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L.A. Marquez-Cedillo · P.M. Hayes · B.L. Jones
A. Kleinhofs · W.G. Legge · B.G. Rossnagel · K. Sato
E. Ullrich · D.M. Wesenberg
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QTL analysis of malting quality in barley based on the doubled-haploid progeny of two elite North American varieties representing different germplasm groups

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Abstract Characterization of the determinants of economically important phenotypes showing complex inheritance should lead to the more effective use of genetic resources. This study was conducted to determine the number, genome location and effects of QTLs determining malting quality in the two North American barley quality standards. Using a doubled-haploid population of 140 lines from the cross of Harrington×Morex, malting quality phenotype data sets from eight environments, and a 107-marker linkage map, QTL analyses were performed using simple interval mapping and simplified composite interval mapping procedures. Seventeen QTLs were associated with seven grain and malting quality traits (percentage of plump kernels, test weight, grain protein percentage, soluble/total protein ratio, α-amylase activity, diastatic power and malt-extract percentage). QTLs for multiple traits were coincident. The loci controlling inflorescence type [*vrs1* on chromosome 2(2H) and *int-c* on chromosome 4(4H)] were coincident with QTLs affecting all traits except malt-extract percentage. The largest effect QTLs, for the percentage of plump kernels, test weight protein percentage, S/T ratio and diastatic power, were coincident with the *vrs1* locus. QTL analyses were conducted separately for each sub-population (six-rowed and two-rowed). Eleven new QTLs were detected in the subpopulations. There were significant interactions between the *vrs1* and *int-c* loci for grain-protein percentage and S/T protein ratio. Results suggest that this mating of two different germplasm groups caused a disruption of the balance of traits. Information on the number, position and effects of QTLs determining components of malting quality may be useful for maintaining specific allele configurations that determine target quality profiles.

Key words *Hordeum vulgare* · Two-rowed · Six-rowed · Quality traits · Quantitative trait loci

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North American Barley Genome Mapping Project
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L.A. Marquez-Cedillo · P.M. Hayes (✉)
Department of Crop and Soil Science, Oregon State University, Corvallis, OR 97331, USA
Fax: +1-541-737-1589
e-mail: patrick.m.hayes@orst.edu

B.L. Jones
Cereal Crops Research Unit, USDA-ARS, 501 N. Walnut St., Madison, WI 53705, USA

A. Kleinhofs · S.E.Ullrich
Department of Crop and Soil Science,
Washington State University, Pullman, WA 99164, USA

W.G. Legge
Agriculture and Agri-Food Canada, Brandon MB R7A 5Y3, Canada

B.G. Rossnagel
Crop Development Centre, University of Saskatchewan, Saskatoon, SK S7N 5A8, Canada

K. Sato
Research Institute for Bioresources, Okayama University, Kurashiki 710-0046 Japan

D.M. Wesenberg
National Small Grains Germplasm Research Facility, P.O. Box 307, Aberdeen, ID 83210, USA

Introduction

Quantitative trait locus (QTL)-analysis tools are useful for dissecting complex traits and for identifying favorable alleles in diverse germplasm. Characterization of the determinants of economically important phenotypes showing complex inheritance should lead to a more effective use of genetic resources. The malting quality of barley (*Hordeum vulgare* L.) is an economically important phenotype that represents the net effects of a number of interacting component traits.

Malting is a process involving controlled germination of the barley grain. Pollock (1962), Burger and La Berge (1985), and Bamforth and Barclay (1993) provide reviews of the malting process. Briefly, cytolitic, amylolytic and proteolytic enzymes dissolve cell walls, allowing diastatic enzymes to degrade starch granules. Polymeric carbohy-

drates and proteins are broken down to component sugars and amino acids or peptides, providing fermentable sugars and nitrogenous compounds as substrates for fermentation (Pollock 1962). Key enzymes in the pathways culminating in malting quality are α-amylase, β-amylase, β-glucanase, and the endo- and exo-proteinases.

α-amylase activity measures the effects of enzymes which initially attack starch, forming the substrates upon which other enzymes can work. β-amylase reduces the ends of glucose chains, breaking glucosidase bonds, and liberating maltose. The joint action of α-amylase and β-amylase and any other carbohydrate-degrading enzymes is termed "diastatic power" (Pollock 1962; Burger and La Berge 1985). Soluble and hydrolyzed forms of endosperm proteins are essential for germination. The ratio of soluble protein to total protein (S/T) indicates the degree of proteolysis and the degree of modification of grain to malt (Hunter 1962; Burger and La Berge 1985). Malt-extract percentage is a measure of the sugars and nitrogenous compounds available for yeast nutrition.

Two indicators of grain physical quality that are prerequisites for malting quality are the percentage of plump grain (kernel plumpness) and test weight. These two traits are important because they indicate uniformity of kernel size and germination. Both attributes are important during the malting process. Barley grain with adequate physical quality has a test weight ≥60 kg hl^{-1} and ≥70% of plump grain (as measured on a 2.38-mm sieve). Grain protein level is the initial commercial specification for malting barley and correlates with many of the other quality traits. Within a narrow acceptable range of protein (10.5–13.5%), there are additional specifications for S/T protein ratio (40–46%), α-amylase activity (35–60 20° DU), diastatic power activity (95–170° ASBC) and malt extract percentage (≥78%). Target quality profiles differ somewhat for the two germplasm groups: two-rowed and six-rowed. These germplasm groups are discussed in greater detail in a succeeding section.

A number of the genes encoding key enzymes in malting quality pathways have been cloned (von Wettstein-Knowles 1992; Hayes 1996). However, the phenotypic distributions for components of malting quality are usually continuous, leading to the generalization that these traits are quantitatively inherited (Bell and Lupton 1962). The components of malting quality have, accordingly, been the subject of QTL analysis (Hayes et al. 1993; Mather et al. 1997). In some cases the map positions of structural genes coincide with QTLs, as in the case of β-amylase 1 and diastatic power (Hayes et al. 1997). In most cases, however, QTLs do not coincide with known genes (Hayes et al. 1997; Mather et al. 1997). These QTLs may represent the effects of regulatory genes. Efforts are underway to determine the genetic basis of these QTLs (Han et al. 1997).

The North American Barley Genome Mapping Project (NABGMP) has focused on QTL analysis of malting and agronomic traits in three populations of doubled-haploid lines derived from the following crosses: Steptoe×Morex (Hayes et al. 1993); Harrington×TR306 (Tinker et al. 1996; Mather et al. 1997) and Harrington×Morex (Hayes et al. 1997). Steptoe and Morex are six-rowed varieties. Harrington and TR306 are two-rowed varieties. The number of kernel rows refers to the number of fertile spikelets per rachis node. In two-rowed barleys only the central spikelet of a triplet is fertile, while in six-rowed barleys all three spikelets are fertile. Two loci – the *vrs1* locus located in the centromeric region of chromosome 2 (2H), and the *int-c* on the short arm of chromosome 4 (4H) – control this trait (Nilan 1964; Franckowiak and Lundqvist 1997; Lundqvist and Franckowiak 1997). Although the number of kernel rows (hereafter referred to as inflorescence type) is simply inherited, two-rowed and six-rowed barleys are distinct germplasm groups (Bell and Lupton 1962; Kjaer and Jensen 1996; Jui et al. 1997). 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 and are therefore classified as "feed" barleys. In the progeny of Steptoe (feed) ×Morex (malt), malting quality QTLs mapped to all seven chromosomes (Hayes et al. 1993). In the progeny of Harrington (malt) ×TR306 (feed), malting quality QTLs mapped to all chromosomes except chromosome 2 (Mather et al. 1997). No QTL analysis of malting quality in two-rowed by six-rowed populations has been reported. Agronomic-trait QTLs in two-rowed×six-rowed crosses were mapped throughout the genome, including the locations of the two loci determining inflorescence type (Kjaer and Jensen 1996).

The objective of the present investigation was to determine the number, location and effects of malting quality QTLs in the North American two-rowed and six-rowed standards. We reasoned that if these genotypes had contrasting alleles at malting quality QTLs, this information could be useful for developing new genotypes with superior malting quality profiles.

Materials and methods

One hundred and forty doubled-haploid (DH) lines were produced from the F_1 of Harrington×Morex by the *H. bulbosum* method, as described by 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). This map was used for mapping malting quality QTLs. We added the *int-c* locus to this map. Lateral florets were scored as homozygous recessive (Harrington alleles) for those DH lines with small laterals, no anthers and no awns, and homozygous dominant (Morex alleles) for those with sessile laterals, anthers and large awns.

The DH lines and parents were grown in eight environments; three locations in 1995 (Pullman, Washington, USA; Saskatoon, Saskatchewan; and Brandon, Manitoba, Canada) and five locations in 1996 (Pullman, Washington; Pendleton, Oregon; and Aberdeen, Idaho USA; Saskatoon, Saskatchewan, and Brandon, Manitoba, Canada) without replication. Plot size and management were in accordance with local practice. The percentage of plump kernels was calculated as the weight of a 100-g sample of grain remaining on a 2.38 mm×1.91 cm slotted sieve after 30 shaking cycles on a Seedburo strand sizer/shaker. Test weight was measured as the weight in grams of grain contained in a one-quart cylinder. This weight was divided by 10.99 to convert to kg hl^{-1}.

A 170-g sample of each DH line and each of the parents from each location was malted and used to measure malting quality [grain protein (%), S/T protein ratio (%), α-amylase activity (20° DU), diastatic power (° ASBC), and malt extract (%)] according to the standard procedures of the USDA/ARS Cereal Crops Research Unit, Madison, Wisconsin. Protocols are available upon request (bljones@facstaff.wisc.edu). The American Society of Brewing Chemists and the European Brewery Convention (EBC) units are equivalent for grain protein percentage, malt extract percentage and α-amylase activity. The S/T protein ratio is equivalent to the Kolbach index. Diastatic power in Dextrinizing units is converted to Windish-Kolbach units as follows: (Dextrinizing units-4/0.3) (Oziel et al. 1996).

QTL analyses were performed using 5000 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 SIM and sCIM peaks, and the SIM peaks exceeded the significance threshold. For those traits where significant ($P<0.05$) QTLs were detected that were coincident with the *vrs1* locus, separate QTL analyses were performed at the whole genome for the two-rowed (72 lines) and six-rowed (68 lines) sub-populations.

Approximate estimates of heritability were calculated as: $h^2=\sigma^2g/)\sigma^2g+\sigma^2e/r)$, where σ^2g is the variance among DH lines, σ^2e is the error variance among DH lines, and r is the number of environments. Environments were substituted for replications because the cost of malting quality analyses precludes replicated testing within environments. Multiple regression procedures (implemented in the Statistical Analysis System, SAS Institute 1989) were used to test the significance of two-locus interactions.

Results and discussion

Malting quality in parents and the doubled-haploid population

Each of the eight environments that were sampled gave distinct quality profiles (Table 1). Because QTL×environment interaction was significant only for two malt-extract-percentage QTLs on chromosome 5 (1H) (Fig. 1), means across environments were used for QTL analysis. Averaged across environments, Harrington and Morex approached the US malting industry specifications (Table 2). Harrington had a lower grain protein percentage and diastatic power activity, and a higher percentage of plump grain, α-amylase activity, and malt-extract-percentage than Morex. Both cultivars had the same test weight. The two-rowed subpopulation had significantly ($P<0.01$) plumper grains than the six-rowed subpopulation. However, the two-rowed and six-rowed subpopulations had malting quality profiles contrary to expectations. The two-rowed subpopulation had a significantly ($P<0.01$) higher diastatic power, and grain protein percentage than the six-rowed subpopulation (Table 2). The malt-extract-percentage values for two subpopulations were not significantly different. Both were lower than the lowest malt-extract-percentage parent (Morex). Both subpopulations had malt-extract-percentage values lower than in-

Table 1 Means and standard deviations for seven grain and malting quality traits in Harrington, Morex and their doubled-haploid progeny in eight environments. See text for definition of the environments[a]

Environment	Kernel plumpness (%)				Test weight (kg hl^{-1}				Grain protein (%)				S/T protein (%)			
	Parents		DH Lines		Parents		DH Lines		Parents		DH Lines		Parents		DH Lines	
	H	M	μ	σ	H	M	μ	σ	H	M	μ	σ	H	M	μ	σ
WA 95	91	84	81	14	67	65	63	2	12	14	14	2	34	31	30	6
SK95	84	85	76	14	66	66	63	2	12	14	13	1	36	32	35	5
MB95	87	80	78	19	64	65	64	3	11	13	13	1	38	37	32	6
WA96	94	72	76	21	68	69	67	2	12	13	14	1	33	37	35	5
OR96	94	90	80	18	71	71	70	2	10	10	10	1	43	44	42	5
ID96	92	93	84	16	70	69	67	2	14	15	14	3	33	33	31	6
SK96	79	67	71	23	64	66	64	4	12	14	14	1	38	37	35	6
MB96	90	96	88	10	66	65	64	2	11	14	14	1	45	34	38	8

Environment	α-amylase (20° DU)				Diastatic power (° ASBC)				Malt Extract (%)			
	Parents		DH Lines		Parents		DH Lines		Parents		DH Lines	
	H	M	μ	σ	H	M	μ	σ	H	M	μ	σ
WA95	47	38	42	9	98	126	130	30	80	77	74	11
SK95	58	47	45	9	121	137	120	25	79	77	75	1
MB95	68	61	56	10	125	145	141	23	79	78	75	1
WA96	59	54	55	6	105	127	122	26	80	79	77	6
OR96	55	46	49	7	90	84	91	18	82	81	80	1
ID96	52	45	46	12	134	164	147	42	79	78	72	1
SK96	47	45	48	8	115	173	154	27	79	76	77	2
MB96	61	55	59	12	104	152	148	26	81	78	77	16

[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)

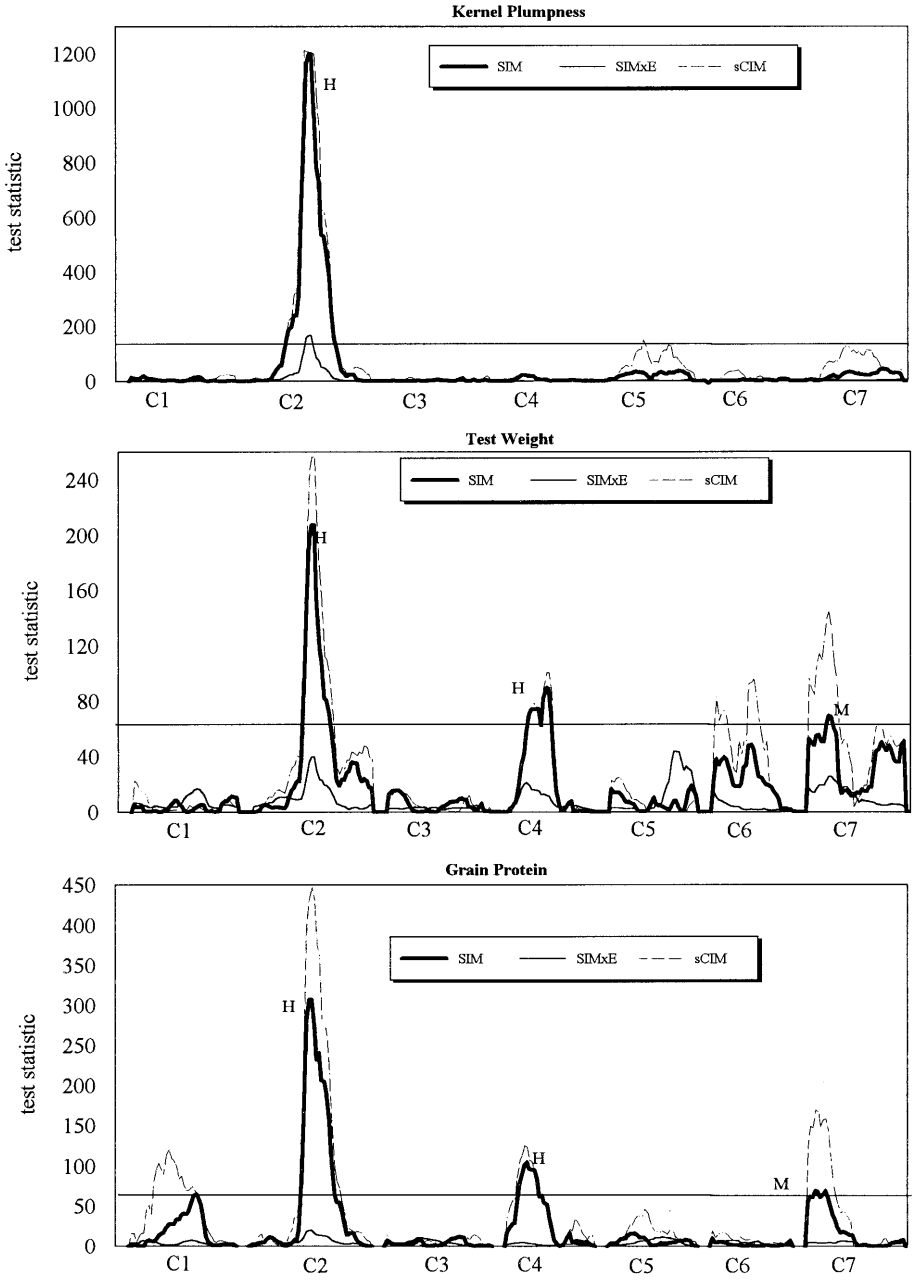


Fig. 1 Scans of test statistics (Y-axis) for simple interval mapping (SIM, *solid wider lines*), simple composite interval mapping (sCIM, *broken lines*) and QTL×environment interaction(solid, *thinner lines*) for the full population of DH lines from Harrington×Morex. Scans are shown for seven malting traits, as indicated. Chromosomes 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 5000 permutations. The parent giving the higher value allele is shown for each QTL peak (*H*=Harrington; *M*=Morex)

Table 2 Grain and malting quality data for Harrington, Morex, their 140 DH progeny, and the two-rowed and six-rowed subpopulations, averaged over eight environments

Trait	Parents		Doubled-haploid progeny						
	Harrington	Morex	Two-rowed	Six-rowed	All lines				
	$\bar{x}$	$\bar{x}$	μ	μ	μ	σ	Min	Max	$h^{2\,a}$
Kernel plumpness	90	85	93	66** [b]	80	18	6	99	98
Test weight	67	67	66	64**	65	3	50	75	97
Grain protein	11	13	14	12**	14	1	11	17	79
S/T protein	38	36	35	38**	36	4	28	46	83
α-amylase	56	49	50	51*	51	6	34	67	84
Diastatic power	111	138	137	122**	130	17	94	178	83
Malt extract	80	78	77	77ns	77	2	69	80	25

[a] Estimated as the percentage of genetic variance attributable to DH lines environments as replications
[b] Significance of *t*-tests comparing the means of the two-rowed and the six-rowed subpopulations: ns=non-significant; * significant at $P<0.05$; and ** significant at $P<0.01$

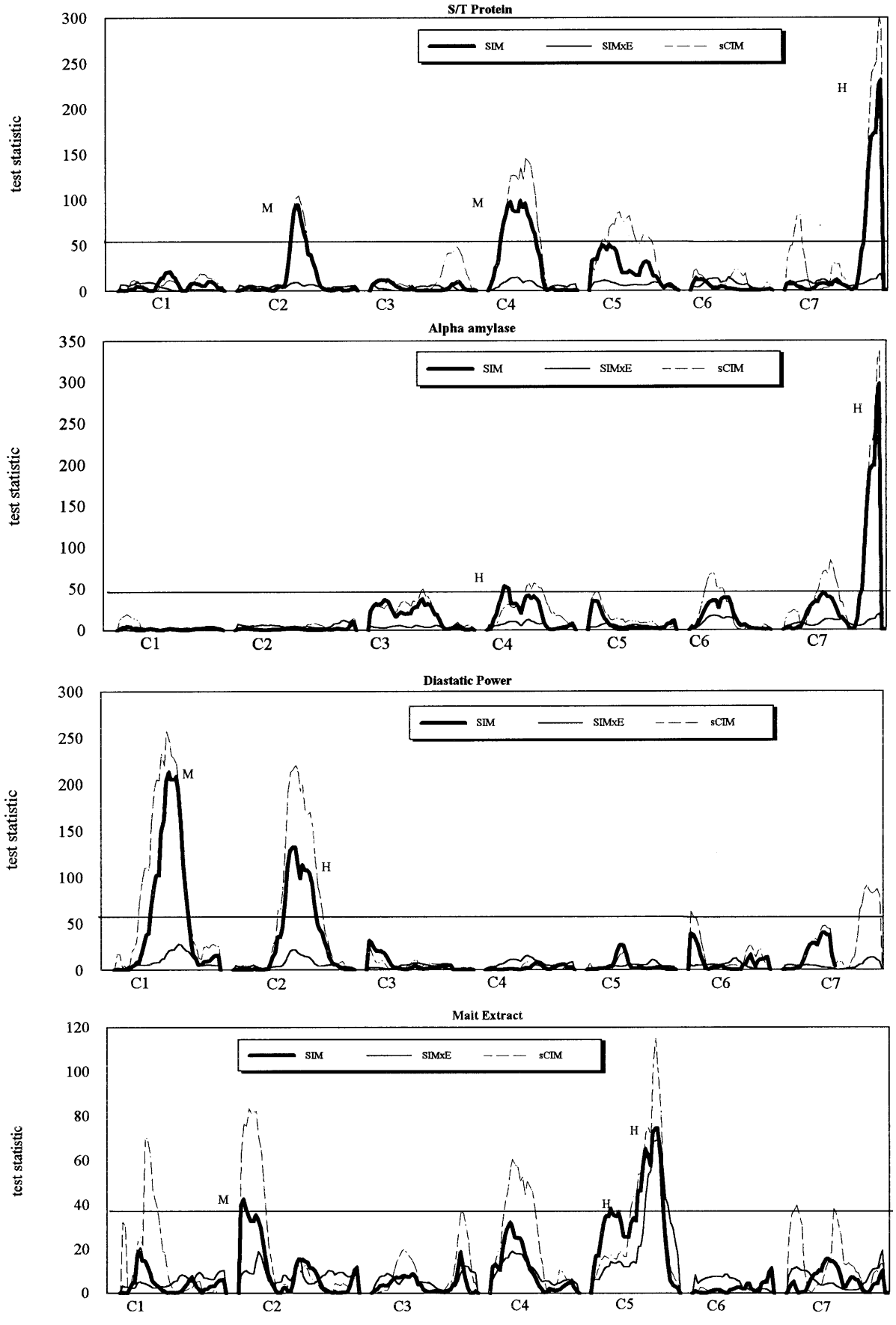


Fig. 1 (continued)

Table 3 QTL location[a], higher value allele[b], and percentage of phenotypic variance accounted for by QTLs in the doubled-haploid progeny of Harrington×Morex, averaged over eight environments

Phenotype	Chromosome					R^2p^c
	1 (7H)	2 (2)	4 (4H)	5 (1H)	7 (5H)	
Kernel plumpness		*vrs1*-MWG503^H				61
Test weight		*vrs1*-MWG503^H	ABG003a-MWGO58^H		MWG635d-ABC302a^M	27
Grain protein		*vrs1*-MWG503^H	*int-c*-HVM40^H		MWG635d-ABC302a^M	38
S/T protein		*vrs1*-MWG503^M	*int-c*-HVM40^M		ABG463-MWG851b^H	32
α-amylase			*int-c*-HVM40^H		ABG463-MWG851b^H	26
Diastatic power	ABC465-MWG2031^M	*vrs1*-MWG503^H		ABC801-CDO99^H		30
Malt extract		KgE35M54.243-BCD175^M		cMWG706-ABG702b^H		15

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

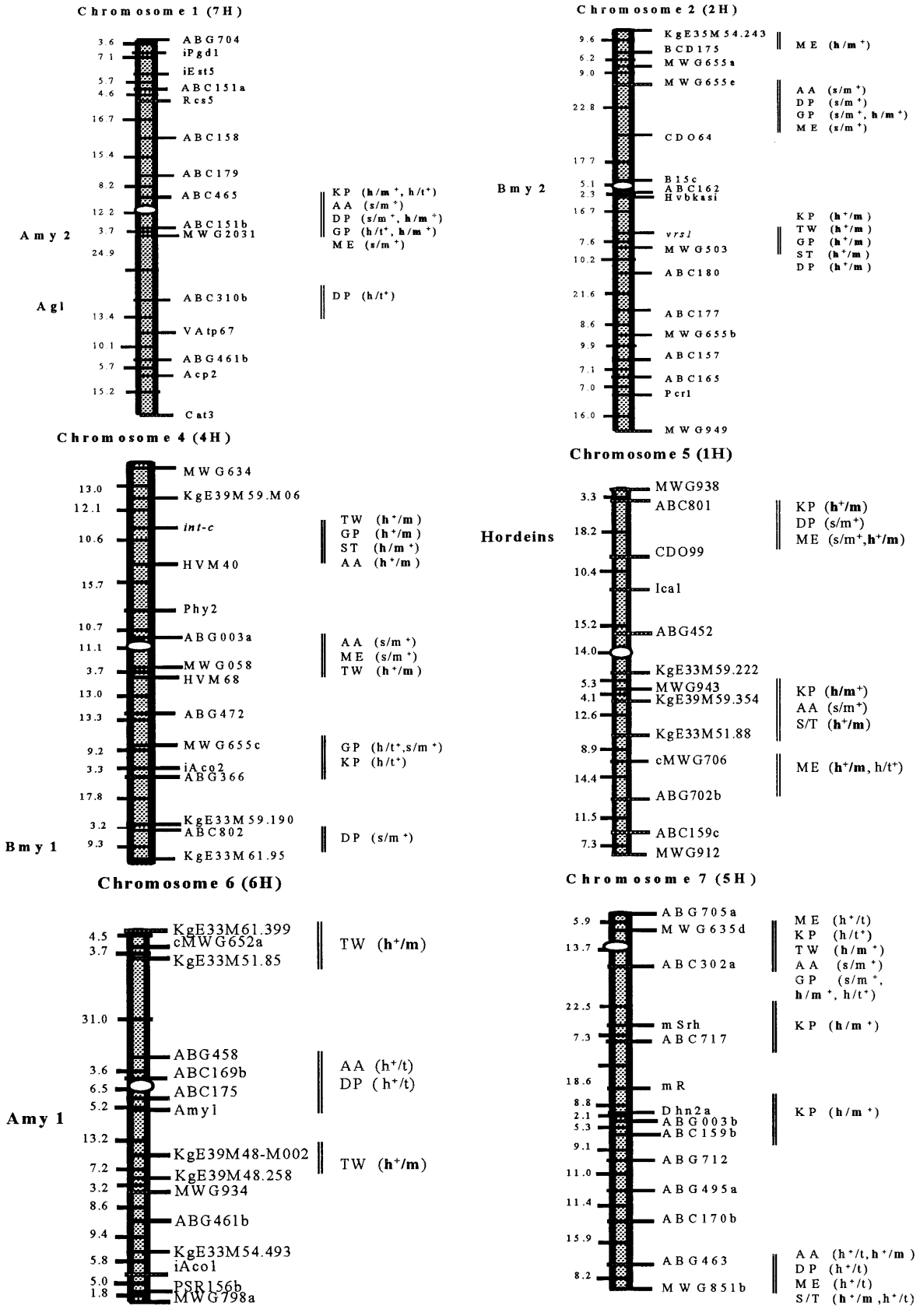
dustry specifications. α-amylase activities were significantly different ($P<0.05$) between the two subpopulations, and both were higher than the lower α-amylase-activity parent (Morex). The estimates of heritability were high for all traits except malt extract percentage (Table 2). The phenotypic data from the DH population suggest a disruption of the balance in malting quality leading to increases in protein and enzymes levels. These increases were not matched by an increase in malt extract percentage, particularly in the two-rowed genotypes.

Detection of malting quality QTLs

All QTLs described in this report were significant with SIM, and corresponding peaks were detected with sCIM. QTLs for the seven components of malting quality were detected in nine regions of the genome on five chromosomes. Coincident QTLs for more than one trait were detected at four of these regions (Table 3, Fig. 1). The subsequent discussion is presented in order of physical grain characteristics, followed by grain protein percentage, S/T protein ratio, enzyme activities, and finally malt extract percentage. Only one QTL for kernel plumpness was detected. It explained 61% of the phenotypic variation for this trait. This kernel plumpness QTL coincided with the *vrs1* locus and QTLs for test weight, grain protein percentage, S/T protein ratio and diastatic power. Harrington contributed larger-value alleles for kernel plumpness, test weight, protein percentage and diastatic power, while Morex contributed higher-values alleles for the S/T protein ratio (Table 3; Fig. 1). In addition to the test-weight QTL on chromosome 2 (2H), two test-weight QTLs were detected, one on chromesome 4 (4H) and one on chromosome 7 (5H). QTLs for grain protein were detected on chromosomes 2 (2H), 4 (4H), and 7(5H).

Coincident QTLs may be due to linkage or pleiotropy. The two cannot be distinguished at the level of resolution afforded by this mapping population. In this case, inflorescence architecture could determine the grain size and the allocation of protein and enzymes to kernels. Alternatively, the grain-protein-percentage, diastatic-power, and S/T-protein ratio QTL effects could be due to a

Fig. 2 Skeleton map of the Harrington×Morex population after (Hayes et al. 1997) but modified for chromosome 4 (*4H*) to include the *int-c* locus. QTL detected in the Harrington×Morex (***h*/*m***); Steptoe×Morex (s/*m*) and Harrington×TR306 (*h*/*t*) populations are shown on the right of each linkage group. QTLs for the h/m population include those detected in the full population and in the two-rowed and six-rowed subpopulations (see test for details). The parent contributing the higher value allele is marked with a^+ sign. *KP*=Kernel plumpness; *TW*=Test weight; GP=Grain protein; *S/T* Soluble/total protein; *AA*=α-amylase; *DP*=Diastatic power, *ME*=Malt extract. QTLs detected in the h/t and s/m populations are based on the report of Hayes et al. (1993) and Mather et al. (1997). Loci on the left of each chromosome correspond to mapped genes that have a role in malting quality. Distances are in Kosambi cM. Chromosome 3 (3H) is not shown because no malting quality QTLs were detected on this chromosome in the Harrington×Morex population


Chromosome 1 (7H)
3.6 ABG704
7.1 iPgd1
iEst5
5.7 ABC151a
4.6 Rcs5
16.7 ABC158
15.4 ABC179
8.2 ABC465
12.2 ABC151b
Amy 2 3.7 MWG2031
24.9
Agl ABC310b
13.4 VAtp67
10.1 ABG461b
5.7 Acp2
15.2 Cat3
KP (h/m+, h/t+)
AA (s/m+)
DP (s/m+, h/m+)
GP (h/t+, h/m+)
ME (s/m+)
DP (h/t+)
Chromosome 4 (4H)
MWG634
13.0 KgE39M59.M06
12.1 int-c
10.6 HVM40
15.7 Phy2
10.7 ABG003a
11.1 MWG058
3.7 HVM68
13.0 ABG472
13.3 MWG655c
9.2 iAco2
3.3 ABG366
17.8 KgE33M59.190
3.2 ABC802
Bmy 1 9.3 KgE33M61.95
TW (h+/m)
GP (h+/m)
ST (h/m+)
AA (h+/m)
AA (s/m+)
ME (s/m+)
TW (h+/m)
GP (h/t+,s/m+)
KP (h/t+)
DP (s/m+)
Chromosome 6 (6H)
4.5 KgE33M61.399
3.7 cMWG652a
KgE33M51.85
31.0
ABG458
3.6 ABC169b
6.5 ABC175
Amy 1 5.2 Amy1
13.2 KgE39M48-M002
7.2 KgE39M48.258
3.2 MWG934
8.6 ABG461b
9.4 KgE33M54.493
5.8 iAco1
5.0 PSR156b
1.8 MWG798a
TW (h+/m)
AA (h+/t)
DP (h+/t)
TW (h+/m)
Chromosome 2 (2H)
9.6 KgE35M54.243
6.2 BCD175
9.0 MWG655a
MWG655e
22.8 CDO64
17.7
Bmy 2 5.1 B15c
2.3 ABC162
Hvbkasi
16.7 vrs1
7.6 MWG503
10.2 ABC180
21.6 ABC177
8.6 MWG655b
9.9 ABC157
7.1 ABC165
7.0 Pcr1
16.0 MWG949
ME (h/m+)
AA (s/m+)
DP (s/m+)
GP (s/m+, h/m+)
ME (s/m+)
KP (h+/m)
TW (h+/m)
GP (h+/m)
ST (h+/m)
DP (h+/m)
Chromosome 5 (1H)
3.3 MWG938
ABC801
18.2 CDO99
10.4 Ica1
15.2 ABG452
14.0
KgE33M59.222
5.3 MWG943
4.1 KgE39M59.354
12.6 KgE33M51.88
8.9 cMWG706
14.4 ABG702b
11.5 ABC159c
7.3 MWG912
Hordeins
KP (h+/m)
DP (s/m+)
ME (s/m+,h+/m)
KP (h/m+)
AA (s/m+)
S/T (h+/m)
ME (h+/m, h/t+)
Chromosome 7 (5H)
5.9 ABG705a
MWG635d
13.7 ABC302a
22.5 mSrh
7.3 ABC717
18.6 mR
8.8 Dhn2a
2.1 ABG003b
5.3 ABC159b
9.1 ABG712
11.0 ABG495a
11.4 ABC170b
15.9 ABG463
8.2 MWG851b
ME (h+/t)
KP (h/t+)
TW (h/m+)
AA (s/m+)
GP (s/m+, h/m+, h/t+)
KP (h/m+)
KP (h/m+)
AA (h+/t, h+/m)
DP (h+/t)
ME (h+/t)
S/T (h+/m, h+/t)

gene, or genes, linked to the *vrs1* locus. Since the *vrs1* locus is located in the centromeric region of the chromosome, recombination is suppressed and conservation of different alleles at loci in the two-rowed and six-rowed germplasm groups could be expected. The allele values at the coincident QTLs support the observed pattern of phenotypes in the two-rowed and six-rowed subpopulations, with a higher test weight, percentage of plump grain, grain protein percentage and diastatic power in the two-rowed subpopulation and a higher S/T protein ratio in the six-rowed subpopulation. The role of a conserved linkage block around the *vrs1* locus and/or pleitropic effects of this locus are supported by the absence of malting quality QTLs at this region in other mapping populations which were derived from six-rowed×six-rowed or two-rowed×two-rowed crosses (Fig. 2).

The *int-c* locus, the second determinant of inflorescence type, mapped between KgE39M59.M06 and *HVM40* at a distance from the centromere corresponding to that reported by Nilan (1964). Coincident QTLs and alternative allele phases were detected for test weight, grain protein percentage, S/T protein ratio and α-amylase activity on chromosome 4 (4H) in the region of the *int-c* locus (Fig. 2). Morex contributed the larger-value allele for S/T protein ratio, while Harrington contributed larger-value alleles for test weight, grain protein percentage and α-amylase activity. Higher-value alleles for grain protein percentage and S/T protein ratio may or may not be favorable for malting quality. Rather than quantity, a specific balance is desired. No malting quality QTLs mapped to this region of the genome in the reference populations where the parents are monomorphic for inflorescence type, suggesting that these QTL alleles, like those coincident with the *vrs1* locus, are unique to this two-rowed×six-rowed population. This also suggests that these two traits are attributable to a pleiotropic effect of the *int-c* locus, or to conservation of linkage blocks, as in the case of kernel plumpness, test weight, grain protein percentage, diastatic power, and S/T ratio on chromosome 2 (2H).

The third region where a grain-protein-percentage QTL was detected was at the centromeric region of chromosome 7 (5H). This QTL was coincident with a test-weight QTL. Morex contributed the higher-value allele for both traits (Fig. 1). QTLs were also detected in this region of the genome for multiple traits in the Steptoe× Morex and Harrington×TR306 populations (Fig. 2). A QTL for grain protein percentage was detected in this region in the Steptoe×Morex and Harrington×TR306 populations, where Morex and TR306 contributed the allele with the larger value respectively (Fig. 2). Of the total grain protein percentage, the soluble fractions and enzymes involved in hydrolysis are favorable for malting quality. In addition to the S/T protein ratio QTLs on chromosomes 2 (2H) and 4 (4H) that coincided with grain protein percentage QTLs, a S/T-protein-ratio QTL was detected on chromosome 7 (5H) that coincided with a QTL for α-amylase activity. In both cases Harrington contributed the larger-value alleles. This capacity of Harrington to produce a higher S/T ratio and α-amylase activity than Morex was also apparent in the multi-environment parental means (Table 2). QTLs for malt extract and kernel plumpness were found at this position in Harrington×TR306, where Harrington also contributed the higher-value allele for malt extract percentage and TR306 for kernel plumpness. In the same way an α-amylase-activity QTL was detected in the Steptoe×Morex population where Morex contributed the higher-value allele.

The α-amylase-activity QTL on chromosome 4 (4H) did not coincide with QTLs detected in Steptoe×Morex or Harrington×TR306, but the α-amylase-activity QTL on chromosome 7 (5H) coincided with a QTL detected in Harrington×TR306. In both cases Harrington contributed the higher-value allele. A diastatic-power QTL was detected in the centromeric region of chromosome 1 and coincided with the region where a diastatic-power QTL was detected in the Steptoe×Morex population (Fig. 2). In Harrington×Morex, Morex contributed the favorable allele, as it did in Steptoe×Morex.

Malt-extract-percentage QTLs were detected on chromosomes 2 (2H) and 5 (1H). Neither coincided with QTLs for other malting quality traits. The chromosome 2 (2H) malt-extract-percentage QTL, where Morex contributed the favorable allele, was distal to a cluster of QTLs determining multiple quality traits. The malt-extract-percentage QTL on the short arm of chromosome 5 (1H), in which Harrington contributed the favorable allele, coincided with a malt-extract-percentage QTL detected in the Steptoe×Morex population, where Morex contributed the favorable allele. This position coincided with a kernel-plumpness QTL in the six-rowed subpopulation and a diastatic-power QTL in the Steptoe×Morex population.

With the exception of kernel plumpness, the proportion of phenotypic variance (R^2_p) accounted for by QTLs for each trait was ≤40%. The largest values of R^2_p were for those traits where QTLs were coincident with the *vrs1* and *int-c* loci (kernel plumpness, test weight, grain protein percentage, S/T protein ratio, α-amylase activity and diastatic power). Considering the high estimates of heritability (Table 2) these QTLs accounted for less than 50% of the genetic variance. The test for two-locus interactions was significant between the *vrs1* and *int-c* loci for both grain protein percentage and S/T protein ratio. The two-locus R^2_p values were 52% and 48%, respectively. These estimates of QTL effect may be biased since larger populations and alternative QTL estimation procedures may give better estimates of the QTL effect (Melchinger et al. 1998).

Fig. 3 Scans of test statistics for simple interval mapping (SIM) from the two-rowed subpopulation (*solid line*) and the six-rowed subpopulation (*broken line*). Scans are shown for malting quality traits for which *vrs1* locus had a significant effects. Chromosomes 1 (7H) to 7 (5H) are shown left to right. *Horizontal lines* show the SIM threshold estimated from 5000 permutations. The parent giving the higher value allele is shown for each QTL peak (*H*=Harrington; *M*=Morex) ▶

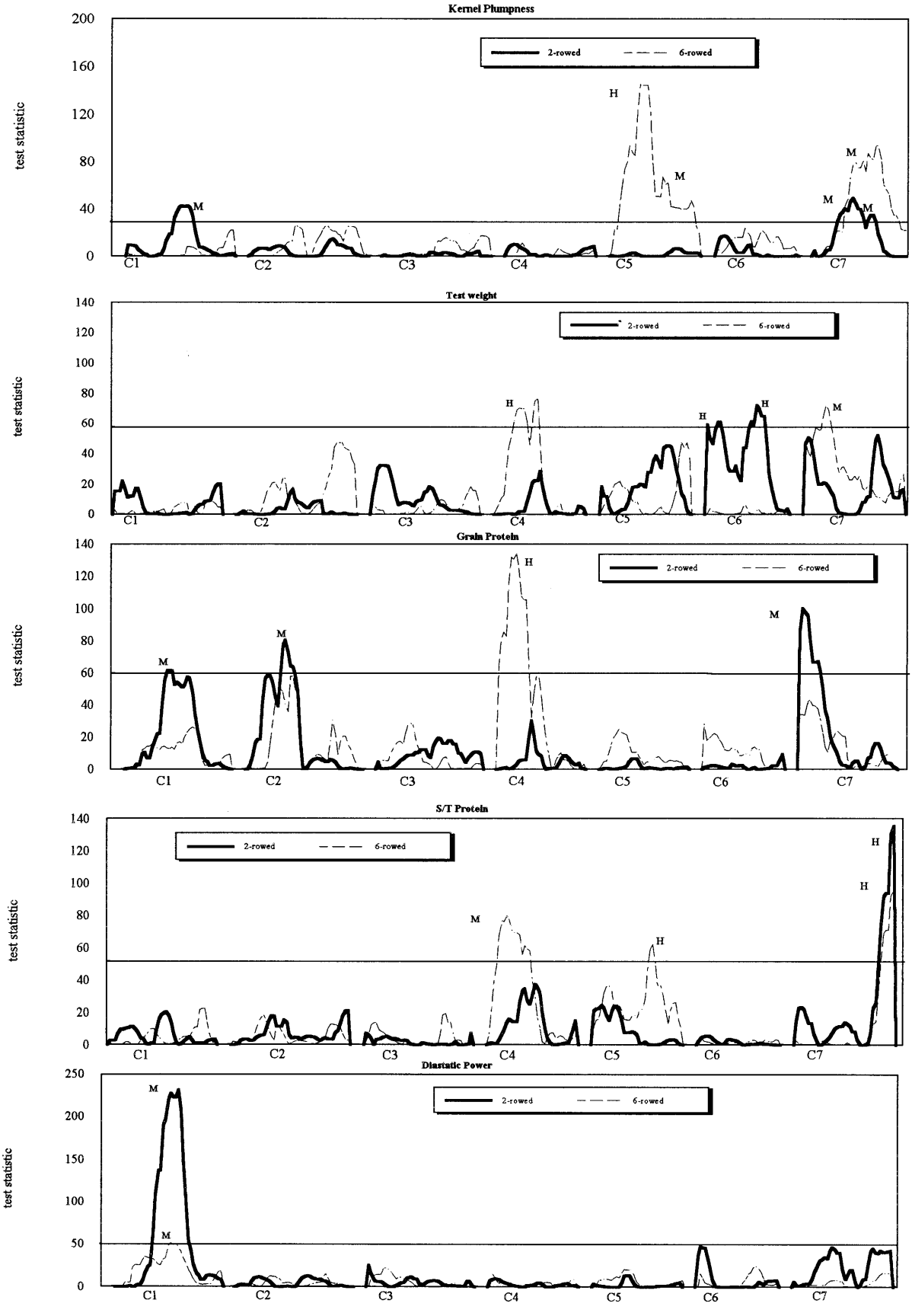

Kernel Plumpness
2-rowed
6-rowed
test statistic
200
160
120
80
40
0
H
M
M
M
M
M
C1
C2
C3
C4
C5
C6
C7
Test weight
2-rowed
6-rowed
test statistic
140
120
100
80
60
40
20
0
H
H
H
M
C1
C2
C3
C4
C5
C6
C7
Grain Protein
2-rowed
6-rowed
test statistic
140
120
100
80
60
40
20
0
H
M
M
M
C1
C2
C3
C4
C5
C6
C7
S/T Protein
2-rowed
6-rowed
test statistic
140
120
100
80
60
40
20
0
H
H
M
H
C1
C2
C3
C4
C5
C6
C7
Diastatic Power
2-rowed
6-rowed
test statistic
250
200
150
100
50
0
M
M
C1
C2
C3
C4
C5
C6
C7

Table 4 QTL location[a] higher value allele[b], and percentage of phenotypic variance accounted for by QTLs in the two-rowed doubled-haploid progeny of Harrington×Morex, averaged over eight environments

Phenotype	Chromosome				R^2p[c]
	1 (7H)	2 (2H)	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
Grain protein	ABC465-MWG2031^M	MWG655e-CD064^M		MWG635d-ABC302a^M	40
S/T protein				ABG463-MWG851b^H	21
Diastatic power	ABC465-MWG2031^M				34

[a] Flanking markers
[b] ^{H}=Harrington; ^{M}=Morex
[c] Multilocus percentage of variance explained

Table 5 QTL location[a] higher value allele[b], and percentage of phenotypic variance accounted for by QTLs in the six-rowed doubled-haploid progeny of Harrington×Morex averaged over eight environments

Phenotype	Chromosome				R^2p[c]
	1 (7H)	4 (4H)	5 (1H)	7 (5H)	
Kernel plumpness			ABC801-CDO99^H MWG943-KgE33M51.88^M	ABG003b-ABC159b^M	35
Test weight		*int-c*-HVM40^H		MWG635d-ABC302a^M	25
Grain protect		*int-c*-HVM40^H			22
S/T protein		*int-c*-HVM40^M	MWG943-KgE3M51.88^H	ABG463-MWG851b^H	37
Diastatic power	ABC465-MWG2031^M				9

[a] Flanking markers
[b] ^{H}=Harrington; ^{M}=Morex
[c] Multilocus percentage of variance explained

Subpopulation analysis classified by the inflorescence type

The only QTLs for grain protein percentage and S/T protein ratio that showed significant (P<0.05) two-locus interactions were those coincident with the *vrs1* and *int-c* loci. In order to further explore this interaction and to determine if the *vrs1* locus was masking other QTLs, we divided the total population into the two-rowed (n=72 lines) and six-rowed (n=68 lines) subpopulations. In the two-rowed subpopulation, two kernel-plumpness QTLs were detected on chromosome 1 (7H) and 7 (5H) (Table 4, Fig. 2). Two new QTLs for test weight were detected on chromosome 6 (6H). No other QTL mapped to this chromosome in this population. QTLs for grain protein percentage on chromosomes 1 (7H) and 2 (2H) were significant and were also detected in the Steptoe×Morex and Harrington×TR306 populations (Table 4, Fig. 2). At the QTL on chromosome 1 (7H), Morex contributed the higher-value allele in both populations. At the QTL on chromosome 2 (2H) Harrington contributed the higher-value allele. In the case of the six-rowed subpopulation, two new kernel-plumpness QTLs were detected on chromosome 5 (1H) (one of them coincident with a malt-extract QTL in the full population) and one on chromosome 7 (5H) (coincident with a kernel-plumpness QTL detected in the two-rowed subpopulation). Two test-weight QTLs were detected, one on chromosome 4 (4H) (in the region of *int-c* locus) and one in the centromeric region of chromosome 7 (5H). This latter QTL was coincident with a QTL for grain protein percentage in the two-rowed subpopulation, and with QTL for test weight and grain protein percentage in the full population. The same S/T protein ratio and diastatic power QTLs were detected on chromosomes 1 (7H), (4H and 7 (5H) that were detected in the full population.

The higher level of grain protein percentage observed in Morex (Table 2), a feature of North American six-rowed malting barleys compared with the two-rowed types, may be due to alleles at the QTL on chromosome 7 (5H) (Table 3). At the grain-protein-percentage QTLs detected in the two-rowed subpopulation, higher-value alleles were contributed from Morex (Table 4). These QTLs are coincident with QTLs detected in the Steptoe×Morex population. In the six-rowed subpopulation, no new QTLs were detected.

In the case of the S/T protein ratio, no new QTLs were detected in the two-rowed subpopulation. In the six-rowed sub-population, a significant QTL was detected on chromosome 5 (1H) in the same region where an α-amylase-activity QTL was detected in Steptoe×Morex

(Table 5, Fig. 2). The S/T-protein-ratio QTL on chromosome 7 (5H) is coincident with a S/T-protein-ratio QTL detected in Harrington×TR306; in both cases Harrington contributed the higher-value allele and it was significant in both subpopulations. For diastatic power, one QTL was detected in the full population and in both subpopulations. This QTL coincided with a diastatic-power QTL detected in the Steptoe×Morex population, and in both cases Morex gave the larger-value allele.

Conclusions

Four out of seven of the malting quality QTLs we detected in the full Harrington×Morex population coincided with malting quality QTLs detected in the Harrington×TR306 and Steptoe×Morex populations. Favorable allele phases were consistent at QTLs common to the three mapping populations. The notable exceptions were QTLs coincident with the *vrs1* and *int-c* loci, confirming the importance of linkage/or pleitropy. In feed×malt crosses (e.g. Steptoe×Morex, Harrington×TR306) most breeders would not expect positive transgressive segregants for malting quality. In the case of Harrington×Morex, since the parents are genetically distinct malt standards, we hypothesized that we would recover positive transgressive segregants. We found transgressive segregants for high-grain-protein percentage and desirable transgressive segregants for high enzyme activity. Favorable transgressive segregants for malt-extract percentage were not found. Malt-extract percentage is the result of a balance of all the malting quality components. Our detection of QTLs in association with, or as a result of, the genes determining inflorescence type suggests that integration of two germplasm groups disturbs the balance of properties leading to malting quality.

Malting quality QTLs detected in this population coincided with the *Amy2* locus on chromosome 1 (7H), the hordein loci on chromosome 5 (1H), and the *vrs1* and the *int-c* loci. Are these candidate genes for the coincident QTLs? On chromosome 1 (7H), recent work suggests that the *Amy2* locus is not the only determinant of malting quality QTLs in the Steptoe×Morex population (Han et al. 1997). The hordein loci code for structural proteins (von Wettstein-Knowles 1992) and their role in determining malt extract is not obvious. The association of the *vrs1* and *int-c* loci with malting quality QTLs could be due to pleitropy or linkage drag. Distinguishing between these alternatives will be complicated by suppressed recombination in the vicinity of the *vrs1* locus on chromosome 2 (2H).

Because of the delicate balance of properties required for malting quality, any modification of the balance that results in higher or lower levels of a specific component may lead to an unacceptable quality profile. In many cases, the malting and brewing industries demand a defined and static quality profile. In such situations, the most-efficient breeding strategy may be one that uses QTL information to maintain the specific configurations of QTL alleles that lead to a target quality profile. To implement such a strategy, markers could be used to retain a particular genomic architecture for regions critical for malting quality during the process of introgressing positive alleles for other target phenotypes, such as disease resistance and productivity traits.

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