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Identification of QTLs for partial resistance to leaf rust (*Puccinia hordei*) in barley

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Abstract The partial resistance to leaf rust in barley is a quantitative resistance that is not based on hypersensitivity. To map the quantitative trait loci (QTLs) for partial resistance to leaf rust, we obtained 103 recombinant inbred lines (RILs) by single-seed descent from a cross between the susceptible parent L94 and the partially resistant parent Vada. These RILs were evaluated at the seedling and adult plant stages in the greenhouse for the latent period (LP) of the rust fungus, and in the field for the level of infection, measured as area under the disease progress curve (AUDPC). A dense genetic map based on 561 AFLP markers had been generated previously for this set of RILs. QTLs for partial resistance to leaf rust were mapped using the "Multiple Interval Mapping" method with the putative QTL markers as cofactors. Six QTLs for partial resistance were identified in this population. Three QTLs, *Rphq1*, *Rphq2* and *Rphq3*, were effective at the seedling stage and contributed approximately 55% to the phenotypic variance. Five QTLs, *Rph2*, *Rphq3*, *Rphq4*, *Rphq5*, and/or *Rphq6* contributed approximtely. 60% of the phenotypic variance and were effective at the adult plant stage. Therefore, only the QTLs *Rphq2* and *Rhpq3* were not plant-stage dependent. The identified QTLs showed mainly additive effects and only one significant interaction was detected, i.e. between *Rphq1* and *Rphq2*. The map positions of these QTLs did not coincide with those of the race-specific resistance genes, suggesting that genes for partial resistance and genes for hypersensitive resistance represent entirely different gene families. Also, three QTLs for days to heading, of which two were also involved in plant height, were identified in the present recombinant inbred population. These QTLs had been mapped previously on the same positions in different populations. The perspectives of these results for breeding for durable resistance to leaf rust are discussed.

Key words Partial resistance · Leaf rust · Barley · QTL mapping · *Puccinia hordei* · *Hordeum vulgare* · Latent period

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Introduction

Leaf rust caused by the pathogen *Puccinia hordei* Otth is one of the most important diseases in barley (*Hordeum vulgare* L.). While in most areas the reduction in yield caused by leaf rust is relatively low, in some areas, it may be close to 30% (Arnst et al. 1979; Feuerstein et al. 1990). Barley leaf rust has been controlled primarily by the use of resistant cultivars carrying genes for hypersensitivity resistance, designated as *Rph* (*Pa*) genes. Rapid adaptation of the *P. hordei* populations, however, has rendered most of the resistance genes ineffective. The recently identified resistance genes, *Rph13* and *Rph14* (Jin et al. 1996), are also unlikely to be durable. Furthermore, sources of leaf rust resistance in cultivated barley are limited (Jin et al. 1995; Jin and Steffenson 1994). In contrast, partial resistance to leaf rust, characterised by a reduced rate of epidemic development despite a susceptible infection type (Parlevliet and Van Ommeren 1975), occurs very frequently in West European spring cultivars (Parlevliet et al. 1980) and Ethiopian barley landraces (Alemayehu and Parlevliet 1996) and is presumably more durable (Alemayehu and Parlevliet 1996; Parlevliet 1983a, b). Partial resistance in the field appears to be strongly correlated with the latent period (LP) and also with other components, such as infection frequency, pustule size, infectious period and spore production. LP can be

evaluated with much greater accuracy than the other components (Parlevliet 1975, 1977, 1979, 1986, 1992, Parlevliet et al. 1985; Neervoort and Parlevliet 1978; Parlevliet and Van Ommeren 1975). Genetic studies indicated that the longer LP in several partially resistant cultivars is governed by six to seven minor genes with additive effects (Parlevliet 1976, 1977, 1978).

Through the use of a dense molecular linkage map, polygenic quantitative traits can be resolved into discrete Mendelian factors (e.g. Paterson et al. 1988). With quantitative trait locus (QTL) mapping, the individual resistance loci can be identified and located on the chromosomes. This is a highly effective tool for studying genetically complex disease resistance such as partial resistance (Young 1996). It will allow the assessment of the race specificity of partial resistance genes, the interactions between resistance genes, and their expression in different growth stages and environments. Many genes conferring hypersensitivity resistance to pathogenic fungi and several QTLs for partial resistance to powdery mildew have already been mapped on the barley genome (Graner 1996). Two QTLs for resistance to *P. striiformis* were detected on chromosomes 7L and 4L (Chen et al. 1994), respectively. In the research presented here, we studied a recombinant inbred population (103 RILs) derived from a cross of L94 (susceptible) × 'Vada' (partially resistant) and mapped QTLs for partial resistance on the barley genome based on a high-density amplified fragment length polymorphism (AFLP) map (Qi et al. 1998).

Materials and methods

Development of recombinant inbred lines

A population of 103 F_9 recombinant inbred lines (RILs) was obtained from a cross of L94 × 'Vada' by single-seed descent. L94 is a line from an Ethiopian landrace, with black and naked seeds, and is extremely susceptible to leaf rust (*Puccinia hordei*) (Parlevliet 1975). 'Vada' is a commercial West European cultivar, with white and covered seeds, previously released by the Department of Plant Breeding, Wageningen Agricultural University, and has a high level of partial resistance to *P. hordei*. Both parents have been included in numerous experiments to characterise aspects of partial resistance of barley to leaf rust (Parlevliet 1975, 1976, 1978, 1979, 1983b; Parlevliet et al. 1985; Niks 1986). The 103 RILs (F_9) and the two parents were used for AFLP marker analysis (Qi et al. 1998) and for disease tests in the greenhouse and in the field.

Disease evaluations

Seedlings in the greenhouse

Seedlings of 103 RILs, L94 and 'Vada' were inoculated with leaf rust isolate 1.2.1. Fresh urediospores were diluted ten times with lycopodium spores and dusted over the adaxial sides of the seedling leaves fixed in a horizontal position. After incubation at a relative humidity of 100% over-night, the seedlings were transferred to a greenhouse where the temperature was set at about 18°C. The latent period (LP) of each plant was evaluated by estimating the period (hours) at which 50% of the ultimate number of pustules became visible. The relative latent period of seedlings (RLP50 S) was calculated relative to the LP of L94 in seedlings, where L94 = 100, as described by Parlevliet (1975). Four experiments were conducted in the course of 3 years. Each experiment consisted of two replications, each with 5–6 plants per line. Because separate analysis of these data did not reveal significant genotype × environment effects and all of the QTLs involved in RLP50 S were found in all experiments, the RLP50 S values were averaged over these four experiments.

Adult plants in the greenhouse

The rust isolate 1.2.1 was also used for evaluation of the RILs in the adult plant stage in the greenhouse. One experiment was carried out with 5 plants per line. The relative LP of young flag leaves (RLP50 A) was measured similar to the RLP50 S.

Adult plants in the field

A randomised complete block design with three replications was applied in a field experiment in 1996. Plot size was $0.75 \times 1.25\ m^2$. Plots of barley lines alternated with plots of oats to limit inter-plot interference (Parlevliet and Van Ommeren 1984). One month after sowing, more than 250 L94 plants were inoculated in the greenhouse, and after 2 weeks the pots with sporulating L94 plants were transferred to the experiment field and placed in the alley ways between the plots. When L94 plants in the plots started to sporulate, the spreader-plants were removed. Three samplings with 7-day intervals between samplings were carried out from the early-heading stage to the late-grain-filling stage. At sampling time, three tillers were sampled from each plot and evaluated for the number of spores according to the scale of Parlevliet and Van Ommeren (1984). The area under the disease progress curve (AUDPC) was calculated using of the mean values from these three observations. In addition, days to heading was evaluated as the number of days from sowing till 50% of plants in the plot had headed. Plant height was also measured in the final stage of plant development.

Genotyping and map construction

From the high-density AFLP map (Qi et al. 1998), a skeletal map with uniformly distributed markers (approximately 5 cM per marker interval) was extracted (Fig. 3) and used for QTL identification.

Statistical analysis

In both the RLP50 S and the field experiment a few observations were missing. Therefore, the least square estimate means of RLP50 S, AUDPC, days to heading and plant height, and ANOVAs, were calculated by using PROC GLM in SAS programme (SAS Institute 1988). Subsequently, wide-sense heritability (h^2) for the four traits was estimated. A computer software package, MAPQTL version 3.0 (Van Ooijen and Maliepaard 1996), was used for interval mapping (Lander and Botstein 1989). In the region of the putative QTLs (LOD > 2.5), the markers with the highest LOD values ("peak markers") were taken as co-factors for running a multiple-QTL mapping programme, the MQM method (Jansen 1993; Jansen and Stam 1994). When LOD values of some markers on other regions reached a significant level, the MQM was repeated by adding those new 'peak markers' as cofactors until a 'stable' LOD

profile was reached. A LOD value of 2.5 was chosen as significant threshold value for declaring a QTL.

Results

Assessment of resistance and plant development traits

The analysis of variance indicated highly significant differences among the 103 RILs for all four evaluated traits (data not shown). Due to the use of one replication, no analysis of variance could be applied for latent period in adult plants in the greenhouse (RLP50A). The frequencies of all three parameters for partial resistance and for days to heading and plant height were approximately normally distributed (Fig. 1). The values of the 103 RILs in three measures for partial resistance fell between the parental values, indicating the absence of transgressive segregation in this population (Fig. 1A–C). In contrast, transgressive segregation was observed for days to heading and plant height (Fig. 1D and E).

The wide-sense heritabilities of the two measures for partial resistance and the two traits were estimated. The heritability for RLP50S was about 0.58 and was 0.82 for both plant height and AUDPC. A very high heritability of 0.94 was found for days to heading.

A strong negative correlation was found between RLP50A and AUDPC (Table 1). Moderate correlations were observed between resistance in the seedling stage (RLP50S) and in the adult plant stage (RLP50A and AUDPC). Plant height was strongly correlated with days to heading. No correlation between the three measures of partial resistance and plant height was observed nor between days to heading and resistance in the seedling stage (RLP50S). However, a moderate

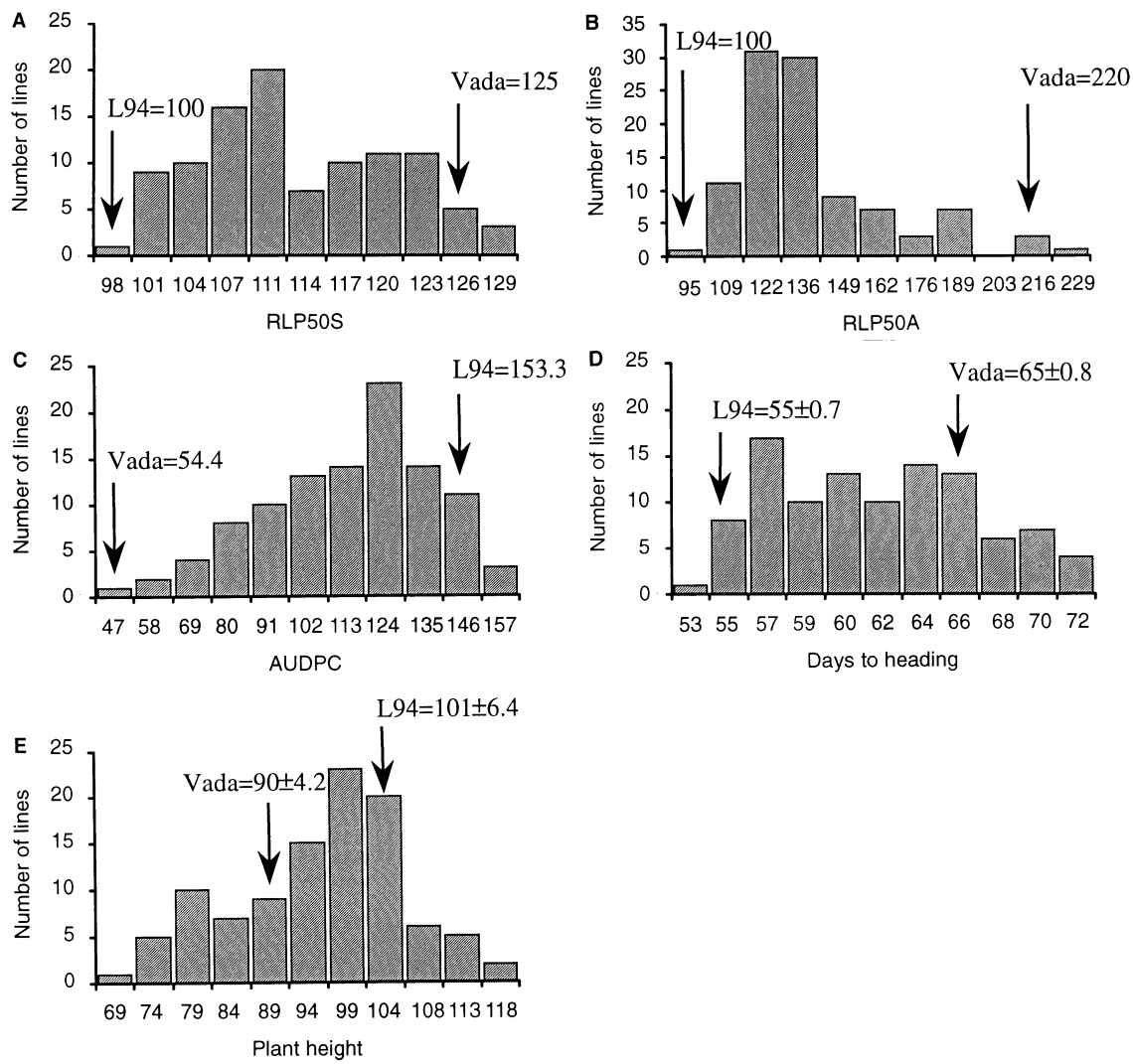

Fig. 1A–E Frequency distribution of phenotypes for the three measures of leaf rust resistance and two agronomic traits in 103 RILs (F_9) derived from a cross L94 × 'Vada'. **A** RLP50S, **B** RLP50, **C** AUDPC, **D** days to heading, **E** plant height (see Methods and materials). Values of L94 and 'Vada' are shown by *arrow*. The values indicated on the *x*-axis are the lower limit of each category

Table 1 Correlation coefficients (*r*) among traits in 103 RILs (F_9) derived from the cross L94 × ' Vada'

Traits	RLP50S	RLP50A	AUDPC	Days to heading
RLP50S				
RLP50A	0.43**			
AUDPC	− 0.43**	− 0.78**		
Days to heading	− 0.07	0.40**	− 0.34**	
Plant height	− 0.15	0.24*	− 0.19	0.68**

* $P \leq 0.5$; ** $P \leq 0.01$

correlation between days to heading and partial resistance in the adult plant stage (RLP50 A and AUDPC) was observed.

QTLs for partial resistance

To map QTLs for partial resistance and plant development traits, we applied interval mapping and MQM methods (Fig. 2). A major improvement in the accuracy of QTL mapping was achieved by using MQM where the "peak" markers were taken as cofactors. Therefore, QTLs identified using MQM methods were considered to be the most reliable (Jansen 1993; Jansen and Stam 1994).

In total, six QTLs for partial resistance to leaf rust were identified in this population (Fig. 3). Some QTLs were identified that were common to each of the parameters of partial resistance, often showing the highest LOD score at exactly the same marker loci. Most likely, the same QTL was involved in different parameters of partial resistance. Three QTLs for RLP50S were identified; these were designated *Rphq1*, *Rphq2* and *Rphq3*. Two major QTLs, *Rphq2* and *Rphq3*, located on chromosomes 2 and 6, respectively, explained a large part of the phenotypic variance (Table 2). *Rphq1*, a minor QTL (explaining 3.4% phenotypic variance) on chromosome 1, was detected with a LOD score of 2.5 which was just above the threshold value. The three QTLs together explained 56 of the phenotypic variance. Four QTLs, *Rphq2*, *Rphq3*, *Rphq4* and *Rphq5*, were identified at the adult plant stage, both in the greenhouse and in the field. *Rphq4* and *Rphq3*, on chromosomes 7 and 6 respectively explained most of the phenotypic variance; *Rphq2* and *Rphq5*, on chromosomes 2 and 4 respectively, contributed moderately to the partial resistance at adult plant stage. In the field experiment the four QTLs explained 63% of total phenotypic variance. In addition, another QTL, *Rphq6* ($R^2 = 0.07$), was found to affect the latent period only at the adult plant stage (RLP50A). It was mapped at the same position as that of a major QTL for days to heading (see next paragraph). In total, 59% of the phenotypic variance for prolonged latent period

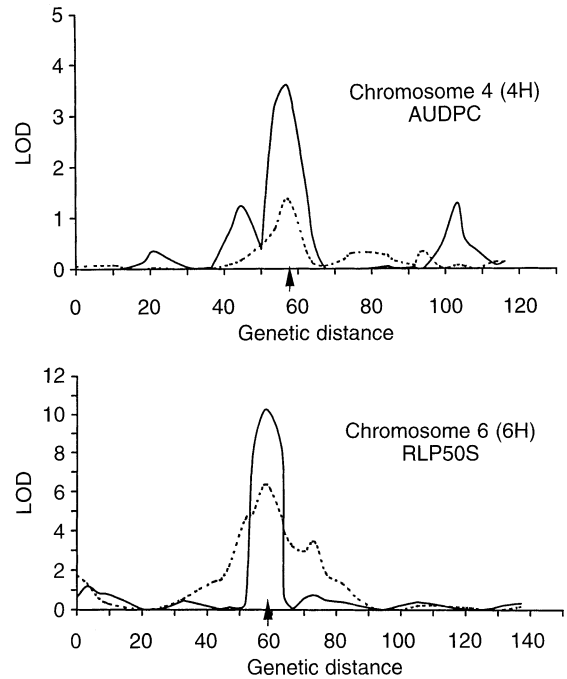

Fig. 2 LOD profiles of QTL on chromosomes 4 (4H) and 6 (6H). *Dotted lines* are based on interval mapping and the *solid lines* are MQM. The *arrow* indicates the position of the markers taken as cofactors for the MQM analysis. Chromosome maps are oriented with the short arm to the *left* and correspond to the maps shown in Fig. 3

at the adult plant stage in the greenhouse was accounted for by the five QTLs.

Disease evaluations for LP in seedlings (RLP50S) were conducted in four experiments by different persons and in different years. When the four data sets were used separately for QTL mapping, the three QTLs (*Rphq1*, *Rphq2* and *Rphq3*) were always identified with an identical ranking order of quantitative effects (data not shown). Moreover, the QTLs found to affect RLP50A were also found to affect AUDPC, and had the same ranking order for size of effect for both parameters. These results indicate that these QTLs for partial resistance to barley leaf rust were relatively

Fig. 3 Locations of QTLs for partial resistance to barley leaf rust and race-specific resistance genes (*Rph*), days to heading and plant height on the skeletal map, based on 103 RILs (F_9) from a cross L94 × 'Vada'. Chromosomes were oriented with the short arms to the *top*. Kosmbi's mapping function was used. Names of QTLs are to the *left* of each QTL. *Boxes inside* the *chromosome bars* are the QTLs for partial resistance to leaf rust (all resistance alleles are from 'Vada'). *Boxes outside* the *chromosome bars* are QTLs for days to heading and plant height, with the positive effects of the alleles from 'Vada' on the *left side* and negative effects of the alleles from Vada on the *right side*. *Length* of bars corresponds to two LOD support intervals (from peak) based on the results of the MQM method. The aproximate locations of race-specific resistance genes (*Rph* genes) are estimated from the literature (see text)

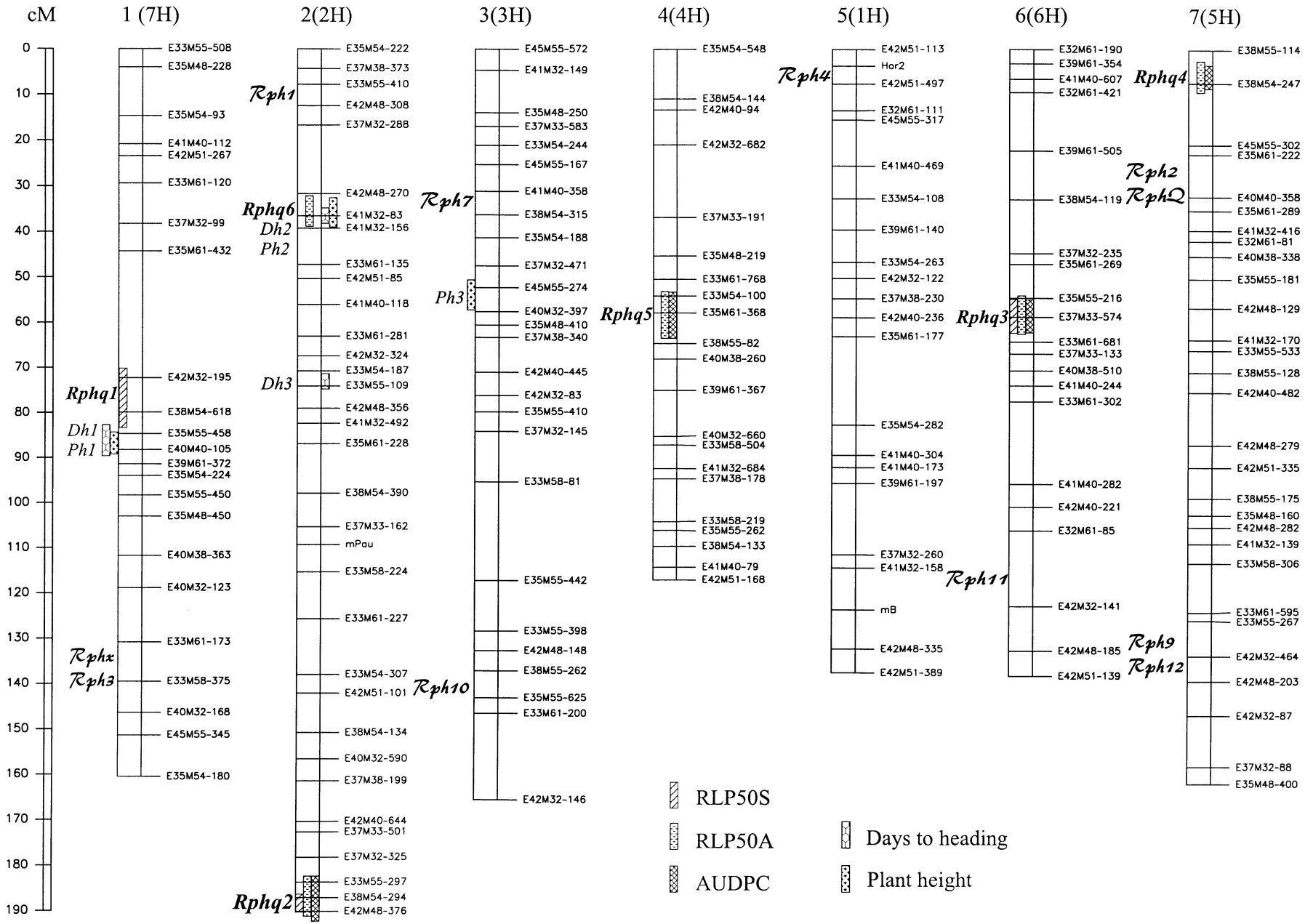
cM
1 (7H)
2(2H)
3(3H)
4(4H)
5(1H)
6(6H)
7(5H)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
E33M55-508
E35M48-228
E35M54-93
E41M40-112
E42M51-267
E33M61-120
E37M32-99
E35M61-432
E42M32-195
Rphq1
E38M54-618
Dh1
E35M55-458
Ph1
E40M40-105
E39M61-372
E35M54-224
E35M55-450
E35M48-450
E40M38-363
E40M32-123
E33M61-173
Rphx
Rph3
E33M58-375
E40M32-168
E45M55-345
E35M54-180
E35M54-222
E37M38-373
E33M55-410
Rph1
E42M48-308
E37M32-288
E42M48-270
Rphq6
E41M32-83
Dh2
E41M32-156
Ph2
E33M61-135
E42M51-85
E41M40-118
E33M61-281
E42M32-324
E33M54-187
Dh3
E33M55-109
E42M48-356
E41M32-492
E35M61-228
E38M54-390
E37M33-162
mPau
E33M58-224
E33M61-227
E33M54-307
E42M51-101
E38M54-134
E40M32-590
E37M38-199
E42M40-644
E37M33-501
E37M32-325
E33M55-297
Rphq2
E38M54-294
E42M48-376
E45M55-572
E41M32-149
E35M48-250
E37M33-583
E33M54-244
E45M55-167
E41M40-358
Rph7
E38M54-315
E35M54-188
E37M32-471
E45M55-274
Ph3
E40M32-397
E35M48-410
E37M38-340
E42M40-445
E42M32-83
E35M55-410
E37M32-145
E33M58-81
E35M55-442
E33M55-398
E42M48-148
E38M55-262
Rph10
E35M55-625
E33M61-200
E42M32-146
E35M54-548
E38M54-144
E42M40-94
E42M32-682
E37M33-191
E35M48-219
E33M61-768
Rphq5
E33M54-100
E35M61-368
E38M55-82
E40M38-260
E39M61-367
E40M32-660
E33M58-504
E41M32-684
E37M38-178
E33M58-219
E35M55-262
E38M54-133
E41M40-79
E42M51-168
E42M51-113
Hor2
Rph4
E42M51-497
E32M61-111
E45M55-317
E41M40-469
E33M54-108
E39M61-140
E33M54-263
E42M32-122
E37M38-230
E42M40-236
E35M61-177
E35M54-282
E41M40-304
E41M40-173
E39M61-197
E37M32-260
E41M32-158
mB
E42M48-335
E42M51-389
E32M61-190
E39M61-354
E41M40-607
E32M61-421
E39M61-505
E38M54-119
Rphq3
E35M55-216
E37M33-574
E33M61-681
E37M33-133
E40M38-510
E41M40-244
E33M61-302
E41M40-282
E42M40-221
E32M61-85
Rph11
E42M32-141
E42M48-185
E42M51-139
E38M55-114
Rphq4
E38M54-247
E45M55-302
E35M61-222
Rph2
RphQ
E40M40-358
E35M61-289
E41M32-416
E32M61-81
E40M38-338
E35M55-181
E42M48-129
E41M32-170
E33M55-533
E38M55-128
E42M40-482
E42M48-279
E42M51-335
E38M55-175
E35M48-160
E42M48-282
E41M32-139
E33M58-306
E33M61-595
E33M55-267
Rph9
Rph12
E42M32-464
E42M48-203
E42M32-87
E37M32-88
E35M48-400
RLP50S
RLP50A
AUDPC
Days to heading
Plant height

Table 2 Summary of QTLs for partial resistance to barley leaf rust

QTLs	RLP50 S			RLP50 A			AUDPC		
	LOD	Exp%[a]	Add[b]	LOD	Exp%	Add	LOD	Exp%	Add
Rphq1	**2.5**	**3.4**	**−1.5**	0.3	0.4	1.8	1.2	0.9	2.6
Rphq2	**18.1**	**35.5**	**−4.9**	**3.0**	**4.1**	**− 5.6**	**4.1**	**3.8**	**4.9**
Rphq3	**10.3**	**16.7**	**−3.5**	**10.7**	**17.4**	**−12.0**	**10.3**	**11.1**	**9.0**
Rphq4	1.0	1.3	−0.9	**14.3**	**25.4**	**−14.3**	**25.4**	**44.7**	**17.4**
Rphq5	0	0	0	**3.1**	**4.3**	**− 5.7**	**3.6**	**3.3**	**4.6**
Rphq6	0	0	−0.1	**5.3**	**7.7**	**− 7.9**	1.5	1.4	3.0
Total[c]		**55.6**	**−9.9**		**58.9**	**−45.5**		**62.9**	**35.9**

[a] The proportion of the explained phenotypic variance
[b] Effects of the alleles from 'Vada'
[c] Sum of the values of the significant QTLs (**Bold** font)

insensitive to environmental conditions. However, distinct clear plant stage-specific effects of QTLs were also identified. *Rphq4* and *Rphq5* were greatly effective at the adult plant stage (RLP50A and AUDPC), but not at the seedling stage (RLP50S). In contrast, *Rphq2* was largely effective at the seedling stage (RLP50S) but only weakly at adult plant stage (RLP50A and AUDPC). One minor QTL, *Rphq1*, was only effective in the seedling stage. *Rphq3* on chromosome 6 was the only QTL with a substantial effect at both the seedling and adult plant stages.

QTLs for days to heading and plant height

In a previous study a moderate correlation between days to heading and partial resistance was found (unpublished data). These two traits might be related to partial resistance. In the present study, four QTLs were detected for days to heading and plant height, two of which were involved in both traits and the other two in only one of these traits (Fig. 3). One major QTL, designated as *Dh2*, on the short arm of chromosome 2 explained 58% of the total phenotypic variance for days to heading (Table 3). A QTL with a moderate effect, *Dh3*, was identified at the putative centromeric region of chromosome 2. The three QTLs for days to heading explained together 70% of the total phenotypic variance. The three QTLs detected for plant height explained 65% of the phenotypic variance. Two main plant height QTLs, *Ph1* and *Ph2*, were mapped at the same positions as *Dh1* and *Dh2*, respectively. Another one, *Ph3*, on chromosome 3, affected only days to heading but not plant height.

Model fitting of QTLs for partial resistance

Model fitting was applied to check to what extent the detected QTLs could account for the observed values (RLP50S and AUDPC). For each QTL, the nearest "peak" marker (normally a "cofactor" marker) was used to determine the QTL genotypes of each line (Tables 4 and 5). The observed mean values per genotype class fitted well with the predicted values, indicating that all major QTLs for partial resistance were correctly identified, despite possible errors of miss-classification of lines by using a single "peak" marker to define the genotype.

Table 3 Summary of QTLs for days to heading and plant height

QTLs	Days to heading (Dh)			Plant height (Ph)		
	LOD	Exp%[a]	Add[b]	LOD	Exp%	Add
Dh1, *Ph1*	**3.6**	**4.2**	**1.0**	**11.2**	**23.7**	**5.5**
Dh2, *Ph2*	**27.5**	**57.8**	**−3.7**	**12.7**	**27.9**	**−6.0**
Dh3	**7.0**	**8.5**	**−1.5**	1.4	2.2	−1.7
Ph3	0.2	0.3	0.2	**7.0**	**13.5**	**4.1**
Total[c]		**70.5**			**65.1**	

[a] The proportion of the explained phenotypic variance
[b] Effects of the alleles from 'Vada'
[c] Sum of the values of the significant QTLs (**Bold** font)

Additive effects of QTL for partial resistance

Three-factor (three QTLs for RLP50 S) and four-factor (four QTLs for AUDPC) analyses of variance (data not shown) based on the values in Tables 4 and 5 gave only one highly significant interaction ($P \leqslant 0.001$) between *Rphq1* and *Rphq2*, the QTLs for partial resistance at the seedling stage (Fig. 4) and no significant interaction among QTLs for resistance at adult plant stage. Previous genetic studies (Parlevliet 1976, 1978) indicated that six unlinked loci could be involved in RLP50A in 'Vada' relative to L94. Furthermore, one of the genes from 'Vada' was supposed to have a larger effect than the others, and with a recessive inheritance. The other genes acted in an additive way. In the present study we detected five QTLs for partial resistance with different

Table 4 Fitted values of three QTLs for partial resistance of seedlings in the greenhouse tests (RLP50 S)

Genotype[a]			Number of RILs	Observed mean[b]	Fitted value[c]
Rphq1	*Rphq2*	*Rphq3*			
B	B	B	23	122.2d	121.4
A	B	B	9	116.3 cd	118.4
B	B	A	5	112.0bc	114.4
B	A	B	14	107.5ab	111.6
A	B	A	9	108.2ab	111.4
A	A	B	20	108.7abc	108.6
B	A	A	5	106.5ab	104.6
A	A	A	13	101.9a	101.6
Mean				111.5	
L94				100.0	
Vada				125.0	

[a] Genotype classes of QTLs are based on the genotypes of the corresponding 'peak' markers; A indicates L94 genotype and B is 'Vada' genotype

[b] Average value of each genotype class; values followed by the same letter do not differ significantly according to Waller–Duncan's test ($P \leq 0.05$)

[c] The theoretical values calculated based on the population mean (μ) and the allelic effect of each QTL; i.e. a genotype class A A B = 111.5 − 1.5 − 4.9 + 3.5 = 108.6

Table 5 Fitted values of four QTLs for partial resistance of adult plants in the field test (AUDPC)

Genotype[a]				Number of RILs	Observed mean[b]	Fitted value[c]
Rphq2	*Rphq3*	*Rphq4*	*Rphq5*			
B	B	B	B	10	74.2a	72.9
B	B	B	A	4	82.8ab	82.1
A	B	B	B	4	71.4a	82.7
B	A	B	B	2	92.0ab	90.9
B	B	A	B	6	107.8bc	107.7
A	B	B	A	6	90.2ab	91.9
B	A	B	A	4	98.6abc	100.1
A	A	B	B	1	100.5	100.7
B	B	A	A	10	117.1 cd	116.9
A	B	A	B	5	113.3c	117.5
B	A	A	B	3	125.8cde	125.7
A	A	B	A	8	108.8bcd	109.9
A	B	A	A	15	130.1de	126.7
B	A	A	A	5	129.4de	134.9
A	A	A	B	6	141.3e	135.5
A	A	A	A	2	142.2de	144.7
Mean					108.8	
L94					153.3	
Vada					54.4	

[a] Genotype classes of QTLs are based on the genotypes of the corresponding 'peak' markers, A indicates L94 genotype, and B is 'Vada' genotype

[b] Average value of each genotype class, values followed by the same letter do not differ significantly according to Waller–Duncan's test ($P \leq 0.05$)

[c] The theoretical values calculated based on the population mean (μ) and the allelic effect of each QTL, i.e. a genotype class B A B A = 108.8 − 4.9 + 9.0 − 17.4 + 4.6 = 100.1

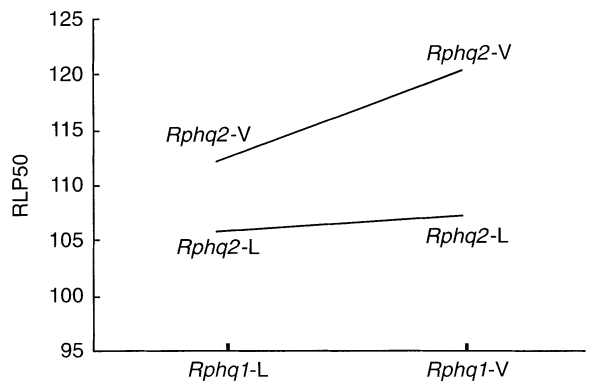

Fig. 4 Interaction of two QTLs, *Rphq1* and *Rphq2* based on seedlings tested in the greenhouse (RLP50S). Letters *L* and *V* following the QTL indicate the alleles of the corresponding QTL from 'Vada', respectively

quantitative effects (RLP50A; Table 2). However, only 55–65% phenotypic variances were explained by the identified QTLs. With respect to the heritabilities of 0.6–0.9, most of the genetic variation were explained by these QTLs. Still it is possible that some QTLs with smaller effects were not identified due to the small population size (103 RILs) and large genetic and/or environmental noises or that epistatic loci contributing to partial resistance can not be detected with interval mapping (or/and MQM) where an additive model is applied.

Discussion

Resolution of QTL mapping

We identified QTLs using a multiple QTL model which combines the interval mapping method with a multiple linear regression method (Jansen 1993). It is now widely recognised that simultaneous mapping of multiple

QTLs is more efficient and more accurate than interval mapping which fits single QTL (Knapp 1991; Jansen and Stam 1994). Indeed, in most cases the QTLs identified by MQM in this research clearly showed higher LOD scores and lower background (sharper peaks) than interval mapping (Fig 2). Moreover, the probability of detecting a QTL may increase using the MQM method. For example, *Rphq5* could not be detected by interval mapping, while by MQM, with taking the "peak" marker for QTL *Rphq4* as a cofactor, it could be identified as being significant (LOD = 3.6).

Though markers giving "peak" LOD values in interval mapping are usually taken as cofactors in MQM, there are no good reasons not to take the imminent markers (within 5 cM) as cofactors. A sharp peak in the LOD profile may shift when imminent markers are applied as a cofactor for a QTL. This illustrates that the sharp LOD peaks obtained with MQM should also be taken with some caution when locating QTLs. To maximise the chance of assigning a QTL to the correct interval, one should apply large LOD differences for the selection of a support interval or flanking markers when the LOD profiles generated by MQM are used. Therefore, we took two LOD support intervals.

Comparison to known major genes and/or mapped QTLs in barley

When the 'Proctor'/'Nudinka' AFLP and restriction fragment length polymorphism (RFLP) combined map (Becker et al. 1995) is used as a 'bridge', the present AFLP map of L94/'Vada' can be aligned with the integrated RFLP map which contains about 880 RFLP markers (Qi et al. 1996). Consequently, previously mapped genes and QTLs can be compared with the QTLs identified in this study. The two earliness QTLs, *Dh2* and *Dh3* on chromosome 2 (2H), may correspond to the two QTLs detected in the 'V. Gold' × 'T. Prentice' cross (Kjær et al. 1995). The chromosome region of *Dh2*/*Ph2* with the largest effects for both traits is likely the site of one of the early maturity (*Ea*) loci (Nilan 1964) and/or a photoperiod-response gene, *Ppd-H1* (Laurie et al. 1994, 1995). *Dh3* mapped in the same region as *eps2S*, a QTL for earliness per se, on chromosome 2 based on the genetic map of the 'Igri' × 'Triumph' cross. *Dh1* and *Ph1* on the short arm of chromosome 1 (7H) may correspond to the two very closely linked QTLs for earliness and plant height identified in the 'Steptoe' × 'Morex' cross (Hayes et al. 1993). These two QTLs were also detected in a two-row barley cross, 'Harrington' × 'TR306' (Tinker et al. 1996). Another QTL (*Ph3*) for plant height on the short arm of chromosome 3 likely mapped at the same region of the plant height QTL detected in the 'Steptoe' × Morex' cross (Hayes et al. 1993). In conclusion: the QTLs for plant height and days to heading identified in the present 'L94' × 'Vada' cross are in agreement with previously mapped QTLs in various barley populations.

In barley, about 14 race-specific resistance genes to leaf rust (designated as *Rph* loci) have been reported (Jin et al. 1996). Recently, several resistance genes have been mapped on barley molecular maps (Fig. 3). By using sequence-tagged site (STS) and microsatellite markers, Borovkova et al. (1997) mapped *Rph9* and *Rph12* at the same region of the long arm of chromosome 7 (5H) and later found them to be allelic. Moreover, on chromosome 7 (5H), *RphQ*, a presumed allele at the *Rph2* locus, was mapped on the short arm, near the centromere (B. Steffenson, personal communication). This location is quite far from the major QTL, *Rphq4*, mapped at the distal part of the short arm of this chromosome. Another leaf rust resistance locus, designated as *RphX*, was mapped to the long arm of chromosome 1 (7H) using RFLP markers (Hayes et al. 1996). It may be allelic to the *Rph3* that was also mapped to the similar position on this chromosome using a morphological marker (Jin et al. 1993). *Rph4* was mapped on the short arm of chromosome 5 (1H) using the Ml-a locus as a genetic marker (McDaniel and Hathcock 1969). *Rph1* and *Rph7* were assigned to chromosome 2 (2H) (Tuleen and McDaniel 1971) and chromosome 3 (3H) (Tan 1978; Tuleen and McDaniel 1971), respectively, by trisomic analysis and were localised on the short arm and centromeric region of the corresponding chromosomes using morphological markers (Roane and Starling 1989). *Rph10* and *Rph11* were assigned to the long arms of chromosome 3 (3H) and chromosome 6 (6H), respectively, using isozyme markers (Feuerstein et al. 1990). Interestingly, there is no indication that map positions are shared between race specific resistance genes (*Rph* loci) and the QTLs for partial resistance identified in the L94/'Vada' population. This implies different sets of genes and/or a different evolutionary origin of these two types of resistance to barley leaf rust. Histological studies showed that the *Rph* resistance acts post-haustorially with hypersensitivity, whereas partial resistance is based on pre-haustorial mechanisms associated with the formation of papillae (Niks 1986).

Latent period is a major factor (or component) for partial resistance

The severity of rust epidemics in the field measured by AUDPC reflects the joint effects of all components for partial resistance, such as infection frequency, latent period, spore production, infectious period and pustule size (Parlevliet 1979; Neervoort and Parlevliet 1978). On the partially resistant parent 'Vada' the barley leaf rust fungus has a lower infection frequency, longer LP and lower spore production than on the susceptible line L94. LP is regarded the most effective of these components of resistance (Zadoks and Schein 1979; Parlevliet

1979). Indeed, all the QTLs that we detected affecting the AUDPC were also found to influence the LP of adult plants in the greenhouse. Because of the moderate to high correlation between LP and the other components of partial resistance (Parlevliet 1986, 1992), we presume that some or all of these genes pleiotropically also govern the other components of partial resistance. We did not find QTLs that affect the AUDPC but not the LP in adult plants. This suggests that in this population there are no genes segregating that substantially affect the epidemic progress without prolonging the LP in adult plants. Therefore, LP of the rust in adult barley plants is a good predictor for partial resistance to leaf rust in the field.

Development-dependent expression of genes for partial resistance

The often reported moderate correlation coefficient values between seedling data and adult plant data for partial resistance components have suggested that during development of the plant, different genes are involved in the latent period and infection frequency of leaf rust in barley (Parlevliet and Kuiper 1977; Parlevliet and Van Ommeren 1975; Parlevliet 1975). By using QTL mapping, we have now resolved the partial resistance (latent period) into six QTLs with different quantitative effects and their dependence on plant development. Three QTLs (*Rphq4*, *Rphq*5 and *Rphq6*) contributed to a longer latent period at the adult plant stage only. In contrast, *Rphq1* contributed to a longer latent period at the seedling stage, but not at the adult plant stage. These results are in accordance with the previously reported relatively weak correlation between seedling and adult plant data.

Pleiotropic effects of QTLs

As has been reported for yield and its components in maize (Veldboom et al. 1994) and rice (Xiao et al. 1996), correlated traits often are associated with the same QTLs. In the present study of barley, we also found that the highly correlated earliness and plant height ($r = 0.67$) were governed by the same two QTLs. Moreover, the allelic effects were in the same directions for the QTLs of both traits (Table 3). Trait correlation may result from either the pleiotropic effects of single genes or from tight linkage of several genes controlling the traits.

The same map position on the short arm of chromosome 2 was shared by the main QTL *Dh*2/*Ph2* for earlier heading and shorter plant height and the moderate QTL *Rphq6* for longer latent period in adult plants in the greenhouse (RLP50A). Minor effects (LOD = approximately 1.5) were detected at this position using AUDPC data. However, on the basis of the present results, it is difficult to conclude whether the same gene regulating plant development also affects partial resistance or whether tightly linked genes are being mapped on the same region that can not be resolved by current QTL mapping.

Utilisation of the mapped QTLs for partial resistance in plant breeding

The present study has clearly demonstrated that QTLs prolonging LP in the adult plants are a major factor for partial resistance. Therefore, evaluation of LP in the flag leaf in the greenhouse is an efficient way to select for partial resistance in the progenies (of individual plants). By using marker-assisted selection (MAS), plant breeders can apply molecular markers associated with the favourable QTL alleles for partial resistance at the early stage of plant development and, consequently, improve the efficiency of selecting for partial resistance to leaf rust in the breeding programme. In view of the large number of polymorphisms that can be detected with AFLP, also within the European and North American barley germplasm (Qi and Lindhout 1997; Schut et al. 1997), the transfer of AFLP-tagged QTL alleles from 'Vada' into other cultivars is now a feasible approach. In addition, more QTL alleles for partial resistance from other sources can be easily combined together using MAS, resulting in a higher level of resistance. Furthermore, in a modern breeding programme, many favourable traits have to be integrated into a cultivar. By use of MAS, QTLs for partial resistance can be more efficiently incorporated in the cultivars to be released, thus offering better prospects for durable resistance as a breeding goal.

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