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Small cross mapping of barley quality characters[1]

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Introduction

A large number of QTL studies have now been carried out in barley with many variates being studied on the same cross (Forster and Thomas, 2003) or a number of different crosses being studied (Langridge and Barr, 2003). Much of this research has, however, not been transferred to the highly successful commercial barley breeding programmes in Europe. One of the major reasons for this lack of transfer has been that much published research has ignored the current elite gene pool that current commercial barley breeders are actively exploiting with the result that the research has explained past breeding success but, apart from a few major genes, has not produced any information that could be deployed in Marker-Assisted Selection (MAS) for barley. The benefits of utilising the structure within plant breeding programmes to identify meaningful QTL associations has been reported (Jansen *et al.,* 2003) and, more recently, has been demonstrated in bread wheat to identify robust QTLs for resistance to yellow rust (Christiansen *et al.,* 2006). We have therefore formed a composite spring barley population from the amalgamation of several small doubled haploid populations, produced from pairwise crosses between contemporary elite genotypes, to analyse the genetics of a range of agronomic and malting quality traits and identify QTLs associated with their genetic control.

Materials and methods

Germplasm

We assembled a tranche of 207 DH lines from the F1s of 11 pair crosses between lines entered into National List Trial 1 in 1999. The lines were grown in a single replicate trial at SCRI in 2001, using a Modified Augmented Design Type 2, and in replicated trials at SCRI in 2002 and 2003, using a row and column design. The same material was grown in unreplicated and replicated trials at the National Institute of Agricultural Botany (NIAB) in 2002 and 2003 respectively, using the same designs as at SCRI but with different randomisations. All trials received prophylatic fungicide regimes that reflected the current standards for conducting official trials and fertiliser was applied to each according to local practise for producing malting barley samples. Apart from the 2001 trial at SCRI, all entries were sown at a constant density of seeds of 380 and 425 seeds m^{-2} at NIAB and SCRI respectively.

Phenotypes

During the growing season, the plots in each trial were scored for heading date and plant height. When the majority of the trial was ripe, all plots were harvested with a small plot combine and the plot weights expressed as yield in t/ha at 15% moisture content. After harvest, grain samples were cleaned and graded over a 2.5 mm sieve (SCRI) or a 2.2 mm sieve (NIAB), reflecting local trading practise, and the screenings percentage calculated. Samples of cleaned and graded seed were micro-malted at NIAB and malt samples analysed by Heriot Watt University (HWU) for wort parameters. Further grain samples were measured at SCRI for: nitrogen content, thousand grain weight, hardness (milling energy), and grain length, width, area and width to length ratio. Hot Water Extract (HWE), Malt Nitrogen/Protein (Dumas), Total Soluble Nitrogen of Wort (Dumas), Fermentability and Diastatic power were measured

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on the NIAB trial samples using standard protocols. The Soluble Nitrogen Ratio was derived by expressing the total soluble nitrogen as a percentage of the total malt nitrogen. Hot Water Extract was also corrected for variation in nitrogen content (HWEc) (Bishop, 1948) to check if the correction had any effect upon the apparent genetic control of the character. Finally, germination tests were carried out on the samples from the NIAB harvest in 2003 by germinating 100 seeds in 4 ml and 8 ml of distilled water to examine the ability to germinate in excesses of oxygen and water respectively. Alpha-amylase, beta-amylase, total limit dextrinase (bound and unbound), beta-glucanase, beta-glucan and free amino nitrogen (FAN) were determined on wort samples using standard protocols. Wort sugars were determined by HPLC using a PA100 ion exchange column. Analysis of epiheterodendrin production was carried out using a diagnostic molecular marker developed at SCRI. Each trial was analysed separately using either AGROBASE (unreplicated trials) or GENSTAT (replicated trials) to derive entry means corrected, where appropriate, for spatial trends. These means were then used to derive overall means for each entry over both years and sites.

Genotypes

SSR genotyping was carried out according to standard protocols using either fluorescent or radio-nucleotide labels for fragment analysis by capillary electrophoresis or polyacrylamide gel electrophoresis respectively. The parents and DHs were screened with a range of Simple Sequence Repeat (SSR) markers, including the standard set of 48 SCRI SSRs (Macaulay *et al.,* 2001) to provide a total of 97 loci that segregated in at least one of the crosses. Many of the additional SSR markers were derived from the SCRI library but some newly developed SSRs were used to extend the direct relevance of these maps. To improve the functionality of the map a few of the supplementary SSRs were derived from EST sequences and thus represent "next generation markers" that broker the move from relatively anonymous marker maps to more functional marker maps. This will thus help "future-proof" the maps and might also help interpret some of the results found. It should be noted that, whilst EST-SSR markers have been found to be polymorphic in wider crosses such as the Oregon Wolfe population, few were polymorphic amongst the elite parental lines examined in this study.

Sequence-Specific Amplified Polymorphism (S-SAP) was performed on the parental lines using three primers designed from LTRs of each of 6 retrotransposon families and MseI and EcoRI (Leigh *et al.,* 2003). All 18 LTR primers were screened in combination with each of 8 Mse primers containing 3 selective bases, from which 12 were selected to analyse variation in the parent lines. After screening, five primer combinations were selected to analyse the populations based upon the number of polymorphic bands and the quality of the profile obtained. A total of 59 bands were scored across the populations as dominant markers, i.e. as present or absent with on average, 11.8 bands scored per primer combination.

Results and discussion

Genetic analysis

Significant genetic variation was detected in at least one of the 11 crosses for all but five of the 29 characters measured (Table 1). There were also highly significant environmental effects due to both site and year differences with only one and two non-significant effects for each respectively. Over all the variates, the percentage of variation due to genetic differences was 5.5% with 31% due to differences between years and 11% due to site differences but error variation was considerable at 48%. Some variates, such as Screenings at the SCRI site, were only measured from trials at one site and the proportion of genetic variation was much higher in these cases at 25%. Significant genotype x environment interactions were detected in just over half of all potential occurrences with genotype x year being slightly more frequent than genotype x site effects. No significant differences were detected between the mid-parental values and means of their respective doubled haploid lines, indicating absence of epistatic interactions. Apart from grain width, wort maltose level, and fermentability, the double haploids transgressed the parental range. This most likely reflects the dispersion of increasing and decreasing factors amongst the parents and thus, despite the apparent narrow genetic base of the crosses, means that breeding progress in the elite gene pool can still be made. This is re-inforced by the fact that significant genetic variation was detected most frequently in the Braemar x Cora cross and least

frequently in Celebra x FDO 92012-540. Braemar and Cora were lines from the same UK breeding programme with similar pedigrees and are thus less diverse parents, a finding that is also apparent in their genetic similarities.

Table 1. Numbers of crosses (from 11) showing significant genetical variation for a range of agronomic, wort and malting quality phenotypes

Agronomic										
Char.	Yield	Head	Height	Screens	Glen	Gwid	Garea	Wid/Len	TKW	MillEn
Crosses	3	9	1	10	8	0	3	0	1	0
Wort										
Char.	%SE	A-Amy	B-Amy	BG-ase	LimDex	Bglucan	Fructose	Glucose	Maltose	Sucrose
Crosses	1	9	7	3	7	0	5	0	1	5
Malt										
Char.	FAN	GrainN	MaltN	TSN	SNR	HWE	HWEc	Ferment	DP	
Crosses	2	1	3	4	6	5	5	1	5	

Principal Component Analysis was used to examine the relationships between the correlations of the measured characters with the first three principal components accounting for over 40% of the variation. From plots of the first two principal components (Fig. 1), limit dextrinase, alpha amylase, soluble nitrogen ratio, beta glucanase, free amino nitrogen and total soluble nitrogen all tended to group together, suggesting some common genetic control of these characters. Similarly, the measurements of grain size (apart from width:length ratio) also grouped together, again indicating some common genetic control, but were separated from height and yield. The three separate measures of malt extract grouped together and screenings was also included in this grouping. There was a noticeable inverse relationship between height and yield.

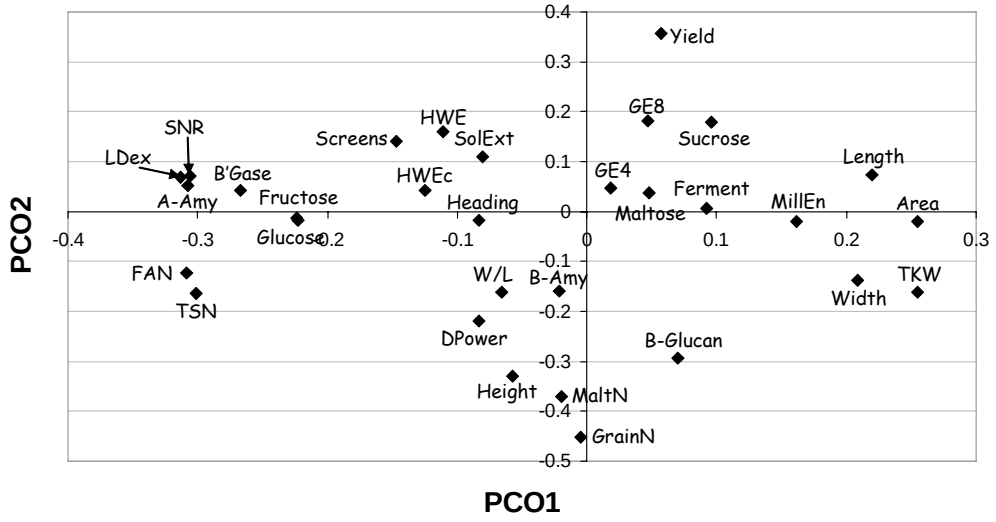

Fig. 1. Loadings on the first two principal component axes (based upon correlations) of 31 agronomic, wort and malting quality phenotypes.

QTL mapping

A number of discrepancies between parental genotypes and the observed segregations amongst

their progeny were apparent, which has been observed before and represents a real problem, even when working with elite germplasm. The breeding cycle for spring barley is now very rapid as it is obvious from pedigrees that UK breeders are often progressing from cross to National List (NL) submission in 4 years. Unless doubled haploids are used to achieve this, and their use is relatively rare in UK spring barley breeding, it is highly likely that there will be at least some degree of residual heterozygosity and/or heterogeneity in NL1 entries. One solution might be to ensure that the parental plants used to make the crosses are also harvested and kept to use in future genotyping studies. With such small populations for each cross, the parent/progeny mismatches caused considerable problems in map construction and, for the purposes of this paper, we have conducted single marker analyses to associate markers with characters.

Among the 31 variates measured, 204 QTLs were detected by a form of multiple regression analysis, ranging from 13 for screenings to one for soluble extract. The percentage of phenotypic variation accounted for by the QTLs ranged from 58 to 5% for limit dextrinase activity and soluble extract respectively. The average percentage of phenotypic variation accounted for by the associations was 41% for the yield, agronomic and grain size parameters, 30% for the malt and wort detailed analyses and 28% for the conventional malting quality parameters. Several "hot spots" were detected with Bmag127(3H) and Bmac40(6H) being significantly associated with 10 and 8 variates respectively. Overall, there were 11 "hot spots" (associations with 5 or more variates) with at least one on six of barley's seven chromosomes, the exception being 7H.

QTLs were detected most frequently on chromosomes 3 and 4H (33 associations each) and least on 1H (14 associations). Most of the associations on 3 and 4H were detected in centromeric regions but there was a noticeable effect of the telomeric portion of the long arm of chromosome 4H. Some of this was associated with allelic variation in an SSR in the beta amylase gene where one particular allele was associated with an increase in beta amylase activity and diastatic power. A neighbouring locus (Bmag419) showed a similar association.

There was some evidence of co-location of QTL for malt enzymes or wort sugars with one locus (Bmag225) associated with HWE also being associated with limit dextrinase and beta glucanase activity. GBMS0032 on 5H was also associated with HWE and beta glucanase activity and both loci were also associated with increased soluble nitrogen ratio. Seven QTLs were detected for wort fermentability with three of the loci also associated with wort sugars. The LDEX60 SSR, for instance, was associated with beta amylase, diastatic power and maltose as well as fermentability.

Conclusions

We have demonstrated that, despite some problems in aligning parental and progeny genotypes, the composite mapping approach using a number of small populations can be applied to identify robust QTLs in elite germplasm. The markers associated with these QTLs are of value in MAS but the challenge is to formulate a strategy to continually update this knowledge pool to take account of new introductions. We have also demonstrated that there is still considerable genetic variation in the elite gene pool that can be exploited by barley breeders without, as yet, the need to introduce more unadapted germplasm.

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Table 2. Numbers of QTLs detected and percentage of variation accounted for by marker associations with 31 phenotypic characters

Character	QTLs	% Variation
Yield	6	32.0
Head	8	35.6
Height	9	47.6
Screens	13	54.1
G_Area	9	46.2
G_Length	9	46.1
G_Width	5	31.2
Wid:Len	8	42.5
TKW	9	42.3
MillEn	7	33.4
SolExt	1	4.6
Aamy	7	43.4
Bamy	8	39.8
B_Gase	6	32.4
L_Dex'ase	12	57.8
B-Glucan	6	30.0
Fructose	6	26.5
Glucose	7	26.3
Maltose	3	14.9
Sucrose	4	21.0
FAN	7	38.8
GrainN	4	22.7
MaltN	5	22.5
TSN	7	34.3
SNR	8	38.7
HWE	7	37.6
HWEc	4	30.7
Ferment	7	32.4
DP	6	33.6
GE4	2	8.3
GE8	5	21.1