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A GENOME-BASED APPROACH TO IMPROVING BARLEY FOR THE MALTING AND DISTILLING INDUSTRIES

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A GENOME-BASED APPROACH TO IMPROVING BARLEY FOR THE MALTING AND DISTILLING INDUSTRIES

by

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This is the final report for a 54-month research project, which started in July 1997. The work was funded under the Ministry of Agriculture, Fisheries and Food **Agro-Food Quality** LINK scheme. The total cost of the project was £483,029 with each organisation contributing the following amounts MAFF (£181,237 - project AFQ109), Advanta Seeds (£47,009), HGCA (£150,000 - project 1573), Mylnefield Research Services Ltd (£22,049), Scotch Whisky Research Institute (£82,734).

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Abstract

The distilling industry utilises around 25% of the UK malting barley crop, equivalent to over 60% of the Scottish malting barley crop. Scotch Whisky is by far the leading export in the Food and Drink sector and is currently the UK's fifth highest export earner. Whilst the distilling industry purchases specified barley cultivars, little or no testing for specific distilling requirements is carried out by breeders or the testing authorities. Distillers therefore have little knowledge about the quality of newly recommended cultivars other than their extract levels. The major requirement of the distilling industry is to produce the maximum amount of spirit per tonne of malt as efficiently as possible. Not all of the components extracted during mashing from a malt are fermentable by yeast so spirit yield is the product of extract and its fermentability. Little was known about fermentability and its relationship to other malting quality characters so the aims of this project were to: understand the genetical and environmental control of fermentability and its relationship to spirit yield and other characters; identify genetic markers that could be used to select for the character without the need for expensive malting quality and fermentability assays; and initiate a programme to produce barley lines for specific use in the distilling industry.

We clearly showed that while fermentability was genetically controlled, it was also liable to be affected by environmental variation. Some fermentability genes were inversely related to some affecting extract so that increasing fermentability without careful selection could actually reduce extract and, therefore, spirit yield. We identified regions of barley chromosomes responsible for the genetic control of fermentability and a range of other malting quality characters. In doing so, we also identified genetic markers that could be used to indirectly select for these characteristics. Plant breeders could use such markers to eliminate poor malting quality lines before initiating an expensive trialling and testing scheme. An example of this was a marker linked to a gene controlling non-production of epi-heterodendrin, a characteristic required by some distilleries and found in cultivars such as Maresi, Delibes, Derkado and Decanter. This was a single major gene and the marker was found to be effective in discriminating between producers and non-producers of epiheterodendrin. Fermentability was a more complex character and a number of genes were found to be controlling the character. Markers were used to select for one gene found to have a major influence on fermentability but this was of limited effectiveness in the absence of selection for the other genes. The gene could also have a different effect when the genetic background is changed. Nevertheless, we were able to develop some lines of potential commercial value within the project and also identify more specific targets that are highly likely to lead to improved barley cultivars for use in the distilling industry.

Summary Report

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 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. Under certain conditions a breakdown product of epi-heterodendrin, a glycosidic nitrile produced in germinating barley, can react with ethanol, catalysed by copper in stills, to produce the putative carcinogen ethyl carbamate (urethane). Barley cultivars that do not produce epi-heterodendrin are essential in grain whisky distilling and also in some malt whisky distilleries.

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. Fermentability is, as noted above, 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 for a specific end-user requirement and is the basis for the project being reported here. The aims of the project were:

1. Determine the genetic control of fermentability and spirit yield and their relationships to other characters
2. Identify molecular markers linked to genes controlling fermentability, hot water extract and spirit yield
3. Combine genes for high spirit yield in a spring barley genotype suitable for Northern Britain.

We collected genetic marker and malting quality data from a spring barley population of random inbred lines that was constructed from a cross between commercially relevant parents. The parents did, however, exhibit contrasting combinations of fermentability and hot water extract and so maximised our chances of revealing QTLs for the former that could be used to improve spirit yield.

Materials & Methods

Mapping Population

Random inbred lines were produced by doubled haploidy from the F1 of a spring barley cross between the genotypes Derkado and B83-12/21/5 to achieve the first two objectives of the project. Derkado had good malting quality and was one of the main Scottish cultivars in the 1990's, principally because it was a non-producer of epi-heterodendrin, and B83-12/21/5 was a breeding line from the Scottish Crop Research

Institute (SCRI). The whole population, together with the parents and some controls, was grown in trials at SCRI from 1995 to 1997 inclusive and at a site near Sleaford, Lincs, UK in 1996 and 1997. Each trial was sown in plots at a normal commercial density, received a typical fertiliser regime, and was kept free of foliar pathogens by the application of fungicides. The fraction passing over a 2.5mm sieve from each plot was retained for phenotypic analysis of quality characters. Adverse weather conditions delayed harvest of the 1997 trial near Sleaford and there was considerable pre-harvest sprouting in a number of samples. No malting analyses were therefore carried out on this trial, leaving four trials for analysis.

A large number of molecular markers, the two major dwarfing genes *sdw1* and *ari-e*GP, the mildew resistance gene *mlo*, and a gene controlling the non-production of epi-heterodendrin (*eph*) were used to generate a genetic map that covered most of the barley genome. A range of malting quality characters was also scored on the population, namely hot water extract, fermentability, predicted spirit yield (PSY), grain nitrogen content, soluble nitrogen content of the malt, soluble nitrogen ratio, grain β-Glucan content and quantitative production of epi-heterodendrin. In addition, malt samples from a subset of lines from two trials were used to estimate the wort contents of the sugars glucose, sucrose, maltose and malto-triose. These data were used to study the genetics of each character (Objective 1) and, when combined with the marker data, to identify QTLs controlling each character (Objective 2).

Breeding Population

We developed a breeding population for the third objective of the project. We selected 8 lines, on the basis of their phenotypic performance, as donors of high fermentability to initiate a progamme to produce first backcross (BC1) inbred lines. The cultivar Landlord and two SCRI breeding lines, B91-47/22 and B91-99/15, were chosen as recipient parents, as there was scope to improve the fermentability of each. We found, however, that our target QTL was closely linked in coupling with the *ari-e*GP dwarfing gene and in repulsion to a hot water extract QTL at the *ari-e* locus. We would therefore need to produce recombinants between the fermentability QTL and the dwarfing gene to develop a successful cultivar within the project. For every 100 BC1DH lines that we produced, we would expect an average of 5 to be recombinants and we would therefore need to develop a very large population to generate a sufficiently large number of desired recombinants within the project. We therefore changed our strategy to a more random one by testing all the BC1DH lines that we developed. Time constraints limited the development of the breeding population so that seed was available from only 255 BC1DH plants in time for sowing in trials in 1999. Selections based on field observations made on the trials were multiplied over winter in New Zealand and returned for large plot ($7m^2$) trials at commercial density with and without fungicide at SCRI and fungicide treated trials near Sleaford and Docking in 2000. Cleaned and sieved samples from the plots were retained for analysis of the malting quality characters hot water extract, fermentability, PSY, grain nitrogen content, soluble nitrogen content of the malt, soluble nitrogen ratio and grain β-Glucan content.

The genetic fingerprints of the BC1DH lines entered into trials at SCRI 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. As well as developing lines of potential commercial merit, 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 also used regression analysis to identify markers that acted together in statistically significant associations with the characters and compared the results to those obtained from the mapping population.

Validation of laboratory tests

An essential question that this project sought to answer was the relevance of the laboratory measures to commercial practice. This applied particularly to the measures of fermentability and epi-heterodendrin. The problem is that methodology based upon commercial practice is resource consuming and cannot be applied to a large number of samples and certainly not on a scale large enough to conduct detailed genetic studies. We therefore selected a stratified set of malt samples for high gravity spirit yield (HGSY) analysis by the Scotch Whisky Research Institute (SWRI). This test gives an estimate of the likely spirit yield under distillery conditions. As the malts for both PSY and HGSY had been prepared under the same conditions, the two measures can be compared to determine the value of PSY in predicting spirit yield under distillery conditions. This test was applied to samples from both the mapping and the breeding populations. Validation of the measures of epi-heterodendrin was also carried out by SWRI using the standard distillery method.

Results

Genetics of the traits

Derkado was generally the better parent for most of the quality characters but B83-12/21/5 had a greater fermentability. In general, DH lines that transgressed, or equalled, the parental means were apparent for all characters, indicating the presence of useful alleles in both parents that potentially could be recombined to produce superior inbred lines. There was highly significant genetic variation for all the characters apart from the wort sugar data, which indicates that most of the characters should be responsive to selection. The high amount of genetic variation found for epi-hetrodendrin reflects the segregation of the major gene controlling production of the compound but the figure is still high when the effects of the gene are excluded by restricting analysis to lines without the *eph* gene. Apart from glucose, there was little indication of genetic variation for the wort sugars but this may reflect the fact that there was not a proper error to test for genetic effects in the project, which may therefore have been obscured by interactions from contrasting sites.

The correlations between the means of the characters show that extract is the major determinant of PSY although fermentability does have a small but significant positive correlation with the character (Table 1). Selection for increased fermentability could improve spirit yield but would need to be applied cautiously due to its higher but negative correlation with hot water extract. QTL mapping of the two traits would identify a suitable locus for selection. The correlations of the wort sugars with hot water extract are as expected but, with the exception of glucose, the wort sugars are not correlated with fermentability. The negative correlation of glucose with fermentability is surprising but could be an indication of over-modification, particularly as there is evidence of a positive correlation between glucose and soluble nitrogen ratio.

Table 1. Correlations between 11 malting quality characters measured on random DHs from Derkado x B83-12/21/5. Figures in bold are significant at P<0.05.

	Ferment	HWE	PSY	N	SNR	β-Glucan[1]	EPH[2]	Glucose[3]	Sucrose[3]	Maltose[3]
HWE	**-0.41**									
PSY	**0.17**	**0.75**								
N	**0.27**	**-0.37**	**-0.21**							
SNR	**-0.49**	**0.49**	**0.21**	**-0.71**						
β-Glucan[1]	**0.25**	-0.09	0.09	**0.25**	**-0.19**					
EPH[2]	0.09	-0.06	0.00	0.10	-0.02	**0.17**				
Glucose[3]	**-0.57**	**0.46**	**0.20**	-0.14	**0.32**	-0.16	-0.03			
Sucrose[3]	-0.01	**0.38**	**0.43**	-0.14	**0.37**	-0.09	-0.06	**0.31**		
Maltose[3]	0.11	**0.31**	**0.36**	-0.06	0.11	-0.08	0.12	**0.33**	**0.44**	
M-Triose[3]	0.05	**0.32**	**0.35**	-0.06	**0.19**	-0.07	-0.03	**0.39**	**0.51**	**0.75**

[1] Based on 3 sites only – 1995 trial not measured
[2] Based on 1995 and 1997 sites only
[3] Based on 98 lines from 1996 Sleaford and 1997 trials

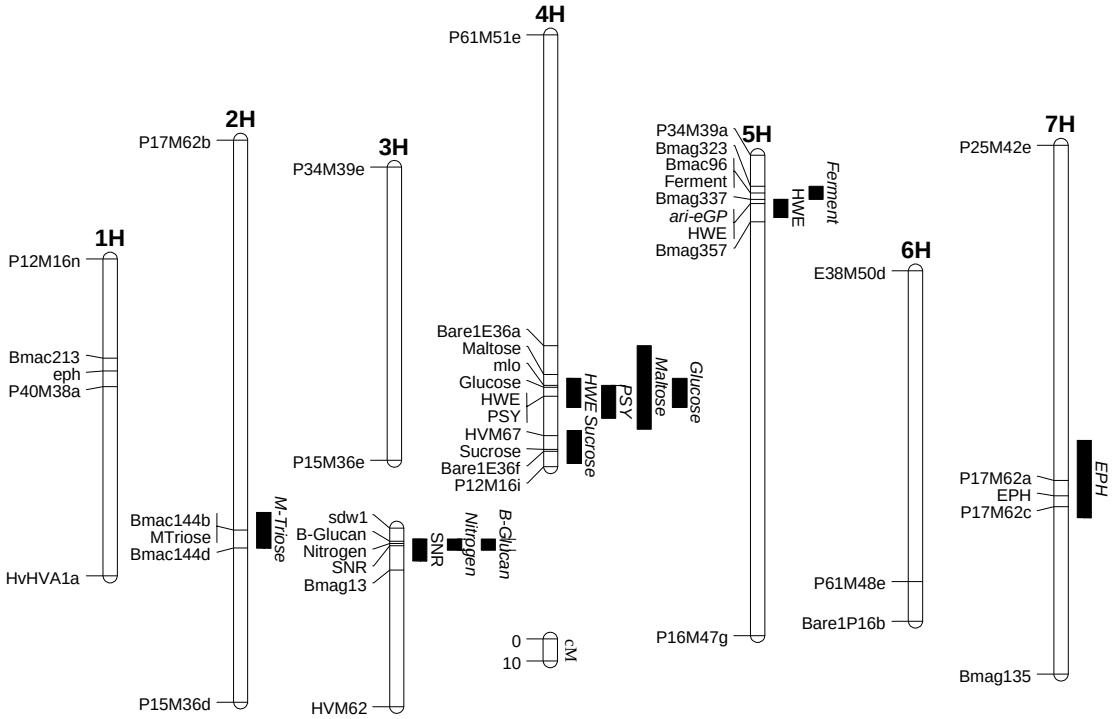
There was a significant positive correlation between PSY and HGSY (r = 0.52; P<0.01), indicating that the laboratory test gives a good estimation of distillery performance. Four lines performed poorly in the predicted spirit yield test but much better under the high gravity spirit yield test. However they all had low hot water extracts and soluble nitrogen ratios and were therefore under modified. The high gravity test uses much more rigorous extraction than the laboratory test and would therefore solubilise more material. The key point, however, is that lines that performed well under the laboratory test also gave a high HGSY, so positive selection for PSY will be effective. All the lines identified as non-producers of epi-heterodendrin gave very low levels of glycosidic nitriles in the spirit. When these were excluded, however, the agreement between epi-heterodendrin and glycosidic nitriles was non-significant (r = 0.1; P >0.05). This means that while the laboratory test can be used to identify non-producers, it cannot reliably differentiate between the relative amounts of glycosidic nitriles likely to be produced by genotypes without the *eph* gene.

Mapping of traits

Eight of the most significant QTLs for each character were associated with two currently important major-genes, *sdw1* on 3H and *mlo* on 4H (Figure 1). In both cases, no markers between the major-genes and the QTL peaks were identified, so there was no opportunity to use marker-assisted selection to identify desirable recombinants. This would be particularly desirable in the case of *mlo*, where the resistant allele was associated with a reduced level of the mono- and disaccharide sugars measured in the study and hence a reduction in hot water extract and PSY. The most significant QTL for fermentability was located in the region of another major-gene, the *ari-e*GP dwarfing gene on 5H, but linked in repulsion to the second most significant QTL for hot water extract. There were, however, markers in this region that could be used to select recombinants with increasing alleles of both. The most significant QTLs for the other two characters are located on 2H and 7H but no known major genes were segregating in these regions of the genome. For six of the 11 characters studied, the most significant QTL accounted for over 10% of the phenotypic variation. Where characters had low amounts of genetic variation (Table 2), the percentage of genetic variation accounted for by the most significant QTL was, not surprisingly, lower. The SSRs Bmag323 and

Bmag337 flank the fermentability QTL on 5H and the Derkado QTL allele accounts for just under 0.5% decrease in %fermentability. This translates, however, into an extra 3 litres of PSY that, if applied over the whole malt whisky industry, translates into an extra 1 million bottles production annually.

Figure 1. Location of most significant QTLs for 11 malting quality traits measured on Derkado x B83-12/21/5 population. QTL peaks are shown on the left hand side of chromosome bars in between their flanking markers. Solid bars on the right hand side represent confidence intervals for the QTLs and italic text denote alleles from B83-12/21/5 increasing expression of a character.



The other major result of the mapping was the location of the locus controlling the non-production of epi-heterodendrin, *eph*, on chromosome 1H, between the SSR Bmac213 and the AFLP P40M38a. Both markers were over 5cM from *eph* and could be used in marker-assisted selection for the gene but there would be an expected error rate of over 6%. The cultivar Cooper, a producer of epi-heterodendrin, is for example linked with the SSR allele associated with non-production in Derkado.

Molecular Breeding

Without selection, we would expect the average donor genome content to be 25%, whereas it was over 30%, but 67% of the BC1 plants possessed the donor target region and might have increased the donor genome contribution. Additionally, the doubled haploid process may have selected some donor genes increasing green plant production, which is possible as the donors were the products of a doubled haploid programme.

Data from the 2000 trials were used to evaluate the merits of the 135 lines in the breeding population and to determine the effect of the transferred region in another genetic background. 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 means values for many of the malting characters (Table 2). Landlord performed 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 there were some lines that malted considerably better than Landlord.

Table 2. Summary statistics of results from two field trials of 135 BC1DH lines and controls grown at SCRI in 2000. Numbers in Bold are significantly different from Landlord.

Character				BC1DHs			
	Landlord	Derkado	B83-12/21/5	Minimum	Mean	Max	SED
Yield(t/ha)	5.02	4.76	5.16	**4.07**	4.84	**5.96**	0.25
Head(days)	19.4	23.4	21.6	**15**	18.7	**28.5**	1.5
Height(cm)	61.8	64.3	60.5	**38.3**	56.9	**75.3**	2.8
Ext(%)-NIR	81.5	82.8	78	**76.5**	**80.0**	83.3	1
Grain Nitrogen(%)	1.25	1.34	1.38	1.27	**1.40**	**1.66**	0.08
HWE(°L/kg)	261	337	325	**210**	268	**317**	21
Fermentability(%)	82.4	78	80.9	**77.9**	80.9	83.7	1.6
PSY(l/t)	333	412	410	**267**	341	**406**	29
Soluble Nitrogen(%)	0.406	0.713	0.699	0.386	**0.514**	**0.665**	0.068
SNR(%)	32.4	56.5	51.9	26.6	38.2	**53.4**	5.7
Viscosity-NIR(sec)	12.6	12.1	18.6	**8.9**	**15.6**	**20.2**	1.2
Wort Viscosity(cP)	1.47	1.39	1.44	**1.36**	1.45	1.54	0.05

There was significant genetical variation for all the characters measured on the 2000 trials apart from fermentability, which had probably been adversely affected by the uneven modification of samples. Apart from soluble nitrogen, the mean of the BC1DHs did not differ significantly from Landlord, confirming that their general behaviour reflected a Landlord background. In contrast, the results from the NIR analysis all showed that the mean of the population was significantly worse than Landlord as grain nitrogen and viscosity was higher and extract lower. Seven lines were selected from their yield and agronomic merit in the 2000 trials for further trialling in 2001. One line showed outstanding yield potential over all three trials and three others had the same or slightly better yields than Optic. The effect of the poor micro-malting performance of the samples from the 2000 SCRI trials can be seen as Landlord and two other lines had very low extracts. None of the other lines had very good extracts although some did have higher fermentabilities.

The majority (103) of the lines can be classified as having the parental genotype in the target region of 5H, i.e. donor genotype with high fermentability and low extract (HF,LE) or recipient genotype with low fermentability and high extract (LF,HE). The remaining 32 lines had recombination events in the 16 cM between Bmag323 and Bmag357 and can be classified into all four combinations of high and low extract and fermentability, but the numbers in some groups are too low to enable accurate comparisons to be made. We therefore cannot test whether we have successfully generated recombinants with high fermentability and high extract QTLs in the target region of 5H but we can amalgamate the data from all 135 lines into separate comparisons of donor and recipient differences at the fermentability and extract QTLs.

The effect of the donor segment can be seen in the summaries of results presented in Table 3. Donor alleles at both the fermentability and extract QTLs have very similar effects upon all the malting 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. Donor alleles decrease extract, whether measured after micro-malting or predicted by NIR analysis. They also produce a slight increase in the grain nitrogen content and a decrease in the soluble nitrogen content of the wort, resulting in a reduced soluble nitrogen ratio. The differences were most pronounced at the SCRI sites and some caution should be exercised in making firm conclusions from the results due to the poor malting performance of samples in

those trials. The soluble nitrogen effects were also apparent at the Docking site, particularly in the contrast for the HWE QTL. This QTL is co-located with the *ari-e*GP dwarfing gene and the results suggest that use of the gene leads to problems in protein breakdown.

Table 3. Differences between means of BC1DH lines grown in three trials in 2000 and classified according to whether they possessed donor or recipient alleles at the fermentability and HWE QTLs in the target region of 5H. Differences are expressed as donor minus recipient means and those in bold type are significantly different.

Site	QTL Mean	NIR Ext	Ferment	HWE	PSY	Grain N	Sol N	SNR	Viscosity
Docking	Ferment	-0.1	0.5	-4	-2	0.01	-0.016	-1.0	-0.2
Docking	HWE	-0.2	**0.8**	**-6**	-3	0.01	**-0.029**	-1.8	0.1
SCRI-F	Ferment	**-1.1**	0.0	**-21**	**-27**	**0.15**	**-0.036**	**-5.8**	**1.1**
SCRI-F	HWE	**-1.1**	0.3	**-26**	**-33**	**0.15**	**-0.048**	**-7.2**	**1.0**
SCRI+F	Ferment	**-1.0**	-0.5	**-28**	**-37**	**0.13**	**-0.047**	**-7.3**	**1.0**
SCRI+F	HWE	**-1.1**	-0.4	**-33**	**-44**	**0.14**	**-0.052**	**-8.0**	**1.1**

Allelic differences at the fermentability QTL did show an increase due to 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 pattern can be seen in the results from the SCRI untreated trial, although the increase was not significant. At the SCRI treated trial, donor alleles actually reduced fermentability but, as malting performance was noticeably worse in the treated than the untreated trial, less credibility can be given to this finding. 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. The mapping trials did show that fermentability was subject to considerable genotype x environment interactions and that the fermentability QTL was not effective in the trial grown near Sleaford.

Regression analysis of the phenotypic and genotypic data collected on the means from the SCRI and the Docking trials revealed a number of significant associations. Apart from fermentability at both SCRI sites, and soluble nitrogen and SNR at the untreated site, the marker associations at SCRI accounted for more variation than at Docking. This probably reflected the range of variation found in the characters measured after micro-malting at SCRI. Each character was associated with at least one marker and there were 25 cases where results agreed between at least two of the trials. There were six notable clusters of associations at HVM54 (2H), Bmag225 (3H), HvBAMY (4H), *ari-e* (5H) and Bmac273a (7H). In each case, the increasing allele associations were generally either recipient or donor, which would be consistent with a particular genomic region affecting a number of malting quality parameters. The association of the *ari-e*GP allele with a decrease in hot water extract is consistent with the findings from the mapping study but contrasting results for the target fermentability locus were obtained from the trials. 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. Other

loci found to be affecting hot water extract in the mapping population on 1H, 3H, 4H and 7H are in similar regions to those found in the BC1DH population at Bmag211, Bmag225, HVM67 and Bmac273a respectively. In addition to the target region, another region affecting fermentability detected on 5H in the mapping population corresponds to that detected at Bmag222 in the untreated BC1DH trial at SCRI. The other character for which some common regions can be observed is SNR, where two regions on 3H and one on 7H detected in the mapping population correspond to HvLTPPB, Bmag013 and Bmac273a respectively.

Conclusions and End-User Relevance

We have clearly shown that there is genetic variation for fermentability but phenotypic selection for the character is likely to be difficult. Although fermentability is negatively correlated with hot water extract the correlation is not great and it should be possible to manipulate both characters to increase Predicted Spirit Yield. The results obtained from our small-scale laboratory tests for fermentability were shown within the project to be relevant to commercial distilleries, despite a large range in the distilling potential of the lines studied. All these findings mean that targeted breeding of cultivars specifically adapted for use in the Scotch Whisky industry can be undertaken.

The *mlo* mildew resistance gene has proved to be durable over 20 years of commercial deployment in spring barley cultivars in the UK and is the main resistance gene found in current recommended cultivars. Within this study, we found that the *mlo* resistance gene was associated with a reduction in malting quality characters, especially wort sugar content leading to an overall reduction in hot water extract. This may be due to adverse genetic linkages, in which case it would be possible to recombine the resistance gene with the allele improving malting performance. It is clear that more detailed studies of this region of the genome are required in order for breeders to devise an appropriate strategy. Meanwhile, genes from other segments of the genome will have to be incorporated to overcome the deleterious effects of the *mlo* gene.

We expected wort sugar levels to have a closer relationship with extract and/or fermentability than our results showed. The correlations between extract and the wort sugars were all significant and positive but less than 0.5, thus accounting for little of the variation in the character. There was some indication from multiple regression studies that both glucose and sucrose act together to influence levels of extract and that glucose and maltose also act together to influence fermentability. In the last case, glucose levels were negatively associated with fermentability and may reflect a general over-modification of the micro-malts in the mapping trials. The two most significant extract QTLs were, however, located in the same region as the two most significant QTLs for glucose and it may well be that the analysis in the current study was not sufficiently detailed to reveal closer associations.

Our aim was to identify a fermentability QTL and test its utility by transferring it into another genetic background but our finding that the target QTL was linked in repulsion to a hot water extract QTL meant that we had to generate recombinants to produce useful lines. We planned to generate recombinant backcross inbred lines through a combination of genotyping and doubled haploidy that would enable us to rapidly develop useful germplasm but also test our strategy. The finding of an undesirable linkage and time constraints meant that we were unable to produce sufficient lines to give us a realistic chance of achieving

our aim. We were able to generate and test enough lines, however, to compare the effect of donor alleles against the recipient alleles in the target region of the genome. The results were inconclusive and interpretation was hampered by poor malting performance of samples from the 2000 SCRI trials. 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.

We attempted to maximise our chances of detecting fermentability QTLs of large effect by using parents that were relatively diverse, but adapted to the target environment. The most significant QTL accounted for just 6% of the phenotypic variation in fermentability, however, and there were a number of other possible loci with smaller effects. To detect such a 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 quantities 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 moderately poor 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.

The abnormal malting performance of the samples from the SCRI trials in 2000 was un-expected as we did not carry out the micro-malting until 5 months after harvest, when we expected dormancy to have been broken. It is a definite genetic effect as replicate samples from a randomised trial perform similarly and appears to be due to the use of Landlord as the main recipient parent. Whilst Landlord performed poorly some other controls, notably Derkado and Optic, malted normally and gave high extracts but Chariot, a parent of Landlord, also did not micro-malt well. Some environmental factors must have induced some water sensitivity at SCRI in 2000, as we found marked water sensitivity in some samples 10 months after harvest. From a stratified set of samples, germination figures in 8ml of water were very highly correlated with hot water extract. As both Landlord and Chariot are very susceptible to *Ramularia* infection, there is the possibility that factors associated with the disease affect malting quality, as it was present in the SCRI trials. This is a problem associated with backcrossing as the recipient parent can be outclassed by the time of release of a new genotype if one chooses an established cultivar as a parent. Choosing a 'high-flyer', such as

Landlord, from early trials information can mean that undesirable effects, such as water sensitivity, become apparent after development of new lines is well advanced. The practice of adaptive backcrossing, in which one changes the parent at each stage is the most practical way to avoid these two problems of backcrossing. If one couples it with marker-assisted selection, then previously mapped SSRs would be of great value as their multi-allelic nature means one can identify not only donor alleles but also the different recipient alleles.

The markers that we identified as flanking *eph* could potentially be used to eliminate epi-heterodendrin producers from distillery malts, either by plant breeders or the testing authorities and thus avoid any future problems with statutory ethyl carbamate tolerance levels. The SSRs linked to *eph* were close enough to the gene to enable their use in marker assisted selection but were not close enough to be diagnostic. One would need to genotype the parents of each cross and know whether or not they were epi-heterodendrin producers before deploying marker-assisted selection. There is a need to develop diagnostic markers for the character so that they can be deployed by the distilling industry. We managed to make some advances during the project but further work is necessary to ideally locate a diagnostic marker within the gene itself.

Our results have shown that extract is the major determinant of predicted spirit yield but opportunities for further improvement in extract appear to be limited, unless hull-less barley is developed for use in malting. The removal of the husk would provide a quantum leap in extract levels but, especially for the distilling industry, some degree of husk retention or mixing with an appropriate husked variety would be required for filtration. Research would also need to be carried out on the agronomic and financial implications of utilising naked barley as there would be an immediate yield loss.

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 thermostability, which are not found in European spring barley, may improve fermentability. Another potential approach would be to explore natural variation to ensure that limit dextrinase remains bound during mashing but is released during fermentation and would therefore increase fermentability. Putting such variants of these two genes 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.

This project has increased the exchange of information between geneticists, breeders and end-users, thus vastly improving each group's mutual understanding of the potential applications of molecular biological methods. We wish to develop these relationships, not only into future research projects, but also to develop tangible benefits all the way along the supply chain to the end-user. We see such networks as being essential to not only a healthy domestic market but also maintaining and extending the export market.

Technical Report

Introduction

The UK produces around 7 million tonnes of barley annually of which approximately 1.9 million tonnes is malted, mainly for brewing and distilling. The uptake of barley for malting is highest in Northern Britain where up to 60% of the spring crop may be used in brewing and distilling, with the distilling industry alone consuming over 500,000 tonnes per annum. The Northern British spring barley crop therefore represents a major component of UK malt and there are also export opportunities. As a product of malted barley, Scotch Whisky is the fifth largest British export and the leading food and drink commodity, earning over £2 billion per annum. Malt whisky is made exclusively from malted barley and grain whisky is made from an un-malted cereal, currently wheat, with a small amount of high enzyme barley malt added to convert the starch in the adjunct to fermentable sugars. High spirit, or alcohol, yield is probably the main quality requirement of the distilling industry. This is 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 amount of alcohol produced per tonne of malt. It may be calculated as the product of the hot water extract and its fermentability. The extract is the amount of material solubilised from barley during processing whilst the fermentability is the degree to which that material can be fermented by yeast. Whilst high spirit yield is not possible without high extract, a high level of extract does not necessarily lead to a high fermentability. In fact, 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. Another requirement of the distilling industry is to minimise levels of the barley-derived glycosidic nitrile epi-heterodendrin. Under certain conditions a breakdown product of epi-heterodendrin can react with ethanol, catalysed by copper in stills, to produce the putative carcinogen ethyl carbamate (urethane). A low level of epi-heterodendrin is thus an essential requirement in barley used for grain whisky distilling.

Improvement of barley quality for the malting and distilling industries is hampered by the multiplicity of the component characters, many of which are genetically complex, inter-related and modified to varying levels by the environment. The development of molecular markers has permitted the dissection of such complex traits and the location of Quantitative Trait Loci (QTL) controlling component parts through whole genome analysis. The success of such an approach depends upon being able to exploit suitable genetic material for accurate phenotypic (character) and genotypic (marker) characterisation.

Up to the 1990's, relatively few studies of the genetics of important malting quality characters had been carried out, especially of hot water extract. The advent of molecular marker maps for barley resulted in a number of studies of hot water extract in Australian (Langridge et al., 1995), European (Thomas et al., 1996; Bezant et al., 1997) and North American (Hayes et al., 1993; Mather et al., 1997) germplasm. None of these studies examined fermentability or predicted spirit yield and, apart from some germplasm assays, even less work has been carried out on epi-heterodendrin. The value of using fermentability in predicting spirit yield was first demonstrated by Dolan et al. (1981) but little genetical research was carried out on the character. Bringhurst et al. (1996) demonstrated that calculation of spirit yield based upon laboratory-scale high gravity

extracts (HGSY) was an accurate reflection of what was likely to be achieved in distilleries, which extended the possibilities for studying fermentability and hence spirit yield. Fermentability 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. MAFF therefore funded SCRI to conduct an investigation into molecular markers for fermentability and spirit yield as part of the Agro/Food LINK programme. Industrial research and funding was provided through partnerships with the Scotch Whisky Research Institute, Home-Grown Cereals Authority, Advanta Seeds UK and Mylnefield Research Services.

The aims of the project were:

1. To determine the genetic control of fermentability and hence spirit yield and their relationship with other characters
2. To identify molecular markers linked to genes controlling fermentability, hot water extract and hence spirit yield
3. Combine, by conventional breeding methods, genes for high spirit yield in a spring barley genotype with a suitable agronomic background for Northern Britain.

We collected genotypic and phenotypic results upon a mapping population that was constructed between parents that exhibited contrasting combinations of fermentability and hot water extract in order to maximise our chances of revealing QTLs for the former that could be used to improve spirit yield. Mixed results have been obtained in studies of the transferability of QTLs between different populations of the same species and even in different populations from the same cross (Han et al., 1997). We therefore needed to verify that we had detected a QTL that could be expressed in contemporary germplasm. We expected to achieve this in the third objective by utilising selections from the mapping population with high fermentability in a back crossing programme to a promising cultivar and some breeding lines. Through genotyping and phenotyping the lines produced, we would be able to evaluate the transferability of the QTL and, in the process, hope to identify lines of merit for commercial development.

Materials & Methods

Mapping Population

A random inbred population of 156 lines was produced by doubled haploidy from the F1 of a spring barley cross between the genotypes Derkado and B83-12/21/5. Derkado originated from Germany, carried the *sdw1* semi-dwarf allele from Diamant, and the *mlo* mildew resistance allele from Grannenlose Zweizeiliege. Derkado had good malting quality and was one of the main Scottish cultivars in the 1990's, principally because it was a non-producer of epi-heterodendrin. B83-12/21/5 was a breeding line from the Scottish Crop Research Institute (SCRI) that carried the *ari-e*GP semi-dwarf gene from Golden Promise and the *Mla13* mildew resistance allele, although the latter could not be assayed in the presence of the *mlo* allele. Swanston et al. (1999) carried out a pilot study on plots of the population grown at SCRI in 1995 and identified potential QTL for both fermentability and hot water extract. In addition, they were able to locate the locus controlling the presence or absence of epi-heterodendrin, which was designated *eph*, on the molecular

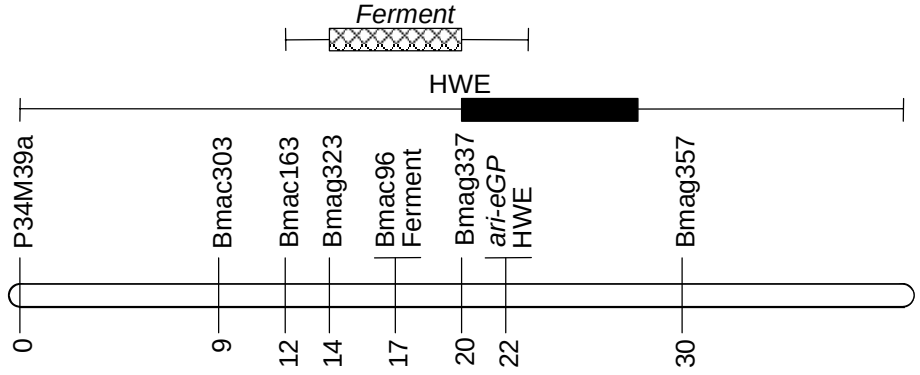
genetic map. The whole population, together with the parents and some controls, was grown in replicated and randomised plot trials at SCRI from 1995 to 1997 inclusive and at a site near Sleaford, Lincs, UK in 1996 and 1997. Each trial received a typical fertiliser regime and was kept free of foliar pathogens by the application of fungicides. When the majority of plots were ripe, the whole trial was harvested with a plot combine and grain samples were cleaned and the fraction passing over a 2.5mm sieve retained for phenotypic analysis of quality characters. Adverse weather conditions delayed harvest of the 1997 trial near Sleaford and there was considerable pre-harvest sprouting in a number of samples. No malting analyses were therefore carried out on this trial, leaving four trials for analysis.

Breeding Population

We developed a breeding population to demonstrate the value of our QTL and, in the process develop lines of potential commercial value. At the start of the project, we had some preliminary fermentability information (Swanston et al., 1999) on the mapping population and we selected 8 donor lines, on the basis of their phenotypic performance (Table 1), to initiate a programme to produce first backcross (BC1) inbred lines. The cultivar Landlord and two SCRI breeding lines, B91-47/22 and B91-99/15, were chosen as recipient parents. Landlord was chosen as a recipient parent because it was newly recommended and appeared to be agronomically well suited to Northern Britain. Landlord had a good level of hot water extract but its fermentability was average for a malting cultivar, so there was potential to improve its spirit yield by improving its fermentability. The two SCRI breeding lines represented developing germplasm that appeared very promising from early trial results.

By the time of BC1 production, we had a strong indication that the largest QTL affecting fermentability was on chromosome 5H and our initial strategy was to use marker-assisted selection to transfer it into another genetic background. Once we had mapped the fermentability trait, it was apparent that it 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 (Thomas et al., 1991). Furthermore, we found that the fermentability

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).



QTL was also linked in repulsion to a hot water extract QTL at the *ari-e* locus (Figure 1). To develop a successful cultivar within the project would therefore mean selecting just recombinants between the QTL and the dwarfing gene. There were neither the resources nor the time to generate a large enough population to ensure that there were a sufficiently large number of desired recombinants within the project. 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 a second stage of genome wide testing of the location of regions controlling fermentability. It also became apparent that at least two of the donors were of the wrong genotype at the fermentability QTL peak (Table 1) but we continued some development of crosses from them as it provide an added control. We continued to monitor the transfer of the segment by genotyping all the BC1 seed produced and this enabled the selection of some plants for anther culture but there was not sufficient time to complete the selections before culturing anthers. Time constraints also meant that we were not able to enter many plants into anther culture so that whilst we produced over 1000 BC1 seed, we were only able to derive doubled haploids from 55 of them, 37 of which were heterozygous for markers in the target region of 5H.

Table 1. Summary of the donor lines and progression of material in deriving the breeding population. Numbers in **bold**, normal, and *italic* type denote Landlord, B91-47/22 and B91-99/15 as recipient parents respectively.

	B91-63/6/1	B91-63/75/1	B91-63/82/1	B91-63/91/1	B91-63/120/1	B91-63/125/3	B91-63/137/1	B91-63/147/1	Totals
Ferment	85.3	84.8	84.8	84.0	84.3	85.6	84.3	84.1	
Bmag323	B83	B83	B83	Der	B83	B83	Der	Der	
Bmag337	B83	B83	B83	Der	B83	B83	Der	B83	
ari-e	B83	B83	B83	Der	B83	B83	B83	Der	
Bmag357	B83	B83	B83	Der	B83	B83	B83	Der	
eph	B83	B83	Der	B83	B83	B83	B83	B83	
BC1's	21, *40*	**293**, *24*	**255**, *39*	**34**, *61*	*52*	*54*	19, *18*	33, *97*	**582**, 73, *385*
Donor Hets	4, *14*	**68**, *12*	**53**, *15*	**0**, *0*	*23*	*27*	0, *0*	0, *0*	**121**, 12, *98*
Anther Culture	2	**24**	**16**	**2**, *3*	*2*	*3*	1	1, *1*	**42**, 4, *9*
BC1DHs	12	**266**	**103**	**14**, *4*	*24*	*33*	3	10, *17*	**383**, 25, *78*
Trial 99	2	**132**	**49**	**7**, *4*	*7*	*39*	2	3, *10*	**188,** 7, *60*
Trial 00		**96**	**29**	*1*	*4*	*1*		*4*	**125**, *10*

The same time constraints further limited the doubled haploid programme so that 486 green plants were produced from the BC1 plants. Seed was available from only 255 BC1DH plants in time for sowing in a late and thinly-sown single replicate trial ($3m^2$ plots) at SCRI in 1999. The plots were sown in a Modified Augmented Design 2 trial (May et al., 1989) and kept free from foliar pathogens by applying a standard

fungicide regime. There was sufficient surplus seed to sow 94 of the BC1DH lines in mini-observation plots ($1m^2$) in a trial near Docking, Norfolk, UK. Both trials were harvested with a small plot combine and cleaned and sieved seed retained for malting quality analyses. From field observations made on the trials, 135 lines showed sufficient merit and produced enough seed to sow in multiplication trials in New Zealand in 1999/2000. Seed was returned from these plots to enable large plot ($7m^2$) trials at commercial density with and without fungicide at SCRI and fungicide treated trials near Sleaford and Docking in 2000.

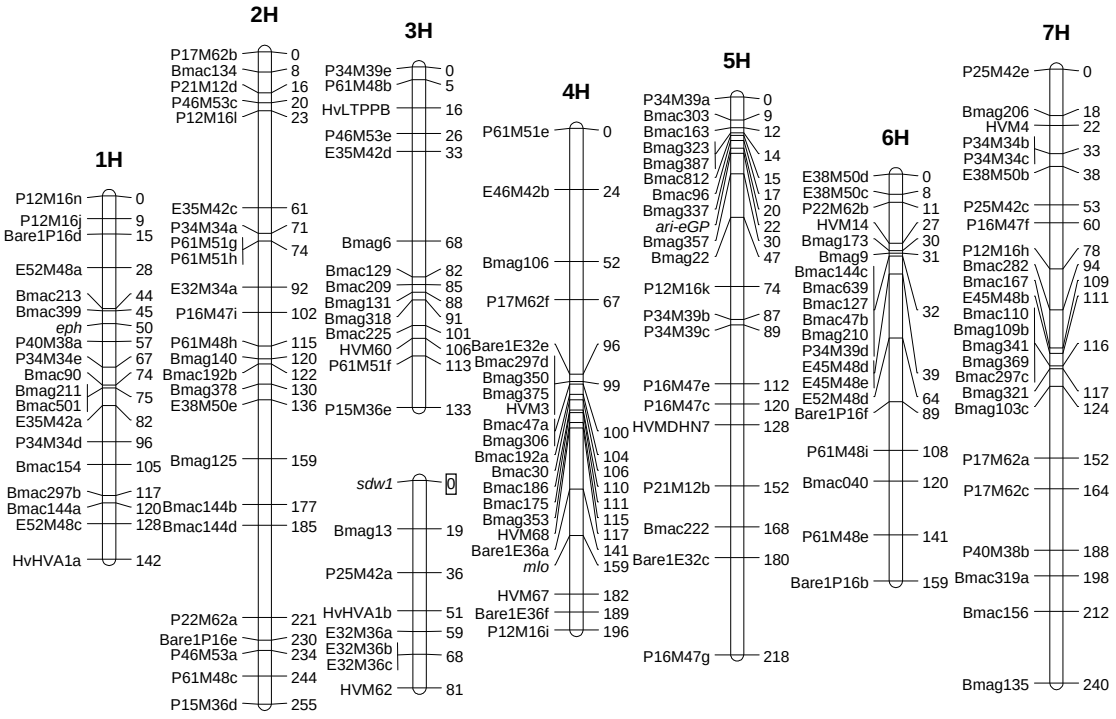
The fungicide treated trials were replicated but the untreated trial was sown in another Modified Augmented Design 2 trial with Landlord as the main-plot control. The plots from each trial were harvested with a small plot combine, yield was recorded and cleaned and sieved samples were retained for malting quality analysis. Table 1 summarises the progression and derivation of lines in the breeding trials.

Genotyping

Mapping Population

For the analysis of QTLs within the project, the entire population was surveyed with 241 markers, including the four phenotypic major-genes *sdw1*, *ari-e*GP, *mlo* and *eph*. The molecular markers consisted of 20 Sequence-Specific Amplified Polymorphisms (S-SAPs) (Waugh et al. 1997b), 127 AFLPs and 90 previously mapped Simple Sequence Repeats (SSRs). The SSRs were largely derived from an enriched library together with some from sequences deposited in public databases (Ramsay et al., 2000) and previously published SSRs (Liu et al., 1996). JOINMAP 2.0™ (Stam and van Ooijen, 1995) was used to construct a genetic map for the population. Using LOD=4 and the map location of the SSRs to establish chromosomal identities,

Figure 1. Genetic map of Derkado x B83-12/21/5 population showing the distribution of markers (major genes in italics) across the seven barley chromosomes. Note that chromosome 3H is made up of two separate groups.



eight linkage groups were formed from the marker data with chromosome 3 being represented by 2 separate groups. Two rounds of the mapping module were used to derive the order for each linkage group and the pairwise output was inspected to eliminate any markers that did not result in a good fit. This process resulted in a map of 153 markers comprising all four phenotypic major-genes, 8 S-SAPs, 66 AFLPs and 75 SSRs (Figure 2). For QTL mapping, markers that mapped within 1cM of each other were eliminated, leaving 127 markers.

After construction of the map for QTL analysis, more markers became available and were also scored on the population. There were 35 SSRs, 6 AFLPs, 7 markers using telomere specific primers (Kilian et al., 1999) and a further 3 SSRs from mapped barley RFLP sequences deposited in public databases (Michalek et al., 1999). Primers were also designed to amplify products from previously mapped RFLP markers estimated to be in the region of *sdw1* and *eph* from the integrated genetic map of Kunzel et al. (2000). Sequencing of the products obtained from the parents revealed single nucleotide polymorphisms (SNPs) at several loci. These SNP loci were surveyed across the whole population by sequencing the products obtained from each DH. Three *mlo* specific markers, two associated with SSRs and one with a mini inverted tandem repeat (MITE) element (L. Ramsay *pers. comm.*) within the published sequence (Buschges et al., 1997) were also scored on the population, making a total of 297 markers. All the mapping results will, however, be based on the initial marker screen of 241 markers.

Breeding Population

All the BC1DH lines entered into the 1999 trial at SCRI were genotyped with 44 previously mapped SSRs, selected to sample regions of the whole barley genome as well as the region surrounding the target QTL. The SSRs were also chosen to be polymorphic between the recipient backcross parent and at least one of the mapping parents, which gave us good genome coverage but meant that some SSR alleles could not be un-ambiguously assigned to one of the three potential parents. The lines were also scanned with 63 AFLP markers, 38 of which were included on the genetic map of the Derkado x B83-12/21/5 population. As AFLP products of the same size can be transferred between populations of the same species (Waugh et al, 1997a), the 38 mapped markers could also be used to survey variation at definite regions of the genome in the BC1DH lines. The remaining 25 AFLP markers were anonymous, as the primers had identified polymorphisms between the recipient parents and both mapping parents. Because of the dominant nature of AFLPs, it was also not possible to un-ambiguously assign all the markers to one of the parents. Some primers also failed to amplify products in a number of the BC1DH lines so the AFLP marker data was incomplete compared to the SSRs. In addition, observations of the juvenile growth habits of the plots enabled the scoring of allelic differences at the *sdw1* and *ari-e*GP loci. There were therefore 84 previously mapped and 25 anonymous loci to monitor genome content of the BC1DH lines.

Phenotyping

The cleaned and sieved samples from the plots of the mapping and breeding trials were micro-malted at SCRI with 30g sub-samples in a semi-automated system using the regime described by Swanston et al. (1999). Hot water extract (HWE) was measured by a scaled down version of the Institute of Brewing (IOB)

recommended method, using 20g grist and modifying the volumes of water accordingly. Extracts were determined by measuring specific gravity and expressed as Lintner degrees per kg (°L). Fermentability of the extracts was then measured using a modified IOB method (Swanston et al., 1999). The equations provided by Dolan et al. (1981) enabled measurements of specific gravity before and after fermentation to be used in determining both fermentablity and fermentable extract. The latter, expressed as a percentage, is the product of HWE and fermentability and is multiplied by a constant to derive Predicted Spirit Yield (PSY), which is expressed in litres per tonne of malt. The malt samples from each trial were assayed for soluble nitrogen content using a UV spectrophotometer (Haselmore and Gill, 1995). Sub-samples of the cleaned and sieved seed from each of the mapping trials were analysed for nitrogen content by the Dumas combustion method (Buckee, 1994). The nitrogen content of the 2000 breeding trials was analysed by Advanta Seeds using NIR. The soluble nitrogen ratio (SNR) was calculated from the estimates of grain and soluble nitrogen in the malt using a constant to correct for conversion of the former into malt nitrogen. Acrospires obtained from germinating seedlings of samples grown in the 1995 and 1997 SCRI trials were assayed for epi-heterodendrin (Cook and Oliver, 1991) at SCRI, using modifications to enable quantification (Swanston, 1999). Grain β-glucan content of the mapping trials was measured at SCRI using the Megazyme kit method, which is based on McCleary and Glennie-Holmes (1985), and omitting the 1995 SCRI trial. For the breeding trials, β-glucan analysis was confined to the 2000 SCRI treated trial due to time constraints at the end of the project. Additional data collected by Advanta Seeds on the 2000 breeding trials consisted of NIR predictions of Extract (%) and extract viscosity (sec) as a prediction of the falling time test (Morgan and Gothard, 1977).

Malt samples from the 1996 Sleaford trial and the 1997 SCRI trial were assayed by the Scotch Whisky Research Institute (SWRI) for the wort sugars glucose, sucrose, maltose and maltotriose using High Performance Anion Exchange Chromatography with a PA100 anion exchange column against a sodium hydroxide and water eluant gradient. Resources were insufficient to analyse all the samples from both trials so it was decided to sample 100 lines, including the parents, from one replicate of each trial, as it was felt important that some idea of environmental variation for the characters was obtained. Due to the lack of a replicate structure, there was not a proper error term to test the Genotype item so the Genotype x Environment item was used instead.

A stratified set of 13 malt samples, representing the whole range of malting quality from the 1996 mapping trials, was evaluated for high gravity spirit yield (HGSY) by SWRI using the methodology described by Bringhurst et al. (1996) on malt supplied by SCRI. This test gives an estimate of the likely spirit yield under distillery conditions. As the malts for both PSY and HGSY had been prepared under the same conditions, the two measures can be compared to determine the value of PSY in predicting spirit yield under distillery conditions. Thirteen genotypes were chosen from the 1996 set of trials for HGSY analysis by SWRI. An additional thirteen genotypes were chosen from the 1997 SCRI trial for HGSY analysis, giving a total of 26. Malt was pooled over replicates to provide sufficient for analysis and resources would not have been sufficient to analyse both replicates anyway. As for the wort sugars, there was not a proper error variance with which to test the significance of the items from the analysis of variance and the Genotype x

Environment item was used. Selected samples from the 2000 breeding trial grown at SCRI were also analysed by SWRI using the HGSY test and the raw spirit analysed for flavour using a tasting panel at SWRI. In the HGSY assay, fermentability can be estimated as the decrease in specific gravity after fermentation expressed as a percentage of the original gravity.

The question of applicability of a small-scale laboratory test also applies to the quantification of epi-heterodendrin based upon the colorimetric test carried out on samples from the acrospires of seedlings. Validation was therefore carried out by SWRI using the standard distillery method (Brown and Morrall, 1996). Malt samples from twenty-five genotypes, some with replication, from the 1997 mapping SCRI mapping trial were sent by SCRI to SWRI for analysis and results compared to the laboratory quantitative test.

Statistics

The replicated trials were sown in a range of designs and were analysed individually with corrections for any spatial variation, according to the design used, to derive genotype means for each character measured at each site. Overall means were derived as the mean of these means. For the HGSY data with incomplete representation, the REML directive within GENSTAT was used to estimate overall means and standard errors. An over-site analysis was also carried out on the fully replicated data sets to estimate the relevant amounts of each component of variation but, because of the different individual designs, a Randomised Complete Block analysis was carried out.

The phenotypic and genotypic data from the mapping population was scanned for QTLs using the software package MQTL (Tinker and Mather 1995) with 1000 permutations of the data to determine a threshold for a 5% genome wide error rate. MQTL fits both main effect QTLs and QTL x Environment interactions to the data and establishes separate significance thresholds for them. In addition the package uses simple interval mapping (SIM) and simplified compound interval mapping (sCIM), in which background markers are used to account for variation in other regions of the genome. The background markers were evenly spread across the genome but included the major gene loci *sdw1, ari-eGP* and *mlo*, as previous experience had revealed that they affected a number of quantitative traits. Primary QTLs were declared where both SIM and sCIM peaks exceeded the threshold. In cases where either the SIM or the sCIM peak exceeded the threshold, we were less confident about the presence of a QTL and so have termed them Secondary QTLs. The means from each environment in which a character was scored were also scanned with the same parameters and Secondary QTLs were only declared when either the SIM or the sCIM scan exceeded the SIM threshold in at least one environment.

The Modified Augmented Design 2 trial used for some of the breeding trials provides methods for adjusting means according to variation between either main- or sub-plot controls (May et al., 1989). In most cases, we used the main-plot control method but, given the poor malting of Landlord in the 2000 trial, we used the sub-plot control method for the malting characters. The error variation from either the main- or sub-plot controls was used to derive approximate standard errors for comparing genotypes. The phenotypic and genotypic data from the breeding population were also combined to detect genomic regions affecting the characters

measured. This could either validate the loci detected in the mapping study or could also reveal new loci, caused by the introduction of the new parents. Because the breeding population did not have a consistent structure, the most appropriate method of detecting QTL was to carry out a marker mean analysis using regression analysis to establish whether or not there were significant differences between the means associated with each marker locus for each character. We wished to detect whether or not the introgressed segment from the donor altered the expression of a character in the recipient and therefore recoded all the genotypic data as being either donor or recipient in origin. Those alleles that could not un-ambiguously be assigned to either donor or recipient were coded as recipient. Forward stepwise multiple regression was used to identify markers that acted together in statistically significant associations with the characters. From this, the effect of alleles at significant marker loci could be estimated.

Results

Genetics of the traits

Derkado was generally the better parent for most of the quality characters but B83-12/21/5 had a greater fermentability (Table 2). There was, therefore, an opportunity to improve spirit yield through the use of fermentability alleles from B83-12/21/5. In general, DH lines that transgressed, or equalled, the parental means were apparent for all characters, indicating the presence of useful alleles in both parents that potentially could be recombined to produce superior inbred lines. There was, however, some evidence of skewness, notably for hot water extract, fermentability and hence predicted spirit yield. This may be indicative of epistatic effects or could also reflect some non-linearity in the malting behaviour of poor genotypes (Table 2).

Table 2. Summary statistics for 11 malting quality characters measured on random DH lines from the F1 of Derkado x B83-12/21/5

	Parental Means		DH Population			Percentage Variation			
Character	Derkado	B83-12/21/5	Min	Mean	Max	Genetic	Env'ment	GxE	Error
HWE	311.3	301.9	255.6	301.3	311.8	19.8	30.5	16.8	32.9
Ferment	83.1	83.9	80.3	83.2	85.6	7.2	35.4	10.5	46.8
PSY	409	400.9	335	396.6	410.9	13	34.2	15.1	37.7
Nitrogen	1.3	1.21	1.16	1.32	2.06	15.9	63.8	3	17.4
SNR	49.2	51.7	34	49.2	58.8	8.6	61.3	0	30.1
β-Glucan	2.93	3.5	2.41	3.2	3.96	6.8	62.5	3	27.7
EPH[1]	0	0.81	0	0.54	1.53	77.1	5.9	8.1	8.9
EPH (prod)[1]	N/A	0.81	0.56	0.98	1.53	23	38.1	10.7	28.2
Glucose	8.48	6.42	4.85	7.38	10.78	17.8	4.8	0	77.4
Sucrose	3.55	2.81	0.94	2.49	4.72	1	40.9	0	58.1
Maltose	33.41	33.41	22.85	31.72	38.59	4.1	1.3	0	94.6
M-Triose	6.37	6.55	4.05	6.43	9.06	0	13.7	0	86.3

[1] EPH (prod) is population restricted to lines without *eph* gene, i.e. epi-heterodendrin producers

There was highly significant genetic variation for all the characters apart from the wort sugar data (Table 2). The high amount of genetic variation found for epi-hetrodendrin reflects the segregation of the major gene

controlling production of the compound. When the non-producing lines are eliminated from the analysis, there is still a substantial amount of genetic variation remaining. In fact, the portion is the highest of all the characters considered in this study but this may reflect the fact that it was only measured on two sites compared to three or four for most of the other characters. Apart from Glucose, there was no apparent significant genetic variation for the wort sugars analysed in this study. The Genotype x Environment interactions were used as the error for the wort sugars but were frequently present and comparable to Genotype effects for the other characters. We concluded that there probably was genetic variation for the wort sugars, as there were some significant differences between the extremes of the population (Table 2), but it had been obscured by the interactions from two very different sites.

Given the large population size, it is not surprising that most of the correlations between the characters are significant. Hot water extract is the major determinant of predicted spirit yield although fermentability does have a small but significant positive correlation with the character (Table 3). This confirms that selection for increased fermentability could have a beneficial effect upon spirit yield but careful consideration would have to be given as to how this might be achieved, as fermentability had a higher but negative correlation with hot water extract. QTL mapping of the two traits would be of great benefit here as it would then be possible to identify suitable loci for selection. All the wort sugars have significant correlations with hot water extract

Table 3. Correlations between 11 malting quality characters measured on random DHs from Derkado x B83-12/21/5. Figures in bold are significant at P<0.05.

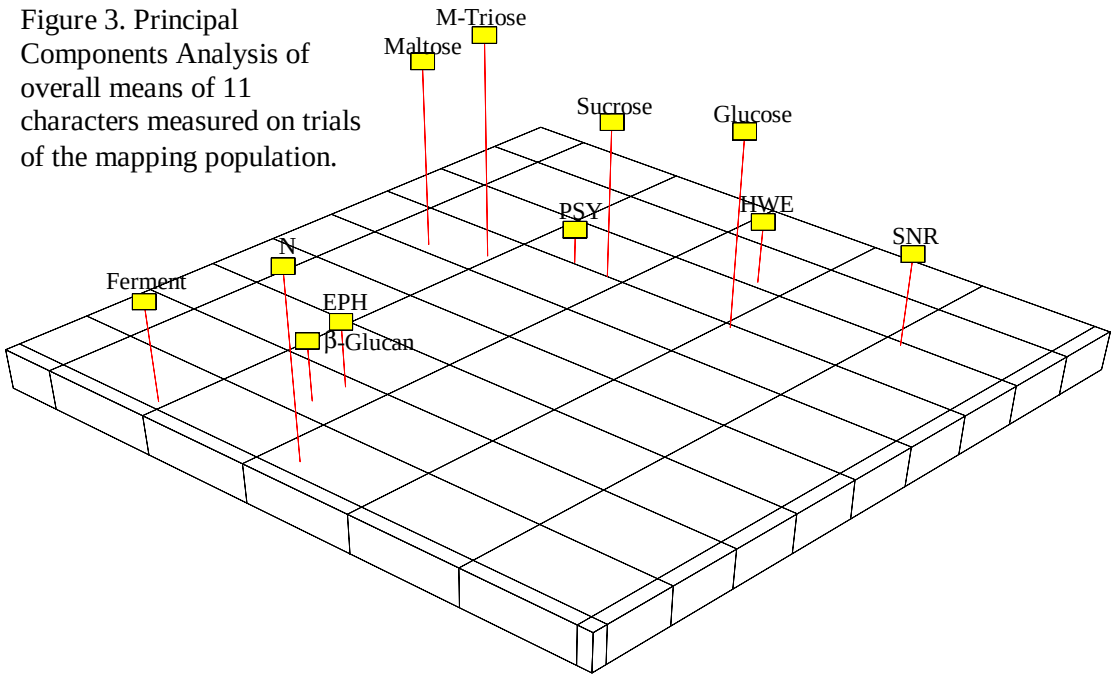
	Ferment	HWE	PSY	N	SNR	β-Glucan[1]	EPH[2]	Glucose[3]	Sucrose[3]	Maltose[3]
HWE	**-0.41**									
PSY	**0.17**	**0.75**								
N	**0.27**	**-0.37**	**-0.21**							
SNR	**-0.49**	**0.49**	**0.21**	**-0.71**						
β-Glucan[1]	**0.25**	-0.09	0.09	**0.25**	**-0.19**					
EPH[2]	0.09	-0.06	0.00	0.10	-0.02	**0.17**				
Glucose[3]	**-0.57**	**0.46**	**0.20**	-0.14	**0.32**	-0.16	-0.03			
Sucrose[3]	-0.01	**0.38**	**0.43**	-0.14	**0.37**	-0.09	-0.06	**0.31**		
Maltose[3]	0.11	**0.31**	**0.36**	-0.06	0.11	-0.08	0.12	**0.33**	**0.44**	
M-Triose[3]	0.05	**0.32**	**0.35**	-0.06	**0.19**	-0.07	-0.03	**0.39**	**0.51**	**0.75**

[1] Based on 3 sites only – 1995 trial not measured
[2] Based on 1995 and 1997 sites only
[3] Based on 98 lines from 1996 Sleaford and 1997 trials

and this is also refelected in their correlations with predicted spirit yield. Interestingly, glucose shows the highest correlation with hot water extract but the lowest with predicted spirit yield, possibly because it has quite a high negative correlation with fermentability. This latter correlation is slightly puzzling but could be an indication of over-modification, particularly as there is evidence of a positive correlation between glucose and soluble nitrogen ratio.

Principal Components Analysis is another way of examining the relationships between the variables measured on the mapping population. Using correlations between the overall means as a measure of association, Figure 3 shows the relationships between the 11 variables measured on the population. The

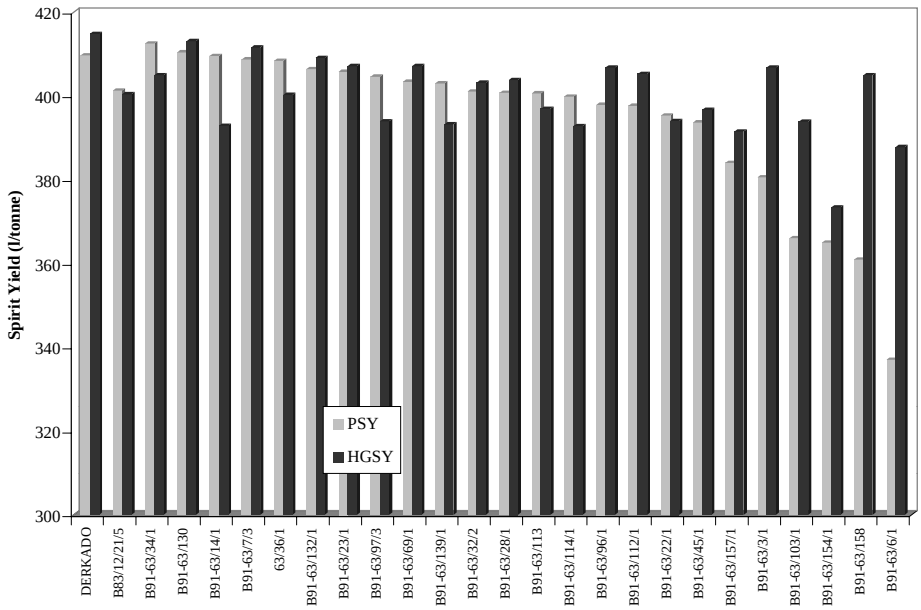
Figure 3. Principal Components Analysis of overall means of 11 characters measured on trials of the mapping population.



distribution of the variables is based on the first three principal components, which account for over 60% of the variation. The second PCP separates all the wort sugar data, HWE, PSY and SNR from the other variables and the third PCP tends to separate the wort sugars and grain nitrogen from the other characters. The only characters showing any close similarity are EPH and β-Glucan, and Maltose and Malto-triose. Hot water extract shows surprisingly little association with fermentability, as does nitrogen with SNR. Of the wort sugars, glucose appears to have some association with HWE and sucrose with PSY.

Significant genetic variation was found for HGSY, similar to PSY and, not surprisingly, there was also

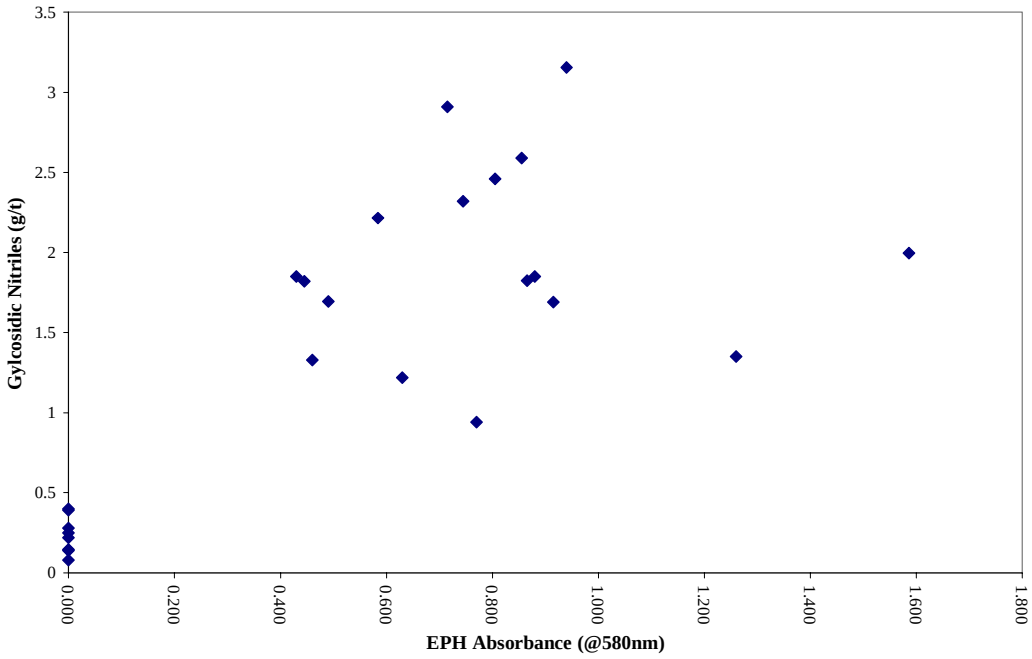
Figure 4. Comparison of Predicted and High Gravity Spirit Yield for Selected Lines from the Mapping Population – 1996-7 means.



highly significant variation between sites. There was a significant correlation between PSY and HGSY (r = 0.52; P<0.01). Figure 4 shows the comparison of predicted spirit yield and high gravity extract spirit yield and it can be seen that there were four lines that performed poorly under the predicted spirit yield test but relatively much better under the high gravity spirit yield test. The high gravity test consists of repeated extractions with increasing temperature and is therefore much more rigorous than the unithermal conditions of the predicted spirit yield test, which also used a coarser grist. Certainly, the four lines where the high gravity spirit yield was much greater than the predicted spirit yield all had low hot water extracts and relatively low soluble nitrogen ratios. This reflects under-modification of these lines and indicates that they were not efficiently extracted under the laboratory system compared to distillery practice.

The comparison of epi-heterodendrin content in acrospires with levels of glycosidic nitriles measured in malt samples by SWRI gave very good agreement over all samples (r = 0.75; P<0.001). All the lines identified as non-producers (absorbance at 580nm <0.4) were confirmed as having very low levels of glycosidic nitriles in the malt (Figure 5). The agreement between the producers was less good, however (r = 0.1; P >0.05). Inspection of the data reveals that there appears to be two groups of producers, one with between 0.9 and 2

Figure 5. Comparison of amounts of epi-heterodendrin (EPH) detected in acrospires of germinating grain with amounts of glycosidic nitriles in spirit.

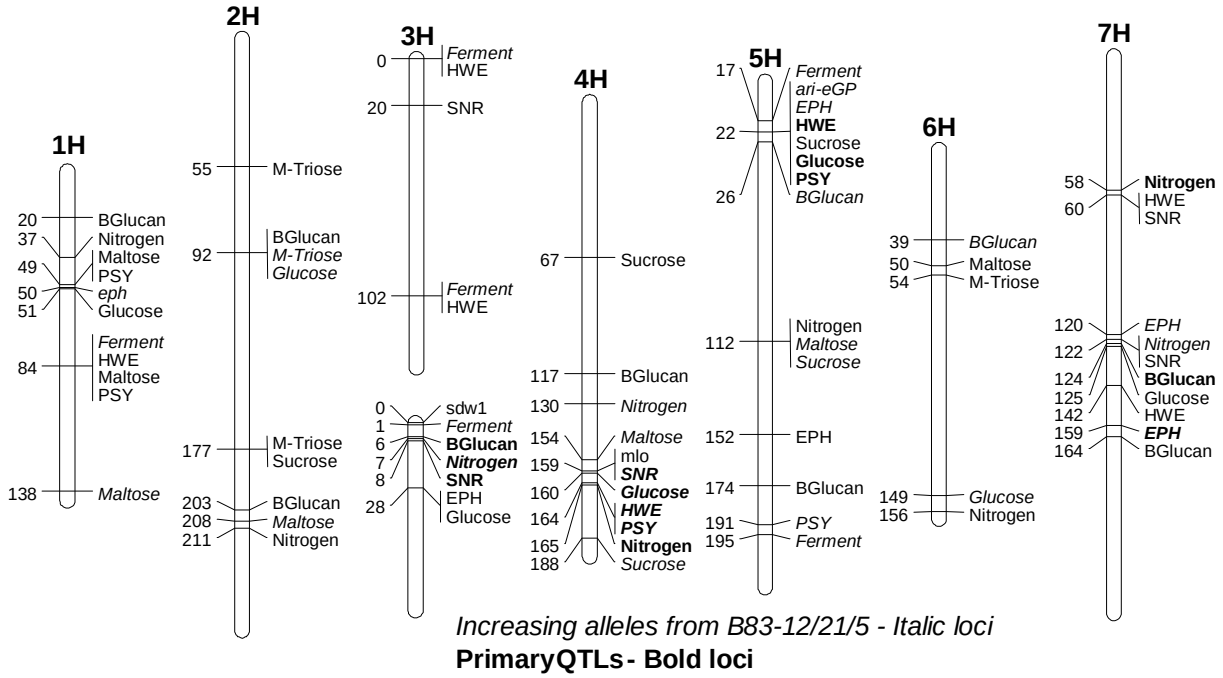


and another with >2 g/t of glycosidic nitriles. This means that results relating to quantitative EPH, whilst significant, should be interpreted with caution. Whilst the allelic constitution of the selected lines was in good agreement with the absorbance values, the agreement with glycosidic nitriles was generally poor. The only exception was at the secondary QTL locus on 5H in the region of *ari-e*GP, where 5 of the 6 lines >2g/t had the Derkado allele but only 5 of 10 lines in the 0.9-2g/t group. Comparison of the parental distribution of alleles revealed that other regions of the genome that may be involved in the control of glycosidic nitrile production were on 2H and in the region of *mlo* on 4H. Only one marker was, however, significantly associated with the character and that was Bare1P16e on the long arm of 2H.

Mapping of Traits

We detected 70 QTLs over all the characters measured in this study (Figure 6). Fifteen of these were primary QTLs in which we could have a lot of confidence. Nine of the primary QTLs were, however, associated with two currently important major-genes, *sdw1* on 3H and *mlo* on 4H, where there were few additional markers to exploit molecular breeding to identify suitable recombinants. This would be particularly desirable in the case of *mlo*, where the resistant allele is associated with a greater nitrogen content and hence under-modification and reduced hot water extract and predicted spirit yield. Another three primary QTLs were clustered in the region of *ari-e*GP on 5H but there were markers in this region which could be used to identify useful recombinants. The other phenotypic major gene in this study, *eph* on 1H, was associated with three secondary QTLs, all of which (Glucose, Maltose and PSY) could be viewed as related, with Derkado contributing the favourable allele. The remaining primary QTLs were located on 7H and there were no known major-genes segregating in the population that may have influenced expression.

Figure 6. Summary of QTLs for 11 malting quality characters mapped in Derkado x B83-12/21/5.



In general, the largest QTL detected for each character was located in the region of one of the phenotypic major-genes segregating in the population (Table 4). The exceptions to this were for epi-heterodendrin (7H), sucrose (distal on 4H) and maltotriose (2H long arm). The largest allelic effect was detected for hot water extract and predicted spirit yield in the region of *mlo* but this probably reflects the scale of measurement for these characters. The amount of phenotypic variation accounted for by the QTLs gives a more accurate indication of the magnitude of their effect and, for six of the 11 characters studied, the largest QTL accounts for over 10% of the phenotypic variation. Where characters had low amounts of genetic variation (Table 2), the percentage of genetic variation accounted for by the largest QTL was, not surprisingly, lower.

Table 4. Summary of the results of QTL mapping of 11 malting quality characters from random DHs from Derkado x B83-12/21/5.

	Largest QTL					Desirable QTLs from	
Character	Chromosome Location	Left-Hand Marker	Distance (cM)	Derkado Effect	Phenotypic Variance	Derkado	B83-12/21/5
Ferment	5H	Bmac96	0	-0.43	5	0	6
HWE	4H	mlo	5	-6.88	18	5	2
PSY	4H	mlo	5	-6.76	12	2	3
Nitrogen	3H	sdw1	7	-0.11	14	3	6
SNR	3H	sdw1	8	3.17	8	3	2
β-Glucan	3H	sdw1	6	-0.26	11	8	2
EPH	7H	P17M62a	7	0.21	13	2	3
Glucose	4H	mlo	1	-0.71	17	3	4
Sucrose	4H	HVM67	6	-0.02	6	2	3
Maltose	4H	Bare1E36a	13	-1.02	7	2	4
M-Triose	2H	Bmac144b	0	-0.37	3	2	2

Considerable clustering of QTLs was evident from Figure 5, which reflects the correlations between characters (Table 3). It is noticeable that hot water extract and predicted spirit yield are often associated in coupling but that fermentability and hot water extract are associated in repulsion. There was evidence of some positive association of the wort sugars with hot water extract and hence predicted spirit yield but there were other regions of the genome where this association was not apparent. The SSRs Bmag323 and Bmag337 flank the fermentability QTL on 5H and the Derkado QTL allele accounts for just under 0.5% decrease in fermentability. Off-setting this decrease would give an extra 3 litres of PSY that, if applied over the whole malt whisky industry, would translate into an extra production of 1 million bottles annually.

Molecular Breeding

The overall mean fermentabilities (Table 1) of the donor lines either exceeded or equalled that of B83-12/21/5, the high fermentability parent, although it was noticeable that the fermentabilities of those with Derkado alleles in the region of the QTL were lower than the others. The initial selection of lines had, therefore been successful and we could expect to improve the fermentability of the recipient parent. The majority of the BC1DHs being tested in the breeding trials resulted from crosses to donors with B83-12/21/5 alleles in the target region of 5H. With many of the BC1s that produced DHs being heterozygous in this region, we therefore expected to generate a large enough number of BC1DHs that would have donor alleles in region.

Table 5. Summary of recipient parent contributions to the genomes of approximately 250 BC1DH lines produced from the backcross programme.

	Landlord	B91 lines
Min	34.0	39.5
Mean	67.7	69.6
Max	84.1	95.7

Table 5 summarises the distribution of all the markers over approximately 250 of the genotyped BC1DH lines. Without selection, we would expect the average donor genome content to be 25% whereas it is slightly

in excess of this amount. However, 67% of the BC1 plants had the introgressed segment of the genome so may have biased the donor genome contribution. Additionally, the donor parents may have carried some genes increasing green plant production in doubled haploidy, which is more than feasible as they were the products of a doubled haploid programme.

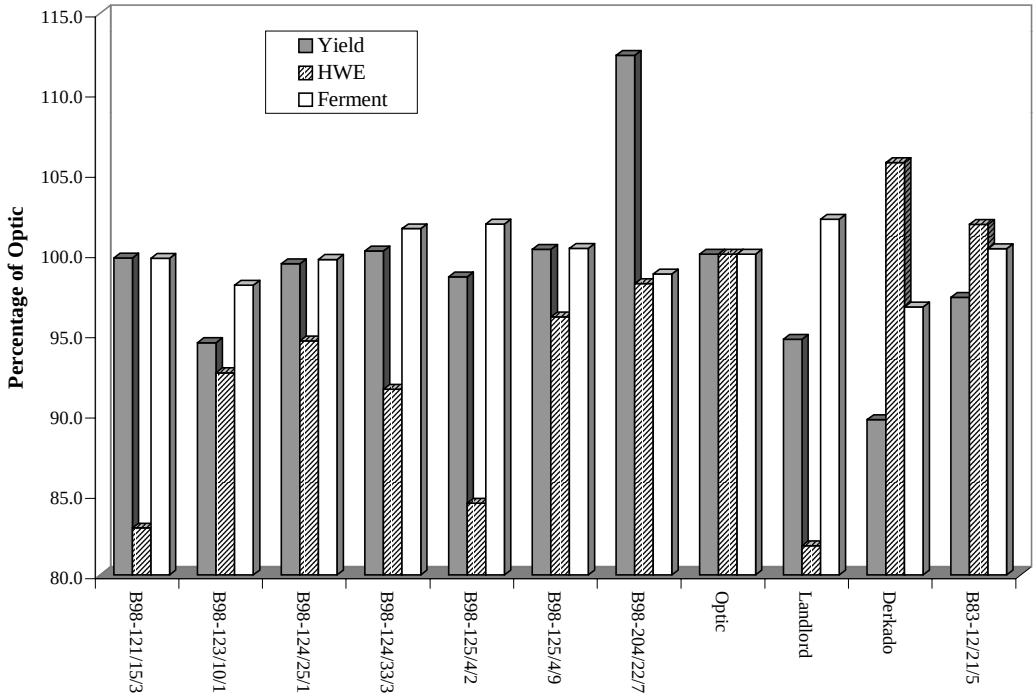
Table 6. Summary statistics of results from two field trials of 135 BC1DH lines and controls grown at SCRI in 2000. Numbers in Bold are significantly different from Landlord.

Character				BC1DHs			
	Landlord	Derkado	B83-12/21/5	Minimum	Mean	Max	SED
Yield(t/ha)	5.02	4.76	5.16	**4.07**	4.84	**5.96**	0.25
Head(days)	19.4	23.4	21.6	**15**	18.7	**28.5**	1.5
Height(cm)	61.8	64.3	60.5	**38.3**	56.9	**75.3**	2.8
Ext(%)-NIR	81.5	82.8	78	**76.5**	**80.0**	83.3	1
Grain Nitrogen(%)	1.25	1.34	1.38	1.27	**1.40**	**1.66**	0.08
HWE(L°/kg)	261	337	325	**210**	268	**317**	21
Fermentability(%)	82.4	78	80.9	**77.9**	80.9	83.7	1.6
PSY(l/t)	333	412	410	**267**	341	**406**	29
Soluble Nitrogen(%)	0.406	0.713	0.699	0.386	**0.514**	**0.665**	0.068
SNR(%)	32.4	56.5	51.9	26.6	38.2	**53.4**	5.7
Viscosity-NIR(sec)	12.6	12.1	18.6	**8.9**	**15.6**	**20.2**	1.2
Wort Viscosity(cP)	1.47	1.39	1.44	**1.36**	1.45	1.54	0.05

Data were collected from all the plots grown in trials at SCRI and Advanta Seeds in 1999 but, because the material was late and thinly sown, it could not be used to do much more than eliminate agronomically poor lines. Data from the selected lines grown in 2000 were of more value in evaluating the effect of the high fermentability QTL in a different genetic background. Despite delaying malting until dormancy had been broken, however, the samples from the SCRI trials generally malted poorly with very low extracts (Table 6). Landlord also performed poorly and inspection of the data reveals that it and many of the samples were under-modified. In contrast, the two parents of the donor lines, Derkado and B83-12/21/5 malted normally and there were some lines that malted considerably better than Landlord.

There was significant genetical variation for all the characters measured on the 2000 trials apart from fermentability, which had probably been adversely affected by the uneven modification of samples. Apart from soluble nitrogen, the mean of the BC1DHs did not differ significantly from Landlord, confirming that their general behaviour reflected a Landlord background. In contrast, the results from the NIR analysis all showed that the mean of the population was significantly worse than Landlord as grain nitrogen and viscosity was higher and extract lower. The presence of lines with the *ari-e*GP gene in the population, which has previously been shown to adversely affect malting quality characters (Thomas et al., 1991), may have biased the results from the NIR analysis. Further evidence of this can be seen in the significant reduction in the mean height, due to the presence of lines expressing both dwarfing genes in the population. The extremes of the BC1DH population transgressed the means of the three parents for each character with many of the differences being significantly different from Landlord (Table 6). This indicates that there is germplasm with breeding potential amongst the lines. Seven lines were selected from the 2000 trials for further trialling in 2001 and a brief summary of their performance relative to Optic is given in Figure 7. The selections were all

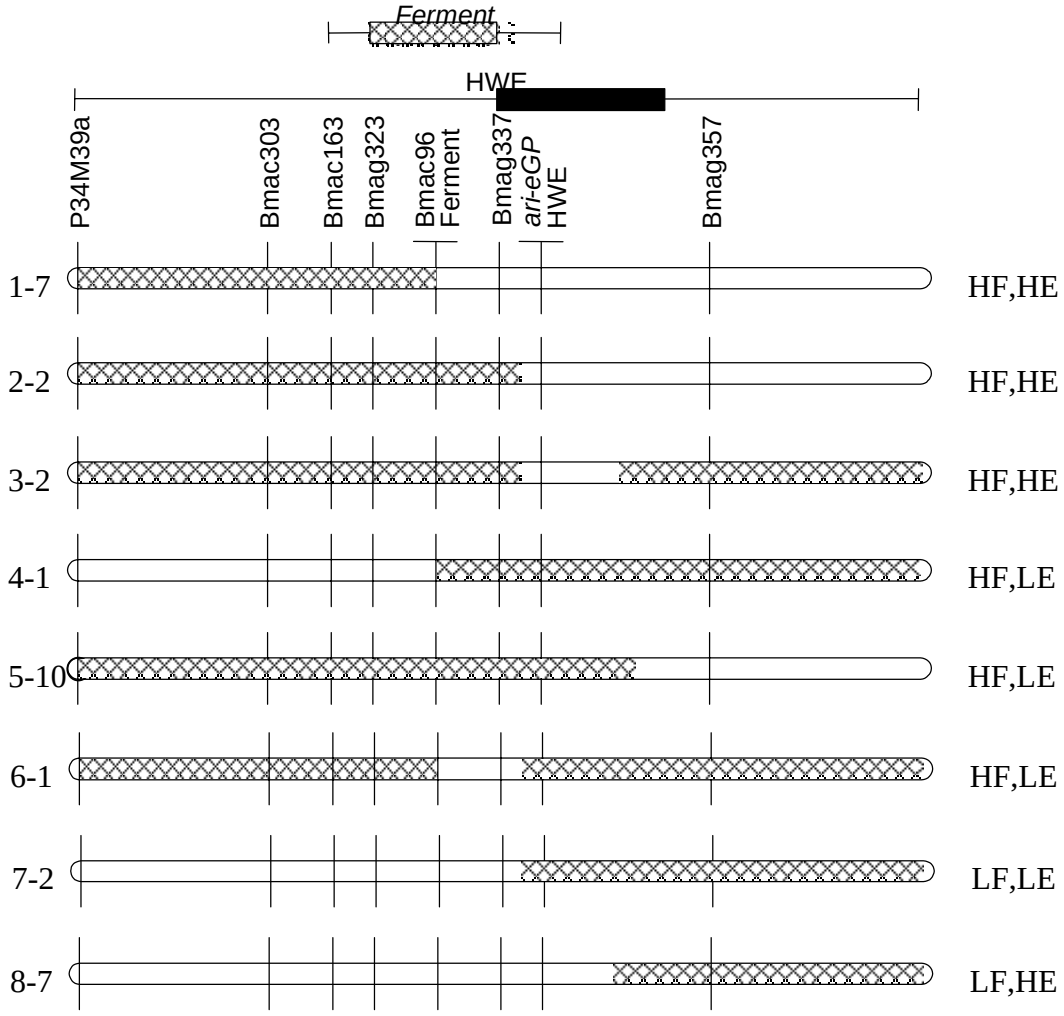
Figure 7. Performance relative to Optic of 7 BC1DH lines selected for further trials in 2001. Characters are means of 3 trials carried out in 2000.



made according to yield and agronomic merit before any malting results were available. All the lines yielded better than either Derkado or Landlord and most exceeded the best parent B83-12/21/5. One line showed outstanding yield potential over all three trials and three others had the same or slightly better yields than Optic. The effect of the poor micro-malting performance of the samples from the 2000 SCRI trials can be seen as Landlord and two other lines had very low extracts. None of the other lines had very good extracts although some did have higher fermentabilities. Whilst Landlord was not noted for high fermentability, it had a higher fermentability than B83-12/21/5 in the 2000 SCRI trial. The fermentabilities of both B83-12/21/5 and Derkado were low, however, and both had high SNRs, which indicates that they were over-modified and could, therefore, have increased levels of non-fermentable protein-derived material in their extracts.

The above statistics give the general properties of the population under study but, more relevant, is the comparison of lines carrying the introgressed segment of genome that we predicted to carry the high fermentability allele from B83-12/21/5. Figure 8 illustrates the portion of chromosome 5H that we were trying to manipulate. The QTL peaks for fermentability and hot water extract are some 5 cM apart with the 1 LOD confidence intervals almost overlapping. With the limited size of the BC1DH population, we did not therefore expect to generate many recombinants within this region. Figure 8 also shows the graphical genotypes of the 8 recombinant groups that we detected within the target region of the genome. Using the position of the QTL peaks, we assigned the 8 groups into the four combinations of high and low extract and fermentability but, because the fermentability peak is equidistant from Bmag323 and Bmag337, we could not be sure which fermentability allele groups 1, 4 and 6 might have. In these cases, we assigned them to high fermentability groups but recognise that they could be low fermentability. The majority (103) of the lines can

Figure 8. Graphical genotypes of BC1DH recombinants in the target region of chromosome 5H. The hatched portion represents the portion of the donor genome introgressed with fermentability (F) and extract (E) predicted to be high (H) or low (L) (right hand side of bar). Numbers of lines in 2000 trials are on the left hand side of the bar. Note two double recombinant groups were detected.



be classified as having the parental genotype in the target region of 5H, i.e. donor genotype with high fermentability and low extract (HF,LE) or recipient genotype with low fermentability and high extract (LF,HE).

From the Docking trial, it is clear that the parental groupings follow the predicted outcome in that the B83-12/21/5 group has a higher fermentability and a lower extract and these differences are significant (Table 6a). The recombinant groups do not, however, follow the predicted patterns for fermentability as all are lower than group 7 (LF,LE), where recombination has taken place between Bmag337 and *ari-e*GP. There does, however, appear to be some agreement in the predicted outcomes for extract, the exceptions being groups 4 and 6 (HF,LE), which have relatively high extracts but are only represented by one line each. Given the low numbers of lines in the recombinant groups and the low heritability of fermentability, it is perhaps not surprising that the means of the recombinant groups do not follow the predicted patterns but there does appear to be some association of the *ari-e*GP dwarfing gene with high fermentability. With the exception of

the LF,HE group (8), the mean fermentabilities of the groups with the dwarfing gene are higher than those without.

Table 6. Means of malting quality characters analysed on samples from 2000 BC1DH trials grown near Docking (a) and at SCRI untreated (b) and treated (c).

a.)

Group	*ari-e*GP[1]	NIR Ext	Ferment	HWE	PSY	Grain N	Sol N	SNR	Viscosity
Donor	Yes(51)	78.8	80.1	310	391	1.67	0.674	43.1	16.8
Recipient	No(53)	79.2	79.3	315	393	1.66	0.707	45.2	16.6
1-HF,HE	No(7)	79.3	79.2	316	395	1.67	0.772	49.2	15.7
2-HF,HE	No(2)	78.8	79.3	321	401	1.71	0.698	43.6	14.9
3-HF,HE	No(2)	80.3	77.1	316	384	1.70	0.701	43.5	14.8
4-HF,LE	Yes(1)	78.4	80.6	317	403	1.71	0.672	41.2	16.8
5-HF,LE	Yes(10)	79.9	80.5	308	391	1.68	0.681	43.6	15.1
6-HF,LE	Yes(1)	79.7	80.5	322	408	1.61	0.767	50.8	11.4
7-LF,LE	Yes(2)	78.5	80.7	306	388	1.70	0.757	47.1	15.1
8-LF,HE	No(7)	78.9	80.2	319	403	1.68	0.657	42.0	16.0
SED		0.9	2.0	8	17	0.15	0.083	7.1	0.5

b)

Group	*ari-e*GP[1]	NIR Ext	Ferment	HWE	PSY	Grain N	Sol N	SNR	Viscosity
Donor	Yes(51)	78.5	81.6	246	316	1.43	0.457	33.4	18.2
Recipient	No(53)	79.8	81.3	274	351	1.27	0.500	39.9	17.1
1-HF,HE	No(7)	79.4	81.8	271	348	1.33	0.491	38.7	16.8
2-HF,HE	No(2)	79.4	78.9	282	351	1.43	0.565	43.3	17.2
3-HF,HE	No(2)	80.5	80.8	282	361	1.31	0.525	47.3	16.9
4-HF,LE	Yes(1)	78.2	84.2	275	372	1.34	0.470	38.1	18.3
5-HF,LE	Yes(10)	79.3	81.5	242	311	1.42	0.407	29.1	16.9
6-HF,LE	Yes(1)	78.2	78.5	316	389	1.50	0.670	48.8	17.9
7-LF,LE	Yes(2)	80.5	83.5	247	322	1.34	0.460	35.2	14.2
8-LF,HE	No(7)	80.4	81.8	264	340	1.24	0.489	42.0	15.5
SED		0.7	1.098	16.860	20.811	0.045	0.071	5.703	1.104

c)

Group	*ari-e*GP[1]	NIR Ext	Ferment	HWE	PSY	Grain N	Sol N	SNR	Viscosity
Donor	Yes(51)	79.7	81.2	229	292	1.35	0.401	31.4	16.0
Recipient	No(53)	80.9	81.7	264	340	1.20	0.455	39.4	14.8
1-HF,HE	No(7)	81.0	82.2	255	330	1.25	0.420	35.3	14.3
2-HF,HE	No(2)	80.7	80.5	263	334	1.30	0.455	37.3	15.1
3-HF,HE	No(2)	80.9	80.9	267	342	1.23	0.458	41.8	15.2
4-HF,LE	Yes(1)	80.0	83.4	257	341	1.30	0.419	34.6	16.2
5-HF,LE	Yes(10)	80.5	81.2	225	288	1.34	0.371	28.7	14.6
6-HF,LE	Yes(1)	80.5	78.8	238	293	1.34	0.461	35.6	14.0
7-LF,LE	Yes(2)	81.5	82.8	224	292	1.26	0.378	31.1	12.8
8-LF,HE	No(7)	81.5	81.6	249	320	1.16	0.433	39.8	13.6
SED		0.9	2.0	22	29	0.07	0.069	5.9	1.2

[1] Numbers in parentheses are the number of lines in each group.

Results from the SCRI trials provide some support for these findings, mainly from the untreated trial (Table 6b). The mean fermentability of the B83-12/21/5 group of BC1DH lines was higher than that of the Derkado group and the mean extract was lower, although only the latter was significant. Amongst the recombinant groups, three of the HF,HE groups had a higher mean fermentability than Derkado, two of which (4 and 5) were the same as detected in the Docking trial. Only one of these differences was, however, significant. Group 7 (LF,LE) also had a significantly higher mean fermentability than Derkado but it was not as high as

that of group 4 (HF,HE). With one exception, the groups with the *ari-e*GP gene again had high fermentabilities. Extract generally followed the predictions with the donor being significantly less than the recipient. The low extract groups were, with the exception of group 6, not significantly greater than the donor group.

Results from the treated trial (Table 6c) provide less support for the action of the 5H fermentability QTL as the recipient group had a higher mean fermentability than the donor group. As in the untreated trial, groups 1 and 4 had a higher mean fermentability than the recipient group, however, and two other groups had mean fermentabilities equal to that of the donor group. As in the two other trials, group 7 (LF,LE) had a high mean fermentability but not quite as high as group 4. Extracts were, again, more consistent with their predicted performance with only group 4 of the low extract groups having a higher figure than any of the high groups. As already noted, the samples from the SCRI trials malted poorly but the untreated trial malted relatively better than the treated trial as can be seen from the higher extracts for each grouping. The SCRI data therefore highlights potential processing problems in malting but probably do not give a true test of the malting potential of the lines.

None of the 17 BC1DH lines submitted for HGSY analysis by SWRI carried the *ari-e*GP dwarfing gene and the lines could therefore be split into four groups. No recombination had occurred across the target region in 7 lines and they resembled the recipient genome. The other three groups were formed from recombination events between Bmag323 and Bmag337, Bmag337 and *ari-e*GP, and *ari-e*GP and Bmag357 and contained 2, 3 and 5 lines respectively. We would expect the groups with 2 and 3 lines to have high fermentabilities. For the HGSY analysis, we can calculate the fementability as being the difference between the initial and residual gravities expressed as a percentage of the initial gravity. Whilst these two groups show a slightly higher mean fermentability than the other two, the results are far from conclusive and their mash-tun extracts are significantly lower so there is no advantage in PSY. In addition, the two groups have a higher sparge loss, indicating possible processing problems with the recombinant groups. The HGSY analysis was conducted on malt produced from the treated trial grown at SCRI in 2000, so will have been affected by the same problems that affected the micro-malting results. It is thus difficult to draw any conclusions but results of the flavour analyses conducted by SWRI on the genotypes showed that none were associated with any adverse tastes.

Table 7. Mean Results of HGSY analysis of selected samples from 2000 SCRI treated trial of BC1DH lines

Group	Ferment	Extract	PSY	Sparge Loss
HF(5)	78.9	72.7	372	2.89
LF(12)	78.7	74.3	380	2.03
SED	2.5	0.8	15	0.49

Regression analysis of the phenotypic and genotypic data collected on the means from the SCRI trials and the Advanta trials revealed a number of significant associations. For clarity, we will just present the results from the most relevant malting quality characters below. Apart from fermentability at both SCRI sites, and soluble nitrogen and SNR at the untreated site, the marker associations at SCRI accounted for more variation than at Docking. This probably reflected the range of variation found in the characters measured after micro-

malting at SCRI. Despite this, it is clear that measurements of hot water extract following micro-malting detect more genetic variation than do NIR predictions of extract and that fermentability is subject to far more environmental variation than hot water extract.

Table 8. Summary results of QTL associations found in BC1DH lines for some malting characters measured on samples from 2000 trials at Docking (a) and SCRI untreated (b) and treated (c).

a)

	Most Sig Marker	Increasing alleles from		
Character		Recipient	Donor	%Variation
NIR-Extract	**Bmag222**	3	1	27.7
Hot water extract	**Bmag211**	3	1	34.9
Fermentability	**Bmag500**	2	1	17.3
Grain Nitrogen	Bmag211	1	1	13.8
Sol Nitrogen	HvLTPPB	5	3	48.7
SNR	**Bmag211**	7	2	50.0

b)

	Most Sig Marker	Increasing alleles from		
Character		Recipient	Donor	%Variation
NIR_Extract	***ari-e*GP**	4	3	42.6
Hot water extract	Bmac273a	3	4	51.1
Fermentability	Bmag222	0	1	5.0
Grain Nitrogen	Bmag323	1	4	57.9
Sol Nitrogen	Bmac273a	1	3	23.6
SNR	***ari-e*GP**	4	3	41.2

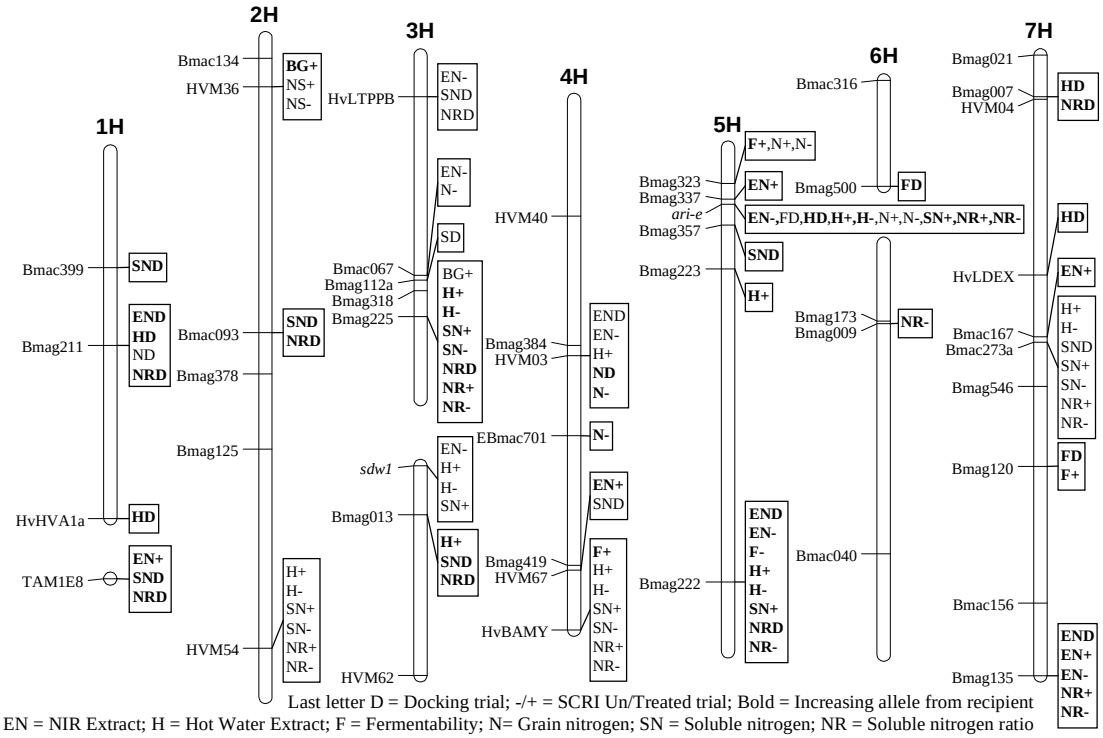
c)

	Most Sig Marker	Increasing alleles from		
Character		Recipient	Donor	%Variation
NIR_Extract	**Bmag337**	5	0	39.7
Hot water extract	***ari-e*GP**	5	5	69.3
Fermentability	**HvBAMY**	3	0	13.5
Grain Nitrogen	*ari-e*GP	0	3	59.1
Sol Nitrogen	Bmac273a	3	4	51.9
SNR	Bmac273a	4	3	63.0
Beta-glucan	**HVM36**	1	1	7.5

Each character was associated with at least one marker and there were 25 cases where results agreed between at least two of the trials (Figure 9). Recipient alleles increasing hot water extract, NIR predictions of extract, soluble nitrogen, and SNR at both SCRI sites and Docking were found at *ari-e* (5H), Bmag135 (7H), Bmac273a (7H), and Bmag225 (3H) respectively. Apart from soluble nitrogen, recipient alleles produced the greatest expression of the characters. The remainders of the agreements were between two of the three trials and were largely between the SCRI trials and involved all the characters apart from fermentability. There were six notable clusters of associations at HVM54 (2H), Bmag225 (3H), HvBAMY (4H), *ari-e* (5H) and Bmac273a (7H). In each case, the increasing allele associations were generally either recipient or donor, which would be consistent with a particular genomic region affecting a number of malting quality parameters. The association of the *ari-e*GP allele with a decrease in hot water extract is consistent with the findings from the mapping study but contrasting results for the target fermentability locus were obtained from the trials. 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.

Figure 9. 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 2000 trials of BC1DH lines.



Last letter D = Docking trial; -/+ = SCRI Un/Treated trial; Bold = Increasing allele from recipient

EN = NIR Extract; H = Hot Water Extract; F = Fermentability; N= Grain nitrogen; SN = Soluble nitrogen; NR = Soluble nitrogen ratio

Some consistency between the results found in the BC1DH population and the mapping population can be observed in other regions of the genome. Loci found to be affecting hot water extract in the mapping population on 1H, 3H, 4H and 7H are in similar regions to those found in the BC1DH population at Bmag211, Bmag225, HVM67 and Bmac273a respectively. In addition to the target region another region affecting fermentability detected on 5H in the mapping population corresponds to that detected at Bmag222 in the untreated BC1DH trial at SCRI. The other character for which some common regions can be observed is SNR, where two regions on 3H and one on 7H detected in the mapping population correspond to HvLTPPB, Bmag013 and Bmac273a respectively.

Discussion

We have successfully carried out a comprehensive analysis of malting quality characters relevant to the distilling industry. We have demonstrated that there is genetic variation for fermentability and that whilst it is negatively correlated with hot water extract there is the possibility to manipulate both to produce an increase in Predicted Spirit Yield. Indeed, this is what commercial maltsters do to produce distilling malt by balancing the development of extract versus fermentability during germination. In addition, the results

obtained from our small-scale laboratory tests for fermentability have been shown to be relevant to commercial distilleries, despite the much wider range in the distilling potential of the lines that we studied. Whilst fermentability was positively correlated with Predicted Spirit Yield, the correlation was low, which probably reflected its low heritability. Fermentability was quite highly negatively correlated with SNR, which probably reflects the high mean SNR observed over all the mapping population trials, indicating relative over-modification in the mapping trials.

The QTLs detected in the mapping population for fermentability and hot water extract reflected the correlation between the characters. Four of the six QTLs found for fermentability were also at, or very close to, loci detected for hot water extract and increasing alleles for one character from one parent were associated with decreasing alleles for the other. The association between SNR and fermentability QTLs was less pronounced with two of the four SNR loci in the region of fermentability QTLs but not very close. The other noticeable feature of the results of the QTL mapping was the clustering of loci around four regions of the genome. Three of these were in the vicinity of the major genes *sdw1*, *mlo* and *ari-e*GP on chromosomes 3H, 4H and 5H respectively. The fourth was on 7H but no major genes were known to be segregating in this region of the genome. This last region does appear to be of major importance in North American germplasm as QTLs affecting extract, grain nitrogen and beta-glucan were detected in its vicinity in Steptoe x Morex, Harrington x TR306 and Harrington x Morex (Zale et al., 2000).

Previous results had indicated that both *sdw1* and *ari-e*GP were associated with effects upon malting quality characters (Thomas et al., 1991, 1996; Bezant et al., 1997) but *mlo* had not previously been associated with any malting quality characters. The association was such that the resistant allele was associated with a reduction in malting quality characters. This may reflect an increase in grain nitrogen content associated with resistance leading to a reduction in modification but the effect of the resistant allele upon nitrogen content is only +0.08% and it is hard to envisage such a small increase having such a drastic effect upon the other characters. There is little evidence of the region having a large effect upon malting quality parameters from the results of other studies, although a QTL for grain nitrogen was detected in the North American barley cross Harrington x TR306 (Zale et al., 2000). It therefore appears that the introduction of the *mlo* gene has either resulted in the introgression of some deleterious malting quality characters through linkage or that the gene has a pleiotropic effect upon malting quality. With the publication of sequences containing the *mlo* gene (Buschges et al., 1997), it is now possible to carry out accurate fine mapping studies in the region of the gene and this should resolve whether or not pleiotropy is the cause.

In addition to the QTLs found in Blenheim x E224/3 (Thomas et al., 1996), a QTL for grain nitrogen was found in the North American cross Steptoe x Morex (Zale et al., 2000) in a region comparable to that found near *sdw1* in the mapping population of the current study. The hot water extract QTL found on 5H in the current study is in the same region as QTLs found in Blenheim x E224/3 and Dicktoo x Morex (Zale et al., 2000) but none of these parents carries dwarfing alleles at the *ari-e* locus. In the current study, the *ari-e*GP allele co-segregates with an allele for reduced hot water extract, which suggests a pleiotropic effect of the dwarfing gene, although Swanston et al. (1990) did not detect any differences in extract between Golden

Promise and its parent cultivar, Maythorpe. If the loci detected in Blenheim x E224/3 and Dicktoo x Morex are the same, then the association may be due to very close linkage, rather than pleiotropy and it should be possible to produce lines with the dwarfing gene and high extract.

Results from the study of wort sugars were inconclusive, probably because of the incomplete data that we were able to generate within the project. The principal components analysis and some of the QTL mapping results indicated an association between glucose levels and hot water extract but there was little other consistent evidence of links between sugar levels and either extract or fermentability. This is a surprising result but may reflect a general over-modification of the micro-malts in the mapping trials. Alternatively, conversion of sugars to starch will continue during fermentation so that not all the fermentable sugars will be measured in the wort dextrin profiles. More complete studies on populations generated from crosses between diverse and similar parents and conducted under a different malting regime are required before some firm conclusion can be drawn about the influence of wort sugars upon malting quality parameters.

The results from the attempt to transfer the fermentability QTL detected on chromosome 5H in the mapping population to a more contemporary cultivar are inconclusive. The mapping results suggested that the fermentability QTL was linked in repulsion to a hot water extract QTL and our strategy was to generate recombinant backcross inbred lines through a combination of genotyping and doubled haploidy that would combine high fermentability and hot water extract. Because of time constraints within the project, we were not able to generate sufficient numbers of doubled haploid lines to give us a chance of achieving our target and we adopted a more random approach in our evaluation of the doubled haploids. Nevertheless, we tested sufficient lines that would enable us to compare the effect of the donor with that of the recipient alleles over the target region of the genome but there were few recombinants in this region. Analysis of the results has been further complicated by the unexpectedly poor malting performance of a number of lines from the SCRI trials.

We found that extracts were very low from the SCRI sites and, in this situation, most of the samples had been under-modified. In contrast, extracts from the Docking site were very good with some degree of over-modification. In this situation we would expect distinguishing between high and low fermentability lines to be difficult from the SCRI data as malting had obviously not proceeded sufficiently to render all the fermentable material extractable. The means of the BC1DH lines show that extract is low and under-modified and, under such circumstances, one would expect a higher mean fermentability. The fact that it is relatively low suggests that all samples had a relatively high amount of unfermentable material, under which circumstances one would not expect to detect many differences in fermentability. Fermentabilities from the Docking results were also low but probably more typical of commercial malts despite some over-modification. We can therefore conclude that the Docking malting results are more reliable for the purposes of interpretation and that the increase in fermentability due to alleles of the donor genome on 5H is probably genuine but a further year of trials would be necessary to verify this conclusion.

The poor malting performance of samples from the SCRI trials in 2000 was surprising as we delayed malting until 5 months after harvest. We even attempted a repeat micro-malting after another 4 months had elapsed

but abandoned it when it was clear that germinations were still abnormally low from a number of lines. The effect appears to be associated with Landlord as it performed poorly but some other controls, notably Derkado and Optic malted normally and gave high extracts. In addition, 10 of the 135 BC1DH lines had a B91-99/15 background and also gave more normal micro-malting results. In contrast, Chariot, a parent of Landlord, also did not micro-malt well so there appears to have been some environmental factors present at SCRI in 2000 that induced some genotypic differences in germination. A water senstivity test of some of the samples from the SCRI treated trial showed that germination was still very poor some 10 months after harvest with 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. It does highlight the problem of using a backcrossing strategy to meet a commercial target. Our strategy of choosing 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 upon such cultivars is, despite a large amount of yield trial data, limited 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.

In the target region of the fermentability QTL on 5H, there is a significant association of the character with *ari-e* (P <0.01) but the associations with the flanking markers Bmag323, Bmag337 and Bmag357 are not quite significant. This suggests that the fermentability locus is more closely associated with the dwarfing gene than results from the mapping study indicated and may even be a pleiotropic effect of the dwarfing gene itself. Again, a more detailed study of the region in a common genetic background is required to address the issues of whether or not the QTLs for hot water extract and fermentability in the region of the dwarfing gene are loci that can be deployed in breeding programmes or just pleiotropic effects of a major gene.

We have used relatively diverse parents in our attempt to detect QTLs for fermentability, which should have maximised our chances of finding robust effects. Despite this choice of parents, the most significant QTL that we detected was subject to interactions with the environment and it was a Secondary rather than a Primary QTL. With the low heritability of the character, the target QTL accounted for just 6% of the phenotypic variation in fermentability, although it was the largest effect detected. Detecting such a QTL in another genetic background will be difficult and one would expect that it may not be significant in a number of instances. From the statistics obtained from the over trials analysis, we estimate the number of genes controlling fermentability to be 14. Producing recombinant inbred lines with desirable alleles at all loci would therefore require a far larger population than we were able to generate within the project. Using marker-assisted selection to reduce the population size down to lines that carried the target QTL would, however, maximise the chances of detecting recombinant inbred lines with high fermentability.

The results of the BC1DH trials provide some support for the successful transfer of the target QTL to another genetic background but appear to confine it to one with the *ari-e*GP dwarfing gene. Whilst the cultivar Golden Promise carried this gene and was used in great quantitities by maltsters and distillers, it was never regarded as a top-class malting quality cultivar. It may well be that QTLs detected in crosses between moderate and good quality cultivars are not sufficiently expressed in a good malting quality background to detect a significant effect. In such cases, we will not therefore be able to derive the benefit anticipated from estimates of the effects at QTL loci, which are notoriously unreliable (Utz et al., 2000). With the sequencing of the complete genome of rice (http://www.rice-research.org), it is now possible to conduct whole genome analysis of complex biological traits such as malting quality and related parameters, which may lead more promising results. In such cases, comparisons are usually made between extreme genotypes for a character and the possible problem of transferring potential beneficial alleles from poor to a good background without losing expression remains. Alternative mapping approaches such as association genetics (Jorde, 2000) may identify more robust loci to deploy in marker-assisted selection programmes for traits of low heritability. It is also possible that the use of a number of small populations from different crosses, an approach we term Small Cross Mapping, may also yield more robust results. This approach would be especially useful in the analysis of crosses between elite parents, which have not been examined in single cross studies due to the relatively low levels of polymorphism between any two parents. By examining a range of parents, Small Cross Mapping overcomes this problem as a pilot study on 10 elite lines has shown that SSRs are polymorphic 50% of the time. This means that studies can be carried out completely on germplasm that is relevant to commercial breeders and the results should be more applicable than in the past.

The discrepancies between the quantitative scores of EPH by absorbance measures and the absolute values from the analysis of samples from the pilot still could reflect a number of factors. Clearly selection of low absorbance values does not guarantee low levels of glycosidic nitriles in the final spirit from malts of epi-heterodendrin producers and further work is necessary to identify factors leading to low glycosidic nitrile levels amongst producers. From the results of this project there is, however, the possibility to select non-producers of epi-heterodendrin either by plant breeders or the testing authorities.

We identified markers that were linked to the *eph* locus on 1H (Swanston et al., 1999). This means that these markers could potentially be used to eliminate epi-heterodendrin producers from distillery malts. The SSRs linked to *eph* were sufficiently close to mean they could be effectively used in selection but a recombination event was found to have occurred between the marker and the locus in the cultivar Cooper. Without an effective flanking marker, this limits the usefulness of a marker-assisted selection scheme for the character. Extra work was done within the project to attempt to locate alternative markers and initially concentrated upon a flanking AFLP product but partial sequencing of the product failed to identify any base differences (SNPs) between the parents. The publication of a combined physical and genetical map of barley (Kunzel et al., 2000) and the availability of primers to amplify RFLP probes (Graner et al., 1999) provided a means of locating alternative markers. We were able to locate the region in the interval between MWG896 and MWG913 on the combined map of chromosome 1H. SNPs from these probes provide flanking markers for *eph* but, for effective marker-assisted selection, there is a need to generate closer markers and, ideally,

markers within the gene itself. ABG500 is located within this region but sequencing of PCR products from the parents failed to reveal any differences. Some fine mapping in a larger population is required to identify markers close to the gene and there would then be the opportunity to identify BACs likely to contain the gene. This would provide a means not only to identify SSRs close to the gene (Cardle et al., 2000) but also lead to cloning the gene itself.

Our results from the genotyping of the BC1DH population show that the donor genome contribution is higher than expected and is also higher than that found in a similar study of stripe rust resistance introgression into the cultivar Steptoe (Toojinda et al., 1998). It is unlikely that the differences reflect different selection strategies as, if anything, the selection pressure in the current study was less and would therefore be likely to have resulted in an decreased donor genome content compared to the stripe rust study. It is more likely that our strategy of using previously mapped markers, especially SSRs, has ensured that we have carried out a reasonably comprehensive genome survey. AFLP markers that were largely anonymous were used in the study of stripe rust resistance and it may well be that many were clustered and did not adequately sample the entire genome. Such a situation could, therefore, artificially reduce the apparent donor genome contribution.

Increased β-amylase thermostability has been reported to lead to increased fermentability as measured by Apparent Attenuation Limit (Eglinton et al. 1998; Kihara et al., 1998). The former study refers to three different β-amylase thermostability alleles, Low Medium and High that can be found in cultivated barley. Using a previous classification, the Sd1 β-amylase allele appears to confer medium thermostability but the Sd2 allele is associated with all three levels of thermostability The major difference appears to be that cultivars with a low thermostability level have a lower mean fermentability than the other two. The parents of the mapping population, Derkado and B83-12/21/5, could not be distinguished by IEF analysis of β-amylase (Forster et al., 1991) and, as ancestral lines of B83-12/21/5 possess the Sd2 allele (Eglington and Evans, 1997), we can assume that Derkado also possesses Sd2. The Sd2H allele is not found in European spring barley cultivars so both must possess Sd2L or SdM. An SSR in the third intron of the published β-amylase sequence (EMBL: HVU301645; Erkkila, 1999) reveals a polymorphism between Derkado and B83-12/21/5. This SSR also revealed a polymorphism between Landlord and the donor lines, which carried the Derkado allele.It is likely that Landlord carries the Sd1 β-amylase allele and would therefore have the medium level of thermostability (Eglington et al., 1998; Kihara et al., 1998) and it is interesting to note that this allele was associated with an increase in fermentability from the analysis of samples obtained from the 2000 BC1DH trial at SCRI. Donor alleles at this locus were associated with increases in SNR and hot water extract and, given the abnormal malting performance of many samples from this trial, the association of Sd1 with increased fermentability needs to be validated and could also reflect differences in β-amylase activity. The results from the Docking site provided no indication of a significant effect upon any of the three characters, neither in multiple nor single marker regressions, although they were in the same direction as the SCRI results.

The work carried out in this project focused upon an anonymous gene, or linked genes, affecting fermentability. There is evidence that the gamma-ray muation of Maythorpe to produce Golden Promise (*ari-e*GP) resulted in an increased rate of modification (Swanston et al., 1990). In cultivars with moderate or poor malting quality, e.g. B83-12/21/5, such a gene could well have a beneficial effect upon malting parameters. In a good malting quality background, the gene could lead to greater malting loss and actually reduce fermentability and this is a weakness of the anonymous approach that we used in our QTL transfer study.

The targeting of specific genes in a whole genome approach may provide a better means of increasing fermentability whilst at least maintaining hot water extract. The introgression of the Sd2H β-amylase allele may be one means of achieving this aim, especially as it is not present in European spring barley cultivars. Eglinton et al. (1998) also described a *Hordeum spontaneum* accession with enhanced β-amylase thermostability, which they term Sd3. A small-scale survey of β-amylase variation in *H. spontaneum* accessions from a number of sites in Israel revealed four novel alleles by iso-electric focusing (Chalmers et al., 1992). *H. spontaneum* grain contains little endosperm compared to cultivated barley making valid comparisons of enzymes levels difficult (Swanston, pers. comm.) but there does appear to be variation that could be of value in increasing β-amylase thermostability. Introgression of the character would require the careful deployment of marker-assisted selection to minimize deleterious effects of the donor genome. Another means of increasing fermentability might be through the targeted manipulation of the binding and release of limit dextrinase. It has recently been shown that bound limit dextrinase can survive mashing and activity of the enzyme can increase through release during fermentation (Walker et al., 2001). Maintaining the binding of limit dextrinase during mashing and facilitating its release during fermentation may provide further increases in its activity and lead to increased fermentability. A detailed study of limit dextrinase and its inhibition would be required to examine the feasibility of this approach and provide a protocol to search for naturally occurring variants that would behave in the desired manner. The removal of the inert husk through the development of naked malting barley would provide a quantum leap in hot water extract, and hence spirit yield (Pearson et al., 1998), especially if the industry adopts new filtration methods that obviate the need for a bed of husks. Even in the absence of new filters, it would be possible to obtain enhanced spirit yield from mixtures of naked and husked barley. Careful evaluation of the suitability of the Scottish climate to the growing of naked barley would be required, as would a feasibility study on a suitable premium that could be paid to farmers to offset the lower yield incurred through the removal of the husk.

Through the publication by SWRI of various reports detailing aspects of the project, this project has increased the dialogue between geneticists, breeders and end-users. This has improved each group's appreciation of the potential uses of molecular biological methods to exploit natural variation for relevant end-user targets. SWRI plans to hold a dissemination event to maintain and develop this information flow in order to identify future targets and projects that will result in tangible benefits all the way along the supply chain to the end-user. Given that other leading exporters of malting barley are investing heavily in levy-board funded Research and Development to improve malting barley (e.g. Australia), we see such networks as being essential to not only a healthy domestic market but also maintaining and extending the export market.

The methodologies developed within this project have demonstrated that markers can be used for effective selection of single gene targets, e.g. *eph*. Here, there is the possibility that breeders and/or the testing authorities could apply the markers identified within the project to develop non-producers of epi-heterodendrin for use within the distilling industry. This methodology can be applied to other single gene targets such as β-amylase thermostability.

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References

Bezant, J.H., D.A. Laurie, N. Pratchett, J. Chojecki and M.J. Kearsey. (1997b). Mapping of QTL controlling NIR predicted hot water extract and grain nitrogen content in a spring barley cross using marker-regression. *Plant Breeding* **116**: 141-145.

Bringhurst, T.A., Brosnan, J.M., McInnes, B. and Steele, G.M. (1996) Methods for determining the fermentability and predicted spirit yield of distilling malts. *Journal of the Institute of Brewing* **102:** 433-437

Brown, A.T., and Morrall, P. (1996). Determination of repeatability and reproducibility of a new rapid enzyme method for the determination of glycoside nitrile in malted barley. *Journal of the Institute of Brewing* **102**: 245-24

Buckee G.K. (1994). Determination of total nitrogen in barley, malt and beer by Kjeldahl procedures and the Dumas combustion method - collaborative trial. *Journal of the Institute of Brewing* **100**: 57-64

Buschges, R., Hollricher, K., Panstruga, R., Simons, G., Wolter, M., Frijters, A., van Daelen, R., van der Lee, T., Diergaarde, P., Groenendijk, J., Topsch, S., Vos, P., Salamini, F., and SchulzeLefert, P. (1997). The barley *mlo* gene: A novel control element of plant pathogen resistance. *Cell* **88**: 695-705.

Cardle L., Ramsay, L., Milbourne, D., Macaulay, M., Marshall, D., and Waugh, R. (2000). Computational and experimental characterisation of physically clustered simple sequence repeats in plants. *Genetics* **156**, 847-854.

Chalmers, K.J., Waugh, R., Watters, J., Forster, B.P., Nevo, E., Abbott, R.J., and Powell, W. (1992). Grain isozyme and ribosomal DNA variability in *Hordeum spontaneum* populations from Israel. *Theoretical & Applied Genetics* **84**, 313-322.

Cook, R., and Oliver, W.B. (1991). Rapid detection of cyanogenic glycosides in malted barley. *European Brewery Convention, Proceedings of the 23rd Congress*, Lisbon, 513-519.

Dolan T.C.S., Dewar, E.T., and Gray, J.D. (1981). Development and use of a method for determination of malt fermentability. *Journal of the Institute of Brewing* **87:** 352-355.

Eglington, J.K., and Evans, D.E. (1997). Identification of the *beta*-amylase isoelectric focusing band pattern in barley (*Hordeum vulgare* L.). *Barley Genetics Newsletter* **27**: 8-12.

Eglinton, J.K., Langridge, P. and Evans, D. E. (1998). Thermostability variation in alleles of barley beta-amylase. *Journal of Cereal Science* **28**, 301-309.

Erkkila, M.J. (1999). Intron III-specific markers for screening of beta-amylase alleles in barley cultivars. *Plant Molecular Biology Reporter* **17**: 139-147.

Forster, B.P., Thompson D.M., Watters J., and Powell, W. (1991). Water-soluble proteins of mature barley endosperm - genetic-control, polymorphism, and linkage with beta-amylase and spring winter habit. *Theoretical & Applied Genetics* **81**: 787-792

Han, F., I. Romagosa, S.E. Ullrich, B.L. Jones, P.M. Hayes and D.M. Wesenberg. (1997). Molecular marker-assisted selection for malting quality traits in barley. *Molecular Breeding* **3**: 427-437.

Haslemore, R.M., and Gill, A.A. (1995). Rapid spectrophotometric measurement of soluble nitrogen in micromalts from a barley breeding program. *Journal of the Institute of Brewing* **101**: 469-472.

Hayes, P. M., B.H. Liu, S.J. Knapp, F. Chen, B. Jones, T. Blake, J. Franckowiak, D. Rasmusson, M. Sorrells, S.E. Ullrich, D. Wesenberg and A. Kleinhofs. (1993). Quantitative trait locus effects and environmental interaction in a sample of North American barley germ plasm. *Theoretical & Applied Genetics* 87: 392-401.

Jorde, L.B. (2000). Linkage disequilibrium and the search for complex disease genes. *Genome Research* **10**: 1435-1444.

Kihara, M., Kaneko, T., and Ito, K (1998). Genetic variation of beta-amylase thermostability among varieties of barley, *Hordeum vulgare* L., and relation to malting quality. *Plant Breeding* **117**:, 425-428.

Kilian, A., Kudrna, D., and Kleinhofs, A. (1999). Genetic and molecular characterization of barley chromosome telomeres. *Genome* **42**: 412-419

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

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

Liu, Z.W., Biyashev, R.M., and Maroof, M.A.S. (1996). Development of simple sequence repeat DNA markers and their integration into a barley linkage map. *Theoretical & Applied Genetics* **93**, 869-876.

Mather, D.E., Tinker, N.A., LaBerge, D.E., Edney, M., Jones, B.L., Rossnagel, B.G., Legge, W.G., Briggs, K.G., Irvine, R.B., Falk, D.E., and Kasha, K.J. (1997). Regions of the genome that affect grain and malt quality in a North American two-row barley cross. *Crop Science* **37:** 544-554.

May, K.W., Kozub, G.C., and Schaalje, G.B. (1989). Field-evaluation of a modified augmented design (type-2) for screening barley lines. *Canadian Journal of Plant Science* **69**: 9-15

McCleary, B.V. and Glennie-Holmes, E. (1985). Enzymic quantification of (1-3), (1-4)-β-D-Glucan in barley and malt. *Journal of the Institute of Brewing* **91**: 285-295.

Michalek, W., Kunzel, G., and Graner, A.(1999). Sequence analysis and gene identification in a set of mapped RFLP markers in barley (Hordeum vulgare). *Genome* **42**: 849-853

Morgan, A.H., and Gothard, P.G. (1977). A rapid, simple viscometric technique for indirect estimation of soluble beta -glucan content of raw barley. *Journal of the Institute of Brewing.* **83**: 37-38.

Pearson, S.Y., Bringhurst, T.A., and Brosnan, J. (1998). Potential of hull-less (aked) barley for distilling. *Proceedings of the fifth Aviemore Confererence on Malting, Brewing and Distilling*, 229-232.

Ramsay, L., M. Macaulay, S. degli Ivanissivich, K. MacLean, L. Cardle, J. Fuller, K. Edwards, S. Tuvesson, M. Morgante, A. Massari, E. Maesti, N. Marmiroli, T. Sjakste, M. Ganal, W. Powell, and R. Waugh. (2000). A Simple Sequence Repeat-based linkage map of Barley. *Genetics* **156**: 1997-2005

Stam, P. and van Ooijen, J.W. (1995) Joinmap™ version 2.0: Software for the calculation of genetic linkage maps, CPRO-DLO, Wageningen, the Netherlands.

Swanston, J.S. (1999). Quantifying cyanogenic glycoside production in the acrospires of germinating barley grains. *Journal of the Science of Food and Agriculture* **79:** 745-749.

Swanston, J.S., Allison, M.J., and Taylor, K.J. (1990). The relationship between grain and malt milling energy in barley cultivars of differing malting quality. *Proceedings of the third Aviemore Confererence on Malting, Brewing and Distilling*, pp. 338-343.

Swanston. J.S., W.T.B. Thomas, W. Powell, G.R. Young, P.E.Lawrence, L. Ramsay and R. Waugh. (1999). Using molecular markers to determine barleys most suitable for malt whisky distilling. *Molecular Breeding* **5**: 103-109.

Thomas W.T.B., Powell, W. and Swanston J.S. (1991). The effects of major genes on quantitatively varying characters in barley. 4. The *GPert* and *denso* loci and quality characters. *Heredity* **66**: 381-399.

Thomas, W.T.B., W. Powell, J.S. Swanston, R.P. Ellis, K.J. Chalmers, U.M. Barua, P. Jack, V. Lea, B.P. Forster, R. Waugh and D.B. Smith. (1996). Quantitative trait loci for germination and malting quality characters in a spring barley cross. *Crop Science* **36**: 265-273.

Tinker, N.A., and D.E. Mather. (1995). MQTL: software for simplified composite interval mapping of QTL in multiple environments. *Journal of Agricultural Genomics* **1**: http://www.ncgr.org/ag/jag/papers95/paper295/indexp295.html.

Toojinda, T., E. Baird, A. Booth, L. Broers, P. Hayes, W. Powell, W. Thomas, H. Vivar and G. Young. (1998). Introgression of quantitative trait loci (QTLs) determining stripe rust resistance in barley: an example of marker-assisted line development. *Theoretical & Applied Genetics* **96**: 123-131.

Utz, H.F., Melchinger A.E., and Schon, C.C. (2000). Bias and sampling error of the estimated proportion of genotypic variance explained by quantitative trait loci determined from experimental data in maize using cross validation and validation with independent samples. *Genetics* **154**: 1839-1849

Walker, J.W., Bringhurst, T.A., Broadhead, A.L., Brosnan, J.M., and Pearson, S.Y. (2001). The survival of limit dextrinase during fermentation in the production of Scotch whisky. *Journal of the Institute of Brewing* **107**: 99-106

Waugh, R., Bonar, N., Baird, E., Thomas, B., Graner, A., Hayes, P. and Powell, W. (1997a) Homology of AFLP products in three mapping populations of barley. *Molecular & General Genetics* **255**: 311-321

Waugh, R., McLean, K., Flavell, A.J., Pearce, S.R., Kumar, A., Thomas, W.T.B., and Powell, W. (1997b). Genetic distribution of Bare-1-like retrotransposable elements in the barley genome revealed by sequence-specific amplification polymorphisms (S-SAP). *Molecular and General Genetics* **253**: 687-694.

Zale, J.M., Clancy, J.A., Ullrich, S.E., Jones, B.L., Hayes. P.M., and the North American Barley Genome Mapping Project. (2000). Summary of barley malting quality QTLs mapped in various populations. *Barley Genetics Newsletter* **30**: 44-54.