A genome based approach to improving barley for the malting and distilling industries

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Background Up to 60% of the Scottish barley crop is used in malting for brewing and distilling. The distilling industry alone uses some 500,000 tonnes per annum, with the total malt purchases in Scotland exceeding 800,000 tonnes. Scotch Whisky is the fifth largest British export and the leading food and drink commodity, earning over £2 billion per annum. Malt whisky can only be made from malted barley and is at the premium end of the market. High spirit yield is probably the main quality requirement of the malt whisky distilling industry, because a 1% increase in spirit yield would lead to a saving of approximately £1.1 million in distilling production costs. Spirit yield is the product of hot water extract (i.e. the total soluble component following malting) and the fermentability of the extract, since not all solubilised components are fermentable. The peak level of fermentability is achieved earlier in the malting process than the peak level of extract, and malting has to be optimised to produce the maximum spirit yield. A further requirement in some malt whisky distilleries is for varieties that do not produce epi-heterodendrin (EPH), a glycosidic nitrile occurring in germinating barley. A breakdown product of EPH can react with ethanol, catalysed by copper in stills, to produce the putative carcinogen ethyl carbamate (urethane).

The development of genetic finger-printing techniques in human genetics has led to applications of the various types of molecular markers, especially in the rapid creation of genetic maps of an organism. The advantage of such maps is that regions controlling complex characters, such as malting quality, can be identified as Quantitative Trait Loci (QTL). This knowledge can then be applied in a targeted manner to improve plant characters for a specific end-user need. As noted above, fermentability is a key character for the distilling industry but its analysis is difficult to carry out in plant breeding and genetical studies. It is, however, an ideal character for exploiting molecular marker methods in plant breeding. Within a MAFF funded Agri-Food LINK project, the most significant QTL that we identified from the Derkado x B83-12/21/5 mapping population accounted for around 6% of the phenotypic variation in the character. The Derkado QTL allele reduced fermentability by just under 0.5% and off-setting this decrease would give an extra 3 litres of spirit yield per tonne. Applied over the whole malt whisky industry, this would translate into an extra production of 1 million bottles annually from the same quantity of malted barley.

On the basis of their phenotypic performance, we used two lines from the mapping population as donors of high fermentability to initiate a programme to produce first backcross (BC1) inbred lines. Our initial strategy was to use marker-assisted selection to transfer the QTL enhancing fermentability into a Landlord genetic background, as it was a promising new cultivar but

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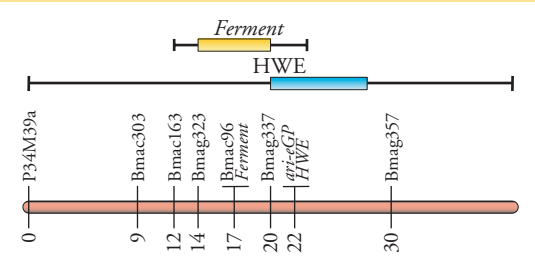

Figure 1 Detailed map of target region of 5H showing location of fermentability and hot water extract QTLs, their 1 LOD confidence intervals (bars) and regions over which a significant effect was detected (lines).

with scope to improve its fermentability. It became apparent, however, that the QTL was linked in coupling with the *ari-e*GP dwarfing gene on chromosome 5H. An increase in the minimum sieving size in trading Scottish grain meant that cultivars with this dwarfing gene were no longer commercially viable as the gene was associated with small grain. Furthermore, we found that the fermentability QTL was also linked in repulsion to a hot water extract QTL at the *ari-e* locus (Fig. 1).

The development of the breeding population is outlined in Figure 2. The donors both carried B83-12/21/5 alleles at all loci within the target region, so it

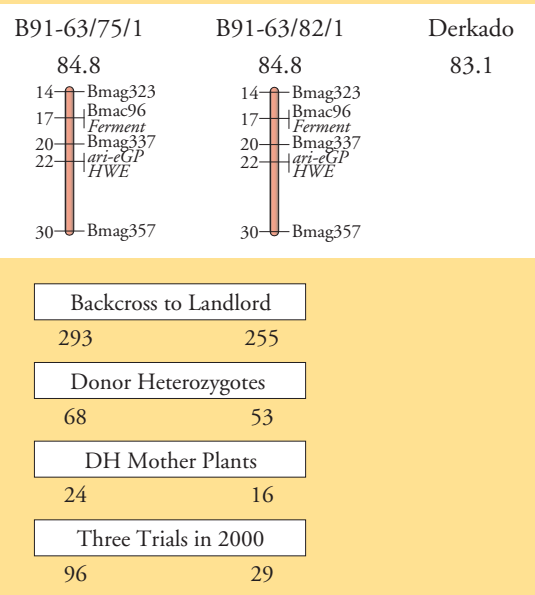

Figure 2 Derivation of Population used to test the effect of the target fermentability QTL in another genetic background. Values under the genotypes are the mean % fermentabilities from four trials conducted from 1995-7.

was necessary to generate recombinants between the fermentability QTL and *ari-e* to develop lines that had high levels of fermentability and extract, and were also agronomically suitable for Scotland. There were not the resources within the project to generate a large enough population with sufficient recombinants from which we could hope to select a line with commercial potential. We therefore changed our strategy to a more random one by testing all the BC1DH lines that we developed. This strategy would have the added bonus of enabling further genome-wide testing of the location of regions controlling fermentability. We therefore tested 125 BC1DH lines in large plot ($7m^2$) trials at commercial density, with and without fungicide at SCRI, and fungicide treated trials near Sleaford and Docking, in 2000. The plots were harvested with a small plot combine and their yields recorded. Cleaned and sieved samples from all the plots, except those from the Sleaford site, were retained for analysis of the malting quality characters, hot water extract and fermentability.

Results

Overall, the mean yield of the BC1DH lines was less than that of Landlord, which may reflect the presence of both the *ari-e*GP and *sdw1* dwarfing genes in a number of lines. Whilst the minimum yielding line from the BC1DH population was significantly lower than Landlord, the highest yielding line was not significantly greater. Despite delaying malting until dormancy had been broken, the samples from the SCRI trials generally malted poorly with very low extracts, leading to abnormal overall mean values for many of the malting characters. Landlord itself performed

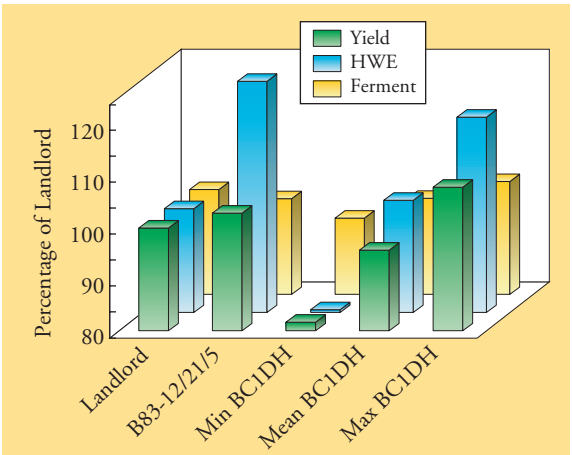

Figure 3 Summary of the BC1DH population for Yield, Extract and Fermentability from performance in trials grown in the year 2000.

poorly as it and many other samples were under-modified. In contrast, the parents of the donors, Derkado and B83-12/21/5, malted normally and thus B83-12/21/5 was significantly better than Landlord, even though it was only of moderate malting quality. The lines with the lowest extract and fermentability were both significantly worse than Landlord and, whilst there were lines that were higher than Landlord, only the extract difference was significant (Fig. 3). The means of the BC1DH lines from the two SCRI sites show that extract was very low. If the lines were under-modified, one might expect a higher mean fermentability. The fact that fermentability is also relatively low indicates the presence of significant amounts of unfermentable material, under which circumstances one might not detect many differences in fermentability. Fermentabilities from the Docking results were also low but samples were more typical of commercial malts, despite some evidence of over-modification.

We found that germinations of Landlord and Chariot, a parent of Landlord, were low and concluded that some environmental factors present at SCRI in 2000 induced some genotypic differences in germination, leading to poor micro-malting performance. A water sensitivity test of some of the samples from the SCRI trial treated with fungicide showed that germination was still very poor some 10 months after harvest and gave a correlation of >0.9 with hot water extract. Both Landlord and Chariot have been found to be very susceptible to *Ramularia* infection but there is, as yet, no evidence that the disease inhibits germination. The results do highlight the problem of using a backcrossing strategy to meet a commercial target. The choice of a newly recommended cultivar as the recipient parent for the major part of our backcrossing programme was correct as, if successful, the cultivar would still be relevant at the time of release of the backcross line. The problem is that malting and agronomic information about such cultivars is limited, despite a large amount of yield trial data, and problems only become apparent when the cultivar is grown on a large scale. A safer system would be an adaptive backcrossing scheme in which one changed the parent at each stage. Previously mapped SSRs would be of great advantage in genotyping such a population, as one would have a good chance of separating out not only the donor alleles but also the different recipient alleles, due to their multi-allelic nature.

The genetic fingerprints of the BC1DH lines entered into trials were established by surveying them with 44 previously mapped Simple Sequence Repeat (SSR) markers, which were selected to sample the whole barley genome as well as the target QTL. In addition, allelic differences at the *sdw1* and *ari-e*GP loci were established from observations of the juvenile growth habits of the plots. We wished to detect whether or not the donor QTL chromosomal segment altered the expression of fermentability in the recipient. We coded all the genotypic data as being either donor or recipient in origin and compared the means of the different genotypes observed in the target region. We could then classify all 125 lines as having either donor or recipient alleles at the fermentability and extract QTLs on chromosome 5H. We predicted, therefore, that donor alleles at the fermentability QTL should result in an increase in the character, whereas donor alleles at the extract QTL should decrease extract.

The effect of the donor segment can be seen in the summaries of results presented in Table 1. Donor alleles at both the fermentability and extract QTLs have very similar effects upon the characters that were measured on the 2000 trials. The similarity of the response is to be expected as the two QTLs are closely linked. The results are generally consistent for each character as well. As expected, donor alleles significantly decreased yield and extract. The differences were more pronounced at the SCRI sites but the poor malting performance of samples in these trials may have exacerbated the differences in extract. Allelic differences at the fermentability QTL did show an increase in the character associated with the donor alleles from the results from the Docking site but the effect was not significant. Donor alleles at the HWE QTL not only significantly reduced extract but also significantly increased fermentability, and the same

Site	Differences at 5H QTL	Yield	Ferment	HWE
Docking	Ferment	**-4.0**	0.7	**-1.2**
Docking	HWE	**-3.1**	**1.1**	**-1.6**
SCRI-F	Ferment	**-12.2**	0.1	**-8.1**
SCRI-F	HWE	**-12.0**	0.5	**-9.6**
SCRI+F	Ferment	**-12.0**	-0.7	**-11.2**
SCRI+F	HWE	**-11.9**	-0.5	**-12.7**

Table 1 Differences between means of BC1DH lines grown in three trials in the year 2000 and classified according to whether they possessed donor or recipient alleles at the fermentability and HWE QTLs on chromosome 5H. Data are expressed as the donor minus recipient means expressed as a percentage of the recipient and those in bold type are significantly different.

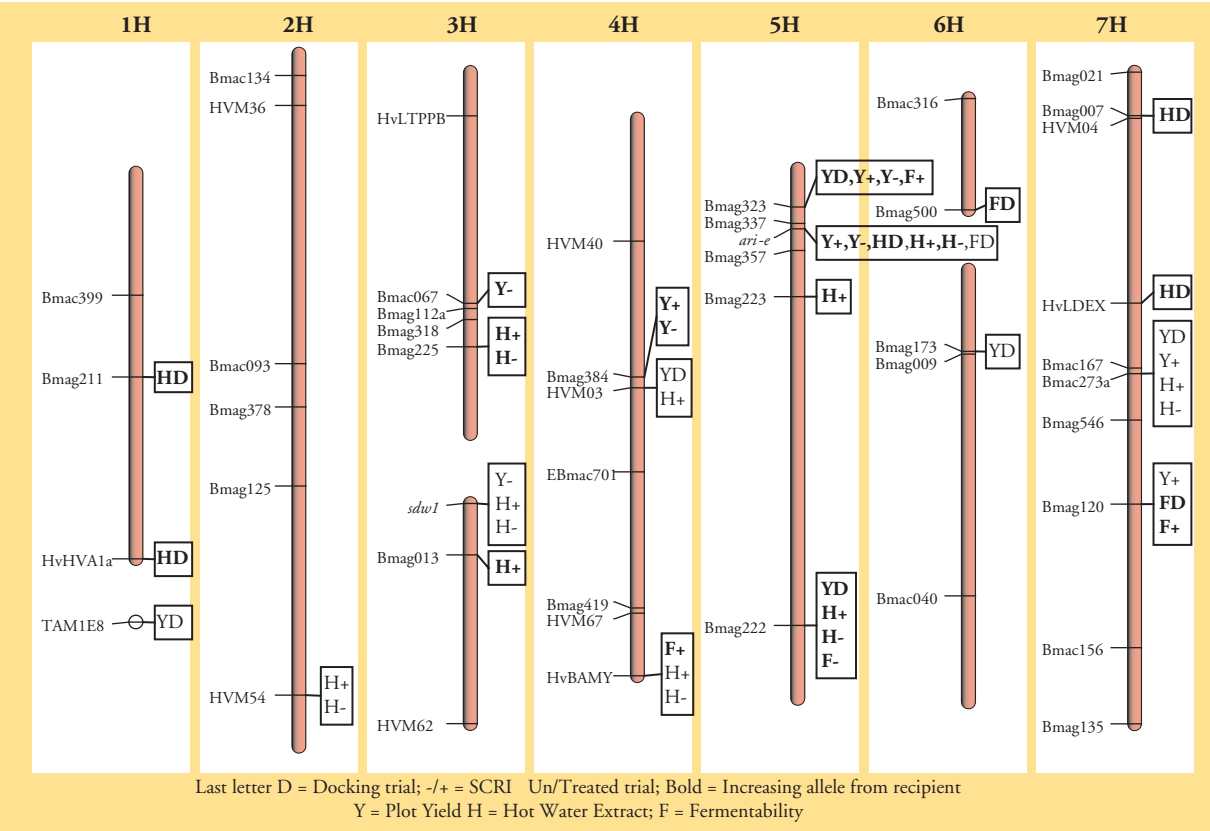

Figure 4 Location of SSR and morphological markers in relation to the genetic map of the mapping population together with significant marker associations detected by multiple regression analysis of data collected from the year 2000 trials of BC1DH lines.

pattern can be seen in the results from the SCRI untreated trial, although the increase was not quite significant (P=0.06). In the SCRI treated trial, donor alleles apparently reduced fermentability but this difference was not significant and could be an artefact of poorer malting performance compared to the untreated trial. The general effect appears to be that the fermentability and extract QTLs are more closely associated than was apparent from the mapping study but we would need more recombinants and malting results from Scottish trials to verify this finding.

We also used forward stepwise multiple regression analysis to identify markers that acted together in statistically significant associations with yield, extract and fermentability. Applying this analysis to the phenotypic and genotypic data collected on the BC1DH means from each of the SCRI and the Docking trials, revealed a number of significant associations. Each character at each site was associated with at least one marker and there were 12 cases where results agreed between at least two of the trials and more confidence can be attached to them. Seven of these 12 marker / trait associations involved extract, with donor alleles at four associated with an increase. Another four of the 12 marker / trait associations where results agreed between two or more sites were for yield, with donor alleles producing a significant increase at one. For fermentability, results were in agreement at just one locus, Bmag120 on 7H with donor alleles significantly decreasing the character. Whilst donor alleles at *ari-e* were significantly associated with an increase in fermentability from results obtained from the Docking site, donor alleles at a nearby locus (Bmag323) were significantly associated with a decrease in fermentability at the SCRI treated site in 2000. No significant associations of fermentability with markers in the target region were detected from the results of the untreated trial at SCRI by either multiple or single marker regression. Donor alleles at *ari-e* did produce an increase in fermentability but the effect was far from significant.

Conclusions and end-user relevance

The one trial that malted normally was grown outside the target environment but did produce evidence of

increased fermentability due to the presence of donor alleles in the target region, and there was some indication of corroborating evidence from the untreated trial grown at SCRI. The data did indicate, however, that the fermentability QTL might be associated with the *ari-e*GP dwarfing gene. Further work is necessary to establish whether or not this is so, as deleterious effects of the dwarfing gene, such as high screenings and reduced extract, mean that it is no longer viable in a commercial cultivar.

Other evidence revealed that fermentability was controlled by a number of genes, each of small effect, and highly subject to modification by the environment. Detecting this QTL in another genetic background requires most of the other increasing loci to be present and using marker assisted selection for just the target QTL means that many of the other increasing alleles are eliminated by chance. This is not just a problem for the current project, but also applies to other characters of low heritability with a number of controlling genes. In such cases, there is no alternative but to generate large populations, use marker-assisted selection to form a pool of 'improved' lines and rely on phenotypic selection to pick out the best lines.

Whilst the cultivar Golden Promise carried the *ari-e*GP gene and was used in great quantitities by maltsters and distillers, it was never regarded as a top-class malting quality cultivar. The fermentability QTL studied in this project either represents the action of an anonymous gene or *ari-e*GP. There is evidence that the gamma-ray mutation of Maythorpe to produce Golden Promise resulted in an increased rate of modification. Such a gene, taken from a moderate malting background, may lead to excessive modification in a good malting quality background and this is a possible weakness of the anonymous approach used in the current project.

There is opportunity to manipulate natural variation in fermentability, however, and the targeting of specific genes of known function may well be a better means of improving barley for use in distilling in the short term. For instance, natural variants of β-amylase with improved stability at extraction temperatures may improve fermentability. As starch breakdown continues into the fermentation stage in a distillery, enhancing the release of the enzyme limit dextrinase during fermentation would be another potential approach that increases fermentability. Putting natural variants of these two enzyme systems together may well provide a further means of improvement. Results from functional genomics programmes could provide better overall understanding of the genetics of complex traits such as malting quality and eliminate some of the problems associated with the single gene approach that we adopted within this project. With functional genomics, one can attempt to establish how various candidate genes interact to produce a given phenotype. After gathering such information from a range of cultivars and associating it with malting quality data, it will be possible to identify targets to manipulate in order to improve performance for specific malting attributes.