

Isolate specificity of quantitative trait loci for partial resistance of barley to *Puccinia hordei* confirmed in mapping populations and near-isogenic lines

Thierry C. Marcel¹, Benoît Gorguet¹, Minh Truong Ta¹, Zuzana Kohutova², Anton Vels¹ and Rients E. Niks¹

¹Laboratory of Plant Breeding, Graduate School for Experimental Plant Sciences, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, the Netherlands; ²Department of Genetics and Breeding, Faculty of Agrobiological Sciences, Czech University of Agriculture, Prague, Czech Republic

Summary

Author for correspondence:

Rients E. Niks

Tel: +31 317 482 508

Fax: +31 317 483 457

Email: rients.niks@wur.nl

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- Partial resistance is considered race-nonspecific and durable, consistent with the concept of 'horizontal' resistance. However, detailed observations of partial resistance to leaf rust (*Puccinia hordei*) in barley (*Hordeum vulgare*) revealed small cultivar × isolate interactions, suggesting a minor-gene-for-minor-gene interaction model, similar to so-called 'vertical' resistance.

- Three consistent quantitative trait loci (QTLs), labelled *Rphq2*, *Rphq3* and *Rphq4*, that were detected in the cross susceptible L94 × partially resistant Vada have been incorporated into the L94 background to obtain near-isogenic lines (NILs). Three isolates were used to map QTLs on seedlings of the L94 × Vada population and to evaluate the effect of each QTL on adult plants of the respective NILs under field conditions.

- *Rphq2* had a strong effect in seedlings but almost no effect in adult plants, while *Rphq3* was effective in seedlings and in adult plants against all three isolates. However, *Rphq4* was effective in seedlings and in adult plants against two isolates but ineffective in both development stages against the third, demonstrating a clear and reproducible isolate-specific effect. The resistance governed by the three QTLs was not associated with a hypersensitive reaction.

- Those results confirm the minor-gene-for-minor-gene model suggesting specific interactions between QTLs for partial resistance and *P. hordei* isolates.

Key words: barley (*Hordeum vulgare*), disease resistance, isolate specificity, near-isogenic line, *Puccinia hordei*, quantitative trait locus, synergistic activity, virulence spectrum.

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Introduction

Plants have developed different resistance strategies to protect themselves against invading pathogens. Such resistance can be qualitative and governed by one major gene or quantitative and governed by one to several minor genes, the so-called quantitative trait loci (QTLs). Resistance conferred by most major genes prevents fungal growth after the parasite has entered the host plant cell, and is accompanied by suicide of

the penetrated cell or cluster of cells surrounding the site of challenge, a phenomenon known as the hypersensitive response (HR). This HR-based resistance occurs upon direct or indirect recognition of a pathogen-specific effector, known as an avirulence (*Avr*) factor, by a cognate receptor encoded by a host resistance (*R*) gene. Genetic variation in pathogen populations for *Avr* genes leads to race specificity of this type of resistance. In partial resistance, minor genes typically stop fungal growth just before or during the process of cell wall penetration (Niks

& Rubiales, 2002; Collins *et al.*, 2007). Unlike in hypersensitive resistance, defended plant cells remain alive. Partial resistance is considered isolate-nonspecific and durable, and therefore consistent with Van der Plank's concept of 'horizontal' resistance (1963, 1968). However, detailed observations of partial resistance to leaf rust (*Puccinia hordei*) in barley (*Hordeum vulgare*) revealed small cultivar \times isolate interactions (Parlevliet, 1978; Parlevliet & van Ommeren, 1985). Parlevliet & Zadoks (1977) explained these interactions by assuming a minor-gene-for-minor-gene interaction, similar to so-called 'vertical' resistance. They even argued that the minor-gene-for-minor-gene interaction would explain the durability of this polygenic resistance (Parlevliet, 2002).

More recently, Qi *et al.* (1998b, 1999) and Niks *et al.* (2000a) mapped QTLs in the L94 \times Vada barley population against two and four different leaf rust isolates, respectively. Qi *et al.* (1998b, 1999) found that the three largest-effect QTLs were consistently effective against both isolates, but seven small-effect QTLs were only effective against one of the two isolates tested, suggesting an isolate-specific effect. Niks *et al.* (2000a), however, found no evidence for isolate specificity after testing this mapping population with four isolates. Isolate specificity of QTLs has also been observed in plant-pathosystems other than barley-leaf rust (Leonards-Schippers *et al.*, 1994; Caranta *et al.*, 1997; Arru *et al.*, 2003; Chen *et al.*, 2003; Rocherieux *et al.*, 2004; Talukder *et al.*, 2004; Jorge *et al.*, 2005).

In all the studies of which we are aware, the QTLs consistently effective against all isolates tested were always those with the greatest effect on resistance. The individual effects of genes controlling plant quantitative traits are often much smaller than the effects of the environment (Pooni & Kearsy, 2002), underlining the importance of confirming a QTL effect across independent experiments. This also raises the question of the reliability of declaring small-effect QTLs isolate-specific while large-effect QTLs have not shown specificity to date. The use of near-isogenic lines (NILs) to test for isolate specificity allows one to test for the effect of several isolates and to use more replications, as fewer plants are needed per experiment. With NILs, the effect of each QTL can also be determined in the absence of interactions with other QTLs and of the variable genetic background of the mapping population lines.

The aim of this research was to investigate whether larger-effect QTLs for partial resistance may show specificity in their reactions when exposed to different isolates of barley leaf rust. NILs containing the consistent QTLs, labelled as *Rphq2*, *Rphq3* and *Rphq4* (Van Berloo *et al.*, 2001; Marcel *et al.*, 2007a), for resistance to *Puccinia hordei* QTL2, QTL3 and QTL4, respectively, were tested with a set of 21 *P. hordei* isolates from which three were selected. The three selected isolates were used to map QTLs at the seedling stage in the recombinant inbred line (RIL) population derived from the cross between L94 and Vada and to test the NILs at the adult plant stage under field conditions.

Table 1 Country of origin and collection date of 21 *Puccinia hordei* isolates

Isolate name	Origin	Collection date
1.2.1+‡	The Netherlands	1971
121–86	Monospore of 1.2.1	1986
3	Wales	1979
5.1	Israel	1979
9	Kenya	1977
13	Greece (Crete)	1979
17+‡	The Netherlands	1973
18	The Netherlands	1974
22	France	1974
24+	The Netherlands	1974
25	Italy	1980
26+‡	Finland	1980
28.1	Morocco	1981
29	Greece	1984
202	Israel	1976
Achterberg'01	The Netherlands	2001
Cordoba	Spain	1999
IVP2000	The Netherlands	2000
M7	Morocco	1986
Uppsala+‡	Sweden	1999
Yellow mutant	Australia	unknown

+*P. hordei* isolates used for mapping quantitative trait loci (QTLs) on seedlings.

‡*P. hordei* isolates used for field experiments.

Materials and Methods

Plant and fungal material

A set of 103 F₂ RILs derived from the cross between the leaf rust susceptible line L94 and the partially resistant cultivar Vada were used to map QTLs for barley leaf rust resistance at the seedling stage (Qi *et al.*, 1998b). Through a marker-assisted backcross programme, Van Berloo *et al.* (2001) and Marcel *et al.* (2007b) incorporated the QTLs *Rphq2*, *Rphq3* and *Rphq4* into the L94 background to obtain NILs. L94-*Rphq2*, L94-*Rphq3* and L94-*Rphq4* contained Vada introgressed fragments of 4.6, 22.6 and 10.8 centiMorgans (cM), respectively. The three NILs were evaluated at the seedling stage in a glasshouse compartment and at the adult plant stage in the field.

A set of 21 leaf rust isolates were applied to seedlings of 13 barley (*Hordeum vulgare* L.) lines and cultivars to determine their virulence spectra (Table 1). Most of the 13 barley accessions tested (Table 2) belong to the regular differential series for barley leaf rust (Clifford, 1985; Niks *et al.*, 2000b). The inoculation was performed as described in the next section, and 10 to 12 d later infection types (ITs) were scored according to the scale of McNeal *et al.* (1971) modified by Shtaya *et al.* (2006a). Lines of ITs 0–3 were regarded as resistant, those of ITs 4–6 as moderately resistant and those of ITs 7–9 as susceptible. All isolates were multiplied in separate glasshouse

Table 2 Differential series of barley (*Hordeum vulgare*) lines carrying different *Rph* genes

Line	<i>Rph</i> gene symbol†	Previous name
L94	–	
Sudan	<i>Rph1.a</i>	<i>Rph1</i>
Peruvian	<i>Rph2.b</i>	<i>Rph2</i>
Estate	<i>Rph3.c</i>	<i>Rph3</i>
Gold	<i>Rph4.d</i>	<i>Rph4</i>
Magnif 102	<i>Rph5.e</i>	<i>Rph5</i>
Bolivia	<i>Rph2.r, Rph5.f</i>	<i>Rph2, Rph6</i>
Cebada Capa	<i>Rph7.g</i>	<i>Rph7</i>
Tunisian 17	<i>Rph7.ac, RphC</i>	
Egypt 4	<i>Rph8.h</i>	<i>Rph8</i>
Hor 2596	<i>Rph9.i</i>	<i>Rph9</i>
Trumpf	<i>Rph9.z</i>	<i>Rph12</i>
Tunisian 34	<i>RphD</i>	

†*Rph* gene symbols are given according to the recommendation of Franckowiak *et al.* (1997), and to the allelic tests of Zhong *et al.* (2003) for *Rph5.f* and Borovkova *et al.* (1998) for *Rph9.z*.

compartments on the susceptible barley line L98. Once collected, the spores were dried in a desiccator and stored at -80°C .

Disease evaluations at the seedling stage

Before inoculation, urediospores were taken from the -80°C freezer and thawed in $38-42^{\circ}\text{C}$ water. The first leaf of each seedling was fixed horizontally on the soil, adaxial side up, and trays were placed in a settling tower. Then 3 mg of urediospores diluted 10 times with lycopodium spores was dusted over each tray, resulting in *c.* 180 rust spores per cm^{-2} . After incubation overnight (8 h) at 100% relative humidity in a dark dew chamber at 18°C , the seedlings were transferred to a glasshouse compartment at $20 \pm 3^{\circ}\text{C}$ with 30–70% relative humidity. The latency period (LP) on each seedling was evaluated by estimating the time (h) at which 50% of the ultimate number of pustules became visible. The relative latency period on seedlings (RLP50S) was calculated relative to the LP on L94 seedlings, where the LP on L94 seedlings was set at 100, as described by Parlevliet (1975).

In a preliminary glasshouse experiment, the NILs were evaluated with the 21 isolates of the pathogenic fungus *Puccinia hordei* Oth (Table 1). Four seeds of each NIL and each parent, L94 and Vada, were sown in trays of 37×39 cm. One tray per isolate was used, and three series of evaluation were necessary to test the 21 isolates. In each series the isolate 1.2.1 was used as a recurrent standard.

A subset of three *P. hordei* isolates, selected for their differential effects among NILs containing different QTLs, were used to map QTLs on seedlings of RILs. For each isolate, four seedlings per RIL and 24 seedlings of L94 and Vada were evaluated in two consecutive experiments. Also, the RLP50S values of the three selected *P. hordei* isolates and of the isolate 1.2.1

were re-evaluated on the NILs and the parental lines. Five seedlings per line were used in each of three consecutive experiments.

Disease evaluations in the field

The NILs were tested in the field in 2003 and 2004 against the three selected leaf rust isolates 17, 26 and Uppsala, and against our standard isolate 1.2.1. The trial design was a split-plot in two to four replicates, depending on seed supply, with isolates on main plots and barley lines on subplots. Replications within plots were arranged as blocks. Within each replication the order of the subplots was randomized. The main plots were separated from each other by a distance of 100–200 m cultivated with oat (*Avena sativa*) in 2003 and with winter wheat (*Triticum aestivum*) in 2004. Each subplot of a barley accession consisted of three rows (*c.* 50 seeds per row) sown at 0.25-m intervals and alternated with similar subplots of oat. Sowing in the field was carried out on 21–23 April for the two experiments. For each isolate, 20 pots containing five seedlings of the susceptible genotype L98 were spray-inoculated in the glasshouse and incubated overnight at a relative humidity of 100%. Before sporulation of these spreader plants, on 1–2 June, the pots were placed uniformly in the field and removed 8 d later. The climatic conditions were more favourable to initiate the epidemics in 2003 than in 2004. In 2003, three disease assessments were performed at 18, 23 and 29 d after placement of the spreader pots (dpi), and in 2004, four disease assessments were performed at 25, 30, 36 and 41 dpi for isolate Uppsala and 25, 36, 41 and 45 dpi for isolates 17, 26 and 1.2.1. Per assessment, three random tillers were sampled per subplot to count the number of mature rust pustules on the three upper leaves. After the last disease evaluation, spores of the four isolates were collected from the field. Their virulence spectrum was determined as described earlier and compared with the virulence spectrum of the isolates used in the glasshouse seedling tests.

A logarithm transformation (\log_e scale) was performed on the data collected according to the formula $T_{\log} = \log_e(P + 1)$, where P is the number of rust pustules, to satisfy the condition of homogeneity of variance. The transformed data were used to calculate the area under disease progress curve (AUDPC) according to the formula $\text{AUDPC} = \sum (t_{(i+1)} - t_i)(y_{(i+1)} + y_i)/2$, where t_i is the first assessment date of two consecutive assessments, y_i is the disease severity on assessment date t_i , $t_{(i+1)}$ is the second assessment date of two consecutive assessments, and $y_{(i+1)}$ is the disease severity on assessment date $t_{(i+1)}$. A Duncan's multiple range test was performed using GENSTAT® release 8.1 (VSN International Ltd, Hemel Hempstead, UK) to compare all pairs of means.

Map construction and QTL mapping

A data set of 958 morphological and molecular markers segregating in the 103 RILs of L94 \times Vada was used to

construct a dense marker map of the barley genome. This data set has previously been used as a component of a high-density consensus map of barley (Marcel *et al.*, 2007a), and is predominantly composed of amplified fragment length polymorphisms (AFLPs) (709 markers) and microsatellites (138 markers). JOINMAP® 3.0 (Van Ooijen & Voorrips, 2001) was used for linkage grouping and map construction. Linkage groups were assigned to the corresponding barley chromosomes according to previously published maps (Qi *et al.*, 1998a; Marcel *et al.*, 2007a). Map distances were calculated using Kosambi's mapping function. A skeletal map with 210 uniformly distributed markers (approx. 5 cM per marker interval) was extracted. All the markers on the skeletal map were fitted to the dense map during the first or exceptionally the second round of JOINMAP® 3.0. This skeletal map was used for QTL analyses in a previous study by Shtaya *et al.* (2006b) and in the present study.

The wide-sense heritability (h^2) for RLP50S was estimated from ANOVA according to the formula $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2/n)$ with the genetic variance $\sigma_g^2 = (MS_g - MS_e)/n$, the environmental variance $\sigma_e^2 = MS_e$, MS_g being the mean square and n being the number of replicates per RIL. ANOVA on RLP50S revealed significant genotype and replication effects with all *P. hordei* isolates tested. Therefore, the genotype effect of each line was extracted from the ANOVA and used to map QTLs on the skeletal map. The ANOVA was performed with the GENSTAT® 8.1 software package (VSN International Ltd). QTL mapping was performed using MAPQTL® 5.0 (Van Ooijen, 2004). Interval mapping (IM) was run and, in the region of the putative QTLs, the markers with the highest likelihood of odds (LOD) values (peak markers) were used as co-factors for running a multiple-QTL mapping (MQM) programme (Jansen & Stam, 1994). When LOD values of some markers on other regions reached the significance level, the MQM was repeated by adding those new 'peak markers' as co-factors until a stable LOD profile was obtained. The restricted-MQM method (rMQM) was used to determine the values of the LOD, phenotypic variation, additive effect and confidence interval for the detected QTLs. After a genome-wide permutation test on each set of data, an LOD threshold value of 2.9 for *P. hordei* 1.2.1 and 26, of 3.0 for *P. hordei* 17 and Uppsala, and of 3.1 for *P. hordei* 24 was set for declaring a QTL.

Results

Characterization of the 21 barley leaf rust isolates

The 21 leaf rust isolates were classified according to their virulence/avirulence pattern on seedlings of the differential series of barley accessions and their LP on the NILs with individual *Rphq* genes. This virulence characterization distinguished 16 races of *P. hordei* (Table 3). None of the resistance genes was effective against all *P. hordei* isolates, because isolate 28.1 was virulent for all the resistance genes tested. The results

of this survey helped to verify the identity of the isolates tested later in the field.

The preliminary evaluation of RLP50S on NILs containing *Rphq2*, *Rphq3* and *Rphq4* distinguished three classes of isolates (Table 3): a first class for which the RLP50S on L94-*Rphq2* was higher than the RLP50S on L94-*Rphq3* (10 isolates), a second class for which the RLP50S on L94-*Rphq2* was similar to the RLP50S on L94-*Rphq3* (nine isolates), and a third class for which the RLP50S on L94-*Rphq2* was lower than the RLP50S on L94-*Rphq3* (two isolates). Qi *et al.* (1998b, 1999) reported that *Rphq4* is ineffective in seedlings against isolates 1.2.1 and 24. However, in the preliminary evaluation, Uppsala had a significantly higher RLP50S on L94-*Rphq4* than on L94 (Table 4). On the basis of these observations, the leaf rust isolates 17, 26 and Uppsala were selected for further studies on the isolate specificity of QTLs. Isolate 17 (second class) had a similar RLP50S on L94 as on the NILs (Table 4). However, the level of partial resistance of the cultivar Vada to isolate 17 was still high, indicating that the resistance of Vada to this isolate might be attributable to a different QTL(s) from those that are effective against other isolates. Isolate 26 (third class) had a significantly higher RLP50S on L94-*Rphq3* than on L94, while the RLP50S on L94-*Rphq2* was similar to that on L94 (Table 4), in contrast to isolate 1.2.1 (our standard isolate). Finally, isolate Uppsala, one of the most aggressive isolates tested, had a significantly higher RLP50S on L94-*Rphq3* and on L94-*Rphq4* than on L94 (Table 4). The virulence spectra of the three selected rust isolates were different, indicating distinct races (Table 3).

The re-evaluation of the RLP50S of isolates 1.2.1, 17, 26 and Uppsala gave results contrasting with the observations made in the preliminary evaluation (Table 4). No differential interaction was observed between the isolate 1.2.1 and the subset of three selected isolates. Indeed, this re-evaluation showed that the RLP50S was always highest on L94-*Rphq2*, intermediate on L94-*Rphq3* and lowest on L94-*Rphq4*. The effect of *Rphq2* was significant against isolates 1.2.1, 26 and Uppsala and the effect of *Rphq3* was significant against isolate Uppsala only.

Construction of a dense marker map of L94 × Vada

The 958 markers segregating in L94 × Vada were assembled into seven linkage groups corresponding to the seven barley chromosomes 1H to 7H, homeologous to the wheat chromosomes 1 to 7. The new L94 × Vada dense marker map had a total map length of 1088 cM with an average distance between two consecutive loci of 1.1 cM. This represents a substantial improvement compared with the previously published linkage map of L94 × Vada that covered 1062 cM with an average distance between two consecutive loci of 1.9 cM (Qi *et al.*, 1998a). The distribution of 235 new *PstI/MseI* AFLP markers was more homogeneous compared with the distribution of the previous 561 *EcoRI/MseI* AFLP markers (Marcel *et al.*,

Table 3 Resistance/susceptibility pattern† of 21 *Puccinia hordei* isolates according to their infection type on a differential series of barley (*Hordeum vulgare*) lines carrying different *Rph* genes and classification of the same isolates according to their latency period on quantitative trait locus (QTL) near-isogenic lines (NILs)

	<i>RphD</i>	<i>Rph7.g</i>	<i>Rph7.ac, RphC</i>	<i>Rph3.c</i>	<i>Rph9.i</i>	<i>Rph9.z</i>	<i>Rph5.e</i>	<i>Rph8.h</i>	<i>Rph2.r, Rph5.f</i>	<i>Rph2.b</i>	<i>Rph1.a</i>	<i>Rph4.d</i>	Race‡	Class§
28.1	S	S	S	S	S	S	S	S	S	S	S	S	1	2 > 3
M7	R	S	R	S	S	MR	S	S	S	S	S	S	2	2 > 3
13	R	R	S	R	MR	S	S	S	S	S	S	S	3	2 = 3
Uppsala	R	R	S	R	MR	MR¶	MR	S	S	S	S	S	4	2 = 3
18	R	R	R	S¶	S	S	S	S	S	S	S	S	5	2 > 3
Achterberg'01	R	R	R	S	S	S	R	R	S	S	S	S	6	2 = 3
9	R	R	R	S	MR	S	S	S	S	S	S	S	7	2 > 3
5.1	R	R	R	S	MR	S	S	S	S	S	S	S	7	2 = 3
202	R	R	R	S	MR	R	S	S	R	S	S	S	8	2 > 3
IVP 2000	R	R	R	R	S	S	S	S	S	S	S	S	9	2 > 3
17	R	R	R	R	S	S	S	S	S	S	S	S	9	2 = 3
121-86	R	R	R	R	S	S	S	S	S	S	S	S	9	2 = 3
25	R	R	MR	R	S	S	S	S	S	S	S	S	9	2 = 3
26	R	R	R	R	S	S	MR¶	S	S	S	S	S	10	2 < 3
Cordoba	R	R	R	R	S	S	R	MR	S	S	S	S	11	2 = 3
29	R	R	R	R	S	R	S	MR	S	S	S	S	12	2 > 3
3	R	R	R	R	S	MR	S	S	S	S	S	S	13	2 < 3
1.2.1	R	R	R	R	S	MR	S	S	S	S	S	S	13	2 > 3
Yellow Mutant	R	R	R	MR	MR	S	MR	S	S	S	S	S	14	2 > 3
24	R	R	R	R	MR	S	MR	MR	S	MR	S	S	15	2 > 3
22	R	R	R	R	MR	MR	S	S	S	S	S	S	16	2 = 3

†Lines with infection types 0–3 are resistant (R); lines with infection types 4–6 are moderately resistant (MR); lines with infection types 7–9 are susceptible (S).

‡Different races are distinguished by different virulence spectra.

§2 > 3: *Rphq2* prolongs the latency period (LP) more than *Rphq3*; 2 = 3: *Rphq2* and *Rphq3* prolong the LP to similar extents; 2 < 3: *Rphq2* prolongs the LP less than *Rphq3*.

¶Different seedlings showed contrasting infection types.

Table 4 Relative latency period of seedlings (relative to L94, estimated using the time at which 50% of the ultimate number of pustules became visible (RLP50S)) of L94+, Vada and near-isogenic lines (NILs) measured against the *Puccinia hordei* isolates 1.2.1, 17, 26 and Uppsala in the preliminary evaluation and in the re-evaluation

	Isolate 1.2.1‡		Isolate 17		Isolate 26		Isolate Uppsala	
	PE§	RE¶	PE	RE	PE	RE	PE	RE
L94	100	100	100	100	100	100	100	100
L94- <i>Rphq2</i>	104*	111*	100	107	102	105*	102	106*
L94- <i>Rphq3</i>	98	104	102	103	108*	104	104*	104*
L94- <i>Rphq4</i>	96*	102	99	102	100	103	105*	102
Vada	128*	125*	119*	127*	125*	119*	127*	119*

†L94 is set at RLP50S = 100.

‡An * indicates that the mean differs significantly from the mean of L94 (LSD_{0.05}).

§RLP50S from the preliminary evaluation (PE) with 21 isolates; estimated on four seedlings per line.

¶RLP50S from the re-evaluation (RE); estimated on 15 seedlings per line.

2007a). The three gaps larger than 20 cM reported by Qi *et al.* (1998a) on chromosomes 1H, 3H and 7H have been reduced in this new map. Only one of them, on chromosome 1H, remained larger than 10 cM. The average marker distance on the extracted skeletal map used for QTL mapping in this

study was 5.2 cM. The map lengths and marker order were highly consistent with those of the high-density molecular map constructed by Qi *et al.* (1998a) and the high-density consensus map of barley constructed by Marcel *et al.* (2007a). All the mapping data and segregation data of this new

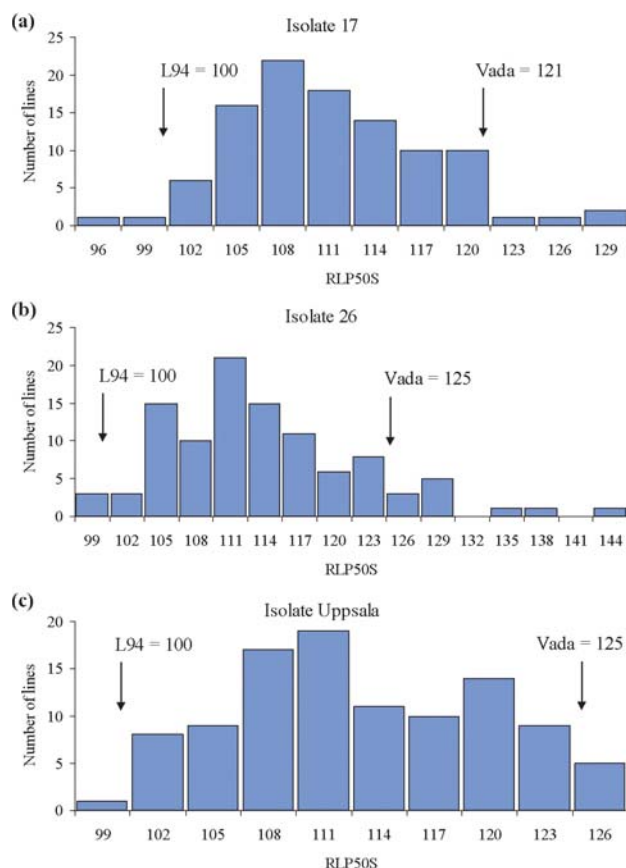


Fig. 1 Frequency distribution of phenotypes for the measures of leaf rust (*Puccinia hordei*) resistance in seedlings (the latency period in seedlings, relative to L94, estimated using the time at which 50% of the ultimate number of pustules became visible (RLP50S)) with (a) isolate 17, (b) isolate 26 and (c) isolate Uppsala, in 103 recombinant inbred lines (RILs) derived from the cross L94 × Vada. Values for L94 and Vada are indicated by arrows. The values indicated on the x-axis are the average values for each category.

L94 × Vada linkage map have been deposited in the GrainGenes 2.0 database (Barley, L94 × Vada, 2006).

QTL mapping confirms the isolate specificity of small-effect QTLs in seedlings

To investigate the isolate specificity of individual QTLs for partial resistance in seedlings, the L94 × Vada segregating population was challenged with the three virulent *P. hordei* isolates 17, 26 and Uppsala (Table 1). Raw data obtained from Qi *et al.* (1998b, 1999) were available for isolates 1.2.1 and 24.

The wide-sense heritability (h^2) for RLP50S was 0.84 with *P. hordei* 17, 0.88 with *P. hordei* 26 and 0.89 with *P. hordei* Uppsala. The RLP50S values of *P. hordei* 17 and 26 slightly exceeded the range of values between the susceptible line L94 and the partially resistant line Vada (Fig. 1a,b), but those for *P. hordei* Uppsala did not (Fig. 1c).

The genotype effect was extracted from ANOVA and used for QTL mapping. A total of eight QTLs were detected with one or more of the leaf rust isolates tested (Table 5). The QTLs with a significant LOD value with IM and/or with rMQM (Table 5) together explained 59, 39, 56, 60 and 72% of the phenotypic variance for isolates 1.2.1, 24, 17, 26 and Uppsala, respectively. The QTLs *Rphq2* and *Rphq3* explained most of the phenotypic variance with all isolates, while the other QTLs contributed moderately to the total level of partial resistance. The resistance alleles of seven QTLs originated from the partially resistant parent Vada, while the resistance allele of *Rphq17* originated from the susceptible parent L94.

Three of the QTLs identified, *Rphq1*, *Rphq2* and *Rphq3*, were effective against all five isolates, confirming the results of Qi *et al.* (1998b) who first identified them against isolate 1.2.1. A fourth QTL, *Rphq7*, was effective against isolate 24, with a LOD value above the threshold (Table 5). At the same linkage group position as indicated for *Rphq7*, we also found consistent LOD peaks between 2 and 3 against the other four isolates (Table 5; Fig. 2). The four other QTLs, *Rphq4*, *Rphq17*, *Rphq20* and *Rphq21*, had an effect against only one or two of the three isolates tested in this study (Table 5). One QTL, *Rphq17*, was at a mapping position similar to that of a QTL reported in the Oregon Wolfe Barleys population (Marcel *et al.*, 2007a). We assume that it is at the same locus and provisionally use the same gene designation as Marcel *et al.* (2007a). *Rphq20* and *Rphq21* were at locations in which no QTLs for resistance to *P. hordei* had been reported before. Surprisingly, the QTLs with an effect against one or two rust isolates but not against the others were not detected with the IM method (LOD values below 2), while they were detected with LOD values up to 8.2 with the rMQM method (Fig. 2).

Synergistic activities of QTLs corroborate an isolate-specific effect

The isolate-specific effect of *Rphq4*, *Rphq17*, *Rphq20* and *Rphq21* found by QTL mapping was associated with strong discrepancies between the results obtained using the IM and rMQM methods (Fig. 2), questioning the reliability of declaring those QTLs isolate-specific. *Rphq17* and *Rphq20* showed the greatest contrast between LOD values calculated by the IM and rMQM methods (Table 5, Fig. 2). *Rphq17* only had a significant effect against isolate 26 and *Rphq20* against isolate Uppsala, but each QTL also had a peak LOD value between 2 and 3 against isolates Uppsala and 26, respectively.

We determined whether the presence of other QTLs in the genetic background had an influence on the effect of *Rphq17* against isolate 26 and of *Rphq20* against isolate Uppsala (Fig. 3a,b). Here, the effect of a QTL refers to the average difference in LP in hours between the RILs carrying the resistance allele and the RILs carrying the susceptibility allele at the LOD peak marker of that QTL. The presence of the resistance allele of *Rphq2*, *Rphq3*, *Rphq4* or even *Rphq20*

Table 5 Summary of quantitative trait loci (QTLs) for partial resistance to five leaf rust (*Puccinia hordei*) isolates at the seedling development stage

QTLs	Chr.	cM‡	<i>P. hordei</i> 1.2.1†		<i>P. hordei</i> 24†		<i>P. hordei</i> 17		<i>P. hordei</i> 26		<i>P. hordei</i> Upp.	
			IM§	rMQM¶	IM	rMQM	IM	rMQM	IM	rMQM	IM	rMQM
<i>Rphq1</i>	7H	46–122	2.9	1.6	3.2	2.5	4.2	5.4	3.4	2.6	3.1	4.4
<i>Rphq2</i>	2H	187–192	9.4	17.1	11.8	19.1	10.8	16.7	9.2	22.3	13.3	26.1
<i>Rphq3</i>	6H	53–63	8.7	14.0	5.9	10.0	4.0	8.0	5.4	12.4	4.6	10.9
<i>Rphq4</i>	5H	5–16	–††	–	–	–	1.5	6.9	2.7	6.8	–	–
<i>Rphq7</i>	5H	101–138	2.1	0.9	3.8	5.3	2.5	2.1	2.1	2.1	2.0	3.0
<i>Rphq17</i> ‡‡	3H	54–60	–	–	–	–	–	–	0.3	8.2	0.0	2.1
<i>Rphq20</i>	4H	76–79	–	–	–	–	–	–	0.4	2.8	1.3	7.6
<i>Rphq21</i>	1H	36–56	–	–	–	–	–	–	–	–	1.3	3.9

†Raw data obtained from Qi *et al.* (1998b, 1999).

‡Position of the two-likelihood of odds (LOD) confidence interval based on the results of restricted multiple-QTL mapping (rMQM) on the L94 × Vada marker map.

§LOD value obtained with the interval mapping (IM) method; values in bold are above the LOD threshold.

¶LOD value obtained with the rMQM method; values in bold are above the LOD threshold.

††Data are presented only when LOD ≥ 2 with IM or with rMQM.

‡‡The resistance allele was contributed by L94.

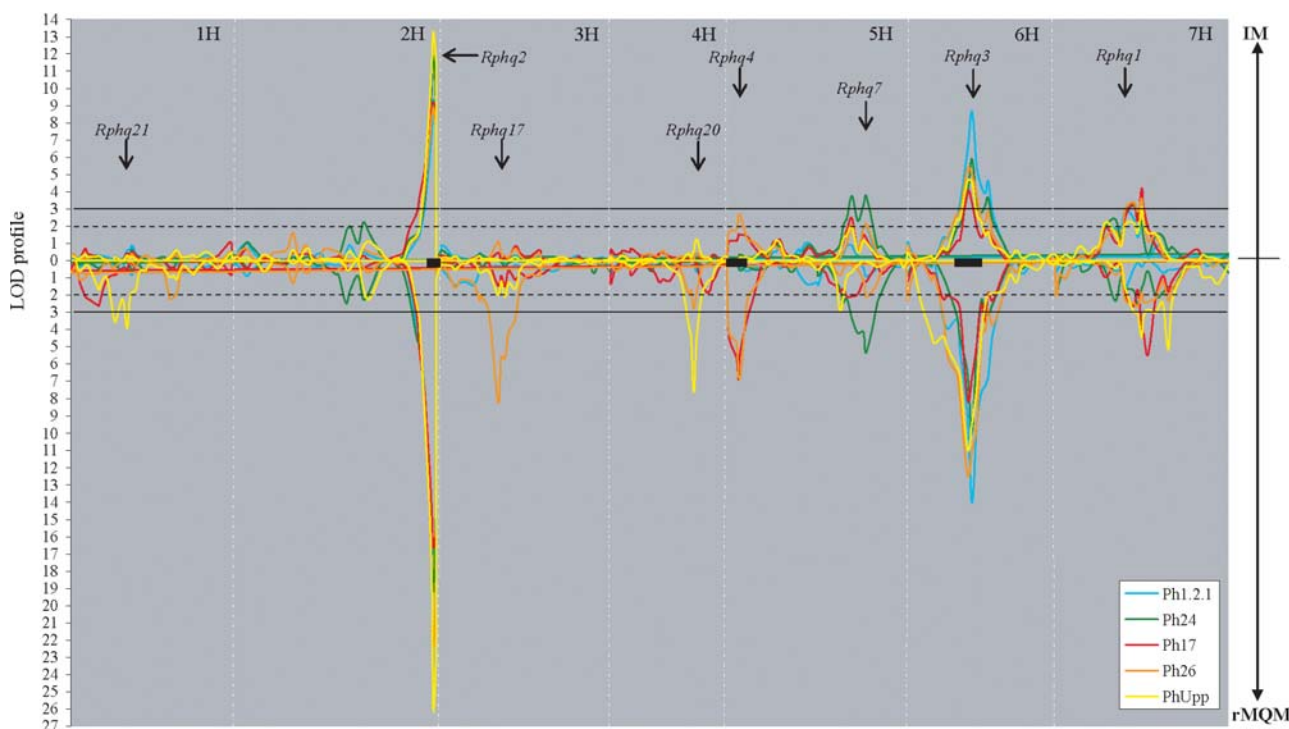


Fig. 2 Likelihood of odds (LOD) profiles of five barley leaf rust (*Puccinia hordei*) isolates obtained by interval mapping (IM), above the x-axis, and by restricted multiple-quantitative trait locus (QTL) mapping (rMQM), below the x-axis, along the linkage map constructed with the L94 × Vada recombinant inbred line (RIL) population. Dashed vertical lines separate the seven barley chromosomes; solid horizontal lines indicate the approximate LOD threshold for significance; dashed horizontal lines indicate a LOD of 2. Names of the identified QTLs are given with an arrow indicating the approximate position of their peak marker. The three boxes on the x-axis represent the Vada fragments introgressed into the L94 background to develop near-isogenic lines (NILs).

increased the effect of *Rphq17* against isolate 26 from 2-fold (*Rphq2*) to 9-fold (*Rphq4*) compared with the presence of the susceptibility allele of each of these QTLs (Fig. 3a). In a similar way, *Rphq20* had an effect against isolate Uppsala only in the

presence of the resistance allele of *Rphq2* or *Rphq17*, and the resistance allele of *Rphq3* increased the effect of *Rphq20* more than 3-fold compared with the presence of the susceptibility allele of this QTL (Fig. 3b). However, the presence of the

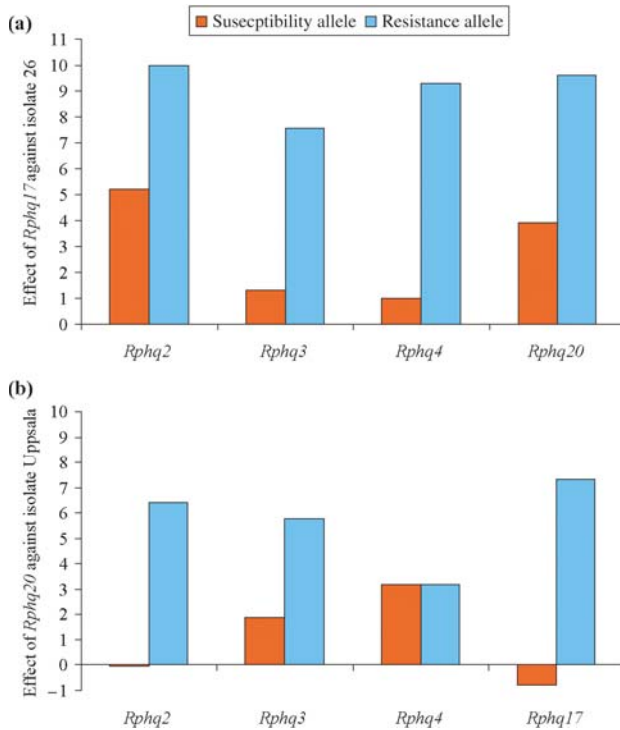


Fig. 3 (a) The effect of the resistance allele of *Rphq17* (prolongation of latency period (LP) in hours) against isolate 26 in the genetic background of recombinant inbred lines (RILs) having the susceptibility or the resistance allele of *Rphq2*, *Rphq3*, *Rphq4* or *Rphq20*, and (b) for *Rphq20* against isolate Uppsala in the genetic background of RILs having the susceptibility or the resistance allele of *Rphq2*, *Rphq3*, *Rphq4* or *Rphq17*.

Vada allele of *Rphq4* did not influence the effect of *Rphq20* against isolate Uppsala. In this study, *Rphq4* was only detected on seedlings challenged with isolates 17 and 26. The fact that *Rphq4* had no influence on the effect of *Rphq20* against isolate Uppsala is consistent with the finding that *Rphq4* was not effective against that isolate. The interactions observed confirm an effect of *Rphq17* against isolate 26 and an effect of *Rphq20* against isolate Uppsala. These results corroborate the isolate-specific effects of *Rphq4*, *Rphq17* and *Rphq20*, and suggest a synergistic activity of any pair of QTLs expressed in the same genetic background.

The isolate specificity of *Rphq4* is confirmed under field conditions

The NILs with individual *Rphq* genes were used to verify the QTLs under agricultural conditions. The three selected isolates, 17, 26 and Uppsala, and the isolate 1.2.1 were used to determine the effects of *Rphq2*, *Rphq3* and *Rphq4* in their NIL background in the field. This experiment was conducted twice, in 2003 and in 2004.

Against isolate 1.2.1, *Rphq3* and *Rphq4* had significant effects in 2003 and 2004, while *Rphq2* had a significant effect

in 2004 only (Fig. 4a,b). The effect of *Rphq3* was always stronger than that of *Rphq4*. These results do not agree with those of Qi *et al.* (1998b), who found that, on adult plants of the mapping population, *Rphq4* was the most effective QTL. This disagreement indicates that *Rphq4* is not consistently expressed in RILs and in its NIL background. Against isolates 17 and 26, *Rphq2* did not always have a significant effect, while *Rphq3* and *Rphq4* always had a clear and significant effect on the level of partial resistance (Fig. 4c–f). Against isolate Uppsala, however, the only QTL having a significant effect in 2003 was *Rphq3* (Fig. 4g), while none of the QTLs had a significant effect in 2004 (Fig. 4h). Nevertheless, L94-*Rphq3* was the NIL with the lowest AUDPC against isolate Uppsala in 2004 (Fig. 4h).

As expected, the partially resistant Vada had the lowest AUDPC against all four isolates (Fig. 4). The AUDPC on L94-*Rphq2* was significantly lower than the AUDPC on L94 for one isolate in 2003 (26; Fig. 4e) and for two isolates in 2004 (1.2.1 and 17; Fig. 4b,d), indicating that the effect of *Rphq2* introduced in the L94 background is weak in adult plants, and that different environmental conditions can influence its phenotypic expression. The AUDPC on L94-*Rphq3* was significantly lower than the AUDPC on L94 for all four isolates in 2003 and for three isolates in 2004. The AUDPC on L94-*Rphq4* was always significantly lower than the AUDPC on L94 for isolates 1.2.1, 17 and 26 but not significantly different for isolate Uppsala, demonstrating the isolate specificity of *Rphq4* at the adult plant stage and confirming the results obtained earlier by QTL mapping on seedlings.

In the field, the contamination of one or several isolates by another local isolate might dramatically change the results and their interpretation. To confirm their identity, samples of the four isolates were collected in the field after the last disease assessment of 2003 (data not shown). Seven barley lines from the differential series evaluated earlier were chosen to compare the virulence spectra of the four isolates. The ITs of the collected isolates corresponded to the ITs observed earlier on the seedlings (Table 3). Because the ITs were similar, the contamination of the experiment by a local leaf rust isolate is not likely. In commercial barley fields in Wageningen, barley leaf rust is indeed not an abundantly occurring pathogen.

Discussion

Since Parlevliet & Zadoks (1977) proposed and discussed the hypothesis of minor-gene-for-minor-gene interactions, it remained to some extent an open question whether individual QTLs for partial resistance can be effective against some races of a pathogen and ineffective against others. Several authors mapped QTLs in a segregating population with different isolates of the same pathogen (Qi *et al.*, 1999; Niks *et al.*, 2000a; Arru *et al.*, 2003; Chen *et al.*, 2003; Rocherieux *et al.*, 2004; Talukder *et al.*, 2004; Jorge *et al.*, 2005). In each case, they found that the QTLs that were effective against all the isolates were always

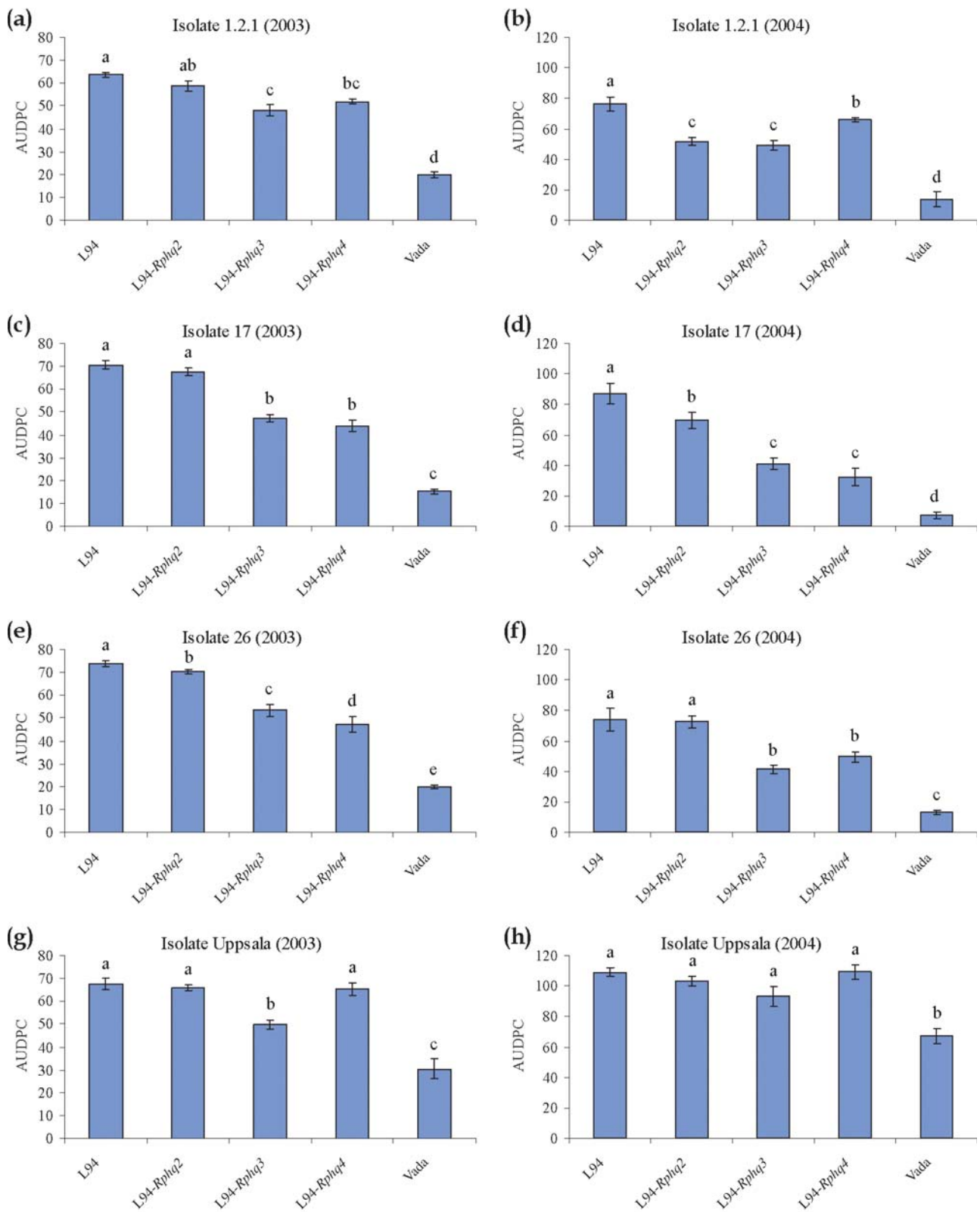


Fig. 4 Histograms of area under disease progress curve (AUDPC) of barley (*Hordeum vulgare*) accessions L94 and Vada and of the near-isogenic lines (NILs) L94-Rphq2, -Rphq3 and -Rphq4 tested against the leaf rust (*Puccinia hordei*) isolates 1.2.1, 17, 26 and Uppsala, during the field evaluations of 2003 and 2004. Error bars, \pm SE; similar letters on bars indicate that the means do not differ significantly according to Duncan's test ($P < 0.05$).

those with the greatest effect on resistance. The present research aimed to determine whether larger-effect QTLs for partial resistance to leaf rust in barley may show specificity in their reaction to different *P. hordei* isolates.

We determined the virulence spectra of 21 isolates on a barley differential series and their LPs on near-isogenic lines that each carried a different minor gene for partial resistance (Table 3). The virulence spectra for isolates, as far as tested before, were generally in agreement with earlier data (Parlevliet *et al.*, 1981; Niks *et al.*, 1989). Our data indicate different specificities for *Rph7.g* (Cebada Capa) and *Rph7.ac* (Tunisian 17), which belong to a multiple allelic series mapped on the short arm of chromosome 3H (Chicaiza *et al.*, 1996; Graner *et al.*, 2000; Isidore *et al.*, 2005). Interestingly, *RphD* had the widest range of effectiveness, and was only overcome by isolate 28.1. *RphD* has been identified in a barley land race from Tunisia (Tunisian 34) and is not allelic to *Rph3*, *Rph7* or *Rph9* (Yahyaoui *et al.*, 1988). As far as we know, this gene has not yet been assigned to a chromosome.

In the preliminary evaluation, the significantly longer LP of isolate Uppsala on seedlings of L94-*Rphq4* compared with seedlings of L94 suggested an isolate-specific effect of this QTL at the seedling stage (Table 4). These results were contradicted by the results obtained on the mapping population and on the NILs evaluated in the field. Isolate Uppsala was selected for the effect of *Rphq4* in seedlings, whereas later this isolate distinguished itself by overcoming *Rphq4* in seedlings and in adult plants. Based on results of the preliminary evaluation, isolate 17 was selected because *Rphq2* and *Rphq3* did not have a significant effect compared with L94, and isolate 26 because only *Rphq3* had a significant effect. However, in the mapping population, *Rphq2* and *Rphq3* had effects against all the isolates, and *Rphq2* always explained a higher percentage of the variation and always had a stronger additive effect than *Rphq3*. It appeared that four seedlings per NIL-*P. hordei* isolate combination were too few to obtain a reliable estimation of the effect of each QTL in its NIL background under glasshouse conditions. Indeed, the re-evaluation of the RLP50S of the selected isolates on a larger number of seedlings produced results contrasting with those of the preliminary evaluation but in agreement with the observations made in QTL mapping and field experiments (Table 4). The power of NILs to detect the effect of individual QTLs is real but should not be overestimated. The individual effects of QTLs are often small and could easily be confounded with the effects of the environment if conclusions are based on an insufficient number of observations. Testing the NILs under field conditions provided more reliable results, as the disease build-up is polycyclic, each life cycle of the pathogen exponentially amplifying the small effect of the individual QTLs.

To investigate the isolate specificity of partial resistance further, the L94 × Vada segregating population was challenged with the three *P. hordei* isolates, namely 17, 26 and Uppsala, and data on isolates 1.2.1 and 24 were re-analysed. With the

interval mapping method, three QTLs (*Rphq1*, *Rphq2* and *Rphq3*) had a significant effect against the five isolates, while one QTL (*Rphq7*) had a significant effect only against isolate 24. This finding has already been reported by Qi *et al.* (1999), who asserted that *Rphq7* is isolate-specific at the seedling stage. However, with the IM method a LOD peak higher than 2 was consistently observed at *Rphq7* with all isolates (Fig. 2), and with rMQM *Rphq7* had a significant effect against two isolates, 24 and Uppsala (Table 5), indicating that *Rphq7* was not really isolate-specific but at most varied quantitatively in its effect against the different isolates. With the rMQM method, the LOD profiles of the five isolates were surprisingly less consistent than with the IM method (Fig. 2). The most surprising result was that four QTLs had a significant effect when rMQM was used while they remained undetected using IM (*Rphq4*, *Rphq17*, *Rphq20* and *Rphq21*). It is remarkable that these four QTLs happened to be the four QTLs detected with one or two rust isolates but not with the other isolates. For the other, constant, QTLs a consistent peak was seen for all isolates with the IM and with the rMQM methods, although the effect for *Rphq1* was not always significant with the rMQM method. At the chromosomal location of *Rphq4*, LOD peaks of 1.5 and 2.7 against isolates 17 and 26 already suggested the effect of this QTL using IM. However, this was not the case for *Rphq17*, *Rphq20* and *Rphq21*. On chromosome 3H, *Rphq17* has previously been reported in a linkage disequilibrium study (RLP4: Kraakman *et al.*, 2006) and in the Oregon Wolfe Barley (OWB) population (Marcel *et al.*, 2007a). In the linkage disequilibrium, OWB and L94 × Vada mapping populations, the peak marker of the LP-prolonging gene mapped to the same position (within 1 cM) of the barley consensus map of Marcel *et al.* (2007a). The linkage disequilibrium population was inoculated with isolate IVP2000 and the OWB population with isolate 1.2.1. Our data suggest that *Rphq17* is not effective against isolate 1.2.1, implying that in different cultivars different alleles are involved in the level of resistance. *Rphq20* and *Rphq21* represent new loci for partial resistance to barley leaf rust. *Rphq20* was co-locating, but in repulsion phase, with the powdery mildew resistance gene *mlo* (Qi *et al.*, 1998a) and with the quantitative resistance to scald gene *Rrsq2* (Shtaya *et al.*, 2006b), which are both segregating in L94 × Vada. The higher number of QTLs detected using the rMQM method than the IM method may be a result of an increase in power or an increase in the type I error rate (i.e. a QTL is indicated at a location where actually no QTL is present) (Jansen, 1994). Simulation studies demonstrated that the chance of a type I or type II error (i.e. a QTL is not detected) is higher in interval mapping than it is in simultaneous mapping of multiple QTLs (Jansen *et al.*, 1994). The use of marker co-factors in MQM mapping strongly reduces the genetic variation induced by nearby QTLs (Jansen, 1994). It is less clear, however, how a marker co-factor determined on one chromosome will affect the detection power of QTLs on other chromosomes. However, one can imagine that co-factor

analysis will be more powerful to detect unlinked QTLs with epistatic effects, as the variation of possible QTLs on other chromosomes is regressed on marker co-factors. In support of this hypothesis, we found that the bigenic combination of *Rphq17* and *Rphq20* with the most consistent QTLs *Rphq2*, *Rphq3* and *Rphq4*, or with each other, synergistically enhanced protection against leaf rust infection (Fig. 3). A synergistic effect means that the combined effect of the administration of two compounds may be greater than the sum of the two effects. The observed synergistic activities of *Rphq17* and *Rphq20* with other QTLs validate the effect of these loci on the level of partial resistance, and suggest that MQM mapping is indeed more powerful to detect QTLs than interval mapping; that QTLs can show isolate specificity at the seedling stage; and that epistasis between QTLs probably plays an important role in partial resistance. A previous report already indicated that bigenic epistasis could be a major component of the genetic control of partial resistance (Talukder *et al.*, 2004). The observation that combined expression of different antifungal proteins *in planta* can lead to synergistic protection against phytopathogenic fungi (Jach *et al.*, 1995; Jongedijk *et al.*, 1995) may be relevant in explaining this phenomenon.

The results obtained in the glasshouse on seedlings of the RIL population and the results obtained in the field on adult plants of the NILs were generally in good agreement with previous reports (Qi *et al.*, 1998b; Niks *et al.*, 2000a). *Rphq2*, having a strong effect in seedlings (Fig. 2) and almost no effect in adult plants (Fig. 4), was clearly plant stage dependent, while *Rphq3* was always effective and not plant stage dependent. The isolate specificity of *Rphq4* on seedlings was also confirmed on adult plants in the field experiments. *Rphq4* had an effect on seedlings against only two isolates, 17 and 26, for which *Rphq4* explained 18 and 10%, respectively, of the total variation explained by the significant QTLs. On NILs in the field, the effect of *Rphq4* on the level of partial resistance was always high against isolates 17 and 26, moderate against isolate 1.2.1 and absent against isolate Uppsala.

The differences found in the effect of *Rphq4* among isolates are consistent with the minor-gene-for-minor-gene model of Parlevliet & Zadoks (1977), which suggests specific interactions between QTLs for partial resistance and *Puccinia hordei* isolates. In their integrated concept of disease resistance, Parlevliet & Zadoks (1977) suggested that all genes for true resistance in the host population, whether they are major or minor genes, interact in a gene-for-gene way with genes for (a)virulence, either major or minor, in the pathogen population. The isolate specificity of *Rphq4* is consistent with the idea that QTLs for partial resistance encode proteins that act as pathogenicity targets interacting with specific elicitor proteins from the pathogen. So, while partial resistance appears isolate-nonspecific on the whole, the individual QTLs composing this resistance appear to interact in a gene-for-gene manner and could be overcome by the pathogen.

Polygenic, quantitative resistance is believed to be highly durable. However, there is hardly any experience with large-scale usage of quantitative resistance over a long period to confirm this statement (Lindhout, 2002). If QTLs for partial resistance function in a gene-for-gene manner, as do nondurable genes associated with a hypersensitive response, the durability of partial resistance becomes questionable. However, the fact that *Rphq2* and *Rphq3* were effective against isolate Uppsala while *Rphq4* was overcome by the same isolate suggests that, if the pathogen succeeds in breaking down the resistance of a QTL, this success does not necessarily imply that it will succeed in neutralizing the effects of the other QTLs. This is of importance for the durability of partial, polygenic resistance because, when one or several genes for quantitative resistance become ineffective against the pathogen, a subset of genes conferring a certain degree of quantitative resistance will always remain. Then, unlike the case of monogenic resistances, the selection pressure exerted by the host plant on the pathogen is divided among multiple partial resistance genes. The sum of the actions of each QTL against a pathogen race or isolate forms a multiple-gene barrier composed of minor genes interacting in a minor-gene-for-minor-gene way with the pathogen. If individual minor resistance genes can be overcome by the pathogen, the remaining genes that form the multiple-gene barrier will confer a sufficient level of resistance to reduce the rate by which the new virulence factors will spread through cultivated areas.

In summary, the results revealed several important features of quantitative resistance against *P. hordei* in barley. Some of the genes underlying QTLs may be involved in defence responses to particular isolates of *P. hordei* and others may be more commonly involved in defence responses to a broader range of isolates. Because partial resistance is governed by several genes with different specificities, the minor-gene-for-minor-gene interaction model seems more stable than the monogenic gene-for-gene interaction model. The apparent synergistic (epistatic) interactions among QTLs are also intriguing in respect of the underlying resistance mechanisms. Knowledge of the gene sequences underlying the QTLs would offer prospects for understanding their potential structural relationship and gaining insights into the basis of this form of durable disease resistance.

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