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Genetic mapping of quantitative trait loci associated with β-amylase and limit dextrinase activities and β-glucan and protein fraction contents in barley*

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Abstract: High malting quality of barley (*Hordeum vulgare* L.) relies on many traits, such as β-amylase and limit dextrinase activities and β-glucan and protein fraction contents. In this study, interval mapping was utilized to detect quantitative trait loci (QTLs) affecting these malting quality parameters using a doubled haploid (DH) population from a cross of CM72 (six-rowed) by Gairdner (two-rowed) barley cultivars. A total of nine QTLs for eight traits were mapped to chromosomes 3H, 4H, 5H, and 7H. Five of the nine QTLs mapped to chromosome 3H, indicating a possible role of loci on chromosome 3H on malting quality. The phenotypic variation accounted by individual QTL ranged from 8.08% to 30.25%. The loci of QTLs for β-glucan and limit dextrinase were identified on chromosomes 4H and 5H, respectively. QTL for hordeins was coincident with the region of silica eluate (SE) protein on 3HS, while QTLs for albumins, globulins, and total protein exhibited overlapping. One locus on chromosome 3H was found to be related to β-amylase, and two loci on chromosomes 5H and 7H were found to be associated with glutelins. The identification of these novel QTLs controlling malting quality may be useful for marker-assisted selection in improving barley malting quality.

Key words: Barley (*Hordeum vulgare* L.), Quantitative trait locus, β-amylase, Limit dextrinase, β-glucan, Protein fraction
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INTRODUCTION

Barley (*Hordeum vulgare* L.) is the major raw material for brewing industries including beer and whiskey, and its grain malting quality is of critical concern. In general, a good malting quality requires a high conversion of starch to fermentable sugars, high malting extract, optimal protein content, and low haze formation. Diastatic power (DP), which represents the starch-degrading ability, is closely associated with the activities of four starch hydrolytic enzymes, i.e., α-amylase, β-amylase (BAM), limit dextrinase (LD), and β-glucosidase (Delcour and Verschaeve, 1987). Among them, BAM (EC 3.2.1.2) catalyses the hydrolysis of 1,4-α-glycosidic linkages of poly-glucan chains at the non-reducing ends to produce maltose, and is highly and consistently correlated with DP. LD (EC 3.2.1.41), the only enzyme specifically cleaving the α-1,6-glucosidic bonds originating from amylopectin component of starch, is more closely associated with wort fermentability (Arends *et al*., 1995; Georg-kraemer *et al*., 2001; Zhang *et al*., 2006; Wang *et al*., 2006). Hence, high BAM and LD activities will be favorable for fermentation. On the other hand,

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β-glucan (BG) and protein contents are another two important indicators of malting quality. High BG content may result in insufficient degradation of cell walls, hamper the diffusion of germination enzymes and kernel reserves, and decrease malt extract. Residual BG will also lead to highly viscous wort and cause chill haze in bright beer (Bamforth, 1982; Wang *et al*., 2004), while too high protein content will also reduce malt extract and deteriorate final beer quality (Rasmusson and Glass, 1965). Most of recent studies confirmed that these negative effects of protein were related to its fraction composition. For example, glutelins and gel protein in the malt will generate a complex during mashing, which causes filtration problem (Celus *et al*., 2006). Disulfide bridges between hordeins can be formed, resulting in mash separation difficulty (Baxter, 1981; Celus *et al*., 2006), while the hydrolyzed and dissolved hordein fraction during malting might also be involved in haze formation.

Quantitative trait locus (QTL) analysis is a method used extensively to detect genomic regions for marker-assisted selection (MAS) in barley breeding. Up to date, the QTL analysis has been conducted to examine these malting quality traits, and a number of relative genes have been cloned and mapped (Hayes and Jones, 2000). At least 286 distinct QTLs affecting malting quality have been reported in literature (Zale *et al*., 2000; Hayes *et al*., 2001; Li *et al*., 2005; Emebiri *et al*., 2005; Panozzo *et al*., 2007; von Korff *et al*., 2008). In some cases, QTLs coincide with functional genes directly correlated with traits, as in the case of BAM and DP (Hayes *et al*., 1997). But in most cases, QTLs are located on unknown positions (Hayes *et al*., 1997; Mather *et al*., 1997). More attention should be paid to these loci, for they may represent the regulatory genes, and genetic polymorphism is largely dependent on them.

The objective of the current research was to determine the number, locations, and effects of QTLs controlling several important malting quality traits. Valuable information is obtained to determine the feasibility of using MAS in breeding new cultivars with superior malting quality. In this study, a doubled haploid (DH) population derived from the cross of "CM72" (six-rowed) and "Gairdner" (two-rowed) was utilized.

MATERIALS AND METHODS

Materials and planting conditions

A segregating DH population consisting of 93 lines, developed by anther culture of the F_1 hybrid between CM72 (six-rowed) and Gairdner (two-rowed), was used for assays of BAM activity, LD activity, BG content, total protein content, and protein fraction content. The DH lines and their two parent cultivars were planted in the experimental farm of Huajiachi campus, Zhejiang University, Hangzhou, China. The experiment was a complete block design with two replicates. All the samples were sown in 2 m long, 30 cm between rows, and 50 seeds per row in November, 2005. All agronomic managements, including fertilization, weed, and disease control, were the same as applied locally.

Protein extraction and separation

Mature grains were dried at 80 °C for 48 h, milled to pass through a 0.5-mm screen, and stored in zip lock bags under a refrigerator of 4 °C for analysis. Protein fraction was separated and analyzed according to Liu *et al*.(2005) with some modification. The 4 protein fractions were sequentially extracted in the order given below by stirring the flour (25 ml solvent for 0.5 g sample) for 2 h at room temperature in the following solvents: albumin (ALB), 10 mmol/L Tris-HCl, pH 7.5; globulin (GLO), 1 mol/L NaCl, 10 mmol/L Tris-HCl, pH 7.5; hordein (HOR), 55% (v/v) *n*-propanol, 10 mmol/L Tris-HCl, pH 7.5; glutelin (GLU), 0.24% (w/v) $CuSO_4$, 1.68% (w/v) KOH, 0.5% (w/v) potassium sodium tartrate, and 50% (v/v) iso-propanol.

Measurement of protein and its fraction contents

Total protein content in barley grains was determined by near infrared reflectance spectroscopy (NIRA, Matrix-1, Bruker Co., Germany) using a previously established calibration curve (Yin *et al*., 2002). After centrifugation at 4000×*g* for 10 min at room temperature, the contents of the ALB, GLO, and HOR were determined according to Bradford (1976), with bovine serum albumin (BSA) as the standard protein. GLU contents were analyzed by the biuret method, using a calibration curve established by the

Kjeldahl method (Holme and Peck, 1998). Each measurement consisted of three replications.

Measurement of β-amylase activity, limit dextrinase activity and β-glucan content

BAM activity was measured using the Betamyl assay kit (Megazyme Ltd., Ireland) according to McCleary and Codd (1989), with three replications.

LD activity was assayed by the method of McCleary (1992) using limit-DextriZyme tablets (Megazyme Ltd., Ireland) as substrate, and each measurement had three replications.

Total BG content in grains was analyzed according to McCleary and Codd (1991), using a commercial kit (Megazyme Ltd., Ireland), and presented on the basis of dry mass with 12% of moisture content. Three replications were taken for each measurement.

Molecular linkage map

A set of DH lines from the same cross was used to build up the linkage map. DNA extraction from barley leaves was done according to mini prep protocol (Dellaporta *et al*., 1983). Polymerase chain reaction (PCR) was carried out in a final volume of 10 μl, containing 3.75 μl H_2O, 3.0 μl 1% (w/v) Cresol red, 1.0 μl 10× Taq polymerase buffer, 0.4 μl 10 mmol/L dNTP, 0.3 μl 50 mmol/L $MgCl_2$, 0.075 μl 10 μmol/L primers (Invitrogen), 0.09 μl 5 U/μl Taq polymerase, and 2.0 μl DNA. A total of 284 DArT (Diversity Array Technology®, Australia) markers and 48 single sequence repeat (SSR) markers were selected to survey DHs.

The genetic linkage map was built by Map Manager QTXb20, and the QTLs were located by QTLNetwork2.0 (Manly *et al*., 2001; Yang *et al*., 2005). The derived DH linkage map spanned 1983.9 cM with an average distance of 5.97 cM between adjacent makers.

Statistical and quantitative trait locus analyses

Population distribution analysis was performed using SPSS 11.0 statistical software. QTL analysis for individual environment was performed using software QTLMAPPER 1.60, which was developed based on the mixed linear model approach (Wang *et al*., 1999) to identify main-effective QTLs. QTL was determined with threshold $P<0.005$. The threshold of likelihood of odds (LOD) >2.5 was chosen for claiming a putative QTL. The nomenclature of QTL followed the system of McCouch *et al*.(1997).

RESULTS

Genetic variation in malting quality traits

Large genetic variations were detected among 93 DH lines and their parents (CM72 and Gairdner) in 8 malting quality traits, including BAM activity, LD activity, BG content, total protein content, and protein fraction content (Table 1). For the two parents, higher BAM (861.1 U/g) and LD activities (114.3 U/kg), but lower levels of BG (3.9%), total protein (6.3%), ALB (5.4 mg/g), GLO (3.3 mg/g), HOR (28.3 mg/g) and GLU (32.6 mg/g) were observed in CM72, compared with those of Gairdner. On an average of all DH lines, all the traits except BAM activity were more close to those of the parent Gairdner. The mean BAM activity of DH lines was a slightly higher than that of CM72.

Table 1 Phenotypic values of malting quality traits in the DH population and their parents

	Activity		Content					
	BAM (U/g)	LD (U/kg)	BG (%)	ALB (mg/g)	GLO (mg/g)	HOR (mg/g)	GLU (mg/g)	TPC (%)
CM72	861.1	114.3	3.9	5.4	3.3	28.3	32.6	6.3
Gairdner	289.5	95.8	4.8	5.8	3.5	31.4	35.2	7.5
DH population								
Mean	891.7	87.0	4.5	5.7	4.0	50.0	39.5	11.3
Range	68.1~2132.1	71.0~107.2	2.6~6.2	4.3~7.3	2.6~6.2	27.9~75.7	25.9~47.6	7.2~18.1
Skewness	0.326	0.295	−0.222	0.058	0.309	0.151	−0.400	0.488

BAM: β-amylase; LD: limit dextrinase; BG: β-glucan; ALB: albumin; GLO: globulin; HOR: hordein; GLU: glutelin; TPC: total protein content

There were approximately normal distributions of all examined traits for the DH lines with the skewness value ranging from −0.4 to 0.488 (Table 1). In addition, strong transgressive segregation could be found in all traits, with minimum and maximum DH values exceeding the parental values, except for LD activity and total protein content.

Trait interrelationship

A total of 21 significant correlations were detected between the 8 malting traits (Table 2). It was indicated that BAM and LD activities were significantly correlated with total protein content. Moreover, they were also significantly and positively correlated with all four protein fraction contents, with the highest correlation coefficient being with GLO and the lowest being with GLU. In addition, no significant correlation was found between BG activity and any other traits.

Quantitative trait locus analysis

For the 8 quality traits analyzed in this study, a total of 9 QTLs were mapped on chromosomes 3H, 4H, 5H, and 7H of barley, respectively (Table 3, Fig.1). A list of the putative QTLs flanked by markers along with their LOD values, phenotypic variance, and additive effects are presented in Table 3. A graphical presentation of QTL locations on the linkage map is shown in Fig.1.

β-amylase activity

One QTL for BAM activity was mapped within the bPb-4564-bPb-3634 region on chromosome 3H (Table 3 and Fig.1). This QTL occupied 12.81% of the total phenotypic variance. The favorable alleles for increasing BAM activity were mostly contributed by CM72, with an additive effect being 183.56 U/g.

β-glucan content

Only one QTL was detected for BG content, which was located on bPb-2305-Bmac0186 interval of chromosome 4H (Table 3 and Fig.1). The qBG4 accounted for 11.06% of the total phenotypic variance, and the Gairdner allele for qBG4 had an additive effect of 0.23% for increasing BG content.

Limit dextrinase activity

For LD activity, one QTL, qLD5, was identified on chromosome 5H (Table 3 and Fig.1). This QTL explained 19.4% of the trait variance and had a positive additive effect from CM72.

Table 2 Simple correlation coefficients among the eight traits in the barley DH populations

	BAM activity	BG content	LD activity	ALB content	GLO content	HOR content	GLU content
BG content	0.028						
LD activity	0.635**	0.047					
ALB content	0.758**	−0.149	0.630**				
GLO content	0.769**	0.083	0.662**	0.817**			
HOR content	0.695**	−0.058	0.651**	0.774**	0.780**		
GLU content	0.239*	−0.043	0.260*	0.350**	0.376**	0.515**	
TPC	0.572**	−0.046	0.510**	0.605**	0.669**	0.855**	0.607**

* Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level

Table 3 Putative QTL with an LOD score >2.4 for 8 traits identified in the barley DH population

Trait	QTL	Chromosome	Marker interval	LOD	Additive effect	Variance explained (%)
BAM activity	qBAM3	3H	bPb-4564-bPb-3634	4.01	183.56	12.81
BG content	qBG4	4H	bPb-2305-Bmac0186	2.63	−0.23	11.06
LD activity	qLD5	5H	bPb-4809-bPb-5766	5.89	3.86	19.40
ALB content	qALB3	3H	bPb-7938-bPb-7989	3.78	−0.19	8.08
GLO content	qGLO3	3H	bPb-7938-bPb-7989	2.61	−0.16	8.94
HOR content	qHOR3	3H	bPb-2553-bPb-2324	4.71	−6.48	30.25
GLU content	qGLU5	5H	bPb-3590-bPb-0171	5.49	−1.47	11.21
	qGLU7	7H	bPb-9825-bPb-5126	5.04	−1.37	9.76
TPC	qTPC3	3H	bPb-7938-bPb-7989	3.28	−0.71	11.51

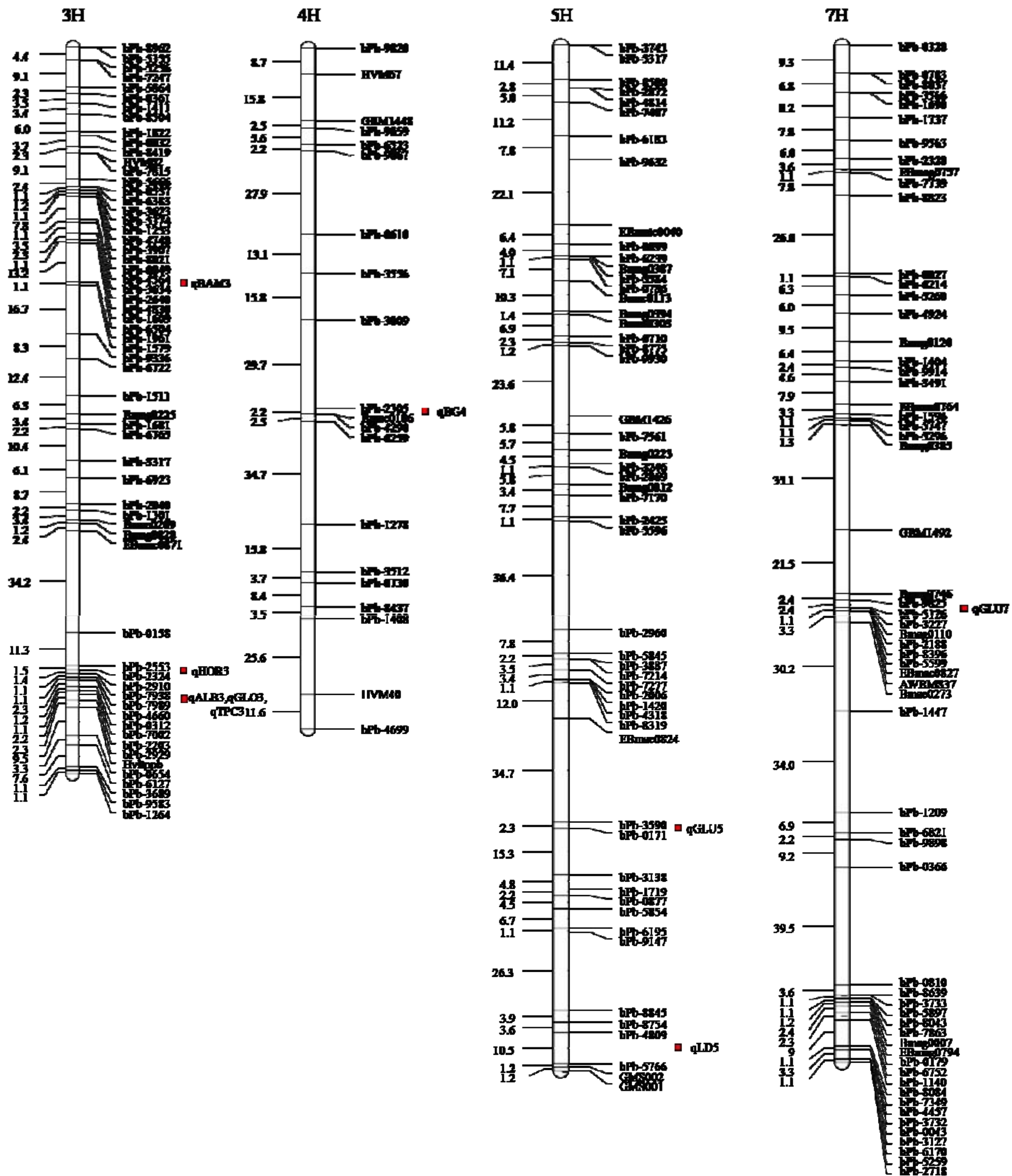

Fig.1 QTL map for eight malting quality traits in CM72/Gairdner DH population

Total protein and protein fraction contents

There was one QTL affecting TPC detected and mapped within the bPb-7938-bPb-7989 region on chromosome 3H (Table 3 and Fig.1). This QTL accounted for 11.51% of the phenotypic variance and the alleles for increasing TPC were contributed by the Gairdner.

In all four protein fractions, one QTL for ALB and GLO was mapped on the same chromosomal location as the QTL for TPC. The QTL for ALB content had a relatively small effect, only explaining 8.08% of the total phenotypic variance. Similarly, the QTL for GLO content explained 8.94% of the total phenotypic variance.

For HOR content, there was only one QTL with magnitude effect within the bPb-2553-bPb-2324 region on chromosome 3H (Table 3 and Fig.1). The qHOR3 accounted for 30.25% of the total phenotypic variance, with an additive effect of the Gairdner allele for increasing HOR content by 6.48 mg/g.

Two QTLs affecting GLU content were identified on chromosomes 5H and 7H. The percentages of phenotypic variance caused by these QTLs were 11.21% and 9.76%, respectively, with the additive effects of 1.47 and 1.37 mg/g, respectively. The Gairdner alleles at both QTLs resulted in an increment of GLU content.

DISCUSSION

Malting quality is one of the most complex and sensitive traits for brewing, which is associated with a set of candidate genes (Zale *et al*., 2000; von Korff *et al*., 2008). Recently, at least 286 QTLs have been reported for 17 malting quality traits in 18 combinations of barley (Zale *et al*., 2000; Hayes *et al*., 2001; Li *et al*., 2005; Emebiri *et al*., 2005; Panozzo *et al*., 2007; von Korff *et al*., 2008). These QTLs are spread across all seven barley chromosomes and primarily concentrated in chromosomes 1H, 2H, 4H, 5H, and 7H (Zale *et al*., 2000).

In this study, a total of 9 putative QTLs were detected for 8 traits in the CM72/Gairdner DH population (Table 1). QTLs of BAM and LD activities were mainly derived from the parent CM72, and positive QTLs from parent Gairdner were found for increased BG, total protein, and protein fraction contents. It is noteworthy that 5 out of the 9 QTLs were identified on chromosome 3H, suggesting an underestimated potential of chromosome 3H to malting quality in the previous researches.

The qBAM3 was located on the bPb-4564-bPb-3634 interval of chromosome 3H, which is coincident with a region previously reported to contain a BAM QTL in Clipper/Sahara and Harrington/TR306 population (Fig.1) (Langridge *et al*., 1996; Clancy *et al*., 2003). However, the region does not overlap the structural genes encoding BAM (*Bmy1* and *Bmy2*) and the QTL explained only 12.81% of BAM variation, indicating a possible minor effect of that QTL (Clancy *et al*., 2003).

LD activity was considered to be more closely correlated with wort fermentability than BAM activity, and a LD locus was mapped to the 10.5 cM interval in the long arm telomere region of chromosome 5H (Li *et al*., 1999). The unique single copy LD gene was previously reported on the short arm of chromosome 1H from Steptoe/Morex population (Li *et al*., 1999). Thus the new LD locus identified in this study might function as regulation of LD activity.

The effect of protein content on malting quality is largely owing to its fraction composition. However, few, if any, of genetic basis of barley protein fractions have been analyzed in detail. Hordeins have been viewed as the main source of haze active proteins isolated from beer and were reported to be encoded by families of genes at a single, linked locus on chromosome 1H (Shewry, 1993). While Robinson *et al*. (2007) demonstrated a type of silica eluate (SE) protein fraction closely associated with haze formation in bright beer, which was mapped to the short arm of chromosome 3H in Chebec/Harrington population. However, the author could not confirm whether SE protein is of hordein origin or not due to limitation of information. In our study, an HOR QTL was located on chromosome 3H, which is approximately within the same region of SE protein (Robinson *et al*., 2007). The qHOR3 occupied 30.25% of the total HOR content variance, showing the largest effect in all 9 QTLs identified in this study. The discovery of qHOR3 provides an evident that SE protein might be HOR origin, which will be useful for improving haze stability in the resultant beer. However, HOR content was also found to be positively correlated with BAM and LD activities (Table 2), suggesting the opposite effect of HOR content.

Meanwhile, there were overlapping QTLs for ALB, GLO, and total protein contents next to the qHOR3 on chromosome 3H (Fig.1). It is assumed to be pleiotropic effects at this location as ALB and GLO are compositions of TPC. In addition, two GLU QTLs were mapped to chromosomes 5H and 7H, respectively. These two loci were overlapped with the regions of QTLs for grain protein in Harrington/TR306 and Dicktoo/Morex population (Mather *et al*., 1997; Oziel *et al*., 1996), revealing that the two QTLs detected in previous experiments might be mainly associated with the production of storage protein (glutelins).

On chromosome 4H, we identified a QTL for BG content around the centromeric region, which is flanked by DNA marker Bmac0186. Once there was a QTL for finished malt α-glucanase mapped in that region in Steptoe/Morex population, it might also be caused by pleiotropic gene effects (Han *et al.*, 1995).

In summary, it is the first time that all four protein fractions in barley were together examined using QTL analysis, and that new loci have been found for LD activity and BG content in the DH population. The identification of these novel QTLs controlling malting quality may be useful for marker-assisted selection in improving barley malting quality. However, as some QTLs appeared to be specific to this population, further clarification is need.

References

Arends, A.M., Fox, G.P., Henry, R.J., Marschke, R.J., Symons, M.H., 1995. Genetic and environmental variation in the diastatic power of Australian barley. *J. Cereal Sci.*, **21**(1): 63-70. [doi:10.1016/S0733-5210(95)80009-3]

Bamforth, C.W., 1982. Barley β-glucans: their role in malting and brewing. *Brew. Dig.*, **57**(6):22-27.

Baxter, E.D., 1981. Hordein in barley and malt. A review. *J. Inst. Brew.*, **87**(2):173-176.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**(1-2):248-254. [doi:10.1016/0003-2697(76)90527-3]

Celus, I., Brijs, K., Delcour, J.A., 2006. The effects of malting and mashing on barley protein extractability. *J. Cereal Sci.*, **44**(2):203-211. [doi:10.1016/j.jcs.2006.06.003]

Clancy, J.A., Han, F., Ullrich, S.E., 2003. Comparative mapping of beta-amylase activity loci among three barley crosses. *Crop Sci.*, **43**(3):1043-1052.

Delcour, J.A., Verschaeve, S.G., 1987. Malt diastatic power. Part II. A modified EBC diastatic power assay for the selective estimation of β-amylase activity. Time and temperature dependence of the release of reducing sugars. *J. Inst. Brew.*, **93**(4):296-301.

Dellaporta, S.L., Wood, J., Hicks, J.B., 1983. A plant DNA minipreparation: version II. *Plant Mol. Biol. Rep.*, **1**(4): 19-21. [doi:10.1007/BF02712670]

Emebiri, L.C., Moody, D.B., Horsley, R., Panozzo, J.F., Read, B.J., 2005. The genetic control of grain protein content variation in a doubled haploid population derived from a cross between Australian and North American two-rowed barley lines. *J. Cereal Sci.*, **41**(1):107-114. [doi:10.1016/j.jcs.2004.08.012]

Georg-kraemer, J.E., Mundstock, E.C., Cavalli-Molina, L., 2001. Developmental expression of amylase during barley malting. *J. Cereal Sci.*, **33**(3):279-288. [doi:10.1006/jcrs.2001.0367]

Han, F., Ullrich, S.E., Chirat, S., Menteur, S., Jestin, L., Sarrafi, A., Hayes, P.M., Jones, B.L., Blake, T.K., Wesenberg, D.M., *et al.*, 1995. Mapping of β-glucan content and β-glucanase activity loci in barley grain and malt. *Theor. Appl. Genet.*, **91**(6-7):921-927. [doi:10.1007/BF00223901]

Hayes, P.M., Jones, B.L., 2000. Malting Quality from a QTL Perspective. *In*: Logue, S. (Ed.), Proceedings of the Eighth International Barley Genet. Symp., p.99-106.

Hayes, P.M., Cerono, J., Witsenboer, H., Kuiper, M., Zabeau, M., Sato, K., Kleinhofs, A., Kudrna, D., Killian, A., Saghai-Maroof, M., Hoffman, D., the North American Barley Genome Mapping Project, 1997. Characterizing and exploiting genetic diversity and quantitative traits in barley (*Hordeum vulgare*) using AFLP markers. *J. Quant. Trait Loci*, **3**(1):1-15.

Hayes, P.M., Castro, A., Marquez-Cedillo, L., Corey, A., Henson, C., Jones, B., Kling, J., Mather, D., Matus, I., Rossi, C., Sato, K., 2001. A Summary of Published Barley QTL Reports. Available from: http://www.barleyworld.org/northamericanbarley/qtlsummary.php (access at Mar. 28, 2005).

Holme, D.J., Peck, H., 1998. Analytical Biochemistry, 3rd Ed. Addison Wesley Longman, New York, p.388-393.

Langridge, P., Karakousis, A., Kretschmer, J., Manning, S., Logue, S., 1996. Barley Mapping and QTL Analysis of Three Populations from the University of Adelaide. Available on line through GrainGenes. Available from: http://wheat.pw.usda.gov/ggpages/maps.html (access at Dec. 19, 2002).

Li, C.D., Zhang, X.Q., Eckstein, P., Rossnagel, B.G., Scoles, G.H., 1999. A polymorphic microsatellite in the limit dextrinase gene of barley (*Hordeum vulgare* L.). *Mol. Breeding*, **5**(6):569-577. [doi:10.1023/A:1009692207966]

Li, J.Z., Huang, X.Q., Heinrichs, F., Ganal, M.W., Röder, M.S., 2005. Analysis of QTLs for yield, yield components, and malting quality in a BC3-DH population of spring barley. *Theor. Appl. Genet.*, **110**(2):356-363. [doi:10.1007/s00122-004-1847-x]

Liu, Z.H., Cheng, F.M., Cheng, W.D., Zhang, G.P., 2005. Positional variations in phytic acid and protein content within a panicle of japonica rice. *J. Cereal Sci.*, **41**(3): 297-303. [doi:10.1016/j.jcs.2004.09.010]

Manly, K.F., Cudmore, R.H., Meer, J.M., 2001. Map manager QTX, cross-platform software for genetic mapping. *Mamm. Genome*, **12**(12):930-932. [doi:10.1007/s00335-001-1016-3]

Mather, D.E., Tinker, N.A., LaBerge, D.E., Edney, M., Jones, B.L., Rossnagel, B.G., Legge, W.G., Briggs, K.G., Irvine, R.B., Falk, D.E., *et al.*, 1997. Regions of the genome that affect grain and malt quality in a North American two-row barley cross. *Crop Sci.*, **37**(2):544-554.

McCleary, B.V., 1992. Measurement of the content of limit dextrinase in cereal flours. *Carbohydr. Res.*, **227**(1): 257-268. [doi:10.1016/0008-6215(92)85076-C]

McCleary, B.V., Codd, R., 1989. Measurement of beta-amylase in cereal flours and commercial enzyme preparations. *J. Cereal Sci.*, **9**(1):17-33. [doi:10.1016/S0733-5210(89)

80018-9]

McCleary, B.V., Codd, R., 1991. Measurement of (1-3),(1-4)-β-D-glucan in barley and oats: a streamlined enzymic procedure. *J. Sci. Food Agric*., **55(2)**:303-312. [doi:10.1002/jsfa.2740550215]

McCouch, S.R., Cho, Y.G., Yano, M., Paul, E., Blinstrub, M., 1997. Report on QTL nomenclature. *Rice Genet. Newslett.*, **14**:11-13.

Oziel, A., Hayes, P.M., Chen, F.Q., Jones, B., 1996. Application of quantitative trait locus mapping to the development of winter-habit malting barley. *Plant Breeding*, **115**(1):43-51. [doi:10.1111/j.1439-0523.1996.tb00869.x]

Panozzo, J.F., Eckermann, P.J., Mather, D.E., Moody, D.B., Black, C.K., Collins, H.M., Barr, A.R., Lim, P., Cullis, B.R., 2007. QTL analysis of malting quality traits in two barley populations. *Aust. J. Agric. Res*., **58**(9):858-866. [doi:10.1071/AR06203]

Rasmusson, D.C., Glass, R.L., 1965. Effectiveness of early generation selection for four quality characters in barley. *Crop Sci*., **5(2)**:389-391.

Robinson, L.H., Healy, P., Stewart, D.C., Eglinton, J.K., Ford, C.M., Evans, D.E., 2007. The identification of a barley haze active protein that influences beer haze stability: the genetic basis of a barley malt haze active protein. *J. Cereal Sci.*, **45**(3):335-342. [doi:10.1016/j.jcs.2006.08.013]

Shewry, P.R., 1993. Barley Seed Proteins. *In*: MacGregor, A.W., Bhatty, R.S. (Eds.), Barley: Chemistry and Technology. American Society of Cereal Chemists, Minnesota, USA, p.131-197.

von Korff, M., Wang, H.J., Léon, J., Pillen, K., 2008. AB-QTL analysis in spring barley: III. Identification of exotic alleles for the improvement of malting quality in spring barley (*H. vulgare* ssp. *spontaneum*). *Mol. Breeding*, **21**(1):81-93.

Wang, D.L., Zhu, J., Li, Z.K., Paterson, A.H., 1999. Mapping QTLs with epistatic effects and QTL×environment interactions by mixed linear model approaches. *Theor. Appl. Genet*., **99**(7-8):1255-1264. [doi:10.1007/s001220051331]

Wang, J.M., Zhang, G.P., Chen, J.X., Wu, F.B., 2004. The changes of β-glucan content and β-glucanase activity in barley before and after malting and their relationships to malt qualities. *Food Chem.*, **86**(2):223-228. [doi:10.1016/j.foodchem.2003.08.020]

Wang, X.D., Yang, J., Zhang, G.P., 2006. Genotypic and environmental variation in barley limit dextrinase activity and its relation to malt quality. *J. Zhejiang Univ. Sci. B*., **7**(5):386-392. [doi:10.1631/jzus.2006.B0386]

Yang, J., Hu, C.C., Ye, X.Z., Zhu, J., 2005. QTLNetwork 2.0. Institute of Bioinformatics, Zhejiang University, Hangzhou, China. Available from: http://ibi.zju.edu.cn/software/qtlnetwork

Yin, C., Zhang, G.P., Wang, J.M., Chen, J.X., 2002. Variation of β-amylase activity in barley as affected by cultivar and environment and its relation to protein content and grain weight. *J. Cereal Sci*., **36**(3):307-312. [doi:10.1006/jcrs.2002.0467]

Zale, J.M., Clancy, J.A., Ullrich, S.E., Jones, B.L., Hayes, P.M., 2000. North American barley genome project. Summary of barley malting-quality QTLs mapped in various populations. *Barley Genet. Newslett*., **30**:44-54.

Zhang, G.P., Chen, J.X., Dai, F., Wang, J.M., Wu, F.B., 2006. The effect of cultivar and environment on beta-amylase activity is associated with the change of protein content in barley grains. *J. Agron. Crop Sci*., **192(1)**:43-49. [doi:10.1111/j.1439-037X.2006.00181.x]