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A partial genome assay for quantitative trait loci in wheat (*Triticum aestivum*) using different analytical techniques

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Abstract F_1 plants between two intervarietal chromosome substitution lines of European spring wheat varieties, 'Sicco' ('Chinese Spring 5B') and 'Highbury' ('Chinese Spring 5B'), were used to produce 114 doubled haploid lines, 45 by the *Hordeum bulbosum* technique and 69 by anther culture. These two sets of lines were characterized for variation at a range of morphological, isozyme and RFLP marker loci, and genetic maps were developed with emphasis on chromosomes 6B, 7A, 7B and 7D. A subset of lines, scored for production traits in field trials in 1986 and 1987, were analysed for quantitative trait loci (QTL). The performance of the lines for the quantitative traits studied showed no overall differences due to the method of production of the lines. QTL were located on the linkage map for ear emergence time, height, tiller weight, yield and 50-grain weight using four analytical methods. Many of these effects showed genotype × year interaction.

Key words Doubled haploids · Genetic maps
QTL analysis · Wheat

Introduction

The study of quantitative trait loci (QTL) in wheat has a long history, based upon the use of morphological and isozyme markers with cytogenetically-derived single chromosome substitution lines (Law 1966, 1967). However, the lack of sufficient polymorphic marker loci hampered analysis until the advent of molecular markers. Restriction fragment length polymorphisms (RFLPs) are now being used to construct a comprehensive molecular marker linkage map of the entire genome (Devos and Gale 1993), although gaps still exist, as wheat is apparently less polymorphic than such cereal species, as maize (Helentjaris et al. 1986), rice or barley, and markers tend to cluster (Devos and Gale 1993). Nevertheless, due to the availability of markers, QTL analysis in wheat is no longer dependent on the use of cytogenetically derived material.

Anther culture techniques and the use of crosses with *Hordeum bulbosum* and maize facilitate the production of doubled haploid (DH) lines, from which highly-replicated families of identical genotypes may be generated. Prior to the advent of molecular markers, populations of such DH lines had been produced and put into large-scale agronomic trials for conventional plant breeding and biometrical studies (Snape et al. 1986). Many of these lines have subsequently been genotyped at biochemical and molecular marker loci. The purpose of the present study was to use these marker loci, together with existing field data, to locate, count and measure QTL effects underlying quantitative, agronomic traits of interest in wheat. A further aim was to compare several methods of QTL analysis.

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Materials and methods

Mapping populations

Two sets of wheat DH lines, produced by contrasting methods from the F_1 of a cross between the intervarietal chromosome substitution lines, 'Sicco' ('Chinese Spring 5B') and 'Highbury' ('Chinese Spring 5B') (Snape et al. 1986), were analysed. These were 45 lines derived from female gametes via the *Hordeum bulbosum* technique (HB lines), and 69 lines produced from male gametes by anther culture (AC lines) (Henry et al. 1988).

Marker loci

Following a screening of the parents for marker locus polymorphisms, the DH lines were genotyped for 17 polymorphic markers,

Table 1 Function and location of polymorphic genetic markers (RFLP loci and known-function isozyme and morphological markers) in the population of DH lines. (*Prefix X* RFLP loci · *S* and *L* short and long arm of the chromosome, respectively)

Marker Locus	Function	Chromosome
XNra	Nitrate reductase	6BS
Xpsr167	Anonymous cDNA	6BS
XCxp-3	Carboxypeptidase	6BS
Xpsr141	Anonymous cDNA	6BS
Xα-Amy-1	α-Amylase-1	6BL
Xpsr119	Anonymous cDNA	7AS
XNra	Nitrate reductase	7AS
Per	Peroxidase	7AS
Wx	Waxy	7AS
Amp	Aminopeptidase	7AS
XPepc	PEP carboxylase	7AL
Xpsr117	Anonymous cDNA	7BL
Xpsr150	Anonymous cDNA	7BS
Xpsr160	Anonymous cDNA	7DS
Xpsr119	Anonymous cDNA	7DS
Xpsr103	Anonymous cDNA	7DS
Xpsr150	Anonymous cDNA	7DS

comprising morphological, isozyme and RFLP loci on chromosomes 6B, 7A, 7B and 7D (Table 1) as described by Snape and Wang (unpublished).

Field trials

In 1986 and 1987, 34 of the HB-DH lines, together with 35 of the AC-DH lines, were grown in field experiments at the Plant Breeding Institute, Cambridge, from a Spring sowing. A randomized complete blocks design was used in both years, with five 1 m single-row microplot replicates per genotype in 1986 and four 3 m × 0.5 m drilled plots in 1987.

Quantitative traits

During the growing season, each plot was scored for the number of established plants, ear emergence time and final plant height. At maturity, 1 leading tiller was randomly selected from each of 4 random plants per plot. These samples were bagged as one unit, weighed, and the number of spikelets, the grain weight after threshing and the weight of 50 random grains recorded. In 1986, the remainder of each plot was cut at ground level, bound into a sheaf and weighed. The grain weight per sheaf was scored after threshing with a standing combine harvester. In 1987, each plot was combined directly. The characters shown in Table 2 were derived from these scores.

Statistical methods

Marker linkage analysis

Segregation and linkage analysis were initially performed separately on each set of lines as described in Snape and Wang (unpublished), and maps were developed using Mapmaker (Lander et al. 1987) with the Haldane (1919) mapping function. Previous mapping data (M. D. Gale personal communication) was also used in choosing the most likely combined maps.

QTL analysis

For each trait, an analysis of variance was used to confirm that the lines were exhibiting significant genetical variation. Significant lines × years interactions were found for all traits; hence the data were analysed independently for each year. Four analytical approaches were used: model-fitting, analysis of variance, Mapmaker/QTL (Lander and Botstein 1989) and regression mapping (Haley and Knott 1992; Martìnez and Curnow 1992). A significance level of 0.05 was applied for individual tests in all methods.

Model-fitting

The trait value of each of the 69 lines was averaged over blocks separately for each year. Within each year, the trait value was averaged over lines for every combination of marker locus, method of DH production and allele. A model was then fitted to these four means in each year (Y_{jk}), which included effects for methods (t), alleles (d) and their interaction (td), all being measured as deviations from the mean (m), as follows:

$$Y_{11} = m + [t] + [d] + [td]$$
$$Y_{12} = m + [t] - [d] - [td]$$
$$Y_{21} = m - [t] + [d] - [td]$$
$$Y_{22} = m - [t] - [d] + [td]$$

where j = 1 for HB, j = 2 for AC and k = 1 for 'Highbury', k = 2 for 'Sicco'.

The parameters of this model were estimated by weighted least squares (Cavalli 1952), with weights based on the total variance of line means. Following the standard rules of parsimony, the simplest model, which yielded significant main effects and an adequate fit to the data, as tested by chi-square, was accepted, with the proviso that allele effects were always tested for significance.

Analysis of variance

A one-way analysis of variance was performed on all 69 line means to test for allele effects at each marker locus. In contrast to the method of model-fitting the error variance was based on the variation between lines within allelic groups and hence differed at each marker locus. A significant *F*-value between allelic classes indicated the presence of a QTL, linked to the marker locus. The position of the QTL was determined by an "approximation" method (Snape et al. 1985). This model assumes the presence of one QTL, the location of which can be estimated if, and only if, the model fits. Failure of the model suggests the presence of more than one QTL.

Mapmaker/QTL

A Mapmaker/QTL analysis was performed, applying the backcross algorithm and a LOD score threshold of 0.83 ($\alpha = 0.05$) for significance for each individual peak tested (Lander and Botstein 1989).

Regression mapping

Initially, the chromosomes were scanned for the presence of QTL by fitting the following model, which incorporates information from

Table 2 Quantitative traits, measured and derived, in the doubled haploid populations

Trait	Designation	Description
Ear emergence time	EET	Mean number of days to first flower appearing in 50% of row-plot from 1 June
Height	HT	Mean final plant height (in cm)
Yield	YLD	Mean mass of grain per plant (1986) or plot (1987) (in g)
50 grain weight	GW50	Mean mass of 50 grains (in g)
Spikelet number	SPNO	Mean number of spikelets per ear
Ear weight	EARWT	Mean mass of grain per ear (in g)
Tiller weight	TWT	Mean mass of leading tiller per plant (in g)

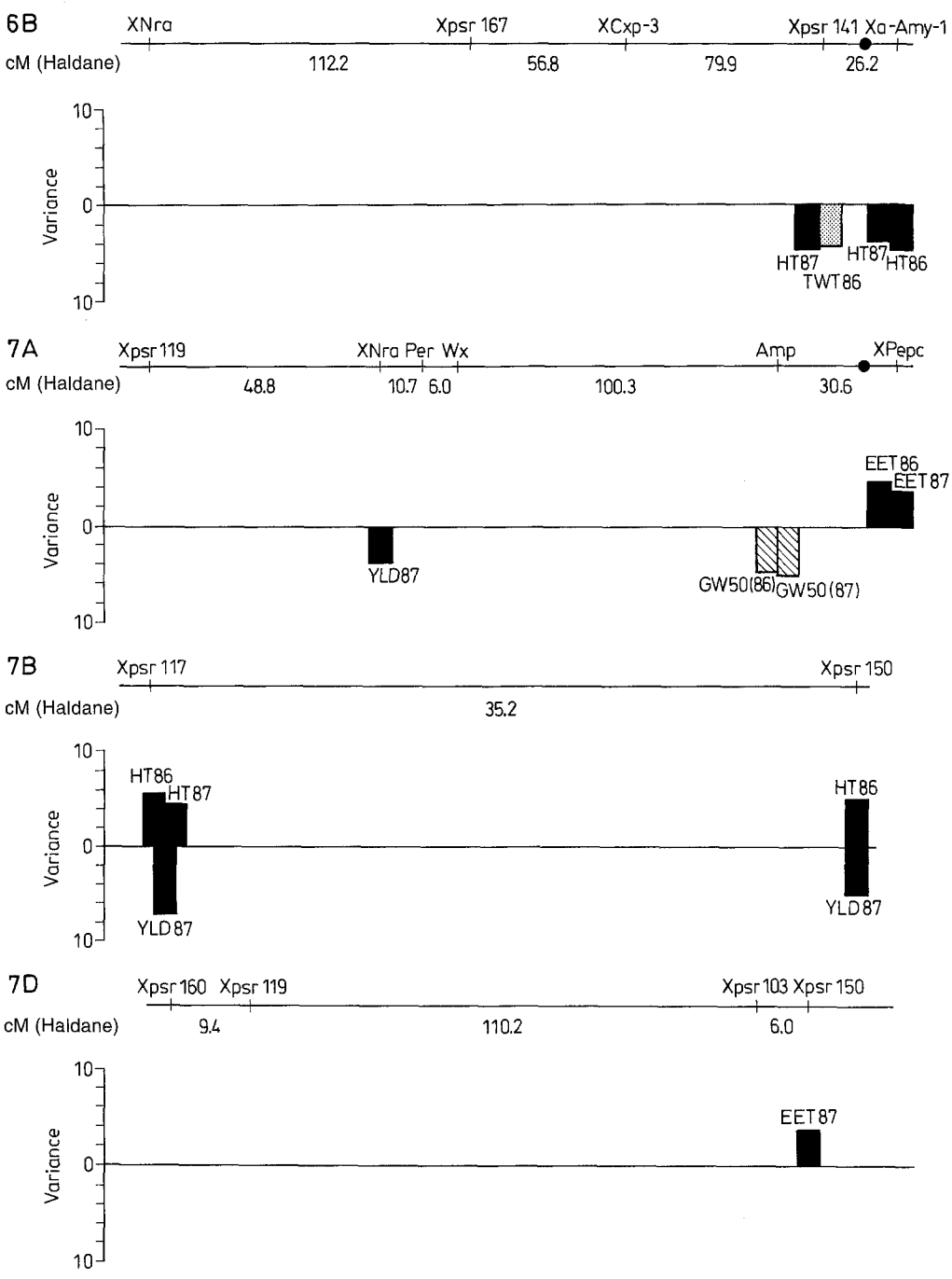

Fig. 1 Linkage maps for chromosomes 6B, 7A, 7B and 7D together with significant QTL effects resulting from the single marker analysis of variance, expressed as the percentage variation explained by the putative QTL at the marker position indicated

pairs of successive markers:

$$Y_{ij} = \beta_0 + \beta_1 \Gamma_j(t) + \varepsilon_{ij}$$

where Y_{ij} is the trait value in the i-th DH line (i = 1–69), classified in the j-th marker class (j = 1–4); β_0 is the mean; β_1 is the additive effect; and $\Gamma_j(t)$ is the probability that a specific QTL genotype is present, t cM from the leftmost marker locus of a pair, given that line i has the genotype j (Haley and Knott 1992; Martìnez and Curnow 1992). The error term ε_{ij} is approximately $N(0, \sigma^2)$. The QTL was moved between marker loci by varying t, and for each linkage group, the mean square error MSE(t) and $F(t)$ of the analysis of variance were plotted against t. A QTL was located where a minimum MSE(t) and a significant $F(t)$ were encountered. A method was also applied to recover information from missing markers (Martìnez and Curnow 1994). Generally, when multiple, non-independent tests are being performed, the threshold for significance of $F(t)$ should be 0.01 for each individual test, giving an approximate overall significance of 0.04. However, for purposes of comparison with the three other analytical techniques, the significance level for each individual test was 0.05.

Results

Marker linkage analysis

In the 45 HB-DH lines segregation was aberrant at 2 of the 17 loci, *XNra* (6B) and *XPepc* (7A), with approximately 2

Table 3 Associations detected between quantitative traits and marker loci by the four analytical methods. (All effects significant at P≤0.05, unless otherwise stated). See text for details

Chromosome	Marker Locus	Trait	Model-Fitting [d]	ANOVA [d]	Mapmaker/QTL [d]	Regression [d]
6B	*Xα-Amy-1*	HT(86)	−3.637	−3.501	−3.566	−3.526
		HT(87)	−4.187	−3.937	−4.220	−4.107
	Xpsr141	HT(87)	−4.594	−4.445	−5.365	−5.130
		TWT(86)	−0.178	−0.173	−0.153	−0.149
7A	*XPepc*	EET(86)	0.684	0.676	0.598 ns	0.540 ns
		EET(87)	0.751 ns	0.795	0.709 ns	0.633 ns
	Amp	GW50(86)	−0.036 ns[a]	−0.055	−0.063	−0.058
		GW50(87)	−0.048	−0.0493	−0.056	0.053
		YLD(87)	−0.398 ns	−0.380 ns	−0.362[b]	−0.368 ns
	Wx	YLD(87)	−0.280 ns	−0.316 ns	−0.333[b]	−0.310 ns
	XNra	YLD(87)	−0.400 ns	−0.443	−0.410[c]	−0.411[c]
7B	*Xpsr117*	HT(86)	3.418	3.144	3.518	3.464
		HT(87)	3.960	4.223	4.389	4.171
		TWT(86)	0.138 ns	0.12 ns	0.145	0.083 ns
		YLD(87)	−0.618[a]	−0.599	−0.590	−0.568
	Xpsr150	YLD(87)	−0.474	−0.477	−0.518	−0.462
		HT(86)	3.696	3.500	3.487	3.204
7D	*Xpsr150*	EET(87)	0.891	0.872	0.815	0.864

[a] Plus other effects
[b] Although not significant at *Wx* and *Amp*, Mapmaker/QTL identified a QTL between these two loci
[c] Although not significant at this locus, the effect was significant at the peak

'Highbury' to 1 'Sicco' alleles. Of the 69 AC-DH lines, 3 loci on 6B exhibited highly distorted segregation, *XCxp-3*, *Xα-Amy-1* and *Xpsr141*, the 'Sicco' allele being the most frequent Snape and Wang (unpublished). In each linkage analysis, the 17 marker loci fell into the expected four linkage groups (Table 1). Since there were no major differences between the pairs of "male" and "female" maps Snape and Wang (unpublished), linkage analysis was performed on the 114 combined lines and the resultant map used for QTL analysis (Fig. 1).

QTL analysis

Table 3 shows the effects obtained after applying the four analytical techniques. The estimate of the additive effect of the QTL, [d], at the marker locus is given for each method, the sign of [d] indicating whether the 'Highbury' allele increases or decreases the trait. Estimates of [d] are significant at $P \leq 0.05$, unless indicated otherwise. Using model-fitting, an allele effect alone was required to give an adequate fit to the model in all but 2 cases. In the 2 exceptions, 50-grain weight (86) at *Amp* and yield (87) at *Xpsr117*, complex interactions with DH methods existed. In total, 17 marker-associated QTL effects were detected in the 2 years, of which 11 were significant by all techniques. The significant effects are shown graphically for the analysis of variance (Fig. 1) as the percentage variation explained by the putative QTL at each marker. A bar above the x-axis, at the marker position, indicates a positive value of [d] ('Highbury' > 'Sicco'). The Mapmaker/QTL results are shown as the LOD score plotted against cM distance along the chromosome (Fig. 2).

Chromosome 6B

QTL effects were detected by all methods in both years for height at *Xα-Amy-1*, the 'Sicco' allele increasing height. The "approximation" method gave two positions for the QTL; in 1986 it was 31 cM distal to *Xα-Amy-1*, and in 1987 it was between *XCxp-3* and *Xpsr141*, 33 cM from the latter (18 cM using Mapmaker/QTL). Mapmaker/QTL revealed two significant peaks in 1986. By means of regression mapping, one QTL was detected between *Xpsr141* and *Xα-Amy-1* in both years, 13.7 cM and 4.2 cM from the former in 1986 and 1987, respectively.

A significant effect on tiller weight was detected at *Xpsr141* by all methods in 1986. Mapmaker/QTL placed a QTL between *Xpsr141* and *Xα-Amy-1*, 14 cM from the latter (8.7 cM using regression mapping). However, the "approximation" method suggested more than one QTL for tiller weight.

Chromosome 7A

Effects on ear emergence time were significant at *XPepc*, in both years, by analysis of variance, the 'Sicco' allele producing earlier flowering. The "approximation" method indicated more than one QTL. With Mapmaker/QTL, the effect was almost significant in 1986 and placed exactly at the marker locus. The effect was not significant in either year using the regression approach and was almost significant in 1987 using model-fitting. 'Sicco' alleles increased 50-grain weight in both years at *Amp*, although the estimated number of QTL responsible was variable – one with Mapmaker/QTL, two with the "approximation" method.

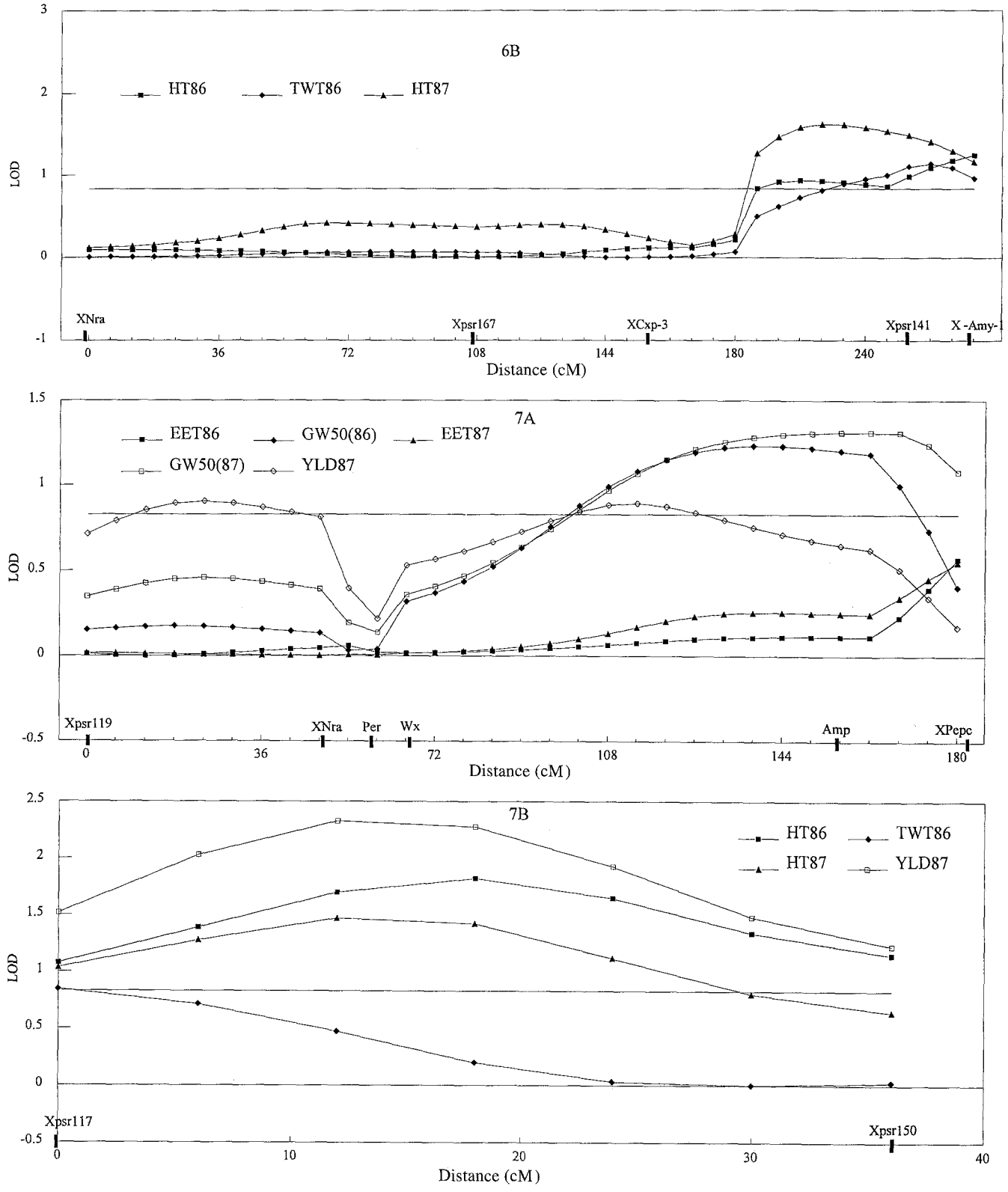

Fig. 2 Results of the Mapmaker/QTL analysis for chromosomes 6B, 7A and 7B plotted as the LOD score against the distance in cM (Haldane) along each chromosome. The line of LOD = 0.83 represents the LOD score threshold for significance at each marker locus and is equivalent to significance at the 5% level

The 1986 effect was not significant using model-fitting. The 1987 yield effect between *Wx* and *Amp* was detected by Mapmaker/QTL only, suggesting a false positive. More than one QTL for yield were identified in 1987 at *XNra* by the "approximation" technique. The association with *XNra* was significant by analysis of variance and almost so by model-fitting. It did not reach significance at *XNra* with Mapmaker/QTL, but the LOD score peaked significantly mid-way between *Xpsr119* and *XNra*, as did regression mapping, indicating a QTL 22.3 cM from *Xpsr119* towards *XNra*.

Chromosome 7B

Effects on height were detected at *Xpsr117* in both years, and at *Xpsr150* in 1986 only, by all methods; the 'Sicco' allele decreasing height. The "approximation" method suggested the presence of one QTL, although the positions varied from 26 cM distal to *Xpsr150* (1986) to 22 cM distal to *Xpsr117* (1987). Mapmaker/QTL and the regression method placed the QTL 18 cM (1986) and 13 cM (1987) from *Xpsr117*. Mapmaker/QTL alone detected a significant QTL for tiller weight at *Xpsr117* in 1986. Yield effects at *Xpsr117* and *Xpsr150* in 1987 were significant using all techniques, although the model-fitting approach indicated effects due to the method of DH production, as well as a method × QTL interaction. This result was due to an aberrantly low 'Highbury' mean from the anther culture lines in 1987. Mapmaker/QTL and the regression technique placed this effect approximately half-way between *Xpsr117* and *Xpsr150*. By the "approximation" method, one QTL for yield was located 21 cM distal to *Xpsr117*. These yield and component effects may have been due to pleiotropy of a QTL for height in this region, in which height and yield are inversely related.

Chromosome 7D

One QTL effect was found in 1987 for ear emergence time at *Xpsr150* by all methods. Mapmaker/QTL located this QTL exactly at *Xpsr150*, the regression method placed it 6.4 cM from *Xpsr103* towards *Xpsr150* and, using the "approximation" method, it could not be located unambiguously.

There was no evidence for the presence of epistasis between the QTL detected for height on chromosomes 6B and 7B, for yield on 7A and 7B, for tiller weight on 6B and 7B and ear emergence time on 7A and 7D.

Discussion

The present work has revealed eight QTL of agronomic interest in wheat. QTL for height were found on chromosomes 6B and 7B, with alleles in dispersion, where the 'Sicco' allele on 6B increased the trait value and decreased it on 7B. Flanking marker mapping placed the QTL approximately mid-way between *Xpsr141* and *Xα-Amy-1* on 6B and mid-way between the only 2 markers on 7B, each QTL responsible for approximately 5–10% of the variation in height. The QTL for height on 7B may have a pleiotropic effect on yield, with which it is negatively correlated. A single QTL for tiller weight occurred on 6B, closely associated and correlated with the QTL for height. A single QTL underlying 50-grain weight was found associated with *Amp* on 7A. Two QTL controlling yield were identified on 7A and 7B, the latter either tightly linked to height genes or indicative of pleiotropy. QTL controlling flowering time occurred at *XPepc* on 7A and at *Xpsr150* on 7D. There are no previous reports of major flowering-time genes on these chromosomes since vernalization genes are located to the homoeologous group 5 chromosomes and photoperiod genes to homoeologous group 2. However, a gene for vernalization response has been located on 7B (Law 1967). For all characters, individual QTL appear to be responsible for around 5% of the phenotypic variance. However, it should be remembered that only 4 of the 20 chromosomes (5B being homozygous for 'Chinese Spring') which are segregating in these DH lines were examined in this study.

The four techniques used in the analysis of this data set were compared, and the results were clearly consistent in the detection and estimation of the size of QTL effects. The results from Mapmaker/QTL and the regression mapping technique were similar, as was expected (Haley and Knott 1992). The model-fitting approach was useful because it gave an insight into the significance of effects other than the QTL. For example, the QTL effect on yield, detected in 1987 at *Xpsr117* as being highly significant by the other three methods, was found to be due entirely to the anomalous value of one allelic group mean, and should be treated with caution.

Evidence for more than one QTL per chromosome was ambiguous across the four methods. Hyne et al. (in preparation), using simulated data, have found that flanking marker methods are is not well able to detect the presence of two QTL with small population sizes. Martìnez and Curnow (1992) recommend the use of three or more marker regression mapping to resolve this problem, but this has the drawback of requiring large population sizes to ensure that each genotypic class is represented. This is not such a problem for DH lines, in which there are just 8 marker classes, as it is for an F_2, in which there are 27. A new method, 'marker regression' (Kearsey and Hyne 1994) overcomes some of these difficulties.

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