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The evidence for abundance of QTLs for partial resistance to *Puccinia hordei* on the barley genome

Xiaoquan Qi[1,*], Fekadu Fufa, Dick Sijtsma, Rients E. Niks, Pim Lindhout & Piet Stam
Graduate School of Experimental Plant Sciences, Dept. of Plant Breeding, Wageningen Agricultural University, P.O. Box 386, 6700 AJ Wageningen, The Netherlands; [1]*Present address: John Innes Centre, Colney Lane Norwich NR4 7UH, UK (*author for correspondence; e-mail xiaoquan.qi@bbsrc.ac.uk)*

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Abstract

Using AFLP markers, a linkage map was constructed based on a recombinant inbred population of barley derived from a cross between a leaf rust susceptible line, L94, and a partially resistant line, 116-5. The constructed map showed a similar marker distribution pattern as the L94 × Vada map. However, it contained more large gaps, and for some chromosome regions no markers were identified. These regions are most likely derived from L94 because 116-5 was selected from the progeny of a cross of L94 × cv. Cebada Capa. Five QTLs for partial resistance to isolate 1.2.1. were mapped on the L94 × 116-5 map. Three QTLs were effective in the seedling stage, jointly contributing 42% to the total phenotypic variance. Three QTLs were effective in the adult plant stage, collectively explaining 35% of the phenotypic variance. Evidence for two additional linked minor-effect QTLs effective in the adult plant stage was also uncovered. The major-effect QTL, *Rphq3*, was the only one that was effective in both developmental stages. Moreover, *Rphq3*, was also identified in the L94 × Vada population, being effective to two rust isolates. The other QTLs were detected in either of the two populations, providing evidence for the existence of many loci for partial resistance to leaf rust on the barley genome. To date, 13 QTLs for partial resistance have been mapped, therefore, a strategy of accumulating many resistance genes in a single cultivar, resulting in a high level of partial resistance, is feasible.

Abbreviations: AFLP, amplified fragment length polymorphism; QTLs, quantitative trait loci; LP, latency period; RILs, recombinant inbred lines

Introduction

In the barley-barley leaf rust (*Puccinia hordei* Otth) plant-pathosystem, two types of resistance have been described. Hypersensitive resistance, based on the *Rph* genes [3, 9, 10, 31], has been used extensively in barley breeding programs. However, the great disadvantage of this resistance is its lack of durable effectiveness. As an alternative, partial resistance to leaf rust, defined as resistance that results in reduced epidemic development despite a compatible infection type [16, 21], is widely present in barley [1, 25]. Partial resistance occurs in numerous plant-pathosystems and is presumed to have durable effectiveness. In the barley-barley leaf rust plant pathosystem, it is associated with various components [17], such as lower infection rate, longer latency period, smaller pustule size and reduced spore production, that can be measured in monocyclic disease tests in the greenhouse. Of these components, the latency period (LP) on mature plants is the best predictor of the level of partial resistance in the field [18, 19].

By use of a high-density AFLP marker linkage map [30], ten QTLs for partial resistance to barley leaf rust in a mapping population from a cross of L94 × Vada have been identified [27, 28]. They are designated as *Rphq* loci. These QTLs act predominantly in an additive fashion. The estimated sizes of the effects

of *Rphq* differ and the expression of several of these QTLs are plant stage specific. In addition, most of these QTLs show a differential expression against two rust isolates, supporting the idea that partial resistance operates according to a 'minor gene-for-minor gene' model [23]. The positions of the identified QTLs on the linkage map do not coincide with those of hypersensitive resistance genes (*Rph* genes). This supports the hypothesis that partial resistance and hypersensitive resistance are two fundamentally distinct types of defense, as has been indicated in histological studies [14].

Genetic mapping of quantitative resistance genes has also been conducted in many other plant-pathosystems [39]. In an experiment to detect QTLs for resistance to gray leaf spot in maize, three populations were used [2]. Among the more than ten QTLs detected, only one was expressed in all three populations and environments. This indicated that many more QTLs for resistance to this fungus could exist in maize germplasm. In studies on partial resistance to barley leaf rust [20, 24], transgressive segregation for partial resistance was observed in the offsprings of a cross between cv. Vada and cv. Cebada Gapa. This implies that at least some of the genes for partial resistance in Cebada Capa are at different chromosome positions. Cebada Capa also possesses a gene (*Rph7*) for hypersensitive resistance. One line, 116-5, was derived from a cross between L94 and Cebada Capa by selection against *Rph7* and for a high level of the partial resistance. Using a recombinant inbred (RI) population derived from a back cross between this line and the susceptible line L94, an AFLP molecular map was constructed, and more QTLs for partial resistance to barley leaf rust were identified.

Materials and methods

Plant materials

A barley line, L94, which is extremely susceptible to leaf rust, was crossed to a partially resistant barley line, 116-5. By applying the single-seed descent (SSD) method, a recombinant inbred (RI) population (F_8) containing 117 lines was derived from this cross. Line 116-5 was derived from a cross between L94 and cv. Cebada Capa. The latter not only has a high level of partial resistance but also has a gene for hypersensitive resistance, *Rph7* [15]. To eliminate *Rph7*, selection against hypersensitive resistance (for high infection type) was carried out in the F_2 generation. By line selection for a high level of partial resistance in the advance generations, the partially resistant line 116-5 was developed.

Disease evaluation in the seedling stage

Seedling leaves of both parental lines, L94 and 116-5, and their progeny, 117 RILs (F_8), were inoculated with the monospore leaf rust isolate 1.2.1. in the greenhouse in three replications. The relative latency period in the seedling stage (RLP50S) was calculated relative to the LP of L94, where L94 = 100, as described by Parlevliet [16]. The procedure for the evaluation of latency period (LP) in the seedling stage was as described in Qi *et al.* [27, 28].

Disease evaluation in the field

Two field experiments were carried out in 1995 and 1997. The experiment of 1995 had no replications, while in 1997, a randomized complete block design with three replications was used. Oat was grown between the barley plots to limit inter-plot interference [22]. The inoculation procedure in the field with the monospore culture derived isolate 1.2.1. was as described by Qi *et al.* [27]. In the experiment of 1995, 10 tillers per plot were sampled on July 10, 13 and 19, respectively, for evaluation of infection frequency according to the scale of Parlevliet and van Ommeren [22]. In 1997, three tillers per plot were sampled, and five observations were conducted on 5, 13, 24 and 30 June and 6 July for all three replications. However, due to dry weather at inoculation time, the epidemics of leaf rust did not develop in some plots or parts of a block. For the calculation of area under disease progress curve (AUDPC) and for further analyses, these plots were not taken into account.

Marker generation and map construction

The AFLP protocol was applied as described previously [26]. Genomic DNA was isolated and digested with the restriction enzymes, *Eco*RI and *Mse*I. The corresponding adapters and primers were the same as described in Qi and Lindhout [26] and are also available via the Internet at 'GrainGenes WWW Page, map data'. Twenty-seven primer combinations were used (Table 1). AFLP marker names were according to the AFLP profiles of 16 reference barley lines (GrainGenes WWW Page, map data). Two qualitative traits, i.e. black/white seeds and two-row/six-row spike (for L94

Table 1. Number of AFLP markers generated in the L94 × 116-5 population.

Primer combinations	Number of markers[1]	Number of marker in common[2]
E32M55	12	–
E38M50	13	–
E38M51	10	–
E38M59	12	–
E38M60	11	–
E38M62	5	–
E39M55	7	–
E40M32	4	–
E45M49	22	–
E45M58	7	–
E32M61	12	8
E33M54	8	3
E33M55	11	7
E33M61	10	6
E35M48	13	8
E35M54	7	2
E35M55	9	7
E35M61	5	4
E37M33	12	6
E37M38	11	3
E38M54	9	7
E39M61	11	7
E41M32	9	7
E41M40	11	9
E42M32	18	9
E42M40	15	7
E45M55	7	5
Total	281	105

[1] Number of AFLP markers in the L94 × 16-5 population.
[2] Number of markers in common with the L94 × Vada population.

and 116-5, respectively) were scored as morphological markers, named m*B* and m*hex-v*, respectively. JoinMap 2.0 [33, 34] was used to group the linked markers and to construct the genetic map. AFLP markers common to the L94 × Vada population were used to assign linkage groups to the corresponding barley chromosomes. Kosambi's mapping function was applied for map distance calculation [11].

Statistical analysis

Because of some missing values, the least square estimate means of RLP50S and AUDPC in the 1995 and 1997 experiments, and the ANOVAs were calculated by using PROC GLM of the SAS package [32]. The wide sense heritabilities (h^2) of two measures of partial resistance were estimated based on the corresponding mean squares from the ANOVA. Both interval mapping [12] and multiple-QTL mapping (MQM) [7, 8], available in a computer software package, MapQTL version 3.0 [38], were used for mapping QTLs. A LOD score of 3.0 was chosen as significance threshold value for declaring a QTL.

Results

Map construction

By using 27 primer combinations, 281 AFLP markers were generated in the present mapping population, yielding an average of 10 markers per primer combination (Table 1). Of these 27 primer combinations, 17 had been used previously for the construction of the L94 × Vada AFLP map [30] resulting in 105 markers in common between the two populations. One marker, E39M61-360 (Figure 1, indicated by *), was formerly mapped to chromosome 7 of the L94 × Vada map [30], was assigned to chromosome 2. The remaining 104 markers were used as 'anchors' to assign marker linkage groups to barley chromosomes. The 283 markers (281 AFLP and two morphological) were split into 16 linkage groups at a LOD threshold grouping value of 4.0. Except for one unlinked marker (E38M51-371) and one group of two markers (E32M55-613 and E42M32-490), the remaining 14 linkage groups, containing 280 markers, with at least one anchor marker per group, were assigned to the barley chromosomes. By using JoinMap 2.0 [34], a linkage map was successfully constructed (Figure 1).

The linkage map covers a total map distance of 857 cM, corresponding to an average density of 3 cM per marker (Table 2). Markers assigned to chromosomes 1 and 4 were grouped into two linkage groups and two separate linkage maps were constructed for each of the chromosomes. Alignment of the present maps with the L94 × Vada chromosome map revealed large gaps around the putative centromeric regions on chromosomes 1 and 4 (Figure 1, the dotted lines). In the distal regions of chromosomes 2, 3, 6 and 7, no dimorphic AFLP markers were found. About one-third of the markers were mapped on chromosome 2. Chromosome 5 was the only chromosome that was equally well-covered in the L94 × Vada map and in the current map. The positions of two morphological

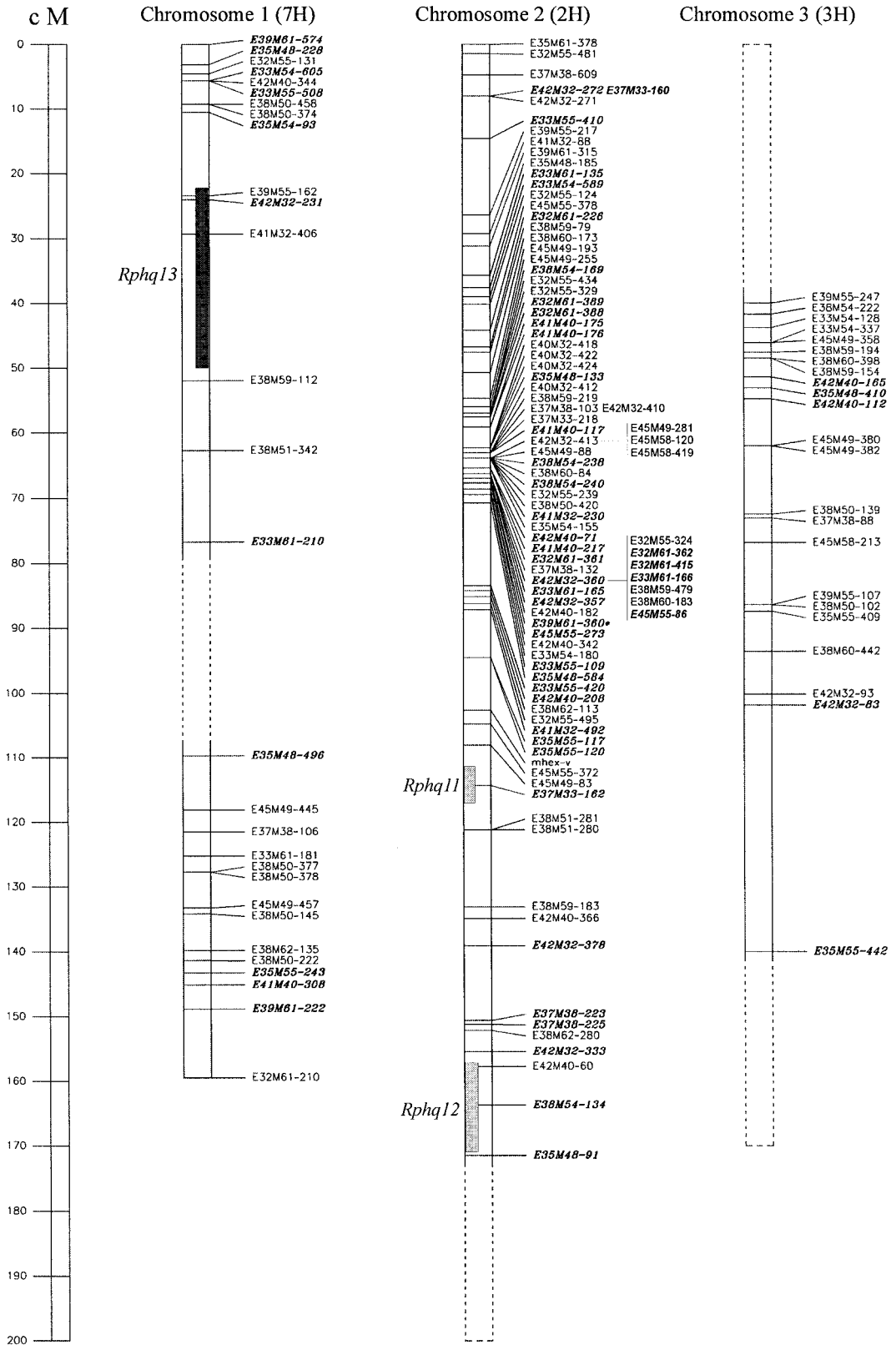

Figure 1. The barley L94 × 116-5 AFLP marker linkage map with the positions of the QTLs for partial resistance. Chromosomes are oriented with the short arm at the top. Kosambi's mapping function was used. Markers in ***bold italic*** font are in common with L94 × Vada map. Groups of markers with identical segregation were aligned to the corresponding representative markers. Lengths of the dotted chromosome bars, indicating absence of markers, were estimated based on the alignment with the L94 × Vada map. Names of QTLs are designated on the left side of each QTL. Length of bars corresponds to two LOD support intervals (from peak) based on the results of MQM.

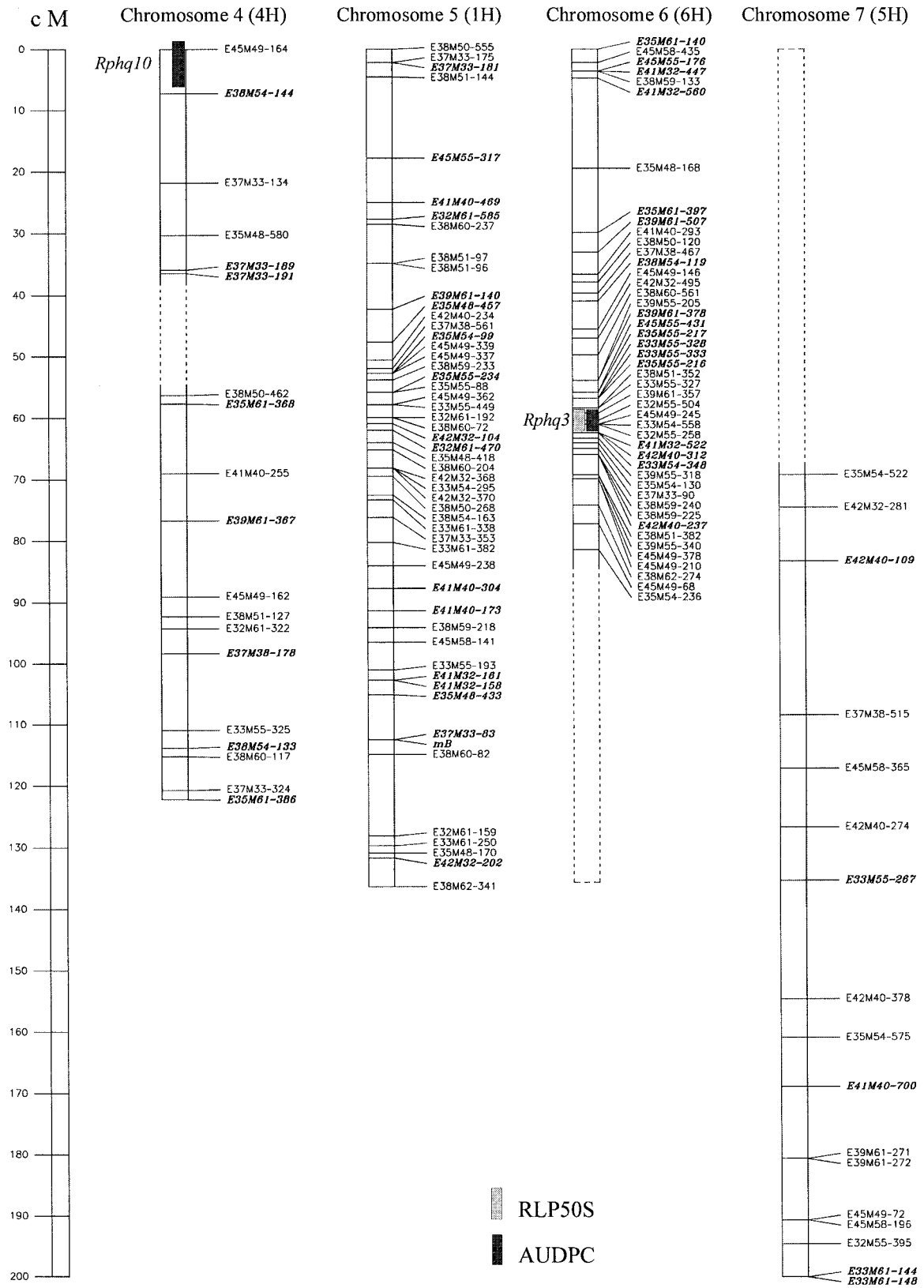

Figure 1. Continued.

Table 2. Summary of L94 × 116-5 mapping data.

Chromosome	Number of markers	Length (cM)
1(7H), short arm	15	77
1(7H), long arm	14	50
2(2H)	93	172
3(3H)	23	100
4(4H), short arm	6	37
4(4H), long arm	13	67
5(1H)	53	137
6(6H)	46	84
7(5H)	17	133
Total	280	857

markers, m*hex-v* and m*B*, on chromosomes 2 and 5, respectively, were in agreement with earlier reports [4, 6, 29, 30].

QTLs for partial resistance

Analysis of variance revealed highly significant differences among the 117 RILs for both AUDPC and RLP50S. Since analysis of the AUDPC data did not show significant 'Year × RIL' interaction and our previous study [28] indicated that the expression of genes for partial resistance to barley leaf rust were insensitive to environmental conditions, the 1995 experiment was treated as another replication. Due to some missing observations, the least square estimate means of AUDPC and RLP50S of the 117 RILs were calculated from the four and three replications, respectively. The frequency distribution of AUDPC and RLP50S were approximately normal (Figure 2). The RLP50S and AUDPC values of the most extreme RILs were similar to those of the two parents, indicating absence of transgressive segregation for partial resistance. The wide sense heritabilities (h^2) in the seedling stage (RLP50S) and in the adult plant stage (AUDPC) were 0.72 and 0.51, respectively. A moderate correlation was observed between RLP50S and AUDPC ($r = -0.52$).

Five QTLs for partial resistance to isolate 1.2.1. were identified (Figure 1 and Table 3). Three QTLs were effective in the seedling stage, jointly contributing 42% of the total phenotypic variance. Two of those QTLs, *Rphq3* and *Rphq11*, had relatively large effects, and were mapped on chromosomes 6 and 2, respectively. Three QTLs were effective in the adult plant stage, together explaining 35% of the phenotypic variance. A major-effect QTL, *Rphq3*, explaining 20% of the phenotypic variance, was mapped to the centromeric region of chromosome 6. Also, *Rphq3* was the only QTL that was effective in both plant development stages. *Rphq11* and *Rphq12* were only effective in the seedling stage, and *Rphq10* and *Rphq13* only in the adult plant stage. All of the resistance-enhancing alleles of the five QTLs originated from the partially

Table 3. Summary of QTLs for partial resistance to barley leaf rust.

	RLP50S			AUDPC		
QTLs	LOD	Exp%[1]	Add[2]	LOD	Exp%	Add
Rphq11	14.2	20.0	2.3	–	–	–
Rphq12	3.5	4.5	1.0	–	–	–
Rphq3	12.8	16.9	2.2	10.1	20.2	−8.6
Rphq13	–	–	–	3.7	9.2	−5.7
Rphq10	–	–	–	3.1	5.5	−4.5
Total[3]	–	**41.9**	**5.5**	–	**34.9**	**−18.8**

[1]The proportion of the phenotypic variance explained.
[2]Effects of the alleles from 116-5.
[3]Sum of the values of the significant QTLs.

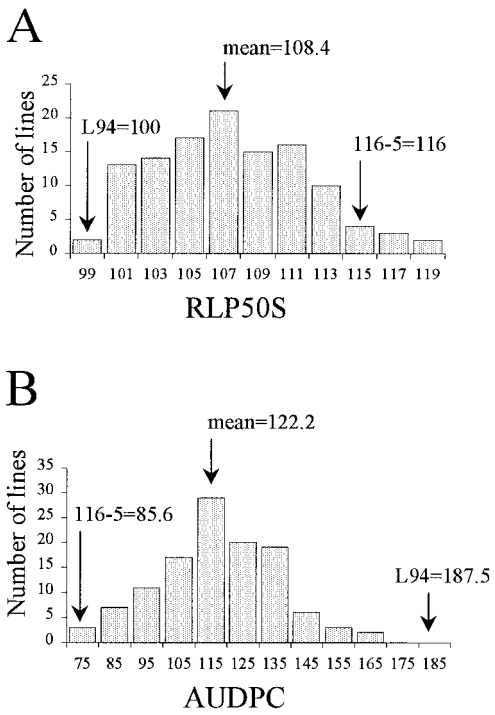

Figure 2. Frequency distribution of phenotypes for the two components of leaf rust resistance in 117 RILs derived from the cross L94 × 116-5. A. RLP50S. B. AUDPC. Values of L94 and 116-5, and population mean values are shown by an arrow. The values indicated on the x-axis are the lower limit of each category.

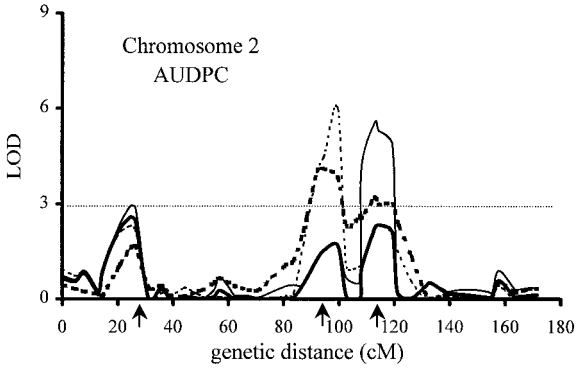

Figure 3. LOD profiles of two linked QTLs on chromosomes 2. The arrows indicate the positions of the markers taken as cofactors for the MQM analysis. The thick dotted line is based on interval mapping and the thick solid line is based on MQM with seven cofactors, including the two at both peak positions (at 95 and 115 cM) simultaneously. The thin dotted and the thin solid lines were from MQM with taking six cofactors, including the one either at the peak position of about 95 cM or at the peak position of about 115 cM. The chromosome is oriented with the short arm to the left and corresponds to the map shown in Figure 1.

resistant parent 116-5. This is in accordance with the absence of transgressive segregation. Three factor analysis of variance based on the genotype classes of three QTLs showed that there were no significant two-way and three-way interactions among the QTLs identified for partial resistance in both development stages (not shown). Therefore, the genes have mainly additive effects on the level of partial resistance.

Minor-effect QTLs for partial resistance

Comparison with the heritabilities (0.72 for RLP50S and 0.51 for AUDPC) showed that about 70% of the genetic variance was explained by the QTLs declared. Actually, in addition to the five declared QTLs, several other chromosome regions showed LOD scores between 2.0 and 3.0, that likely correspond to even more minor-effect QTLs.

There is strong evidence for two linked QTLs for AUDPC on chromosome 2 near the map position of 100 cM, within a distance of about 20 cM. One of these QTLs coincides with *Rphq11*, which is also affecting RLP50S. Figure 3 shows LOD profiles for chromosome 2 obtained with interval mapping and MQM mapping, the latter by using cofactors at varying positions in the region of interest. Interval mapping gives a profile with two, not clearly separated, peaks, both above the threshold value 3.0. In order to verify whether these peaks corresponded to two QTLs, we introduced cofactors at either of these peak positions and also at both peak positions simultaneously. With all these cofactor configurations, the LOD profile clearly showed two separate peaks. Although the two peaks are not simultaneously above the threshold value (3.0), this pattern was taken as a strong evidence for the existence of two QTLs. Since there is no clear guideline for the significance threshold for such a configuration of QTLs, we have further investigated this by means of simulation. Analysis of the simulated data (using a population of the same size and QTL effects of similar size as the estimated effects) showed that in the case of a single QTL, the LOD profiles, obtained by changing the choice of markers as cofactors, do not show two clearly separated peaks. Thus we hypothesize that there is another QTL affecting AUDPC on chromosome 2, at a map position of about 95 cM. Therefore, the QTL at the position of ca. 115 cM (*Rphq11*) not only has an effect in the seedling stage (Table 3, Figure 1), but also in the adult plant stage. In addition, at a map position of 95 cM there may be a minor-effect QTL contributing to the partial resistance of adult plants. However, to verify and designate these minor-effect QTLs further experiments are required.

Discussion

Alignment and comparison of two AFLP linkage maps

Since L94 was a parent for the two mapping populations, L94 × Vada and L94 × 116-5, the two corresponding linkage maps have a large number of markers in common. These common markers enabled the alignment of the present map with the L94 × Vada AFLP linkage map [30]. However, the L94 × 116-5 map contained large gaps and even some missing chromosome regions (Figure 1, dotted lines). The line 116-5 was derived from a cross of L94 × Cebada Capa, and any L94 derived locus or chromosome segment in 116-5 will not segregate in progeny of the cross L94 × 116-5. Consequently, these regions cannot be identified by markers. Thus, seven large segments from the line 116-5 remained unidentified. These seven segments covered ca. 210 cM, compared to 857 cM of identified regions.

Cebada Capa possesses a gene for hypersensitive resistance, *Rph7* [15, 20, 24], on the short arm of chromosome 3 [35, 37]. 116-5 does not have *Rph7*, and indeed, a segment of about 35 cM on the short arm of chromosome 3 of 116-5 is derived from L94. In the absence of any selection, one would expect an equal

proportion of the two parent (L94 and Cebada Capa) genomes in 116-5. The larger proportion (80%) of the Cebada Capa genome in 116-5 is most likely due to the presence of at least five genes for partial resistance in 116-5 and the associated linkage drags.

Markers were not evenly distributed over the genetic map. Similar to the L94 × Vada map, some gaps and a strong clustering of markers were found. Regression of the number of markers in the 21 corresponding segments (bins) of the L94 × Vada and the L94 × 116-5 maps is high ($r^2 = 0.9045$), indicating a very similar marker distribution along the seven chromosomes on the two maps. Clear clustering of markers around the centromeres and a low-density in certain distal regions were observed on both the integrated RFLP linkage map [29] and the L94 × Vada AFLP linkage map [30]. These results indicate that the distribution pattern of molecular markers is not specific for a certain type of markers (RFLP vs. AFLP with using similar restriction enzymes) nor depending on the mapping population, but rather reflects the distribution of recombination over the barley chromosomes. The clustering of markers is possibly due to the centromeric suppression of recombination [5, 36], whereas the gaps in certain chromosome regions could correspond to recombination 'hotspots' [13] in the barley genome or lack of polymorphism for the markers used.

Comparison of QTLs for partial resistance in two populations

Comparison of QTLs for partial resistance showed that a QTL on chromosome 6 which was identified in the L94 × 116-5 mapping population coincided with *Rphq3* which was previously detected on the L94 × Vada map [27, 28]. The exact same position of the QTL on the two maps and the similar magnitudes of effect to the same rust isolate in both the seedling and the adult plant stages provided strong evidence for the same QTL on both maps. Consequently, we named the gene on this locus *Rphq3*, as we did in our earlier study [28]. However, the other four QTLs mapped to different regions, and hence were assigned with different symbols. Interestingly, a QTL, *Rphq10*, on the distal part of the short arm of chromosome 4 of the L94 × 116-5 map, which was effective to isolate 1.2.1., has also been mapped on the same chromosome region of the L94 × Vada map, but was effective to isolate 24, but not to isolate 1.2.1. [27, 28]. In both populations, this QTL was effective only in the adult plant stage. We hypothesize that this is one locus with different alleles, i.e., an allele from Vada being effective to isolate 24, but not to isolate 1.2.1., whereas another allele from 116-5 is effective to isolate 1.2.1.

Parlevliet and his colleagues [1, 25] have shown that partial resistance to leaf rust occurs very frequently in West-European spring barley cultivars and Ethiopian barley landraces. The present research clearly demonstrates that several genes are involved in partial resistance in each barley line, that different loci are involved in each line. Although our results are based on only two resistant lines, we assume that these results can be extrapolated, and that many loci for partial resistance are present on the barley genome.

Development of durable resistant cultivars by MAS

Partial resistance in barley to barley leaf rust is likely based on a minor gene-for-minor gene interaction as proposed by Parlevliet and Zadoks [23]. Such a gene-for-gene interaction for partial resistance does not necessarily result in low durability, but may even enhance durability [23, 27]. Accumulation of genes for partial resistance in breeding programs may be the most durable way to protect crops from pathogens with race specificity in modern agriculture. Most genes for partial resistance in two partially resistant lines, Vada and 116-5, were mapped to different chromosome regions, supporting a strategy for accumulating many resistance genes in a single cultivar [20, 24]. In a phenotypic selection experiment, Parlevliet *et al.* [25] demonstrated that selection for a high level of partial resistance could be effectively carried out in the seedling stage and in the adult plant stage. Still, the polygenic nature of the resistance and the relatively small effects of individual genes have hampered an effective accumulation of genes in commercial breeding programs. Our results obtained from the current and the previous [27, 28] studies have demonstrated that some genes for partial resistance were expressed in different plant development stages. Therefore, in breeding programmes, phenotypic selection for resistance should take place in the adult plant stage. However, accumulation of genes for partial resistance that are effective in the adult plant stage can be achieved by marker assisted selection in the seedling stage. By conversion of the AFLP markers flanking the QTL regions mapped into simple PCR markers, the resistance-enhancing QTL alleles can easily be introgressed into elite breeding lines.

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