Experimental design and phenotypic value estimations

Malting quality data were generated from grain samples obtained from yield trials conducted by each breeding program in randomized complete block designs in multiple locations between 2006 and 2010. Each breeding program used two to nine common check cultivars in all years to account for environmental effects and to allow for phenotypic adjustment across trials. Phenotypic adjustment for individual breeding programs (hereafter called panels) as well as the joint 6R and 2R panels was conducted using Proc Mixed procedure in SAS v 9.2 (SAS Institute 2008) as described below. Pearson's correlation coefficients among traits were calculated in R environment 2.15.3 (R Development Core Team 2013). To calculate a single phenotypic value for each breeding line, we fit a mixed-model for each trait within each panel by treating lines as fixed effects and trials and interaction of lines and trials as random effects. The common set of check cultivars in each panel allowed for extraction of solutions for trial effects as well as interaction of trials and lines effects. Line effects were significant ($P < 0.01$) for all traits in each panel. Then, we used the LSMEANS statement in SAS to obtain BLUEs for each trait and line (Pozniak et al. 2012) within each panel. This process is similar to the two-step adjustment procedure, which uses best linear unbiased prediction (BLUP) solutions of trial effects followed by numerical adjustment of means. The combined 6R panel was comprised of 33 trials from AB6, MN6, and ND6. From these 33 trials, two trials were adjusted with only one common check cultivar and the remaining trials shared a minimum of three common

Table 1 Characteristics of the trait and marker data sets for ten association mapping panels

Characteristics	AB2[a]	MT2	ND2	WA2	2R	AB6	MN6	ND6	6R	OR6
Number of trials	6	5	12	15	38	6	14	22	59	17
Number of entries assessed in trials	182	351	155	323	1,011	98	474	194	966	200
Number of entries used for AM	182	315	151	290	938	98	474	192	764	160
Number of grain samples analysed	417	471	306	525	1,719	156	1,420	544	3,257	1,137
Number of markers used for AM	2,068	2,201	2,042	2,046	2,190	1,922	2,115	1,839	2,252	2,236
Average LD for adjacent markers (R^2)	0.38	0.36	0.35	0.33	0.29	0.40	0.35	0.38	0.34	0.35

[a] USDA at Aberdeen, ID (AB6), University of Minnesota (MN6), North Dakota State University (ND6), and Oregon State University (OR6) and two-row spring breeding programs i.e., USDA at Aberdeen, ID (AB2), Montana State University (MT2), North Dakota State University (ND2), and Washington State University (WA2). The 6R association panel is a joint panel of the three six-row spring breeding programs i.e., AB6 + MN6 + ND6 and the 2R association panel is a joint panel of the four two-row spring breeding programs i.e., AB2 + MT2 + ND2 + WA2

check cultivars. The combined 2R panel consisted of 38 trials from AB2, MT2, ND2, and WA2, of which seven trials shared only one common check cultivar and the rest shared a minimum of three common check cultivars. Similar mixed-model analyses were performed for the combined 6R and 2R datasets and the LSMEANS statement in SAS was used to obtain BLUEs across lines and traits. Table 1 summarizes the number of trials and lines for each of the mapping panels. The differences between the total number of entries assessed in each panel and the number of entries used for association mapping analysis are primarily due to the removal of genetic stocks and food barley lines. Our intent was to retain only malting barley lines in the analysis, however, in the case of WA2, some feed barley lines were retained in the analysis because for WA2 panel, the majority of lines (186) were feed barley and only 104 were designated as malt barley.

Molecular marker data and linkage disequilibrium

DNA extracted from a single plant from each line in the study was genotyped. All of the lines included in the study were advanced to at least the F_4 generation and genotyped with 3,072 SNP markers using two Illumina Golden Gate (Illumina Inc., San Diego, CA) Oligonucleotide Pool Assays known as BOPA1 and BOPA2 (Close et al. 2009; Szűcs et al. 2009). For each individual panel and for the joint 6R and 2R panels, we extracted genotype data from The Triticeae Toolbox database (http://www.triticeaecap.org/; Blake et al. 2012) by querying for line names with the genetic map developed by Muñoz-Amatriaín et al. (2011). Markers with greater than 20 % missing genotype data across the germplasm in each AM panel and markers with conflicting genotyping data were excluded from further analysis. Assessment of LD was conducted by calculation of R^2 statistics between markers per chromosome and subsequently aggregated across all chromosomes using TASSEL (Bradbury et al. 2007; http://www.maizegenetics.net).

Association mapping

We performed association mapping analysis on individual breeding programs and combined 6R and 2R panels. The prior knowledge of population structure in U.S. barley germplasm reported by Hamblin et al. (2010) indicates that most of the individual membership in subpopulations may be explained by differences in inflorescence row type and growth habit, suggesting the existence of three subpopulations i.e., spring six-row malting barley, winter six-row malting barley, and spring two-row malting barley. The same research group has also concluded that the best fit was a model describing subpopulations without admixture roughly representing the individual breeding programs. Therefore, we used an additive kinship matrix (K model) to account for individuals' relatedness in each breeding program and in the joint populations characterized by row type. After eliminating monomorphic markers and those with more than 20 % missing data, the remaining markers in each association panel were used for estimating individuals' relatedness matrix. Our association genetics model included markers and kinship as fixed effects and the polygenic effect of line and error as random effects. Association mapping was conducted using the EMMA package in R environment 2.7.0 (R Development Core Team 2008), which uses the Efficient Mixed Model Association (EMMA) algorithm (Kang et al. 2008). The EMMA package returns the maximum likelihood estimation of marker effect and likelihood ratio test of significance for marker-trait associations. For control of false discovery rate (FDR), we considered a Bonferroni correction and a 5 % false discovery rate as described by Storey and Tibshirani (2003). A conservative Bonferroni $-\log P$ value of nearly 4.6 was derived by dividing $P = 0.05$ by the average total number of mapped markers across panels $N = 2{,}091$. A less conservative $-\log P$ value of 2.8 was derived, based on the average of several panels and traits using the Storey and Tibshirani method. We therefore selected a $-\log P$ value of

Table 2 Pearson's correlation coefficients of nine malting quality traits for the six-row combined association panel (6R) with 745 individuals (upper right triangle) and for the two-row combined association panel (2R) with 938 individuals (lower left triangle)

Traits[a]	KW	PL	ME	BP	WP	ST	DP	AA	BG
KW		0.573[b]	ns[c]	ns	0.284	0.169	-0.072	ns	0.088
PL	0.555		ns	ns	ns	ns	ns	ns	ns
ME	0.372	0.271		-0.600	ns	0.525	-0.433	0.183	-0.091
BP	0.085	ns	-0.464		0.252	-0.423	0.654	-0.065	ns
WP	0.252	0.182	0.649	0.067		0.641	0.108	0.501	ns
ST	0.118	0.099	0.798	-0.464	0.830		-0.333	0.641	-0.209
DP	0.113	0.096	0.245	0.338	0.446	0.231		ns	-0.106
AA	0.078	0.091	0.645	-0.132	0.813	0.804	0.458		-0.215
BG	0.150	0.085	-0.404	0.483	-0.328	-0.560	-0.181	-0.456	

[a] *KW* Kernel weight, *PL* kernel plumpness, *ME* malt extract, *BP* barley protein, *WP* wort protein, *ST* soluble/total ratio, *DP* diastatic power, *AA* α-amylase and *BG* β-glucan

[b] Magnitudes of correlations were color coded so to show correlations from low to high positive in orange to dark red color schemes and correlations from low to high negative in lime to dark green color schemes

[c] Significance test of Pearson's correlations was performed using $t = r/\text{sqrt}[(1\text{-}r\text{^}2)/(N\text{–}2)]$ distribution with $df = N-2$. Non-significant values were denoted by "ns"

4.0 as a threshold for QTL detection that was on the conservative side of this range. Marker effect was estimated using the rrBLUP package in R (Endelman 2011). For each significant marker-trait association, we passed the vector of a given significant marker alone and K matrix as the covariance structure of the random effect in 'mixed.solve' function of rrBLUP package (Endelman 2011) to obtain the allele substitution effect when only that marker is in the model. Once we summarized all marker-trait associations for each trait, we identified those that were in close proximity (within 5 cM) and determined the LD between them. If the LD was greater than ($R^2 = 0.97$), we concluded that the markers could be identifying the same QTL. We footnoted two such cases where two adjacent markers that were in high LD such that the two markers likely represent two distinct haplotypes at a single locus rather than two tightly linked loci with opposite marker effects.

We used the SNP-based barley map reported by Muñoz-Amatriaín et al. (2011) as reference to build our tables containing mapped malting quality trait QTL. The relative map positions of previously identified malting quality QTL were determined by aligning markers with the genetic map developed by Muñoz-Amatriaín et al. (2011) with prior QTL studies (Han et al. 1997, 2004; Hayes et al. 1993; Hayes and Jones 2000; Marquez-Cedillo et al. 2000, Mather et al. 1997). We used the genomic data repository available at the GrainGenes database (http://www.graingenes.org) to obtain historical molecular markers associated with previously reported malting quality traits QTL. We then used the approximate position of flanking markers to highlight the genomic regions specifying these QTL in our tables. Pearson's correlation coefficients among $-\log P$ values between pairs of breeding programs were calculated using the "pairwise.complete.obs" option in the "cor" function in R environment 2.15.3 (R Development Core Team 2013).

Results

Trait correlations

We calculated pair-wise trait correlations for each panel based on the trait estimates obtained from mixed-model analyses (Table 2). The summary statistics and variance components for each trait in each breeding program are given in Supplemental Table S1. The correlation matrices for the individual panels are given in Supplemental Table S2. Protein content was negatively correlated with malt extract and positively correlated with diastatic power in all association panels consistent with previous studies (Rasmusson and Glass 1965; Ullrich et al. 1981; Eagles et al. 1995). The relationship between malt extract and diastatic power varied among the panels. The correlation was positive in AB2, negligible in MT2, ND2, WA2, and OR6, and negative in MN6, ND6, and AB6. Diastatic power was found to be negatively correlated with malt extract in some studies (Hartog and Lambert 1953; Arends et al. 1995), although some modest positive genotypic correlations have also been reported (Eagles et al. 1995). The correlation between malt extract and wort protein and the correlation between α-amylase and diastatic power were positive and

Table 3 Distribution of significant marker-trait associations obtained for nine malting quality traits across ten association mapping panels

Trait[a]	AB6[b]	MN6	ND6	OR6	6R	AB2	MT2	ND2	WA2	2R
KW	0[c]	0	0	0	0	1	18	0	1	2
PL	0	6 (2)[d]	0	0	7 (2)	0	1	0	0	0
ME	0	1 (1)	1	4 (3)	4 (1)	6	3	0	5	5
BP	0	3	32 (26)	0	8 (5)	0	0	0	0	1
WP	1	4	0	1	5	8	4	0	9	7
ST	0	6 (6)	0	0	7 (5)	5 (5)	5 (5)	0	11 (7)	4 (3)
DP	7	0	33 (2)	3 (3)	6	0	0	2	2 (2)	1
AA	4 (4)	5 (3)	1	0	7 (4)	6 (6)	5 (5)	0	7 (7)	4 (4)
BG	3 (3)	1	0	0	1 (1)	2	2	0	4	3
Total	15 (7)	26 (12)	67 (28)	8 (6)	45 (18)	28 (11)	38 (10)	2	39 (16)	27 (7)

[a] *KW* Kernel weight, *PL* kernel plumpness, *ME* malt extract, *BP* barley protein, *WP* wort protein, *ST* soluble/total ratio, *DP* diastatic power, *AA* α-amylase and *BG* β-glucan

[b] USDA at Aberdeen, ID (AB6), University of Minnesota (MN6), North Dakota State University (ND6), and Oregon State University (OR6) and two-row spring breeding programs i.e., USDA at Aberdeen, ID (AB2), Montana State University (MT2), North Dakota State University (ND2), and Washington State University (WA2)

[c] Numbers in the table are the counts of marker-trait association signals where $-\log P \geq 4$ and the frequency of rare variants ≥10

[d] Shown in parentheses are the number of associations coincident with QTLs identified in previous bi-parental studies

substantial in two-row panels while negligible in six-row panels. Several correlations were consistent across all panels. Barley protein content was positively correlated with diastatic power, wort protein and negatively correlated with soluble/total protein ratio. Alpha-amylase was positively correlated with wort protein and soluble/total ratio.

Markers and linkage disequilibrium

Association mapping was conducted to identify QTL for malting quality traits in the ten association panels. The number of markers included in the analyses of the ten mapping panels ranged from 1,829 to 2,252 (Table 1). In general, our LD analysis revealed that LD persisted for up to 10 cM (data not shown) as has been shown previously in other analyses of this germplasm (Hamblin et al. 2010; Massman et al. 2011). Given the total genetic map of 1,127 cM (Muñoz-Amatriaín et al. 2011) used in this study, about 113 (1,127/10) polymorphic SNPs, evenly distributed across the genome are required to cover the entire genome for association mapping (Zhou et al. 2014). Thus, an average of 2,091 SNPs per panel used in this study provides sufficient coverage for association mapping. Linkage disequilibrium decayed more rapidly in the combined panels (2R and 6R) compared to the individual panels. Nordborg et al. (2002) have shown that the decay of LD depends on the demographic history of the population and that LD in local populations (e.g., in our case individual breeding programs) may be stronger than global population (e.g., combined 6R and 2R panels). Consistent with our observation, Laido et al. (2014) reported a rapid decay of LD upon pooling of durum wheat varieties with wild and domesticated durum accessions. The average LD for adjacent markers among the single program panels ranged from a minimum of 0.33 in WA2 to a maximum of 0.40 in AB6 (Table 1). In the combined panels, the average adjacent marker LD was slightly higher for 6R compared to 2R. For each association panel, we report the results for only those markers with $-\log P$ value equal or greater than 4 and with at least 10 individuals homozygous for the minor allele to insure adequate reliability of the estimate of the mean of the rare variants.

Comparison of six-row and two-row breeding germplasm

The genetic architecture of malting quality revealed by the two-row and six-row barley panels differed considerably (Fig. 1). We identified more marker-trait associations in the 6R panel (45) compared to the 2R panel (27; Table 3). A complete listing of the results for all markers across individual breeding programs and in the combined six-row and two-row panels is given in Supplemental Table S2. In addition, the numbers of associations detected for specific traits were different between the 6R and 2R panels (Table 3).

No associations in the 6R panel and two associations in the 2R panel were identified for kernel weight. No associations in the 2R panel were identified for kernel plumpness. Only one association was detected for barley protein and one for diastatic power in the 2R panel. Numerous associations were detected for these three traits in the 6R panel. Similar numbers of associations were detected for wort protein, soluble/total protein, and α-amylase in the 6R and 2R panels.

The most striking result is the general lack of coincident QTL between six-row and two-row germplasm (Fig. 1). Only two regions were identified that had common marker-trait associations between 6R and 2R. The telomeric region of the long arm of 5H was associated with kernel plumpness, wort protein, soluble/total protein, α-amylase in the 6R panel. The last three traits (wort protein, soluble/total protein, α-amylase), and malt extract and β-glucan were also detected in the 2R panel in that same region. The other region with coincident QTL was located on 7H where kernel plumpness and malt extract were identified in 6R and kernel plumpness was identified in 2R. All of the other QTL detected were specific to either two-row or six-row germplasm.

Since the allele frequencies and LD could differ between the 2R and 6R panels, it is possible that identical markers may not be significant in both panels, but closely linked markers may be significant. We identified 51 marker-trait associations, with $-\log P > 4$ and minor allele frequency >0.05 that were identified in either the 6R or 2R panel, but not in both (Table S2). These included associations for all traits except for kernel weight and kernel plumpness. In 29 out of the 51 cases (57 %), a significant association with a marker within 5 cM was detected, indicating the presence of the QTL in both panels although associated with different SNP markers. In 22 cases (43 %), no marker-trait association was identified within 5 cM, indicating that the QTL is specific to only one panel. These 22 marker-trait associations represent a total of seven regions of which two were identified in 2R but not in 6R and five were identified in 6R but not in 2R. The two regions identified only in 2R include the telomeric region of chromosome 5H associated with malt extract and β-glucan and 3H (95 cM) associated with diastatic power. The five regions identified only in 6R include chromosome 4H (28 cM) associated with barley protein and wort protein, 6H (50 cM) associated with wort protein, the short arm of 5H (1 cM) associated with diastatic power, 6H (50 cM) associated with diastatic power, and 6H (81 cM) associated with α-amylase.

Consistency of marker-trait associations among individual breeding programs

The number of markers associated with one of the traits in individual breeding programs ranged from zero in many cases to 33 for diastatic power in the ND6 program (Table 3). Many of the markers associated with diastatic power were also associated with barley protein in the ND6 program and were localized to a region spanning from 50 to 65 cM on 6H (Table S2). This region appears to be of particular importance to only the ND6 program for these two traits. Overall, the ND2 panel detected the fewest marker-trait associations while ND6 detected the most (Table 3).

In the six-row breeding programs, there were two regions where QTL for the same trait were detected by more than one program (Table 4). One region on 5H was detected for α-amylase by markers that are about 2 cM apart (12_31292 and11_10401) in the MN6 and AB6 programs, respectively. However, the effect of the allele is in the opposite direction between the two programs suggesting that these may be two tightly linked QTL. Similarly, a region on 4H was detected for wort protein in the AB6 and OR6 panels. These markers (12_30540 and 11_10221) are 8 cM apart and thus they could also represent tightly linked QTL. In the two-row breeding programs, there were three regions where QTL for the same trait were detected by more than one program. The region on 5H that was associated with the five traits (wort protein, soluble/total protein, α-amylase, malt extract and β-glucan; 183–188 cM), was consistently detected across all four two-row panels for four of the five traits. Nearby on 5H (172–176 cM), two QTL were detected for wort protein in the WA2 and AB2 panels. The third region on 4H (142–144 cM) is associated with diastatic power in the MT2 and ND2 panels.

To quantify consistency of marker-trait associations between pairs of breeding programs, we calculated the correlation of $-\log P$ values for markers across the genome between each pair of breeding programs. We removed those with a $-\log P$ less than 1.3 (corresponding to a comparison-wise P value of $P = 0.05$) in any of the programs and displayed it as a heatmap (Fig. 2). In general, correlations of marker scores were low indicating that marker-trait associations are generally specific to each breeding program. An exception was for Wort protein, α-amylase and beta-glucan among the AB2, MT2, and WA2 panels where the correlations were quite high. This suggests that genetic control of these three traits is very similar for these three breeding programs. However, for the vast majority of traits different markers are associated with the traits in different breeding programs.

A possible explanation for the lack of consistency of QTL detection among breeding program panels is variation in minor allele frequency affecting power of detection. There are numerous cases where a QTL is detected in one breeding program panel and that marker is fixed or at a very low allele frequency in other programs (Table 5). For example, a strong association was detected for kernel weight on chromosome 2H (98 cM) in the MT2 program. However, this marker was fixed for the high kernel weight allele in the other two-row programs. Another example is the barley protein QTL on 4H (28 cM). In this case, the QTL was detected in the MN6 program and fixed for the high protein allele in the other six-row programs. In contrast, the QTL for alpha-amylase on 6H (11_10331) was segregating in the MN6, AB6 and OR6 breeding programs, but only detected in MN6 suggesting that either the genetic

Table 4 Significant marker-trait associations identified in each breeding program for nine malting quality traits

Program[a]	SNP	Chr	Map position[b]	−log*P* (*N*[c])	Trait[d]	PM[e]	ME[f]	Resides in
AB6								
	11_20145[h]	4H	1.19	5.13 (12)	BG	240.9	−85.3	×[g]*QBgnm.StMo-4H(malt)*
	11_10208[h]	4H	2.58	4.98 (14)	BG	240.9	78.3	×*QBgnm.StMo-4H (malt)*
	12_30540	4H	14.74	4.52 (27)	WP	4.4	−0.2	*QDp.StMo-4H; QBgnm.StMo-4H*
	11_10401	5H	185.51	4.54 (43)	AA	62.8	−5.2	×*QAa.StMo-5H.2;* ×*QAa.HaMo-5H;QS/T.HaMo-5H*
	12_10857	5H	187	4.57 (47)	AA	62.8	−4.8	×*QAa.StMo-5H.2;* ×*QAa.HaMo-5H;QS/T.HaMo-5H*
	11_10003	6H	55.55	4.61 (46)	DP	117.0	8.9	*QGpc.DiMo-6H*
	11_20835	6H	60.1	4.34 (46)	DP	117.0	8.6	*QGpc.DiMo-6H*
	11_20184	6H	60.63	4.47 (48)	DP	117.0	8.7	*QGpc.DiMo-6H*
MN6								
	11_10176	1H	58.06	5.06 (66)	BG	190.9	32.5	
	11_20302	4H	27.75	6.07 (141)	BP	13.2	0.3	
	11_11200	5H	108.49	4.34 (31)	PL	89.9	2.6	
	11_10310	5H	181.49	5.66 (15)	PL	89.9	−4.0	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_31292	5H	183.33	4.78 (156)	ST	43.3	1.0	*QAa.StMo-5H.2; QAa.HaMo-5H;* ×*QS/T.HaMo-5H*
	12_31292	5H	183.33	5.01 (156)	AA	71.2	2.4	×*QAa.StMo-5H.2;* ×*QAa.HaMo-5H;QS/T.HaMo-5H*
	12_30360	5H	185.51	4.49 (78)	WP	5.5	−0.2	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	11_10401	5H	185.51	5.53 (84)	ST	43.3	−1.4	*QAa.StMo-5H.2; QAa.HaMo-5H;* ×*QS/T.HaMo-5H*
	12_30360	5H	185.51	4.71 (78)	ST	43.3	−1.3	*QAa.StMo-5H.2; QAa.HaMo-5H;* ×*QS/T.HaMo-5H*
	11_10401	5H	185.51	4.79 (84)	AA	71.2	−3.1	×*QAa.StMo-5H.2;* × *QAa.HaMo-5H;QS/T.HaMo-5H*
	12_30382	5H	186.35	4.46 (18)	ST	43.3	−2.2	*QAa.StMo-5H.2; QAa.HaMo-5H;* × *QS/T.HaMo-5H*
	12_30382	5H	186.35	4.26 (18)	AA	71.2	−5.1	×*QAa.StMo-5H.2;* ×*QAa.HaMo-5H;QS/T.HaMo-5H*
	12_10857	5H	187	4.42 (82)	WP	5.5	−0.2	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_10857	5H	187	5.96 (82)	ST	43.3	−1.5	*QAa.StMo-5H.2; QAa.HaMo-5H;* × *QS/T.HaMo-5H*
	11_10331	6H	91.86	4.72 (33)	AA	71.2	4.8	
	11_21404	6H	93.33	6.15 (29)	AA	71.2	6.1	
	12_30879	7H	55.14	5.28 (10)	ME	78.7	−1.5	×*QMe.StMo-7H; QBgnm.StMo-7H.1, QKp.HaMo-7H; QDp.HaMo-7H;QGpc.HaMo-7H*
ND6								
	12_30948	1H	15.76	4.16 (10)	BP	13.7	−0.4	*QMe.StMo-1H.2; QKp.HaMo-1H.1*
	12_30456	5H	106.22	5.38 (12)	ME	78.5	−0.8	
	12_30456	5H	106.22	4.01 (12)	AA	76.6	6.5	
	11_10954	6H	58.72	6.06 (20)	BP	13.7	0.9	×*QGpc.DiMo-6H*
	12_30346	6H	65.24	5.68 (21)	BP	13.7	0.8	
	11_10954	6H	58.72	4.79 (20)	DP	175.7	24.2	*QGpc.DiMo-6H*
	12_30346	6H	65.24	6.13 (21)	DP	175.7	24.2	
OR6								
	12_31152	1H	104.82	4.46 (41)	ME	78.5	−0.7	×*QMe.StMo-1H.4;* ×*QMe.HaMo-1H.2; QMe.*Gutiérrez et al. (2011)
	12_10905	1H	115.12	4.61 (29)	ME	78.5	−0.8	×*QMe.HaMo-1H.2*
	11_10338	1H	117.91	4.29 (24)	ME	78.5	−0.8	×*QMe.HaMo-1H.2*
	11_10221	4H	22.72	4.8 (72)	WP	4.2	0.2	*QAa.StMo-4H.1; QMe.StMo-4H; QDp.StMo-4H; QGpc. HaMo-4H; QS/T.HaMo-4H; QAa.HaMo-4H*
	11_20013	4H	145.16	4.98 (13)	DP	120.2	−16.0	*QGpc.DiMo-4H*
	11_10331	6H	91.86	4.25 (68)	ME	78.5	0.7	
AB2								
	11_10837	2H	41.63	4.4 (13)	KW	39.7	1.6	*Qme.DiMo-2H*
	11_10283	3H	178.5	4.46 (29)	ME	80.4	−0.5	
	11_20022	5H	176.3	4.01 (39)	WP	4.4	−0.2	*QAa.StMo-5H.2*

Table 4 continued

Program[a]	SNP	Chr	Map position[b]	−log*P* (*N*[c])	Trait[d]	PM[e]	ME[f]	Resides in
	12_30360	5H	185.51	4.82 (36)	WP	4.4	−0.3	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_30360	5H	185.51	6.14 (36)	AA	66.6	−8.2	*×QAa.StMo-5H.2; ×QAa.HaMo-5H;QS/T.HaMo-5H*
	12_30382	5H	186.35	4.8 (56)	ME	80.4	−0.6	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_30382	5H	186.35	8.03 (56)	WP	4.4	−0.3	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_30382	5H	186.35	12.61 (56)	ST	41.8	−3.4	*QAa.StMo-5H.2; QAa.HaMo-5H; × QS/T.HaMo-5H*
	12_30382	5H	186.35	9.04 (56)	AA	66.6	−8.9	*×QAa.StMo-5H.2; ×QAa.HaMo-5H;QS/T.HaMo-5H*
	12_30382	5H	186.35	4.29 (56)	BG	230.8	57.8	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_10322	5H	187.52	7.86 (68)	ME	80.4	0.8	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_10322[i]	5H	187.52	12.9 (68)	WP	4.4	0.4	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_10322[i]	5H	187.52	18.88 (68)	ST	41.8	4.0	*QAa.StMo-5H.2; QAa.HaMo-5H; × QS/T.HaMo-5H*
	12_10322[i]	5H	187.52	18.85 (68)	AA	66.6	12.4	*×QAa.StMo-5H.2; ×QAa.HaMo-5H;QS/T.HaMo-5H*
	12_10322	5H	187.52	4.08 (68)	BG	230.8	−55.8	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_31123[i]	5H	188.18	12.27 (67)	WP	4.4	0.4	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_31123[i]	5H	188.18	16.41 (67)	ST	41.8	3.8	*QAa.StMo-5H.2; QAa.HaMo-5H; ×QS/T.HaMo-5H*
	12_31123[i]	5H	188.18	16.85 (67)	AA	66.6	11.9	*×QAa.StMo-5H.2; ×QAa.HaMo-5H;QS/T.HaMo-5H*
MT2								
	12_30336	1H	34.8	5.38 (22)	PL	84.2	5.5	*QMe.StMo-1H.3*
	11_20340	2H	97.85	7.22 (15)	KW	37.6	−5.7	*Qme.DiMo-2H*
	11_10065	2H	146.05	4.78 (20)	KW	37.6	−3.5	
	12_10915	2H	153.2	5.4 (14)	KW	37.6	−5.0	
	11_10380	3H	62.68	7.22 (15)	KW	37.6	−4.4	*QBgnm.StMo-3H*
	11_10631	3H	152.22	4.24 (31)	KW	37.6	1.9	*QS/T.DiMo-3H*
	12_30239	4H	142.06	4.24 (71)	BG	313.9	60.3	*QDp.DiMo-4H*
	12_30382	5H	186.348	6.4 (135)	WP	4.4	−0.3	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_30382	5H	186.348	6.14 (135)	ST	37.1	−2.2	*QAa.StMo-5H.2; QAa.HaMo-5H; ×QS/T.HaMo-5H*
	12_30382	5H	186.348	7.32 (135)	AA	59.7	−7.2	*×QAa.StMo-5H.2; ×QAa.HaMo-5H; QS/T.HaMo-5H*
	11_20402	5H	187	7.7 (139)	ME	78.1	0.7	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	11_20402	5H	187	15.57 (139)	WP	4.4	0.4	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	11_20402	5H	187	14.74 (139)	ST	37.1	3.1	*QAa.StMo-5H.2; QAa.HaMo-5H; ×QS/T.HaMo-5H*
	11_20402	5H	187	16.46 (139)	AA	59.7	10.0	*×QAa.StMo-5H.2; ×QAa.HaMo-5H; QS/T.HaMo-5H*
	12_10322	5H	187.52	7.99 (136)	ME	78.1	0.7	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_10322	5H	187.52	16.96 (136)	WP	4.4	0.4	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_10322	5H	187.52	15.29 (136)	ST	37.1	3.2	*QAa.StMo-5H.2; QAa.HaMo-5H; ×QS/T.HaMo-5H*
	12_10322	5H	187.52	19.37 (136)	AA	59.7	10.9	*×QAa.StMo-5H.2; ×QAa.HaMo-5H; QS/T.HaMo-5H*
	12_31123	5H	188.18	7.37 (138)	ME	78.1	−0.7	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_31123	5H	188.18	15.24 (138)	WP	4.4	0.4	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_31123	5H	188.18	12.8 (138)	ST	37.1	2.9	*QAa.StMo-5H.2; QAa.HaMo-5H; ×QS/T.HaMo-5H*
	12_31123	5H	188.18	16.89 (138)	AA	59.7	10.2	*×QAa.StMo-5H.2; ×QAa.HaMo-5H; QS/T.HaMo-5H*
	11_11348	7H	64.4	4.92 (14)	KW	37.6	−4.3	*QKp.HaMo-7H;QDp.HaMo-7H;QGpc.HaMo-7H*
	11_10534	7H	81.67	4.76 (48)	BG	313.9	−81.1	*QMe.StMo-7H.3; QDp.StMo-7H.12; QAa.StMo-7H.1; QKp.HaMo-7H; QDp.HaMo-7H; QGpc.HaMo-7H*
ND2								
	11_20089	4H	144.39	4.27 (36)	DP	120.2	−8.0	*×QDp.DiMo-4H*
	11_20013	4H	145.16	4.61 (36)	DP	120.2	−8.3	*×QDp.DiMo-4H*
WA2								
	11_21038	1H	121.24	4.28 (10)	WP	4.1	−0.4	*QMe.HaMo-1H.2*
	11_10387	4H	129.64	4.04 (108)	DP	110.3	−6.0	*×QDp.DiMo-4H*
	11_21130	4H	135.98	6.39 (37)	DP	110.3	−9.5	*×QDp.DiMo-4H*
	11_20553	5H	1.9	4.4 (42)	KW	38.4	0.9	

Table 4 continued

Program[a]	SNP	Chr	Map position[b]	−log*P* (*N*[c])	Trait[d]	PM[e]	ME[f]	Resides in
	12_11450[j]	5H	167.62	4.42 (57)	ST	32.9	−1.7	×*QAa.StMo-5H.2*
	12_30656[j]	5H	168.19	4.62 (55)	ST	32.9	1.6	×*QAa.StMo-5H.2*
	11_10736	5H	170.03	5.75 (54)	ST	32.9	1.976	×*QAa.StMo-5H.2*
	12_30504	5H	171.94	4.1 (78)	WP	4.1	−0.2	*QAa.StMo-5H.2*
	12_31292	5H	183.329	4.05 (104)	WP	4.1	−0.1	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_31292	5H	183.329	5.91 (104)	ST	32.9	−1.5	*QAa.StMo-5H.2; QAa.HaMo-5H; ×QS/T.HaMo-5H*
	12_31292	5H	183.329	4.52 (104)	AA	56.4	−4.7	×*QAa.StMo-5H.2; ×QAa.HaMo-5H; QS/T.HaMo-5H*
	11_10401	5H	185.512	4.75 (91)	ME	76.9	−0.5	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	11_10401	5H	185.512	8.47 (91)	WP	4.1	−0.2	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	11_10401	5H	185.512	8.36 (91)	ST	32.9	−1.8	*QAa.StMo-5H.2; QAa.HaMo-5H; ×QS/T.HaMo-5H*
	11_10401	5H	185.512	9.23 (91)	AA	56.4	−6.9	×*QAa.StMo-5H.2; ×QAa.HaMo-5H; QS/T.HaMo-5H*
	11_10401	5H	185.512	5.44 (91)	BG	200.0	38.6	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	11_20402	5H	187	5.38 (77)	ME	76.9	0.5	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	11_20402	5H	187	12.56 (77)	WP	4.1	0.3	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	11_20402	5H	187	12.08 (77)	ST	32.9	2.3	*QAa.StMo-5H.2; QAa.HaMo-5H; ×QS/T.HaMo-5H*
	11_20402	5H	187	16.28 (77)	AA	56.4	9.5	×*QAa.StMo-5H.2; ×QAa.HaMo-5H; QS/T.HaMo-5H*
	11_20402	5H	187	4.23 (77)	BG	200.0	−36.0	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_10322	5H	187.52	5.04 (76)	ME	76.9	0.5	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_10322	5H	187.52	12.57 (76)	WP	4.1	0.3	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_10322	5H	187.52	12.15 (76)	ST	32.9	2.2	*QAa.StMo-5H.2; QAa.HaMo-5H; ×QS/T.HaMo-5H*
	12_10322	5H	187.52	16.68 (76)	AA	56.4	9.7	×*QAa.StMo-5H.2; ×QAa.HaMo-5H; QS/T.HaMo-5H*
	12_10322	5H	187.52	4.13 (76)	BG	200.0	−34.8	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_31123	5H	188.18	10.01 (84)	WP	4.1	0.2	*QAa.StMo-5H.2; QAa.HaMo-5H;QS/T.HaMo-5H*
	12_31123	5H	188.18	13.04 (84)	AA	56.4	8.3	×*QAa.StMo-5H.2; ×QAa.HaMo-5H; QS/T.HaMo-5H*

[a] USDA at Aberdeen, ID (AB6), University of Minnesota (MN6), North Dakota State University (ND6), and Oregon State University (OR6) and two-row spring breeding programs i.e., USDA at Aberdeen, ID (AB2), Montana State University (MT2), North Dakota State University (ND2), and Washington State University (WA2)

[b] Map positions are based on Muñoz-Amatriaín et al. (2011)

[c] Number of lines homozygous for the minor allele

[d] *KW* Kernel weight, *PL* kernel plumpness, *ME* malt extract, *BP* barley protein, *WP* wort protein, *ST* soluble/total ratio, *DP* diastatic power, *AA* α-amylase and *BG* β-glucan

[e] PM represents population average of BLUE estimates for each trait and association panel combination

[f] ME stands for marker effect

[g] "×" denotes the QTL controlling the same trait that has been identified in the previous bi-parental mapping populations

[h] The LD between 11_20145 and 11_10208 in AB6 is $D' = -1$ ($R^2 = 1$). These markers likely represent two distinct haplotypes at a single locus rather than two tightly linked loci with opposite marker effects

[i] The LD between 12_10322 and 12_31123 in AB2 is $D' =$ ~0.98 ($R^2 =$ ~0.98). These markers likely represent two distinct haplotypes at a single locus

[j] The LD between 12_11450 and 12_30656 in WA2 is $D' = -1$ ($R^2 =$ ~0.98). These markers likely represent two distinct haplotypes at a single locus rather than two tightly linked loci with opposite marker effects

background or genotype by environment interaction could be important.

QTL detection in individual programs compared to combined programs

In general, combining breeding lines from individual programs to form the 6R or 2R panels resulted in losing the signal of many markers that were detected in individual programs (Fig. 3). Of 105 marker-trait associations detected in individual six-row breeding programs, only 21 remained significant in the combined 6R panel analysis. Similarly, when we combined the four two-row breeding programs to make the joint 2R panel, out of 72 significant marker-trait associations in individual two-row breeding programs, only 19 remained significant. However, combining individual programs also resulted in detecting 23 and 8 new marker-trait associations in the 6R and 2R panels, respectively.

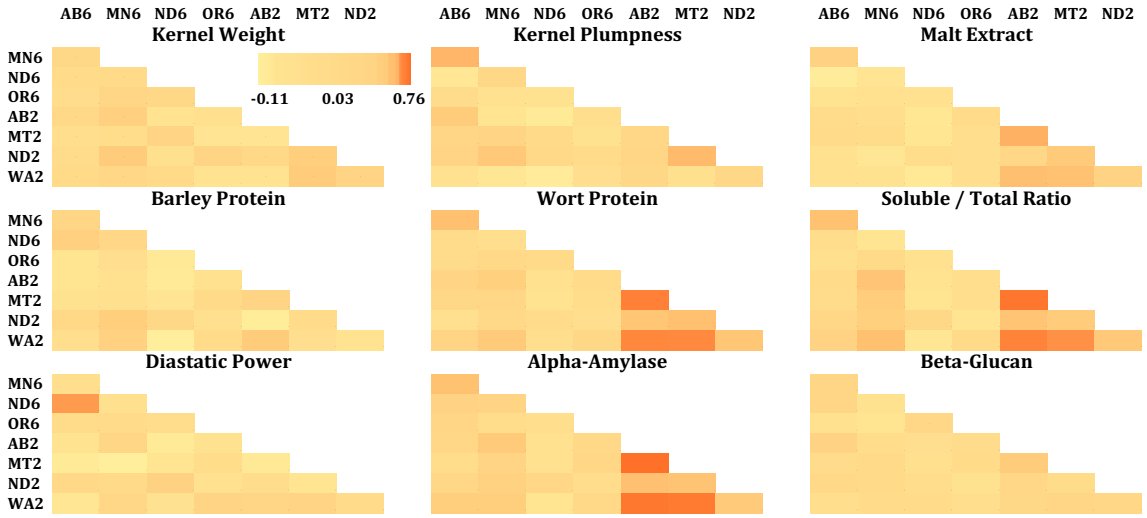

Fig. 2 Heatmap of pair-wise Pearson's correlation coefficients of $-\log P$ values for marker associations between pairs of breeding programs for malt quality traits

Detection of QTL reported in previous studies

We detected marker-trait associations in 11 of the 70 QTL previously reported for malting quality traits in the three bi-parental populations, Dicktoo × Morex, Steptoe × Morex, and Harrington × Morex (Fig. 1). Six QTL (i.e., *QAa.HaMo-5H, QAa.StMo-5H.2, QBgnm.StMo-4H (malt), QS/T.HaMo-5H, QMe.StMo-7H,* and*QGpc.DiMo-6H*) were identified in at least one individual breeding program and one of the combined panels. Three QTL (i.e., *QDp.DiMo-4H, QMe.HaMo-1H.2,* and *QMe.StMo-1H.4*) were specific to individual association panels while two QTL (i.e., *QKp.HaMo-7H*and *QKp.StMo-3H*) were specific to the 6R panel. The kernel plumpness QTLs we detected on 3H and 7H using the 6R panel were also detected in the Steptoe x Morex population (*QKp.StMo-3H* and *QKp.HaMo-7H*). We detected a region associated with α-amylase and soluble/total protein in both the 6R and 2R panels that was previously detected in Steptoe × Morex and Harrington × Morex. The barley protein QTL we detected on 6H using the 6R panel was previously mapped in Dicktoo × Morex. Last, the malt extract QTL we detected on 7H using the 6R panel was previously identified in Steptoe × Morex. Most malt quality QTL detected in previous wide cross mapping populations were not detected in the 6R and 2R panels.

Similarly, when we examine the results of the individual breeding programs, less than half of the marker-trait associations identified in our study were identified in the previous bi-parental mapping studies (Table 3). In the 6R panel, 53 of the 116 associations we detected were identified in previous studies. In the 2R panel, 37 of the 107 associations we detected were identified in previous studies. Thus, in this study, we failed to detect many QTL that were identified in prior mapping studies, but also identified numerous QTL that have not been previously described.

Interestingly, some of the QTL identified in previous bi-parental studies for a given trait were significant for other traits in our study. Markers significantly associated with more than one malting quality trait are listed in Supplemental Table S5. For example, we identified a QTL for grain protein and malt extract in the 6R panel at 12_10811 (49 cM) on 6H, which was previously identified for grain protein content (*QGpc.DiMo-6H*). We also identified a QTL for diastatic power in the 2R panel at 11_21493 (114 cM) on 3H, which was previously associated with only grain protein content (*QGpc.StMo-3H.1*).

In a recent association mapping study Gutiérrez et al. (2011) analysed three populations of sizes 79, 71, and 96 from the Oregon State University program that overlap with the data that we used to construct OR6. They mapped marker-trait associations for five malting quality traits (barley protein, malt extract, diastatic power, α-amylase, and β-glucan). For malt extract, they identified three SNPs located at 101–130 cM of chromosome 1H. Similarly, we identified three SNPs associated with malt extract in OR6 located at 104, 110, and 115 cM on chromosome 1H. They also detected four markers associated with β-glucan, which we did not identify in our analysis. Similar to our study, they did not find any associations for barley protein, diastatic power, or α-amylase.

Table 5 Distribution of marker alleles that were significant in at least one breeding program mapping panel, but fixed in other panels

SNP	Chr	Map pos	Trait[a]	Panels[b]	−log*P*	Allele[c]	Num. of lines with minor allele[d]
11_20340	2H	97.85	KW	AB2		**G**	
				MT2	7.22	T/**G**	15 (T)
				ND2		**G**	
				WA2		**G**	
11_10065	2H	146.05	KW	AB2		**C**	
				MT2	4.78	T/**C**	20 (T)
				ND2	0.35	T/**C**	1 (T)
				WA2	3.16	T/**C**	6 (T)
12_30680	3H	61.38	KW	AB2	0.45	A/**G**	3 (A)
				MT2	7.22	A/**G**	15 (A)
				ND2	0.31	A/**G**	8 (A)
				WA2		**G**	
12_30367	3H	148.11	KW	AB2	0.50	**A**/T	6 (T)
				MT2	7.22	**A**/T	15 (T)
				ND2	0.08	**A**/T	1 (T)
				WA2		**A**	
11_11348	7H	64.40	KW	AB2	0.01	T/**C**	2 (T)
				MT2	4.92	T/**C**	14 (T)
				ND2	0.48	T/**C**	11 (T)
				WA2		**C**	
11_20302	4H	27.75	BP	AB6		**C**	
				MN6	6.07	T/**C**	141 (T)
				ND6	0.05	T/**C**	1 (T)
				OR6	0.49	T/**C**	21 (T)
12_10199	6H	49.23	BP	AB6	2.87	A/**G**	42 (A)
				MN6		**G**	
				ND6	5.99	A/**G**	20 (A)
				OR6		**G**	
12_11353	6H	55.55	BP	AB6	1.34	T/**C**	42 (T)
				MN6		**C**	
				ND6	5.99	T/**C**	20 (T)
				OR6	0.28	T/**C**	12 (T)
12_31003	6H	64.07	BP	AB6	0.51	A/**C**	39 (A)
				MN6		**C**	
				ND6	5.68	A/**C**	21 (A)
				OR6	0.25	A/**C**	19 (A)
12_10199	6H	49.23	DP	AB6	3.27	A/**G**	42 (A)
				MN6		**G**	
				ND6	4.77	A/**G**	20 (A)
				OR6		**G**	
12_11353	6H	55.55	DP	AB6	4.49	T/**C**	42 (T)
				MN6		**C**	
				ND6	4.77	T/**C**	20 (T)
				OR6	0.23	T/**C**	12 (T)
12_31003	6H	64.07	DP	AB6	2.55	A/**C**	39 (A)
				MN6		**C**	
				ND6	6.13	A/**C**	21 (A)

Table 5 continued

SNP	Chr	Map pos	Trait[a]	Panels[b]	−log*P*	Allele[c]	Num. of lines with minor allele[d]
				OR6	0.31	A/**C**	19 (A)
11_10331	6H	91.86	AA	AB6	0.64	**T**/C	24 (C)
				MN6	4.72	**T**/C	33 (C)
				ND6		**T**	
				OR6	0.51	**T**/C	68 (C)
11_10176	1H	58.06	BG	AB6		**G**	
				MN6	5.06	C/**G**	66 (C)
				ND6		**G**	
				OR6		**G**	

[a] *KW* Kernel weight, *PL* kernel plumpness, *ME* malt extract, *BP* barley protein, *WP* wort protein, *ST* soluble/total ratio, *DP* diastatic power, *AA* α-amylase and *BG* β-glucan

[b] USDA at Aberdeen, ID (AB6), University of Minnesota (MN6), North Dakota State University (ND6), and Oregon State University (OR6) and two-row spring breeding programs i.e., USDA at Aberdeen, ID (AB2), Montana State University (MT2), North Dakota State University (ND2), and Washington State University (WA2)

[c] Alleles present in the panel. A single nucleotide state indicates that allele is fixed. The allele responsible for higher trait level is shown in bold

[d] Number of lines carrying the minor allele. The nucleotide state of minor allele is in parentheses

Discussion

Using breeding data to map QTL

Breeding data sets are extremely valuable and yet highly underutilized resources for the exploration of genetic architecture for a wide array of crop traits. This study involved 1,862 breeding lines, 97 field trials, and nearly 5,000 grain samples that were analysed for malting quality. A conservative cost estimate of 100 dollars per sample to conduct the micro-malting analysis alone would make a de novo study of this sort impractical. Exploiting historical breeding data for malting quality allowed us to confirm previously mapped QTL and identify new QTL. Comparing results across breeding programs gives further support and independently validates QTL. One challenge of using historical data sets is that they are frequently unbalanced and therefore could result in false identification of QTL (Wang et al. 2012). Therefore, a comprehensive review of QTL results across mapping panels taking into account population size and allele frequencies is necessary to identify QTL with confidence. The AB6 panel had the smallest population size (98) and also detected relatively few QTL. Small population sizes not only affect the estimates of LD (Slate and Pemberton 2007), but also can result in low power to detect QTL and inflated QTL effects (Vales et al. 2005; Beavis 1994, 1997; Melchinger et al. 1998). Increasing population size would likely identify QTL with smaller effects, QTL that are not in high LD with the markers, and QTL associated with marker alleles at lower frequencies (Zhu et al. 2008).

We increased population size by combining six-row breeding populations and by combining two-row breeding populations with the primary goal of increasing the power to detect marker-trait associations. As a consequence of combining samples, we lost about 66 % of the signals that were detected in individual breeding programs suggesting that increasing sample size by pooling samples from different breeding programs does not necessarily increase the power to detect marker-trait associations. On the other hand, we did detect some new QTL using the combined data sets. This suggests that multiple approaches to analysing data sets such as these are warranted. Because marker allele classes are often highly skewed, combining data sets will change allele frequencies and possibly affect detection power.

Diversity for QTL among breeding programs

The eight breeding programs included in this study devote significant resources to developing malting barley cultivars. Malting barley, which must meet industry standards for a complex set of traits, is clearly distinguishable from barley developed for feed or food end-uses. The most significant difference between malt and feed barley is that feed barley fails to produce moderate to high levels of hydrolytic enzymes needed during malting and mashing to break down endosperm (Fox et al. 2004). Feed barley checks included in malting quality evaluations typically exhibit malt parameters far outside acceptable industry standards. There is general agreement among malt end-users on several malt parameters (e.g., higher malt extract, lower

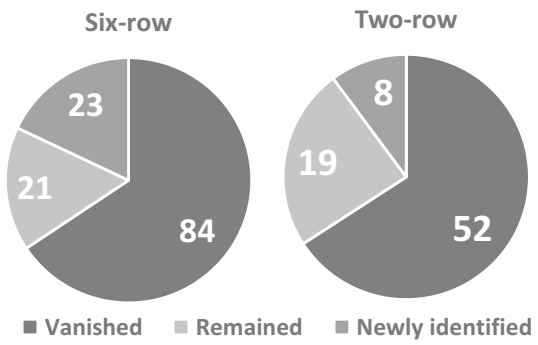

Fig. 3 Number of marker-trait associations that (a) vanished—detected in the individual program panels and not detected in the combined panels, (b) remained—detected in both combined and individual panels, or (c) newly identified—only identified after combining individual breeding programs

β-glucan, and moderate grain protein concentration). However, differences in beer style and the use of adjuncts have resulted in different requirements for some malt parameters (e.g., diastatic power). It is therefore interesting that multiple breeding programs emphasizing similar targets for malt quality would result in different QTL segregating within their programs. In many cases, this was the result of fixation of the favorable allele within some breeding programs but not others. There is typically limited germplasm exchange among breeding programs. Thus, even with similar selection pressure for malting quality, the combination of different parental founders and drift due to small breeding population sizes could lead to differences in the alleles that become fixed in each of the programs. Also, even though selection for malting quality may be similar for all of the programs, selection for adaptation to target regions for variety deployment is not similar and could therefore lead to differential selection of quality alleles through linkage to local adaptation genes.

Implications for breeding and selection

This study revealed that some markers are associated with multiple malting quality traits in the germplasm tested and therefore impose some challenges to trait improvement. For example, a genomic region on 5H is associated with soluble/total protein and α-amylase. The SNP 12_31123 was highly associated ($-\log P = 7.37$) with malt extract in two-row barley germplasm from MT2. The same marker was significantly ($-\log P = 16.89$) associated with α-amylase. Selection for the high allele for malt extract at this marker would decrease α-amylase to a large extent (Table 3). Depending on the malt end-user, this could be desirable or undesirable. If it is necessary to increase the level of both traits, it will be necessary to determine if the association is due to tight linkage or pleiotropy. Generating large numbers of recombinant progeny in this region may be necessary to separate linked alleles and reassemble them in a favorable linkage block.

One of the goals of the Barley CAP was to create a national infrastructure for barley breeding and a shared trait and marker database to facilitated germplasm exchange. Typically elite lines are exchanged among breeding programs based on performance in regional trials. The easy availability of trait and genotype information on a large set of U.S. elite barley breeding germplasm coupled with QTL information on target traits allows for more informed exchange of germplasm. Breeders can select high performing lines that carry alleles at QTL for target traits that are more favorable than those present in their current breeding germplasm.

A general strategy for using this QTL information in breeding would be to enrich or fix favorable alleles that are segregating in the current breeding program germplasm. This could be done by screening parents for informative markers and designing crosses that fix or enrich the favorable allele in the resulting progeny. An example of this would be selecting for the low β-glucan allele using the marker 11_10176 in the MN6 program (Table 5) which would result in about 15 % reduction in β-glucan content (Table 4). Since the other six-row programs lack the low β-glucan allele for this marker, they could select a parent from the Minnesota program with the low allele to introduce it into their breeding population.

Breeders are generally cautious about making wider crosses such as between two-row and six-row lines or winter and spring habit. In this study, we did not generate a combined two-row and six-row panel because these two sets of lines were evaluated in entirely different trials without any common checks. However, the general lack of congruence of malting quality QTL detected in the six-row and two-row panels suggests the opportunity to make gains by crosses between the two germplasm groups. However, it is also likely that such crosses will produce segregation at numerous quality QTL and that large populations will be necessary to recover recombinants with the favorable allele at all or most of the loci. It should be possible to use the marker data to select combinations of two-row and six-row parents that carry the favorable allele at as many QTL as possible to minimize the number of loci that will segregate. Of course it is also important to note that there will likely be segregation at loci for other traits like yield and disease resistance that will further complicate selecting favorable recombinants.

Malting quality is genetically complex and the acceptable range of values for malting quality traits can vary depending on the end-user. Developing new varieties that meet industries changing needs and are productive in the target growing region will require continuous breeding

effort. The enhanced understanding of the genetic architecture of malting quality traits provided through this large collaborative study should help breeders maintain favorable alleles currently fixed in breeding populations and identify opportunities to introduce new genetic diversity to further improve malting quality.

Author contribution statement KS, PH, RD, DO, TB, and SU developed the breeding lines. AB conducted the malting quality analysis. SC conducted the marker genotyping. MM conducted the data analysis and generated figures and tables. KS and MM wrote the manuscript. All the authors have read manuscript, provided feedback and approved the final manuscript.

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Conflict of interest The authors declare that they have no conflict of interest.

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