Published July, 2009

ORIGINAL RESEARCH

An Integrated Resource for Barley Linkage Map and Malting Quality QTL Alignment

Péter Szűcs, Victoria C. Blake, Prasanna R. Bhat, Shiaoman Chao, Timothy J. Close, Alfonso Cuesta-Marcos, Gary J. Muehlbauer, Luke Ramsay, Robbie Waugh, and Patrick M. Hayes*

Abstract

Barley (*Hordeum vulgare* L.) is an economically important model plant for genetics research. Barley is currently served by an increasingly comprehensive set of tools for genetic analysis that have recently been augmented by high-density genetic linkage maps built with gene-based single nucleotide polymorphisms (SNPs). These SNP-based maps need to be aligned with earlier generation maps, which were used for quantitative trait locus (QTL) detection, by integrating multiple types of markers into a single map. A 2383 locus linkage map was developed using the Oregon Wolfe Barley (OWB) Mapping Population to allow such alignments. The map is based on 1472 SNP, 722 DArT, and 189 prior markers which include morphological, simple sequence repeat (SSR), Restriction Fragment Length Polymorphism (RFLP), and sequence tagged site (STS) loci. This new OWB map forms, therefore, a useful bridge between high-density SNP-only maps and prior QTL reports. The application of this bridge concept is shown using malting-quality QTLs from multiple mapping populations, as reported in the literature. This is the first step toward developing a Barley QTL Community Curation workbook for all types of QTLs and maps, on the GrainGenes website. The OWB-related resources are available at OWB Data and GrainGenes Tools (OWB-DGGT) (http://wheat.pw.usda.gov/ggpages/maps/OWB/).

Published in The Plant Genome 2:134–140. Published 10 July 2009.
doi: 10.3835/plantgenome2008.01.005
© Crop Science Society of America
677 S. Segoe Rd., Madison, WI 53711 USA
An open-access publication

All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Permission for printing and for reprinting the material contained herein has been obtained by the publisher.

BARLEY (*Hordeum vulgare* L.) is a diploid (2n = 14) economically important crop and genetic model system. Just over forty years ago, linkage data were available for only 79 loci in barley (Nilan, 1964). Since then, there has been steady progress in building increasingly dense linkage maps. Most of these maps are curated and available at GrainGenes (http://wheat.pw.usda.gov/GG2/maps.shtml#barley; verified 27 April 2009). Marker systems are increasingly gene-based, with the most recently published high-density map having 1032 expressed sequence tag (EST)-based loci (Stein et al., 2007). A 3000-EST locus map is in press (K. Sato, personal communication, 2009) and a consensus , single nucleotide polymorphism (SNP) map with 2943 loci is available at HarvEST (www.harvest-web.org; verified 27 April 2009). The primary impetus for these linkage mapping efforts was (and remains) gene discovery, which is complicated by the lack of a detailed physical map. The large genome size of barley (5000 Mb) has precluded whole-genome sequencing although recent developments in technology may remove this impediment, as reported by the International Barley Sequencing Consortium (ISBC; http://

P. Szűcs, A. Cuesta-Marcos, and P.M. Hayes, Dep. of Crop and Soil Science, Oregon State Univ., Corvallis OR 97331; V.C. Blake, Dep. of Plant Sciences and Plant Pathology, Montana State Univ., Bozeman, MT 59717; P.R. Bhat and T.J. Close, Dep. of Botany and Plant Sciences, Univ. of California, Riverside, CA 92521; S. Chao, USDA-ARS, Fargo, ND 58105; G.J. Muehlbauer, Dep. of Agronomy and Plant Genetics, Univ. of Minnesota, St. Paul, MN 55108; L. Ramsay and R. Waugh, Genetics Programme, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5NE, Scotland.
Received 18 Jan. 2009. *Corresponding author (patrick.m.hayes@oregonstate.edu).

Abbreviations: DArT, diversity arrays technology; DH, doubled haploid; OPA, oligonucleotide pool assay, EST, expressed sequence tag; MAS, marker-assisted selection; OWB, Oregon Wolfe Barley; OWB-DGGT, Oregon Wolfe Barley Data and GrainGenes Tools; QTL, quantitative trait locus; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; STS, sequence tagged site.

www.public.iastate.edu/~imagefpc/IBSC%20Webpage/IBSC%20Template-home.html; verified 27 April 2009).

Even when the whole-genome sequence is available, a complete genetic tool kit will still include linkage maps. These maps will be most useful when the marker loci can be directly related to DNA sequence. The most recent generation of barley linkage maps fulfills this criterion as the mapped loci are based on SNPs identified in ESTs. Bioinformatics tools allow for identification, or prediction, of the genes in which the mapped SNPs reside. This provides a direct way to systematically assign genes to linkage maps. However, these mapped ESTs, which represent only a subset of the genes in barley, do not provide perfect markers for all traits. For determining the genetic basis of quantitative traits, abundant SNP-based loci provide a useful catalog of markers for quantitative trait locus (QTL) mapping. A subset of the informative SNPs used for QTL mapping can then be readily adapted for subsequent marker-assisted selection (MAS). A concerted international collaborative effort is underway to use association analysis tools for mapping quantitative traits in barley using extensive germplasm arrays genotyped with large numbers of SNPs (reviewed by Hayes and Szűcs, 2006). A necessary foundation for this effort is integration of QTLs mapped via association analysis with the 300+ QTLs reported in the literature. Integration of QTLs identified via association mapping with QTLs detected previously via bi-parental population mapping can be achieved by combining the markers historically used for QTL analysis [e.g. morphological, restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR), and diversity arrays technology (DArT)] with the current generation of SNPs. Automated map alignment tools, e.g. CMap (http://wheat.pw.usda.gov/cmap/; verified 27 April 2009) can then be used to align various maps with subsets of markers in common. For example, QTLs detected using a population genotyped with SSRs can be objectively positioned on a SNP-only map if an intermediate population is used that has both SSRs and SNPs.

The Oregon Wolfe Barley (OWB) population [(Costa et al., 2001) and described at BarleyWorld (http://barleyworld.org/oregonwolfe.php; verified 27 April 2009)] is a particularly useful resource in this regard because its high polymorphism rate has made it an international resource for collaborative mapping of all types of markers. These markers range from twelve easily-scored morphological traits to the SNP and DArT loci described in this report. The objective of this research was thus to integrate new SNP and DArT loci with (i) morphological loci and (ii) representative SSR, RFLP, and STS markers in order to access prior QTL information. Malting quality QTLs were chosen to model this approach, due to their economic and scientific importance. Ultimately, our goal is to provide access to all types of QTLs via the Barley QTL Community Curation Workbook (http://wheat.pw.usda.gov/ggpages/barleyQTLworkbook.xls; verified 27 April 2009).

Materials and Methods

Mapping Population

Allele data for 2383 markers scored on 93 doubled haploid (DH) lines from the OWB mapping population were used for linkage map construction. The OWB mapping population (Costa et al., 2001) was derived from the F_1 of the cross of the Wolfe Dominant × Wolfe Recessive multiple marker stocks using the *Hordeum bulbosum* chromosome elimination technique (Chen and Hayes 1989). The development of the Wolfe Dominant and Recessive marker stocks was described by Wolfe and Franckowiak (1991).

Markers

Of the 2383 markers on the current OWB map, 1472 SNPs and 722 DArTs were mapped de novo. These were added to a framework map consisting of 189 morphological, RFLP, SSR, and STS markers. The process of developing the high confidence SNP markers will be described in detail in a forthcoming report (Tim Close, personal communication, 2009). Briefly, SNPs observed in EST contigs were used to design three Illumina 1536-plex pilot Oligonucleotide Pool Assays (pilot OPAs; POPA1, POPA2, POPA3) (Illumina, San Diego, CA). The three POPAs were used to genotype the OWB population using the Illumina GoldenGate BeadArray SNP detection platform. The genotyping assays were conducted at the Southern California Genotyping Consortium at the University of California, Los Angeles. The SNP loci are designated by their POPA numbers (e.g. 1_1311), where 1 = the POPA number (POPA1 in this case) and the subsequent four digits correspond to the SNP order in the corresponding POPA. The locus designations can be directly referenced to assembly #35 unigene numbers by referring to the barley SNP consensus map at HarvEST (http://harvest.ucr.edu and www.harvest-web.org). For example, 1_1311 maps to the short arm of chromosome 1H and corresponds to assembly #35 unigene 5087. A spreadsheet translating the POPA SNP locus names used in this report to names used on two compressed "production" barley OPAs (BOPA1 and BOPA2) is available at the Oregon Wolfe Barley Data and GrainGenes Tools (OWB-DGGT) site.

The development of barley DArT markers is described in Wenzl et al. (2004). Of the 722 bPb-type DArT marker mapped in the OWB, 458 were used to develop the barley DArT consensus map (Wenzl et al. 2006). The DArT genotyping assays were conducted at Triticarte Pty Ltd, Canberra. The DArT loci were named using bPb designations (e.g. bPb-0018) or by the clone identification numbers [e.g. (104834)] provided by Triticarte.

The 189 non-SNP and non-DArT markers used as a framework are a high-confidence subset of data available at (OWB-DGGT) (http://wheat.pw.usda.gov/ggpages/maps/OWB/). These markers provide anchor points for map integration and landmarks for map comparisons. Standard nomenclature employed in prior reports was used for these loci.

Mapping

JoinMap 4 (Van Ooijen, 2006) was used for map construction. At linkage LOD score 5, the 2383 markers formed seven linkage groups. Linkage groups were assigned to the corresponding barley chromosomes on the basis of the non-SNP and non-DArT markers. The Monte Carlo maximum likelihood (ML) mapping algorithm was used to determine the marker order in each of the seven linkage groups. Recombination frequencies were converted to centiMorgans (cM) using Haldane's mapping function, as implemented in JoinMap 4.

QTL Summary Procedures

Malt quality QTLs, originally compiled and posted at BarleyWorld (http://www.barleyworld.org; verified 27 April 2009), were subsequently curated into GrainGenes QTL records and placed on each of the seven linkage groups. Since there was no precedent, nor policy formulated, for naming QTLs at the onset of this project, we developed the nomenclature based on the following rules (Blake and Blake, 2008). Barley QTL names consist of a "Q", a trait abbreviation, the population abbreviation, and a chromosome number. For example, *QAa.StMo-2H* is a QTL for alpha amylase activity mapped in the Steptoe × Morex population on chromosome 2H.

When the significant marker(s) reported for QTLs were on the OWB map, placement was straightforward. In some cases, the significant marker co-localized with an OPA marker on the HarvEST Integrated Barley Map (http://www.harvest-web.org/hweb/bin/gmap.wc?wsize=1477x851). Most often, it was necessary to determine the approximate position of the QTL on the OWB map by utilizing GrainGenes to find alternative barley maps with both the QTL markers and markers in common with the OWB map. These placement notes, and the bibliography of malting quality QTL reports, are available at OWB-DGGT (http://wheat.pw.usda.gov/ggpages/maps/OWB/).

Results and Discussion

The OWB linkage map described in this report combines the current generation of EST-based SNP markers as reported at HarvEST (http://harvest.ucr.edu) with DArT markers (Wenzl et al. 2006) and different types of molecular and morphological markers, described at BarleyWorld (http://barleyworld.org/) and by Costa et al. (2001). All linkage map images and the marker segregation data are available at OWB-DGGT (http://wheat.pw.usda.gov/ggpages/maps/OWB/).

The 2383 loci (primarily SNP and DArT markers) were mapped in a single population. Therefore, every map position is based on actual recombination data. A large number of OPA and DArT markers co-segregated, as is expected given the large number of loci and the relatively limited scope for recombination in the mapping population. These co-segregating markers can be used to infer corresponding map positions in SNP-only and DArT-only maps. The 2383 markers represent a high quality data set: only 0.24% of the total allele calls were missing. The POPA markers had the least missing data (0.006%). The corresponding percentages of missing data for the DArT and other markers were 0.46 and 1.29%, respectively. The 2383 markers spanned a total linkage distance of 1280 cM. There were no singleton allele calls, i.e., apparent double crossovers in short intervals. Chromosome 5H had the highest number of markers (398), whereas chromosome 1H had the least (253). Excluding the co-segregating markers, the average two-locus interval was 2 cM. Two of the two-locus intervals exceeded 20 cM (24 cM on 2H and 23 cM on 7H) though these distances are probably inflated through the use of the Haldane mapping function. Seventy five percent of the markers (1793) co-segregated with one or more other markers. The largest marker clusters were on chromosomes 7H, 5H, 6H, and 4H (53, 50, 46, and 43 co-segregating markers, respectively). These clusters included markers from all the three marker classes (OPA, DArT, and other). The order of the markers and genetic distances were consistent with previously published OWB maps (Costa et al. 2001; http://wheat.pw.usda.gov/ggpages/maps/OWB/; verified 27 April 2009) and the DArT consensus map (Wenzl et al. 2006).

Using common or nearby markers, 154 malt quality QTLs were placed on the OWB map. Table 1 reports the traits and QTLs on a per-chromosome basis. In some cases, QTLs for the same trait were mapped to the same chromosome location in more than one population. Coincident QTLs could be due to the alleles at the same locus or to tightly linked loci, however, determining QTL allelism is beyond the scope of this research. Therefore, each QTL in each report was assigned a unique identifier and as a consequence the numbers of QTLs reported in Table 1 are likely to be inflated. Grain protein content was the trait with the most QTLs (21). Alpha amylase activity and malt extract were also well-represented, with 20 QTLs each. There was only one QTL for Beta amylase activity (on 4H), which corresponds with the location of the *Bmy1* locus. Beta amylase activity is a component of diastatic power (DP), a trait for which there were 13 QTLs. Chromosome 5H had the most malt-quality QTLs (40) whereas 3H and 6H had the least (12 each).

An example illustrating the utility of the integrated map/QTL resource is shown in Figure 1 and relates to developing malting barley varieties with superior low-temperature tolerance. The principal low-temperature tolerance QTLs (*Fr-H1* and *Fr-H2*) are located on chromosome 5H, with *Fr-H1* being important for both vegetative- and reproductive-stage cold tolerance (Reinheimer et al., 2004; Skinner et al., 2006). Recent studies have identified *Vrn-H1* and a cluster of *HvCBF*-genes as candidate genes for *Fr-H1* and *Fr-H2*, respectively (Francia et al. 2004; Skinner et al. 2005; Skinner et al., 2006; von Zitzewitz et al., 2005). Because low temperature-tolerant germplasm is often deficient in malting quality, more effective breeding strategies for developing low

temperature-tolerant varieties could be based on knowledge of genetic relationships between target traits. As shown in Figure 1, an alignment of the malting quality QTL/OWB map with the abiotic stress QTL/consensus map revealed QTLs for multiple quality traits coincident with *Fr-H1* and *Fr-H2* (between the common markers scsnp06144 and MWG 877). If favorable alleles at the coincident QTL allele trace to parents that have contrasting phenotypes (e.g. good quality/poor cold tolerance vs. poor quality/good cold tolerance), simultaneous improvement for both traits would be a significant challenge. However if the alternative favorable allele configuration is caused by linkage rather than pleiotropy, targeted marker-assisted breeding could be used to break the repulsion linkage.

There is some evidence that repulsion linkage, rather than pleiotropy, underlies the coincident QTLs in this region of chromosome 5H in some winter barley germplasm as breeding lines developed by the Oregon State University Barley Project have good malting quality coupled with good cold tolerance. On the basis of the results of association mapping using the high density OPA SNP consensus map from HarvEST (http://harvest.ucr.edu) these lines have favorable alleles at the 5H malting quality QTL and the low-temperature tolerance QTL (Hayes lab, unpublished data). Presumably, in this germplasm the preexisting negative linkage (and perhaps epistatic) relationships have been broken by repeated cycles of intermating. Another example of sub-optimal combinations of genes controlling malting quality and winter-hardiness in some European material is the tight linkage of *Bmy1* and *Vrn-H2* in the distal region of the long arm of 4H (Forster et al., 1991). *Bmy1* encodes the Beta amylase enzyme whose activity is a component of diastatic power. *Vrn-H2* may be a determinant of low-temperature tolerance in some germplasm (Karsai et al., 2005; von Zitzewitz et al., 2005). Repulsion linkages between these two genes, whose effects appear as coincident QTLs, could also be broken by marker-assisted breeding for target alleles. The selection of sub-optimal combinations of winter-hardiness and malting quality on 4H and 5H probably reflects the results of previous breeding that necessarily focused initially on agronomic performance prior to addressing end-user traits. The integration of molecular marker and QTL data provided in this report provides a means by which such issues can be addressed less empirically in the future.

A second example of the utility of the integrated map/QTL resource relates to the possible integration of the map with the Affymetrix (Santa Clara, CA) Barley I GeneChip as features on the GeneChip are represented in the current OWB map as POPA SNPs. The GeneChip has been used to establish an atlas of gene expression (Druka et al., 2006) against which expression assays of barley genotypes during germination and/or the preliminary steps of malting could be referenced. This would allow for the objective alignment of expression level differences with malting quality QTLs.

Table 1. Summary of malting quality-related QTL assigned map locations using the integrated 2383-locus Oregon Wolfe Barley population map. QTL citations are available at the Oregon Wolfe Barley Data and GrainGenes Tool site (http://wheat.pw.usda.gov/ggpages/maps/OWB/).

Malting quality trait	Chromosome							
	1H	2H	3H	4H	5H	6H	7H	QTL/trait
Alpha amylase activity	2	2	1	3	7	3	2	20
Beta amylase activity				1				1
Beta glucan (grain)	1	2						3
Beta glucan (malt)	2		3	1	2		2	10
Beta glucan (wort)					1			1
Beta glucanase activity	2			1	2			5
Beta glucanase activity (green malt)	1							1
Beta glucanase activity (kilned malt)	1						2	3
Diastatic power	1	2	1	2	3	2	2	13
Extract viscosity						1	1	2
Fine coarse difference			1		3	1	1	6
Grain nitrogen	1	1			1			3
Grain protein content	1	5	3	5	5	1	1	21
Kernel plumpness	2	2	1	3	3		3	14
Kernel weight				1	2		4	7
Malt extract	9	5		1	5			20
Soluble/Total protein	1	2	1	1	3		1	9
Test Weight	1	3	1	2	3	4	1	15
QTL/chromosome	25	24	12	21	40	12	20	

Finally, the inclusion in POPA3 of genes with known (or predicted) roles in malting quality allows for direct assessment of the relationship of these candidate genes with malting quality QTLs. As shown in Table 2, one or two SNPs in each of nine genes with known (or predicted) roles in malting quality were included in POPA3. Of these, three genes (*Bmy1*, *DTDP,* and *SS1*) could be mapped directly in the OWB population and each gene is coincident with one or more malting quality QTLs. Six of the SNPs were not polymorphic in the OWB population but have been placed on the SNP consensus map (http://harvest.ucr.edu). Three of these are coincident with QTLs reported in the literature. Two of the three genes that were not coincident with QTLs (*CAT1* and *PDI*) were identified in expression profiling of germinating barley grain (Potokina et al., 2004). The one gene that does not correspond with any reported QTL, or expression level polymorphism, is *Aglu2*. Alpha-glucosidases (E.C. 3.2.1.20 alpha-D-glucoside glucohydrolases) catalyze the exolytic removal of a glucose residue from the non-reducing terminus of the substrate (http://www.chem.qmul.ac.uk/iubmb/; verified 27 April 2009). During malting and mashing, alpha glucosidase enzymes participate in conversion of native starch, gelatinized starch, and alpha-glucans into glucose (Sun and Henson, 1990). Members of the gene family were mapped in order

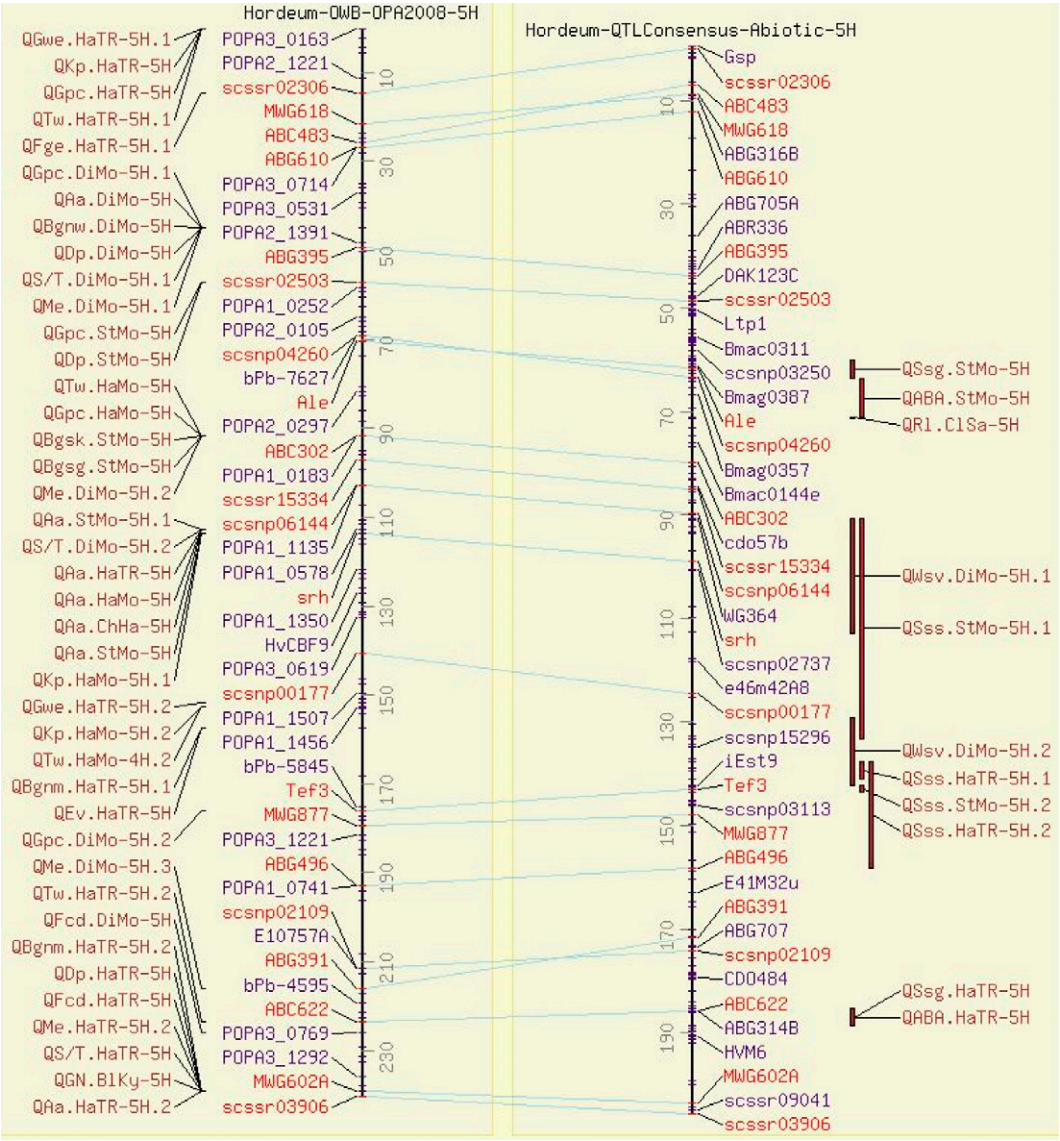

Figure 1. cMAP alignment of malting quality QTL on the OWB map (left panel) with abiotic stress QTL on the consensus map of Rostoks et al. (2005) (right panel). Locus designations for OPA SNPs are described in the Materials and Methods of this report. Prior markers follow standard locus nomenclature. QTL definitions are described and QTL names are defined in the Materials and Methods of this report and at OWB-DGGT (http://wheat.pw.usda.gov/ggpages/maps/OWB/).

to determine which, if any, could be targets for MAS for malting quality. However it appears, based on these results, that there is not significant functional variation for *Aglu2* in the current elite germplasm, and thus the gene may not be a high priority target for MAS at this time. While MAS has, in principle, been a practical technology for more than a decade, the improvement of malting quality by this approach has proven to be an elusive goal for many breeders. One exception is the successful use of MAS for specific *Bmy1* alleles in Australian germplasm (Coventry et al., 2003; Evans et al., 2008). Placement of 154 malting quality QTL on the current OWB map provides breeders with a new tool to apply to MAS for malting quality.

The malting-quality summary reported herein is part of an ongoing systematic cataloging of QTL, initiated by Hayes et al. (2003). An abiotic stress QTL summary is also available on GrainGenes http://wheat.pw.usda.gov/report?class=mapdata;name=Barley,+Abiotic+QTL+Consensus (verified 27 April 2009) and summary map sets for agronomic and biotic stress QTLs are in preparation. In many cases several QTL for different traits were mapped to the same marker (or marker interval), implying either pleiotropy or linkage. Coincidence may also be an artifact of the sparse nature of the early generation barley maps. Comparative placement of QTLs on multiple maps should enable researchers to more efficiently discover and utilize the genes underlying economically important quantitative traits.

The 2383-marker map is a milestone in the development of the many OWB-based collaborative teaching and research tools described at BarleyWorld (http://barleyworld.org/oregonwolfe.php). This availability of seed of the OWB population, together with the high-quality

Table 2. Malting quality related genes with one or more SNPs represented on Barley OPAs and their coincidence with malting quality QTL reported in the literature. QTL citations are available at OWB-DGGT (http://wheat.pw.usda.gov/ggpages/maps/OWB/).

Gene	Enzyme/ protein	OPA locus (BOPA_C)†	OWB map position‡	Consensus map position¶	QTL
Aglu2	α-glucosidase	12_30818	NP	3H – 13.4	None
Aglu3	α-glucosidase	12_30820	NP	1H – 84.8	QBgn.StMo-1H, QBgsg.StMo-1H, QBgsk.StMo-1H.1
Aglu5	α-glucosidase	12_30822 12_30823	NP	2H – 227.4	QGpc.StMo-2H.3
Bmy1	β-amylase	12_30824 12_30825	4H – 125.4	4H – 177.8	Qba-GaHN-4H, QDp.DiMo-4H
CAT1	catalase 1	12_30826 12_30827	NP	7H – 231.2	None
DTDP	d-TDP-glucose dehydratase	12_30839 12_30840	4H – 54.4	4H – 80.3	QBgsg.StMo-4H
HSP70	70 kd heat shock	12_30843	NP	6H – 35.9	QFcd.HaTR-6H, QTw.HaMo-6H.1
PDI	protein disulfide isomerase	12_30878	NP	4H – 69.8	None
SS1	sucrose synthase 1	12_30879 12_30880	7H – 60.9	7H – 85.1	QAa.StMo-2H.3, QDp.StMo-2H.3 QBgnm.StMo-7H.1, QBgsk. StMo-7H.2, QS/T.HaTR-7H

†More than one locus name indicates different SNPs in the same gene.

‡Not polymorphic in OWB.

¶SNP-only consensus map position per Close et al. www.harvest-web.org.

marker data set described in this report, provides the genetics research and instruction communities with tools for map-based cloning, consensus map construction, and integration of historical QTL data.

Acknowledgments

This work was made possible by the vision, skill, and dedication of Dr. Robert Wolfe. This research was supported by USDA-CSREES-NRI Grant No. 2006-55606-16722, "Barley Coordinated Agricultural Project: Leveraging Genomics, Genetics, and Breeding for Gene Discovery and Barley Improvement", UK SA LINK 302 / BB/D522003/1 "Association Genetics of Elite UK Barleys", and NSF DBI Grant No. 0321756 "Coupling EST and Bacterial Artificial Chromosome Resources to Access the Barley Genome." We extend our sincere gratitude to all who, over the years, have contributed data to the OWB map. Special thanks to Ann Corey for curation of the OWB genetic stocks and to Tanya Filichkina for assistance in genotyping and resequencing of malting quality related genes.

References

Blake, V.C., and T.K. Blake. 2008. Barley QTL: a compilation of known loci and the discovery of new QTL for digestibility. Proc. 10th Intl. Barley Genetics Symp. April 5–10, 2008. Alexandria, Egypt.

Chen, F., and P.M. Hayes. 1989. A comparison of *Hordeum bulbosum*-mediated haploid production efficiency in barley using *in vitro* floret and tiller culture. Theor. Appl. Genet. 77:701–704.

Costa, J.M., A. Corey, P.M. Hayes, C. Jobet, A. Kleinhofs, A. Kopisch, S.F. Kramer, D. Kudrna, M. Li, O. Riera-Lizarazu, K. Sato, P. Szűcs, T. Toojinda, M.I. Vales, and R.I. Wolfe. 2001. Molecular mapping of the Oregon Wolfe Barleys: a phenotypically polymorphic doubled-haploid population. Theor. Appl. Genet. 103:415–424.

Coventry, S.J., H.M. Collins, A.R. Barr, S.P. Jefferies, K.J. Chalmers, S.J. Logue, and P. Langridge. 2003. Use of putative QTLs and structural genes in marker assisted selection for diastatic power in malting barley (*Hordeum vulgare* L.). Aust. J. Agric. Res. 54:1241–1250.

Druka, A., G. Muehlbauer, I. Druka, R. Caldo, U. Baumann, N. Rostoks, A. Schreiber, R. Wise, T. Close, A. Kleinhofs, A. Graner, A. Schulman, P. Langridge, K. Sato, P. Hayes, J. McNicol, D. Marshall, and R. Waugh. 2006. An atlas of gene expression from seed to seed through barley development. Funct. Integr. Genomics 6:202–211.

Evans, E., C. Li, and J. Eglinton. 2008. Improved prediction of malt fermentability by measurement of the diastatic power enzymes beta-amylase, alpha-amylase, and limit dextrinase: I. Survey of the levels of diastatic power in commercial malts. J. Am. Soc. Brew. Chem. 66:223–232.

Forster, B.P., D.M. Thompson, J. Watters, and W. Powell. 1991. Water-soluble proteins of mature barley endosperm genetic control, polymorphism, and linkage with b-amylase and spring winter habit. Theor. Appl. Genet. 81:787–792.

Francia, E., F. Rizza, L. Cattivelli, A.M. Stanca, G. Galiba, B. Tóth, P.M. Hayes, J.S. Skinner, and N. Pecchioni. 2004. Two loci on chromosome 5H determine low temperature tolerance in the new 'winter' x 'spring' ('Nure' x 'Tremois') barley map. Theor. Appl. Genet. 108:670–680.

Hayes, P.M., A. Castro, L. Marquez-Cedillo, A. Corey, C. Henson, B.L. Jones, J. Kling, D. Mather, I. Matus, C. Rossi, and K. Sato. 2003. Genetic diversity for quantitatively inherited agronomic and malting quality traits. *In* R. Von Bothmer et al. (ed.) Diversity in barley. Elsevier Science Publishers, Amsterdam, The Netherlands.

Hayes, P.M., and P. Szűcs. 2006. Disequilibrium and association in barley: Thinking outside the glass. Proc. Natl. Acad. Sci. USA 49:18385–18386.

Karsai, I., P. Szűcs, K. Meszaros, T. Filichkin, P.M. Hayes, J. Skinner, L. Lang, and Z. Bedo. 2005. The Vrn-H2 locus is a major determinant of flowering time in a facultative × winter growth habit barley (*Hordeum vulgare* L.) mapping population. Theor. Appl. Genet. 110:1458–1466.

Nilan, R.A. 1964. The cytology and genetics of barley. Washington State University Press, Pullman, WA.

Potokina, E., M. Caspers, M. Prasad, R. Kota, H. Zhang, N. Sreenivasulu, M. Wang, and A. Graner. 2004. Functional association between malting quality trait components and cDNA array based expression patterns in barley (*Hordeum vulgare* L.). Mol. Breed. 14:153–170.

Reinheimer, J.L., A.R. Barr, and J.K. Eglinton. 2004. QTL mapping of chromosomal regions conferring reproductive frost tolerance in barley (*Hordeum vulgare* L.). Theor. Appl. Genet. 109:1267–1274.

Rostoks, N., S. Mudie, L. Cardle, J. Russell, L. Ramsay, A. Booth, J.T. Svensson, S.I. Wanamaker, H. Walia, E.M. Rodriguez, P.E. Hedley, H. Liu, J. Morris, T.J. Close, D.F. Marshall, and R. Waugh. 2005. Genome-wide SNP discovery and linkage analysis in barley based on genes responsive to abiotic stress. Mol. Genet. Genomics 274:515–527.

Skinner, J.S., J. von Zitzewitz, P. Szűcs, L. Marquez-Cedillo, T. Filichkin, K. Amundsen, E. Stockinger, M.F. Thomashow, T.H.H. Chen, and

P.M. Hayes. 2005. Structural, functional, and phylogenetic characterization of a large CBF gene family in barley. Plant Mol. Biol. 59:533–551.

Skinner, J.S., P. Szűcs, J. von Zitzewitz, L. Marquez-Cedillo, T. Filichkin, M.F. Thomashow, E.J. Stockinger, T.H.H. Chen, and P.M. Hayes. 2006. Mapping of barley homologs to genes that regulate low temperature tolerance in Arabidopsis. Theor. Appl. Genet. 112:832–842.

Stein, N., M. Prasad, U. Scholz, T. Thiel, H. Zhang, M. Wolf, R. Kita, R. Varshney, D. Preovic, I. Grosse, and A. Graner. 2007. A 1,000-loci transcript map of the barley genome: new anchoring points for integrative grass genomics. Theor. Appl. Genet. 111:823–839.

Sun, Z., and C. Henson. 1990. Degradation of native starch granules by alpha-glucosidase. Plant Physiol. 94:320–327.

Van Ooijen, J.W. 2006. JoinMap4, Software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen, The Netherlands.

von Zitzewitz, J., P. Szűcs, J. Dubcovsky, L. Yan, E. Francia, N. Pecchioni, A. Casas, T.H.H. Chen, P.M. Hayes, and J. Skinner. 2005. Structural and functional characterization of barley vernalization genes. Plant Mol. Biol. 59:449–467.

Wenzl, P., J. Carling, D. Kudrna, D. Jaccoud, E. Huttner, A. Kleinhofs, and A. Kilian. 2004. Diversity arrays technology (DArT) for whole-genome profiling of barley. Proc. Natl. Acad. Sci. USA 101:9915–9920.

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

Wolfe, R.I., and J.D. Franckowiak. 1991. Multiple dominant and recessive genetic marker stocks in spring barley. Barley Genet. Newsl. 20:117–121.