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Malting quality and winterhardiness in barley are "ultimate" phenotypes composed of component, quantitatively inherited traits. A 69-point genome map of the seven chromosomes of barley was used, in conjunction with multi-environment phenotypes for grain yield and malting quality, to determine the chromosome locations of quantitative trait loci (QTLs). A combined analysis of the two environments identified QTLs that were both common and unique to each environment. Dispersed QTLs with positive relationships provide ready targets for marker-assisted selection. Overlapping QTLs for agronomic and malting quality QTLs with favorable alleles contributed by alternate parents will require further, higher resolution mapping to determine if negative relationships are due to linkage or pleiotropy. There is preliminary evidence for orthologous agronomic trait and malting QTLs in barley. This QTL analysis will hopefully assist in the rapid development of winter malting varieties that will maximize the profitability of Oregon barley production.

MAPPING OF QUANTITATIVE TRAIT LOCI FOR MALTING QUALITY IN A WINTER X SPRING BARLEY (HORDEUM VULGARE, L.) CROSS

by

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MAPPING OF QUANTITATIVE TRAIT LOCI FOR MALTING QUALITY IN A WINTER X SPRING BARLEY (HORDEUM VULGARE, L.) CROSS

INTRODUCTION

The greatest economic return to farmers comes from the production of malting type, as opposed to feed type, barley (Hardwick, 1977; Wilson, 1985). However, as malting barley varieties must meet a strict set of criteria established by the malting and the brewing industries, selection for malting types is a significantly greater challenge to plant breeding than the development of feed types, where the principal selection criteria is yield (Burger and LaBerge,1985).

All malting varieties grown in the U.S. are of spring habit. Winter habit varieties would provide both growers and industries with an attractive crop alternative. While significant effort has been devoted to the development of winter habit malting barley in Europe, winter types have yet to meet the quality profile of spring types (Schildbach, 1987). The only comprehensive winter malting barley breeding effort in the U.S. is at Oregon State University.

The challenge in developing winter habit malting barley lies in the complexity of the many component traits that determine malting quality and winterhardiness (Rutger et al, 1967; Dantuma,1958). Principal components of malting quality are total grain protein, diastatic power, alpha-amylase, soluble to total protein ratio, and malt extract. A comprehensive review of the biochemical basis of malting quality in the context of barley improvement is presented by Burger and LaBerge (1985). Briefly, malting is a carefully controlled germination process in which complex hydrolytic and proteolytic pathways are manipulated to develop an ideal substrate for subsequent fermentation. Kernel carbohydrates are hydrolyzed by α and β-amylase, and the selection criteria of α-amylase and diastatic power are thus measures of individual (α) and combined (α plus β) enzymatic activity.

Proteinase activity is expressed as the ratio of soluble to total protein. Malt extract percentage is a measure of soluble sugars and proteins and thus is the final consequence of the overall efficiency of the malting process. Differences in brewing procedure and desired end product properties in North America and Europe, the principal malting barley production regions of the world, have led to distinct target quality profiles. As a consequence, culling levels and selection weights in North American and European breeding programs may vary. Each of the malting quality component traits is quantitatively inherited (Peterson and Foster, 1973). Principal components of winterhardiness are vernalization requirement, photoperiod sensitivity, and cold tolerance (Hayes et al., 1993) and each of these traits has a complex genetic basis (Grafius,1981; Takahashi and Yasuda, 1971).

The development of molecular marker mapping techniques and accompanying procedures for location of quantitative trait loci (QTLs) (reviewed by Paterson et al., 1991) promise to reduce the complexity of quantitative expression to more tractable, Mendelian types of analyses. The recent development of molecular maps in barley (Graner et al., 1991; Heun et al., 1991; Kleinhofs et al., 1993) together with accompanying QTLs analyses (Hayes et al., in press) allows for the application of these techniques to the development of winter habit malting barley varieties.

Our objectives were therefore to locate QTLs for malting quality traits in the doubled haploid progeny of a winter feed X spring malt cross evaluated under fall and spring-planted conditions in order to (i) determine linkage relationships of malting quality characters with previously mapped QTLs for cold tolerance, (ii) measure the effect of environment on trait expression, and (iii) determine the extent of orthologous QTL expression for malting quality characters. Our goal was to identify molecular markers flanking QTLs that could be used to accelerate the development of winter habit malting quality cultivars.

MAPPING OF QUANTITATIVE TRAIT LOCI FOR MALTING QUALITY IN A WINTER X SPRING BARLEY (HORDEUM VULGARE, L.) CROSS

Abstract

Malting quality and winterhardiness in barley are "ultimate" phenotypes composed of component, quantitatively inherited traits. A synthesis of molecular marker linkage information and field phenotyping to reveal the location of quantitative trait loci (QTLs) may assist in the development of winter habit malting barley varieties and serve as a model system for the application of QTLs analysis techniques to complex breeding problems. One hundred doubled haploid progeny from a winter x spring cross were evaluated under fall and spring-seeded conditions. Malting quality phenotypes and a 69-point map were used in a non-linear interval mapping procedure to identify QTLs, and to assess the effect of environment on QTL expression. A combined analysis of the two environments identified QTLs that were both common and unique to each environment. Dispersed QTLs with positive relationships provide ready targets for marker-assisted selection. Overlapping QTLs for agronomic and malting quality traits with favorable alleles contributed by opposing parents will require further and higher resolution mapping to determine if negative relationships are due to linkage or pleiotropy. There is preliminary evidence for orthologous agronomic trait and malting QTLs in barley.

Key words: barley, QTL, malting quality, winterhardiness, orthologous genes.

Introduction

The grain of barley (*Hordeum vulgare L.*) is used principally as an animal feed and for the manufacture of malt (Hardwick,1977). As there is little or no premium paid for nutritional quality (Wilson,1985), the principal selection criterion in the development of feed barley varieties is yield. The exigent requirements of the malting and brewing industries, in contrast, demand simultaneous selection for a number of quality characteristics in addition to agronomic performance (Burger and LaBerge,1985). As a rule, malting varieties are usually lower yielding than feed barley varieties. Market discrimination or an additional premium negate or compensate for, respectively, the yield penalty (Wilson, 1985).

Principal selection criteria in a malting barley breeding program are malt extract percentage, total grain protein percentage, soluble to total protein ratio, α-amylase, and diastatic power. A comprehensive review of the biochemical basis of malting quality in the context of barley improvement is presented by Burger and LaBerge (1985). Briefly, malting is a carefully controlled germination process in which complex hydrolytic and proteolytic pathways are manipulated to develop an ideal substrate for subsequent fermentation. Kernel carbohydrates are hydrolyzed by α and β-amylase, and the selection criteria of α-amylase and diastatic power are thus measures of individual (α) and combined (α plus β) enzymatic activity. Proteinase activity is expressed as the ratio of soluble to total protein. Malt extract percentage is a measure of soluble sugars and proteins and thus expresses the overall efficiency of the malting process. Differences in brewing procedure and desired end product properties in North America and Europe, the principal malting barley production regions of the world, have led to distinct target quality profiles. As a consequence, culling levels and selection weights in North American and European breeding programs may vary.

Nonetheless, the multiple components of malting quality, each of which can be considered quantitatively inherited (Peterson and Foster, 1973), equally challenge North American and European Plant Breeders. Given the necessity of simultaneously improving agronomic and quality performance, the tendency to exploit very narrow genetic germplasm bases is understandable. In the interest of genetic diversity, agricultural sustainability, and continued selection response, it would be desirable to broaden this genetic base and even to expand the range of options for both farmers and industry to include winter malting varieties.

No adapted and approved winter malting varieties are currently in commercial production in North America. In Europe, considerable progress has been made in winter malting barley improvement, although winter types in general have not achieved the level of quality found in spring genotypes (Schildbach, 1987). Winterhardiness, which is determined by a number of quantitatively inherited component traits (Grafius,1981, Datuma,1958), including growth habit, vernalization response, photoperiod reaction, and cold tolerance (Hayes et al., 1993), is in and of itself as great a challenge as malting quality. As a consequence, simultaneous improvement for malting quality, winterhardiness, and agronomic performance is a formidable challenge.

The recent development of comprehensive medium density maps in barley (Graner et al., 1991; Heun et al., 1991; Kleinhofs et al., 1993) coupled with the statistical tools for locating quantitative traits loci (QTLs) (reviewed by Paterson et al., 1991) and demonstrated by Hayes et al. (in press) should clarify the genetic basis of trait expression and relationship and thus allow for a more systematic approach to complex breeding problems, such as the development of winter habit malting barley varieties.

Our objectives were, therefore, to locate QTLs for malting quality traits in the doubled haploid progeny of a winter feed X spring malt cross evaluated under fall and spring-planted conditions in order to (i) determine linkage relationships of malting quality characters with

previously mapped QTLs for cold tolerance, (ii) measure the effect of environment on trait expression, and (iii) determine the extent of orthologous QTL expression for malting quality characters. Our goal was to identify molecular markers flanking QTLs that could be used to accelerate the development of winter habit malting quality cultivars.

Materials and Methods

Population development and genetic analysis

The development of the reference population and marker analysis protocols were described in detail by Hayes et al. (1993). It is important to reiterate that the accession of Dicktoo we have used is cold tolerant and that it displays typical winter growth habit under field conditions as a consequence of extreme photoperiod sensitivity. It has no vernalization requirement. This allowed for evaluation of both parents and their doubled haploid progeny under both fall and spring-seeded conditions. Briefly, a population of 100 doubled haploid (DH) lines was developed by the *Hordeum bulbosum* technique as described by Chen and Hayes (1989). Dicktoo is a six-row winter feed barley of unknown ancestry and mixed description released by the Nebraska Agricultural Experiment Station in 1952. Morex, the US six-row spring malting barley standard, was released by the Minnesota Agricultural Experiment Station in 1978 (Rasmusson and Wilcoxson,1979).

Data on sixty nine markers, shown in Figure 1, were cooperatively generated at Linkage Genetics, Inc. (Salt Lake City, Utah), Montana State University (Bozeman, Montana) and Oregon State University (Corvallis, Oregon). Restriction Fragment Length Polymorphism (RFLP) protocols were essentially as described by Kleinhofs et al. (1993), with minor variations between laboratories. Full protocols are available upon request. Polymerase Chain Reaction (PCR) markers were assayed as described by Tragoonrung et al. (1992). Isozymes and storage proteins markers were assayed according to Nielsen and Johansen (1986). Morphological markers were scored visually or under a stereo-microscope. Marker nomenclature follows that used by Hayes et al. (1993) and Kleinhofs et al (1993). The prefix "m" designates morphological markers; the prefix "i" designates isozyme markers; the prefixes "WG" and "BCD" designate wheat and barley

genomic clones, respectively, developed at Cornell University (Heun et al., 1991); the "ABC" and "ABG" prefixes designate barley cDNA and genomic clones, respectively, developed by the North American Barley Genome Mapping Project (Kleinhofs et al., 1993); and the prefix "ap" designates specific amplicon polymorphisms. Additional information on the "ap" class markers is available from T. Blake at Montana State University.

The linkage map was produced by G-MENDEL (Liu and Knapp, 1990) with the following constraints : recombination $\leq$ 34 % and p = .001. When revelant for the purposes of discussion, the recombination values shown in Figure 1 were converted to cM using the Kosambi mapping function (Kosambi,1944). QTL analyses were conducted using QTL STAT (B.H. Liu and S.J. Knapp, pers.comm.). QTL parameters were estimated using least squares interval mapping methods (Knapp et al., 1990). QTL genotype means were estimated and the hypothesis of "no QTL" was tested against the hypothesis of one QTL for every marker bracket. Rather than estimating QTL parameters and test statistics at 1 cM intervals within every marker bracket and selecting the location which maximized the likelihood ratio or minimized the sum of squares, as is often done in interval mapping, we estimated the QTL location directly using non-linear least squares (Knapp et al., 1990). Hypotheses about QTLs and QTLs effects were tested using Wald statistics (Knapp and Bridges, 1990). QTLs and QTL X E effects were considered significant if they exceeded a Wald statistic of 10.0, which is approximately equal to p = .001. Wald Support Intervals (WSIs) > 90% were specified at Wald = 10, following the LOD Support Interval (LSI) concept described by van Ooijen (1992).

Phenotype assessment

As plant survival QTL in three environments mapped to the same interval on the long arm of chromosome 7 (Hayes et al., 1993) we have used the data from the most extreme

environment - Montana - as the reference in the current analyses. Malting quality and agronomic traits were measured in field tests of the doubled haploid population under fall and spring planted conditions near Corvallis, Oregon. Agronomic practices including seeding rate and date, fungicide control of *Rhynchosporium secalis* (fall-seeded) and *Puccinia recondita* (spring-seeded), as well as fertilizer application timing and amount were in accordance with standard recommended practice for each environment. Full field management protocols are available on request. Briefly, for the fall-seeded and spring-seeded experiments, a single replicate of the 100 DH lines and the two parents was sown. The plot size was 4.32 m^2. The use of a single replication was justified on the grounds that the primary determinant of the power of hypothesis tests regarding QTL genotype means are the number of replications of QTL genotypes, not the number of times each line is replicated (Knapp et al., 1990). Supplemental irrigation was applied as needed. Plots were harvested at maturity with a plot binder and bundles were treshed in a stationary tresher. Malt quality analyses were performed on samples from both the fall and spring-seeded experiments, and grain β glucan content was determined for the fall-planted experiment only, by the USDA/CRS Cereal Crop Research Unit as described by Tragoonrung et al (1990).

Results and Discussion

Phenotypic trait expression and correlation

Due to an exceptionally mild winter, no appreciable winter-kill was observed in the fall-seeded experiment. Despite large differences in heading date of test materials (data not shown; a full genetic analysis of growth habit, vernalization response, and photoperiod reaction in this material is in progress), relatively humid conditions at harvest minimized shattering losses in the fall-seeded experiment, and the use of a plot binder and stationary tresher allowed for near-optimum grain recovery in the spring-seeded experiment. Thus, the agronomic performance of this test population, while certainly outside the range of normal breeding practice, is probably as robust as can be obtained under such extreme environments. As shown in Table 1, Dicktoo was higher yielding than Morex under both fall and spring-seeded conditions. For all malting quality characters, Dicktoo was consistently much worse than Morex, indicating the overriding effect of genotype on the expression of malting quality. Even under fall-seeded conditions, Morex achieved near-acceptable levels of all characters except malt extract. The performance of Morex under spring-seeded conditions was fairly typical. As shown in Table 1, the DH population means in both environments were intermediate between the two parents for all characters. Frequency distributions for all traits were approximately normal (Figures 1a and 1b). There were positive transgressive segregants for total grain protein, soluble to total protein ratio and diastatic power under spring-seeded conditions; and for malt extract and yield under fall-seeded conditions. For the spring-seeded experiment, we observed 11 DH lines with diastatic power greater than Morex, 16 DH lines with soluble to total protein ratio greater than Morex, and 16 DH lines with total grain protein greater than Dicktoo. For the fall-seeded experiments, we observed 63 DH lines with malt extract greater than Morex, and 26 DH lines with grain yield greater than Morex.

The magnitude and sign of phenotypic correlations among malting quality characters in each environment (Table 2) generally conform to previous reports (Burger and LaBerge, 1985; Rutger et al, 1967; Smith,1990), and are reasonable in view of the biochemical basis of trait expression described in the Introduction. As expected, diastatic power and alpha amylase are positively correlated, as the latter measure is included in the computation of the former. Both enzymatic characters, in turn, were positively correlated with malt extract, the soluble to total protein ratio, and total grain protein. Malt extract was negatively correlated with total grain protein, and the largest negative correlation was found in the fall-seeded experiment, where even the Morex parent had a grain protein well outside of the acceptable US standards. With the exception of malt extract in both environments and soluble to total protein ratio in the spring-seeded experiment, modest negative correlations were observed between malting quality traits and grain yield. Inconsistent correlations among traits in the two environments were seen for diastatic power/malt extract, diastatic power/yield, total grain protein/soluble total protein ratio, and soluble total protein ratio/yield. The phenotypic correlations of Montana field survival and malting quality present an intriguing pattern. With the exception of malt extract, which was negatively correlated with field survival in both environments, malting traits were positively correlated with field survival under spring-seeded conditions and negatively correlated with field survival under fall-planted conditions. The consistently negative association of malt extract and cold tolerance is not encouraging from the standpoint of phenotypic selection response. A principal attribute of the QTL analyses that will subsequently be presented is the dissection of the genetic basis of traits showing phenotypic correlations. In summary, trends consistent with breeding experience for correlated trait expression were observed in this relatively wide cross evaluated under extreme environmental conditions. Nonetheless, simultaneous improvement for all components of malting quality, yield and cold tolerance, based on phenotypes alone, would be an enormous challenge.

Therefore, we have taken an alternative approach to these data, namely QTL analysis, in an effort to determine the genetic basis of trait expression.

Map construction

The 69 markers shown in Figure 1 provide reasonably complete genome coverage , with the exception of chromosome 3. Intervals greater than the specified allowable recombination value occurred on chromosomes 1,3, and 5. However, we can confidently assign these distinct blocks of linked markers to chromosomes based on their correspondence, in terms of both order and relative distance, to the more saturated North American Barley Genome Mapping Project map (Kleinhofs et al., 1993). Overall, locus orders and distances are in excellent agreement with previous map (Heun and Kennedy, 1991; Kleinhofs et al.,1993). Despite the relatively wide genetic base of this cross, significant segregation distortion ($p=.001$) was detected only at 7 loci.

QTL X environment interaction

As described in the preceding section on phenotypic expression, environment was critical in determining trait expression, but within the limits established by genetic background. Although the malt extract and grain protein values for Morex under fall seeded conditions were unacceptable, Morex was consistently superior for all malting traits to the winter feed parent under both fall and spring-planted conditions.

In a QTL-based analysis and selection program, as in a conventional breeding program, selection decision must be made in view of the interaction of genotypes and environments. Significant G X E interactions have been observed for the malting quality traits and yield expression (Finlay and Wilcoxson, 1963; Rasmusson and Glass, 1967; Sparrow, 1971).

Paterson et al. (1991) addressed QTL X environment interaction by tabulating the number of QTLs that were detected in each environment in which an F2 population of tomato and its F3 progeny were evaluated vs. these QTLs that appeared in more than one environment. QTLs common to more than one environment were considered to be those conferring broad adaptation while those that were detected in a single environment were considered to be those conferring environment specific advantage. These authors went on to argue that molecular marker-assisted selection could allow for the pyramiding of broad adaptation and environment specific QTLs to develop truly stable varieties. As the non-linear analysis procedure approach we have employed also allows for formal testing of hypotheses regarding QTL X environment interaction, we have presented data from the two types of analyses.

QTL X E interaction would be expected to be one of the two types: (i) alternative QTL alleles could be favorable in distinct environments, or (ii) QTL could vary in magnitude of effect across environments. With these data, QTL X E effects $\geq$ Wald 10 were detected only for WSIs for total grain protein on chromosomes 4 (spanning a WSI from *apGlu2* to *ABG366*) and 7 (spanning a WSI from *Rrn2* to *ABG391*), and in both cases Morex contributed the larger value allele. Thus, QTL X E interaction was negligible and when it occurred, it was a change in magnitude of response phenomenon. This justifies the main effects approach to QTLs analysis that we employ in a succeeding sections on average QTLs effects and orthologous gene expression. Nonetheless, it is of interest to relate the results of formal hypothesis testing of QTL X E with the approach taken by Paterson et al. (1991).

Prior to an enumeration of numbers of QTLs detected in each environment and their corresponding r^2 values, it is appropriate that we reiterate, and more extensively justify, the conservative criteria we employed in associating a marker interval with a QTL. Two issues are of concern: the number of QTLs that are detected as a consequence of the specified α threshold

and the resolution of QTLs. The issue of LOD thresholds was addressed by Lander and Botstein (1989) and Paterson et al. (1991) supply an extensive justification of their choice of a LOD 2.4 threshold value. Our specified Type I error rate , approximately $\alpha = .001$, limits our ability to detect small effect QTL, which may represent the effects of minor genes (Heun, 1992). However, QTL genotype mean differences for such small effect QTLs are so negligible as to preclude their detection in most field tests (Hayes et al., in press). In this report we will make occasional reference to QTLs with Wald ≥ 5; ≤ 10.

Interval mapping is known to generate multiple adjacent peaks (Paterson et al., 1991) and may in the case of adjacent, linked QTLs, generate centrally positioned, maximum value peaks where in reality no QTL exists (Haley and Knott, 1992; Martinez and Curnow, 1992). The latter authors refer to such artefacts as "ghost QTLs". Our specification of a WSI $\geq$ 90% maximizes QTL amplitude and consequently hampers resolution. Nonetheless, from an applied molecular marker-assisted selection context, QTL intervals of up to 30% would be allowable, provided that flanking markers are used for selection and that there are no coincident peaks or overlapping support intervals for alternative favorable QTL alleles. Thus, by specifying stringent Type I error rates and confidence intervals we should be able to "capture" a QTL even in the event that its predicted location may be somewhat biased as a consequence of the estimation procedure. Finally, we view population-based QTL mapping as a preliminary procedure that can be used to guide breeding practice and further, more detailed mapping in the alternative genetic stocks that are required for higher resolution mapping (van Ooijen, 1992).

Numbers of QTLs detected by the various analysis procedures and the corresponding r^2 values are presented in Table 3. For the purposes of enumeration, individual QTL were defined, as described above, by a Wald peak $\geq$10 and the accompanying WSI = 10. When there were two adjacent obvious peaks with overlapping WSIs, these were considered as separate QTL, for

purposes of enumeration and calculation of the proportion of phenotypic variance accounted for by each marker interval. The r^2 values were determined by linear regression of trait phenotype on Wald peak markers. Of the individual analyses, the fall-seeded experiment revealed the largest number of QTLs for the five quality traits. In both individual environment analyses, most QTLs were environment-specific. The number of common QTLs for diastatic power, alpha-amylase, total grain protein, and the soluble to total protein ratio were 2, 1, 0, 1 and 1, respectively. The combined analysis revealed the maximum number of QTLs (24) and explained the largest proportion of phenotypic variance for each trait. The QTLs detected in the combined analysis corresponded to those identified in the single environment analyses, with three exceptions. A QTL for malt extract under fall-planted conditions, with the positive allele contributed by Dicktoo (chromosome 3: WSI = *ABG460 - ABC176*) was not significant at Wald $\geq$ 10 in the combined analysis. Significant effects (Wald $\geq$ 10) were detected in the combined analysis that did not correspond to individual environment effects at the same α level in the individual environment analyses for diastatic power on chromosome 3 at WSI = *ABG4 - ABC 174* and for soluble to total protein ratio on chromosome 5 at WSI = *BCD265c - mRh*.

Environment-specific malting quality QTLs may be a consequence of environmentally regulated gene expression (Henry, 1990) or a consequence of the relatively greater information provided by certain test environments. In any event, in QTLs analysis, as in conventional phenotype evaluations, multi-environment testing is obviously required to generate reliable data for selection.

Given the lack of QTL X E interaction and the apparent power of the combined analysis to consistently detect the greatest number of large effect QTLs, the subsequent discussion will be based on the results of the combined analysis.

Chromosome location, magnitude, and relationships of QTLs

As described in the Introduction, and supported by the phenotypic correlations, the five malting quality characters are the consequence of interrelated phenomena. For the sake of clarity and exposition, we therefore present our discussion on individual chromosomes rather than a trait basis.

Chromosome 1

Significant QTLs effects for diastatic power, α amylase, total grain protein and grain yield were detected. The diastatic power WSI spanned a 40.6 cM interval from *ABC158* to *ABC455*, with a peak at *Brz* - *ABC465* that accounted for a 14.2 deg difference. Morex contributed the favorable allele. The WSI for α amylase overlapped with the diastatic power WSI, spanning a 39.4 cM interval from *ABC167* to *ABC 465*. The favorable allele was contributed by Morex, with a QTL genotype mean difference of 2.6 20 deg units at the *ABC 158* - *Brz* peak. The total grain protein was well-resolved, with a coincident peak and WSI at *Brz* - *ABC465*. The larger value allele was again contributed by Morex.

A QTL ($5 \leq$ Wald ≤ 10) for soluble to total protein was also detected in the *ABC158* - *ABC455* interval, with Morex contributing the favorable allele. The genetic basis of these coincident or overlapping QTLs effects with the positive allele contributed by the same parent cannot, at this stage of our analysis, be attributed to linkage or pleiotropy, but one can generalize that QTLs for key enzymatic characters are located on chromosome 1. Unfortunately, from a selection standpoint, these enzymatic trait QTLs overlap with a yield QTL, where Dicktoo contributed the favorable allele. The WSI for this QTL extended from *ABG380* to *ABC455*, with a QTL genotype mean difference of 437 kg ha^{-1} at the *ABC158* - *Brz* peak. Whether this is a

negative pleiotropic or linkage relationship cannot be determined without additional markers in the *ABC158* - *Brz* interval.

Chromosome 2

The only QTL effect ≥ Wald = 10 was for malt extract. Two peaks were detected at *ABG8* - *ABC311* and *ABC156* - *ABC170*. The QTL genotype mean differences were 1.0 % and 0.9 %, respectively, with Morex contributing the favorable alleles. The WSIs for these two peaks overlapped and spanned a 19 cM interval from *BCD175* to *ABC170*. Additional resolution of this interval would not be necessary for molecular marker-assisted selection, provided that flanking markers were employed. As no corresponding QTL for enzymatic characters were detected, it would be of interest to further characterize this chromosome region in terms of its role in carbohydrate synthesis, deposition, and storage.

Chromosome 3

The limited number of markers mapped to this linkage group precludes a definitive discussion of the number and magnitude of QTLs effects. The only significant effect was for soluble to total protein ratio, with a coincident peak and WSI spanning the 21.2 cM interval between *ABG4* and *ABC174*. As no corresponding QTL for other malting quality traits were detected, this region would be of interest for further characterization in terms of proteinase activity.

Chromosome 4

QTLs for diastatic power and soluble to total protein ratio, with Morex contributing the favorable allele, were well-resolved with coincident peaks and WSIs in the *ap1920* - *WG1026b*

interval. the QTL genotype mean differences were 9.6 deg and 3.4 %, respectively. There was a corresponding peak for total grain protein, with the higher value allele contributed by Morex, in this same interval. The WSI for this QTL extended from *apGlu2* - *WG1026b*.

QTLs ≥ 5; ≤ 10 for malt extract and α amylase, with Morex contributing the favorable alleles, were also detected in the *ap1920* - *WG1026b* interval. These coincident enzymatic trait QTL and smaller effect malt extract QTL support the observed phenotypic correlations, and provide a relatively defined interval for further characterization in terms of protein activity. In a coincident peak and WSI in the *mHs* - *Bmy1* interval, Dicktoo contributed a favorable allele for diastatic power (10.5 deg) that was not reflected in coincident QTL for other protein-related traits or malt extract.

Bmy1 is a β amylase probe (Kreis et al., 1987) and as diastatic power is a joint measure of α and β amylase activities, this provides an example of coincident known function genes and QTL effects. The contribution of a favorable allele for a malting quality trait by the feed parent provides genetic evidence for the observed phenotypic transgressive segregation and offers some hope for the recovery of superior progeny in a fairly wide, feed x malting type cross.

Chromosome 5

Larger value alleles for total grain protein (WSI = *Horc* - *iGpi*) and soluble to total protein ratio (WSI = *BCD265c* - *apHrth*) were contributed by Morex but were not reflected in malt extract or hydrolytic enzyme QTL. The presence of a QTL effect for total grain protein in the vicinity of one of the hordein storage protein loci (Bunce,1986) provides an additional example of coincident QTLs and known function genes.

Chromosome 6

Dicktoo contributed larger value alleles for total grain protein at two peaks (*Amy1 - Nar7* and *WG223 - ABG1*) with overlapping support intervals. QTL genotype mean differences were 0.6 % at both peaks, and the total WSI spanned 37.5 cM from *ABG387* to *Nar7*. Morex contributed a same favorable allele for yield in the same WSI, with a peak at *ABG1 - Amy1* and a QTL genotype mean difference of 340 kg ha^{-1}. A QTL for malt extract significant at Wald ≥ 5; $\leq$ 10 spanned a WSI from *WG223* to *Nar7*. Within this region lies the *Amy1* locus (Muthukrishnan and Chandra, 1983; Muthukrishnan et al, 1984; Rogers, 1983), providing additional evidence in support of coincident QTLs effects and known function genes. The presence of these yield and malt extract QTLs also support the positive phenotypic correlations observed for malt extract and yield. From a selection standpoint, the relative importance of the yield and extract contributions would have to be weighed against the total grain protein contribution of Dicktoo. However, as there is an allowable upper limit of 13 % total grain protein in malting barley (Burger and LaBerge,1985) and this grain protein QTL was in fact in repulsion phase with a small effect QTL for malt extract, molecular marker-assisted selection would logically be in favor of the Morex alleles.

Furthermore, the only grain β glucan QTL detected in this analysis coincided with the total grain protein QTL WSI. The peak was at *Amy1 - Nar7* and accounted for a 0.6 % QTL genotype difference with Dicktoo contributing the larger value allele. High grain β glucan is an underisable property in both malt and feed barleys (Molina-Cano et al, 1989; Edney et al, 1991). As cold tolerance is reported to involve cell wall carbohydrates (Olien,1978), we were also interested in determining the QTLs relationship of β glucan and cold tolerance. As reported by Hayes et al (1993) and summarized in the next section, cold tolerance QTLs effects were detected only on the long arm of chromosome 7. Thus, it would appear that β glucan is not directly

related to cold tolerance. The contribution of a favorable QTL for grain yield from the consistently lower yielding parent is additional genetic evidence for the observed phenotypic transgressive segregation.

Chromosome 7

QTLs for all traits were detected on this chromosome, with both parents contributing favorable alleles. The repulsion phase of these allelic contributions indicates that tighter resolution will be required for effective molecular marker introgression of target regions on this chromosome. Morex contributed favorable alleles for diastatic power, malt extract, α amylase, soluble to total protein ratio, and total grain protein with multiple peaks and broad WSIs for each trait. There were two peaks for malt extract at *BCD298* - *ABC302* and *mR* - *BCD265b*, accounting for 0.9 % and 0.8% QTL genotype mean differences, respectively. The overlapping WSIs for these peaks spanned a 124 cM region extending from *aADH* to *ABG391*. There were two peaks for diastatic power, at *Rrn2* - *Ipa* and *aADH* - *BCD298*. QTL genotype mean differences were 20.0 deg and 20.4 deg,respectively. The overlapping WSIs for these peaks spanned a 91.5 cM interval extending from *apNAR7l* to *mR*. The WSI for α amylase spanned the same interval, except that Wald peaks were located at *Ipa* - *aADH* and *BCD298* - *ABC302* intervals. QTL genotype mean differences at these peaks were 5.6 and 5.1 20 deg units, respectively. The WSI for soluble to total protein ratio extended from *Rrn2* to *mS*, with a peak at *Ipa* - *aADH*, where the QTL genotype mean difference was 4.2 %. The total grain protein QTL WSI extended from *apNar7l* to *mS*. Thus, on a significant proportion of chromosome 7, there were overlapping and sometimes coincident peaks for the full spectrum of malting quality characters.

A yield QTL, with the positive allele contributed by Dicktoo spanned a WSI extending from *apNar7l* to *mS*. At the peak, *Ipa - aADH*, the QTL genotype mean difference was 371 kg ha^{-1}, and this interval corresponds exactly with the peak intervals for soluble to total protein ratio and α amylase and is adjacent to peak intervals for diastatic power and malt extract. These repulsion associations of yield with malting quality characters present a significant impediment to molecular marker-assisted selection. Finer structure mapping, based on lines near-isogenic for segments of chromosome 7, may assist in more rigorously defining QTLs boundaries and thus permit simultaneous selection for yield and malting quality QTLs.

As reported by Hayes et al (1993) the only QTL for cold tolerance in this population was detected on chromosome 7 at a peak interval defined by *mR - BCD265b*. Dicktoo contributed the favorable allele and the QTL genotype mean difference for Montana field survival was 47 %. The WSI for this QTL extends from *WG364b* to *ABG391*, the latter marker having been added to the map since the first report by Hayes et al (1993). As described in the preceding paragraph, a QTL peak for malt extract was also detected at *mR - BCD265b*, with Morex contributing the favorable allele. As with the remainder of this chromosome, higher resolution mapping would be required to determine if the negative relationship of malt extract and cold tolerance is due to linkage or pleiotropy. At this point, molecular marker selection in favor to cold tolerance in this large (24.8 cM) interval would be at the expense of malt extract. This negative QTL association was reflected in the negative phenotypic correlation of malt extract and Montana survival.

Orthologous gene expression

A principal issue in QTL-based breeding strategies is cross specificity : are QTLs detected in one genetic reference population genotype-specific or will they be present in diverse germplasm arrays ? Paterson et al. (1991) reported coincident QTLs in diverse species of

Lypocersicum and suggested that even interspecific variation could be due to allelic variation at a limited number of orthologous QTLs. Fatokun et al. (1992) have recently presented evidence for orthologous gene expression for seed weight in cowpea and mung bean. Given the limited reports on barley QTLs, at this point we can only draw tentative conclusion regarding orthologous expression.

The North American Barley Genome Mapping Project map (Kleinhofs et al., 1993) and the subsequent QTL analysis (Hayes et al., in press) allows for fairly direct comparison as the two maps employ a number of common markers. However, extension of these conclusions to barley germplasm as a whole is limited by the fact that the two maps employ a common parent, Morex. QTLs for malt extract on chromosomes 2 and 4, total grain protein on chromosomes 4 and 7, diastatic power on chromosomes 1 and 7, and α-amylase on chromosomes 1,5 and 7 have coincident peaks or WSIs in the two populations. In all cases, Morex contributed the favorable allele at each QTL. In both population, the non-malting parents (Dicktoo or Steptoe) contributed a favorable allele for diastatic power on chromosome 4, in intervals adjacent to the *Bmy1* marker. In both populations the feed parents contributed favorable alleles for yield at QTLs in the chromosome 1 interval defined by *ABC167-ABC158* and on chromosome 2 in the interval defined by *apWG110s-WG645*. Evidence for coincident QTLs, but with alternative favorable alleles, was found for malt extract on chromosome 5 (WSI = *ap23BIa - Hor1*), where Dicktoo contributed the favorable allele at Wald $\geq$ 5; $\leq$ 10 in the Dicktoo x Morex population, and Morex the favorable allele in the Morex X Steptoe population. On chromosome 2, Dicktoo contributed the favorable alleles for diastatic power in the *ABC170-CDO88* interval at a Wald $\geq$ 5; $\leq$ 10 in the Dicktoo x Morex population and Morex the favorable allele in the Morex x Steptoe population. As the effect were only marginally significant in the Dicktoo x Morex population analysis,

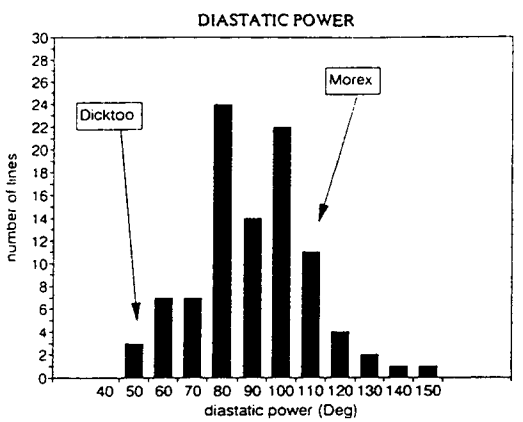
rigorous testing of hypotheses regarding a series of alternative favorable alleles at a QTL locus would require a corresponding analysis in the progeny of Dicktoo x Steptoe.

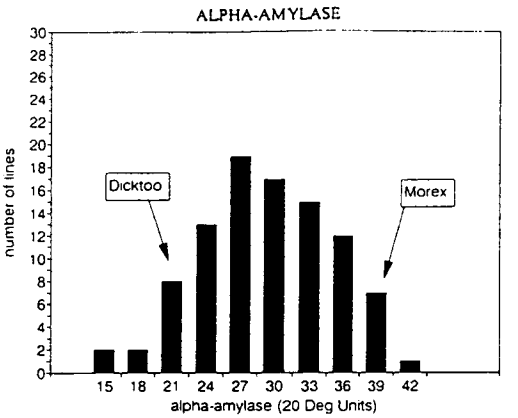
Evidence for orthologous gene expression in other genetic backgrounds is more tenuous, due the paucity of common markers. However, some preliminary parallels can be drawn based on approximate chromosome regions. Giese et al. (1993) reported that the *Ml(la)* locus was linked to a milling energy QTL on chromosome 2. Milling energy is an indirect measure of malt extract (Swanston, 1987; Allison et al.,1979) , and we detected a malt extract QTL in the region bounded by *BCD175-ABC170* in Dicktoo x Morex, and a malt extract QTL was also reported in this region in Steptoe x Morex (Hayes et al, in press). Powell et al. (1992) reported the presence of an additional milling energy QTL linked to *Rrn2* on chromosome 7. Diastatic power and α-amylase QTL, but not malt extract QTL, were found in the vicinity of Rrn2 in the Dicktoo x Morex population. Roberts (1990) and Sutka and Snape (1989) mapped cold tolerance QTL on wheat chromosome 5, which is homologous to barley chromosome 7 (Islam et al., 1981). We detected QTL for cold tolerance on chromosome 7.

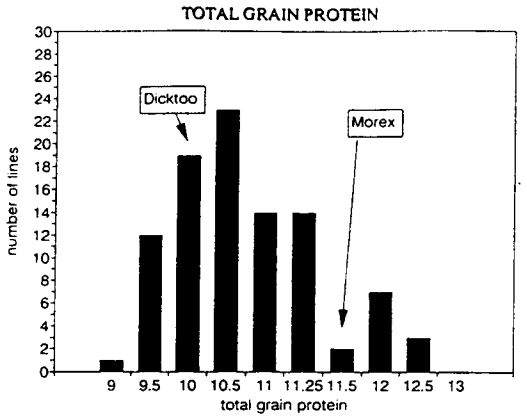
In conclusion, QTL analyses should be of assistance in meeting the challenge of simultaneously improving malting quality and cold tolerance. While the results of this analysis are of necessity limited to the genetic population studied, there is preliminary evidence for orthologous gene expression of QTLs that may justify extrapolation of our results to the development of winter malting barley as a whole. Coincident or adjacent repulsion relationship of yield QTL alleles with malting QTL alleles, and of cold tolerance with a malt extract QTL, may explain the hard-fought gains achieved in developing winter habit malting barley. Higher resolution mapping in these key chromosome regions will be required to determine if the negative associations are due to linkage or pleiotropy.

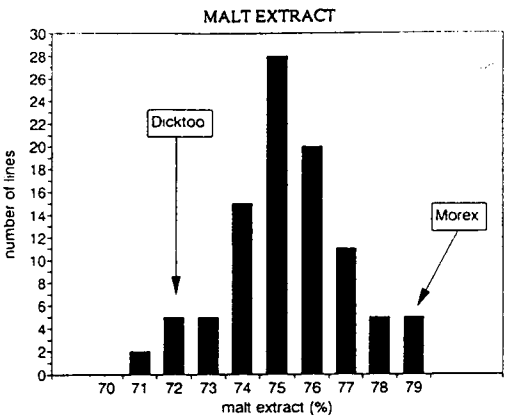
Nonetheless, a number of malting quality and yield QTLs were located elsewhere in the genome that present unambiguous targets for molecular marker-assisted selection. The distribution of QTLs effects confirms observed phenotypic correlations. Our analyses confirm the overriding effect of genotype on the expression of malting quality. Although environment is, of course, a key determinant of the level of the malting quality achieved, QTL X E interaction was minimal, and when it occurred it was due to a difference in magnitude of response. Whether QTLs detected only in one environment are indicative of environmentally-mediated control of gene expression or simply the ability of tests to generate discriminant phenotypes is open to question. In any event, formal testing of hypotheses regarding QTL X E interactions via the non-linear model approach followed by analyses based on main effects detected in the combined analysis over environments should be more powerful than enumeration and visual comparison of common vs. environment-specific QTLs. There is excellent evidence for QTLs affecting malting quality characters in barley, but these observed effects must be validated in selection response experiments.

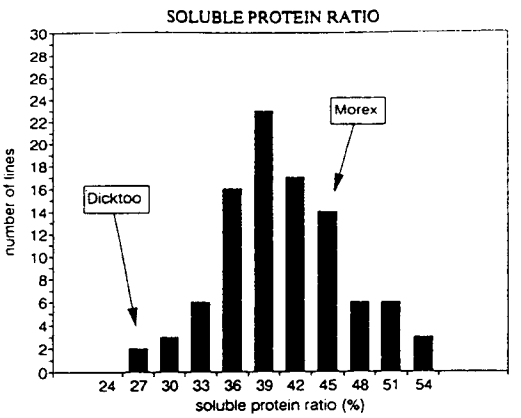
Figure 1a : Frequency distributions for malting quality traits and grain yield in a population of 100 doubled haploid lines evaluated under spring-seeded conditions











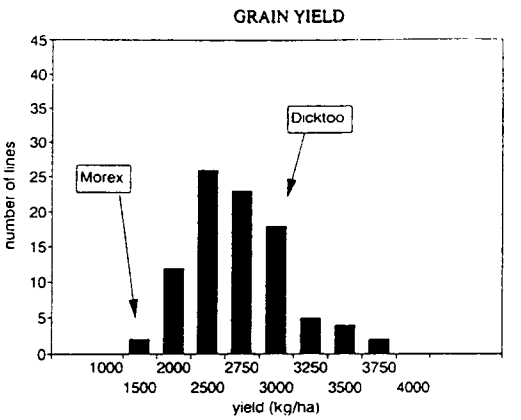


Figure 1b : Frequency distributions for malting quality traits and grain yield in a population of 100 doubled haploid lines evaluated under fall-seeded conditions

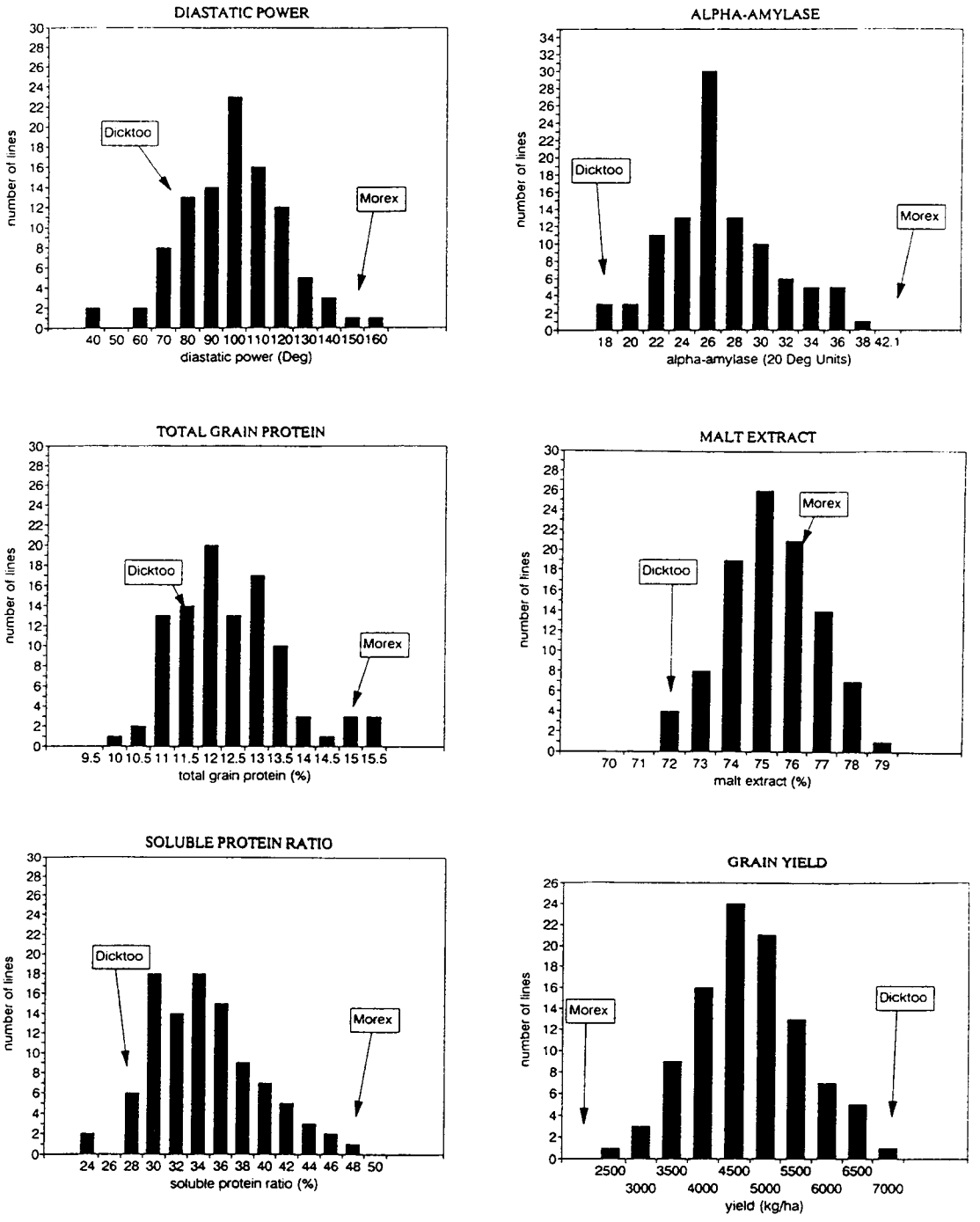


Figure 2 : Linkage map based on sixty nine markers assayed in 100 F1-derived doubled haploid progeny from the cross of Dicktoo X Morex. Distances are given in percent of recombination. Dotted lines indicate clusters of linked markers presumed to be located on the same chromosome but unlinked in the current analysis. See text for details of mapping procedure and marker nomenclature.

Figure 2 :

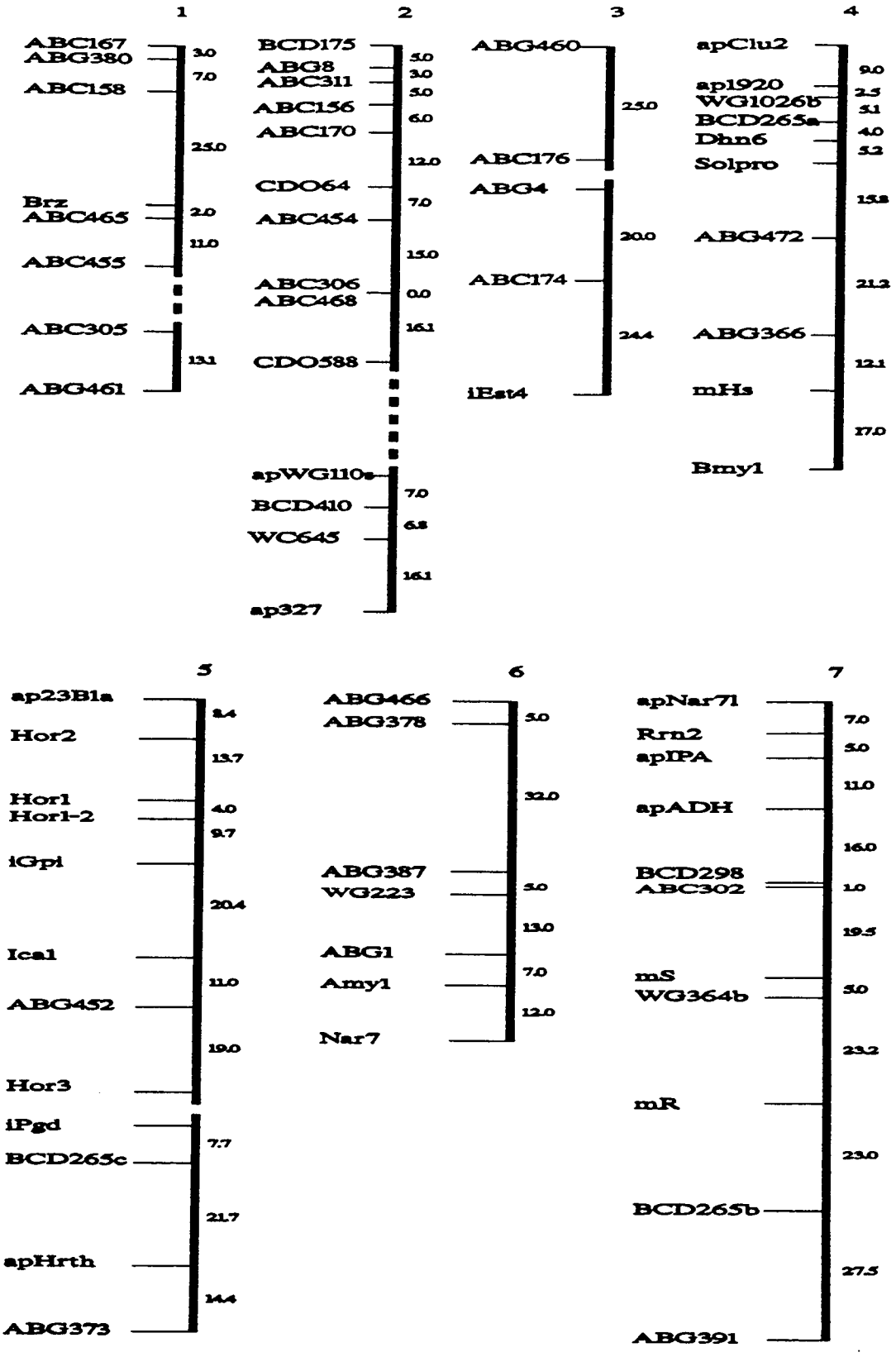

1
ABC167
ABG380
ABC158
Brz
ABC465
ABC455
ABC305
ABG461
3.0
7.0
25.0
2.0
11.0
13.1
2
BCD175
ABG8
ABC311
ABC156
ABC170
CDO64
ABC454
ABC306
ABC468
CDO588
apWG110a
BCD410
WC645
ap327
5.0
3.0
5.0
6.0
12.0
7.0
15.0
0.0
16.1
7.0
6.8
16.1
3
ABG460
ABC176
ABG4
ABC174
iEst4
25.0
20.0
24.4
4
apClu2
ap1920
WG1026b
BCD265a
Dhn6
Solpro
ABG472
ABG366
mHs
Bmy1
9.0
2.5
5.1
4.0
5.2
15.8
21.2
12.1
17.0
5
ap23B1a
Hor2
Hor1
Hor1-2
iGpi
Ica1
ABG452
Hor3
iPgd
BCD265c
apHrth
ABG373
8.4
13.7
4.0
9.7
20.4
11.0
19.0
7.7
21.7
14.4
6
ABG466
ABG378
ABG387
WG223
ABG1
Amy1
Nar7
5.0
32.0
5.0
13.0
7.0
12.0
7
apNar71
Rrn2
apIPA
apADH
BCD298
ABC302
mS
WG364b
mR
BCD265b
ABG391
7.0
5.0
11.0
16.0
1.0
19.5
5.0
23.2
23.0
27.5

Table 1 : Malting quality and agronomic traits means and ranges for a population of Doubled Haploid (DH) lines derived from the cross of Morex (M) and Dicktoo (D) evaluated under fall (F) and spring (S) seeded conditions.

	Diastatic Power Deg		Alpha-amylase 20 Deg units		Malt Extract %		Total Grain Protein %		Soluble Protein Ratio		Yield Kg/ha	
	F	S	F	S	F	S	F	S	F	S	F	S
D	77	61	16.5	20.5	71.2	71.5	11.2	10	29.9	27.4	6388	2781
M	145	105	42.1	37.7	74.4	78.5	14.6	11.4	47.2	44.7	1753	1883
DH mean	96	85	25.9	27.8	74.8	74.9	12.2	10.9	33.7	39.2	4496	2319
DH range	54 156	42 143	16.5 37.5	14.4 39.4	71.4 78.1	70.4 78.9	9.9 15.4	8.9 13	23.4 47.3	24.7 53.9	2881 6673	1041 3549

Table 2 : Phenotypic correlations among agronomic and malting quality traits measured in fall ( ) and spring [ ] seeded experiments (upper part of the table) and for overall values (below the diagonal) of 100 DH lines derived from the cross of Dicktoo X Morex.

	Diastatic Power	Alpha-Amylase	Malt Extract	Total Grain protein	Soluble Protein Ratio	Yield	Montana survival
Diastatic Power		[68.8] (71.0)	[39.4] (2.64)	[50.0] (67.0)	[38.6] (40.8)	[-3.3] (-34.5)	[16.1] (-4.3)
Alpha-Amylase	69.8		[59.3] (32.8)	[18.4] (43.1)	[46.5] (66.3)	[-3.0] (-18.2)	[11.5] (-10.1)
Malt Extract	28.9	56.3		[-23.0] (-27.9)	[52.5] (44.2)	[23.9] (33.17)	[-27.8] (-10.4)
Total Grain Protein	61.8	34.5	-14.4		[-12.6] (14.4)	[-11.3] (-35.0)	[20.7] (-13.0)
Soluble Protein Rtaio	46.4	66.4	65.4	2.4		[-11.9] (5.0)	[18.2] (-6.8)
Yield	-31.7	-17.3	27.2	-37.6	-5.5		[-32.6] (-9.2)
Montana survival	5.5	2.2	-22.6	0.6	8.4	-25.0	

Table 3 : Numbers of QTLs and corresponding r^2 values for malting quality traits and grain yield detected in the individual (fall-seeded vs spring-seeded) and combined analysis of 100 doubled haploid lines derived from the cross Dicktoo X Morex.

Trait	QTLs detected in fall-planted experiment		QTLs unique to fall-planted experiment		QTLs detected in spring-planted experimentt		QTLs unique to spring-planted experiment		Total of QTL detected in combined analysis	
	QTL#	r^2	QTL#	r^2	QTL#	r^2	QTL#	r^2	QTL#	r^2
Diastatic power	4	56.9	2	32.3	3	56.6	1	18.2	6	72.0
Alpha-amylase	1	31.0	0	-	2	45.3	1	11.6	3	53.0
Malt extract	3	34.5	3	31.5	2	39.0	2	3.9	4	31.5
Total grain protein	6	54.3	5	48.2	2	35.0	1	9.4	7	55.2
soluble protein to total ratio	2	38.8	1	13.3	2	21.3	1	10.0	4	51.4

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CONCLUSION

Preliminary evidence was found for orthologous gene expression of certain malting quality QTLs that may justify generalization of results to winter malting barley development. Coincident or adjacent repulsion relationships of yield QTL alleles with malting QTL alleles, and of cold tolerance with a malt extract QTL, may explain the modest gains achieved in developing winter habit malting barley. Higher resolution mapping in these key chromosome regions will be required to determine if the negative associations are due to linkage or pleiotropy. Nonetheless, a number of malting quality and yield QTLs were located elsewhere in the genome that present clear targets for molecular marker-assisted selection. The distribution of QTLs effects confirm the observed phenotypic correlations. The data confirm the overriding effect of genotype on the expression of malting quality. Although environment is, of course, a key determinant of the level of malting quality achieved, QTL X E interaction was minimal, and when it occurred it was due to a difference in magnitude of response. Whether QTLs detected in one environment are indicative of environmentally-mediated control of gene expression or simply the ability of tests to generate discriminant phenotypes is open to question. Formal testing of hypothesis regarding QTL X E interaction via the non-linear model approach followed by analysis based on main effects detected in the combined analysis over environments should be more powerful than enumeration and visual comparison of common vs. environment-specific QTLs . There is excellent evidence for QTLs affecting malting quality characters in barley, and these observed effects will be validated in selection response experiments. The QTLs analysis procedure allowed for a dissection of the genetic basis of complex phenotypes, and may servs as a model system for comparable analyses in other crops.

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