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ORIGINAL PAPER

The phenotypic expression of QTLs for partial resistance to barley leaf rust during plant development

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Abstract Partial resistance is generally considered to be a durable form of resistance. In barley, *Rphq2*, *Rphq3* and *Rphq4* have been identified as consistent quantitative trait loci (QTLs) for partial resistance to the barley leaf rust pathogen *Puccinia hordei.* These QTLs have been incorporated separately into the susceptible L94 and the partially resistant Vada barley genetic backgrounds to obtain two sets of near isogenic lines (NILs). Previous studies have shown that these QTLs are not effective at conferring disease resistance in all stages of plant development. In the present study, the two sets of QTL–NILs and the two recurrent parents, L94 and Vada, were evaluated for resistance to *P. hordei* isolate 1.2.1 simultaneously under greenhouse conditions from the first leaf to the flag leaf stage. Effect of the QTLs on resistance was measured by development rate of the pathogen, expressed as latency period (LP). The data show that *Rphq2* prolongs LP at the seedling stage (the first and second leaf stages) but has almost no effect on disease resistance in adult plants. *Rphq4* showed no effect on LP until the third leaf stage, whereas *Rphq3* is consistently effective at prolonging LP from the first leaf to the flag leaf. The changes in the effectiveness of *Rphq2* and *Rphq4* happen at the barley tillering stage (the third to fourth leaf stages). These results indicate that multiple disease evaluations of a single plant by repeated inoculations of the fourth leaf to the flag leaf should be conducted to precisely estimate the effect of *Rphq4*. The present study confirms and describes in detail the plant development-dependent effectiveness of partial resistance genes and, consequently, will enable a more precise evaluation of partial resistance regulation during barley development.

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Introduction

Plant disease resistance depends on many factors, including environmental conditions, the genotypic combination of host species and pathogen, the nature of the infected tissues and plant developmental stages. An increasing number of studies have shown that disease resistance governed by major genes (R genes) or minor genes (quantitative trait loci, QTLs) is, in some cases, plant stage-specific (Whalen 2005; Develey-Rivière and Galiana 2007). In recent years, the mode of action of several host growth stage-dependent

R genes has been studied in more or less detail (Century et al. 1999; Kus et al. 2002; Panter et al. 2002; Goggin et al. 2004). QTL mapping on quantitative resistance has shown that the phenotypic expression of an individual QTL is often plant stage-dependent (e.g., Steffenson et al. 1996; Qi et al. 1998; Chu et al. 2009). However, owing to the polygenic nature of quantitative resistance, the effects of individual QTLs throughout plant development remain largely unknown. QTL-near isogenic lines (QTL–NILs) allow the evaluation of effects of single QTLs in a nearly uniform genetic background. In a QTL–NIL, the target QTL becomes the major genetic source of variation because of the absence of other segregating QTLs, and the QTL is considered Mendelized (Alonso-Blanco and Koornneef 2000). With NILs, the effect of each QTL can be determined in the absence of interactions with other QTLs and is undisturbed by the variable genetic background as occurs in mapping populations. Pairs of QTL–NILs allow measurements of the QTL effect by using only two plant genotypes, rather than more than hundred plant genotypes when QTL effects are measured on the basis of a mapping population. As a consequence, pairs of NILs are more efficient, and allow one to use more experimental replications compared with mapping populations, as fewer plants are required per experiment. This has a distinct advantage in, for example, field evaluations (Marcel et al. 2008). QTL–NILs are further instrumental in fine mapping of the QTL responsible for the phenotypic effect (Marcel et al. 2007). We set out to use the QTL–NILs to characterize the dependence of each QTL on the host plant growth stage.

Partial resistance to leaf rust (*Puccinia hordei* Otth) in barley is a quantitative resistance that is inherited polygenically and is not based on hypersensitivity (Parlevliet 1978). In a previous study (Qi et al. 1998), QTL mapping was performed in a recombinant inbred population derived from a cross between the susceptible L94 barley line and the partially resistant Vada barley cultivar, at the seedling stage and adult plant stage. After challenge with *P. hordei* isolate 1.2.1, six QTLs were identified, named *Rphq1*, *Rphq2*, *Rphq3, Rphq4*, *Rphq5* and *Rphq6*, that contributed to the partial resistance of Vada at either of the two plant stages. Three QTLs, *Rphq2*, *Rphq3* and *Rphq4*, which were mapped at the distal part of the long arm of the chromosome 2H, near the centromere of the chromosome 6H and at the distal part of the short arm of chromosome 5H, respectively, showed the largest and most consistent effect (Qi et al. 1998). *Rphq2* was found to be effective in seedlings but almost not in adult plants, while *Rphq3* was effective both in seedlings and in adult plants. *Rphq4* was effective only at the adult plant stage in the greenhouse and in the field (Qi et al. 1998). Recently, the Vada alleles of the three largest-effect QTLs from the above-described study were introgressed into the susceptible L94 line, and the L94 alleles were introgressed into the resistant cultivar Vada by marker-assisted backcrossing to obtain NILs (Van Berloo et al. 2001; Marcel et al. 2007). The QTL effects found in the L94 × Vada mapping population were consistently confirmed in the two complementary sets of NILs, including their dependence on plant developmental stage (Marcel et al. 2007, 2008).

Appearance of resistance at different stages of host development may be driven by diverse defense mechanisms. Investigation of the dynamic changes in phenotypic expression of *Rphq2*, *Rphq3* and *Rphq4* at different developmental stages is the first step in exploring the intricate mechanisms underlying this durable partial resistance and will facilitate map-based cloning of the genes involved. For example, pinpointing when *Rphq4* starts to express may help in detecting its expression earlier than the heading stage and will likely hasten map-based cloning of this QTL.

Here, we determine the effectiveness of the three QTLs, *Rphq2*, *Rphq3* and *Rphq4,* in conveying partial resistance to *P. hordei* during plant development. The susceptible L94 line, the partially resistant Vada cultivar and two complementary sets of NILs were evaluated for susceptibility to *P. hordei* isolate 1.2.1 simultaneously under the same greenhouse conditions, minimizing complicating environment effects. The estimation of the latency period (LP) of the rust fungus, which has been shown to be the most reliable and effective method for quantifying levels of partial resistance in a greenhouse test (Neervoort and Parlevliet 1978), was used to quantify levels of resistance to *P. hordei* isolate 1.2.1.

Materials and methods

Plant materials

L94-*Rphq2*, L94-*Rphq3*, L94-*Rphq4* and L94-*Rphq2+3* carried the Vada allele of a QTL or a combination of Vada QTLs in the susceptible L94 genetic background. The reciprocal NILs, Vada-*rphq2*, Vada-*rphq3*, Vada-*rphq4* and Vada-*rphq2+3*, carried the L94 allele of a QTL or combination of L94 QTLs in the partially resistant Vada genetic background. L94-*Rphq2*, L94-*Rphq3* and L94-*Rphq4* contained Vada introgressed fragments of 4.6, 22.6 and 10.8 centiMorgans (cM), respectively. Vada-*rphq2*, Vada-*rphq3* and Vada-*rphq4* contained L94 introgressed fragments of 5.2, 45.8 and 12.7 cM, respectively (Van Berloo et al. 2001; Marcel et al. 2007). In each NIL, the target QTL was the major source of genetic variation for partial resistance to leaf rust because of the absence of other segregating QTLs.

The susceptible line L94, the partially resistant cultivar Vada, and the NILs were tested in a plastic film-covered

solar greenhouse in the spring of 2008 and 2009 against the leaf rust isolate 1.2.1. To obtain uniform germination, 30 plants of each line were germinated in Petri dishes. Four days later, 15–20 seeds with uniform germination were sown in soil. Subsequent sowing at about 14-day intervals was conducted to produce eight series of plants corresponding to different plant stages. In 2008, at the time of inoculation, the eight series of plants were grown to the first, second, third, fourth, fifth, sixth, seventh and the flag leaf stage. In 2009, the eight series of plants were grown to the first, second, third, fourth, fifth, sixth, seventh, penultimate (flag-1) and the flag leaf stage at the time of inoculation. The inoculation was performed as described in the next section and only the main tillers were used. To minimize the influence of leaf maturity on LP, only the uppermost leaves that were just fully unfolded were inoculated. For each line per stage, 10–15 plants were selected for inoculations.

Disease evaluations and statistical analyses

Puccinia hordei isolate 1.2.1 is a monospore culture derived from isolate 1.2 (Parlevliet 1976). Fresh urediospores were collected from susceptible L94 plants. To make a countable and even density, the spores were diluted 15 times in 2008 and 30 times in 2009 with inert lycopodium spores as carriers and applied to the leaves using a soft-hair brush. After inoculation, the greenhouse was kept 12 h in darkness and saturated relative humidity and was subsequently returned to normal conditions. In the spring of 2008, the temperature in the greenhouse was 8–23°C, and the photoperiod was 12–14 h of natural light. In the spring of 2009, the temperature in the greenhouse was 10–25°C, and the photoperiod was 12–14 h of natural light. When the first urediospore was visible, a linear area of 2 cm was marked in the middle part of the leaves. The mature spore pustules within the delimited areas were counted at 24 h intervals until the number no longer increased. The LP on each leaf was evaluated by estimating the period (in hours) at which 50% of the ultimate number of pustules became visible (Neervoort and Parlevliet 1978).

Latency periods (LP) for each line at each stage were estimated by averaging the LP values for the 10–15 inoculated leaves. L94-*Rphq2+3* and Vada-*rphq2+3* were only included in the 2009 experiment. The analyses of variance with LP on L94, Vada and NILs were performed using Microsoft Excel®. At each leaf stage, LSD tests ($P < 0.05$) were used to compare the L94 background NILs with L94 and the Vada background NILs with Vada. SPSS11.5 software (SPSS Inc, Chicago, IL, USA) was used for LSD tests.

Results

The disease evaluations in the spring of 2008 and 2009 showed that LP increased with the progression of plant development from the first leaf to the flag leaf. Also, the LP determined for the susceptible parent L94 was less affected by growth stage than for lines that carried partial resistance (Tables 1, 2; Fig. 1). As expected, the Vada-derived alleles (*Rphq*) tended to prolong the LP in the susceptible L94 background, while the L94-derived alleles (*rphq*) tended to reduce the LP in the resistant Vada background (Tables 1, 2). The two sets of QTL–NILs formed two complementary sets that mirrored each other in the plant development stages during which each *Rphq*-gene was effective (Fig. 1a, c compared to Fig. 1b, d).

Rphq2's effect declines from the third leaf stage

At the first leaf stage, *Rphq2* was the most effective of the three QTLs. The LP in L94-*Rphq2* and Vada-*rphq2* were significantly different than in L94 and Vada, respectively (Tables 1, 2). *Rphq2* prolonged the LP by

Table 1 Mean latency periods in hours at eight growth stages of *P. hordei* isolate 1.2.1 in L94, Vada and NILs in 2008

Leaf stage	L94 and L94 background NILs				Vada and Vada background NILs			
	L94	L94-*Rphq2*	L94-*Rphq3*	L94-*Rphq4*	Vada	Vada-*rphq2*	Vada-*rphq3*	Vada-*rphq4*
First leaf	182[a,*]	211[c]	202[b]	187[a]	282[a]	250[c]	258[b]	278[a]
Second leaf	185[a]	209[c]	196[b]	191[ab]	301[a]	269[c]	273[c]	290[b]
Third leaf	197[a]	204[a]	258[c]	238[b]	327[a]	308[b]	240[c]	232[d]
Fourth leaf	214[a]	227[b]	289[c]	301[d]	351[a]	312[b]	278[c]	259[d]
Fifth leaf	217[a]	221[a]	262[b]	312[c]	349[a]	326[b]	310[c]	260[d]
Sixth leaf	219[a]	224[a]	266[b]	320[c]	351[a]	339[b]	311[c]	267[d]
Seventh leaf	234[a]	239[a]	296[b]	349[c]	377[a]	360[b]	337[c]	285[d]
Flag leaf	241[a]	247[a]	313[b]	359[c]	398[a]	383[b]	343[c]	287[d]

* At each leaf stage, L94 background NILs were compared with L94, and Vada background NILs were compared with Vada. Means followed by a common letter are not significantly different according to LSD test ($P < 0.05$)

Table 2 Mean latency periods in hours at eight growth stages of *P. hordei* isolate 1.2.1 in L94, Vada and NILs in 2009

Leaf stage	L94 and L94 background NILs					Vada and Vada background NILs				
	L94	L94-*Rphq2*	L94-*Rphq3*	L94-*Rphq4*	L94-*Rphq2+3*	Vada	Vada-*rphq2*	Vada-*rphq3*	Vada-*rphq4*	Vada-*rphq2+3*
First leaf	200[a,*]	220[c]	214[b]	204[a]	228[d]	259[a]	236[c]	243[b]	256[a]	228[c]
Second leaf	210[a]	225[c]	217[b]	214[a]	226[c]	265[a]	247[c]	254[b]	260[ab]	227[d]
Third leaf	214[a]	220[ab]	223[b]	222[b]	225[b]	270[a]	252[b]	232[c]	239[d]	231[c]
Fourth leaf	227[a]	232[ab]	239[b]	245[c]	241[b]	276[a]	270[b]	260[c]	250[d]	250[d]
Fifth leaf	226[a]	231[ab]	246[b]	260[d]	252[c]	296[a]	281[b]	272[bc]	262[c]	265[c]
Seventh leaf	241[a]	246[a]	280[b]	324[c]	284[b]	460[a]	423[b]	382[c]	308[e]	332[d]
Flag-1 leaf	248[a]	253[a]	284[b]	326[d]	292[c]	468[a]	461[b]	437[c]	319[e]	403[d]
Flag leaf	252[a]	256[a]	292[b]	386[c]	294[b]	542[a]	510[b]	468[c]	347[e]	401[d]

* At each leaf stage, L94 background NILs were compared with L94, and Vada background NILs were compared with Vada. Means followed by a common letter are not significantly different according to LSD test ($P < 0.05$)

29 h in L94-*Rphq2* in 2008 and by 20 h in L94-*Rphq2* in 2009 compared with the LP in L94. In contrast, the *rphq2* allele shortened the LP by 32 h in Vada-*rphq2* in 2008 and by 23 h in Vada-*rphq2* in 2009 compared with the LP in Vada. At the second leaf stage, *Rphq2* was less effective than at the first leaf stage, but it was still the most effective QTL at conferring disease-resistance. As of leaf stage 3, the decreasing differences in LP between L94/L94-*Rphq2* and Vada/Vada-*rphq2* indicated that the effect of *Rphq2* had declined (Fig. 1). In L94-*Rphq2*, the effect of *Rphq2* became insignificant at leaf stage 5. However, replacing *Rphq2* in Vada with *rphq2* resulted in a significant reduction in LP even during the latest development stages (Tables 1, 2). It seemed that *Rphq2* was more effective in the Vada background than in the L94 background.

Rphq4's effect is highly enhanced beginning with the third leaf stage

At the first and second leaf stages, LP in L94-*Rphq4* and Vada-*rphq4* were similar to those in L94 and Vada, respectively, indicating that *Rphq4* conferred little or no resistance in those leaf layers. The first indication of the adult plant resistance conferred by *Rphq4* was at the third leaf stage when the LP in L94-*Rphq4* and Vada-*rphq4* were significantly different than in L94 and Vada, respectively (Tables 1, 2). In the 2008 experiment, the enhancement of *Rphq4* effects in the L94 background was obvious by the third leaf stage, which was mirrored by the sudden drop in LP in Vada-*rphq4* compared with Vada at the same leaf stage (Fig. 1a, b). In 2009, the enhancement of the effect of *Rphq4* was more gradual (Fig. 1c, d). In both years, the effect of *Rphq4* on LP was the largest of all individual *Rphq* QTLs as of the fourth leaf layer. At the highest leaf layers (flag leaves), this QTL explained at least 50% of the LP difference between L94 and Vada (Tables 1, 2).

Rphq3's effect is consistent at all plant stages

Our observations during the course of these experiments suggested that the phenotypic expression of *Rphq3* is plant stage-independent. This gene conferred a moderate level of resistance at all investigated plant stages. In the seedlings, *Rphq3* was effective, but LP in L94-*Rphq3* was shorter than in L94-*Rphq2,* and LP in Vada-*rphq3* was longer than in Vada-*rphq2*. On the first leaf, then, *Rphq3* had a smaller effect than *Rphq2* on LP. Starting with the fourth leaf stage, *Rphq3* had a significant effect on LP but was consistently less effective than *Rphq4* at increasing the LP (Tables 1, 2). In both backgrounds, the effect of *Rphq3* increased considerably at the third leaf stage in 2008. This effect, however, was not observed in the 2009 experiment (Fig. 1).

Additive effects of QTLs for partial resistance

Compared with the LP in the corresponding lines with single QTLs, the LP in lines L94-*Rphq2+3* and Vada-*rphq2+3* were longer and shorter, respectively. This result indicates that the combination of *Rphq2* and *Rphq3* in the same NILs background resulted in levels of resistance higher than the corresponding NILs with the single *Rphq2* or *Rphq3* (Table 2).

Discussion

The genes underlying QTLs are generally sensitive to genetic background and environmental factors (Mackay 2001). To accurately compare the effects of individual QTLs, it is necessary to minimize the influence of these factors. QTL-NILs, as used in the present study, constitute the appropriate material to more precisely estimate the effects of single QTL alleles. The susceptible line L94, the

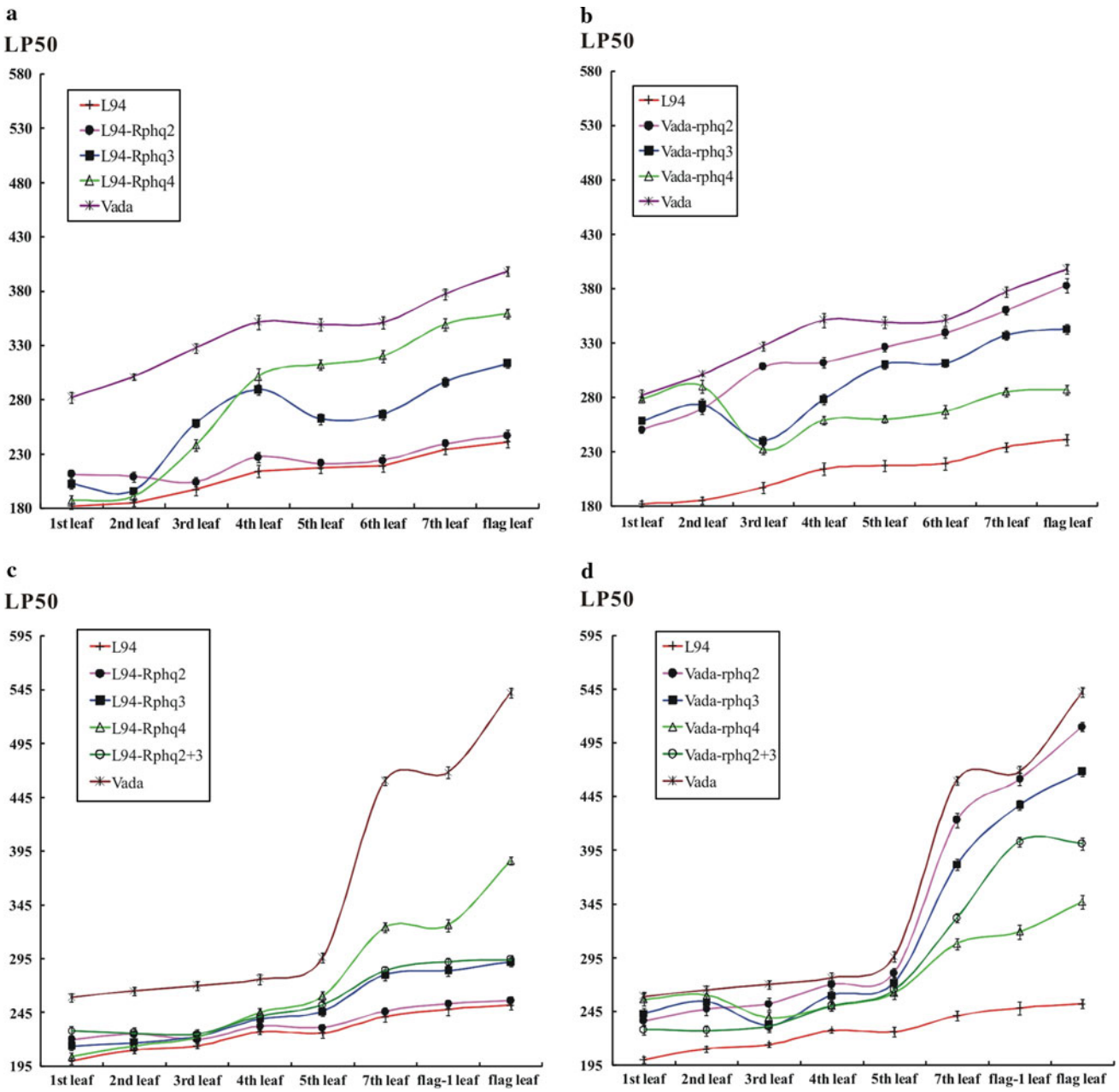

Fig. 1 Graphs of the latency period (*LP*, in hours) of *Puccinia hordei* isolate 1.2.1 in L94, Vada and the NILs. **a** LP graphs of *Puccinia hordei* isolate 1.2.1 in L94 and L94 background NILs in 2008. **b** LP graphs of *Puccinia hordei* isolate 1.2.1 in Vada and Vada background NILs in 2008. **c** LP graphs of *Puccinia hordei* isolate 1.2.1 in L94 and L94 background NILs in 2009. **d** LP graphs of *Puccinia hordei* isolate 1.2.1 in Vada and Vada background NILs in 2009. The *Y* axes represent the values of LP which was evaluated by estimating the period (in hours) at which 50% of the ultimate number of pustules became visible. The *X* axes represent the leaf stages. The value of LP was the mean of LP on 10–15 inoculated leaves. *Bars* indicate one standard error of the mean

partially resistant cultivar Vada and the NILs at eight barley life stages were evaluated concurrently under the same greenhouse conditions, providing an accurate and detailed insight into the interactions between QTLs and plant stages. The present results confirm that *Rphq2* has a strong effect in seedlings and almost no effect in adult plants. However, *Rphq4* is only strongly effective in adult plants, while *Rphq3* is consistently effective in both seedlings and adult plants. In the L94 × Vada mapping population, also Qi et al. (1998) and Marcel et al. (2008) found a strong effect of *Rphq2*, a medium effect of *Rphq3* and no or hardly significant effect of *Rphq4* in the seedling stage. In adult plants, *Rphq2* was reported to give a very low and just significant effect in both greenhouse and field test

(Qi et al. 1998) if measured in the mapping population. In a field test on the QTL–NILs, *Rphq2* also gave a small effect on infection severity (Marcel et al. 2008), which varied according to the isolate tested. In the QTL–NILs test by Marcel et al. (2008), the effect of *Rphq4* tended to be smaller than that of *Rphq3*, in contrast with results on the mapping population reported by Qi et al. (1998). This indicates that phenotypic expression of *Rphq4* may not be consistently expressed. Our data generally confirm the results of both studies on the relative effect size of these three *Rphq*-genes and plant stage in which they are effective. The present research expands upon these studies by including the effects of genetic background and the effects on all leaf layers of the plant. The data reveal that the third to fourth leaf stage is the critical stage in the decline of the effect of *Rphq2* and the increase of the effect of *Rphq4* (Tables 1, 2; Fig. 2).

The results obtained both in 2008 and 2009 showed that *Rphq4* had significant effects as of the seventh leaf stage (Fig. 1), indicating that the seventh leaf will be useful for the reliable evaluation of *Rphq4*-conferred adult plant resistance in individual plants. Moreover, multiple disease evaluations can be conducted for *Rphq4* in individual plants by inoculation of the fourth leaf to the flag leaf, respectively, enabling a more reliable evaluation of *Rphq4* in a single plant. Barley stripe mosaic virus-induced gene silencing (BSMV-VIGS) is an important tool for the analysis of gene function in barley (Holzberg et al. 2002; Lacomme et al. 2003) and has been successfully used to define the function of important genes (Hein et al. 2005; Shen et al. 2007). In BSMV-VIGS system, photobleaching symptoms were usually observed approximately 3 weeks after the viral inoculation. This result suggests that if the virus is applied to the first leaves, the VIGS effect will only start to become effective as of the third leaf. The low phenotypic expression of *Rphq2* in advanced barley developmental stages suggests that VIGS might not be a promising system to study the functions of the candidate genes for *Rphq2*. But for *Rphq3* and *Rphq4*, where the resistance persists throughout the rest of the barley developmental stages, the BSMV-VIGS system will be a useful tool for the functional analysis of the candidate genes.

The disease evaluations were conducted in spring twice, in March 2008 and April 2009. The leaf rust developed more rapidly in 2008 than in 2009 (Fig. 1). This difference may have been due to the influence of inoculation density, which was two times higher in 2008 than in 2009. Also, temperature may have played roles in causing the difference, since inoculation and disease development in 2008 coincided with sunny weather, whereas a few days of rain followed the inoculation in 2009. At the third and fourth leaf stages, the effects of *Rphq3* and *Rphq4* in 2008 were somewhat different than in 2009. Such variations in the effects suggest a possible sensitivity of the two QTLs to environmental conditions. LP increased as plant development progressed. This phenomenon was also observed by Parlevliet and Kievit (1986). Moreover, LP in the susceptible

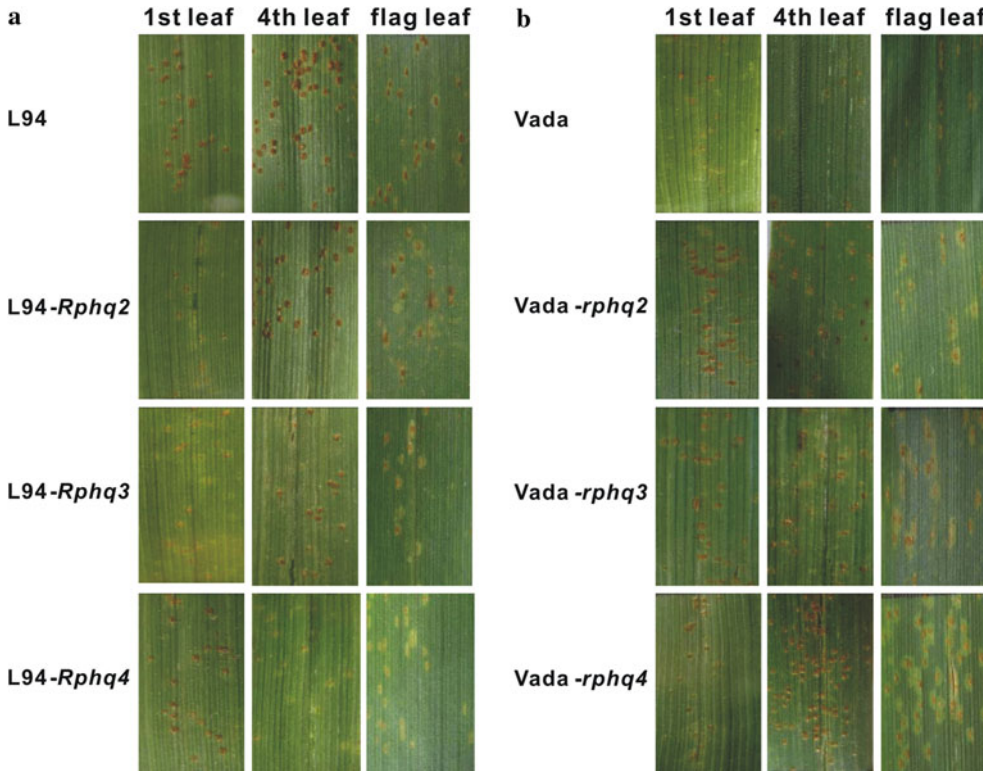

Fig. 2 The leaves of **a** the susceptible line L94, L94 background NILs and **b** the resistant line Vada and Vada background NILs after inoculation with *Puccinia hordei*. The pictures were taken 228 h after inoculation (hai) in the first leaf, 302 hai in the fourth leaf and 372 hai in the flag leaf, respectively

parent (L94) was less prolonged by advanced growth stages than that in the partially resistant cultivar Vada (Fig. 1), which is in accordance with the finding of Pretorius et al. (1988) in wheat–wheat leaf rust interaction.

The present results indicate that *Rphq2* switches off, and *Rphq4* switches on, at the third to fourth leaf stage, which coincides with the tillering stage. Tillering was reported to be associated with the onset of adult plant resistance to *Pyrenophora teres* in barley (Tekauz 1986; Douiyssi et al. 1998), *Puccinia striiformis* in wheat (Ma and Singh 1996) and *Xanthomonas campestris* pv. *oryzae* in rice (Qi and Mew 1985). Tekauz (1986) tested 12 Canadian barley cultivars at the seedling, tillering and heading stages for their reaction to *Pyrenophora teres* and found that changes of resistance between seedling and headed plants were clear at the tillering stage. Ma and Singh (1996) found that the adult plant resistance to stripe rust in wheat cultivars may begin at mid tillering and start even earlier (beginning of tillering) in highly resistant cultivars. Qi and Mew (1985) observed adult resistance to *Xanthomonas campestris* pv. *oryzae* in rice cultivars at the tillering stage. Besides tillering, other developmental transitions, such as the juvenile-adult transition and the onset of floral development, have been reported to be associated with a constant increase in resistance levels (Hunter et al. 1977; Lazarovits et al. 1981; Leisner et al. 1993; Heath 1994; Abedon and Tracy 1996; Coelho et al.1998; Century et al. 1999; Hugot et al. 1999; Panter and Jones 2002; Panter et al. 2002; Rusterucci et al. 2005). The coincident switching off of *Rphq2* and switching on of *Rphq4* at tillering suggests that the two QTLs are involved in different defense pathways.

Only a few reports have dealt with the mechanisms responsible for plant development-related resistance (Kus et al. 2002; Cameron and Zaton 2004; Hugot et al. 2004; McDowell et al. 2005). Phytohormones, which are growth regulators, are involved in plant development as well as in defense pathways (Whalen 2005; Develey-Rivière and Galiana 2007; Bari and Jones 2009). Gibberellins (GAs), promote seed germination and stem elongation (Poethig 2003; Bäurle and Dean 2006) by stimulating the degradation of negative regulators called DELLA proteins (Peng et al. 1997, 1999). Recently, DELLA proteins have been regarded as integrators and regulators of plant defense pathways (Navarro et al. 2008; Grant and Jones 2009; Peng 2009). Developmental changes or transitions, such as tillering and flowering, are associated with changes in the levels of different phytohormones (Gray 2004). Such changes also may result in alterations in the expression of defense related genes and perturbations in plant defense pathways (Develey-Rivière and Galiana 2007; Chung et al. 2008; Navarro et al. 2008; Zhao and Qi 2008; Bari and Jones 2009; Grant and Jones 2009). The complex networks regulating both development and defense remain enigmatic. In the L94 × Vada population, the three QTLs for days to heading and the partially resistant QTLs, *Rphq2 and Rphq4*, inherit independently (Qi et al. 1998). Therefore, the transitions to advanced stages of plant development, like anthesis, may have less impact on disease resistance than the earlier stages like tillering. We are working towards elucidating the mechanisms underlying partial resistance throughout barley development by cloning the developmental stage-dependent QTLs, *Rphq2* and *Rphq4*.

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