Evidence for a Minor Gene-for-Minor Gene Interaction Explaining Nonhypersensitive Polygenic Partial Disease Resistance

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ABSTRACT

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Partial resistance is a quantitative type of resistance that, by definition of Parlevliet, is not based on hypersensitivity. It is largely pathotype nonspecific, although some minor isolate-specific responses have been reported. In order to elucidate the isolate specificity of individual genes for partial resistance, three barley recombinant inbred line mapping populations were analyzed for resistance to the leaf rust fungus *Puccinia hordei*. The mapping populations were inoculated with one isolate avirulent and two isolates virulent to resistance gene *Rph7g*. Six sig-

nificant quantitative trait loci (QTLs) were detected. Of these, two (*Rphq3* and *Rphq11*) were detected with only the avirulent isolate (1.2.1.) and one (*Rphq18*) only with both virulent isolates (CO-04 and 28.1). The effectiveness of these QTLs was tested with 14 isolates, using a tester set of genotypes containing alleles for resistance or susceptibility for these QTLs. QTL *Rphq18* was effective to only two isolates, CO-04 and 28.1, whereas *Rphq3* and *Rphq11* were ineffective to CO-04 and 28.1 but effective to all other isolates, except one. This resulted in a significant Person's differential interaction, which is a hallmark of a gene-for-gene interaction. The minor gene-for-minor gene interaction is not based on hypersensitivity and there is no evidence that the resistance is based on genes belonging to the nucleotide-binding leucine-rich repeat class.

In many biotrophic plant pathosystems, plants respond to infection through two types of immune responses: a hypersensitive response (HR) or a nonhypersensitive-mediated resistance (29,30,42,51). An HR is usually conferred by major genes. Tissue immediately at the site of pathogen ingress undergoes rapid programmed cell death. Most molecularly characterized major genes for hypersensitive resistance to biotrophic pathogens show a remarkably general modular structure over a wide range of plant pathosystems, consisting of a nucleotide-binding leucine-rich repeat (NB-LRR) domain (31,50,61). Major genes for HR may cause either complete or incomplete resistance to pathogens. Adaptation by the pathogen through loss-of-function mutations may easily erode the effectiveness of this resistance, causing low durability of this type of resistance (36,48,51).

Resistance that is not based on HR is referred to by various terms, including quantitative, incomplete, partial, complex, polygenic, horizontal, field, slow-rusting, and durable resistance. Most of these terms refer to the fact that this host plant resistance leads to a reduction in disease rather than the absence of disease. With the availability of molecular markers, chromosome regions determining levels of partial (quantitative) resistance have been mapped for many plant pathosystems. This led to a wide array of candidate genes that have been implicated to underlie the quantitative resistance (42,51). However, too few minor genes for partial resistance have been cloned to know whether they also

constitute or represent a certain gene family (9,20,42,51). Here, we will use the term "partial resistance" to describe the observed nonhypersensitive disease phenotypes without assumptions about their underlying genetic basis. Partial resistance is largely pathotype nonspecific, although some minor isolate-specific responses have been reported in several pathosystems.

The barley leaf rust fungus (*Puccinia hordei* G. H. Oth) and barley (*Hordeum vulgare* L.) constitute a fruitful system to study the genetic basis of the interactions between plants and their pathogens because HR-based resistance occurs side by side with nonhypersensitive quantitative partial resistance (29,30). HR-based resistance of barley to *P. hordei* is governed by major genes (designated as *Rph* genes) that are typically pathotype specific (60). This resistance is often complete, and associated with necrosis of plant cells that are attacked by the pathogen sporulating. At present, at least 20 major pathotype-specific genes for resistance to leaf rust, named *Rph1* to *Rph20*, have been described in barley (13). Unfortunately, most *Rph* genes have been rendered ineffective by adaptation of the pathogen. *Rph7g*, derived from barley accession 'Cebada Capa', is the most effective *Rph* gene for resistance to *P. hordei* in Europe (30). Virulence for this gene has been reported to occur in Israel (11), Morocco (39), the United States (52), and, more recently, in southern Spain (49).

Partial resistance of barley to *P. hordei* is inherited quantitatively (33,35,45). In the greenhouse, the infection appears at a slower rate, with smaller and fewer uredinia formed than on the most susceptible checks. Those uredinia have, however, a compatible appearance. Therefore, partially resistant barley genotypes are identified by a long latency period (LP) of the fungus and a low infection frequency (IF) compared with the susceptible reference line L94. For a long time, this resistance was considered to be pathotype nonspecific and durable (36). However, although many quantitative trait loci (QTLs) were shown to be effective

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against a broad range of isolates, some QTLs were shown to be isolate specific (23,44). Such isolate specificity was already shown to occur as a significant cultivar-isolate interaction in barley-*P. hordei* by Parlevliet and co-workers (35,40). Parlevliet and Zadoks presumed that the observed weak cultivar-isolate interactions were due to minor gene-for-minor gene interactions (36,41), in a similar way as the major gene-for-major gene interaction for hypersensitivity resistance. Gene-for-gene interactions result in a reversed ranking called the Person's differential interaction (47,56). Parlevliet and Zadoks (41) even argued that minor gene-for-minor gene interactions can explain the durability of polygenic partial resistance (29).

More than 20 QTLs responsible for partial host resistance to *P. hordei* have been reported in several barley populations and at several stages of plant development (23,24,43-45). In this and other plant pathosystems, recent studies report race specificity of some resistance QTLs, including those with relatively large effects (3-5,19,23,48,53,62). All cases previously reported involved one or more genes that were significantly effective to some but not to other pathogen isolates. Thus far, significant reversed ranking between QTLs for nonhypersensitive resistance and pathogen isolates has not been well documented.

Several fungi, including *P. hordei*, enter the host leaf through stomata to form intercellular hyphae that produce haustorial mother cells upon contact with a host cell surface within the lamina (37). Partial resistance of barley to leaf rust fungal infection is based on the formation of cell wall appositions (synonym: papillae) at the fungal penetration sites, preventing the formation of fungal haustoria (8). Partial resistance looks like a weak form of nonhost basal resistance to nonadapted rust and powdery mildew fungi. We hypothesize that partial resistance constitutes a quantitative pathogen-associated molecular pattern-triggered defense response, due to incomplete suppression of basal defense by the effectors of the pathogen (18,29).

In barley, unlike some other plant species, there is no indication that map positions are shared between pathotype-specific resistance genes (*Rph* loci) and minor genes for partial resistance to *P. hordei* (22,45). Instead, we found a significant association between QTLs for partial and nonhost resistance and plant defense gene homologues (24); in particular, peroxidase genes (12). This, and the difference in mechanisms observed, suggests that hypersensitive and partial resistances in barley to *P. hordei* rely on different types of genes having different evolutionary origins, although more evidence is necessary to confirm that minor genes are not members of the NB-LRR gene family.

Many studies on pathogen population structure and host resistance are based on qualitative host-pathogen interactions. The effects of the interactions between major resistance genes and avirulence genes are large and well known in barley (30). On the other hand, the effects of single minor genes for partial resistance are too small to test their effectiveness to many isolates. Until now, the evidence for single minor genes (actually QTLs) being effective or not to certain isolates emerged from the screening of mapping populations (23,44) and sets of near-isogenic lines (NILs) (22) with different pathogen isolates.

In this article, we consider both qualitative and quantitative information on the interaction between the pathogen and the host. In a first experiment, three isolates were selected to map QTLs at the seedling stage in three recombinant inbred line (RIL) mapping populations: isolates 1.2.1, CO-04, and 28.1. Isolate 1.2.1 is a long-time standard isolate collected in the Netherlands. Isolate CO-04, which has virulence to most known *Rph* genes, including *Rph7g* (49), was collected in Córdoba (Spain) and may have reached southern Spain from North Africa, where virulence to *Rph7g* is known to occur (27,39). The Moroccan *Rph7g* virulent isolate 28.1 (27) was found to have an abnormal morphology of the substomatal vesicles (SSV). However, germlings of CO-04 showed the typical SSV morphology of *P. hordei* (26), similar to

that of isolate 1.2.1 (49), implying that isolates CO-04 and 28.1 are distinct genotypes.

We set out to test the hypothesis that there is a differential interaction between partial resistance genes and pathogen isolates, as often observed between major resistance genes and avirulence genes in the pathogen. For this, we tested the effectiveness of isolate-specific genes for partial resistance to 14 isolates of various geographic origins and belonging to different pathotypes. This test was performed on selected RILs that contained resistance or susceptibility alleles of QTLs that had shown specificity in the mapping studies. By these experiments, we could identify and confirm clear differential interactions between QTLs and pathogen isolates. We could also determine the spectrum of effectiveness of individual QTLs for partial resistance to a large number of pathogen isolates.

MATERIALS AND METHODS

RILs. Three mapping populations were used to map QTLs for partial resistance. The populations consisted of RILs derived from crosses between L94 and Vada ($L \times V$, 103 RILs) (44,46), L94 and 116-5 ($L \times 116-5$, 117 RILs) (43), and Cebada Capa and SusPtrit ($CC \times Su$, 113 RILs) (15). Vada is a commercial West European cultivar that has a high level of partial resistance to *P. hordei*. Cebada Capa carries the *Rph7g* major gene for hypersensitivity resistance and has a high level of partial resistance in its genetic background. Line 116-5 was derived from a cross between the very susceptible L94 and Cebada Capa by selection against *Rph7g* and for a high level of partial resistance. Therefore, 116-5 should possess most or all of the QTLs for partial resistance to *P. hordei* that occur in Cebada Capa but not *Rph7g* (43).

Quantification of levels of resistance to *P. hordei*. Seedlings of the three mapping populations with the parents and control lines were grown in 37-by-39-cm boxes and were inoculated with the *P. hordei* single urediniospore isolates CO-04 and 28.1 (both virulent to *Rph7g*). $CC \times Su$ was also inoculated with isolate 1.2.1 (avirulent to *Rph7g*). Six boxes were required to accommodate one replication of each mapping population per isolate. For each RIL population-isolate combination, three replications were performed, with three seedlings per RIL per experiment. To monitor the effectiveness of inoculation, we added to each box the parents of the particular cross, the susceptible line L94, and the NIL L94-*Rph7g* (Table 1). L94-*Rph7g* carries the Cebada Capa-derived resistance gene (28). Phenotypic data for $L \times V$ and $L \times 116-5$ with the isolate 1.2.1 were obtained from Qi et al. (43,45) and used to remap the QTLs.

The inoculation of the first seedling leaves was performed as described by Qi et al. (45) with ≈ 240 spores/cm². On partially resistant cultivars, the fungus requires a longer time between infection and maturation of the uredinia (i.e., LP) than on susceptible cultivars. In addition, fewer uredinia of the fungus appear per square centimeter of leaf area (IF), and those uredinia are usually smaller and produce fewer spores. From the day the first uredinia were just visible, they were counted daily using a pocket lens ($\times 10$), until the number did not increase anymore (5 or 6 days). The LP of the pathogen on each seedling was evaluated by estimating the number of hours from inoculation to the moment at which 50% of the ultimate number of uredinia was visible. The final number of uredinia was counted in a 1-cm² frame to determine the IF calculated as the number of uredinia per square centimeter. The relative LP (RLP) on seedlings and relative IF (RIF) were calculated relative to the LP and IF on L94, where L94 = 100, as described by Parlevliet (32). The Duncan test was used to compare barley lines with the most susceptible reference for each isolate.

QTL mapping. Linkage maps of the populations used for QTL mapping have been reported previously. The linkage maps have been used as components of a high-density integrated genetic map

posted on the GrainGenes website, “Barley, Integrated, Marcel 2009” (<http://wheat.pw.usda.gov>) (1). The maps used for QTL mapping contain 957 amplified fragment length polymorphism (AFLP) and simple-sequence repeat (SSR) markers for the L × V population (24), 280 AFLP markers for L × 116-5 (43), and 495 AFLP and SSR markers for CC × Su (15).

For each RIL, the mean of the three replications of the RLP50 was used in the QTL analysis. The computer software package MapQTL5 (57) was used for interval mapping (21). In the regions of the putative QTLs (log of the likelihood ratio [LOD] > 3.0), the markers with the highest LOD values (peak markers) were taken as co-factors for running a multiple-QTL mapping program, the MQM method (16). When LOD values of some markers at other regions reached the significance level, MQM was repeated by adding those new peak markers as co-factors until a stable LOD profile was reached. The restricted-MQM (rMQM) method was used to determine the values of the LOD, the proportion of explained phenotypic variation, and the additive effect. QTL confidence intervals were defined based on the projection of the LOD-1 and LOD-2 cut-offs of the LOD profile on the linkage map (21). A LOD value of 3.0 was taken as significance threshold value for declaring a QTL.

Virulence spectrum of the isolates. Fourteen leaf rust isolates were applied to seedlings of a differential set of 15 barley lines and cultivars to determine their virulence spectra to *Rph* major genes. One experiment consisted of two replications, and each replication consisted of three to five seedlings per barley accession. In each experiment, the plant materials were inoculated with 1 of the 14 selected isolates and, 10 to 12 days after inoculation, infection types (ITs) were scored according to the scale of McNeal et al. (25) modified by Shtaya et al. (49). Lines with ITs 0 to 3 were regarded as resistant, those with ITs 4 to 6 as moderately resistant, and those with ITs 7 to 9 as susceptible. All isolates were multiplied from one urediniospore in separate glass-house compartments on the susceptible barley line L94. Collected spores were dried in a desiccator and stored at -80°C.

Tester set of RILs to evaluate QTL effects against a range of isolates. Evaluation of the level of partial resistance of all RILs in the mapping populations is too labor intensive to be done for a large set of isolates. To evaluate the effect of the QTLs detected in L × 116-5 and CC × Su against the 14 isolates, we visually inspected the graphical genotypes of RILs from both populations with the GGT 2.0 package (54). GGT 2.0 was used to visualize data of markers with known map positions, filtering for the donor alleles in the region of the relevant QTLs. We selected a set of RILs having the resistance allele for the QTLs of interest and the susceptibility alleles for all the other QTLs, and RILs combining the susceptibility alleles for all QTLs or combining the resistance alleles for all QTLs (an example is provided in Supplementary Figure 1). In case the mapping populations contained several RILs with the same QTL allele combination, all RILs were selected in the tester set. The selected RILs represent the “tester set”. RILs from the tester set were inoculated with the 14 leaf rust isolates as described above and evaluated in two consecutive

experiments. Assays with the different isolates were carried out at different times to prevent contamination of the rust isolates. The experiments for each isolate included parental lines Vada, Tunisian 17, Tunisian 34, and L94-*Rph7g* as controls. In each tray, 10 to 12 seedlings per RIL and 3 seedlings per parent and control line were included.

RESULTS

Partial resistance in the parental and control barley lines. Isolate 1.2.1 elicited an HR, as expected, on Cebada Capa and L94-*Rph7g* due to its avirulence to *Rph7g* (Table 1). Isolates CO-04 and 28.1 caused a compatible infection on all parental genotypes and L94-*Rph7g*, confirming their virulence to *Rph7g*. The low RLP and high RIF on L94 and SusPtrit confirmed these two lines as the most susceptible to the three isolates. The presence of *Rph7g* in L94 background did not result in any significant resistance due to a possible residual effect of this gene when challenged by virulent isolates. On Vada, RLP tended to be the highest and RIF the lowest, as evidence of its high level of partial resistance. Interestingly, 116-5 showed a relatively high RLP to 1.2.1 but much lower RLP to CO-04 and 28.1. Also, the low RLP and high RIF measured on Cebada Capa to the virulent isolates CO-04 and 28.1 suggested a lower level of partial resistance than in Vada and not significantly higher than in L94. Both western-Mediterranean *Rph7g* virulent isolates CO-04 and 28.1 always showed similar RLP values.

LP and IF are components of partial resistance of barley against leaf rust (33,35,38). For both components, converted to relative values, there were significant differences among genotypes that indicate a clear cultivar effect. RLP and RIF were negatively correlated, as expected. The different experiments were compared to check the repeatability of the measurements of both components. Repeatability was better and experimental error lower for RLP than for RIF, as in previous studies of partial resistance of barley to *P. hordei*. Therefore, we used RLP as criterion to assess the level of partial resistance of barley cultivars.

QTLs for partial resistance in the three crosses. The RLP of the three *P. hordei* rust isolates exhibited continuous variation in all three mapping populations, suggesting polygenic inheritance (Fig. 1). The frequency distribution for isolate 1.2.1 in the cross CC × Su pertains to only part of the RIL population (only 44 of 113 RILs), because the other part carried *Rph7g* and could not be used to measure LP due to hypersensitive resistance.

To map QTLs for quantitative resistance, we applied interval mapping and rMQM (Supplementary Figure 2). The data presented in Table 2 were obtained with the rMQM method. For the L × V and L × 116-5 populations, we used the phenotypic data for isolate 1.2.1 obtained by Qi et al. (43,45). We remapped the QTLs and the results obtained were very similar to those obtained by Qi and coworkers.

L × V. Three QTLs for RLP were identified in the cross L × V for isolate 1.2.1 (Table 2). These QTLs were designated *Rphq1* (on 7H), *Rphq2* (on 2H), and *Rphq3* (on 6H). *Rphq2* and *Rphq3*

TABLE 1. Mean relative latency period (RLP) at seedling stage and relative infection frequency (RIF) in the genotypes used as controls in the mapping experiment for the three crosses studied^a

Genotype	RLP			RIF		
	1.2.1	CO-04	28.1	1.2.1	CO-04	28.1
L94	100.0 a	100.0 a	100.0 a	100.0 b	100.0 c	100.0 bc
L94- <i>Rph7g</i>	HR	101.9 a	100.6 a	HR	99.2 c	84.6 b
SusPtrit	99.6 a	101.3 a	100.3 a	92.6 b	119.1 d	107.2 c
Cebada Capa	HR	104.5 a	103.4 a	HR	84.3 b	84.3 b
116-5	119.3 b	104.2 a	102.6 a	46.3 a	76.8 b	91.5 bc
Vada	124.0 b	119.3 b	120.4 b	34.5 a	46.6 a	52.4 a

^a RIF referred to L94 = 100. Values in each column followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test. HR = hypersensitive response.

were also effective to the isolates CO-04 and 28.1. The effect of *Rphq1* was just significant against 1.2.1 but not significant against CO-04 and 28.1, despite the appearance of peaks in the LOD profiles obtained with interval mapping. Those peaks disappeared completely from the LOD profiles obtained with the rMQM method. For all isolates, *Rphq2* explained at least 35% of the variation and *Rphq3* at least 16%. All of the resistance-enhancing alleles of the three QTLs originated from the partially resistant parent Vada.

L × 116-5. In this RIL mapping population, three QTLs were detected for isolate 1.2.1 (Table 2). These QTLs were reported before by Qi et al. (43) and designated as *Rphq11*, *Rphq12* (both on chromosome 2H, with almost overlapping confidence intervals), and *Rphq3* (on 6H). *Rphq3* and *Rphq11* had highly significant effects (with rMQM LOD values of 16.9 and 9.4, respectively). However, with the isolates CO-04 and 28.1, none of these three QTLs were detected. Instead, we found only one QTL to be effective, *Rphq18*, which was not effective to 1.2.1. *Rphq18* was located on chromosome 2H, with a LOD score of 4.5 for CO-04 and 4.7 for 28.1. *Rphq18* was named after a QTL mapped in the same interval in the mapping population OWB (24). The resistance alleles of the four QTLs all originated from the partially resistant parent 116-5. These results indicate a stronger isolate specificity of the QTLs in 116-5 than of those in Vada.

CC × Su. Because the partial resistance of line 116-5 should derive from Cebada Capa (43), we expected the same QTLs to segregate in this mapping population as in L × 116-5 (SusPrit and L94 are susceptible lines and do not contain resistance alleles). Indeed, we found *Rphq11*, which was effective to isolate

1.2.1 only, and *Rphq18*, which was effective to isolate CO-04 with a LOD of 3.1 (Table 2). However, the LOD peak at *Rphq18* was below the significance threshold for isolate 28.1 (LOD 2.1). To map QTLs with isolate 1.2.1, only the RILs without the *Rphq7g* gene could be used. This limited population size may explain why *Rphq12* and *Rphq3* were not detected with isolate 1.2.1 in this population.

Assembly of the tester set. We assembled a tester set of seven lines and 30 RILs from the L × 116-5 and CC × Su mapping populations, each selected RIL containing no *Rphq* resistance alleles, a single *Rphq* resistance allele (*Rphq18*), or a combination of *Rphq* resistance alleles (*Rphq11* and -3 or *Rphq11*, -3, and -18). The assembled tester set was used to evaluate the specificity of the QTLs to 1.2.1, CO-04, and 28.1 (Table 3 for RLP; Supplementary Table 1 for RIF).

RLP and RIF observed in the tester set confirmed the QTL specificity observed in the mapping populations. The duplicate RILs, having the same combination of QTLs, gave similar results (data not shown); therefore, we present results of only two RILs with the same combination of alleles in the QTLs of interest (i.e., one RIL per cross).

The effect of *Rphq11* and *Rphq3* for isolate 1.2.1 and the effect of *Rphq18* for isolates CO-04 and 28.1 were confirmed in the tester set (Table 3). RILs that possessed the resistance allele of *Rphq18* gave a significantly longer RLP for isolates CO-04 and 28.1 than for 1.2.1, whereas those with resistant alleles for *Rphq3* and *Rphq11* gave a longer RLP for 1.2.1 than for CO-04 and 28.1. This resulted in a clear quantitative differential interaction (highlighted in Table 3).

Fig. 1. Frequency distribution of phenotypes for relative latency period (RLP) of isolates 1.2.1, CO-04, and 28.1. The RLP was calculated as the number of hours from inoculation until the time that 50% of the ultimate number of pustules had matured relative to the LP of L94, where L94= 100. The actual average LP values for L94 are indicated between brackets. **A**, Recombinant inbred lines (RILs) derived from the cross L94 × Vada, $n = 103$. **B**, RILs derived from the cross L94 × 116-5, $n = 117$. **C**, RILs derived from the cross Cebada Capa × SusPrit, $n = 113$ (except for isolate 1.2.1, for which only 44 RILs were evaluated due to the hypersensitive response reaction of the other 69 RILs). Values of parents and population mean values are indicated and positioned by arrows. Values indicated on the x-axis are the average values of each category.

Effectiveness of QTLs to other *P. hordei* isolates. The two isolates, CO-04 and 28.1, that showed a different QTL responsiveness than our standard isolate, 1.2.1, have a relatively rare virulence to *Rph7g* and are from adjacent geographical regions (southern Spain and Morocco). Therefore, we included additional isolates that are virulent to *Rph7g* (*avrRph7g*) but originating from elsewhere: isolates 5.1 (Israel) and US30 (United States); and some isolates with avirulence to *Rph7g* from various geographic origins, including southern Spain and Morocco: isolates M7, CO-99, AL-02, 202, Uppsala, Achterberg, IVP-2000, and 25. The virulence spectra of the isolates (Table 4) were generally in agreement with earlier data (23,27). Isolates CO-04 and 28.1 were the only isolates virulent for all *Rph* genes tested, with the exception of CO-04, that caused an intermediate IT on Sudan (*Rph1a*).

The *AvrRph7g* isolates, including those from the western-Mediterranean region, were homogeneous with regard to the effectiveness of *Rphq11* and *Rphq3* and ineffectiveness of *Rphq18*. Nonetheless, depending on the isolate, the magnitude of the effect of each QTL varied.

On the other hand, we distinguished several patterns of QTL effectiveness among the four *avrRph7g* isolates. First, the *avrRph7g* isolate US30 (United States) showed the same pattern as the *AvrRph7g* isolates (that is, only *Rphq11* and *Rphq3* were effective). Then, the *avrRph7g* isolate 5.1 from Israel showed a unique behavior: all QTLs controlling the partial resistance in the mapping populations (*Rphq11*, *Rphq3*, and *Rphq18*) were ineffective. However, the average level of resistance of Vada to isolate 5.1 was not impaired, indicating that *Rphq1*, *Rphq2*, and *Rphq3* of Vada are effective against this isolate, or that Vada

TABLE 2. Summary of quantitative trait loci (QTLs) in the crosses L94 × Vada (L × V), L94 × 116-5 (L × 116-5), and Cebada Capa × SusPtrit (CC × Su) for partial resistance to three isolates of *Puccinia hordei*^w

QTL	LG	cM ^x	Isolate 1.2.1			Isolate CO-04			Isolate 28.1		
			LOD	Exp% ^y	Add ^z	LOD	Exp%	Add	LOD	Exp%	Add
L × V											
<i>Rphq2</i>	2H	147–152	19.8	35.1	4.8	14.3	38.4	4.3	16.8	38.2	4.4
<i>Rphq3</i>	6H	53–63	12.4	18.2	3.6	7.5	16.1	2.8	9.1	16.7	2.9
<i>Rphq1</i>	7H	46–122	3.1	3.7	1.6	0.2	0.3	0.4	0.8	1.1	0.8
L × 116-5											
<i>Rphq18</i>	2H	50–60	2.2	5.2	1.0	4.5	16.9	2.0	4.7	16.0	1.9
<i>Rphq11</i>	2H	95–114	9.4	21.2	2.3	0	0.1	0.2	0.0	0.1	0.1
<i>Rphq12</i>	2H	119–130	3.3	6.8	1.2	0	0.2	0.2	0.1	0.3	0.3
<i>Rphq3</i>	6H	55–66	16.9	37.9	2.9	2.1	7.5	1.3	1.8	5.7	1.1
CC × Su											
<i>Rphq18</i>	2H	45–56	0.1	0.6	0.5	3.1	13.7	1.4	2.1	8.7	1.1
<i>Rphq11</i>	2H	92–100	5.8	37.2	3.9	0	0	0	0.4	1.5	0.3

^w Log of the likelihood ratio (LOD) values in bold indicate significant QTLs at relative multiple-QTL mapping (rMQM). Raw data for isolate 1.2.1 are from Qi et al. (45) for L × V and Qi et al. (43) for L × 116-5.

^s Position of the two-LOD confidence interval in centimorgans (cM) based on the results of rMQM.

^y Proportion of the explained phenotypic variance.

^z Effects of the alleles from resistant parent.

TABLE 3. Relative latency period (RLP) to measure the effectiveness of quantitative trait loci (QTLs) against 14 *Puccinia hordei* isolates avirulent (*avr*) or virulent to *Rph7g* in the tester set of recombinant inbred lines (RILs) selected from L94 × 116-5 and Cebada Capa × SusPtrit (CC × Su) populations, and control barley lines carrying different *Rph* genes or other QTLs

QTL, genotype ^w	RLP (location, isolate) ^x													
	NL 1.2.1	Spain, CO-04	Morocco, 28.1	NL, Achterberg	NL, IVP2000	Sweden, Uppsala	Italy, 25	Greece, 13	Spain, AL-02	Spain, CO-99	Morocco, M7	Israel, 202	Israel, 5.1	U.S., US30
None														
L94	100	98	95	100	100	100	100	100	98	100	100	100	100	100
SusPtrit	102	100	97	104	95	103	102	98	99	101	100	103	100	99
RIL 70	100	100	96	98	102	102	101	97	98	101	100	99	98	98
RIL 71	101	102	96	103	97	101	101	100	100	101	102	102	100	98
<i>Rphq18</i>														
RIL 8	102	109	107	100	97	103	103	104	101	100	101	100	101	103
RIL 58	98	110	108	101	98	102	104	102	102	102	100	101	103	100
<i>Rphq11</i> and 3														
RIL 53	114	102	101	127	114	115	112	114	115	116	115	117	102	112
RIL 29	115	102	101	115	112	111	112	116	115	115	116	117	102	113
<i>Rphq11</i> , 3, and 18														
116-5	120	104	103	129	119	118	116	122	121	115	116	118	104	115
Cebada Capa	HR	105	104	HR	HR	HR	HR	HR	HR	HR	HR	HR	103	115
RIL 119	121	108	108	129	126	120	120	117	117	123	113	126	105	111
RIL 108	117	109	108	116	113	113	119	118	115	115	114	117	105	113
<i>Rphq1</i> , 2, and 3														
Vada ^y	122	120	120	122	122	123	125	124	122	125	127	131	122	117
TU17 ^z	143	132	111	I	I	120	139	138	143	I	I	I	I	HR
TU34 ^z	I	104	105	I	I	I	HR	I	I	I	I	I	HR	I

^w RILs 70, 8, 53, and 119 come from L × 116-5 cross; RILs 71, 58, 29, and 108 come from CC × Su cross.

^x RLP is expressed as values relative to L94 (=100%) with isolate 1.2.1. RLPs in bold are significantly higher than RLP on L94. Isolates in bold are *avrRph7g*. NL = The Netherlands, Spain = southern Spain, and U.S. = United States; I = immune and HR = hypersensitive response; boxed frame highlights a quantitative differential QTL-isolate interaction.

^y Vada carries *Rphq1*, *Rphq2*, and *Rphq3* that did not show isolate specificity.

^z Tunisian17 carries *Rph7ac* or *RphC*, Tunisian34 *RphD*, and Cebada Capa *Rph7g*.

possesses other minor resistance genes effective to this isolate. Finally, *Rphq11* and *Rphq3* were ineffective and *Rphq18* was effective to the two *avrRph7g* isolates, CO-04 and 28.1. The different patterns found for *avrRph7g* isolates suggest that the particular pattern of CO-04 and 28.1 to overcome *Rphq3* and *Rphq11* but not *Rphq18* was not associated with their virulence to *Rph7g*. Effectiveness of *Rphq18*, *Rphq3*, and *Rphq11* was not associated with the presence of any known avirulence factor in the set of these isolates.

We also used the differential barley lines Tunisian 17 and Tunisian 34, which carry other *Rph* genes. Tunisian 17 carries *Rph7ac*, which is a member of the same multiple allelic series as *Rph7g*. Both genes were mapped to the short arm of chromosome 3H (7,14). However, Tunisian 17 must possess minor genes or incompletely acting *Rph* genes effective to all *Rph7ac*-virulent isolates that were tested (Table 3) to explain the relatively high RLP on this cultivar. The resistance gene of Tunisian 34 (named *RphD*) has a very wide spectrum of effectiveness (Table 4). It is not allelic to *Rph3*, *Rph7*, or *Rph9* (59) and has not yet been assigned to a chromosome. These lines share a geographic origin with isolate 28.1 (North Africa) and, interestingly, *RphD* was only overcome by the isolates CO-04 and 28.1 (Table 4). This similarity between both isolates suggests that the virulence to *Rph7g* of CO-04 comes from North Africa, and that CO-04 and 28.1 are closely related strains of the pathogen. Tunisian 34 constitutes, with Cebada Capa, a valuable source for complete, albeit probably not durable, resistance.

DISCUSSION

Barley–barley leaf rust (*P. hordei*) has been used since the early 1970s as a model system to investigate the genetics, epidemiological components, mechanism, and specificity of partial resistance (23,30,34,35,40,43,44). The isolate specificity of this resistance is somewhat controversial. Certain cultivars (notably Vada) are consistently more resistant than other accessions (L94 and Akka), even after testing >20 isolates from all over the world (2,24,30,44) (Table 3). This is in accordance with the theory of van der Plank (55), who defined horizontal resistance as a resistance that is determined by many “minor” resistance genes and is effective against all pathotypes of a pathogen species. However, after additional detailed analyses, small cultivar–isolate interactions were observed, indicating that certain minor genes might not be effective to all *P. hordei* isolates. Such observations prompted Parlevliet and Zadoks (41) to publish their seminal paper on the

possibility of a “minor gene–for–minor gene interaction” between plant and pathogen. Such small cultivar–isolate interactions have been demonstrated in field tests on adult plants, as well as in seedling tests (23,34,40). The latter developmental stage is logistically more convenient to study the genetic basis of this specificity.

The present research identified QTLs for partial resistance to *P. hordei* with strong specificity in their effect to the three isolates 1.2.1, CO-04, and 28.1. Of the three QTLs identified, two, *Rphq3* and *Rphq11*, were effective to almost all the isolates but not to isolates CO-04, 28.1, and 5.1; and *Rphq18* was only effective to CO-04 and 28.1 (Table 3). This isolate specificity of QTLs leads to a reversed ranking of resistance parameters. Such a differential interaction is one of the hallmarks of a gene-for-gene interaction (47,55). Our quantitative differential interaction at the individual QTL level (Table 3) supports the hypothesis that partial resistance is under a minor gene–for–minor gene interaction. The final step in clinching the minor gene–for–minor gene interaction is to demonstrate that the difference between CO-04/28.1 and 1.2.1 is based on two genes, one that interacts with *Rphq18* and the other with *Rphq3* or *Rphq11*.

The results obtained in this study show, once more, the great diversity of *Rphq* genes for partial resistance in barley, as was already reported by Qi et al. (43) and Marcel et al. (24). Indeed, mapping of genes for nonhypersensitive quantitative resistance leads, in almost each mapping population, to a different set of QTLs being effective to the same isolate, except when the parents are closely related, as for 116-5 and Cebada Capa. Several of the QTLs detected in this study showed an isolate-specific effect. The most spectacular specificity we found was for *Rphq11/Rphq12* on chromosome 2H. That gene (pair) is, according to the LOD profile, clearly effective to 1.2.1 and definitely not effective to CO-04 and 28.1. For other genes that appeared to be isolate specific, the apparent specificity may be rather a marginal difference in effect size (namely, the LOD profile being just above the significance threshold for one isolate and just below this threshold for another isolate). This seems to be the case for *Rphq1* on 7H, *Rphq18* on 2H, and *Rphq3* on 6H. The latter QTL was the only one in common between Vada and 116-5. However, in *L* × *V*, *Rphq3* was effective to all tested isolates but, in *L* × 116-5, it was effective to all except CO-04 and 28.1. This may indicate that 116-5 and Vada carry different alleles of *Rphq3* or else there could be different genes in the same QTL interval, if this is not merely a marginal difference in effect size as discussed above. Therefore, the isolate specificity of *Rphq3* occurring in Cebada Capa and 116-5 needs further confirmation.

TABLE 4. Resistance or susceptibility pattern of 14 *Puccinia hordei* isolates according to their infection types on a differential series of barley (*Hordeum vulgare*) lines carrying different *Rph* genes

		Infection type (location, isolate) ^y													
Line	Gene	NL, 1.2.1	NL, IVP2000	NL, Achterberg	Sweden, Uppsala	Greece, 13	Italy, 25	Spain, AL-02	Spain, CO-99	Spain, CO-04	Morocco, M7	Morocco, 28.1	Israel, 5.1	Israel, 202	U.S., US30
L94	...	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Sudan	<i>Rph1a</i>	9	9	9	9	9	8–9	9	9	5	2	9	9	9	8
Peruvian	<i>Rph2b</i>	9	9	9	9	8	9	8	9	9	2	9	8	9	7
Estate	<i>Rph3c</i>	2	1	9	1	1	1	4	1	9	9	9	9	9	2
Gold	<i>Rph4d</i>	9	9	9	9	8	9	9	9	9	9	9	9	9	8
Bolivia	<i>Rph2r+6f</i>	9	9	9	9	9	9	9	9	9	9	9	9	1	9
Quinn	<i>Rph2r+5e</i>	6	9	9	6	9	9	7	8	9	8	8	9	1	2
Cebada Capa	<i>Rph7g</i>	2	2	2	2	2	2	2	2	9	2	9	9	2	9
L94 + <i>Rph7g</i>	<i>Rph7g</i>	2	2	2	2	1	2	2	2	9	2	9	9	2	9
Tunisian 34	<i>RphD</i>	1	0	0	1	0	0	0	1	9	0	9	2	1	1
Tunisian 17	<i>Rph7ac</i>	3	1	1	9	9	6	4–5	1	9	0	9	1	1	2
Egypt IV	<i>Rph8h</i>	9	8	1–2	9	9	9	9	6	9	9	9	9	9	8
Hor 2596	<i>Rph9i</i>	9	9	8	6	4	7	9	8	9	7	8	8	6	4
Triumpf	<i>Rph12l</i>	5	9	8	5–9 ^z	7	7	9	9	9	7	9	9	3	7
Magnif 102	<i>RphG</i>	9	8	2	5	9	9	8	3	9	9	9	2	9	8

^y Lines with infection types 0 to 3 are resistant (white cells), lines with infection types 4 to 6 are moderately resistant (light gray cells), and lines with infection types 7 to 9 are susceptible (dark gray cells). NL = The Netherlands, Spain = southern Spain, and U.S. = United States.

^z Different seedlings showed contrasting infection type.

The use of a tester set of barley genotypes allowed us to determine accurately the specificity of the quantitative resistance genes at the relevant QTLs to a set of 14 different *P. hordei* isolates. The results of those 14 isolates indicate that the effectiveness or ineffectiveness of *Rphq18*, *Rphq3*, and *Rphq11* was associated with neither the virulence to *Rph7g* nor with any other known virulence factor and, hence, we could not associate the presence of any of the avirulence factors with a quantitative virulence of the pathogen to *Rphq3* and *Rphq11*.

The resistance in Vada seems to fit the classical definition of durability (i.e., remaining effective despite widespread cultivation in an environment favoring the disease) (17). Vada and its descendants have been grown commercially in Western Europe since the early 1960s. In accordance with this perceived durability, the QTLs for partial resistance derived from Vada were generally effective to all the isolates. The LOD profiles in the mapping population L $\times$ V were almost identical for the three isolates. *Rphq2* always had the highest LOD peak, followed by a lower but significant peak for *Rphq3*; whereas, at rMQM, a significant peak for *Rphq1* was detected only against isolate 1.2.1. The barley lines 116-5 and Cebada Capa had a lower level of partial resistance to isolates CO-04 and 28.1 than Vada. The LOD profiles in the mapping populations L $\times$ 116-5 and CC $\times$ Su were very similar, which is consistent with the derivation of 116-5 from Cebada Capa (see Materials and Methods).

Similar isolate specificities of minor genes for partial resistance have previously been reported in other host-pathogen systems (3,6,10,19,62). Such an isolate specificity of individual QTLs seems in conflict with the apparent isolate nonspecificity and perceived durability of quantitative resistance of barley to the barley leaf rust fungus in cultivars like Vada. Why would the isolate-specific effectiveness of the *Rphq* genes not lead to obvious erosion of this quantitative resistance? Niks and Marcel (29) gave several arguments to explain the perceived durability of quantitative partial resistance despite the fact that single *Rphq* genes may not be effective to all isolates. (i) Overcoming the resistance conferred by one *Rphq* gene may require a specific gain-of-function sequence change in one or more of the effector genes of the pathogen, which should be rare. (ii) Such a mutation in the pathogen will result in only an incremental enhancement in its fitness, which is probably not sufficient to result in a fast replacement of conspecifics by the mutant that overcame a particular *Rphq* gene. (iii) There is great diversity in genes for partial resistance between plant genotypes, at least in the cultivated barley-*P. hordei* system (24). Therefore, each partial resistance gene that the mutant pathogen overcomes is likely to be present in a small proportion of the host population or crop acreage. This would further limit the selective advantage of the microbial mutant. Thus, the selective advantage of mutants that overcome *Rphq* genes is much lower than of mutants that overcome an *Rph* gene for complete resistance, which is based on a loss-of-function mutation (29).

The tester set used in this work and the availability of QTL-NILs (22,58) for partial resistance of barley make it possible to further investigate the importance of isolate specificity of these minor genes for resistance. A progeny from a cross between pathogen isolates 1.2.1 and CO-04 may be generated and phenotyped on the tester set, to determine the inheritance of the ability to overcome certain *Rphq* genes for partial resistance, and the possible association with certain *Rph* virulences or avirulences.

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