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Background
Barley (*Hordeum vulgare* L.) is an important cereal crop species ranking fifth in crop production worldwide after maize, wheat, rice, and soybean (area harvested, FAO 2005, [http://faostat.fao.org](http://faostat.fao.org)). The crop belongs to genus *Hordeum*, which is a moderately sized genus with ca. 32 species and altogether ca. 45 taxa (see Von Bothmer et al., 1995, for a review). Together with wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), and other important forages, barley belongs to the tribe of Triticeae. This tribe represents a highly successful evolutionary branch in the grass family (*Poaceae*) and comprises a vast number of species and genera. All species in *Hordeum* have a similar set of diagnostic morphological characters, particularly with three, one-flowered spikelets at each rachis node. The two lateral florets are pedunculate, or sessile and may be sterile (as in two-rowed barley) or fertile (as in six-rowed barley). The glumes are setaceous of flattened and placed on the adaxial side of (and not surrounding) the spikelet (Von Bothmer et al., 2003a).

**Importance of barley**

Barley has a long history as a domesticated crop, as one of the first species to be adopted for cultivation. 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. Migration of people together with their seed crops led to a major diversification and adaptation to new areas, and the crop is now virtually found worldwide. Conscious selection of desired
Introduction

genotypes by farmers at an early stage, together with natural selection, increased the diversity and created the rich gene pool source of variation found today in local varieties. These landraces also formed the basic material for modern plant breeding, which started some 100 years ago.

Today the crop is grown and used in fertile as well as in marginal areas under extreme conditions, including locations at altitudes up to 4,500 m in the Himalayas, flooded areas of Southeast Asia, and the arid regions of the Mediterranean basin. Barley thus shows a very wide spectrum of adaptation. The development of new technologies and methods increased the genetic diversity even further and turned barley into the universal, highly diverse crop it is today (Von Bothmer et al., 2003b). 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 growth environment (such as vernalization and/or photoperiod response), or they may be defined by evolutionary bottlenecks and by 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. 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.

Over the centuries, barley has been planted for many different purposes. It was initially used as source of human food and
animal feed. As a human staple food it has persisted until today in large regions in the mountainous areas of Central and Southwest Asia, and Northern Africa, and in East Asia. Its importance as an animal feed has increased over the years and barley is now one of the most important feed crops in temperate areas. Early on in the history of agriculture, man invented the process of mating and brewing. For example, barley beer was one of the most important drinks in ancient Egypt. The processes have been refined and beer produced from pure malt as well as good malting barley varieties are currently in demand all over the world. Special uses are the characteristic barley tea of Japan and Korea, and “barley grass” as functional food (Von Bothmer et al., 2003b).

The other side of the importance of barley is due to its genetic architecture. Barley is a diploid species with the basic chromosome number of Triticeae (n = 7) possessing large chromosomes, named series 'H' of the Triticeae, extensively colinear to those of the polyploid species of durum and bread wheat. Its genome comprising more than 5,000 Mbp equals approximately 12 times the size of the rice genome and it consists for about 80% of repetitive DNA. Due to its importance as a staple crop and because of its model character for other Triticeae genomes including wheat, *Triticum aestivum* L. and rye, *Secale cereale* L., comprehensive genetic and genomic resources have been established for barley over the past decades. These include a large number of well-characterized genetic stocks and mutant collections (http://www.untamo.net/cgibin/ace/searches/basic) (Caldwell et al. 2004; Lundqvist et al. 1996), various genetic linkage maps (Varshney et al. 2004a), large insert bacterial artificial
chromosome (BAC) libraries (Isidore et al. 2005; Yu et al. 2000), and a large collection of expressed sequence tag (EST) presently comprising more than $4 \times 10^5$ entries in dbEST (dbEST summary July 21st, 2006, http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html).

Barley, apart from being an important agricultural crop for food and feed, has also been used virtually worldwide as a model species for biological research. This is due to a number of factors, partially cited above, that here are resumed:

- The diploid ploidy level of the crop;
- The inbreeding habit and the diploid system, which makes the inheritance studies easy to perform;
- Large chromosomal synteny and colinearity in the *Triticeae* and in the entire grass family. The results obtained in *Hordeum vulgare* can be applied to other species;
- The wild progenitor *Hordeum vulgare* L. ssp. *spontaneum* (Koch) belongs to the primary genepool of the crop and crosses are hence easy to perform and progeny fertile;
- A large number of well-documented mutations and accessions are available in genetic stocks and germplasm collections;
- Most genotypes are homozygous, which makes it easy to increase seed lots and to repeat experiments;
- The uniqueness of barley is largely due to its long history as a crop, and accentuated by the multitude of uses and the development of many different, often quantitatively inherited characters.
Mapping quantitative variation in barley

The position of genes affecting economically important characters is of great importance to plant breeders, and molecular marker technology has made this increasingly possible. Many characters of economic interest show a continuous range of values, rather than distinct phenotypes. This is due to the joint effects of several genetic loci (quantitative trait loci, or QTLs) and the environment. The individual loci contributing to a quantitative trait cannot be observed, and because of this, quantitative traits cannot be studied in the same way as for single gene characters. The first reported linkage of a trait to a major gene was by Sax (1923). He deduced that in bean (*Phaseolus vulgaris*) the locus for seed colour was linked to a locus affecting seed weight. He observed, in fact, significant differences in seed weight between the three seed-colour genotypes (PP, Pp and pp) in a F2 population raised from a pigmented parent (P) and a not-pigmented one (p). Subsequently, there were comparatively few such studies, mainly due to the small number of suitable major genes. During the last decade the development of molecular markers has transformed this situation, and linkage maps of molecular markers are now available for the main cereal species.

**Single-marker analysis by linear regression**

Once again this approach dates back to Sax (1923), and has been widely used by several groups. In a DH population the analysis involves, for each marker in turn, classifying the offspring into one of two classes depending on their genotype at the marker, calculating the mean trait value associated with each
class of offspring and comparing the mean trait values for each
class to see if they are significantly different. However, the
marker may be linked to a QTL rather than being coincident with
it. In this case we need to consider recombination between the
marker and the QTL. Regression on marker genotypes gives a
great deal of information about marker-trait associations, but
there are some problems with this approach (Lander and
Botstein, 1989):

1. The approach only considers the marker positions, and
   has less power to detect a QTL between the markers.
2. We cannot estimate the QTL effect $\alpha$ and the
   recombination frequency ($\theta$) separately.
3. There is a large amount of variation within each marker
   class and some of this will be due to other QTLs
   affecting the trait.

Despite these problems, regression on marker genotypes is a
good first step in the analysis. It identifies associations without
knowing the position of the marker on the map and it may be
adapted for use in any type of population (Hackett, 2002).

**Simple interval mapping**

In interval mapping, the trait values are related to the genotype
of a putative QTL at different locations along each chromosome.
Information from a linkage map of molecular markers is used to
infer the probability of each possible QTL genotype.

One method to estimate these parameters is by maximum
likelihood estimation, i.e. derivation of the parameter values that
maximize the likelihood of obtaining our observed set of trait
values. A map of the likelihood of a QTL along an entire
chromosome may be derived by combining analyses of
neighboring intervals. At each marker locus the model simplifies to a regression on the marker genotype, so that the likelihood is continuous along the chromosome. The procedure is referred to as interval mapping (Lander and Botstein, 1989), or sometimes as simple interval mapping. Many QTL mapping programs such as MapQTL (van Ooijen and Maliepaard, 1996), QTL Cartographer (Basten et al., 1999) use this approach for QTL mapping.

Interval mapping by maximum likelihood estimation is computationally demanding, and a regression approach to QTL mapping, using least-squares estimation, has been proposed (Haley and Knott, 1992; Martinez and Curnow, 1992). This approach is a close approximation to interval mapping by maximum likelihood estimation, and it is extremely flexible. The regression mapping approach is used by the PLABQTL software (Utz and Melchinger, 1996).

**Multiple QTL mapping**

When a single QTL is fitted, the residual variance includes both environmental variance and genetic variance due to other QTLs. If we could account for all the QTLs affecting a trait simultaneously, the residual variance would be reduced and so the power to detect QTLs would be increased. It has also been shown that the estimates of QTL locations and QTL effects may be biased if the effects of other QTLs are not taken into account (Utz and Melchinger, 1994). Linked QTLs with effects of opposite signs may cancel each other, while linked QTLs of the same sign may lead to a maximum in the LOD profile at a position between the two true QTL locations (a ‘ghost’ QTL). A good approximation is to combine a mixture model and a linear regression model.
This approach has several variants, known as multiple QTL mapping (Jansen, 1994) or composite interval mapping (Zeng, 1994).

In this way the genome can be scanned for the optimal location for each QTL, while using markers flanking the other QTLs to remove their effects and reduce the residual variation. One question with this approach is the choice of the set of markers which are known as cofactors. It would seem better to eliminate redundant markers, and to use as cofactors only those thought to be associated with QTLs.

**Analysis of multiple traits**

A new area of statistical research is the simultaneous search for QTLs affecting several traits. Many QTL studies have been published in which a trait-by-trait analysis often shows that the most likely location of QTLs for the different traits are in very similar locations. The dilemma facing geneticists and breeders is whether such results indicate linkage or pleiotropy (Hackett, 2002).

The methods for analyzing several traits simultaneously by interval mapping all require complicated programming. Software is now available for the methods of Jiang and Zeng (1995), in program JZmapqtl of QTL Cartographer (Basten et al., 1999), and for those of Korol and Ronin, in program MultiQTL (http://www.multiqtl.com/).

In general, QTLs accounting for a fairly high proportion of the trait variance tend to be detected in several environments, but other QTLs are not detected consistently. One explanation might be that the power of the experiment to detect QTLs is too low, and so the QTL is not detected in all the environments. However,
it is also possible that there is QTL × environment interaction, with some QTLs determining the adaptation to particular environments. The question of the power of an experiment to detect QTLs is important: in an experiment with low power, QTL effects may be biased and QTLs may be detected in some environments but not others, giving a false impression of QTL × environment interactions. Current methods of locating QTLs give a confidence interval that may be adequate for marker-assisted backcrossing (Hackett, 2002), but are inadequate for map-based cloning. An increase in the number of meioses seems thus important to locate QTLs more precisely.

**From quantitative trait loci to quantitative trait nucleotides**

The genomics revolution of the past 10 years has improved our understanding of the genetic make up of living organisms. Although traits of plant breeding interest are mainly quantitative in nature, the relationships between quantitative trait variation and molecular diversity of genes can be studied based on a genomic approach (Figure 1; adapted from Morgante and Salamini, 2003). QTL detection has been successful in species where inbred strains are available, owing to the simplicity of the genetics of the system and because tests associating significant phenotypic effects to chromosomal intervals are straightforward to carry out. A simple architecture of crop trait complexity is emerging, with only few loci controlling most of the variation (Kearsey and Farquhar, 1998). The resolution of these experiments is, however, poor (10–30 cM). This represents a major drawback in successive attempts to identify genes responsible for QTLs. QTL experiments can be carried forward to a sufficient resolution to attempt a positional cloning effort (1 cM
or less). In such cases, mapping is based on near isogenic lines (NILs) differing for the QTL region and on the analysis of thousands of progenies from their cross. Fine mapping of QTLs has sometimes revealed the presence of tightly linked loci affecting the same trait, and several genes responsible for quantitative trait variation have been identified in plants so far (see Morgante and Salamini, 2003 for a recent review).

Figure 2 (from Morgante and Salamini, 2003): Genomic approaches used to decode the molecular basis of trait quantitative variation. EST, expressed sequence tag; SNP, single nucleotide polymorphism.

A new paradigm

With the recent advances in DNA sequencing and single nucleotide polymorphism (SNP) genotyping, new approaches to QTL mapping and quantitative trait nucleotide (QTN) identification are available. As reported by Rafalski, 2002, the emerging concept is to exploit the possibility of looking at variation directly in genes and not at anonymous markers (candidate gene association studies), as well as to saturate the genome with markers (whole genome scan). Both approaches
rely on the detection of LD (i.e. non-random association between alleles at linked loci) and take advantage of recombination events accumulated over many generations to restrict a mapping interval. Association studies are based on existing populations/germplasm collections, which is a major advance for species where experimental populations are difficult to access. Although the whole genome scan approach has not yet been used, the candidate gene approach is being applied to crops. Both procedures benefit, in different ways, from the level/amount of LD present in a species or in a population (LD is a function of history and recombination and seems population- rather than species-specific). When LD is low, high-resolution mapping is achievable. A low LD, however, has a drawback: a high number of SNPs is required to detect an association. Conversely, if LD is high, a lower mapping resolution becomes evident but less markers are necessary to apply the genome scan approach. The choice of proper genes is the key issue if the candidate approach to QTL mapping is taken. The first example of a success in using the candidate gene approach to identify determinants of quantitative variation by association mapping was reported by Thornsberry et al., (2001). Candidates can be identified either by mutant analysis or from expression profiling experiments, under the assumption that genes that show genotype-specific differences in their level of expression could be the causative agents for the variation in a trait. With the integration of QTL mapping, comparative mapping information, growing EST databases, expression (including microarray) results, and the identification of more and more genes in the future, the candidate gene approach will become an important and powerful tool to
uncover the determinants lying behind the expression of quantitative traits.

**Barley seed: development and differentiation**

Growing seeds of barley are heterogeneous, highly organized systems, consisting of maternal tissues (i.e. pericarp) that surround the two filial organs: the diploid embryo and the triploid endosperm. Their development occurs subsequently, starting with the maternal and followed by the filial organs. The latter differentiate from meristem-like tissues into highly specialized storage organs. Differentiation is a sequential and continuous process involving cessation of cell division followed by cell expansion, sucrose uptake, greening, gaining photosynthetic activity and accumulation of storage products (Borisjuk et al. 2004).

The barley grain is a typical starch and protein storing sink organ. Nutrients are unloaded from the vascular bundles into the pericarp cells, which in turn, reach the underlying filial tissues in an apoplastic step (Patrick and Offler 2001). How and by which mechanisms, maternal and filial tissues interact to drive and coordinate normal seed development are largely unknown.

Seed development is a highly complex process, genetically programmed and can be correlated to changes in metabolite levels. Sugars and nitrogen, besides their nutritive role, act as signals which regulate and influence development (Kock 1996; Wobus and Weber 1999; Stitt 1999).

Based on growth characteristics, starch accumulation patterns, and metabolite profiles, Sreenivasulu et al. (2004) described a slightly different staging scheme for whole caryopses in the barley variety ‘Barke’ (Weschke et al. 2000, 2003). The first
phase is defined as pre-storage phase (0-5 days after flowering - DAF) and is characterized mainly by the ongoing cell division, morphogenesis and absence of starch in the endosperm. During the following days, an initial accumulation of starch grains occurs in the endosperm (6-9 DAF), followed by a linear increase of storage product biosynthesis and deposition (10-20 DAF), which levels off thereafter (around 20 DAF). The accumulation of storage compounds (starch and storage proteins) is based mainly on imported sucrose, and it dominates endosperm development.

With the recent development of high-throughput technologies allowing the concomitant analysis of thousands of genes, proteins, and/or metabolites (Fiehn et al. 2001), the analysis of complex networks governing developmental and metabolic processes has become possible for the first time (Lee et al. 2002; Ruuska et al. 2002). To investigate regulatory networks operating in barley grains during the pre-storage and early storage phases, the analysis of expressed sequence tags (ESTs) (Michalek et al. 2002; http://pgrc.ipk-gatersleben.de) and cDNA macro arrays for transcriptome analysis (Potokina et al. 2002; Sreenivasulu et al. 2002) have been started.

These analyses provide an eclectic overview of gene expression in the maternal and filial tissues during early caryopsis development, and they have identified sets of genes that are preferentially expressed in one or the other tissue (Sreenivasulu et al. 2002).

Physiological studies, including enzyme activity and metabolite measurements, have been published (Weschke et al. 2000, 2003) and detailed histological analyses of seed development
including 3D-model building, as well as medium-scale *in situ* hybridisation studies, are made (Sreenivasulu et al. 2004).

![Figure 2](from Weschke et al. 2000): Schematic representation of the histological organization of a barley caryopsis. Median cross-section of a developing caryopsis at 6 DAF. The different parts of the caryopsis are indicated as follows: ET, endospermal transfer cells; II, inner integument; NE, nucellar epidermis; NP, nucellar projection cells; OI, outer integument; P, pericarp; SE, starchy endosperm; VT, vascular tissue.

A newly defined intermediate phase in the caryopsis development, between pre-storage and storage phases, is of special interest during seed growth and characterised by dramatic stepwise re-programming of the transcriptional machinery (Sreenivasulu et al 2004). Maternal tissue undergoes degradation characterized by lipid and transient storage compound mobilization. Gene expression profiles during this intermediate phase, suggest an important role for photosynthetic oxygen production and ATP provision for subsequent storage processes. Expression patterns of filial tissue are also indicative of changes in energy provision with respect to development and cellular compartmentation. An early event of differentiation in
seed storage organs is the formation of transfer cells, in the outer cell rows of the endosperm facing the nucellar projection of the barley grain (Weschke et al. 2000; Figure 2). Mature transfer cells of barley express specific sucrose transporter genes (Weber et al. 1997a; Weschke et al. 2000). Borisjuk et al. (2004) have hypothesised that the sudden increase in sucrose generates a specific signal initiating maturation in seed storage organ.

**Malting quality**

As already said, a significant high-value use of barley is for malting, to produce malt as a raw material for the brewing of beer and fermentation and for the distillation and production of whisky.

Barley production to supply the diverse end uses require barley breeding programs to provide varieties with the combination of reliable and efficient production characteristics and grain quality attributes suited to these uses. Selection of varieties with the complex range of traits necessary for efficient processing to produce high quality products such as beer is a difficult process. Testing of end product quality for each line is not only expensive but requires the availability of larger quantities of barley than is available from single plants or lines at an early stage in barley breeding. Biochemical or molecular tests that predict likely feed or malting and brewing quality are therefore needed to allow rapid development of barley varieties. The biochemical basis of several barley quality traits is not well understood. Many of the traditional testing and evaluation methods aim to ensure a consistency of quality without the link between the attribute measured and the end-use quality being known. This process may discriminate against barley with a superior processing traits and will only be overcome by improved understanding of the
basis of barley quality, especially at the biochemical and genetic levels.

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”. These procedures have had limited practical applications, and nowadays information on allelic diversity in the genes that determine malting quality is limited.

The brewing industry considers seven parameters to be most important in defining malt quality (Table 1). There are hot water extract, protein content, viscosity, Kolbach index, wort β-glucan, fermentability, and diastatic power.
<table>
<thead>
<tr>
<th>Trait</th>
<th>Malting range</th>
<th>Feed range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Barley industry specifications (hulled grain)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grain size</td>
<td>&gt;70%</td>
<td>&gt;40%</td>
</tr>
<tr>
<td>&gt;2.5 mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2.2 mm</td>
<td>&gt;5%</td>
<td>&lt;15%</td>
</tr>
<tr>
<td>Protein</td>
<td>10.0–12.0%db</td>
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<tr>
<td>Moisture</td>
<td>12.5% max.</td>
<td>12.5% max.</td>
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<tr>
<td>Weather damage</td>
<td>&gt;300 s</td>
<td>n.a.</td>
</tr>
<tr>
<td>Falling number</td>
<td>150 units</td>
<td>n.a.</td>
</tr>
<tr>
<td>RVA</td>
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<tr>
<td>Test weight</td>
<td>65.0 kg/L</td>
<td>62.5 kg/L</td>
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<tr>
<td><strong>Non-industry specifications (desirable breeding priorities)</strong></td>
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<tr>
<td>Husk</td>
<td>8.0–10.0% db</td>
<td></td>
</tr>
<tr>
<td>β-Glucan (%)</td>
<td>3.0 – 5.0</td>
<td>&gt;5, &lt; 6 (poultry)</td>
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<tr>
<td>Hardness</td>
<td>&lt;250</td>
<td>&gt;40</td>
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<tr>
<td>Compact mill</td>
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<td></td>
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<tr>
<td>SKCS</td>
<td>&lt;30</td>
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</tr>
<tr>
<td>Particle size</td>
<td>1000 – 2000 µm</td>
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<tr>
<td>Starch</td>
<td>55.0 – 65.0% db</td>
<td>&gt;60% db</td>
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<tr>
<td>Digestibility (DMD)</td>
<td>&gt;50 (20–60)</td>
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<tr>
<td>Nitrogen</td>
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<tr>
<td>ADF</td>
<td>5.0 – 10.0% db (lower better)</td>
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<tr>
<td>NDF</td>
<td>15.0 – 30.0% db</td>
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</tr>
<tr>
<td>% Starch digested</td>
<td></td>
<td>52 – 76%</td>
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<tr>
<td>Enzyme digestion</td>
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<td>37 – 53%</td>
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<tr>
<td>Hot water extract: EBC (fine)</td>
<td>80.0 – 83.0%</td>
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<tr>
<td>Kolbach index</td>
<td>35.0 – 49.9</td>
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<tr>
<td>Diastatic power</td>
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<tr>
<td>EBC WK</td>
<td>200 – 350</td>
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<tr>
<td>U/g</td>
<td>300 – 600</td>
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<tr>
<td>Viscosity</td>
<td>1.55 – 1.65cP</td>
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<td>Fermentability</td>
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<tr>
<td>Wort β-glucan</td>
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<tr>
<td>α-Amylase</td>
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<tr>
<td>U/g</td>
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<tr>
<td>DU</td>
<td>&gt;60</td>
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<tr>
<td>Free amino N</td>
<td>140 – 180 mg/L</td>
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<tr>
<td>Friability</td>
<td>Min. 70%</td>
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<tr>
<td>β-Glucanase</td>
<td>&gt; 250 U/g</td>
<td></td>
</tr>
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</table>

n.a., not available.

**Table 1** (from Fox *et al.*, 2003): Barley quality specifications for malting and feed end uses.
Introduction

The malting process

Malting could be defined as 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 2.

**Barley malting quality traits**

**Grain Protein**

Barley protein accounts for 8-13% (dry basis) of malting quality barley, while anecdotal evidence suggests that 12-13% protein is most efficient for ruminant animals. Barley protein has a
Table 2 (from Fox et al., 2003): Barley genes, character or trait, and chromosomal location relating (after Søgaad and von Wettstein-Knowles 1986; von Wettstein-Knowles 1992; reference loc. cit. and GrainGenes) Where possible the recommended system for gene locus nomenclature has been used (see Franckowiak et al. 1998).
complex interaction with quality. High protein is undesirable because of the strong correlation with low carbohydrate levels and thus low extract values. However, if the protein content of malt is too low, brewing performance may be impaired through poor yeast amino acid nutrition. Protein levels in packaged beer are important, positively enhancing foam stability and negatively influencing shelf life by contributing to chill hazes. Many proteins have been identified with specific functions in terms of grain and malt quality, whereas a number have yet to have their function clearly defined.

Storage protein

Storage proteins exist in all cereals. This protein component forms a matrix around starch granules in the endosperm and provides a source of nitrogen for the growing embryo if germination occurs. These proteins are generally rich in the amino acids proline and glutamine (hence the term prolamine). In barley, the major storage protein is called hordein, and this comprises 40-50% of total grain protein. This component is soluble in aqueous alcohol and comprises 4 fractions designated D, C, B and A. The diversity in the hordein family has made the analysis of these fractions very useful in varietal identification.

There was a negative relationship between protein and hot water extract, however each hordein group had some relationship to extract or final beer quality (Holopainen et al. 2005). In particular, several studies have describes a negative correlation between D hordein and hot water extract (Molina-Cano et al. 2000b). In contrast, Brennan et al. (1998) found that with D hordein isogenic lines there was no effect on extract, although the presence or absence of a D hordein allele had an
Introduction

impact on gel protein formation. Recently, Celus et al. (2006) have highlighted the formation, during mashing, of an aggregate composed of the sulfur-rich B hordeins in which C hordeins (sulfur-poor) are entrapped and this aggregation should form gels which cause filtration problems in brewing.

Non-storage protein

Many non-storage proteins exist within the cell walls as well as within the protein matrix. However, very few have been identified as having an impact on quality. Cell walls contain glycine-rich protein, as well as threonine-rich and hydroxyproline-rich glycoproteins (Cassab and Varner 1988; Kieliszewski et al. 1990). A structural protein, friabilin, has been isolated from barley endosperm. This protein has been identified with wheat puroindoline antibodies (Darlington et al. 2000). It has a molecular weight of around 15000, which is similar to the wheat protein. Currently, the function of this protein remains unclear. However, it has been proposed that friabilin may have a role in grain hardness, by binding the protein matrix with starch granules. Darlington et al. (2000) reported an increased level of friabilin extracted in soft wheat and in ‘soft’ barley. In contrast, more friabilin remained attached to the starch granules in hard wheat and barley.

Protein Z

Protein Z comprises a family of serine proteinase inhibitors (Dahl et al. 1996), which have been associated with specific effects on beer quality, in particular the stabilisation of beer foam. However, Lusk et al. (1995) demonstrated that protein Z alone was not the main foam-stabilising protein in beer foam. It is evident that many factors are involved in the formation and
stabilisation of beer foam. Protein Z can be found in bound form as a hetero-dimer with β-amylase, which can be cleaved by malt endopeptidase to produce a free form and thus activate the β-amylase (Guerin et al. 1992).

Lipid transfer proteins

Lipid transfer protein 1 (LPT1) is another structural protein involved in the binding of lipid and starch within the endosperm. LPT1 has been found in beer foam and it is likely implicated in foam retention, although there is a minor modification from the protein form in barley to that detected in beer (Sorensen et al. 1993). It has been suggested that LPT1 inhibits cysteine endoproteinase (Jones and Marinac 1995). A second cysteine inhibitor, named LPT2, has been identified by the same authors. This barley protein survived kilning and, like LPT1, may have a role in controlling the rate of storage protein hydrolysis during malting and mashing (for a detailed review see Jones 2005).

Barley Carbohydrates

Barley carbohydrate composition has been one of the most studied aspects in terms of barley quality and its relation to feed, malt, and beer quality.

Starch

Starch is the most abundant component of the endosperm, comprising around 60% of total grain weight. Starch consists of two polymers, amylose and amylopectin. Amylose is a linear polymer made up of glucose molecules linked via ρ-(1-4) glucosidic bonds. Amylopectin is the larger polymer with ρ-(1-4) glucosidic and ρ-(1-6) glucosidic linkages, which form the branched structure (Hough 1985). The ratio of amylopectin to
amylose is round 3:1 (Palmer 1983). Both amylose and amylopectin polymers are present in the barley endosperm starch granules. The large granules, designated A type, are round in shape and contain 70-80% amylose. The small, spherical, B type granules, contain 40-80% amylose (Evers et al. 1999).

During malting, limited starch breakdown occurs, while, during mashing, starch is degraded more by the hydrolytic enzymes α-amylase, β-amylase, α-glucosidase, and limit dextrinase. High temperature infusion mashes readily solubilise the starch but limit the activity of thermolabile enzymes, in particular α-glucosidase and β-amylase (Osman et al. 1996a). Recently, the gelatinisation properties of starch were reviewed by Evers et al. (1999). For barley, gelatinisation temperature plays an important role in the quality of malt and hot water extract. The temperature at which gelatinisation occurs varies between 55 and 65°C.

Non-starch polysaccharides

The major constituent of barley endosperm cell walls are β-D-(1-3), (1-4) glucans (75%), with a minor component identified as arabinoxylans (20%) (Fincher 1975; Fincher and Stone 1986; Henry 1987). The arabinoxylan fraction is usually referred to as pentosan. The solubility of β-glucan in beer varies according to the number and arrangement of (1-3) and (1-4) linkages (Izawa et al. 1993) as well as the size of the molecules. The range in barley for β-glucan is 2-10% of total grain weight (Henry 1987). Both genotype and environment influence the content of β-glucan (Zhang et al. 2001; Molina-Cano et al. 2004; Yalcin et al. 2007). Recent study have presented models of the structure of endosperm cell walls. Bamforth and Kanauchi (2001) presented
a model of barley seed whereby the outer cell wall was made up of xylan, arabinose, and ferulic acetic acids, with the inner layer composed of β-glucan. These results indicate that enzymic hydrolysis of the arabinoxylan layer would be critical to ensure that β-glucanase could hydrolyse the β-glucan layer. The enzymic breakdown of β-glucan and pentosan during malting is critical for efficient brewing. The level of β-glucan has been shown to have a relationship with other malting quality traits. Importantly, high β-glucan levels may not result in higher or lower extract but relate to other malt quality traits such as Kolbach Index (ratio of soluble to total protein), viscosity or the speed of filtration (Evans et al. 1999).

Grain Size

Grain size is an important descriptive trait based on the physiology of the grain. The final grain size is determined by several environmental effects as well as biochemical components within the grain itself (Coventry et al. 2003b). For thousand of years when grain was used specifically for human consumption, it has been selected based on size. Within the last century, barley breeders have continued to target large grain genotypes in association with improved yield and other attributes. The measurement of grain size is generally based on 4 fractions: < 2.2 mm (screenings), > 2.2 mm, > 2.5 mm, and > 2.8 mm.

Smaller grain generally has lower starch and higher protein levels, thus reducing the extract potential. Large grain generally have increased levels of starch and therefore more potential extract. However, excessively large grain could impact on malting quality particularly on the rate of water hydration and modification during malting.
Introduction

**Dormancy**

Malting barley is one of the few grains where the seed is required to germinate for product development, i.e. production of malt. The failure of barley grain to germinate at an acceptable level, i.e. > 95%, could introduce problems during the malting process.

If we define dormancy as the lack of seed germination when the seed is put in optimal germination conditions, we understand that it is another crucial parameter for the malting process.

The physiological and biochemical components of dormancy may be as complex as grain yield. The role of hormones and enzymes in dormancy has been documented in recent review by Benech-Arnold (2002). Several biochemical mechanisms have been associated with dormancy, including an antagonistic effect between abscisic acid (ABA) and gibberellic acid (GA).

**Grain Hardness (milling energy or friability)**

Hardness describes the texture of the grain endosperm. Starch granules are either readily separated from the protein matrix (soft) or else the granules resist separation (hard). In general, malting barley varieties can be classified as soft, whereas non-malting or feed varieties are classified as hard (Alison *et al.* 1976). Hardness has also been associated with the level of modification of malt, which would imply that grain components within the endosperm directly affect modification. Specific proteins that interact with starch granules such as hordoindolines, including friabilin, have also been implicated in grain hardness (Darlington *et al.* 2000).

Milling energy or friability have been used as a measure of barley grain and malt hardness, thereby providing an indication of
malt endosperm modification. Fox et al. (2007) showed that hardness is a trait can be determined in barley, and while there are genetic and environmental effects, it is possible to select for hardness as well as identify genetic markers for hardness, using conventional methodology (milling energy or friability) or by using surrogate methods such as NIR. Many studies have demonstrated the relationships between barley hardness (milling energy) and grain and malt quality parameters (Swanson et al. 1995). Grain protein and β-glucan have been positively correlated with hardness, and malt extract and endosperm modification correlated negatively to hardness.

**Alpha-amylase**

α-Amylase (EC 3.2.1.1) is an endohydrolase that randomly cleaves α-(1-4) glucosidics bonds in starch. The level of this enzyme is not usually detectable in barley but increases once germination commences (Bathgate and Palmer 1973; Georg-Kraemer et al. 2001). In most cereals, the α-amylase I (Amy2) form appears shortly after anthesis. The level declines during grain maturation. When the germination commences, a second form, α-amylase II (Amy1), appears. In the first hours of germination, α-amylase II is released from the scutellum. After the first day of germination, the aleurone becomes the main source of α-amylase (Munck et al. 1981). The level of α-amylase II formation is highly dependent upon GA (Freeman 1984). In the presence of GA, both enzyme groups continue to be secreted by the aleurone (MacGregor 1987; Murray et al. 2006). The optimal temperature for α-amylase II is around 65°C (Briggs et al. 1981; Hoseney 1986), which would allow the enzyme to perform efficiently under most mashing conditions. 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).

**Beta-amylase**

β-Amylase (EC 3.2.1.2) is one of the key enzymes involved in the production of the fermentable sugar, maltose, which is utilised by yeast during fermentation. This enzyme is found in mature grain in two forms, free and bound. Three beta-amylase genes exist in cultivated barley, Bmy1, 2 and 3 (Li et al. 2002). The level of grain protein has an impact on the level of β-amylase. β-Amylase is found in a bound form as a hetero-dimer with protein Z. The activation of the β-amylase is mediated through cleavage with the malt endopeptidase (Mep1) (Guerin et al. 1992). β-Amylase is an exoenzyme that cleaves the disaccharide maltose from the non-reducing end of amylose and amylpectin. The enzyme activity alone catalyses the hydrolyses of ~70% of the amylose and ~50% of the amylpectin fractions of barley starch. The stability of β-amylase decreases rapidly at temperatures > 55°C.

**Limit Dextrinase**

Limit dextrinase (EC 3.2.1.41) catalyses the hydrolysis the α-(1-6) glucosidic linkages of amylpectin. The enzyme produces an increased number of smaller linear oligosaccaride chains that are subsequently rapidly hydrolysed by α-amylase and β-amylase. Limit dextrinase (Ldx) has been detected in, and extracted from, ungerminated barley (Sissons et al. 1993). The action of limit
dextrinase, thought to be synthesised in the aleurone layer during germination, with maximum activity obtained after 8 days, and exported to the starchy endosperm, is critical for the supply of carbohydrates to the germinating cereal embryo, and is also of great importance in the malting industry for the production of fermentable sugars from barley grain (MacGregor 2002). In its inactive soluble state, limit dextrinase is thought to be combined with a inhibitor, called the limit dextrinase inhibitor (LDI), which is encoded for by a small multigene family (Stahl et al. 2007). Purified limit dextrinase has been found to have an optimal pH of 5.5 and temperature 60-63°C (Stenholm and Home 1999).

**Alpha-glucosidase**

α-Glucosidase (EC 3.2.1.20) is the fourth enzymic activity involved in hydrolysis of starch during mashing. The enzyme catalyses the release of glucose from maltose and higher sugars. Like as α-amylases, α-glucosidase is synthesised during germination and dependent upon GA. The pH optimum for α-glucosidase appears to depend upon substrate: 4.5-4.6 for maltose substrate, but 5.0 for starch as a substrate (Agu and Palmer 1997).

**Beta-glucanase**

β-(1-3), (1-4)-Glucan-4-glucanohydrolases (EC 3.2.1.73) or β-glucanase has the function to hydrolyse β-glucan during germination. Two isoenzymes, EI (Glb1) and EII (Glb2), have been identified and their functional properties reported (Woodward and Fincher 1982). β-Glucanase is produced during grain germination in the aleurone and scutella, with EII and EI produced in the aleurone and EI produced from the scutella.
(Stuart et al. 1988). The level of the EII enzyme increases upon the addition of GA. The first stage of endosperm modification is the breakdown of cell walls. This is one of the critical steps in producing good quality malt. The hydrolysis of β-glucan during germination has a significant impact on the final malt quality. The mechanism and enzymes involved in cell wall modification remain somewhat unclear. However, it is clear that an increase in malt β-glucanase levels results in reduced levels of β-glucan in wort. Slow and/or incomplete breakdown of barley β-glucan has been shown to have a negative impact on hot water extract (Stuart et al. 1988), as well as causing viscosity and filtration problems in the brewhouse (Stewart et al. 2000). β-Glucanase, as with most enzymes in cereals, is inactivated at high temperature, and the optimal temperatures for EI and EII are ~37°C and ~45°C respectively. β-Glucanase activity is considerably reduced during the kilning process, and in the initial stages of high temperature mashing (Woodward and Fincher 1982; Wang et al. 2004); all activity is lost after ~15 min under this conditions. Hence, commercial brewing requires either high levels of β-glucanase or an increase in thermostability of the existing enzymes.

**Secondary malting quality traits (traits derived/influenced by processing)**

Some of the most important traits in terms of malting quality are expressed only during processing. However, most of these vary depending upon the conditions. The final result is highly dependent upon the above primary traits.
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Hot Water Extract

The hot water extract of wort, commonly called malt extract (ME), is the most important trait whether selecting potential new malting varieties or trading malt. The quality of the extract is influenced by several factors (for a detailed review see Collins et al. 2003). The first group of them is environmental, such as growing conditions, temperature, fertiliser, available nitrogen, or moisture. These factors do not impact on extract directly but rather affect traits that influence extract, particularly protein and starch levels and composition. The second group of factors is represented by several genetic biochemical components that influence the final level of extract. These include 2- or 6-row types, husk thickness, grain size, protein, starch, non-starch polysaccharides, and enzyme production. The third factor that influences extract is the malting process itself. Most aspects of grain modification affect final beer quality, including important aspect such as clarity and foam stability. During malting, enzymes that have an impact on the degradation of substrates are either synthesised during germination or enzymically cleaved from their bound forms. The range of enzymes produced included those that degrade cell wall components, proteins, and starch. The process of malt production varies between countries, with 4-day germination schedules in Australia and 5-6-day germination schedules in many other countries. Mashing is the fourth factor that influences extract. Within the mashing process, there are several physical factors that affect the resultant extract. There are pH, mash time, mash temperature, grist/particle size, and grist to liquor ratio.
Introduction

Diastatic Power

Diastatic power is the term used to describe the collective activity of starch degrading enzymes in malt. Industry methods used to measure diastatic power vary considerably in several aspects including substrate, pH, and assay temperature. The value of diastatic power as currently measured in assessing barley quality in industry or breeding is questionable.

Wort Viscosity

The importance of low levels of wort viscosity has increased with the introduction of membrane filtration in breweries. High levels of viscosity reduce the efficiency of breweries. Viscosity, like hot water extract, cannot be related to a single trait within barley. The breakdown of β-glucan during malting has been shown to have a direct impact on extract viscosity in high temperature infusion mashing. High molecular weight fractions have been implicated as one of the main components in increasing wort viscosity. Other barley cell wall polysaccharides (arabinoxylan) have been demonstrated to have an impact on viscosity and, thereby, beer filtration (Stewart et al. 1998, 2000).

QTL analysis of malting quality

Characters that affect barley malting quality (i.e. malt extract content, α- and β-amylase activity, diastatic power, malt β-glucan content, malt β-glucanase activity, grain protein content, kernel plumpness, and dormancy) are quantitatively inherited and variously influenced by the environment (E). Conventional genetic analyses have provided little useful information, while molecular technologies have opened the door for better
understanding of these and other quantitatively inherited traits. In particular, quantitative trait locus (QTL) analysis provides a better understanding of the genetic factors that influence complex traits such as malting quality. This analysis can identify chromosome regions, linked molecular markers, gene effects, QTL x environment (QTL x E) and QTL x QTL interactions that are important in plant improvement. The ability to detect chromosome regions that affect two or more traits also provides an understanding of the genetic basis for correlation between traits. A long-term goal of QTL analysis is to maintain or improve malting quality in barley cultivars through molecular marker assisted selection.

The biochemistry, physiology, and genetics of malting quality is extremely complex. The current status of known genes and their alleles influencing malting and feed quality have been reviewed (MacGregor and Bhaty 1993; Ullrich 2002; Kleinhofs and Han 2002) and summarized in Table 2. The first systematic QTL mapping in barley was reported by Hayes et al. (1993), in which 62 QTLs underlying 8 traits were mapped. Since then, QTL mapping in barley has received worldwide attention and considerable QTL analyses have been performed in recent years on a number of crosses. The main results are summarized in Table 3, including malting quality traits, populations used to map the QTLs, and chromosomal locations of QTLs (Fox et al. 2003). Although various populations have been used by different groups, construction of consensus linkage maps makes it possible to compare the QTLs mapped in the different populations (Langridge et al. 1995; Qi et al. 1996). A barley bin-map developed by Kleinhofs and Graner (2000)
**Table 3:** (from Fox et al., 2003) QTLs mapped for barley malting quality.

<table>
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<tr>
<th>Traits</th>
<th>Populations</th>
<th>Number of QTLs</th>
<th>Chromosomes</th>
<th>References</th>
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Hayes et al. (2001) have summarized QTLs determining economically important traits in barley (agronomic traits, malting quality, and biotic stress resistance) using the barley bin-map developed by Kleinhofs and Graner (2000) and the physical map of Künzel et al. (2000) (Figure 3). The regions which are targeted in the quality footprint are apparent in Figure 3. 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 and this may be due to multi-locus clusters, pleiotrophic effects, or reduced recombination in regions of the genome with increased marker density (Hayes et al. 1996).

Zale et al. (2000) performed a study with the objective to review the literature on malting quality QTLs in barley and determine whether similar or unique QTLs have been identified among different mapping populations. Data came from crosses of germplasm sources originating from North America, Europe, Australia and Asia. They have considered a minimum of 168 malting quality QTLs representing 19 malting quality traits that had been mapped in nine populations. Additional molecular

Figure 3 A-G (from Hayes et al., 2001) Next pages: 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 da Künzel et al. (2000). The criteria used for QTL assignment to map locations were describes by Hayes et al. (2001).
Figura 3A: Chromosome 1H (5)
Figura 3B: Chromosome 2H (2)
Figura 3C: Chromosome 3H (3)
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Figura 3D: Chromosome 4H (4)
Figura 3E: Chromosome 5H (7)
Figura 3F: Chromosome 6H (6)
Figura 3G: Chromosome 7H (1)
markers from an integrated map were used to anchor specific QTLs across mapping populations. In particular, new markers necessary for locating reported QTLs were added to the Harrington/Morex skeleton map of Hayes et al. (1993) based on the consensus markers of Qi et al. (1996).

QTL regions are spread across each of the seven barley chromosomes with concentrations especially within chromosomes 1H, 2H, 4H, 5H, and 7H (Figure 4). Whereas, there is remarkable QTL conservation in some chromosome regions among crosses, some regions hold unique QTLs as well. It is also noteworthy that there are many overlapping QTLs, especially but not surprisingly, of related traits. Malt extract QTLs are almost always coincident with component traits such as carbohydrate hydrolytic enzyme activities, while diastatic power QTLs are often associated with $\alpha$- and/or $\beta$-amylase activity QTLs (Clancy et al. 2003). It is likely that pleiotropy is the cause, but gene clusters cannot be ruled out at this time. Given that malting quality determinants are widely distributed across the barley genome, care must be taken in choosing QTLs for selection in breeding programs, and magnitude of effect, of course, is one criterion that can be applied. In term of breeding strategies, the net effect is that multiple phenotypes will be inherited as a unit. The availability of markers that define such key regions is of value in classifying germplasm and designing breeding strategies. For malting quality, this has both positive and negative effects. For example, the QTLs for high levels of enzyme activity, high diastatic power, and high malting extract were inherited as one unit, which will increase the breeding efficiency for improvement of malting quality. On the
other hand, the QTLs for high grain nitrogen concentration were consistent with most QTLs for high levels of enzyme activity.

Figure 4 (from Fox et al. 2003): Chromosomal locations of mapped QTLs controlling malting quality related to the barley consensus linkage map (Qi et al. 1996). AA, α-amylase; AB, starch granule A/B; BG, β-glucan; BGS; β-glucanase; DP, diastatic power; EV, early vigor; FAAN, free amino acid nitrogen; FCD, fine-coarse difference; FM, fermentability; GP, grain protein; KP, kernel plumpness; ME, malt extract; MEN, milling energy; SD, seed dormancy; SP, soluble protein; TW, test weight; VS, wort viscosity.
QTLs for the same traits have been mapped to different chromosomes or regions by different researchers. These could be due to environmental factors and methods for quality assessment (Henry et al. 1996). Hayes et al. (1996) suggested that the different QTLs in different populations for a same trait are due to genotype-specificity of the QTLs. This limits straightforward application of the results from one population to another. On the other hand, it implies that not all favourable QTLs are fixed in elite germplasm. An alternative to attempting to extend the QTL results from one population to another is to integrate information from a range of germplasm with the aim of identifying regions of the genome that may affect target-trait expression. This information can be used to classify germplasm and design matings that will maximise the probability of accumulating favourable alleles.

While the location of QTLs for malting traits throughout the barley genome is rather constant, the effects of QTLs alleles may vary depending on the degree of influence of environmental factors. The extent of the interaction between QTL x E may be the direct effect of environmental conditions during grain filling or the direct expression of a particular gene to an environmental stimulus. Hayes et al. (1993) concluded that malting quality traits in Steptoe x Morex population were relatively free of environmental interaction effects, and with the exception of grain protein, interactions were limited to agronomics traits. Emebiri et al. (2004 and 2005) in a doubled haploid population derived from a cross of parents (VB9524 and ND11231*12) with inherently low grain protein content showed that the QTL x E was highly significant, but was largely due to differences in magnitude of QTL effects rather than changes in the direction of the effects.
As regards the other malting quality traits, where a significant QTL x E was noted, this was due to a difference in magnitude rather than a change in rank of a phenomenon or could be explained as the interaction of minor genes with the environment. Difference in the response of QTLs over a range of environments provide an opportunity for a plant breeder to select QTLs that consistently respond and develop a specific phenotype that is buffered against the unpredictable nature of environment factors.

**Genetics of barley adaptation to the environment**

Exposure of plants to abiotic stresses (i.e. temperature or water stress) trigs a number of inducible mechanisms that allow the cells to cope with the negative effects of the abiotic constrains. This stress response, known as acclimation or 'hardening', occurs when plants are exposed to a mild stress condition and it contributes significantly to strength plant stress tolerance. Acclimation is an extremely complex process where many different highly co-ordinated biochemical and molecular changes are activated in response to stress perception. The molecular mechanisms leading to the plant response involve three main steps: (1) the perception of external changes; (2) the transduction of the signal to the nucleus including the activation of stress-regulated transcription factors and (3) the stress-related gene expression. In the recent years a number of genes whose expression is induced or enhanced by environmental changes have been cloned in many species including barley and wheat (Cattivelli et al., 2002). On the contrary most of the recent knowledge on stress resistance derives from works carried out in plant species other than barley. Nevertheless it is generally believed that many molecular pathways, including those
controlling stress tolerance, are well conserved in all plant species and, consequently, the basic work carried out in model plants (mainly Arabidopsis thaliana) can be exported to other species such as barley. Genetic information can also be transferred particularly within the Triticeae genomes due to their high degree of synteny (Mastrangelo et al., 2004).

**Growth habit and frost tolerance**

A better understanding of the genetics of cold tolerance could have a significant impact on world food supply, since low-temperature-related stresses limit the productivity of many plants of agronomic and horticultural value. Barley (*Hordeum vulgare* subsp. *vulgare*) is an excellent model system for genetic analysis of the molecular basis of low-temperature tolerance in fall-sown cereals. There is indeed abundant genetic variation for this trait within the primary gene pool and an ever-expanding set of tools for genetic analysis, ranging from mapping populations to arrays (Hayes et al., 1993).

The ability of *Triticeae* species to survive low winter temperatures is a phenomenon entailing a number of factors, including freezing duration and severity, alternation of freeze and thaw periods, synthesis of toxic substances affecting recovery capacity, plant growth stage during the stress, and the duration of the hardening period prior to freezing temperatures. Because the traditional breeding strategies to improve frost resistance in winter cereals have been rather inefficient (Limin and Fowler 1993), increased attention has been given to understand the molecular genetic mechanisms that plants have evolved to tolerate this environmental stress (Pecchioni et al., 2002). In this view, studies undertaken to unravel the molecular basis of cold acclimation in
model plants and in crops have led on one hand to the isolation of many cold-regulated (COR) genes, and on the other hand to the identification of genomic regions which exert a major measurable effect on the tolerance, i.e. quantitative trait loci (Cattivelli et al., 2002).

Plant growth habit and heading date are the basic traits involved in the adaptation of cereals to environments since they allow the synchronization of the plant life cycle with seasonal changes. As shown in Figure 6 (from Cattivelli et al., 2002), a number of well defined loci is known to control the plant response to seasonal changes. The genetic factors determining the flowering time in barley can be divided, according to their interactions with environmental signals, into: photoperiod-responsive (Ppd-H) genes, vernalization-responsive (Vrn-H) genes, and ‘earliness per se’ (Eps-) or ‘early anthesis maturity’ (Eam-) genes largely independent of both day-length and low temperature. In barley, as in other members of the Triticeae, there is also genetic variation for growth habit. Winter growth habit is due to the requirement of an external signal to the plant to shift from vegetative to reproductive growth: this signal can be completion of a vernalization requirement (Limian and Fowler 2002) and/or daylength of sufficient duration (Karsai et al., 1999). The genetic basis of vernalization response in cultivated barley can be described by using a two loci (Vrn-H1 and Vrn-H2) epistatic model, whose candidate genes (HvBM5A and ZCCT-H) have been recently characterized (von Zitzewitz et al., 2005). The loci Vrn-H2 and Vrn-H1 are located on the long arm of chromosomes 4H, 5H respectively (Laurie et al., 1995).

Besides vernalization requirement, resistance to low temperature is necessary for overwintering genotypes grown in areas with
subzero winter temperatures. Maximum low-temperature tolerance is achieved after hardening, i.e. exposure to moderately low temperatures, and it is achieved at vegetative growth stages (Hayes et al., 1997). Frost tolerance is recognized as a complex quantitative character. The interrelationships of vernalization, photoperiod and low-temperature are most likely attributable to linkage rather than pleiotropy. In barley QTLs controlling traits associated with winterhardiness, such as field winter survival and crown fructan content, were mapped in the Dicktoo x Morex (winter x spring) cross only on the long arm of chromosome 5H (Figure 6) (Hayes et al., 1993; Pan et al., 1994). The authors found evidence for a multi-locus cluster of linked QTLs in this region rather than a single QTL with pleiotropic effects. No other genomic regions exceeded the threshold of significance.

From a molecular point of view, during the physiological processes of cold acclimation, a number of stress-related genes are up-regulated (Cattivelli et al., 2002). Among the barley COR (COld-Regulated) genes there are cor14b and tmc-ap3. Cor14b encodes a soluble protein of unknown function localized in the stroma compartment of the chloroplast (Crosatti et al., 1995), whereas tmc-ap3 encodes a putative channel protein of the chloroplast outer envelope selective for amino acids (Baldi et al., 1999). Likewise, the existence of genes regulating COR genes has been hypothesized. Besides, a notable advance in plant cold-tolerance research was the discovery of the CBF (C-repeat Binding Factor) family of genes. In Arabidopsis, these transcription factors have been shown to be key determinants of low-temperature tolerance (Thomashow et al., 2001).
Figure 6 (from Cattivelli et al., 2002): Summary of abiotic stress tolerance QTLs mapped on the homoeologous Triticeae chromosome groups. Summary map, chromosome length and map comparisons are based on the barley consensus map of Qi et al., (1996). Chromosome length scale (Kosambi cM) is reported on the left side. Group 1 and group 5 chromosome long arms are longer than originals (dashed) to allow mapping of telomeric loci. Centromere (C) positions have been calculated from the consensus map of Langridge et al., (1995). On the left side of the chromosomes small characters indicate RFLP and RAPD (underlined) marker loci; italics in larger fonts show the map position of known stress-related genes. Loci belonging to the consensus map are indicated by a line on the chromosome bars; the positions of all other loci have been calculated by bridging maps and weighting distances between common markers. Square brackets indicate loci whose map location is uncertain or contrasting with known locations of anchor RFLP loci reported on consensus maps. Round parentheses indicate synonymous markers and loci. Boxes on the right side of chromosomes indicate map intervals (length-weighted) where QTLs have been mapped by interval mapping procedures. Capitats indicate QTL peaks and most significant marker loci associated with QTLs: CT, cold tolerance; DT, drought tolerance; ST, salt tolerance; ABAQTL, ABA accumulation QTL. Earliness per se (eam), photoperiod response loci (Ppd), vernalization-responsive (Vrn), and frost tolerance (Fr) loci are reported. Different colors indicate the stress specificity of markers, loci, stress-related genes and QTLs: blue, cold; green, drought; orange, salt; black, none. Markers carrying an asterisk of a different color are ‘multiple stress’ markers tagging QTLs for tolerance to different stresses.

Genes with CBF signature sequences have been reported in the Triticeae and have been characterized in terms of their map location, coding sequence, and expression in barley (Choi et al., 2002; von Zitzewitz 2003).

The mapping of candidate genes, such as transcription factors, can reveal their genetic relationships with previously detected QTLs (Ishimaru et al., 2001).

In the Triticeae genome, the long arm of chromosome group 5 is the region most frequently associated with two traits critical for overwinter survival: low temperature tolerance and vernalization requirement (Hayes et al., 1993; Vagujfalvi et al., 2003; Francia et al., 2004; Reinheimer et al., 2004). In particular,
in barley, the position of the two major low-temperature tolerance QTLs, *Fr-H1* and *Fr-H2*, was reported in the ‘Nure’ (winter) x ‘Tremois’ (spring) (NxT cross) (Francia *et al.*, 2004). They are located, approximately 25 cM apart, on the long arm of chromosome 5H. The NxT *Fr-H2* QTL is orthologous to the frost tolerance QTL *Fr-Am2* found in diploid wheat (*Triticum monococcum* L.) by Vagujfalvi *et al.* (2003); in both species these QTL also cosegregate with COR gene product accumulation. Francia *et al.* (2004) showed that HvCBF4 is the peak marker of *Fr-H2* in the NxT population (Francia *et al.*, 2004 and 2007), and more recently, that five additional HvCBFs were shown to be tightly linked to HvCBF4 and *Fr-H2* (Tondelli *et al.* 2006). At the same position in the D x M barley population, Skinner *et al.* (2006) mapped eleven HvCBF genes into two clusters (approximately 1 cM apart), while at the homoeologous *Fr-Am2* region in *Triticum monococcum* Miller and co-workers identified eleven TmCBF genes (Miller *et al.*, 2006). The cluster of group 5 CBFs is currently the most likely set of candidate genes to explain cold tolerance at *Fr-2* in Triticeae (Tondelli *et al.*, 2006).

**Figure 7** (from Francia *et al.* 2004) Next page: QTL LOD plots of chromosome 5H obtained with CIM analysis of the indicated measures of low-temperature tolerance. The 3.0 LOD score threshold is indicated by a hatched line. Chromosome 5H is oriented with short arm at the left; distances are in Kosambi cM; COR and CBF genes, as well as relevant markers, are highlighted.
Chapter 1

Marker assisted selection

Plant breeding, in its conventional form, is based on phenotypic selection of superior genotypes within segregating progenies obtained from crosses. Application of this methodology often encountered difficulties related principally to genotype x environment (G x E) interactions. In addition, several phenotyping procedures are often expensive (i.e. for malting quality traits), time consuming or sometimes unreliable for particular traits (i.e. for some traits related to abiotic stress tolerance).
Molecular marker-assisted selection (MAS) is an approach that has been developed to avoid the problems connected with conventional plant breeding changing the selection criteria from selection of phenotypes towards selection of genes, either directly or indirectly. Molecular markers are clearly not environmentally regulated and are unaffected by the conditions in which the plants are grown and are detectable in all stages of plant growth. With the availability of an array of molecular markers and genetic maps, MAS has become possible both for traits governed by major genes as well as for quantitative trait loci (QTLs).

A successful application of molecular markers to assist breeding procedures rely on several factors: - a genetic map with tight molecular markers linked to the major gene(s) or QTLs of agronomic interest; - adequate recombinations between the markers associated to the trait(s) of interest and the rest of the genome and - the possibility of analyzing a large number of individuals in a time and cost effective manner.

The success of MAS also depends on the localization of the marker with respect to the target gene. In a first case, the molecular marker can be located directly within the gene of interest. This kind of relationship is clearly the most favorable and in most cases requires the availability of the target gene cloned. In a second case, the marker is genetically associated to the trait of interest. In this case lower is the genetic distance between the marker and the gene and more reliable is the application of the marker in MAS because only in few cases the selected marker allele will be separated from the desired trait by a recombination event. In a third case, the target gene(s) can be represented by one or more QTLs. In this case genomic regions to be selected
are often chromosome segments; it is therefore preferable either
to have two polymorphic markers flanking the target QTL, and/or
one or more markers within the QTL genomic region.

For plant breeders, the most useful application of MAS is to use
DNA-based markers for basically three purposes:

- tracing favorable allele(s) (dominant or recessive) across
generations; in order to accumulate favorable alleles,
- identifying the most suitable individuals among segregating
progenies, based on the allelic composition of a part or of
the entire genome and
- breaking the possible linkage of favorable alleles with
undesirable loci.

When the expression of a target trait is regulated by a single
gene, or by a gene responsible for a high percentage of the
phenotypic variance of the trait, the transfer of a single genomic
region from a donor to a recipient line can produce significant
trait improvement. MAS is now increasingly employed for
accelerating the recovery of the recurrent parent in backcross
(BC) programmes.

Compared with conventional backcrossing, the use of
molecular markers can improve the efficiency of BC breeding at
least in three ways: - for traits that are difficult to phenotype,
selection for a marker allele from the donor parent at a locus near
the target gene can increase the efficiency and accuracy of
selection; - markers can be used to select BC progeny with less
amount of donor parent germplasm in the genome outside the
target region and to select rare progenies resulting from
recombination near the target gene, thus minimizing the effects
of linkage drag and - in the transfer of recessive genes through
conventional breeding, additional selfing generations after every
backcross are required, leading to a procedure that is prohibitively low for most breeding purposes.

The probability of selecting superior genotypes is low for low-to-moderate heritability. In classical breeding, plant breeders cope with this problem by producing and testing progeny from numerous crosses, using low selection pressure, using replicate testing and testing advanced generations. Breeders selecting for low to moderate heritability traits have the following dilemmas:

- when the heritabilities of the traits to be selected are low or moderate and small samples of progeny are tested, the probability of selecting an outstanding genotype is very low;
- large numbers of progeny must be selected (low selection intensities must be used) to ensure the presence of one or more superior genotypes in the selected sample and
- even when low selection intensities are used, the most outstanding genotypes produced by a cross might not be present in the selected sample when heritability is low and samples are small.

MAS has therefore emerged as a strategy for increasing selection gains with respect to phenotypic selection alone and quantitative genetic theory suggests that the effectiveness of MAS is inversely proportional to the heritability of a given trait (Lande and Thompson, 1990; Knapp, 1998). Knapp (1998) developed a theory to estimate the probability of selecting one or more superior genotypes by MAS and defined a parameter to estimate the cost efficiency of MAS relative to phenotypic selection. Depending on the selection pressure, the genotypic superiority target and the trait heritability, it is estimated that a breeder using phenotypic selection must test 1.0 to 16.7 times more progeny than a breeder using MAS to be assured of
selecting one or more superior genotypes. Thus, MAS can substantially decrease the resources needed to accomplish a selection goal for a low to moderate heritability trait when the selection goal and the selection intensity are high. The parameter defined by Knapp (1998) predict that MAS is most efficient than phenotypic selection when breeders use high selection intensities and set high selection goals. Selection intensity can be increased to exclude inferior genotypes when the heritability is increased by using MAS.

**MAS for the improvement of quantitative traits**

Most of the traits of agronomic importance, such as yield, some classes of disease resistance genes, several abiotic stress tolerance genes and quality traits, are complex and regulated by several genes. Difficulties in manipulating these traits are derived from their genetic complexity, principally the number of genes involved, the interactions between genes (epistasis) and environment-dependent expression of genes. Quantitative traits often have a low heritability, with many QTLs segregating for the trait, each with small effect individually. The result is that effects of individual regions are not easily identified, and multiple genomic regions must be manipulated at the same time in order to have a significant impact. For this reason, replicates of field tests are required to characterize accurately the effects of QTLs and to evaluate their stability across environments. Although significant QTL effects should be detected across several environments, variation in expression due to QTL by environmental interactions (Q x E) remains a major constraint to the discovery of QTL that will confer a consistent advantage across a wide range of environments; on the other side the
identification of G x E as well as Q x E effects, it may permit identification of genotypes adapted to specific environments (Fox et al., 1997).

Given this complexity, integrated approaches are therefore required to increase the probability of an useful application of MAS for QTLs (Figure 5). In fact, despite the proliferation of QTL mapping works in recent years, a number of constraints have determined severe limitations on an efficient utilization of QTL mapping information in plant breeding through MAS (for a detailed review see Francia et al. 2005 and Collard et al. 2008). These constraints include the identification of major QTLs controlling the trait of interest; uncertainty of the QTL position, notably for those with a small effect (the confidence interval for QTLs location determined with current QTL analysis techniques sometimes is up to 30 cM for small populations); deficiencies in QTL analysis leading either to an overestimation or underestimation of the number and effects of QTLs; problems connected with the identification of QTL-marker associations applicable over different sets of breeding materials; possibility of loosing the target QTL during MAS through double-cross-overs between markers (this possibility is increasing with increased length of the marker interval analyzed); difficulty in precisely evaluating epistatic effects and difficulty in evaluating Q x E interactions.

Improved field designs (Gleeson, 1997) and statistical approaches for QTL can led to a better characterization of the target genes map position. In recently devised mathematical methods, such as composite interval mapping (CIM), field data from different environments can be integrated into a joint analysis to evaluate Q x E and thus identify QTLs that are stable across
environments (Jang and Zeng, 1995). Besides, with a detailed linkage map, CIM allow a better identification of linked QTLs (in coupling phase) from the same parental line. In addition, analysis methods have been proposed to accommodate QTL mapping data for the effects of G x E interactions (Crossa et al., 1999), of epistasis (Boer et al., 2002) and of G x E interactions and epistasis at the same time (Podlich et al., 2004). It is possible that such integrated approaches would better allow estimation of QTL effect for MAS application in breeding programs.

**Figure 5** (from Francia et al. 2005): An integrated approach for a possible exploitation of QTL data in MAS
The concept of the winter habit malting barley

In the recent years, winter barley acreage has increased in geographic area and a certain progress has been made to release winter malting varieties. Winter varieties that can survive winter would warrant, in the autumn sowing, a higher yield in respect to spring type cultivars, thanks to their longer growing cycle (Cattivelli et al., 1994); nevertheless, in Central and Northern Europe the spring-sowing of malting barley is the most common practice (Pržulj et al. 1998). There are currently some winter malting varieties recommended for 2007 by the UK Home-Grown Cereals Authority (HGCA; http://www.hgca.com/), known as ‘Flagon’, ‘Pearl’ and ‘Cassata’ (all two-rowed). In spite of this, no winter malting varieties have been recommended up to now by the American Malting Barley Association (AMBA) in America (http://AMBAinc.org/). Since the first report of Schildbach (1990), that indicated winter barleys as generally of lower malting quality, it is still debated if presently available winter malting lines are really of equal quality than spring ones (Spunar et al., 2000).

Moreover, few studies have been done on the level of frost tolerance of malting barley genotypes. It has been recently reported the development of a new barley map derived from a two-rowed cross ‘winter’ x ‘spring’ (Francia et al. 2004). The winter parent ‘Nure’ is a frost tolerant feed cultivar, whereas the spring parent ‘Tremois’ is a frost susceptible malting variety. The genetic map built on the ‘Nure’ x ‘Tremois’ (NxT) doubled-haploid (DH) is currently the only one where malting quality traits were expected to segregate together with winterhardiness and vernalization requirement, and this represented a valuable tool
to investigate in a unique genetic system with multiple agronomical traits, such as malting quality and tolerance to abiotic stresses.

By using the DH population NxT, the objectives were to study the genetic basis of the quantitative variation observed for malting quality, to validate the genetic determinants of Fr-H1 and Fr-H2 and to identify a molecular marker set to select winter malting barley.
Introduction

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