Theor Appl Genet (2001) 103:625–637

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QTL analysis of agronomic traits in barley based on the doubled haploid progeny of two elite North American varieties representing different germplasm groups

Received: 18 August 2000 / Accepted: 15 December 2000

Abstract A better understanding of the genetics of complex traits, such as yield, may be achieved by using molecular tools. This study was conducted to estimate the number, genome location, effect and allele phase of QTLs determining agronomic traits in the two North American malting barley (*Hordeum vulgare* L.) quality variety standards. Using a doubled haploid population of 140 lines from the cross of two-rowed Harrington×six-rowed Morex, agronomic phenotypic data sets from nine environments, and a 107-marker linkage map, we performed QTL analyses using simple interval mapping and simplified composite interval mapping procedures. Thirty-five QTLs were associated, either across environments or in individual environments, with five grain and agronomic traits (yield, kernel plumpness, test weight, heading date, and plant height). Significant QTL×environment interaction was detected for all traits. These interactions resulted from both changes in the magnitude of response and changes in the sign of the allelic effect. QTLs for multiple traits were coincident. The *vrs1* locus on chromosome 2 (2H), which determines inflorescence row type, was coincident with the largest-effect QTL determining four traits (yield, kernel plumpness, test weight, and plant height). QTL analyses were also conducted separately for each sub-population (six-rowed and two-rowed). Seven new QTLs were detected in the sub-populations. Positive transgressive segregants were found for all traits, but they were more prevalent in the six-rowed sub-population. QTL analysis should be useful for identifying candidate genes and introgressing favorable alleles between germplasm groups.

Keywords *Hordeum vulgare* · Two-rowed · Six-rowed · Agronomic traits · Quantitative trait loci

Communicated by P. Langridge

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Oregon Agricultural Experiment Station Journal No. 11694

Introduction

In many crop species there is limited genetic variation for economically important traits due to domestication bottlenecks and intensive post-domestication selection (Ladizinsky 1985). In order to maintain rates of gain from selection for agronomic performance traits, to meet challenges posed by new biotic stresses, and to increase productivity in the face of abiotic stresses, it may be necessary to introgress new alleles from distinct germplasm groups within the cultivated germplasm pool, from exotic germplasm, or from crop relatives (Tanksley et al. 1989). Examples of distinct germplasm groups within cultivated germplasm pools include the flint and dent germplasm pools in maize (Mangelsdorf 1974) and the two-rowed and six-rowed germplasm pools in barley (Powell et al.1990; Takahashi et al. 1975).

In barley each rachis node has three spikelets, each of them bearing one floret. The two-rowed and six-rowed germplasm groups are defined by the number of fertile florets per rachis node. In two-rowed barley there is one fertile floret per rachis node, whereas in six-rowed barley all three florets are fertile (Hitchcock 1971). Lateral

floret fertility is determined by alleles at the *vrs1* and *int-c* loci on chromosomes 2 (2H) and 4 (4H), respectively (Franckowiak and Lundqvist 1997; Komatsuda et al. 1999). There are epistatic interactions among alleles at these loci such that the *Vrs1Vrs1 int-cint-c* genotype has a two-rowed inflorescence phenotype, the *vrs1vrs1 Int-cInt-c* has a six-rowed phenotype, and the heterozygotes exhibit a range of intermediate phenotypes (Franckowiak and Lundqvist 1997; Lundqvist and Franckowiak 1997; Nilan 1964). Most barley varieties of commercial importance are inbred lines and are thus six-rowed or two-rowed. This simple genetic system defines the two principal germplasm groups of barley, and these germplasm groups have historically defined end use. Two-rowed barleys are favored for malting throughout most of the world except the USA and Mexico, where six-rowed barleys are used extensively for this purpose (Riggs and Kirby 1978).

Malting quality is a complex multi-component trait (Thomas et al. 1996). Stringent quality specifications have led to a very narrow genetic base within the two-rowed and six-rowed malting germplasm pools and a higher degree of genetic distance between the germplasm pools. There has recently been a focus on breeding for defined feed and human food quality specifications in barley (Blake and Bowman 1999; Harten 1998) but historically the primary objective of feed barley improvement was grain yield, and to some extent kernel quality, as measured by test weight and kernel size. As a consequence, genetic diversity studies have revealed less variability in malting barley germplasm than in feed barley germplasm (Hayes et al. 1997; Saghai-Maroof et al. 1994).

Two-rowed varieties usually have a higher number of tillers per plant and larger, heavier seed than six-rowed varieties. Six-rowed varieties, on the other hand, usually have more seeds per inflorescence. Thus the compensatory effects of yield components lead to similar levels of yield potential. However, historical patterns of geographic distribution and end-use of the two-rowed and six-rowed germplasm groups have led to the idea that the two germplasm groups carry different alleles at other loci in addition to those determining lateral floret fertility (Takahashi et al. 1975). Accordingly, crosses between the two germplasm groups could be expected to produce positive transgressive segregants for economically important phenotypes. The experience of plant breeders, however, has generally been that two-rowed×six-rowed crosses are not suitable for variety development (Kjaer and Jensen 1996). Allard (1988) concluded that ''Evidently the two-row/six-row locus affects developmental processes in ways that leave few quantitative characters untouched" and ''this locus had large effects on survival and adaptedness". Large pleiotropic effects on multiple phenotypes are attributed to alleles at the *vrs1* locus based on studies of the progeny of two-rowed×six-rowed crosses (Kjaer and Jensen 1996; Jui et al. 1997). However, it is possible that the correlated phenotypes are due to linkage rather than pleiotropy. It is difficult to distinguish between these phenomena in the case of the *vrs1* locus which is located, on linkage maps, near the centromere (Robertson et al. 1965). On the physical map of Kuenzel et al. (2000), however, the *vrs1* locus is located in one of the higher recombination regions on the long (minus) arm of chromosome 2 (2H). Powell et al. (1990), in a comparative analysis of two types of cross progeny from a six-rowed×two-rowed cross, concluded that some associations between quantitative phenotypes and the *vrs1* locus were due to linkage rather than pleiotropy.

Molecular markers have facilitated the dissection of quantitative traits via quantitative trait locus (QTL) analysis procedures. QTLs for a range of economically important phenotypes are reported throughout the genome (Hayes et al. 1993; Mather et al. 1997; Powell et al. 1990; Tinker et al. 1996) including the region of chromosome 2 (2H) where *vrs1* is located (Zhu et al. 1999). QTL studies based on progeny of two-rowed×six-rowed crosses consistently report determinants of agronomic and malting quality traits in regions coincident with the *vrs1* and *int-c* loci (Jui et al. 1997; Kjaer and Jensen 1996; Marquez-Cedillo et al. 2000; Powell et al. 1990).

The North American Barley Genome Mapping Project (NABGMP) has supported QTL analysis of malting and agronomic traits in three reference populations of doubled haploid lines derived from the following crosses: Steptoe×Morex (Hayes et al. 1993), Harrington×TR306 (Mather et al. 1997; Tinker et al. 1996); and Harrington×Morex (Hayes et al. 1997; Marquez-Cedillo et al. 2000). Steptoe and Morex are six-rowed varieties. Harrington and TR306 are two-rowed varieties. Harrington and Morex, respectively, are the two-rowed and six-rowed malting quality standards for North America. Steptoe and TR306 do not have acceptable malting quality profiles, but they have desirable agronomic attributes. In the progeny of Steptoe×Morex and Harrington×TR306, agronomic and malting quality QTL mapped to all chromosomes (Hayes et al. 1993; Mather et al. 1997; Tinker et al.1996).

The objectives of the investigation reported here were to estimate the number, genome location, effect and allele phase of QTLs for grain and agronomic traits in the Harrington×Morex population and to use this information to determine (1) the relationships of the loci determining inflorescence type with agronomic traits and (2) the consistency of QTL significance and allele phase among three mapping populations, Steptoe×Morex, Harrington×TR306 and Harrington×Morex.

Materials and methods

One hundred and forty doubled haploid (DH) lines were produced from the F_1 of Harrington×Morex by the *Hordeum bulbosum* method (Chen and Hayes 1989). One hundred and six markers were used for construction of a base map, with a target density of 10 cM (Hayes et al. 1997). The *int-c* locus was added to this map. To map this locus, we scored DH lines as homozygous recessive (Harrington alleles) when laterals were small and had no anthers or awns. DH lines were scored as homozygous dominant (Morex alleles) when laterals were large, sessile, and had anthers and long

awns. The 107-point map and multiple environment malting quality data sets were used for QTL analysis of malting quality (Marquez-Cedillo et al. 2000). We used the same map for the QTL analysis of agronomic traits.

The DH lines and parents were grown in nine environments: four locations in 1995 [Pullman, Wash. (WA95), and Klamath Falls, Ore. (OR95), USA; Saskatoon, Sask. (SK95), and Brandon, Man. (MB95), Canada] and five locations in 1996 [Pullman, Wash. (WA96); Pendleton, Ore. (OR96), and Aberdeen, Idaho (ID96), USA; Saskatoon, Sask. (SK96) and Brandon, Man. (MB96), Canada]. Plot size, seeding rate, and management were in accordance with local practice. Plots at Klamath Falls and Aberdeen were irrigated, while plots at other locations received no supplemental irrigation. Two replications were used in 1996 at Aberdeen, Pendleton, and Pullman; a single replication was used at the other sites.

Four agronomic traits – yield, kernel plumpness, test weight, and plant height – were scored in nine environments. Heading date was measured at all sites except Pendleton. Yield was measured as the weight of grain harvested per plot and converted to kilograms per hectare. The percentage of plump kernels was determined by weighing grain remaining on a 0.24×1.91-cm slotted sieve after 30 cycles of shaking a 100-g sample on a *Seedburo Strand* sizer/shaker. Test weight was measured as the weight of grain contained in a 1-quart cylinder and converted to kilograms per hectoliter. Heading date was measured as the number of days from planting until emergence of 50% of the inflorescences in each plot. Plant height was measured, in centimeters, from the soil surface to the tip of the inflorescence (excluding awns).

QTL analyses were performed using 5,000 permutations for the simple interval mapping (SIM) and simplified composite interval mapping (sCIM) procedures of MQTL (Tinker and Mather 1995). Individual and joint additive effects of QTLs were used to estimate the percentage of phenotypic variation (R^2_p) accounted for by significant QTLs. In this report we focus on primary QTLs (*sensu* Mather et al. 1997). These are QTLs where there were coincident peaks with both SIM and sCIM analysis, and the SIM peaks exceeded the significance threshold ($P<0.05$). When significant ($P<0.05$) QTLs were coincident with the *vrs1* locus, separate QTL analyses were performed for both the two-rowed (*Vrs1Vrs1*; $n=72$) and six-rowed (*vrs1vrs1*; $n=68$) sub-populations and the *Int-c Int-c* ($n=69$) and *int-c int-c* ($n=71$) sub-populations. Estimates of heritability were calculated as $h^2=\sigma^2_g/(\sigma^2_g+\sigma^2_{ge}/e+\sigma^2_e/re)$, where r=number of replications; e=number of environments; σ^2_g is the additive genetic variance among DH lines; σ^2_{ge}=genetic×environment interaction variance; and σ^2_e=non-genetic variance. Since the number of replicates was not equal at all locations, the re coefficients and estimates of σ^2_g and σ^2_{ge} were obtained, respectively, from VARCOMP Type-I and REML procedures implemented in SAS (SAS Institute 1989). Multiple regression procedures (implemented in SAS) were used to test the significance of two-locus interactions.

Results and discussion

Quantitative traits

There were large differences, in terms of both mean and standard deviation, for all agronomic traits (Table 1). Averaged over environments, Harrington was higher yielding, had a higher percentage of plump kernels, was later to head, and shorter than Morex (Tables 1, 2). Averaged across environments, frequency distributions were continuous, with the exception of percentage of plump kernels (Fig. 1). The phenotypic frequency distributions and mean values for the two-rowed (*Vrs1Vrs1 Int-cInt-c* and *Vrs1Vrs1 int-cint-c*) and six-rowed (*vrs1vrs1 Int-cInt-c* and *vrs1vrs1 int-cintc*) sub-populations of DH lines were significantly different, and in some cases dif-

Table 1 Means and standard deviations for five agronomic traits in Harrington, Morex, and their 140 doubled haploid (DH) progeny in nine environments[a]

Environment	Yield (kg ha^{-1})				Kernel plumpness (%)				Test weight (kg hl^{-1})			
	Parents[b]		DH Lines		Parents[b]		DH Lines		Parents[b]		DH Lines	
	H	M	μ	σ	H	M	μ	σ	H	M	μ	σ
WA 95	4,737	3,647	4,287	528	91	84	81	14	67	65	63	2
SK 95	4,,822	4,665	4,342	665	84	85	76	14	66	66	63	2
MB 95	4,745	3,079	4,041	759	87	80	78	19	64	65	64	3
OR 95	4,822	3,899	4,235	998	94	72	76	21	68	69	67	2
WA 96	4,424	3,432	3,554	547	94	90	80	18	71	71	70	2
SK 96	4,676	3,674	3,982	559	79	67	71	23	64	66	64	4
MB 96	6,371	6,627	6,020	737	92	93	84	16	70	69	67	2
OR 96	6,502	3,776	4,042	714	94	90	80	18	71	71	70	2
ID 96	8,775	6,726	6,738	941	90	96	88	10	66	65	64	2

Environment	Heading date (days)				Plant Height (cm)			
	Parents[b]		DH Lines		Parents[b]		DH Lines	
	H	M	μ	σ	H	M	μ	σ
WA 95	172	165	167	3	86	94	98	13
SK 95	190	187	189	2	80	97	89	8
MB 95	193	190	191	2	64	83	80	7
OR 95	200	186	193	4	75	85	84	10
WA 96	187	182	185	2	87	94	92	8
SK 96	194	190	193	2	96	111	108	9
MB 96	182	177	180	2	69	95	90	8
OR 96	–	–	–	–	75	90	90	10
ID 96	187	181	176	2	94	111	101	8

[a] For consistency with Tinker et al. (1996) and Mather et al. (1997) environments are coded as follows: letters identify the Canadian province or US state and numerals identify the year (1995 or 1996). Heading date was measured in eight environments only. See Materials and methods for definition of codes

[b] H, Harrington; M, Morex

Table 2 Means, standard deviations and heritabilities for five agronomic traits in Harrington, Morex, their 140 DH progeny, and the two-rowed and six-rowed sub-populations, averaged over nine environments

Trait	Parents[a]		Doubled haploidprogeny						
	H	M	Two-rowed sub-population	Six-rowed sub-population	All lines				
	$\bar{x}$	$\bar{x}$	μ	μ	μ	σ	Minimum	Maximum	h^2 (%)[b]
Yield (kg ha $^{-1}$)	5,539	4,391	4,386	4,791**[c]	4,582	595	3,166	5,693	83
Kernel plumpness (%)	90	85	93	66**	80	18	6	99	98
Test weight (kg hl^{-1})	67	67	66	64**	65	3	50	75	97
Heading date (days)	188	182	185	183**	184	9	160	200	92
Plant height (cm)	81	96	95	89**	92	12	55	132	95

[a] H, Harrington, M, Morex
[b] Estimated as the percentage of phenotypic variance attributable to DH lines using environments as replications
[c] Significance of t-tests comparing the means of the two-rowed and the six-rowed subpopulations: ** P<0.01

Table 3 Agronomic trait QTL location[a], higher value allele[b], and percentage of phenotypic variance explained by QTL(s) (R^2_p[c]) in the DH progeny of Harrington×Morex. Analyses are based on nine environments for yield, kernel plumpness and test weight, and eight environments for heading date

Phenotype	Chromosome				R^2_p (%)
	2 (2H)	3 (3H)	4 (4H)	7 (5H)	
Yield	*vrs1*-MWG503^M				8
Kernel plumpness	Hvbkasi-*vrs1*^{H}				61
Test weight	Hvbkasi-*vrs1*^{H}		ABG003a-MWGO58^H	MWG635d-ABC302a^M	27
Heading date	B15c-ABC162^H				32
Plant height	Hvbkasi-*vrs1*^{H}	ABG462-PSR156a^M	*int-c*-Phy2^H	MWG635d-ABC302a^M	44

[a] Flanking markers [b] H, Harrington; M, Morex [c] Multilocus percentage of variance explained by QTLs

ferent, from the corresponding parent. The six-rowed sub-population was significantly (P<0.05) higher yielding, later and shorter than the two-rowed sub-population (Table 2, Fig. 1). Kernel plumpness and test weights of the six-rowed sub-population were significantly lower than those for the two-rowed sub-population and were lower than the six-rowed parent. There were positive and negative transgressive segregants for all traits. Estimates of heritability across environments were high for all traits (Table 2). Heritabilities for individual environments where replications were used were also high. For example, grain yield heritability estimates ranged from 55% (ID96) to 76% (OR96), and plant height estimates ranged from 61% (WA96) to 86% (OR96).

In summary, the agronomic phenotypic data were quite consistent and, with the exception of kernel plumpness, support complex inheritance. In the case of kernel plumpness, there was a continuous distribution of values within the six-rowed sub-population. In the two-rowed sub-population, the sieve size used precluded separation of the positive phenotypic transgressive segregants falling into the 100% plump grain class. The significant differences between the two-rowed and six-rowed sub-populations for all agronomic traits indicated that loci determining inflorescence type (*vrs1* and the *int-c*) and/or linked loci affect multiple characters. The distribution of values within each sub-population suggests that loci in addition to *vrs1* and *int-c* (and/or linked loci) are also involved.

QTL analysis

In the analysis of the five agronomic traits across environments, a total of ten significant QTLs were detected (Table 3, Fig. 2). The number of QTLs detected for each trait ranged from one to four. The ten QTLs were significant (P<0.05) with the SIM analysis, and coincident peaks were detected with the sCIM analysis. In addition, there were QTLs that approached, but did not reach, the SIM significance threshold. Higher sCIM peaks were coincident with SIM peaks, but as indicated by Tinker and Mather (1995), sCIM significance thresholds cannot be established with multiple environment data sets. Single QTLs were detected for yield, kernel plumpness, and heading date. All were on chromosome 2 (2H). The yield, kernel plumpness, test weight, and plant height QTLs were flanked by the *vrs1* locus. The heading date QTL was 19 cM from the yield QTL and 2.3 cM from the kernel plumpness, test weight, and plant height QTL (Fig. 3). In addition to the QTL on chromosome 2 (2H), two other QTLs for test weight were detected, one on chromosome 4 (4H) and one on chromosome 7 (5H). The chromosome 4 (4H) test weight QTL was 43 cM

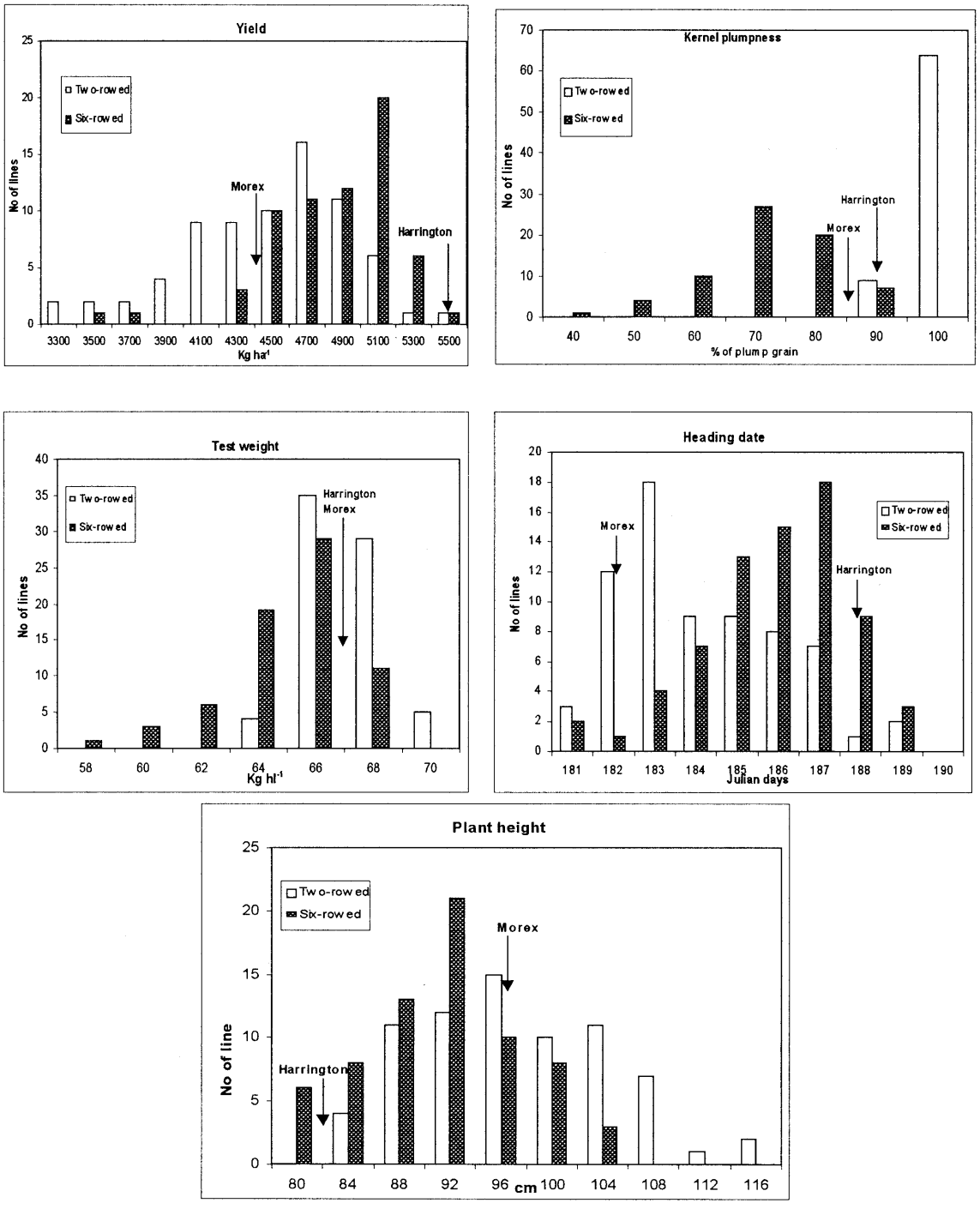

Fig. 1 Phenotypic frequency distributions for five agronomic traits in the doubled haploid (DH) progeny of Harrington×Morex divided by inflorescence row type into two-rowed and six-rowed sub-populations. Distributions are based on nine environments of data for yield, kernel plumpness, test weight, and plant height and eight environments for heading date

from the *int-c* locus. Four QTLs were detected for plant height, one each on chromosomes 2 (2H), 3 (3H), 4 (4H), and 7 (5H) (Table 3).

The percentage of variance explained by the significant QTLs (R^2_p) was low for grain yield (8%) and reached a maximum of 61% for kernel plumpness. Considering the high heritability (h^2) estimates (Table 2), a substantial portion of the genotypic variance for grain yield remains unexplained. This could be due to bias attributable to small population size (Melchinger et al. 1998; Utz et al. 2000) and/or effects of undetected QTLs, and/or QTL interaction. When the grain yield QTL on chromosome 1 (7H) and chromosome 3 (3H), which approached the SIM significance threshold, were included in the calculation of R^2_p, the value reached 15%. The R^2_p values for test weight, heading date, plant height, and kernel plumpness were progressively higher,

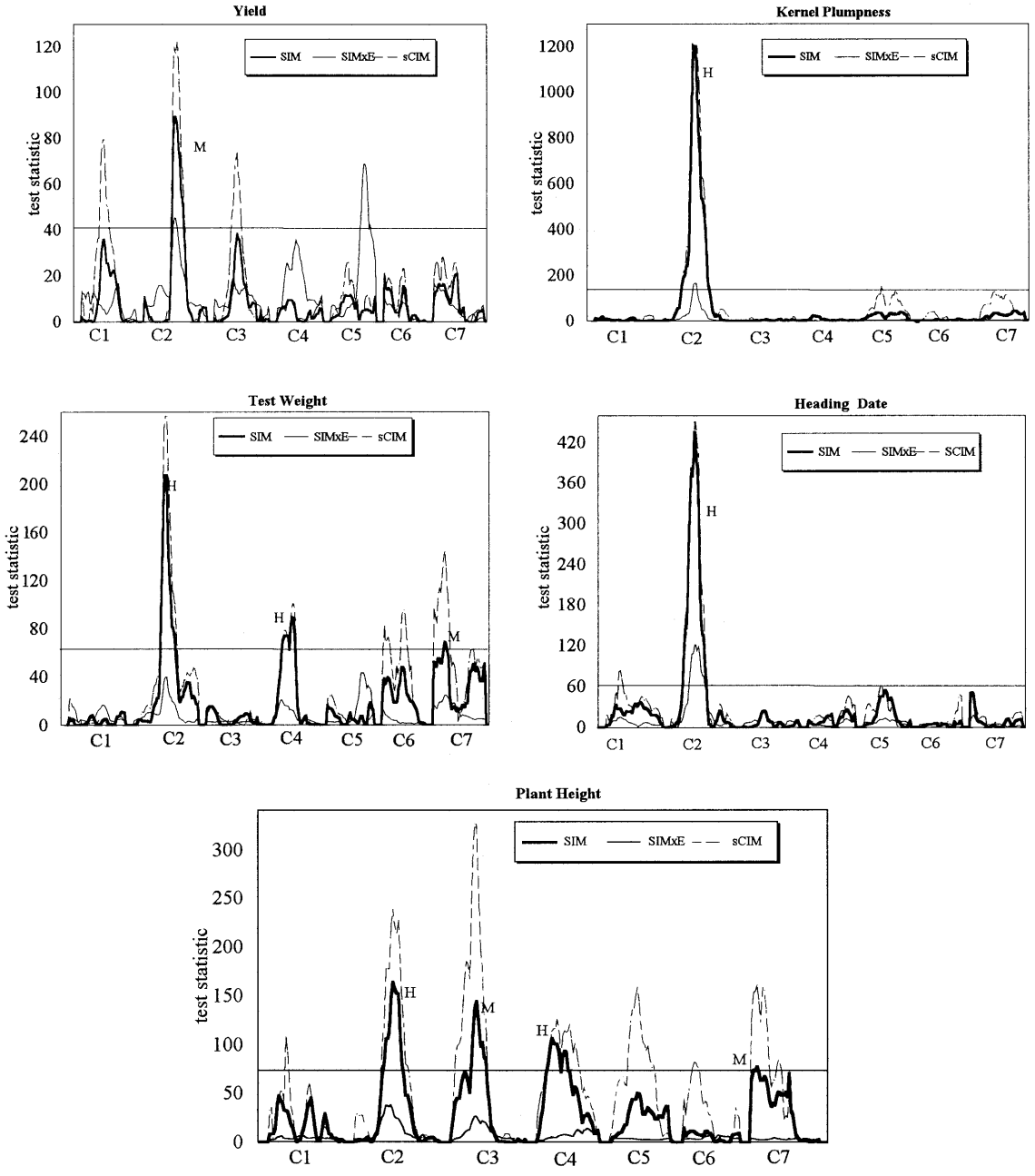

Fig. 2 Scans of test statistics (*Y-axis*) for simple interval mapping (SIM, *solid wider lines*), simple composite interval mapping (sCIM, *broken lines*) and QTL×environment interaction (*solid, thinner lines*) for the full population of Harrington×Morex DH lines. Scans are shown for five agronomic traits as indicated. Chromosomes (*C*) 1 (7H), *2* (2H), *3* (3H), *4* (4H), *5* (1H), *6* (6H), and 7 (5H) are shown *left to right* on the *X-axis.* Horizontal lines indicate thresholds for testing SIM, estimated from 5,000 permutations. The parent giving the higher value allele is shown for each QTL peak (*H* Harrington, *M* Morex)

ranging from 27% to 61% (Table 3). The coincident QTLs, flanked by the *vrs1* locus, for all traits except for heading date support the role of the *vrs1* locus, or of tightly linked loci, in multiple traits. Nevertheless, the presence of additional significant QTLs, and of the genetic variance that remains unexplained, suggests that other genes are also determinants of these traits. The chromosome 2 (2H) heading date QTL – 26.6 cM from the *vrs1* locus – is not as likely a candidate for pleiotropy versus linkage with *vrs1*. Allele phases at the chromosome 2 (2H) QTL are consistent with the parental phenotypes. Morex contributed the higher value allele for grain yield, and six-rowed genotypes have three fertile florets per rachis node whereas the two-rowed genotypes have only one. Likewise, Harrington contributed the higher value alleles for kernel plumpness and test weight, and two-rowed genotypes typically have higher values for these traits than six-rowed genotypes, due to their more uniform kernel size and weight. Harrington was, on average, 6 days later than Morex, and it contributed the later allele for heading date. The exception to this pattern was plant height where Harrington – the shorter parent – con-

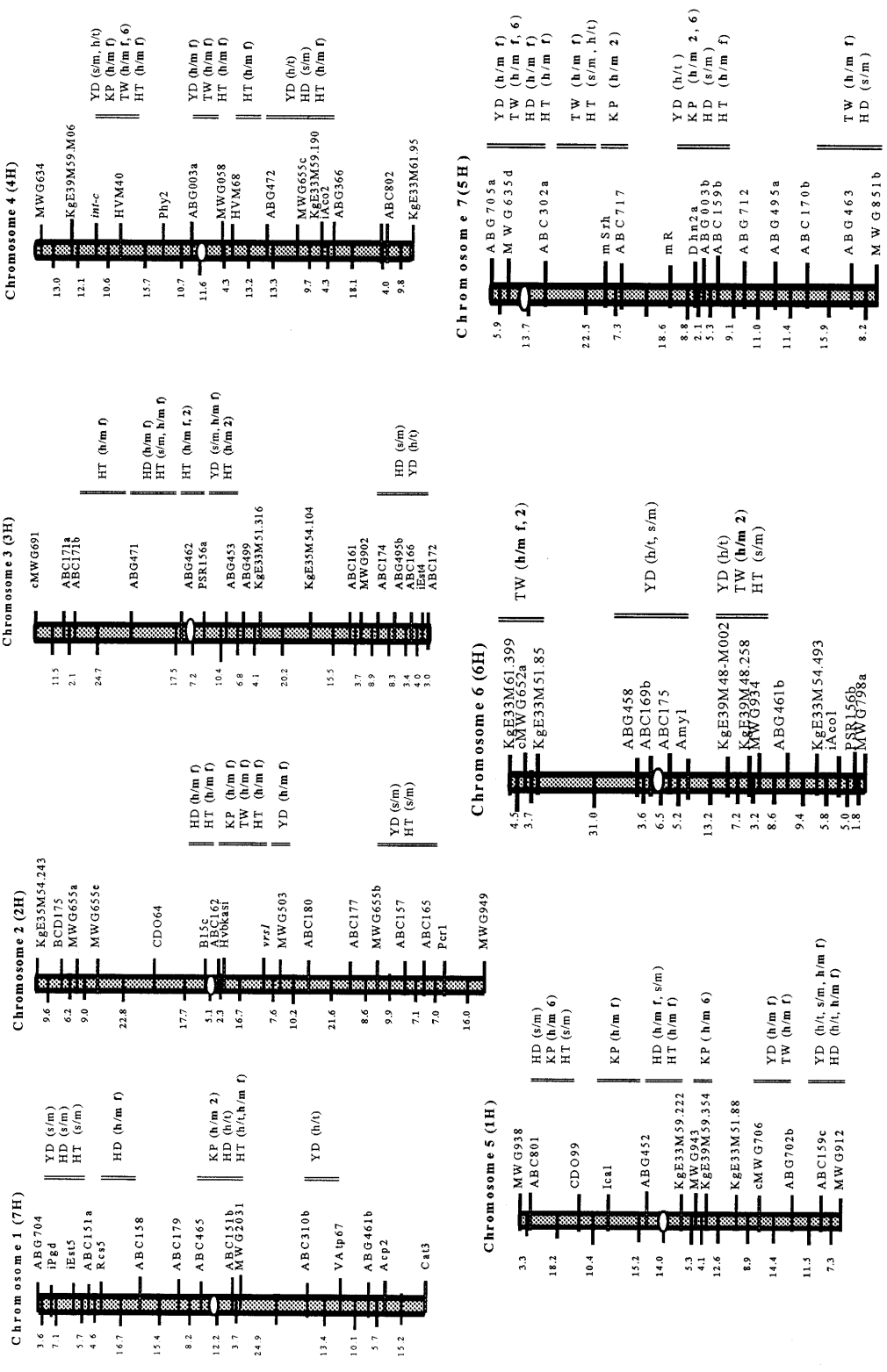

Fig. 3 Summary of agronomic QTL regions detected in three mapping populations: Harrington×Morex (*h/m*), Steptoe×Morex (*s/m*), and Harrington×TR306 (*h/t*), based on Hayes et al. (1997) but modified for chromosome 4 (4H) to include the *int-c* locus. *YD* Yield, *TW* test weight, KP kernel plumpness, *HD* heading date, *HT* plant height. QTL for the **h/m** population include those detected in the full population (*f*) and in the two-rowed (*2*) and six-rowed (*6*) sub-populations. Distances are in Kosambi centiMorgans

tributed the higher value alleles at two out of four plant height QTLs.

Pairs of significant QTLs for each trait were tested for significant (P value <0.05) two-locus interaction. Only the interaction between *vsr1* and *int-c* loci for plant height was significant (P value <0.01). The model that included this interaction, plus the four significant main effect QTLs, explained 62% of the phenotypic variance.

Even with a robust set of environments, a large population, and a trait of high heritability, there may still be bias in QTL estimation (Utz et al. 2000). We sampled a broad and representative set of environments. Heritabili-

Table 4 QTL location[a], higher value allele[b], and percentage of phenotypic variance (R^2_p[c]) explained by QTL(s) in the DH progeny of Harrington×Morex for five agronomic traits in multiple environments

Phenotype	Chromosome						R^2_p (%)
Environment	2 (2H)	3 (3H)	4 (4H)	5 (1H)	6 (6H)	7 (5H)	
Yield							
WA 95							
SK 95	*vrs1*-MWG503^M						32
MB 95				cMWG706-ABG702b^M			11
OR 95			ABG003a-MWG058^M				10
WA 96	*vrs1*-MWG503^M					MWG635d-ABC302A^H	28
SK 96		PSR156a-ABG453^H		cMWG706-ABG702b^M ABC159c-MWG912^M			41
MB 96				cMWG706-ABG702b^M			12
OR 96	*vrs1*-MWG503^M	PSR156a-ABG453^H					18
ID 96	*vrs1*-MWG503^M						15
Kernel plumpness							
WA 95	Hvbkasi-*vrs1*^{H}						59
SK 95	Hvbkasi-*vrs1*^{H}						32
MB 95	Hvbkasi-*vrs1*^{H}						68
OR 95	Hvbkasi-*vrs1*^{H}						63
WA 96	Hvbkasi-*vrs1*^{H}						78
SK 96	Hvbkasi-*vrs1*^{H}		*int-c*-Phy2^H				70
MB 96	Hvbkasi-*vrs1*^{H}			Ica1-ABG452^M			61
OR 96	Hvbkasi-*vrs1*^{H}						68
ID 96	Hvbkasi-*vrs1*^{H}						61
Test weight							
WA 95	Hvbkasi-*vrs1*^{H}			cMWG706-ABG702b^H			31
SK 95	Hvbkasi-*vrs1*^{H}					ABG463-MWG851b^M	24
MB 95	Hvbkasi-*vrs1*^{H}		*int-c*-Phy2^H				30
OR 95	Hvbkasi-*vrs1*^{H}			cMWG706-ABG702b^H	KgE33m61.399-KgE33m51.85^H		36
WA 96	Hvbkasi-*vrs1*^{H}		ABG003a-MWG058^H				20
SK 96	Hvbkasi-*vrs1*^{H}					ABC302a-mSrh^H	46
MB 96	Hvbkasi-*vrs1*^{H}		ABG003a-MWG058^H		KgE33m61.399-KgE33m51.85^H		31
OR 96			ABG003a-MWG058^H		KgE33m61.399-KgE33m51.85^H		21
ID 96	Hvbkasi-*vrs1*^{H}						12
Heading date							
WA 95		B15c-ABC162^H			ABG452-KgE33M59.222^M		56
SK 95		B15c-ABC162^H	ABG471-ABG462^M				52
MB 95		B15c-ABC162^H					32
OR 95		B15c-ABC162^H				ABG705a-MWG635d^H	55
WA 96		B15c-ABC162^H			ABC159c-MWG912^H		19
SK 96		B15c-ABC162^H	ABG471-ABG462^M		ABG452-KgE33M59.222^M		45
MB 96	dRcs5-ABC158^H	B15c-ABC162^H					36
ID 96		B15c-ABC162^H			ABG452-KgE33M59.222^M		51

Table 4 (continued)

Phenotype	Chromosome						R^2_p (%)
Environment	2 (2H)	3 (3H)	4 (4H)	5 (1H)	6 (6H)	7 (5H)	
Plant height							
WA 95		Hvbkasi-*vrs*^{H}	ABC171b-ABG471^{M}				47
			ABG462-PSR156a^{M}				
SK 95	ABC465-MWG2031^{M}		ABG462-PSR156a^{M}	*int-c*-Phy2^{H}		MWG635d-ABC302a^{M}	44
MB 95		Hvbkasi-*vrs1*^{H}		*int-c*-Phy2^{H}		MWG635d-ABC302a^{M}	30
OR 95		B15c-ABC162^{H}	ABG462-PSR156a^{M}				35
		Hvbkasi-*vrs*^{H}					
WA 96		Hvbkasi-*vrs1*^{H}	ABG462-PSR156a^{M}	ABG003a-MWG058^{H}			28
SK 96		Hvbkasi-*vrs1*^{H}	ABG462-PSR156a^{M}	*int-c*-Phy2^{H}			47
				ABG003a-MWG058^{H}			
				HVM68-ABG472^{H}			
MB 96		B15c-ABC162^{H}		*int-c*-Phy2^{H}		MWG635d-ABC302a^{M}	33
		Hvbkasi-*vrs1*^{H}					
OR 96		Hvbkasi-*vrs1*^{H}	ABC171b-ABG471^{M}	*int-c*-Phy2^{H}		ABG003b-ABC159b^{M}	53
				ABG003a-MWG058^{H}			
			ABG462-PSR156a^{M}	ABG472-MWG655c^{H}			
ID 96		Hvbkasi-*vrs1*^{H}		*int-c*-Phy2^{H}	ABG452-KgE33M59.222^{M}	MWG635d-ABC302a^{M}	39
				ABG003a-MWG058^{H}			

[a] Flanking markers [b] H, Harrington; M, Morex [c] Multilocus percentage of variance explained by QTLs

ty estimates were high but our population size was small according to the criteria of Utz et al. (2000). Recognizing this limitation, we present individual environment data in Table 4 to make several points. First, limited testing can lead to a failure to detect "large-effect" QTL. For example, the yield QTL coincident with the *vrs1* locus was only significant in four of nine environments. Second, measurement of related, or component, phenotypes is warranted. For example, although the chromosome 2 (2H) yield QTL was significant in only a subset of environments, the QTLs for kernel plumpness and test weight, related with yield, were significant in all and eight out of nine environments, respectively. Finally, QTLs may be significant in only a subset of the total number of test environments, but these QTLs coincide with QTL for the same trait detected in related populations. For example, the chromosome 3 (3H) yield QTL was significant in only two of nine environments, but it coincides with the largest-effect QTL detected in the Steptoe×Morex population (Table 4, Fig. 3). In the individual environment analyses, we found 25 additional QTLs that were undetected in the combined analysis. In general, R^2_p values were higher in the individual analyses than in the joint analysis (Table 4). In the combined analysis (Table 3), only two QTL regions had QTLs coincident for two or more traits – on chromosome 2(2H) and 7 (5H) – but in the individual environment analysis (Table 4) there were additional QTL regions associated with multiple traits. For example, the yield QTLs on chromosomes 4 (4H) and 5 (1H) were also coincident with test weight QTLs. Morex contributed the favorable allele for yield, while Harrington contributed the favorable allele for test weight. The heading date QTL on chromosome 2 (2H) was also coincident, in two environments, with a plant height QTL. In this case Harrington contributed the larger value alleles for both traits. The heading date QTLs on chromosomes 3 (3H) and 7 (5H) have common flanking markers with a plant height QTL.

QTL×environment (E) interaction

QTL×E interaction was significant for all traits, and QTL×E peaks generally coincided with QTL main effect peaks, except for a grain yield QTL on chromosome 5 (1H) (Fig. 2). At this QTL, Morex contributed the larger value allele in six environments, while Harrington contributed the larger value allele in the other three environments (WA95, SK95, and OR95). In this case, there was no significant main effect QTL in the joint analysis of all environments. For the chromosome 2 (2H) yield QTL coincident with the *vrs1* locus, Morex contributed the higher value allele in eight environments and Harrington the larger value allele in one environment (WA95). In this case, there was a significant main effect QTL in the joint analysis of all environments. QTL×E due to changes in magnitude of QTL effect characterized most of the interactions in this experiment. With this type of interaction, it is necessary to decide if a QTL has

Table 5 QTL location[a] higher value allele[b], and percentage of phenotypic variance (R^2_p[c]) explained by QTL(s) in the two-rowed doubled haploid progeny of Harrington×Morex, across eight environments for heading date and nine environments for kernel plumpness, test weight, and height. No yield QTLs were detected in this sub-population

Phenotype	Chromosome				R^2_p (%)
	1 (7H)	3 (3H)	6 (6H)	7 (5H)	
Kernel plumpness	ABC465-MWG2031^M			MSrh-ABC717^M ABG003b-ABC159b^M	12
Test weight			KgE33M61.399-KgE33m51.85^H KgE39M48-M002-KgE39m48.258^H		18
Height		ABG462-PSR156a^H			30

[a] Flanking markers [b] H, Harrington; M, Morex [b] Multilocus percentage of variance explained by QTLs

Table 6 QTL location[a], higher value allele[b], and percentage of henotypic variance (R^2_p[c]) explained by QTL(s) in the six-rowed DH progeny of Harrington×Morex, across nine environments for kernel plumpness and test weight. Neither yield nor plant height QTLs were detected in this sub-population

Phenotype	Chromosome			R^2p (%)
	4 (4H)	5 (1H)	7 (5H)	
Kernel plumpness		ABC801-CDO99^H MWG943-KGE33M51.88^M	ABG003b-ABC159b^M	35
Test weight	*int-c*-Phy2^H		MWG635d-ABC302a^M	25

[a] Flanking markers [b] H, Harrington; M, Morex
[c] Multilocus percentage of variance explained by QTLs

sufficient effect on the phenotype to warrant selection. In cases where the favorable allele phase changes, breeding objectives will be different for environments in which alternative alleles have favorable effects. Zhu et al. (1999) reported favorable allele phase changes at yield QTLs within a sample of environments that was relatively homogeneous geographically. This degree of interaction will further complicate breeding schemes built on QTL information.

Two-row/six-row sub-population analyses

In order to explore the effects of the two loci that determine inflorescence morphology on the quantitative traits studied, we performed QTL analyses in the sub-populations sorted by the allelic combinations at these two loci. In the first case, QTL analyses were performed on the full population (140 DH lines) sorted by *vrs1* (72 *Vrs1Vrs1* and 68 *vrs1vrs1* lines, respectively) for traits where the *vrs1* locus had a significant effect (yield, kernel plumpness, test weight, and height) (Tables 5 and 6, Fig. 4). No new yield QTLs were detected in either of the sub-populations (Tables 5 and 6). In the two-rowed sub-population, three new kernel plumpness QTLs were detected, one on chromosome 1 (7H) and two on chromosome 7 (5H) (Table 5, Fig. 4). One of the QTLs on chromosome 7 (5H) was coincident with a kernel plumpness QTL in the six-rowed sub-population. In the six-rowed sub-population, two additional kernel plumpness QTLs were detected on chromosome 5 (1H) (Table 6, Fig. 4). Two test weight QTLs were detected in the two-rowed sub-population on chromosome 6 (6H). One of these QTLs was coincident with a test weight QTL detected in the full population in three environments (Table 3). Two test weight QTLs were detected in the six-rowed sub-population. One of these was flanked by the *int-c* locus, and the other mapped to the short arm of chromosome 7 (5H), where a coincident test weight QTL was detected in the full population. Only one plant height QTL was detected in the two-rowed sub-population, and it was coincident with a plant height QTL in the full population on chromosome 3 (3H). No plant height QTLs were detected in the six-rowed sub-population.

Although the only significant QTL coincident with *int-c* was for plant height, in order to further explore the effects of this locus (and linked loci) on agronomic traits, we sorted the full population by *int-c* allele configuration (69 *Int-cInt-c* and 71 *int-cint-c*). No new QTLs were detected in these sub-populations. For all traits except plant height, the only significant QTLs were on chromosome 2 (2H) and coincided with the locations of the QTLs detected in the full population. For plant height, QTLs were detected on chromosomes 2 (2H) and 3 (3H) and coincided with those in the full population (data not shown).

Coincidence of QTLs in Harrington×Morex with QTLs in related populations

One of the objectives of this study was to determine the consistency of QTL significance in the three NABGMP mapping populations sharing common parents. QTLs for agronomic traits significant in each of the three mapping populations are shown on the Harrington×Morex map in

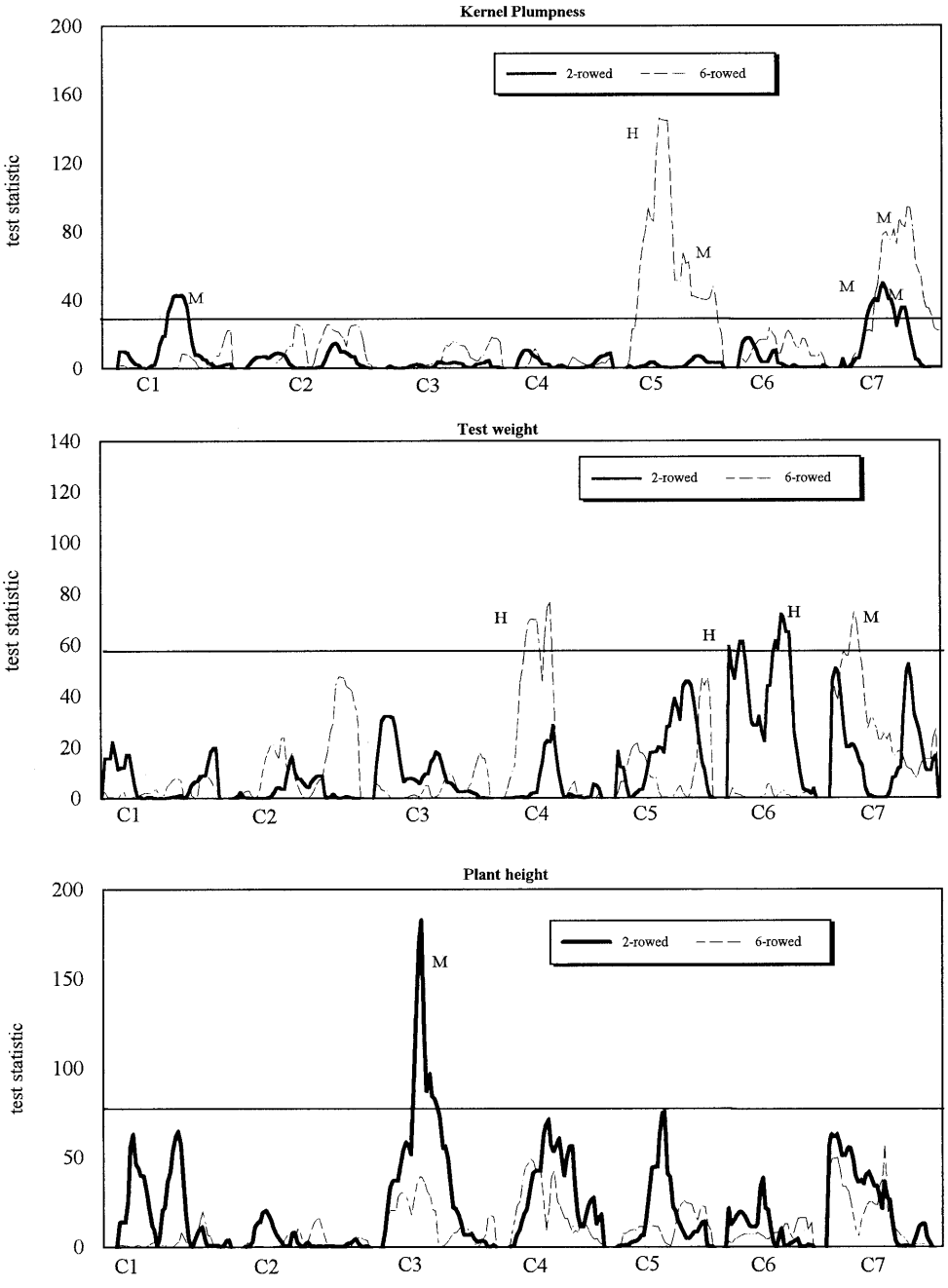

Fig. 4 Scans of test statistics for simple interal mapping (SIM) from the two-rowed population (*solid line*) and the six-rowed population (*broken line*) of the Harrington×Morex DH lines. Scans are shown for three agronomic traits for which *vrs1* locus had a significant effect. No yield QTL were detected in either subpopulation. Chromosomes (*C*) *1* (7H), *2* (2H), *3* (3H), *4* (4H), *5* (1H), *6* (6H), and *7* (5H) are shown *left to right. Horizontal lines* show SIM threshold estimated from 5,000 permutations. The parent giving the higher value allele is shown for each QTL peak (*H* Harrington, *M* Morex)

Fig. 3. A total of 31 QTL regions were detected when the three populations and the QTLs detected in the Harrington×Morex two-rowed and six-rowed sub-populations were considered. Out of 31 regions, 14 were coincident in at least two populations, considering all agronomic traits, and nine were coincident for the same trait. The only QTL coincident in the three populations was the chromosome 5 (1H) yield QTL bracketed by ABC159c and MWG912. The QTLs on chromosome 2 (2H) coincident with the *vrs1* locus were unique to the Harrington×Morex population. These data suggest that these two accessions, representing the two-rowed and six-rowed germplasm groups, may carry alternative alleles at agronomic trait loci throughout the genome. However, the largest and most consistent QTL effects were associated with the *vrs1* locus.

Conclusions

The results presented in this study are consistent with previous reports on the relationship of inflorescence type and multiple agronomic traits in barley (Jui et al. 1997; Kjaer and Jensen 1996; Powell et al. 1990) on the effects of the loci determining inflorescence type (*vrs1* and *int-c*) on multiple agronomic traits. Our QTL analysis of agronomic traits could not distinguish between linkage and pleiotropy for yield, kernel plumpness, test weight,

Table 7 Agronomic data[a] and allele architecture for the five highest and five lowest yielding DH lines in the Harrington×Morex population

Line no.	Head type	Yield (kg ha^{-1})	Kernel plumpness (%)	Test weight (kg hl^{-1})	Heading date (days)	Plant height (cm)	Closest marker and parent contributing the allele for each marker[b]					
							vsr1 **M**[c]	PSR156a **H**[c]	ABG0003a **M**[c]	CMWG706 **M**[c]	ABC159c **M**[c]	MWG635d **H**[c]
Five DH lines high yielding												
31	Six-rowed	5,693	70	64	186	88	**M**	**H**	**M**	**M**	**M**	**H**
22	Six-rowed	5,413	75	65	182	77	**M**	**H**	**M**	**M**	**M**	**H**
83	Six-rowed	5,386	62	66	183	88	**M**	**H**	**H**	**M**	**M**	**M**
84	Two-rowed	5,379	88	66	186	84	**H**	**H**	**H**	**H**	**H**	**H**
116	Six-rowed	5,353	67	64	183	86	**M**	**M**	**M**	**M**	**M**	**H**
Five DH lines low yielding												
122	Two-rowed	3,166	88	67	186	93	**H**	**M**	**H**	**H**	**H**	**M**
106	Two-rowed	3,270	95	65	188	113	**H**	**M**	**H**	**M**	**M**	**M**
35	Two-rowed	3,391	89	66	186	106	**H**	**M**	**H**	**H**	**H**	**M**
91	Six-rowed	3,452	78	67	184	96	**M**	**M**	**M**	**H**	**H**	-
75	Two-rowed	3,483	95	65	185	95	**H**	**M**	**M**	**M**	**M**	**H**

[a] Averaged over nine environments for yield, kernel plumpness, test weight and plant height, and eight environments for heading date

[b] **M**, Morex ; **H**, Harrington

[c] Parent contributing the higher value allele

and plant height. Phenotypic analyses of *vrs1* near-isogenic lines have not been able to distinguish between the effects of linkage and pleiotropy (Takahashi et al. 1975). Molecular marker analysis of these stocks is warranted and could assist in cloning the *vrs1* and *int-c* genes. This would allow for complementation tests involving these loci and characterization of linked loci. Our data suggest that the QTL determining heading date is distal to the *vrs1* locus on the long arm of chromosome 2 (2H).

Other QTLs in addition to those associated with the *vrs1* and *int-c* loci were detected, suggesting that the two-rowed and six-rowed North American malting quality standards varieties carry different alleles at loci distributed throughout the genome. Analysis of the two-rowed and six-rowed sub-populations, and of the data from individual environments, revealed additional QTLs, but these estimates may be biased by sampling and population size.

Previous reports described difficulties in obtaining agronomically acceptable lines from two-rowed×six-rowed crosses (Jui et al. 1996; Powell et al. 1990; Takahashi et al 1975). Our data indicate that the yield of six-rowed genotypes should be improved by the introgression of alleles at QTLs where Harrington contributed favorable alleles. However, due to the consistent advantage of Morex alleles at the *vrs1* locus and/or linked regions, it would be difficult to improve yield of two-rowed genotypes. Four out of the five highest yielding lines were six-rowed, while four out of the five lowest yielding were two-rowed (Table 7). Higher value allele phases for those genotypes generally follow predicted patterns except for the anomalous two-rowed and six-rowed types in the high-yielding and low-yielding groups, respectively. The other determinant of inflorescence morphology (*int-c*) does have an effect on yield, but in this case it is limited and its interaction with the *vrs1* locus is not significant. Other QTLs in addition to those associated with determinants of inflorescence morphology had an effect on yield in this sample of germplasm. One of these is a QTL on chromosome 3 (3H), where Harrington contributed the favorable allele. A yield QTL was reported at this location by Larson et al. (1997), in the Steptoe×Morex germplasm, and they attributed the yield QTL to the propensity of Morex to shatter in dry environments. The positive effects of Harrington alleles at this QTL may explain the transgressive segregation for yield in six-rowed genotypes in the Harrington×Morex population.

QTL analysis can localize determinants of complex traits to specific regions of the genome. This is an improvement over previous quantitative analysis tools, which only allowed estimation of number of genes and allele values (Allard 1988). Bias due to limited population size is an important issue in QTL analysis (Melchinger et al. 1998). However, the resources available to most breeding and research programs will limit the number of genotypes and environments that can be analyzed. Therefore, QTL analysis based on segregating populations can be a useful first step toward identifying regions of the genome to target in marker-assisted selection experiments, understanding the basis of correlated responses, characterizing the genes defining mayor germplasm groups, and toward identifying candidate genes underlying complex phenotypes.

Acknowledgements This research was supported by the U.S. and Canadian components of the North American Barley Genome Mapping Project. Support for the senior author was provided by the Mexican Government through the National Institute of Forest Agriculture and Livestock Research (INIFAP) and the National Council of Science and Technology (CONACYT).

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