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AFLP mapping of quantitative trait loci for yield-determining physiological characters in spring barley

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Abstract An amplified fragment length polymorphism (AFLP) map covering 965 cM was constructed using 94 recombinant inbred lines of a cross between the spring barley varieties Prisma and Apex. This map was employed to identify quantitative trait loci (QTLs) controlling plant height, yield and yield-determining physiological characters using an approximate multiple-QTL model, the MQM method. The seven physiological traits were parameters used in a process-based crop-growth model that predicts barley biomass production as affected by daily temperature and radiation. The traits were measured in experiments conducted over 2 years. Except for the relative growth rate of leaf area, all traits examined had at least one QTL in each year. QTLs and their effects were found to vary with developmental stages for one trait, the fraction of shoot biomass partitioned to leaves, that was measured at several stages. Most of the traits were associated, though to different extents, with the *denso* dwarfing gene (the height-reducing allele in Prisma) located on the long arm of chromosome 3. Some of the QTLs were mapped to similar positions in both years. The results in relation to effects of the dwarfing gene, the physiological basis for QTL × environment interaction, and the relative importance of the parameter traits with respect to yield, are discussed.

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Introduction

Most crop traits are quantitative in nature and are controlled by multiple genes. The advent of molecular markers has made it possible to localise individual genes for this type of trait. By the use of statistical analysis, the variation of a quantitative trait can be dissected into the effect of individual genome regions, the quantitative trait loci (QTLs), linked to markers on a molecular-marker map (Paterson et al. 1988). Barley, as one of the model crops for molecular genetic studies in monocots, has been extensively studied either for constructing a marker map *per se* (Shin et al. 1990; Graner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993; Becker et al. 1995; Sherman et al. 1995; Qi et al. 1998) or for using the marker map to identify QTLs for different quantitative traits (Barua et al. 1993; Hayes et al. 1993; Backes et al. 1995; Kjær et al. 1995; Laurie et al. 1995; Kjær and Jensen 1996; Bezant et al. 1996, 1997; Thomas et al. 1996; Tinker et al. 1996; Powell et al. 1997).

Process-based ecophysiological crop-growth models have been developed during recent decades by integrating knowledge across disciplines (Loomis et al. 1979), and are being increasingly applied in problem-oriented agricultural research (Boote et al. 1996). One of the applications is the explanation of differences in the yield potential of cultivars on the basis of individual physiological parameters, and the use of this knowledge for evaluating and designing plant types (Kropff et al. 1995). This is possible because, as they become more mechanistic and comprehensive, crop-growth models can be used to mimic the genetic characteristics

of plants (Boote et al. 1996). We have recently demonstrated that this type of model can be used to identify key physiological characteristics that determine yield differences among individuals in a genetic mapping population of recombinant inbred lines (RILs) in barley (Yin et al. unpublished). Like other crop traits, physiological parameters in a crop model are of a quantitative nature. They are also often referred to as 'genetic coefficients' (e.g. Hoogenboom et al. 1997), reflecting the awareness that the variation of these parameters is under genetic control (Stam 1996).

Advances in the use of molecular markers to evaluate the inheritance of quantitative traits indicate that such techniques are now eminently suitable for elucidating the inheritance of physiological traits (Hoogenboom et al. 1997; Stam 1998). There is a growing interest in the genetic mapping of various plant physiological traits (e.g. Price et al. 1997; Teulat et al. 1997). However, knowledge of the genetic basis of variation in the physiological parameters used in crop-growth models remains virtually absent (Aggarwal et al. 1997). The objective of the present study is to map QTLs for individual yield-determining parameters applied to a crop-growth model, using an AFLP marker map. For this purpose, the genetically well-studied crop, spring barley, was used.

Materials and methods

The recombinant inbred-line population

Although QTL analysis can be performed using any type of segregating population derived from a single cross between two parents, inbred-line populations are most suitable for the analysis of physiological model parameter traits since the measurement of these traits often needs many plants. A population of 94 RILs was produced by eight generations of single-seed descent from a cross of the two-row spring barley cultivars, Apex and Prisma. The two parents are commercial cultivars in the Netherlands. They have contrasting morpho-physiological characteristics and Prisma usually outyields Apex (Schut 1992). Prisma is also shorter than Apex, largely due to a recessive dwarfing gene called *denso* present in Prisma. RILs with the *denso* gene can be unambiguously identified under field conditions, as this gene confers the distinctive prostrate juvenile growth habit (Haahr and von Wettstein 1976). This character is used to classify the lines into allelic groups for the *denso* gene which can be mapped as a morphological marker (Bezant et al. 1996).

AFLP analysis

Plant DNA from the RILs and the two parents was extracted from freeze-dried tissue of leaves obtained at the late tillering stage of field-grown crops following the CTAB protocol of Qi and Lindhout (1997). AFLP methodology was performed essentially as described by Vos et al. (1995) with the minor modifications suggested by Qi and Lindhout (1997). Briefly, genomic DNA was restricted with *Eco*RI and *Mse*I enzymes. Specific double-stranded adapters were ligated to the restricted fragment ends. The digested and ligated DNA was then pre-amplified using *Eco*RI primer E01 and *Mse*I primer M02, which have one selective nucleotide each. Amplification was performed with combinations of [γ^{33}P]ATP-labelled *Eco*RI primers and unlabelled *Mse*I primers, which have three selective nucleotides each, using diluted pre-amplified DNA as a template. The 24 primer combinations were: E32M55, E32M61, E33M54, E33M55, E33M58, E33M61, E35M48, E35M54, E35M55, E35M61, E38M50, E38M51, E38M54, E38M55, E39M55, E39M61, E42M48, E42M51, E44M49, E44M54, E44M58, M45M49, E45M55 and E45M58. These primer combinations are known to give a high degree of polymorphism in barley (Qi and Lindhout 1997). The PCR product from each primer combination was mixed with an equal volume of formamide loading buffer. The samples were denatured for 5 min at 90°C; 3 µl of each sample was loaded onto a denaturing polyacrylamide gel and then electrophoresed at a constant power of 80 W for about 1 h 45 min. Gels were transferred to Whatman paper and dried for about 1.5 h at 70°C, and subsequently exposed to autoradiographic film to visualise the results.

The markers obtained were designated according to the AFLP profile of the parents (Qi and Lindhout 1997; also see map data on GrainGenes WWW page: http://wheat.pw.usda.gov/ggpages/), and were scored for presence/absence of a given band for individual RILs.

Quantitative traits and field evaluation

The quantitative traits examined in this study are those parameters used in a process-based barley growth model (Yin et al. unpublished), which was based on the widely used models SUCROS (Goudriaan and van Laar 1994) and ORYZAI (Kropff et al. 1994). In brief, the model quantifies barley growth as affected by radiation, temperature and plant nitrogen (N) status. Leaf photosynthesis is calculated based on radiation flux and specific leaf N (SLN), an integrated measurement of leaf N content (LNC) and specific leaf area (SLA). The vertical distribution of both radiation and SLN in the canopy is described by an exponential extinction function. Total daily crop CO_2 assimilation is calculated by integrating instantaneous rates of CO_2 assimilation over the leaf area index (LAI) and over the day. Daily growth rate is estimated after subtraction of dark respiration. The biomass produced is distributed among various organs based on partitioning coefficients that depend on the developmental stage (DS). These coefficients are derived from the periodically measured weight of the organs. The DS is defined to be 0 at emergence, 1 at flowering and 2 at maturity, and is calculated as the accumulation of daily development rates which increases proportionally with the effective temperature between 0°C and 26°C. Leaf-area development is simulated as an exponential function of temperature before canopy closure. The slope of this relation, the relative growth rate of leaf area (RGRL), is obtained by linear regression of the log-transformed LAI against the accumulated daily effective temperatures. After canopy closure, the increase in LAI is estimated from the increase in leaf biomass and SLA.

Important model parameters include pre-flowering duration (Pre-F), post-flowering duration (Post-F), LNC, SLA, RGRL, and the fraction of biomass partitioned to various growing organs. They were measured in field trials conducted in 1996 and 1997 in Wageningen, The Netherlands. Plants were grown in plots of ten rows, spaced at 13 cm, 9 m (1996) or 8 m (1997). The randomized incomplete block design was used, with two replicates for each RIL. The N application, all as basal, was higher in 1997 (104 kg ha^{-1}) than in 1996 (50 kg ha^{-1}) in order to create two different crop-growth environments.

Three stages (emergence, flowering and maturity) were recorded for each RIL. Destructive samplings were used to measure the dry matter of individual shoot organs and LAI during crop growth. Dry matter was determined after oven drying at 105°C for at least 15 h. Leaf area was measured with a LI-1000 leaf area meter. SLA was calculated as leaf area divided by leaf dry weight. LNC at flowering (LNC_{flw}) was determined on the blades of green leaves by micro-Kjeldahl digestion and distillation. Grain yield was expressed at the

Table 1 The quantitative traits examined in this study

Trait	Code	Unit	Year measured
Plant height	HT	cm	1996
Pre-flowering duration	Pre-F	Degree·days (°Cd)	1996, 1997
Post-flowering duration	Post-F	°Cd	1996, 1997
Leaf nitrogen content at flowering	LNC_{flw}	g N (kg leaf)$^{-1}$	1996, 1997
Specific leaf area at flowering	SLA_{flw}	m^2 leaf (kg leaf)$^{-1}$	1996, 1997
Fraction of shoot biomass partitioned to leaves	FP_L	kg kg^{-1}	1997
Fraction of shoot biomass partitioned to ears	FP_E	kg kg^{-1}	1997
Relative growth rate of early leaf area	RGRL	(°Cd)$^{-1}$	1997
Yield (14% moisture content)	–	t ha^{-1}	1996, 1997

14% moisture content for all RILs. As the *denso* dwarfing gene was segregating in the population, plant height (HT) was also measured, as the mean distance from ground to ear tips (excluding awns) over eight randomly chosen mature plants.

Not all the traits were measured in both years (Table 1). Estimation of each parameter-trait was given by Yin et al. (unpublished). As root weight was not measured in the experiments, the QTL analysis for biomass-partitioning between root and shoot was not considered here. Regarding the fraction of the shoot biomass partitioned to leaves, stems and ears (FP_L, FP_S and FP_E), the QTL analysis was performed only for FP_L and FP_E, but not for FP_S because FP_S is calculated as $(1 - FP_L - FP_E)$ in the model. Our measurements allowed estimation of FP_L at four stages during the pre-flowering growth and the estimation of FP_E at one stage after flowering (see Table 3). For SLA, results are given here only for the measurement at flowering (SLA_{flw}). The complete analysis of this trait over the whole growth period provides a new insight about the role of crop-growth models in QTL analysis and is presented in a separate report (Yin et al. 1999).

Data analysis

The software package JoinMap 2.0 (Stam and van Ooijen 1995) was used for constructing an AFLP-marker linkage map. Linkage groups were formed with a LOD of 3.0. Groups were merged and assigned to chromosomes on the basis of homology in the L94 × Vada barley population (Qi et al. 1998). Each marker of a linkage group was tested against the expected segregation ratio using a χ^2 goodness of fit. Map distances were calculated using the Kosambi function. In the first round of running JoinMap, markers that were mapped at the same position were identified. The final map was obtained by re-running JoinMap with the exclusion of redundant markers clustered at the same position.

Analysis of variance was carried out for all traits, using the General Linear Model (SAS 1988). The mean value over the replicates was used for QTL analysis of each trait. The QTL analysis was carried out with MapQTL 3.0 (van Ooijen and Maliepaard 1996). An approximate multiple-QTL model, the MQM mapping method (Jansen 1993; Jansen and Stam 1994), was performed at 2-cM intervals to identify QTLs. In this method, background markers are selected to take over the role of the putative QTLs as co-factors to reduce the residual variance. First, the *denso* locus was deliberately chosen as a co-factor for all traits, as previous studies had highlighted its importance in the control of many traits (Powell et al. 1985, 1997; Thomas et al. 1991, 1996; Laurie et al. 1993; Bezant et al. 1997). Secondly, other background markers closest to the indicated region of possible QTLs (LOD $\geqslant$ 2.5) were gradually added as co-factors, until the LOD profile stabilized. In the final LOD profile, a threshold LOD value of 3.0 was used to declare the presence of a QTL. Regions with a LOD between 2.5 and 3.0 were considered as 'suggestive' for QTLs.

Results

AFLP-marker map construction

With the 24 primer combinations, a total of 252 polymorphic AFLP markers were obtained; 51 of them were identical in size with AFLP bands detected in the L94 × Vada population (Qi et al. 1998). Together with the *denso* locus, linkage analysis was done using JoinMap. By employing a series of increasing LOD thresholds between 2 and 8, with a step size of 0.5, the markers were assigned at LOD 3.0 to 20 linkage groups, each containing at least one distinctive common marker. These 20 groups were assigned to the seven chromosomes on the basis of the chromosome-specific common markers. One marker that did not belong to any group was discarded.

Many markers clustered on chromosomes 3 and 7. Since clustered markers do not provide any additional information, only one marker with the least missing observations was retained at a position while dropping the other redundant ones. In total, 26 markers on chromosome 3 and 35 markers on chromosome 7 were dropped. The resulting map contained 191 markers, covering a total map length of 965.3 cM (Fig. 1). The *denso* gene was mapped on the long arm of chromosome 3, in agreement with earlier reports about the position of this gene in the barley genome (Barua et al. 1993; Laurie et al. 1993; Bezant et al. 1996) based on RFLP or both RFLP and RAPD markers.

Among the 191 mapped markers, 132 segregated in the expected 1:1 ratio ($\alpha = 0.05$). Among the 59 markers with distorted segregation, 17 were skewed in favour of the Apex alleles. On chromosomes 1 and 5, the distortion was not unidirectional. Loci showing distorted segregation were confined to some segments on chromosomes 1 to 5, whereas markers clustered on the upper part of chromosome 7 all showed distorted segregation towards the Prisma alleles (Fig. 1).

Comparison of our map with the L94 × Vada map (Qi et al. 1998) showed that the order of all common markers on chromosomes 3, 4, 5, 6 and 7 was almost identical and distances between linked common

markers were similar, in support of the often-reported co-linearity of the markers. However, the position of the common markers on chromosomes 1 and 2 does not agree between the two maps. Use of the fixed-order option of JoinMap (Stam and van Ooijen 1995) for the common markers did not produce a consensus map as the χ^2 value of the map in this case increased substantially. Since the chance that co-migrating AFLP bands map to different positions in different barley populations is very low (Waugh et al. 1997; Qi et al. 1998), the map for chromosomes 1 and 2 in Fig. 1 should be regarded in tentative. Because the largest gap between markers on these two chromosomes is more than 20 cM in our map and in the map of Qi et al. (1998), a more reliable map might be obtained by either including markers that can fill the gaps, or developing an integrated map based on data from several populations.

QTL mapping

The mean, minimum and maximum trait values, and the phenotypic correlation coefficients between the traits are shown in Table 2. All traits, except for FP_L at the 0.10 DS ($FP_{L,DS0.10}$) and RGRL, showed significant differences ($P < 0.001$) among RILs. QTLs were found for all the traits, except for $FP_{L,DS0.10}$; for RGRL, however, there was only an indication of a minor effect for the *denso* locus (Table 3).

Plant height

As expected, a strong additive effect of 8.5 cm at the *denso* locus on HT with the decreasing allele from Prisma was detected. This locus accounted for 71.5% of the variation for HT in the 1996 experiment. Besides the *denso* locus, one QTL on chromosome 5, three 'suggestive' QTLs on chromosome 1, and one 'suggestive' QTL on chromosome 7 were identified. The shorter-plant alleles of all these five QTLs came from Apex. These QTLs had only a small effect on HT relative to the *denso* gene.

Pre-flowering duration

The height-reducing allele of the *denso* locus was associated with late-flowering time. This locus explained 67.3 and 72.0% of the variation in Pre-F in 1996 and 1997, respectively. The additive effect of this locus exceeded 30°Cd in both years. For 1996 and 1997, five and three additional QTLs were detected, some of them only 'suggestive'. Two QTLs, located on chromosome 5, were detected in both years. Except for the QTL on chromosome 2 for 1996, the late-flowering allele of all these QTLs came from Apex.

Post-flowering duration

Two QTLs were found for Post-F in each year. In 1996, one QTL was located on chromosome 1 with the late allele from Prisma. The other QTL was located on chromosome 5 with the late allele from Apex. In 1997, one QTL mapped to chromosome 2 and the other close to the *denso* locus on chromosome 3. Both QTLs had the late allele from Prisma.

Leaf nitrogen content at flowering

For LNC_{flw}, two QTLs were found in 1996 and three QTLs (including a 'suggestive' one on chromosome 7) in 1997. The QTL shared for both years was closely linked with the *denso* locus, with the decreasing allele from Prisma. This QTL accounted for the variation of LNC_{flw} more in 1996 than in 1997. The other QTLs identified had the increasing allele from Prisma.

Specific leaf area at flowering

For SLA_{flw}, two QTLs were detected on chromosomes 2 and 3 in 1996 and a different QTL on chromosome 4 in 1997. The QTL on chromosome 3 for SLA_{flw} in 1996 is in close proximity to the *denso* locus.

Fraction of biomass partitioned to leaves and ears

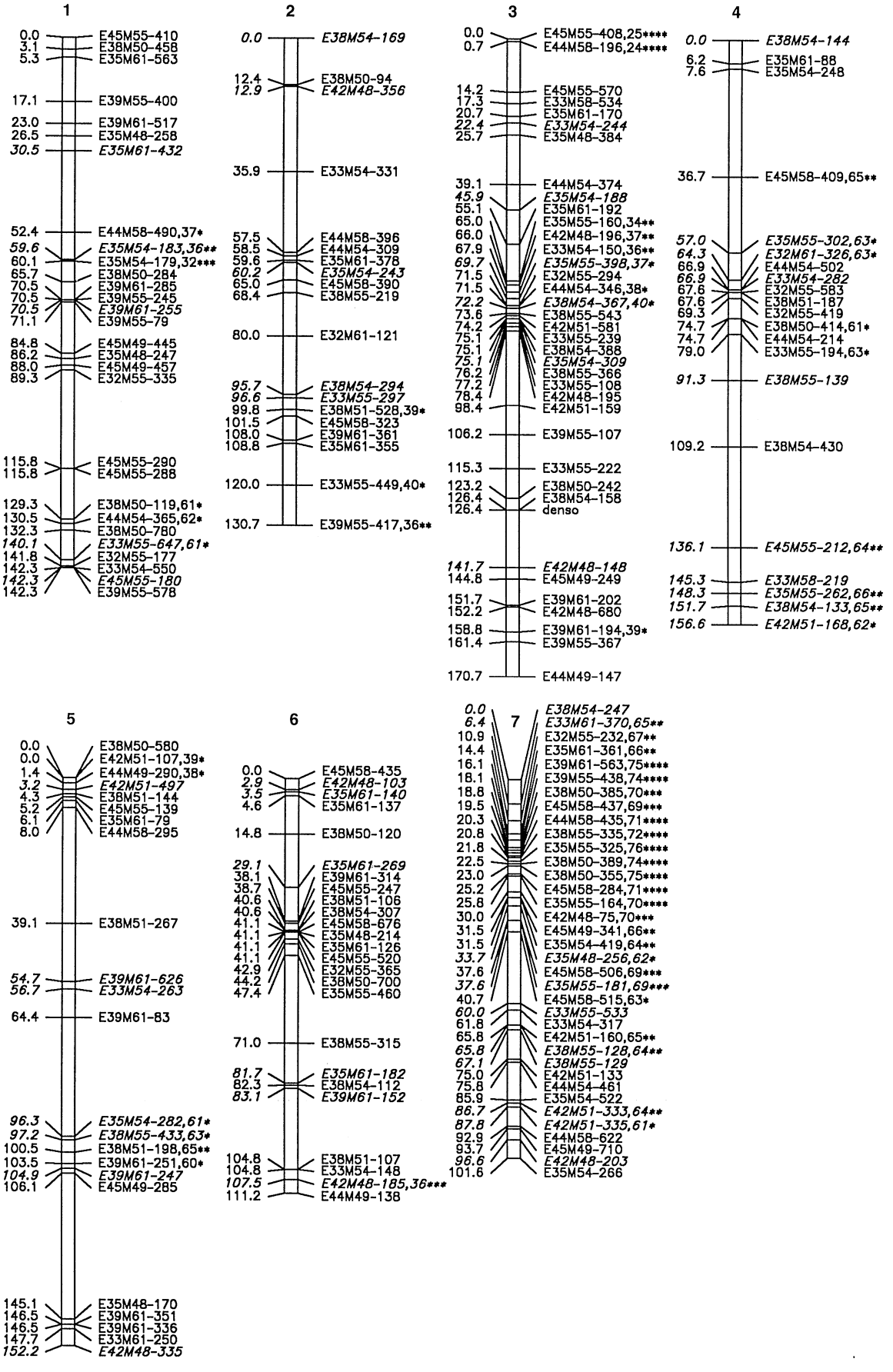
For FP_L, no QTLs were found at the 0.10 DS. For FP_L measured at all the three later stages, a QTL with the decreasing allele from Apex was found in close proximity to the *denso* locus. However, the magnitude of the additive effect differed among the stages. The percentage of variation explained by the QTL was 12.1% at the 0.27 DS, increased up to 71.4% at the 0.47 DS and then declined to 21.7% at the 0.59 DS. For FP_L at the 0.27 DS, an additional QTL with the decreasing allele from Prisma was located on chromosome 1. This QTL accounted for 13.9% of the variation in FP_L at this stage.

For FP_E at the 1.15 DS, the region close to the *denso* locus, with the increasing allele from Prisma, had a strong effect, accounting for 41.2% of the variation. The other 'suggestive' QTL with the increasing allele from Apex, accounting for 8.3% of the variation, mapped to chromosome 1, near the QTL on this chromosome for FP_L at the 0.27 DS.

Yield

For yield, six QTLs (including two 'suggestive') were detected in 1996. The two 'suggestive' QTLs had the high-yield allele from Apex, and the other four QTLs had the high-yield allele from Prisma.

Four QTLs (including two 'suggestive' ones on chromosomes 5 and 6) were detected for yield in 1997. Except for the 'suggestive' QTL on chromosome 5


1
0.0 E45M55-410
3.1 E38M50-458
5.3 E35M61-563
17.1 E39M55-400
23.0 E39M61-517
26.5 E35M48-258
30.5 E35M61-432
52.4 E44M58-490,37*
59.6 E35M54-183,36**
60.1 E35M54-179,32***
65.7 E38M50-284
70.5 E39M61-285
70.5 E39M55-245
70.5 E39M61-255
71.1 E39M55-79
84.8 E45M49-445
86.2 E35M48-247
88.0 E45M49-457
89.3 E32M55-335
115.8 E45M55-290
115.8 E45M55-288
129.3 E38M50-119,61*
130.5 E44M54-365,62*
132.3 E38M50-780
140.1 E33M55-647,61*
141.8 E32M55-177
142.3 E33M54-550
142.3 E45M55-180
142.3 E39M55-578
2
0.0 E38M54-169
12.4 E38M50-94
12.9 E42M48-356
35.9 E33M54-331
57.5 E44M58-396
58.5 E44M54-309
59.6 E35M61-378
60.2 E35M54-243
65.0 E45M58-390
68.4 E38M55-219
80.0 E32M61-121
95.7 E38M54-294
96.6 E33M55-297
99.8 E38M51-528,39*
101.5 E45M58-323
108.0 E39M61-361
108.8 E35M61-355
120.0 E33M55-449,40*
130.7 E39M55-417,36**
3
0.0 E45M55-408,25****
0.7 E44M58-196,24****
14.2 E45M55-570
17.3 E33M58-534
20.7 E35M61-170
22.4 E33M54-244
25.7 E35M48-384
39.1 E44M54-374
45.9 E35M54-188
55.1 E35M61-192
65.0 E35M55-160,34**
66.0 E42M48-196,37**
67.9 E33M54-150,36**
69.7 E35M55-398,37*
71.5 E32M55-294
71.5 E44M54-346,38*
72.2 E38M54-367,40*
73.6 E38M55-543
74.2 E42M51-581
75.1 E33M55-239
75.1 E38M54-388
75.1 E35M54-309
76.2 E38M55-366
77.2 E33M55-108
78.4 E42M48-195
98.4 E42M51-159
106.2 E39M55-107
115.3 E33M55-222
123.2 E38M50-242
126.4 E38M54-158
126.4 denso
141.7 E42M48-148
144.8 E45M49-249
151.7 E39M61-202
152.2 E42M48-680
158.8 E39M61-194,39*
161.4 E39M55-367
170.7 E44M49-147
4
0.0 E38M54-144
6.2 E35M61-88
7.6 E35M54-248
36.7 E45M58-409,65**
57.0 E35M55-302,63*
64.3 E32M61-326,63*
66.9 E44M54-502
66.9 E33M54-282
67.6 E32M55-583
67.6 E38M51-187
69.3 E32M55-419
74.7 E38M50-414,61*
74.7 E44M54-214
79.0 E33M55-194,63*
91.3 E38M55-139
109.2 E38M54-430
136.1 E45M55-212,64**
145.3 E33M58-219
148.3 E35M55-262,66**
151.7 E38M54-133,65**
156.6 E42M51-168,62*
5
0.0 E38M50-580
0.0 E42M51-107,39*
1.4 E44M49-290,38*
3.2 E42M51-497
4.3 E38M51-144
5.2 E45M55-139
6.1 E35M61-79
8.0 E44M58-295
39.1 E38M51-267
54.7 E39M61-626
56.7 E33M54-263
64.4 E39M61-83
96.3 E35M54-282,61*
97.2 E38M55-433,63*
100.5 E38M51-198,65**
103.5 E39M61-251,60*
104.9 E39M61-247
106.1 E45M49-285
145.1 E35M48-170
146.5 E39M61-351
146.5 E39M61-336
147.7 E33M61-250
152.2 E42M48-335
6
0.0 E45M58-435
2.9 E42M48-103
3.5 E35M61-140
4.6 E35M61-137
14.8 E38M50-120
29.1 E35M61-269
38.1 E39M61-314
38.7 E45M55-247
40.6 E38M51-106
40.6 E38M54-307
41.1 E45M58-676
41.1 E35M48-214
41.1 E35M61-126
41.1 E45M55-520
42.9 E32M55-365
44.2 E38M50-700
47.4 E35M55-460
71.0 E38M55-315
81.7 E35M61-182
82.3 E38M54-112
83.1 E39M61-152
104.8 E38M51-107
104.8 E33M54-148
107.5 E42M48-185,36***
111.2 E44M49-138
7
0.0 E38M54-247
6.4 E33M61-370,65**
10.9 E32M55-232,67**
14.4 E35M61-361,66**
16.1 E39M61-563,75****
18.1 E39M55-438,74****
18.8 E38M50-385,70***
19.5 E45M58-437,69***
20.3 E44M58-435,71****
20.8 E38M55-335,72****
21.8 E35M55-325,76****
22.5 E38M50-389,74****
23.0 E38M50-355,75****
25.2 E45M58-284,71****
25.8 E35M55-164,70****
30.0 E42M48-75,70***
31.5 E45M49-341,66**
31.5 E35M54-419,64**
33.7 E35M48-256,62*
37.6 E45M58-506,69***
37.6 E35M55-181,69***
40.7 E45M58-515,63*
60.0 E33M55-533
61.8 E33M54-317
65.8 E42M51-160,65**
65.8 E38M55-128,64**
67.1 E38M55-129
75.0 E42M51-133
75.8 E44M54-461
85.9 E35M54-522
86.7 E42M51-333,64**
87.8 E42M51-335,61*
92.9 E44M58-622
93.7 E45M49-710
96.6 E42M48-203
101.6 E35M54-266

which was the only one with the increasing allele from Apex and had the least additive effect, the other three had been detected for yield in 1996 as well. The magnitude in the effect of these three QTLs, however, differed between the 2 years. The *denso* locus accounted for much more of the yield variation in 1997 than in 1996. The QTLs located on chromosomes 2 and 6 explained a lesser percentage of the yield variation in 1997, compared to their contribution to yield in 1996.

Discussion

Crop-growth models have conventionally been used to predict yields of a cultivar in various environments. This type of model is now increasingly being used in breeding programmes (Aggarwal et al. 1997), e.g. to assist in the design of new plant types (Kropff et al. 1995; Haverkort and Kooman 1997). For a better use of the information from these modelling studies by breeders, the current study presents, for the first time, a QTL analysis of physiological parameters used in a model that quantifies the growth of a genetically well-studied crop, spring, barley, as affected by N input, radiation and temperature.

There was at least one QTL detected for each trait except RGRL, for which there was only a weak indication for its association with *denso*, one of the most widely used dwarfing genes in European barley breeding programmes (Thomas et al. 1991; Laurie et al. 1993; Bezant et al. 1996). The *denso* gene has been reported not only to affect HT but to be associated with flowering time and a number of agronomic traits (Barua et al. 1993; Laurie et al. 1993; Bezant et al. 1996). Our study shows that this gene is also associated with a number of physiological traits. Almost all the high correlation between the traits (Table 2) is caused by the effect of this gene on them. The magnitude of the effect of this gene in reducing HT and in delaying flowering time found in our study is in agreement with those earlier reports. Our results also showed a positive effect of this gene on yield in both years. Other positive effects of this gene included increased leaf thickness (i.e. decreased SLA_{flw} as found in 1996), increased FP_L during the vegetative phase which may stimulate the formation of photosynthetic area, and increased FP_E at early kernel filling which promotes an increased harvest index. However, this gene resulted in a decreased LNC_{flw}, especially in

Fig. 1 AFLP marker linkage map of spring barley based on the RIL population from the cross between Prisma and Apex. Map positions are given in centimorgans (cM), using the Kosambi function. The map is oriented with the short arm of each chromosome at the top. Markers shown in *italic* font are shared by the L94 × Vada map (Qi et al. 1998). The frequency (%) of the allele from Prisma is shown, following the marker name, for markers with significant distorted segregation (*, **, *** and **** signify statistical significance at the 0.05, 0.01, 0.001 and 0.0001 probability level, respectively)

Table 2 Means and ranges (minimum-maximum) of RILs for different traits, and correlation coefficients between the traits (units of the traits are as given in Table 1)

Trait	Mean	Range	Correlation coefficient													
1996			HT	Pre-F	Post-F	LNC_{flw}	SLA_{flw}	Yield								
HT	63.5	39.8–82.8	1.00	− 0.87	− 0.20	0.51	0.66	− 0.39								
Pre-F	744.2	633.7–813.2		1.00	0.27	− 0.63	− 0.72	0.44								
Post-F	562.6	468.5–631.2			1.00	− 0.12	− 0.13	0.44								
LNC_{flw}	22.8	18.7–29.1				1.00	0.47	− 0.08								
SLA_{flw}	25.4	20.1–33.6					1.00	− 0.18								
Yield	5.5	4.7–6.5						1.00								
1997			Pre-F	Post-F	LNC_{flw}	SLA_{flw}	$PF_{L,DS0.10}$	$PF_{L,DS0.27}$	$PF_{L,DS0.47}$	$PF_{L,DS0.59}$	$PF_{E,DS1.15}$	RGRL	Yield			
Pre-F	738.5	673.9–800.5	1.00	0.17	− 0.44	− 0.22	− 0.02	0.26	0.76	0.36	0.71	− 0.30	0.78			
Post-F	657.6	573.7–710.6		1.00	− 0.01	− 0.12	0.10	0.25	0.34	0.23	0.09	− 0.04	0.27			
LNC_{flw}	32.6	26.0–39.7			1.00	0.19	0.10	− 0.14	− 0.33	− 0.17	− 0.43	0.07	− 0.34			
SLA_{flw}	37.4	32.4–42.3				1.00	0.07	− 0.25	− 0.21	− 0.11	− 0.10	0.08	− 0.18			
$PF_{L,DS0.10}$	0.83	0.79–0.89					1.00	0.18	0.01	0.11	0.11	− 0.19	− 0.05			
$PF_{L,DS0.27}$	0.70	0.64–0.78						1.00	0.24	0.12	0.27	− 0.09	0.27			
$PF_{L,DS0.47}$	0.54	0.46–0.64							1.00	0.64	0.49	− 0.29	0.69			
$PF_{L,DS0.59}$	0.38	0.22–0.50								1.00	0.15	− 0.19	0.25			
$PF_{E,DS1.15}$	0.43	0.26–0.62									1.00	− 0.15	0.64			
RGRL	0.015	0.012–0.017										1.00	− 0.13			
Yield	7.6	6.0–9.5											1.00			

Table 3 QTLs identified for different traits (those shown in *italic* font are considered as 'suggestive' for QTLs)

Trait code	Year	Chromosome	Position (cM)	LOD	Add. effect[a]	Var. (%)[b]
HT	1996	*1*	*23.0*	*2.7*	*1.46*	*1.9*
		1	*79.1*	*2.7*	*1.75*	*2.7*
		1	*123.8*	*2.5*	*1.75*	*2.5*
		3	126.4	34.8	− 8.49	65.8
		5	103.5	3.9	1.91	3.9
		7	*77.8*	*2.6*	*1.82*	*3.0*
Pre-F	1996	*1*	*141.8*	*2.8*	*− 5.60*	*1.4*
		2	114.8	4.1	9.66	3.2
		3	*45.9*	*2.5*	*− 5.61*	*1.4*
		3	126.4	42.0	38.99	67.3
		5	100.5	6.0	− 9.06	3.5
		5	*152.2*	*2.8*	*− 5.71*	*1.6*
	1997	3	126.4	40.4	31.52	72.0
		5	100.5	4.9	− 7.16	3.2
		5	*151.7*	*2.8*	*− 5.08*	*1.8*
		7	*96.6*	*2.6*	*− 4.93*	*1.4*
Post-F	1996	*1*	*23.0*	*2.9*	*10.08*	*7.8*
		5	51.1	4.7	− 15.63	17.9
	1997	2	57.5	4.0	11.27	14.4
		3	132.4	3.4	12.27	18.1
LNC_{flw}	1996	2	101.5	4.0	0.809	11.5
		3	125.2	9.4	− 1.319	31.6
	1997	3	125.2	3.7	− 0.838	10.6
		5	122.1	4.3	1.363	26.8
		7	*77.8*	*2.6*	*0.928*	*11.2*
SLA_{flw}	1996	2	8.0	4.8	1.49	15.4
		3	125.4	17.0	2.91	56.9
	1997	4	4.0	3.2	0.79	15.7
$FP_{L,DS0.10}$	1997	No QTL detected				
$FP_{L,DS0.27}$	1997	1	88.0	3.4	− 0.0113	13.9
		3	*128.4*	*2.5*	*0.0106*	*12.1*
$FP_{L,DS0.47}$	1997	3	125.2	27.2	0.0383	71.4
$FP_{L,DS0.59}$	1997	3	123.2	5.7	0.0249	21.7
$FP_{E,DS1.15}$	1997	*1*	*93.3*	*2.7*	*− 0.0253*	*8.3*
		3	125.2	14.5	0.0568	41.2
RGRL	1997	*3*	*126.4*	*2.3*	*− 0.0003*	*9.3*
Yield	1996	1	32.5	4.5	0.15	13.1
		2	*68.4*	*2.8*	*− 0.10*	*5.9*
		2	126.0	5.2	0.16	15.1
		3	126.4	5.6	0.14	12.8
		4	*136.1*	*2.6*	*− 0.11*	*5.7*
		6	61.4	4.9	0.16	16.2
	1997	2	126.0	3.7	0.19	5.3
		3	126.4	28.2	0.64	64.5
		5	*62.7*	*2.5*	*− 0.15*	*3.2*
		6	*57.4*	*2.8*	*0.18*	*5.2*

[a] (Mean of the 'Prisma' allele genotypes – mean of the 'Apex' allele genotypes)/2
[b] The percentage of phenotypic variation accounted for by each QTL

1996 when less N was applied. Although the *denso* gene could be tightly linked to loci controlling these traits, our result with the RIL population that the major QTL for so many different traits mapped at the same position as the *denso* gene is in support of the pleiotropy of this gene. This provides direct evidence for the genetic background and the interdependence of various model parameters, which has generally received little attention from crop modellers (Aggarwal et al. 1997).

Although many of the QTLs identified were mapped to a similar position in both years, some QTLs were expressed in one year but not in the other. The inconsistency of detected QTLs for a given trait across environments may be due to chance, i.e. QTLs of relatively small effect, which are constant over environments, may simply go unnoticed in one experiment but show up in another. However, this inconsistency is more likely to be caused by real QTL × environment interaction, as it can be explained physiologically. For example, the expression of QTLs for Post-F strongly varied between years (Table 3). The physiological basis for such interaction is that Post-F can vary depending

on plant N status as a result of the N translocation from vegetative organs to meet N requirement for grain growth, the phenomenon known as 'self-destruction' (Sinclair and de Wit 1975). This could have been the case in our experiments where N application was higher in 1997 than in 1996, causing a faster maturity in 1996 (Table 2). The different expression of QTLs for SLA_{flw} in years (Table 3) can also be due to the plant N status, since leaves are thicker in low-N than high-N environments (Grindlay 1997), as reflected by our result of a greater SLA_{flw} in 1997 than in 1996 (Table 2). The more striking yield advantage of the *denso* gene in high-N (1997) than low-N (1996) environments (Table 3) is also in good agreement with the long-recognised fact that the yield advantage of the dwarfing gene can be more fully expressed in high-N than low-N environments (Evans 1994).

Our results for FP_L measured at four stages indicate that QTLs detected for this trait varied with the developmental stage (Table 3). The magnitude of the effect of the *denso* locus, which showed up for FP_L at three stages, also differed among stages. The stage-dependence of QTLs and their effects is not surprising from a physiological viewpoint, given that the fraction of assimilates allocated to organs has long been known to vary with developmental stages (Kropff et al. 1994). No QTLs were detected for FP_L at the 0.10 DS because, during early juvenile growth, the vast majority of assimilates are allocated to leaves for all RILs so that little difference in FP_L among RILs was found (Table 2). From physiological considerations, quantitative trait values of all individuals in a mapping population are better measured at the same DS for the QTL analysis of traits, like FP_L, which vary with stages (Yin et al. 1999).

Yield is a complex crop trait. Physiologically, crop yield can be considered to be the result of many dynamic processes during crop ontogeny. A logical way to understand yield formation is to use ecophysiologically based crop-growth models (Kropff et al. 1994). The model whose parameters were analysed in this study is based on well-tested routines for simulating biomass production in SUCROS (Goudriaan and van Laar 1994) and ORYZA1 (Kropff et al. 1994). Using this model, we have shown that, relative to other physiological traits, FP_E, Pre-F and FP_L are important for determining yield differences among RILs of our mapping population (Yin et al. unpublished). Their importance can be largely due to effects of the *denso* gene since both yield and these three traits were strongly associated with this gene (Table 3). Except for the *denso* locus, however, the QTLs identified for the model-parameter traits were found to be poorly associated with the QTLs for yield. Also, there was no significant correlation between yield and other physiological traits (Table 2). This can be due to either the low chance of QTL detection caused by random noise of trait values or the inadequacy of the crop-growth model, or both. Random noise in the phenotypic values of physiological model-parameter traits could be large as the total variance explained by QTLs detected for physiological traits was often smaller than for agronomic traits (unpublished results). As for the inadequacy of the model, our early analysis (Yin et al. unpublished) indicated that there are other important factors for yield differences among RILs in our population that have not yet been incorporated into the model. These factors have to be identified from separate physiological studies.

A number of studies have identified QTLs controlling plant height, flowering time, and yield in barley (Barua et al. 1993; Hayes et al. 1993; Backes et al. 1995; Kjær et al. 1995; Laurie et al. 1995; Bezant et al. 1996, 1997; Kjær and Jensen 1996; Tinker et al. 1996; Powell et al. 1997). Though not our main objective, a comparison of our results with QTLs identified from these studies would be useful. Most of these studies, however, used a map of RFLP markers or a combination of RFLP and RAPD markers, making it difficult, if not impossible, to carry out such comparisons. To do that, a certain number of probes or primers could be deliberately taken to develop some RFLP or RAPD markers for the Apex × Prisma population. Since AFLP markers tend to sample some different regions of the genome compared to RAPDs and RFLPs (Becker et al. 1995; Powell et al. 1997), an inclusion of RFLPs and RAPDs might also fill gaps in the current AFLP map. This would resolve the earlier mentioned uncertainty about the relative position of markers on chromosomes 1 and 2 in our AFLP map.

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