5

General discussion

Malting quality and winterhardiness in barley: genetic approaches

Winterhardiness and malting quality are the consequence of a number of complex and interacting component characters. In particular winterhardiness in cereals is the final expression of a number of interacting component traits, including low temperature tolerance, growth habit, photoperiod response, and crown fructan content (Hayes *et al.*, 1996). On the other hand, malting quality depends from malt extract content, malt friability, wort viscosity, acrospire growth index, α- and β-amylase activity, diastatic power, malt β-glucan content, malt β-glucanase activity, grain protein content, kernel plumpness, and dormancy . These are all quantitative traits variously influenced by the environment and putatively based on many genes. Classical molecular approaches have generated a strong basis of genetic information in barley. However, modern genomics approaches have now begun to expand this knowledge base significantly. Maps of the barley genome are now starting to be complemented by data generated by genomic analysis. The sequence of the *Arabidopsis* and more specifically the rice genome (Goff *et al.*, 2002) provide a significant base of data for barley research. The volume of barley sequence data continues to grow rapidly. A complete genome sequence for barley is unlikely to be completed in the very near future but gene sequences and the sequences of gene rich regions are rapidly becoming available. To reach the goal of a genomic sequence of barley it was recently decided to form the International. Barley Genome Sequencing Consortium (IBSC, http://barleygenome.org/). Sequencing of cDNA libraries from

many barley tissues has generated large amounts of EST data with barley-specific sequences. A significant proportion of the barley genome is now covered by EST data. These cDNA clones, together with the informations derived, have been used to produce microarrays, allowing detailed analysis of patterns of gene expression. Barley transformation provides another important tool for analysis of the molecular basis of quantitative traits: modulation of expression of candidate genes in transgenic barley allows testing of their role.

The genetic material derived from the ‘Nure’ (winter) x ‘Tremois’ (spring) cross underscores the advantage of having different agronomic traits segregating in a single population, e.g. yield stability in droughted environments, malting quality and beta-glucan content (this work: Laidò *et al.*, submitted), vernalization requirement (von-Zitzewitz *et al.*, 2005) and low-temperature tolerance (Francia *et al.*, 2004 and 2007).

The present work of mapping malting quality QTLs (Laidò *et al.*, submitted) is the first one until now made on a 'winter x spring' segregating population. As it was discussed in Chapter 2, QTL analyses of the malting quality traits GPC, grain β-glucan, friability, acrospire growth, wort viscosity and HWE revealed two very important regions in the genome. In the first region on chromosome 1H nine major malting quality loci in clusters have been mapped and, among these, eight loci showed the allelic contribution of the parent ‘Tremois’. The two QTL clusters with highest LOD, localized in the intervals Bmac0399-Bmag0211 (grain β-glucan, wort viscosity and acrospire growth) and Bmag0211-Bmac0032 (friability and HWE) are colinear with two

genomic regions described in the QTL consensus mapping work of Fox *et al.,* (2003).

The long arm of chromosome 5H in the 'Nure' x 'Tremois' cross in particular in two regions between markers HvCBF8 and HvCBF4 and Hv635P2.4-E38M50-215 is very important for malting quality, vernalization requirement and frost tolerance. Two malting quality loci (HWE and wort viscosity) have been mapped: the first is a HWE QTL that is colinear with a GPC QTL mapped in the 'Steptoe' x 'Morex' population by Hayes *et al.* (1993) and the second is a wort viscosity QTL corresponds with a α-amylase activity QTL mapped by Hayes *et al.* (1993). It is notherworty that in the same region of this wort viscosity locus two putative candidate genes (CesA2 and A3) involved in cellulose synthesis (Burton *et al.*, 2004) have been identified. Francia *et al.* (2004) have identified in these regions two major quantitative trait loci for low-temperature tolerance, which determine frost tolerance in the 'Nure' x 'Tremois' cross. The *Fr-H2* locus (proximal) is bracketed by HvCBF4 and OPA17a, whereas the *Fr-H1* locus (distal) is bracketed by dhn1 and Hv635P2.4, with rather balanced and almost completely additive effects. In this view, the phenotypic effects of these loci controlling frost resistance in barley (see Chapter 4 - Table 1 and Figure 2) has been independently validated in F_2-derived F_3 families. Since the difference in Fv/Fm between reciprocal classes of F_3 families can be considered as a rough measure of the allelic substitution effect at *Fr-H1* and *Fr-H2*, present data suggest an additive effect for the two Fr-H genes in the 'Nure' x 'Tremois' system, although incomplete. The large population of 1,849 NxT recombinants developed in this study not only represents a useful advance towards isolating the genetic

determinants of the Fr-H loci, but is also a valuable tool to study their genetic and molecular interactions.

Mapping Bmag0223, MWG583, HvCBF4B, and HvMYB1 in the population of 1,849 F2 individuals, allowed us to refine the genetic distances around *Fr-H2*. In particular, adding the new CAPS marker for MWG583, the resolution of the *Fr-H2* region improved, now targeting a 14.6 cM interval (see Chapter 4 - Figure 3c). Subsequently, to determine the genetic order of the six HvCBF genes polymorphic between 'Nure' and 'Tremois' at *Fr-H2*, a high-resolution genetic map of the HvCBF cluster on 5H was generated. The large segregating population of 1,849 individuals was screened for recombinants between each of the six HvCBF genes. This allowed us to produce the fine map (see Chapter 4 - Figure 3c) in which the HvCBF cluster spans in barley a total genetic distance of 0.81 cM, with the largest distance between Hv*CBF4B* and Hv*CBF12* (0.32 cM, 12 recombinants), and the shortest recombination interval between Hv*CBF12* and Hv*CBF13* (0.03 cM, 1 recombinant). The same large F2 population could be used for the fine mapping of any of the malting quality QTLs, since it was produced from the 'Nure' and 'Tremois' parentals.

In barley, as in other members of the *Triticeae*, 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 and daylength of sufficient duration (Dubcovsky *et al.*, 2006). The 'Nure' x 'Tremois' population a valuable genetic system to study vernalization requirement because it is segreganting at both the

Vrn-H1 and *Vrn*-H2 loci. Vernalization is a period ranging from about one to eight weeks of cold-temperature exposure required to induce reproductive development during a normal, annual growing season life cycle (Takahashi and Yasuda 1971). The underlying genetic effects have been reported as QTLs because they show complex, rather than Mendelian, inheritance. Francia *et al.* (2004) have identified only one QTL for vernalization requirement, and it was on chromosome 5H in a region corresponding with the inferred position of *Vrn-H1*. The cold tolerance *Fr-H1* QTL is coincident with *Vrn-H1* (Hayes *et al.*, 1993; Laurie *et al.*, 1995; Francia *et al.*, 2004). Limin and Fowler (2006) hypothesized a direct role of *Vrn-A1* locus in frost tolerance of winter type wheats, however results of Sutka *et al.* (1999) indicate that *Vrn-A1* and *Fr-A1* are physically separated. In barley, and in particular in the 'model' population ‘Nure’ x ‘Tremois’, it remains to be determined whether linkage or pleiotropic effects of *Vrn-H1* are the molecular basis behind *Fr-H1*. According to von Zitzewitz *et al.* (2005) 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 (Hv*BM5A* and *ZCCT-H*) have been recently characterized. On the bases of this genetic model, all the lines carried a ‘Nure’ allele at *Vrn-H1* locus have classified as ‘facultative’ and ‘winter’, depending on allele at *Vrn-H2* candidate gene (*ZCCT-H)*.

Marker-assisted selection for winter growth habit, frost tolerance and high malting quality in barley

The recent progress in the area of plant molecular biology and plant genomics have the potential to initiate a new Green revolution. However, these discoveries need to be implemented in new cultivars to realize that potential. Some potential limitations of MAS strategies have been highlighted for both simply and complex inherited traits. A cautious optimism has been expressed about MAS of complex traits. Although molecular markers have been successfully associated with QTLs, these associations have actually demonstrated limited usefulness in plant breeding programs. Complex traits are the most difficult to handle during a breeding program, but are responsible for most breeding progress in critical traits such as yield, malting quality and cold tolerance.

In the present thesis, marker-assisted selection was conducted among progeny from the same cross for which the frost tolerance and malting quality QTLs have been mapped. This study confirm that the spring, malting barley variety ‘Tremois’ carries desirable QTL for malting quality, but also indicates that favorable malting alleles may be found in germplasm not conventionally used in malting barley breeding programme like the winter type variety ‘Nure’. The results of the present work indicate that by using the molecular markers set identified in this work, the selection for winter, frost tolerance and high malting quality lines is possible. At least in the genetic material derived from the ‘Nure’ x ‘Tremois’ cross. Marker-assisted selection may be an effective way to introduce the winter growth habit, frost tolerance and malting quality alleles

into elite germplasm. The introduction of winter growth habit, frost tolerance and malting quality alleles using advanced backcrossing with 'Nure' as donor parents it should then be possible because the malting quality QTLs mapped on chromosome 5H are partially coincident with the two major low-temperature tolerance QTLs, *Fr-H2* and *Fr-H1*, and *Fr-H1* locus is coincident with *VrnH1*. Therefore it is possible with the MAS following *Fr-H2*, *Fr-H1/Vrn-H1*, *Vrn-H2* to select winter, frost tolerance lines. The partially coincidence of the malting quality QTLs mapped on chromosome 5H with the frost tolerance loci *Fr-H2* and *Fr-H1/VrnH1* mapped in the NxT population (Francia *et al.*, 2004), with alleles in *cis*, is an useful result for the development of winter, frost tolerance and high malting quality lines. In fact as expected, all the selected lines with superior malting aptitude (BNT 155, BNT 196 and BNT 221) kept the 'Nure' allele at the *Fr-H1/VrnH1* frost tolerance locus. These lines have been analysed at the *Vrn-H2* locus on chromosome 4H. As a consequence, the BNT 196 and BNT 221 lines were classified as winter lines while the BNT 155 was classified as facultative. The use of marker information in selection does not eliminate the need to gather reliable phenotypic data but it should permit breeders to allocate resources to the evaluation of smaller numbers of progenies that are more likely to carry favorable alleles at interest loci. The molecular markers set identified has resulted efficient to select by means of MAS superior barleys. Further studies are necessary to understand which additional alleles among the ten molecular markers are present in other varieties, to extend their use in other 'winter' x 'spring' combination cross.

In the immediate future, the key for the efficiency of MAS in large breeding populations will depend from the implementation and integration of different critical points. The first crucial point is the availability of cost-efficient and high-throughput genotyping methods. A variety of high-throughput genotyping technologies are just becoming sufficiently inexpensive to allow their use in plant breeding (Rafalski, 2002). A new generation of molecular markers based on the detection of SNPs promises high-throughput assays at relatively low costs, along with the potential for high levels of multiplexing. Implementation of this multiplexing technology in plant improvement strategies can provide cost-effective tools for selection of multiple traits in breeding populations.

A second crucial point will be the exploitiation of the highest number of information, derived from comparative genetic maps, and from genomic regions conferring positive traits across syntenic species. These might be directly applied across species, after validation, for the improvement of the different traits. Comparative genetic maps show that chromosomal segment structure (orthology or conserved synteny) and marker order (colinearity) are conserved across plant species over substantial evolutionary distances (Paterson *et al.*, 2000). When genetic mapping in collinear genomes pinpoints similar traits to the same chromosomal regions, there is a good reason to suspect that these loci are encoded by orthologous genes. Even in the absence of an orthologous candidate gene, the information from the model plant of the family (e.g. rice for the grass family and tomato for the nightshade family) can be applied to increase the marker saturation of a defined chromosome region, as it may be required for the identification

of markers suitable for MAS or for the fine mapping of a gene for positional cloning.

A third important point is represented by the capacity of exploitation of the increasing amount of information that are publicly available, both genomics and phenomics. Recent large-scale sequencing projects have produced a large amount of single-pass sequences of cDNAs from different plant species. Because SSRs and SNPs based markers can be obtained quite easily from ESTs (Morgante *et al.*, 2002; Rafalski, 2002), the development of molecular markers has recently shifted from anonymous DNA fragments to genes. Transcription-based genetic maps have thus been obtained from different crop species including wheat (Gao *et al.*, 2004), barley (Graner *et al.*, 2004) and rice (Wu *et al.*, 2002). The availability of marker-dense transcriptional maps has two important implications for the improvement of complex traits in plant breeding. As a first use, they can contribute candidate genes during the mapping of QTLs. Also for the tools directly useful for MAS, publicly available information are increasing.

As a fourth point it has to be mentioned that the availability of comprehensive cDNA and oligoncleotide arrays is now providing an option for the development of functional genomics-based strategies for the investigation of quantitatively inherited traits, using at least two strategies. The first is a functional association strategy, namely a strategy of 'genetical genomics'. A cDNA array can reveal that gene expression within a given tissue varies between genotypes differing for a given trait. Genetic mapping of identified candidate genes can then reveal congruency between the map position of the candidate gene and the presence of a QTL (Graner *et al.*, 2004). The second

strategy allow the identification of QTLs by a methodology called as eXtreme Array Mapping (XAM). This method can estimate the differences in allele frequency between pools of lines, selected for extreme phenotypes, by hybridization of total genomic DNA to a GeneChips (Wolyn *et al.*, 2004). Thus, because different approaches are improving the strategies on which MAS rely on, an increased complementarity between molecular technologies and conventional breeding is expected in the near future for a more efficient improvement of the crop plants.

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