Chapter10

Genetic diversity for quantitatively inherited agronomic and malting quality traits

P.M. Hayes[a], A. Castro[a], L. Marquez-Cedillo[a], A. Corey[a], C. Henson[b,c], B.L. Jones[b,c], J. Kling[a], D. Mather[d], I. Matus[a], C. Rossi[a], K. Sato[e]

[a] Department of Crop and Soil Science, Oregon State University, Corvallis, OR, 97331, USA
[b] Cereal Crops Research Unit, USDA-ARS, 501 Walnut St., Madison, WI 53705, USA
[c] Department of Agronomy, University of Wisconsin, Madison, WI 53706, USA
[d] Department of Plant Science, McGill University, Ste Anne de Bellevue, QC H9X 3V9 Canada
[e] Research Institute for Bioresources, Okayama University, Kurashiki 710-0046, Japan

Introduction

Agronomic and quality traits were undoubtedly key issues for the domesticators of barley. According to the archeological record, these early farmers used both wild and cultivated (non-brittle rachis) forms of barley (Harlan, 1995). Crop productivity would clearly have been an attribute of key interest, and the selection of shattering resistant mutants probably led to a quantum leap in yield. Because barley has been used as both a food and as a principal ingredient of fermented beverages from the earliest times, there may well have been conscious selection for end-use properties. The selection of hull-less mutants in areas of the world where barley was a principal foodstuff underscores the importance of end-use properties in domestication. The malting and brewing properties of wild barley accessions and land-races have not been well described and are, in fact, extremely difficult to measure. There are no absolute definitions of malting and brewing quality, due to differences in malting and brewing practices and consumer preferences. Both direct consumers of barley and the first brewers would have selected for uniform, plump kernels. However, only the latter would have selected for the higher levels of enzymatic activity necessary for satisfactory malting and brewing performance.

Plant breeding efforts are directed primarily at traits exhibiting quantitative variation. However, diversity and quantitative traits are not terms that are often encountered together in the plant breeding and genetics literature. This is certainly true for barley, a crop with a rich legacy of genetics research and an impressive record of variety improvement. Genetics was limited to models where phenotypes allowed for Mendelian analysis. Genetic tools, when applied to variety development, were of a statistical nature. In the mid-1980's, the availability of abundant DNA-level markers, accompanied by the development of computer hardware and software, caused these parallel lines of investigation to change course and head toward intersection. Breeders and geneticists were now able to collaborate in developing and testing hypotheses regarding the number, location, effect, and interactions of genes influencing quantitative traits. These genes were termed "Quantitative Trait Loci" (QTL). In this chapter, we will review (1) diversity in agronomic traits; (2) diversity in malting quality traits; and (3) the current status of QTL analysis in barley and the application of QTL tools to the analysis of genetic diversity in barley and crop improvement.

Agronomic traits

Barley is one of the most widely adapted cereal crops. It is grown in a range of extreme environments that vary from northern Scandinavia to the Himalayan mountains to monsoon paddies. No barley variety is adapted to all environments and, in fact, very different gene pools have evolved in the major barley production areas of the world. The gene pools may be defined by essential physiological parameters that determine adaptation to a production environment - such as vernalization and/or photoperiod response - or they may be defined by evolutionary bottlenecks and the accidents of history, such as regional preferences for two-rowed or six-rowed varieties. Within these gene pools, agronomic performance will be determined by the entire allelic architecture of each genotype. The actual number of genes in barley (with a genome size of 5.3 billion base pairs) is not known at this point. There are at least 26,588 genes in the euchromatic regions of the human genome (2.91 billion base pairs) and there is some evidence for an additional 12,000 genes (Venter *et al*., 2001). The first higher plant genome – *Arabidopsis thaliana*, with an estimated genome size of 125 million base pairs – was sequenced and the number of genes was estimated at 25,800 (Pennisi, 2000). Surely, most of the genes in barley are involved in one way or another with yield – the "ultimate" fitness trait. Likewise, a large number of them must be involved in malting quality, which is the end result of the fundamental processes of seed carbohydrate deposition and hydrolysis.

In terms of agronomic traits, growth habit genes may be major "specious" yield genes (see also Chapter 2). For example, highly significant genotype x environment interaction will occur when the progeny derived from a cross of spring x winter habit parents are evaluated under spring and fall-sown conditions. A genetic analysis of such germplasm would likely reveal vernalization requirement, photoperiod reaction, and low temperature tolerance genes as primary determinants of yield. This was indeed the case when Quantitative Trait Locus (QTL) tools were applied to a population derived from the cross of North American winter and spring genotypes (Oziel *et al.,* 1996). Likewise, resistance to biotic stresses can be the primary determinant of yield, if resistance to the disease in question is endemic to a production zone. A "cost", or lower yield potential of resistant vs. disease susceptible germplasm in disease-free environments, could be due to linkage of resistance and "yield determining" genes or pleiotropic effects of the resistance genes (Hayes *et al.,* 1996).

Effects of inflorescence type on yield

The role of inflorescence type in determining yield is complex. In barley each rachis node has three spikelets, each of them bearing one floret. The two-rowed and six-rowed germplasm groups are defined by the number of fertile florets per rachis node. In two-rowed barley, there is one fertile floret per rachis node, whereas in six-rowed barley all three florets are fertile (Hitchcock, 1971; see also Chapter 2). Most barley varieties of commercial importance are six-rowed or two-rowed inbred lines. This simple genetic system defines the two principal germplasm groups of barley, and these germplasm groups have historically defined end uses. Two-rowed barleys are favored for malting throughout most of the world, except for the USA and Mexico, where six-rowed barleys are used extensively for this purpose (Riggs and Kirby, 1978).

Two-rowed varieties usually have a higher number of tillers per plant and larger, heavier seed than six-rowed varieties. Six-rowed varieties on the other hand, usually have more seeds per inflorescence. Thus the compensatory effects of yield components lead to similar levels of yield potential. However, historical patterns of geographic distribution and end-use of the two-rowed

and six-rowed germplasm groups have led to the idea that the two germplasm groups carry different alleles at other loci in addition to those determining lateral floret fertility (Takahashi *et al.,* 1975). Accordingly, crosses between the two germplasm groups might be expected to produce positive transgressive segregants for economically important phenotypes. The experience of plant breeders, however, has generally been that two-rowed x six-rowed crosses are not suitable for variety development (Harlan, 1957; Kjær and Jensen, 1996). Allard (1988) concluded that "Evidently the two-row/six-row locus affects developmental processes in ways that leave few quantitative characters untouched" and "this locus had large effects on survival and adaptedness". Large pleiotropic effects on multiple phenotypes have been attributed to alleles at the *vrs1* locus, based on studies of the progeny of two-rowed x six-rowed crosses (Kjær and Jensen, 1996; Jui *et al.*, 1997). However, it is possible that the correlated phenotypes are due to linkage rather than pleiotropy. It is difficult to distinguish between these phenomena in the case of the *vrs1* locus, which is located near the centromere on linkage maps. On the physical map of Künzel *et al.* (2000), however, the *vrs1* locus is located in one of the higher recombination regions on the long (minus) arm of chromosome 2H. Powell *et al.* (1990), in a comparative analysis of two types of progeny from a six-rowed x two-rowed cross, concluded that some associations between quantitative phenotypes and the *vrs1* locus were due to linkage rather than pleiotropy. Marquez-Cedillo *et al.* (2001) used QTL tools to analyze a two-rowed x six-rowed mapping population for agronomic traits. The major QTL related with agronomic traits coincided with the loci that determined inflorescence type.

Two-rowed barley in Japan provides an example of the role of growth habit and maturity genes within a germplasm class. Yasuda *et al.* (1992) analyzed a collection of two-rowed barleys from Japan, Turkey, Ethiopia and Europe and found that Japanese two-rowed malting cultivars, which were introduced from Europe less than 130 years ago, were quite different from the other germplasm groups. Adaptation to Japanese conditions was achieved within a relatively short period, and a major distinguishing attribute of the Japanese germplasm is early maturity. In Japan, barley should be harvested before the rainy season, when the fields are converted to rice paddies. Yasuda (1964) used hybrid populations derived from winter/spring barley crosses to demonstrate selection for adaptation to Japanese conditions and later showed the effect of four spring growth habit genes on yield performance (Yasuda, 1977; see also Chapter 2).

Identifying factors that affect yield

Once the more obvious "yield-limiting genes" are accounted for, what determines yield in barley, and how much genetic diversity is there at these loci? There have been three principal approaches to the study of "yield genes". Plant physiologists have defined at the cellular, organ, and organism level the fundamental physiological processes that lead to yield in barley (Kirby and Faris, 1972; Gallagher *et al.*, 1975; Lauer and Simmons, 1989; Dreccer *et al.*, 1997). Crop physiologists and plant breeders have defined physiological parameters for production level phenotypes – e.g. yield components, harvest index, and ideotypes – that determine yield (Rasmusson, 1987; Hamblin, 1993). This research has led to a better understanding of fundamental plant processes and better *a posteriori* understanding of crop performance, but in many cases the two approaches have not led to predictive tools that are useful for barley improvement (Beldford and Sedgley, 1991; Rasmusson, 1991). A problem has been that, often of necessity, these studies were conducted in agronomically irrelevant genetic stocks and environments. Furthermore, it has been exceptionally difficult, as shown in the examples of growth habit and inflorescence type, to separate the effects of individual genes with large effects

from "genetic background".

Because of these challenges, and the fact that most agronomic traits showed inheritance patterns too complex for Mendelian inheritance, barley breeders and crop geneticists approached the issue of the genetics of yield using biometrical tools. However, as stated by Allard (1988) "Most quantitative genetic models require numerous assumptions, many of which are invalid, thus causing estimates of genetic parameters to be imprecise and even to exceed their theoretical limits. Whatever the cause or causes, the laborious biometrical experiments I conducted provided little information about the numbers of alleles per locus, numbers of loci, types of gene action (additive, dominance, and epistatic), the impact of various single locus or multilocus genotypes on fitness, and other genetic factors about which we must learn more if we are to understand the evolution of adaptedness".

For over a decade, molecular approaches have been used to map and describe Quantitative Trait Loci that determine yield in barley. As discussed in a subsequent section of this chapter, our understanding of QTL and their effects on agronomic traits is still incomplete. Nonetheless, developments in molecular biology have provided the necessary tools for determining the functional basis of yield and adaptation in barley.

Malting quality traits

Historically, the biochemistry and genetics of malting quality have been parallel areas of study. The former has focused on the systematic characterization of the deposition and hydrolysis of starch and proteins. This research has provided a more comprehensive understanding of the underlying processes, but has not provided breeders with a better tool kit for improving malting quality. Genetic studies of malting quality, to date, have provided perspectives on allelic diversity at only a few key loci. Consequently, barley breeders must still conduct expensive tests to determine the malting quality of their experimental germplasm and, because of the expense, can only carry out a limited number of assays. This has precluded the use of extensive population-based analyses of malting quality genetics. Furthermore, when such analyses have been conducted, malting quality phenotypes have shown frequency distributions that defy Mendelian analysis. Breeders have therefore relied upon phenotypic selection of malting quality in agronomically relevant germplasm, with occasional attempts to estimate genetic variances and numbers of "effective factors". As is evidenced by the quotation from Allard (1988) cited in the previous section on "Agronomic traits", these procedures have had limited practical applications. Information on allelic diversity in the genes that determine malting quality is limited. Accordingly, we will focus on providing a brief summary of the malting process and an enumeration of the genes known to be involved in these processes. The reader is referred to earlier reviews of the malting process (Burger and LaBerge, 1985; Bamforth and Barclay, 1993) and biochemical aspects of barley seed germination (Briggs, 1992; Fincher and Stone, 1993) for further background information. Expressed Sequence Tag (EST) projects have recently been initiated for malting quality in several laboratories around the world, but results are not yet available. Characterization of the structure and expression of genes expressed at key times and in key tissues relevant to the malting process will provide a foundation for efficient characterization and use of allelic diversity for malting quality traits.

The malting process

Malting is an exercise in applied biochemistry, especially enzymology. The starch, protein and nucleic acid molecules that are stored in barley grains are not good nutrients for brewing yeast

nor do they support the fermentation reactions performed by brewing yeasts. These large and structurally complex compounds must be partially or, in some instances, fully degraded into their component sugars, amino acids, and nucleotides before the yeast can use them. When barley seeds germinate, hydrolytic enzymes are synthesized or converted to active forms that can readily degrade these large compounds.

During "malting", barley seeds are germinated under controlled conditions so that degradative enzymes form and begin to hydrolyze the starch, protein, and nucleic acid molecules into small molecules that are needed at appropriate stages of the brewing process. To arrest the malting process, the green malt is kilned (gently dried, with heat) and the rootlets are removed. By this stage, little of the starch has been converted to sugars, but about 70% of the protein that needs to be solubilized during malting and mashing has already been rendered soluble. There is still some question as to how much free amino nitrogen (FAN) is released during malting. Modification is a collective term that is used to refer to all of the polymer-degrading processes that occur during malting. If malting is allowed to continue too long, the malt obtained will be overmodified and will not produce beers of optimal quality.

The malt is treated with water under appropriate conditions (a process called "mashing") to obtain an extract (wort) that must perform several critical functions. The extract must provide adequate nourishment to the yeast so that fermentation can occur. Secondly, the extract must provide sufficient fermentable sugars to enable the yeast to produce the desired levels of alcohol. A high quality malt will contain the right amount of hydrolytic enzymes and metabolites to fulfill these requirements and will have the right degree of friability to allow many of its components to be readily solubilized during mashing. During malting and mashing, the barley starch should be almost completely degraded into sugars that can be utilized by the brewing yeasts, whereas only about 45% of the barley protein should be solubilized. Too much protein solubilization is thought to result in beers with poor foaming characteristics. When insufficient protein hydrolysis occurs, the remaining proteins may interact with polyphenols to form beer haze precipitates.

The malting process, accordingly, involves a host of interacting genes involved in the fundamental processes of seed germination, growth and development. Domestication and selection have accumulated favorable alleles at multiple loci that determine malting quality. The specific alleles that have been accumulated in the major malting barley germplasm groups may differ, based on regional preferences and genetic drift. A summary of current knowledge of the genes and enzymes that control malting quality, and key citations, are presented in Table 10.1.

Enzymes and genes that control carbohydrate degradation

Four amylolytic enzymes are generally thought to participate in converting the starch in malted barley into fermentable sugars: these are α-amylase, β-amylase, α-glucosidase and limit dextrinase. Alpha-amylase and α-glucosidase can release α-glucans from native (nongelatinized) starch granules (Sun and Henson, 1990; Sissons and MacGregor, 1994). All four enzymes can hydrolyze gelatinized starch and/or glucan fragments. A fifth carbohydrase, isoamylase, has recently been discovered and it may have a role in degrading starch and/or producing fermentable sugars during mashing (Sun *et al*., 1999), but the precise role of this enzyme is not yet known.

Table 10.1. Genes and gene products that control malting quality in barley.

Enzyme/protein	Function	Gene	Chr.	Citations
Carbohydrate-degrading enzymes and inhibitors				
α-amylase	Converts native starch, elatinized starch and glucans into sugars	*Amy1* *Amy2*	7H 6H	Bamforth and Barclay, 1993; Henson and Stone, 1988; Ko and Henry, 1994; Ko *et al*., 1996
β-amylase	Converts gelatinized starch and glucans into sugars; acts as storage protein (globulin)	*Bmy1* *Bmy2*	4H 2H	Bamforth and Berclay, 1993; Eglinton *et al*., 1998 ; Erkkila *et al*., 1998
α-glucosidase	Converts native starch, gelatinized starch and glucans into sugars	*Agl*	7H	Henson and Sun, 1995 ; Im and Henson, 1996 ; Konishi *et al*., 1994
limit dextrinase	Breaks down branched starch and amylopectin molecules	LD	7H 4H	Burton *et al*., 1999; Langridge *et al*., 1996; Li *et al*., 1999; MacGregor *et al*., 1994
isoamylase	May convert starch to sugars, but precise role not known			Sun and Henson, 1999
β-glucanase	Degrades cell wall β-glucans	*Glb1* *Glb2* *Glb31-37*	5H 7H 3H	Loi *et al*., 1988 ; Li *et al*., 1999; MacLeod *et al*., 1991; Viëtor *et al*., 1993
barley amylase subtilisin inhibitor (BASI)	Inhibits α-amylase encoded by *Amy2*	*Isa1*	2H	Cannel *et al*., 1992; Munck *et al*., 1985
limit dextrinase inhibitor (LDI)	Inactivates limit dextrinase			MacGregor *et al*., 1995
Protein Z	β-amylase binding protein; acts as storage protein (globulin)	*Paz1*	4H	Cannel *et al*., 1992
Storage proteins, protein-degrading enzymes and inhibitors				
B hordein	Storage protein (prolamin)	*Hor2*	1H	Shewry, 1993
C hordein	Storage protein (prolamin)	*Hor1*	1H	
D hordein	Storage protein (prolamin)	*Hor3*	1H	
hordenin (GluA, GluB, GluC)	Storage and structural protein (glutelin)	GluA *GluB* *Glu.c1*	1H 1H 1H	Giese *et al*., 1994; Kleinhofs *et al*., 1993
Carboxpeptidase (exopeptidase)	Hydrolyzes storage protein	*Cxp1* *Cxp3*	3H 6H	Kleinhofs *et al*., 1993
malt cysteine endoproteinase	Hydrolyzes hordeins	*Cys1* *Cys2*	 3H	Cannel *et al*., 1992; Jones, 1999; Jones and Budde, 1999; Mikkonen *et al*., 1996
phytepsin; aspartic class endoproteinase	Hydrolyzes storage protein			Runeberg-Roos *et al*., 1991
lipid transfer protein (LTP1); aka Probable Amylase Protease Inhibitor (PAPI)	Regulates activities of malt cysteine class endoproteinases	*Ltp1*	5H	Jones and Marinac, 2000b; Kleinhofs *et al*., 1993; Lusk *et al*., 1995; Sorensen *et al*., 1993
lipid transfer protein (LTP2)	Inhibits endoproteinase	*Ltp2*		Jones and Marinac, 2000b
limit dextrinase inhibitor (LDI)	Inactivates limit dextrinase			MacGregor *et al*., 1995
Protein Z	β-amylase binding protein Also acts as storage protein (globulin)	*Paz1*	4H	Cannel *et al*., 1992; Evans *et al*., 1999

The α-amylases are particularly important in the production of fermentable sugars during mashing because they are the only amylolytic enzymes present that are sufficiently thermostable to retain at least some level of activity for the full duration of mashing (Bamforth and Barclay, 1993). Furthermore, most fermentable sugars are produced during the late stages of mashing when temperatures are quite high. The *Amy1* and *Amy2* loci encode two α-amylase isozymes that differ somewhat in their biochemical and biophysical characteristics. Some allelic variation has been documented in the *Amy2* locus of barley (Ko and Henry, 1994; Ko *et al*., 1996), although the usefulness of this variation is not clear.

Potentially useful allelic variation in the genes that encode the β-amylases have recently been identified. Erkkila *et al*. (1998) identified three alleles of β-amylase1, including one which might be responsible for very high enzyme activity. Eglinton *et al*. (1998) identified β-amylase1 alleles that encode proteins with enhanced thermostabilities.

Limit dextrinase activity is thought to be necessary for the complete degradation of starch (MacGregor *et al*., 1994). Li *et al*. (1999) identified five limit dextrinase alleles in cultivated barley and an additional 10 different genes or alleles in non-cultivated barley species. It is not yet known if the alleles identified confer useful characteristics for malting and brewing.

Inhibitors of carbohydrate-degrading enzymes

There are proteins in barley that interact with and inhibit the activities of some of the enzymes that are involved in degradation of starch and arabinoxylans. The best studied of these proteinaceous inhibitors is the barley amylase subtilisin inhibitor (BASI), which specifically inhibits the α-amylase encoded by *Amy2* (Mundy *et al*., 1986). A portion of the β-amylase in barley and malt is in a bound form that is rendered soluble, and thereby active, via the action of reducing agents or endoproteinases. Genes coding for these β-amylase-solubilizing-proteinases, which have yet to be identified, might affect the amount of β-amylase activity in a malt or mash. One of the proteins that is bound to β-amylase in malt may be "Protein Z". Additional information on, and citations for, BASI and Protein Z are presented in Table 10.1. Proteins have been isolated from wheat that inhibit the activity of barley malt arabinoxylanases and barley has been shown to contain proteins that cause the same effect (Debyser *et al*., 1999). The presence of these inhibitors in barley could reduce the rate of arabinoxylan degradation during malting and mashing, leading to problems of high viscosity in the mash. The barley arabinoxylanase inhibitor has not yet been purified, and nothing is known about the gene that codes for its synthesis.

Enzymes and genes that control protein degradation

The hordeins (prolamins) and hordenins (glutelins) are the major storage proteins of barley, but Protein Z and β-amylase, which are globulins, also appear to behave as storage proteins (Shewry, 1993). Relatively large amounts of hordenin occur in some cultivars, but since these proteins generally serve structural purposes, they may not play much of a role in determining malting quality. The enzyme systems that reduce the barley storage proteins to 'soluble protein' (proteins, peptides and amino acids that are soluble in warm water) are very complicated, and involve both endoproteinases and exopeptidases (mainly carboxypeptidases). The rate-limiting step for protein degradation is the hydrolysis of the original proteins into soluble protein by the endoproteinases, so it is the activities of these enzymes in malt that will usually determine whether a barley genotype is acceptable for malting. There are at least 40 endoproteolytic activities, including representatives of all four of the common endoprotease classes, in green malt (Zhang and Jones, 1995). It seems likely that the cysteine proteinases play a major role in the degradation of storage

proteins during mashing and malting, and that the aspartic and metalloproteinases also contribute significantly to this process (Jones, 1999). A gene coding for one aspartic class endoproteinase called phytepsin has been cloned (Runeberg-Roos *et al*., 1991). Its map location has not been reported. The small peptides and amino acids that are released by the exopeptidases comprise the majority of the free amino nitrogen (FAN) fraction. FAN concentration is measured to indicate how well the original protein material can be utilized by yeasts during brewing. Three carboxypeptidase (Cxp) genes have been cloned and two have been mapped (see also Table 10.1).

Inhibitors of protein-degrading enzymes

The activities of the cysteine class malt endoproteinases are strongly inhibited by a series of proteinaceous inhibitors that occur in both barley and malt, with malt containing about 2.5 times as much inhibitory activity as barley. These inhibitors appear to play a role in controlling the rate of protein solubilization during mashing (Jones and Marinac, 2000a). Two of these inhibitors are lipid transfer proteins - LTP1 and LTP2. LTP1 was initially purified and described as a Probable Amylase Protease Inhibitor (PAPI) (Mundy and Rogers, 1986) and its locus, *Ltp1,* has been mapped to chromosome 5H (Kleinhofs *et al*., 1993; see also Table 10.1).

Future improvement in malting quality

By manipulating the carbohydrate and protein-degrading enzymes that occur in malts, as well as their inhibitor proteins, it should be possible to produce even better malting barleys. Because of the large number of enzymes and inhibitors involved, however, it is clear that this will not be an easy process. Similar to the situation for grain yield, malting quality is an economically important phenotype with complex genetic inheritance. Many of the component determinants of malting quality such as malt extract and α-amylase activity are known. However, we are still in the process of developing a comprehensive understanding of how the individual determinants interact to determine the final phenotypes. In terms of genetic diversity, we have yet to develop comprehensive catalogs of the alleles that determine these traits.

Quantitative Trait Loci (QTL)

The QTL concept represents an incremental step forward in understanding traits showing quantitative variation. The “QTL” acronym, and the often-used term “quantitative inheritance” may perpetuate the idea that the genes affecting quantitative traits are somehow different from the genes that determine qualitative traits that show Mendelian inheritance. Because alleles at both quantitative and qualitative trait loci can ultimately be reduced to DNA sequences, what are the distinguishing features of QTL? Defining QTL based on assumptions regarding the number of loci determining the target phenotype is not appropriate, since the number of loci that can be characterized in an unbiased fashion is dictated by the terms of a given experiment. These terms include population size, heritability, and the number and types of environments that are sampled. A simpler operative definition may be to consider QTL as genes underlying phenotypes that (i) defy Mendelian classification and (ii) are measured by a quantitative scale. Thus defined, QTL approaches are appropriate for most agronomic and malting quality traits. As elegantly demonstrated in rice and tomato, with sufficient resources and the appropriate genetic stocks, QTL can be "converted" to Mendelian loci and cloned (Yano *et al*., 1997, 2000; Yamamoto *et al*., 1998; Frary *et al*., 2000).

In addition to map-based cloning, a goal of many QTL projects in barley, and other crops, is

to discover, dissect, and manipulate the genes that determine quantitatively inherited phenotypes that pose particular challenges for plant breeders. In essence, this involves a systematic characterization of the existing genetic diversity. The first QTL reports were particularly promising in this regard, as complex phenotypes such as yield, malting quality, and quantitative disease resistance were reduced to relatively few loci that showed little evidence of epistasis or interaction with the environment. Based on such results, it seemed likely that the effectiveness of marker-assisted selection for QTL alleles would be limited only by the availability and cost of markers flanking target QTL. However, as more data were generated on more germplasm combinations, and QTL detection and characterization methodologies were improved, QTL mapping has turned out to be nearly as complex as the phenotypes it was meant to simplify. The essentially descriptive genome mapping and QTL detection projects of the late 20th century have been followed by the functional genomics, proteomics, and large-scale sequencing projects of the early 21st century. With our genetics horizons now limited only by our ability to phrase interesting questions, and to afford the technology, the time is right to summarize results from over ten years of barley QTL studies (Hayes *et al*., 2000).

The successful cloning of QTL in two model systems (rice and tomato) justifies an attempt to systematically dissect quantitative traits in barley. However, investigators need to recognize that the initial estimates of the number, genome location, and effects of QTL from mapping populations are probably crude representations of very complex biological processes. Finer structure analyses employing alternative genetic stocks and more refined measures of target phenotypes will be required for precise and accurate QTL analysis. When alleles at multiple QTL in multiple germplasm accessions are isolated and characterized at the DNA sequence, expression, and protein levels, techniques for describing, quantifying and classifying genetic diversity can be applied to these data. This will permit hypotheses to be developed and tested regarding the evolution, distribution, and function of QTL alleles in barley germplasm. The results of these experiments will provide a rational basis for germplasm conservation and utilization. The current barley QTL data, while extensive, are too fraught with bias and low precision to undertake such formal analyses of QTL allelic diversity. Additional limitations of the available data are: (1) inadequate sampling of the global diversity in barley germplasm; (2) the use of different QTL analysis procedures; and (3) a lack of common markers. Accordingly, we approached the question of QTL diversity in barley from descriptive and enumerative perspectives.

Summary of QTL diversity

We have summarized the available barley QTL data and addressed the levels of diversity in Figure 10.1 using the following criteria: (1) germplasm sampled; (2) phenotypes sampled; (3) distribution of QTL across and within linkage groups; and (iv) distribution of QTL on the physical map of barley that was reported by Künzel *et al*. (2000). QTL were assigned to the Bin Map, herein referred to as the "BM", of Kleinhofs and Graner (2000) as follows: if markers flanking a QTL were also in the BM, we were able to unequivocally assign the QTL to a bin or region. Binned QTL were assigned to the physical map of Künzel *et al*. (2000) - referred to as the "PM" - as follows. First, we identified markers that occurred in both the BM and the 'Igri'/'Franka' map, referred to as "IF". The IF map was used in developing both the BM (Kleinhofs and Graner, 2000) and the PM (Künzel *et al*., 2000). We used markers in common to the BM and IF map as our starting framework. Then, IF markers without BM positions were assigned to the latter using the proportional distances to common flanking markers in the IF and

CM maps. This generated an adjusted "Linkage Bin Map" (LBM). We then aligned the LBM and the PM to generate a "Physical Bin Map" (PBM). The LBM and PBM bins were assigned recombination values, following the nomenclature of Künzel *et al*. (2000), to generate a "Recombination Bin Map" (RBM). We then aligned the RBM with the PM. As shown in Figure 10.1, this allows for approximations of the physical size of the bins to which QTL are assigned and an estimate of the degree of recombination in each bin. Finally, as shown in Figure 10.1, QTL assigned to each bin in the LBM were drawn proportional to the bin size in the PBM.

For the five trait groups – abiotic stress resistance, agronomic traits, biotic stress resistance, quality, and other – 757 QTL were distributed across the seven chromosomes, with the most QTL on chromosomes 2H and 5H and the fewest on chromosomes 1H and 6H (Table 10.2). The high number of QTL on chromosome 2 is attributable to QTL coincident with, or located near, the *vrs1* locus.

There are QTL for all traits on all chromosomes that are located in bins where there is a low physical:linkage map ratio (Figure 10.1). These QTL are sufficiently resolved to represent defined targets for marker assisted selection, finer structure mapping, and cloning. However, there are also QTL mapping to large physical distances, which often correspond to the centromeric regions on linkage maps. Additional resolution will be required before these QTL can be considered as targets for map-based cloning. For the purposes of marker-assisted selection, linkage drag is clearly an issue. However, procedures for the marker-assisted selection of QTL alleles in such regions are no different from those for QTL in high recombination regions, since the issue is recombination, not physical distance.

The world barley community, particularly in Europe and North America, has been very active in QTL mapping. Of the 757 QTL, the greatest number of reports – 389 – is for agronomic traits, followed by 180 for quality traits (Table 10.3). However, despite the abundance of reports, the wealth of genetic diversity in barley has barely been characterized. Germplasm from important barley production areas of the world, including Africa, Asia, the former USSR, and the Middle East, is underrepresented. Despite the vigorous barley genomics research effort in Australia, there is only one report of these studies (Jefferies *et al*., 1999). The breadth of the Australian mapping and QTL effort is, however, apparent in the summary posted on GrainGenes (Langridge *et al*., 1996). Even in the case of the best-characterized germplasm groups, there are only preliminary data that allow for a comprehensive characterization of allelic diversity in any particular set of accessions.

Table 10.2. Summary of QTL reports in barley, sorted by chromosome and phenotype. Phenotype classifications correspond to those used by Hayes *et al*. (2000).

Chromosome	Number of QTL					Total
	Abiotic stress resistance	Agronomic traits	Biotic stress resistance	Quality	Other	
1H	8	35	9	24	1	77
2H	7	81	16	35	6	145
3H	3	70	17	19	4	113
4H	8	52	15	21	1	97
5H	14	56	14	47	4	135
6H	15	36	13	10	1	75
7H	12	59	19	24	1	115
Total	67	389	103	180	18	757

Applications of QTL analyses

Information about the QTL that influence economically important traits is useful, necessary, and valuable. QTL need to be assigned to regions of the genome – either as a platform for map-based cloning or as a way of assigning functional roles to sequences. Genetic and physical map coordinates for the determinants of phenotypic variation will be essential pieces of information for characterizing and using genetic diversity. The QTL summary we have generated is based on a limited sampling of germplasm, but it is a useful model for addressing two of the key questions in the conservation and use of barley germplasm. "How many genes determine a phenotype?" and "How much allelic diversity is there at these loci?" Answers to these questions will allow conservators to develop representative, non-redundant collections and they will allow plant breeders to make informed decisions regarding the accumulation of favorable alleles in single genotypes. In the following section we have highlighted some of the ways in which QTL research can be of assistance in answering the two fundamental questions we have posed, and we have drawn on the QTL summary to illustrate these applications.

Table 10.3. Summary of barley QTL reports. See also Hayes *et al.* (2000) for criteria employed in compiling this summary.

Trait	Number of phenotypes measured	Number of QTL[1]	Number of populations[2]	Number of citations[3]
Abiotic stress resistance	26	67	7	9
Agronomic traits	58	389	16	24
Biotic stress resistance	15	103	10	15
Quality traits	27	180	8	22
Other	5	18	3	4

[1]The number of QTL per trait is the number of unique QTL per population. Please refer to Hayes *et al.* (2000) for QTL for the same trait mapping to the same bin in different populations. [2]The number of populations is unique for each trait, but multiple traits are often measured on the same reference population. [3]The number of citations per trait is unique, but multiple traits are often described in the same citation.

Catalogs of mapped loci for economically and evolutionarily important phenotypes

A goal of germplasm collection programs is to systematically catalog allelic diversity at key loci. To date, this cataloging has focused, of necessity, on simply scorable morphological descriptors and molecular markers. However, these indicators are often of limited biological utility. What is needed is a characterization of allelic diversity at loci that determine phenotypes showing complex inheritance, and QTL analysis is a first step in this direction. An example of a powerful and immediate application of this is developing catalogs of allelic diversity at genes that confer resistance to biotic stresses. A wealth of theoretical and empirical studies demonstrate that, from the standpoint of probable durability, quantitative resistance may be more desirable than qualitative resistance (Vanderplank, 1978; Johnson, 1981; Parlevliet, 1983). QTL mapping allows for an indirect allelism test via the classification of different germplasm accessions showing the same phenotype. This "QTL allelism" data can be used as a basis for constructing resistance gene pyramids or deploying resistance genes. This strategy has been used to catalog genes that confer quantitative resistance to barley stripe rust (incited by *Puccinia striiformis* fsp. *hordei*) (Chen *et al*., 1994; Hayes *et al*., 1996; Toojinda *et al*., 2000) and to pyramid quantitative resistance alleles in single genotypes (Castro *et al*., 2000). QTL conferring resistance to six of the

most important foliar pathogens of barley are reported on all seven chromosomes and in bins corresponding to low, middle, and high recombination regions (Figure 10.1). The proximity of these disease resistance QTL to QTL for agronomic and malting quality traits can be used as a guide for future selection studies, considering the potential for linkage drag. The relationship between physical and linkage map distances can indicate QTL that would be good candidates for finer structure mapping and isolation.

Understanding correlated responses to selection

QTL analysis can be used to determine, at a gross level, if trait associations are due to linkage or pleiotropy. In the case of linkage, repulsion phase linkages can be broken via marker-assisted selection, or the QTL information can be used to develop populations that will generate the desired recombinant phenotypes for selection. In the case of coincident QTL, breeding objectives may need to be re-defined, pending a finer dissection of the genetic structure of the region. As discussed in the previous section, the genome-wide distribution of disease resistance QTL, and their associations with agronomic and malting quality QTL can assist in defining breeding objectives. As demonstrated by Zhu *et al*. (1999), plant architecture, phenology, and morphology have important implications for breeding for fusarium head blight (FHB) resistance. Finer structure analysis will be required to determine if, in the case of FHB resistance, function follows form (pleiotropy) or if coincident QTL are due to tight linkages. The assignment of QTL to bins that correspond to the degree of recombination on the physical map will assist in prioritizing targets for finer structure mapping and gene isolation. For example, the FHB resistance QTL on chromosome 2H were reported in populations that were derived from crosses between six-rowed genotypes ('Chevron' /M64) and between two-rowed genotypes ('Gobernadora'/CMB). The bin at which QTL are coincident in the two populations contains the *vrs1* locus. In progeny of crosses between two-rowed and six-rowed genotypes, the principal FHB rust resistance QTL maps to this region. This is commonly assumed to be a pleiotropic effect of inflorescence morphology (reviewed by Zhu *et al*., 1999). However, inflorescence type is not segregating in these populations, which suggests that there is potential for recombination between the genes that determine inflorescence type and FHB resistance. The bin to which the FHB resistance QTL and the *vrs1* locus map lies at the juncture of middle to high recombination regions, and is thus a reasonable target for genetic dissection.

Characterization of blocks of the genome that are necessary for essential phenotypes

Certain complex phenotypes – e.g. quality profiles, resistance to a spectrum of stresses – may be requisites for barley production in some environments. If these regions can be defined via QTL analysis, better efficiencies can be achieved in breeding programs by using a high throughput genotypic screening procedure to identify progeny carrying the target blocks of alleles. Phenotypic and/or genotypic selection can then be carried out for genes that reside outside the target blocks. This strategy has been proposed for malting quality in North American barley germplasm, based on malting quality QTL alleles that are contributed by the reference variety 'Morex' in multiple mapping populations (Marquez-Cedillo *et al*., 2000). The regions which are targeted in the quality footprint are apparent in Figure 10.1. The two regions with the most consistent effects on malting quality are the large blocks on chromosomes 7H and 5H that span the centromeric regions. QTL for multiple malting quality traits map to each of these regions.

Assessment of alternative procedures for measuring the same phenotype

Many complex phenotypes are difficult to improve through breeding because phenotyping is laborious or expensive. When new procedures are developed for measuring a given phenotype, the commonality of QTL detected by the new and the standard procedure can be used as a measure of the correspondence of the two assays. For example, Iyamabo and Hayes (1995) used the consistency of QTL detection as a criterion for comparing grain yield in hill plots and row plots and concluded that the former were useful for detecting large-effect QTL. This has important implications for large-scale germplasm assessment and screening, where the costs of full yield plot assessments are prohibitive. Experiments are underway to compare malting quality QTL that are detected by Near Infrared Spectroscopy (NIR) and conventional wet chemistry analyses, and the summary of observed QTL will be useful in establishing relationships between the two approaches. The efficiencies of NIR would make it an attractive tool for characterizing extensive germplasm arrays for phenotypic diversity in malting quality parameters, and the genetic determinants of this diversity can be assigned to map positions with QTL tools.

Introgression of exotic and/or ancestral germplasm

Exotic germplasm can be a source of alternative alleles at known loci or of entirely new genes, in cases where there is no allelic variation at these loci in cultivated germplasm. Tanksley and Nelson (1996) stimulated widespread interest in discovering and using favorable QTL alleles in phenotypically unattractive tomato germplasm, and this research has borne fruit with the recent cloning of a fruit size QTL (Frary *et al.*, 2000). In barley, there are encouraging preliminary data on the systematic introgression of favorable alleles from ssp. *spontaneum* into cultivated barley (Matus *et al.*, 2000). The QTL summary does not yet include data from such introgression projects. When QTL are mapped in these studies, a priori information about other loci that are found in the introgression region will be invaluable for minimizing linkage drag and establishing allelism.

Conclusions and Outlook

Despite evidence that there is diversity for traits of economic importance in barley, our understanding of allelic architecture at the many loci determining agronomic performance and malting quality is incomplete. It does not yet provide a basis for systematic classification and utilization of barley germplasm resources in breeding programs.

The focus of future QTL studies could be to thoroughly characterize multiple phenotypes and to develop dynamic profiles of QTL expression in different tissues and at different points in crop development. Most QTL studies have been based on single or end-point measures of a phenotype. To obtain a more complete understanding of the genetic basis of complex traits, it will be necessary to decompose complex traits into their components. For example, QTL analyses of quantitative disease resistance revealed that multiple regions of the genome were associated with symptom expression. Some associations may be the consequence of alleles that condition host pathogen interactions, whereas others may reflect variation in alleles that determine crop architecture, phenology, and morphology. Information that relates QTL to underlying mechanisms of resistance will be invaluable for understanding the genetics of disease resistance and in achieving selection response. The full genetic potential of an organism may not be realized due to temporal and spatial changes in gene expression. Functional genomic tools may be developed to measure these changes in any given tissue or at any point in time. Understanding how developmental expression of alleles determines quantitative traits may reveal

complexities, such as changes in favorable allele phase, that explain some of the unexpected results obtained from selection experiments.

There is a pressing need for more comprehensive sampling and reporting of QTL in barley germplasm. Important barley production areas of the world, with much to offer in terms of genetic diversity, are conspicuously underrepresented in the barley QTL literature. This is, in large part, due to economic constraints that face barley researchers outside of Europe, North America, and Oceania. Every effort should be made to stimulate collaborative and mutually rewarding analysis of the world's barley genetic resources.

In general, the trend in crop improvement programs has been to rely on ever-narrower germplasm bases to satisfy increasingly stringent yield and quality expectations of producers and consumers. Barley breeders are faced with the challenge of developing cultivars that satisfy multiple end-use requirements for brewing, animal feed, and human consumption. Malting quality standards for barley impose particularly rigid constraints on breeding programs. As a result, the barley industry tends to be conservative about adopting new varieties and exploiting genetic diversity. Nonetheless, impressive progress has been made through the use of narrow germplasm bases, although the genetic mechanisms that account for selection response remain a matter of conjecture (Rasmusson and Phillips, 1997). Cultivars developed from the North American six-row barley germplasm have exceeded the expectations of growers and the malting and brewing industries. However, the extreme susceptibility of these cultivars to fusarium head blight clearly demonstrates the risk of reliance on such a narrow germplasm base. In response to recent devastating epidemics of this disease in the Midwest, breeders have screened exotic germplasm and are incorporating diverse sources of resistance into new cultivars adapted to the region. This is eloquent testimony to the need for systematically characterizing diversity in barley and maintaining barley germplasm resources.

Barley possesses enormous genetic diversity that enables it to grow under a wide range of environmental conditions and to tolerate stresses such as drought and salinity. In addition to its utility in malting and brewing, barley can provide food security in regions where other cereal crops cannot be produced. It can be readily crossed with its wild ancestor (ssp. *spontaneum*), which may also represent a tremendous reservoir of variation in disease resistance and stress tolerance that remains to be exploited. Although it is difficult to quantify the variation in agronomic and malting quality traits that is present in barley, due to the complex inheritance of these phenotypes, progress is being made in this regard. Efficient strategies for discovering, characterizing, and using alleles in exotic and ancestral germplasm will help to ensure the sustainability, productivity, and profitability of barley production.

References

Allard, R.W., 1988. Genetic changes associated with the evolution of adaptedness in cultivated plants and their wild progenitors. J. Hered. 79: 225-238.

Bamforth, C.W., and A.H.P. Barclay, 1993. Malting technology and the uses of malt. In: A.W. Macgregor and R.S Bhatty (eds), Barley Chemistry and Technology AACC, St. Paul, Minnesota, pp. 297-354.

Beldford, R.K., and R.H. Sedgley, 1991. Conclusions: ideotypes and physiology: tailoring plants for increased production. Field Crops Res. 26: 221-226.

Briggs, D.E., 1992. Barley germination: biochemical changes and hormonal control. In: P.R. Shewry (ed), Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology. CAB International, UK, pp. 369-401.

Burger, W.C., and D.E. LaBerge, 1985. Malting and brewing quality. In: D.C. Rasmusson (ed), Barley, ASA, Madison, Wisconsin, pp. 367-401.

Burton, R.A., X.Q. Zhang, M. Hrmova and G.B. Fincher, 1999. A single limit dextrinase gene is expressed both in the developing endosperm and in germinated grains of barley. Plant Physiol. 119: 859-871.

Cannell, M., A. Karp, P.G. Isaac and P. Shewry, 1992. Chromosomal assignment of genes in barley using telosomic wheat-barley addition lines. Genome 35: 17-23.

Castro, A.J., A. Corey, T. Filichkin, P. Hayes, J.S. Sandoval-Islas and H. Vivar, 2000. Stripe rust resistance QTL pyramids in barley. In: S. Logue (ed), Proc. of the 8th International Barley Genetics Symposium. Adelaide University, Glen Osmond South Australia, pp. 86-88.

Chen, F., D. Prehn, P.M. Hayes, D. Mulrooney, A. Corey, A. and H. Vivar, 1994. Mapping genes for resistance to barley stripe rust (*Puccinia striiformis* f. sp. *hordei*). Theor. Appl. Genet. 88: 215-219.

Debyser, W., W.J. Peumans, E.J.M. van Damme and J.A. Delcour, 1999. *Triticum aestivum* xylanase inhibitor (TAXI), a new class of enzyme inhibitor affecting breadmaking performance. J. Cereal Sci. 30: 39-43.

Dreccer, F., C. Grashoff and R. Rabbinge, 1997. Source-sink ratio in barley (*Hordeum vulgare* L.) during grain filling: effects on senescence and grain protein concentration. Field Crops Res. 49: 269-277.

Eglinton, J.K., P. Langridge and D.E. Evans, 1998. Thermostability variation in alleles of barley β-amylase. J. Cereal Sci. 28: 301-309.

Erkkila, M.J., R. Leah, H. Ahokas and V. Cameron-Mills, 1998. Allele-dependent barley grain β-amylase activity. Plant Physiol. 117: 679-685.

Evans, D.E., M.C. Sheehan and D.C. Stewart, 1999. The impact of malt derived proteins on beer foam quality. Part II: the influence of malt foam-positive proteins and non-starch polysaccharides on beer foam quality. J. Inst. Brew. 105: 171-177.

Fincher, G.B. and B.A. Stone, 1993. Physiology and biochemistry of germination in barley. In: A.W. Macgregor and R.S Bhatty (eds), Barley Chemistry and Technology AACC, St. Paul, Minnesota, pp. 247-295.

Frary, A., T.C. Nesbitt, A. Frary, S. Grandillo, E. van der Knaap, B. Cong, J. Liu, J. Meller, R. Elber, K.B. Alpert, S.D. Tanksley, 2000. *fw2.2*: a quantitative trait locus key to the evolution of tomato fruit size. Science 289: 85-88.

Gallagher, J.N., P.J. Biscoe and R.K. Scott, 1975. Barley and its environment. V. Stability of grain weight. J. Appl. Ecol. 12: 319-336.

Giese, H., A.G. Holm-Jensen, H. Mathiassen, B. Kjær, S.K. Rasmussen, H. Bay and J. Jensen, 1994. Distribution of RAPD markers on a linkage map of barley. Hereditas 120: 267-273.

Hamblin, J., 1993. The ideotype concept: useful or outdated? In: D.R. Buxton, R. Shibles, A. Forsberg, B.L. Blad, K.H. Asay, G.M. Paulsen and R.F. Wilson (eds), International Crop Science I, CSSA, Madison, Wisconsin, pp. 589-597.

Harlan, J.R., 1995. The living fields: our agricultural heritage. Cambridge University Press, Cambridge, England and New York, NY, USA.

Harlan, H.V., 1957. One man's life with barley. Exposition Press, New York.

Hayes, P.M., A. Castro, A. Corey, L. Marquez-Cedillo, B. Jones, D. Mather, I. Matus, C. Rossi, and K. Sato, 2000. A summary of published barley QTL reports. http://www.css.orst.edu/barley/nabgmp/qtlsum.htm

Hayes, P.M., D. Prehn, H. Vivar, T. Blake, A. Comeau, I. Henry, M. Johnston, B. Jones and B. Steffenson, 1996. Multiple disease resistance loci and their relationship to agronomic and quality loci in a spring barley population. J.QTL http://probe.nalusda.gov:8000/otherdocs/jqtl/index.html

Henson, C.A., and J.M. Stone, 1988. Variation in α-amylase and α-amylase inhibitor activities in barley malts. J. Cereal Sci. 8: 39-46.

Henson, C.A., and Z. Sun, 1995. Barley seed α-glucosidases: Their characteristics and roles in starch degradation. In: J.N. Saddler and M.H. Penner (eds), Enzymatic Degradation of Insoluble Carbohydrates. ACS Symposium, Series 618, pp. 51-58.

Hitchcock, A.S., 1971. Manual of the grasses of the United States, 2nd ed. (rev. by A. Chase). Dover Publications, New York.

Im, H., and C.A. Henson, 1995. Characterization of high pI alpha-glucosidase from germinated barley seeds: Substrate specificity, subsite affinities and active-site residues. Carbohydrate-Research 277: 145-159.

Iyamabo, O.E., and P.M. Hayes, 1995. Effects of plot type on detection of quantitative trait locus effects in barley (*Hordeum vulgare* L.). Plant Breed. 114: 55-60.

Jefferies, S.P., A.R. Barr, A. Karakousis, J.M. Kretschmer, S. Manning, K.J. Chalmers, C. Nelson, A.K.M.R. Islam and P. Langridge, 1999. Mapping of chromosome regions conferring boron toxicity tolerance in barley (*Hordeum vulgare* L.). Theor. Appl. Genet. 98: 1293-1303.

Johnson, R., 1981. Durable resistance: definition of, genetic control, and attainment. Phytopathology 71: 567-568.

Jones, B.L., 1999. Malt endoproteinases and how they affect wort soluble protein levels. In: Proc.9th Australian Barley Technical Symposium, Pathway into the 21st Century, Melbourne, Australia, the Barley Technical Symposium Steering Committee, pp. 2.39.1-8.

Jones, B.L., and A.D. Budde, 1999. Endoproteinases and the hydrolysis of malt proteins during mashing. In: European Brewery Convention: Proceedings of the 27th Congress, Cannes, France, 1999. Published by the European Brewery Convention, Zoeterwoude, The Netherlands, pp 611-618.

Jones, B.L., and L.A. Marinac, 2000a. Endogenous inhibitors of the endoproteinases and other enzymes of barley. In: T. Simoinen and M. Tenkanen (eds), Proc. 2nd European Symp. on Enzymes and Grain Processing Helsinki, Finland, 1999. Published by Technical Research Centre of Finland, Espoo, Finland, pp. 39-46.

Jones, B.L., and L.A. Marinac, 2000b. Purification and partial characterization of a second cysteine proteinase inhibitor from ungerminated barley (*Hordeum vulgare* L.). J. Agric. Food Chem. 48: 257-264.

Jui, P.Y., T.M. Choo, K.M. Ho, T. Konishi and R.A. Martin, 1997. Genetic analysis of a two-row x six-row cross of barley using doubled-haploid lines. Theor. Appl. Genet. 94: 549-556.

Kirby, E.J.M., and D.G. Faris, 1972. The effects of plants density on tiller growth and morphology in barley. J. Agric. Sci. 78: 281-288.

Kjær, B., and J. Jensen, 1996. Quantitative trait loci for grain yield and yield components in a cross between a six-rowed and a two-rowed barley. Euphytica 90: 39-48.

Kleinhofs, A., and A. Graner, 2000. The Barley BIN map (March 2000 posting). http://barleygenomics.wsu.edu/

Kleinhofs, A., A. Kilian, M.A. Saghai-Maroof, R.M. Biyashev, P. Hayes, F.Q. Chen, N. Lapitan, A. Fenwick, T.K. Blake and V. Kanazin, 1993. A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor. Appl. Genet. 86: 705-712.

Ko, H.L., and R.J. Henry, 1994. Identification of barley varieties using the polymerase chain reaction. J. Inst. Brew. 100: 405-407.

Ko, H.L., S. Weining and R.J. Henry, 1996. Application of primers derived from barley α-amylase genes to the identification of cereals by PCR. Plt. Varieties and Seeds 9: 53-62.

Konishi,Y., A. Okamoto, J. Takahashi, M. Aitani and N. Nakatani, 1994. Effects of Bay m 1099, and α-glucosidase inhibitor, on starch metabolism in germinating wheat seeds. Biosci. Biotech. Biochem. 58: 135-139.

Künzel, G., L. Korzun and A. Meister, 2000. Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. Genetics 154: 397-412.

Langridge, P., A. Karakousis, J. Kretschmer, S. Manning, K. Chalmers, R. Boyd, C.D. Li, R. Islam, S. Logue, R. Lance and SARDI, 1996. RFLP and QTL analysis of barley mapping populations. http://greengenes.cit.cornell.edu/WaiteQTL/

Lauer, J.G., and S.R. Simmons, 1989. Canopy light and tiller mortality in spring barley. Crop Sci. 29: 420-424.

Li, C.D., X.Q. Zhang, P. Eckstein, B.G. Rossnagel and G.H. Scoles, 1999. A polymorphic microsatellite in the limit dextrinase gene of barley (*Hordeum vulgare* L.). Mol. Breed. 5: 569-577.

Loi, L., B. Ahluwalia and G.B. Fincher, 1988. Chromosomal location of genes encoding barley (1 → 3, 1 → 4)-β-glucan-4-glucanohydrolases. Plant Physiol. 87: 300-302.

Lusk, L.T., H. Goldstein and D. Ryder, 1995. Independent role of beer proteins, melanoidins and polysaccharides in foam formation. J. Am. Soc. Brew. Chem. 53: 93-103.

MacGregor, A.W., L.J. Macri, S.L. Bazin and G.W. Sadler, 1995. Limit dextrinase inhibitor in barley and malt and its possible role in malting and brewing. In: European Brewery Convention: Proc. of the 25th Congress. Brussels, Belgium. pp. xx-xx

MacGregor, A.W., L.J. Macri, S.W. Schroeder and S.L. Bazin, 1994. Purification and characterisation of limit dextrinase inhibitors from barley. J. Cereal Sci. 20: 33-41.

MacLeod, L.C., R.C.M. Lance and A.H.D. Brown, 1991. Chromosomal mapping of the *Glb 1* locus encoding (1 → 3, 1 → 4)-β-D-glucan 4-glucanohydrolase EI in barley. J. Cereal Sci. 13: 291-298.

Marquez-Cedillo, L.A., P.M. Hayes, B.L. Jones, A. Kleinhofs, W.G. Legge, B.G. Rossnagel, K. Sato, S.E. Ullrich, D.M. Wesenberg and the NABGMP, 2000. QTL analysis of malting quality in barley based on the doubled haploid progeny of two elite North American varieties representing different germplasm groups. Theor. Appl. Genet. 101: 173-184.

Marquez-Cedillo, L.A., P.M. Hayes, A. Kleinhofs, W.G. Legge, B.G. Rossnagel, K. Sato, S.E. Ullrich, D.M. Wesenberg and the NABGMP, 2001. QTL analysis of agronomic traits in barley based on the doubled haploid progeny of two elite North American varieties representing different germplasm groups. Theor. Appl. Genet. 103: 625-637.

Matus, I., A. Corey, T. Filichkina, P.M. Hayes, O. Riera-Lizarazu, K. Sato and M.I. Vales, 2000. In: S. Logue (ed), Proc. of the 8th International Barley Genetics Symposium Adelaide University, Glen Osmond South Australia, pp 86-88.

Mikkonen, A., I. Porali, M. Cercos and T.D. Ho, 1996. Plant Mol. Biol. 31: 239-254.

Munck, L., H. Mundy and P. Vaag, 1985. Characterization of enzyme inhibitors in barley and their tentative role in malting and brewing. ASBC J. 43: 35-38.

Mundy, J., and J.C. Rogers, 1986. Selective expression of a probable amylase/protease inhibitor in barley aleurone cells: comparison to the barley amylase/subtilisin inhibitor. Planta 169: 51-63.

Oziel, A., P.M. Hayes, F.Q. Chen and B. Jones, 1996. Application of quantitative trait locus mapping to the development of winter habit malting barley. Plant Breed. 115: 43-51.

Parlevliet, J.E., 1983. Race-specific resistance and cultivar-specific virulence in the barley-leaf rust pathosystem and their consequences for the breeding of leaf rust resistant barley. Euphytica 32: 367-375.

Pennisi, E., 2000. *Arabidopsis* comes of age. Science 290: 32-35.

Powell, W., R.P. Ellis and W.T.B. Thomas, 1990. The effects of major genes on qualitatively varying characters in barley. III. The two row/six row locus (*V-v*). Heredity 65: 259-264.

Rasmusson, D.C., 1987. An evaluation of ideotype breeding. Crop Sci. 27: 1140-1146.

Rasmusson, D.C., 1991. A plant breeder's experience with ideotype breeding. Field Crops Res. 26: 191-200.

Rasmusson, D.C., and R.L. Phillips, 1997. Plant breeding progress and genetic diversity from *de novo* variation and elevated epistasis. Crop Sci. 37: 303-310.

Riggs, T.J., and E.J.M. Kirby, 1978. Developmental consequences of two-row and six-row ear type in spring barley. J. Agric. Sci. Camb. 91: 199-205.

Runeberg-Roos, P., K. Tormakangas and A. Ostman, 1991. Primary structure of a barley-grain aspartic proteinase. Eur. J. Biochem. 202: 1021-1027.

Shewry, P., 1993. Barley seed proteins. In: A.W. MacGregor and R.S. Bhatty (eds), Barley Chemistry and Technology AACC, St. Paul, Minnesota, pp. 131-197.

Sissons, M.J., and A.W. MacGregor, 1994. Hydrolysis of barley starch granules by α-glucosidases from malt. J. Cereal Sci. 19: 161-169.

Sorensen, S.B., L.M. Bech, M. Muldbjerg, T. Beenfeldt and K. Breddam, 1993. Barley lipid transfer protein 1 is involved in beer foam formation. MBAA Tech. Q. 30: 136-145.

Sun, Z., and CA. Henson, 1990. Degradation of native starch granules by barley α-glucosidases. Plant Physiol. 94: 320-327.

Sun, C., P. Sathish, S. Ahlandsberg and C. Jansson, 1999. Analyses of isoamylase gene activity in wild-type barley indicate its involvement in starch synthesis. Plant Mol. Biol. 40: 431-443

Takahashi, R., J. Hayashi and I. Moriya, 1975. Basic studies on breeding barley by the use of two-rowed and six-rowed varietal crosses. Barley Genetics III, Proceedings of the Third International Barley Genetic Symposium, Garching, pp. 662-677.

Tanksley, S.D., and J.C. Nelson, 1996. Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor. Appl. Genet. 92:191-203.

Toojinda, T., E. Baird, L. Broers, X.M. Chen, P.M. Hayes, A. Kleinhofs, J. Korte, D. Kudrna, H. Leung, R.F. Line, W. Powell and H. Vivar, 2000. Mapping quantitative and qualitative disease resistance genes in a doubled haploid population of barley. Theor. Appl. Genet. 101: 580-589.

Vanderplank, J.E., 1978. Genetic and Molecular Basis of Plant Pathogenesis. Springer-Verlag, Berlin, Heidelberg, New York.

Venter, J.C. *et al.*, 2001. The sequence of the human genome. Science 291: 1304-1351.

Viëtor, R.J., A.G.J. Voragen and S.A.G.F. Angelino, 1993. Composition of non-starch polysaccharides in wort and spent grain from brewing trials with malt from a good malting quality barley and a feed barley. J. Inst. Brew. 99: 243-248.

Yamamoto, T., Y. Kuboki, S.Y. Lin, T. Sasaki and M. Yano, 1998. Fine mapping of quantitative trait loci *Hd*-1, *Hd*-2 and *Hd*-3, controlling heading date of rice, as single Mendelian factors. Theor. Appl. Genet. 97: 37-44.

Yano, M., Y. Harushima, Y. Nagamura, N. Kurata, Y. Minobe and T. Sasaki, 1997. Identification of quantitative trait loci controlling heading date in rice using a high-density linkage map. Theor. Appl. Genet. 95: 1025-1032.

Yano, M., Y. Katayose, M. Askikari, U. Yamanouchi, L. Monna, T. Fuse, T. Baba, K. Yamamoto and T. Sasaki, 2000. http://www.intl-pag.org/pag/8/abstracts/pag8819.html

Yasuda, S., 1964. Experimental studies on natural selection for time of heading and its inner factors in some barley hybrid populations. II. Genotypic constitutions of selection lines derived from the populations grown at different locations. Ber. Ohara Inst. Landw. Biol. Okayama Univ. 12: 197-215.

Yasuda, S., 1977. Effect of four different spring genes on earliness of heading, grain yield and some yield components in barley. Proc. 3rd Int. Cong. SABRAO 1: 30-32.

Yasuda, S., J. Hayashi and I. Moriya, 1992. Regional specialities in ecological agronomic characters of two-rowed barley cultivars from Japan and other countries. Bul. Res. Inst. Bioresour., Okayama Univ. 1:61-74 (in Japanese with English summary).

Zhang, N., and B.L. Jones, 1995. Purification and partial characterization of a 31-kDa cysteine endopeptidase from germinated barley. J. Cereal Sci. 21: 145-153.

Zhu, H., L. Gilchrist, P. Hayes, A. Kleinhofs, D. Kudrna, Z. Liu, L. Prom, B. Steffenson, T. Toojinda and H. Vivar, 1999. Does function follow form? QTLs for Fusarium Head Blight (FHB) resistance are coincident with QTLs for inflorescence traits and plant height in a doubled haploid population of barley. Theor. Appl. Genet. 99:1221-1232.

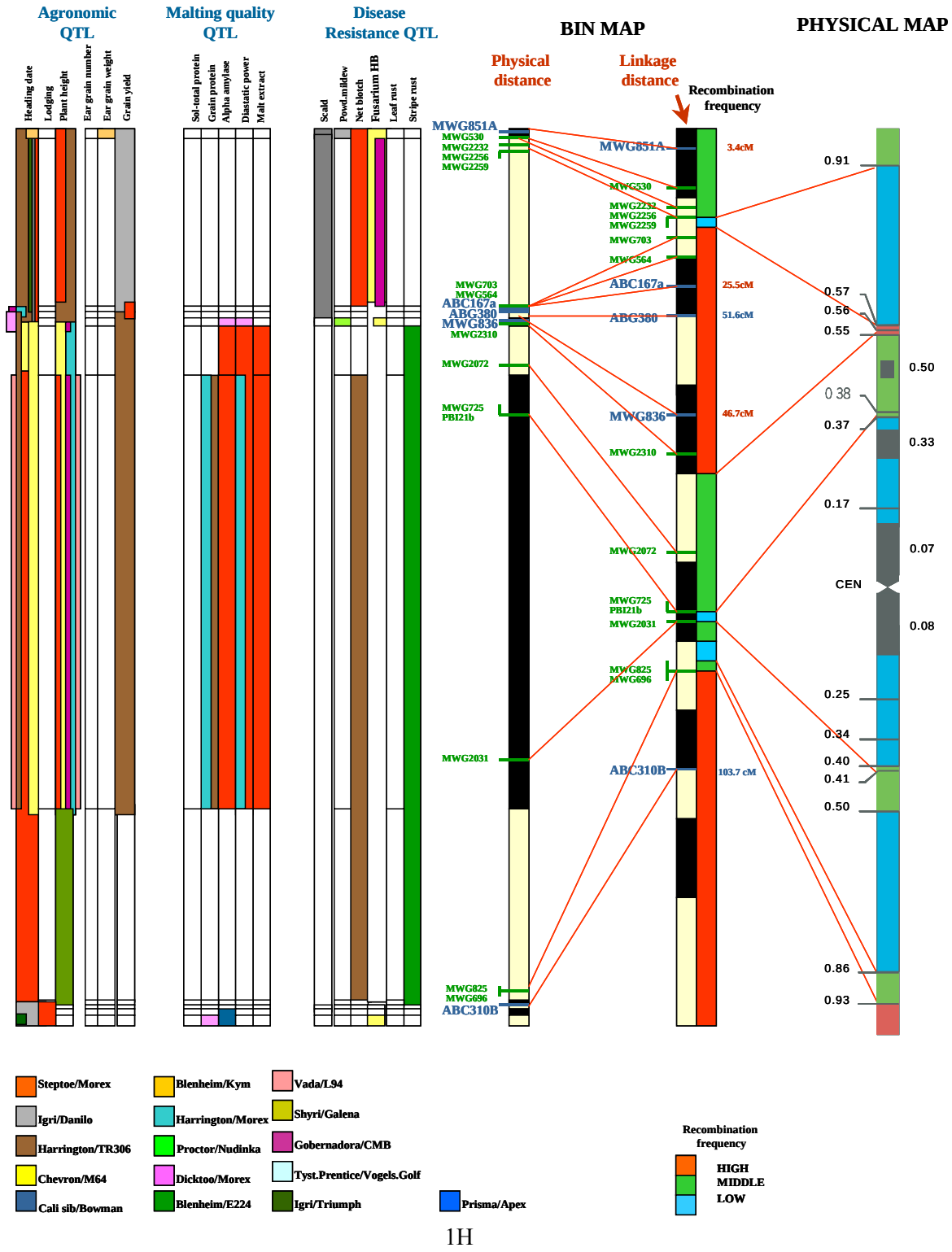
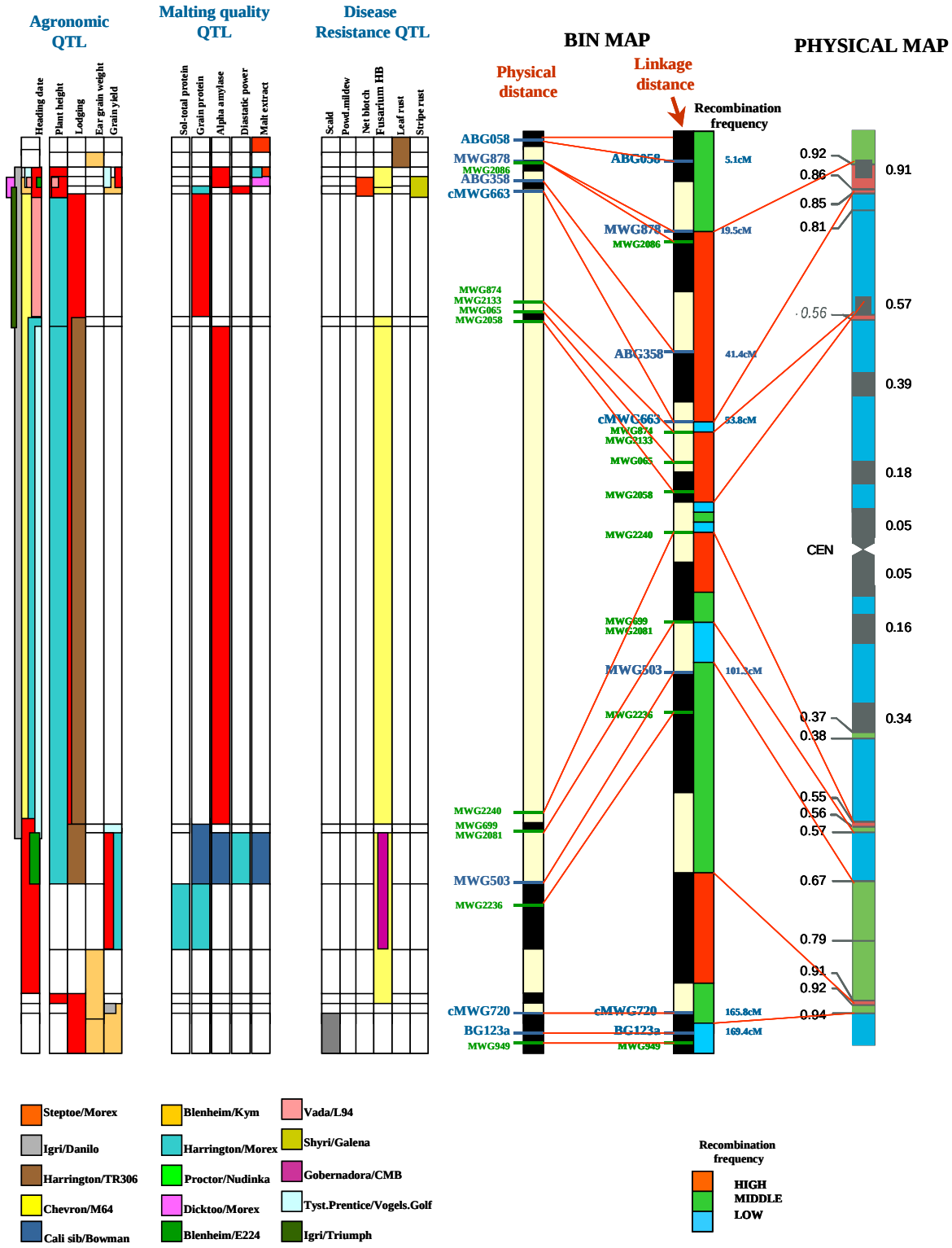
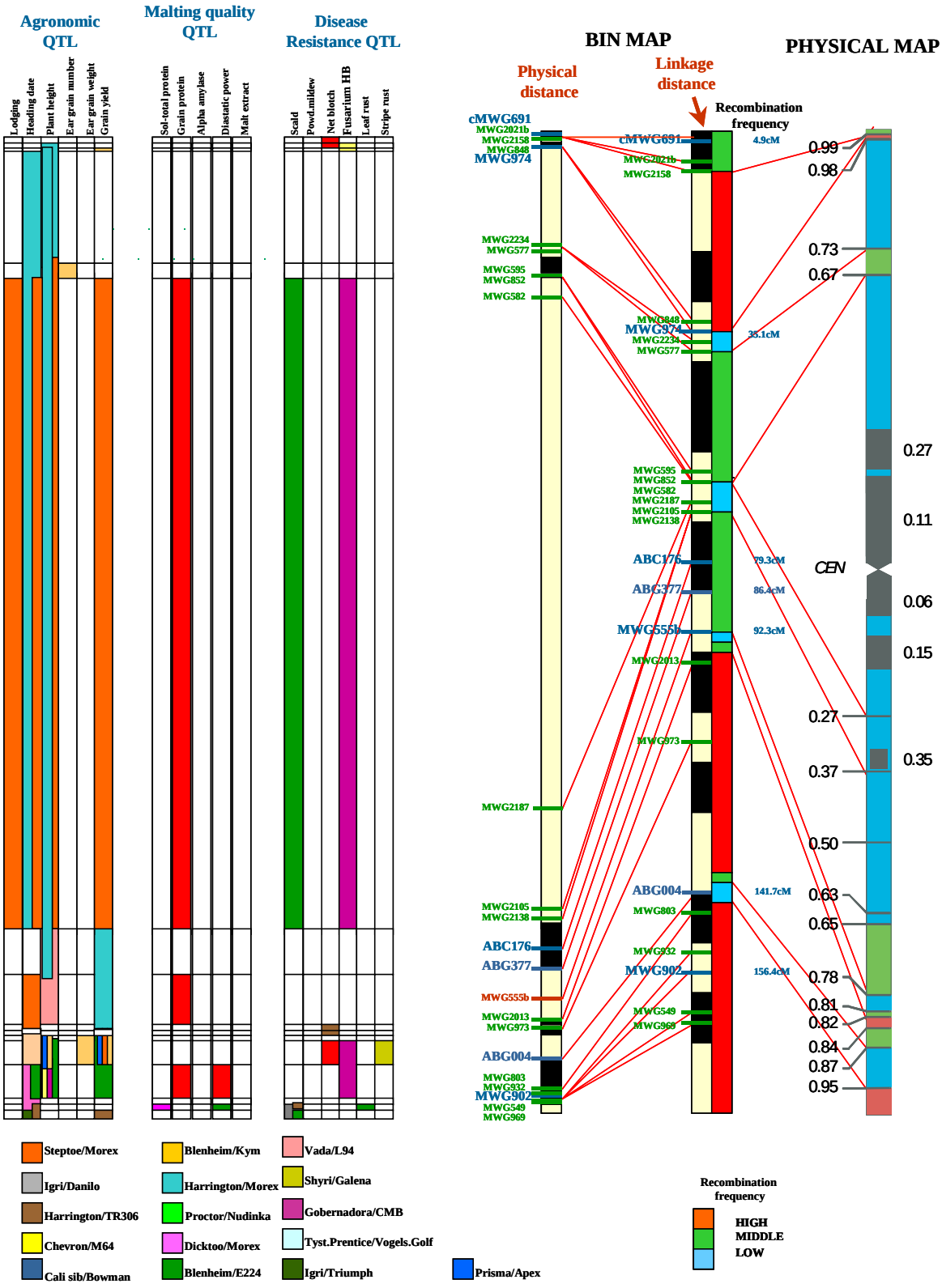


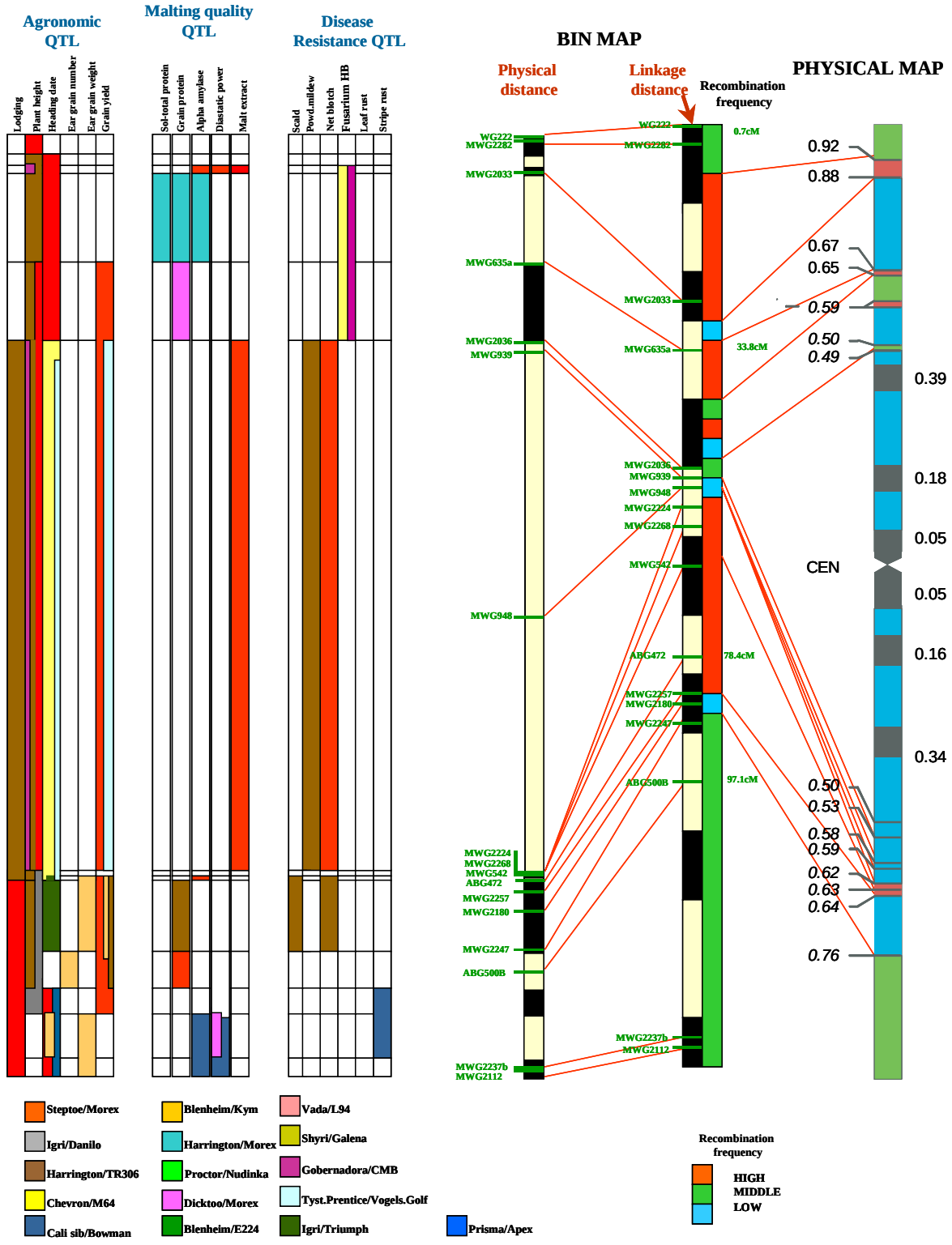
Figure 10.1. Quantitative Trait Loci (QTL) determining economically important traits in barley, assigned to bins on the consensus linkage map (Kleinhofs and Graner, 2000) and the physical map of Künzel *et al.* (2000). The criteria used for QTL assignment to map locations were described by Hayes *et al.* (2000).


Agronomic QTL
Heading date
Plant height
Lodging
Ear grain weight
Grain yield
Malting quality QTL
Sol-total protein
Grain protein
Alpha amylase
Diastatic power
Malt extract
Disease Resistance QTL
Scald
Powd.mildew
Net blotch
Fusarium HB
Leaf rust
Stripe rust
BIN MAP
PHYSICAL MAP
Physical distance
Linkage distance
Recombination frequency
ABG058
MWG878
MWG2086
ABG358
cMWG663
MWG874
MWG2133
MWG065
MWG2058
MWG2240
MWG699
MWG2081
MWG503
MWG2236
cMWG720
BG123a
MWG949
5.1cM
19.5cM
41.4cM
53.8cM
101.3cM
165.8cM
169.4cM
0.92
0.86
0.85
0.81
0.91
0.56
0.57
0.39
0.18
0.05
CEN
0.05
0.16
0.37
0.38
0.34
0.55
0.56
0.57
0.67
0.79
0.91
0.92
0.94
Steptoe/Morex
Igri/Danilo
Harrington/TR306
Chevron/M64
Cali sib/Bowman
Blenheim/Kym
Harrington/Morex
Proctor/Nudinka
Dicktoo/Morex
Blenheim/E224
Vada/L94
Shyri/Galena
Gobernadora/CMB
Tyst.Prentice/Vogels.Golf
Igri/Triumph
Recombination frequency
HIGH
MIDDLE
LOW

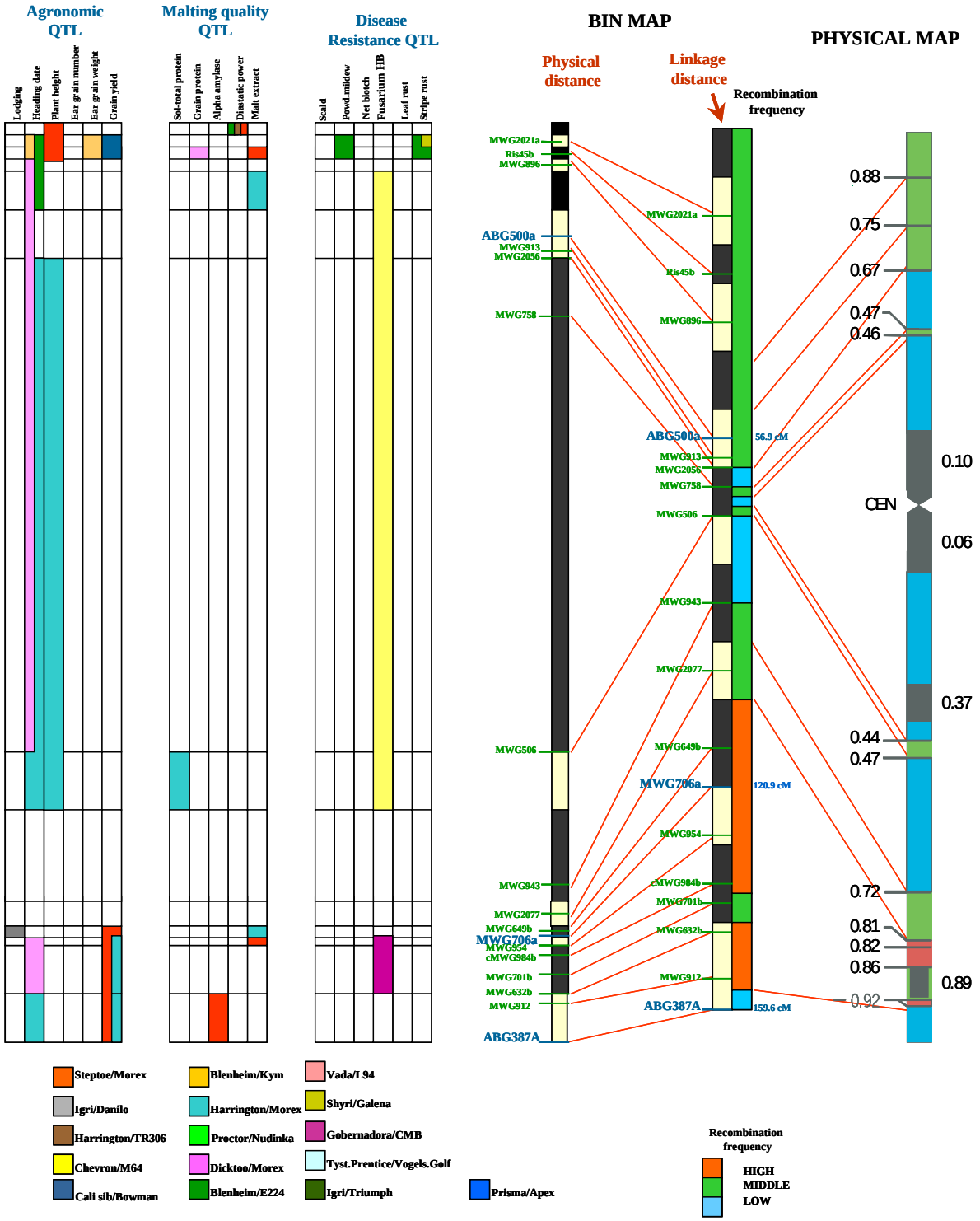
2H


Agronomic QTL
Malting quality QTL
Disease Resistance QTL
Lodging
Heading date
Plant height
Ear grain number
Ear grain weight
Grain yield
Sol-total protein
Grain protein
Alpha amylase
Diastatic power
Malt extract
Scald
Powd.mildew
Net blotch
Fusarium HB
Leaf rust
Stripe rust
BIN MAP
PHYSICAL MAP
Physical distance
Linkage distance
Recombination frequency
cMWG691
MWG2021b
MWG2158
MWG848
MWG974
MWG2234
MWG577
MWG595
MWG852
MWG582
MWG2187
MWG2105
MWG2138
ABC176
ABG377
MWG555b
MWG2013
MWG973
ABG004
MWG803
MWG932
MWG902
MWG549
MWG969
4.9cM
35.1cM
79.3cM
86.4cM
92.3cM
141.7cM
156.4cM
0.99
0.98
0.73
0.67
0.27
0.11
CEN
0.06
0.15
0.27
0.37
0.35
0.50
0.63
0.65
0.78
0.81
0.82
0.84
0.87
0.95
Steptoe/Morex
Igri/Danilo
Harrington/TR306
Chevron/M64
Cali sib/Bowman
Blenheim/Kym
Harrington/Morex
Proctor/Nudinka
Dicktoo/Morex
Blenheim/E224
Vada/L94
Shyri/Galena
Gobernadora/CMB
Tyst.Prentice/Vogels.Golf
Igri/Triumph
Prisma/Apex
Recombination frequency
HIGH
MIDDLE
LOW

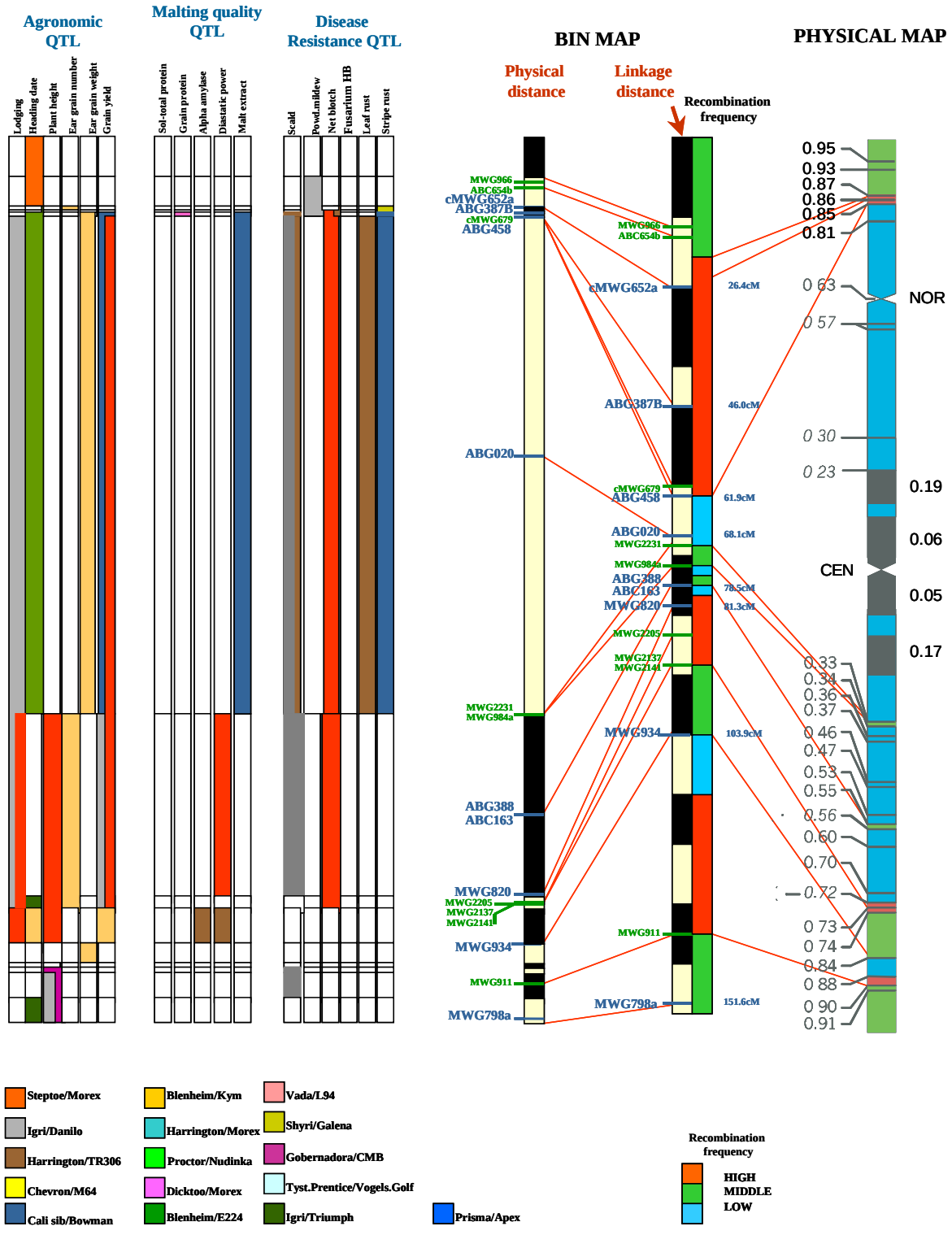
3H


Agronomic QTL
Malting quality QTL
Disease Resistance QTL
Lodging
Plant height
Heading date
Ear grain number
Ear grain weight
Grain yield
Sol-total protein
Grain protein
Alpha amylase
Diastatic power
Malt extract
Scald
Powd.mildew
Net blotch
Fusarium HB
Leaf rust
Stripe rust
BIN MAP
Physical distance
Linkage distance
Recombination frequency
PHYSICAL MAP
WG222
MWG2282
MWG2033
MWG635a
MWG2036
MWG939
MWG948
MWG542
ABG472
MWG2257
MWG2180
MWG2247
ABG500B
MWG2224
MWG2268
MWG2237b
MWG2112
0.7cM
33.8cM
78.4cM
97.1cM
0.92
0.88
0.67
0.65
0.59
0.50
0.49
0.39
0.18
0.05
CEN
0.05
0.16
0.34
0.50
0.53
0.58
0.59
0.62
0.63
0.64
0.76
Steptoe/Morex
Igri/Danilo
Harrington/TR306
Chevron/M64
Cali sib/Bowman
Blenheim/Kym
Harrington/Morex
Proctor/Nudinka
Dicktoo/Morex
Blenheim/E224
Vada/L94
Shyri/Galena
Gobernadora/CMB
Tyst.Prentice/Vogels.Golf
Igri/Triumph
Prisma/Apex
Recombination frequency
HIGH
MIDDLE
LOW

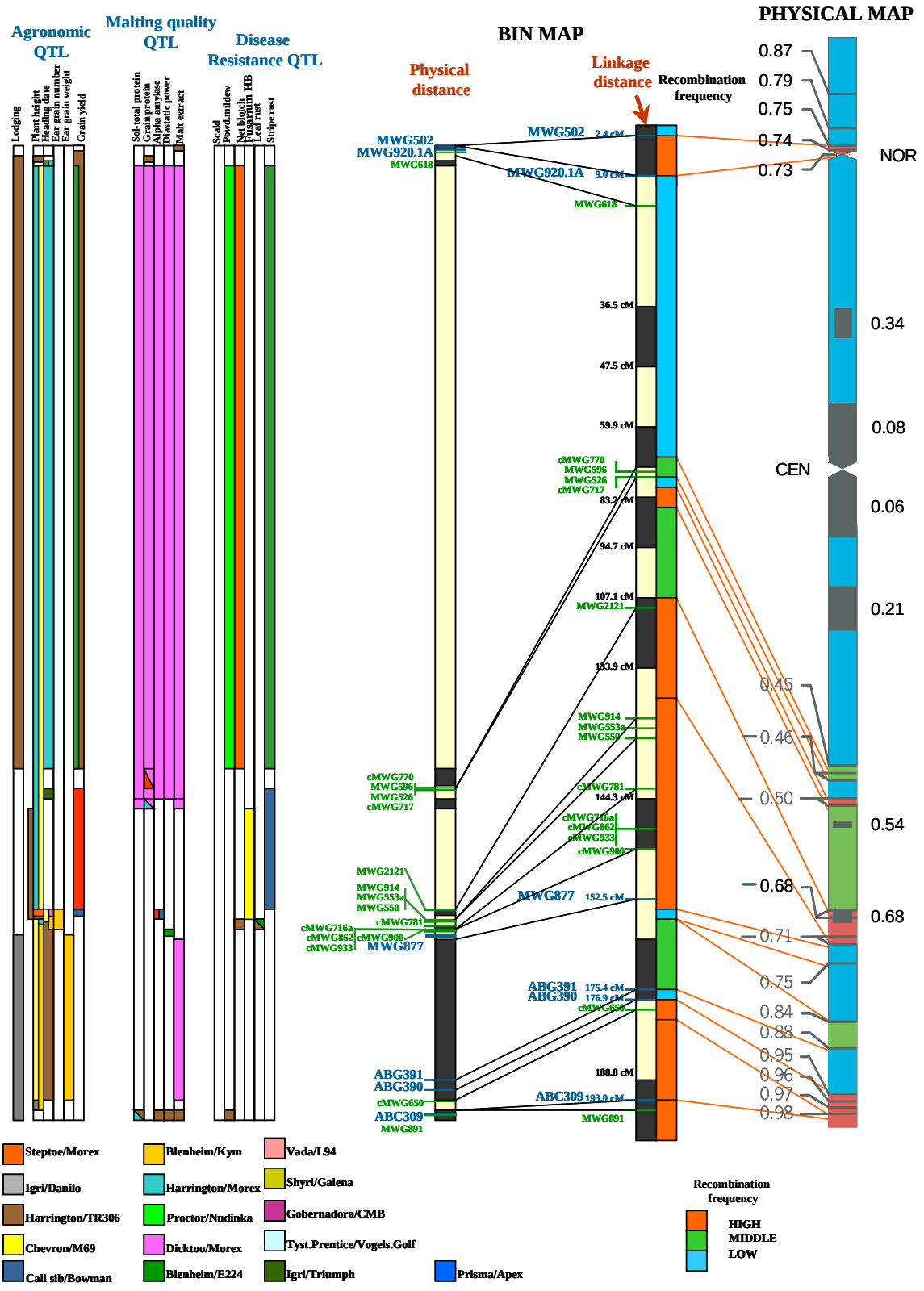
4H


Agronomic QTL
Lodging
Heading date
Plant height
Ear grain number
Ear grain weight
Grain yield
Malting quality QTL
Sol-total protein
Grain protein
Alpha amylase
Diastatic power
Malt extract
Disease Resistance QTL
Scald
Powd.mildew
Net blotch
Fusarium HB
Leaf rust
Stripe rust
BIN MAP
PHYSICAL MAP
Physical distance
Linkage distance
Recombination frequency
MWG2021a
Ris45b
MWG896
ABG500a
MWG913
MWG2056
MWG758
MWG506
MWG943
MWG2077
MWG649b
MWG706a
MWG954
cMWG984b
MWG701b
MWG632b
MWG912
ABG387A
56.9 cM
120.9 cM
159.6 cM
0.88
0.75
0.67
0.47
0.46
0.10
CEN
0.06
0.37
0.44
0.47
0.72
0.81
0.82
0.86
0.89
0.92
Steptoe/Morex
Igri/Danilo
Harrington/TR306
Chevron/M64
Cali sib/Bowman
Blenheim/Kym
Harrington/Morex
Proctor/Nudinka
Dicktoo/Morex
Blenheim/E224
Vada/L94
Shyri/Galena
Gobernadora/CMB
Tyst.Prentice/Vogels.Golf
Igri/Triumph
Prisma/Apex
Recombination frequency
HIGH
MIDDLE
LOW

5H


Agronomic QTL
Malting quality QTL
Disease Resistance QTL
Lodging
Heading date
Plant height
Ear grain number
Ear grain weight
Grain yield
Sol-total protein
Grain protein
Alpha amylase
Diastatic power
Malt extract
Scald
Powd.mildew
Net blotch
Fusarium HB
Leaf rust
Stripe rust
BIN MAP
PHYSICAL MAP
Physical distance
Linkage distance
Recombination frequency
MWG966
ABC654b
cMWG652a
ABG387B
cMWG679
ABG458
ABG020
MWG2231
MWG984a
ABG388
ABC163
MWG820
MWG2205
MWG2137
MWG2141
MWG934
MWG911
MWG798a
26.4cM
46.0cM
61.9cM
68.1cM
70.5cM
81.3cM
103.9cM
151.6cM
0.95
0.93
0.87
0.86
0.85
0.81
0 63
0 57
0 30
0 23
NOR
CEN
0.19
0.06
0.05
0.17
0.33
0.34
0.36
0.37
0.46
0.47
0.53
0.55
0.56
0.60
0.70
0.72
0 73
0 74
0.84
0 88
0 90
0.91
Steptoe/Morex
Igri/Danilo
Harrington/TR306
Chevron/M64
Cali sib/Bowman
Blenheim/Kym
Harrington/Morex
Proctor/Nudinka
Dicktoo/Morex
Blenheim/E224
Vada/L94
Shyri/Galena
Gobernadora/CMB
Tyst.Prentice/Vogels.Golf
Igri/Triumph
Prisma/Apex
Recombination frequency
HIGH
MIDDLE
LOW

6H


Agronomic QTL
Malting quality QTL
Disease Resistance QTL
Lodging
Plant height
Heading date
Ear grain number
Ear grain weight
Grain yield
Sol-total protein
Grain protein
Alpha amylase
Diastatic power
Malt extract
Scald
Powd.mildew
Net blotch
Fusarium HB
Leaf rust
Stripe rust
BIN MAP
Physical distance
Linkage distance
Recombination frequency
PHYSICAL MAP
NOR
CEN
MWG502
MWG920.1A
MWG618
cMWG770
MWG596
MWG526
cMWG717
MWG2121
MWG914
MWG553a
MWG550
cMWG781
cMWG716a
cMWG862
cMWG933
cMWG900
MWG877
ABG391
ABG390
cMWG650
ABC309
MWG891
Steptoe/Morex
Igri/Danilo
Harrington/TR306
Chevron/M69
Cali sib/Bowman
Blenheim/Kym
Harrington/Morex
Proctor/Nudinka
Dicktoo/Morex
Blenheim/E224
Vada/L94
Shyri/Galena
Gobernadora/CMB
Tyst.Prentice/Vogels.Golf
Igri/Triumph
Prisma/Apex
Recombination frequency
HIGH
MIDDLE
LOW

7H