The *R_{Pi-mcd1}* Locus from *Solanum microdontum* Involved in Resistance to *Phytophthora infestans*, Causing a Delay in Infection, Maps on Potato Chromosome 4 in a Cluster of NBS-LRR Genes

M. Y. Adillah Tan, Ronald C. B. Hutten, Carolina Celis, Tae-Ho Park, Rients E. Niks, Richard G. F. Visser, and Herman J. van Eck

Laboratory of Plant Breeding, Wageningen University and Research Centre, P.O. Box 386, 6700 AJ, Wageningen, The Netherlands

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The distinction between field resistance and resistance based on resistance (*R*) genes has been proven valid for many plant–pathogen interactions. This distinction does not seem to be valid for the interaction between potato and late blight. In this study, a locus involved in late blight resistance, derived from *Solanum microdontum*, provides additional evidence for this lack of distinction. The resistance is associated with a hypersensitive response and results in a delay of infection of approximately 1 to 2 weeks. Both a quantitative as well as a qualitative genetic approach were used, based on data from a field assay. Quantitative trait locus (QTL) analysis identified a QTL on chromosome 4 after correction of the resistance data for plant maturity. A qualitative genetic analysis resulted in the positioning of this locus on the short arm of chromosome 4 in between amplified fragment length polymorphism marker pCTmACG_310 and cleaved amplified polymorphic sequence markers TG339 and T0703. This position coincides with a conserved *Phytophthora* *R* gene cluster which includes *R2*, *R2-like*, *R_{Pi-blb3}*, and *R_{Pi-abpt}*. This implies that *R_{Pi-mcd1}* is the fifth *R* gene of this nucleotide-binding site leucine-rich repeat cluster. The implications of our results on *R*-gene-based and field resistance are discussed.

The disease late blight, caused by the oomycete pathogen *Phytophthora infestans*, is a serious worldwide threat to potato cropping. In order to reduce the damaging effects from *Phytophthora* infection, the use of genetically resistant plants is a potentially viable approach for disease management (Strange and Scott 2005). Eleven resistance (*R*) genes originating from the wild species *Solanum demissum* have been discovered in the last century: *R1*, *R2*, *R3*, and *R4* by Black and associates (1953); *R5* and *R6* by Eide and associates (1959); *R7*, *R8*, and *R9* by Malcolmson and Black (1966); and *R10* and *R11* by Malcolmson (1969). All these resistance genes offer race-specific hypersensitive resistance but have been proven not to be durable (Malcolmson and Black 1966).

To look for new sources of resistance that potentially could be more durable, researchers have turned to other wild potato

species besides *S. demissum*. The resistance gene *R_{Pi-ber}* from *S. berthaultii* mapped on chromosome 10 (Ewing et al. 2000; Rauscher et al. 2006). *R_{Pi-ber}* proved to be a new *R* gene after testing with a set of *Phytophthora* strains capable to identify each of the *R* genes *R1* to *R11*. Four *R* genes have been identified in the wild potato species *S. bulbocastanum*. The *RB* locus has been mapped on chromosome 8 by Naess and associates (2000). One allele of this locus has been cloned by Song and associates (2003) and another allele, *R_{Pi-blb1}*, by van der Vossen and associates (2003). Another gene that was cloned from *S. bulbocastanum* was *R_{Pi-blb2}*, located on chromosome 6 (van der Vossen et al. 2005). These two genes, *RB/R_{Pi-blb1}* and *R_{Pi-blb2}*, have tentatively been described as “broad-spectrum resistance genes.” This new terminology may imply that a larger number of elicitors is being recognized, or may just indicate the current absence of a single compatible race. Finally, two race-specific resistance genes, *R_{Pi-blb3}* and *R_{Pi-abpt}*, have been mapped to chromosome 4 (Park et al. 2005a and b).

In addition to the dominant *R*-gene-based resistance conferring the hypersensitive response (HR), the so-called field resistance also is known. This type of resistance is race nonspecific, quantitative, and considered to be polygenically inherited. It has been assumed that this type of resistance is more durable than *R*-gene-based resistance. Race-nonspecific foliage resistance is currently differentiated in either maturity-corrected resistance (Bormann et al. 2004), or maturity-associated resistance, as was shown by Toxopeus (1958), Van Eck and Jacobsen (1996), Collins et al. (1999), Oberhagemann et al. (1999), Costanzo et al. (2005), and Visker et al. (2005). The general importance of a locus near marker GP21 on chromosome 5 involved in both maturity and maturity-related resistance was reviewed by Simko (2002).

In addition to *S. bulbocastanum* and *S. berthaultii*, the late blight resistance offered by *S. microdontum* has been used in commercial potato breeding during the last decade, although little is known about its inheritance. Resistance derived from *S. microdontum* genotype MCD167 (accession BGRC 24981/CGN20597) has initially been studied using area under the disease progress curve (AUDPC) data from a field trial (Sandbrink et al. 2000). Their analysis of the quantitative inheritance of this resistance launched two assumptions, that the *S. microdontum* resistance i) was polygenic and ii) conferred race-nonspecific resistance. Sandbrink and associates (2000) identified a major quantitative trait locus (QTL) on chromosome 4 explaining 40% of the phenotypic variation,

Corresponding author: H. J. van Eck; E-mail: Herman.vanEck@wur.nl

Current address of T.-H. Park: Brassica Genomics Team, National Institute of Agricultural Biotechnology, 224 Suinro Gwonseon-gu, Suwon 441-707, Republic of Korea.

and two QTL on chromosome 5. An additional major QTL was identified in the same study in genotype MCD 178 (accession BGRC 24981/CGN20597). Bisognin and associates (2005) have also described a field resistance derived from *S. microdontum* (BGRC 27353/CGN20640); however, the linkage group containing the only QTL could not be assigned to a specific potato chromosome. Last, it is known that the foliage resistance observed in *S. microdontum* BGRC 18302 (CGN21342) is also effective in the tuber (Park et al. 2005d).

In this article, we describe the genetic and phenotypic analysis of late blight resistance derived from *S. microdontum*. Our plant material represents a different accession than the accessions described by Sandbrink and Bisognin. The accession used in this study is also used in commercial breeding programs in the Netherlands and consistently displays a 1- to 2-week delay in infection in the field (*unpublished data*). Furthermore, we have mapped our locus on chromosome 4 and narrowed down its position to a conserved *Phytophthora* R gene cluster which includes *R2*, *R2-like*, *R_{Pi-blb3}*, and *R_{Pi-abpt}*.

RESULTS

In vitro and detached-leaf assay.

Because of its efficiency and previous successes, the in vitro late blight resistance assay (Huang et al. 2005) was used to classify 371 seedlings of mapping population RH94-076. The

Fig. 1. Pedigree of the *Solanum microdontum*-derived mapping population RH94-076, derived from accession BGRC 18302, backcrossed with susceptible diploid *S. tuberosum* clones.

susceptible control Bintje sporulated after 3 days. The resistant control 707TG11-1 showed an HR and no sporulation. Most of the genotypes of RH94-076 were sporulating within 7 days. In all, 128 genotypes sporulated without an HR and 186 genotypes showed a weak, incomplete HR before sporulation. At the end of the observation period, 12 genotypes showed no symptoms and 45 genotypes gave an HR and no sporulation; therefore, those 57 could be considered as resistant. These results indicated that some form of resistance is present. Based on these observations, unambiguous classification of seedlings as resistant or susceptible was impossible. The most important problem was that no apparent Mendelian segregation ratio could be identified, preventing a BSA approach. For these reasons, we proceeded with a detached-leaf assay.

In the detached-leaf assay, the *S. microdontum*-derived BC1 parent RH90-038-21 was resistant, showing HR, and the *S. tuberosum* parent RH88-025-50 was susceptible. However, clones of the mapping population showed inconsistent phenotypes, where sometimes only one or two leaves of the four leaves per genotype were infected and showed sporulation, whereas the others were without symptoms. When the entire detached-leaf assay was repeated, again with four leaves per genotype, the newly obtained results poorly correlated with the first assay. The phenotypic differences observed among the progeny displayed a continuous distribution and could not be differentiated in a resistant or susceptible class. The resistance derived from *S. microdontum* appeared to be quantitative in nature, which could be much better evaluated using a field experiment. In summary, the value of the in vitro assay and the detached-leaf assay is limited, and only allows us to conclude that these tests require plant material with strong effect *R* genes, as shown before (Huang et al. 2005; Vleeshouwers et al. 1999).

Quantitative genetic analysis of disease resistance and plant maturity.

The mapping population (Fig. 1), consisting of 224 clones, was evaluated for the level of resistance in a field test in 2001. Observations ranged from 7 until 29 days postinfection (dpi). When rAUDPC (relative area under disease progress curve) was used as trait value in Kruskal-Wallis-based QTL detection, three QTL peaks were identified near markers eATGmCAG_235 (at

Fig. 2. Kruskal-Wallis curve showing the quantitative trait loci peak at the position of marker eATGmCAG_235 on chromosome 4 of *Solanum microdontum* parent (BC1) RH90-038-21, marking a locus with a major effect on percentage of leaf area infected by *Phytophthora infestans*.

chromosome 4), eACAmCAC_19, and Sti032 (both at chromosome 5) with K^* values of 78, 28, and 24, respectively. When percentage of infected foliage at a given day was used as trait value, higher K^* values were obtained, with QTL peaks at the same three markers.

The most prominent QTL was identified by any of the markers along maternal chromosome 4. Amplified fragment length polymorphism (AFLP) marker eATCmCAG_235 identified this QTL regardless of observation time during the whole time range of the experiment. The percentage of infected foliage at 9 to 11 dpi was the most informative trait value, showing the highest K^* values ($K^* = 90$ to 91) at AFLP marker eATGmCAG_235. At these days (9 to 11 dpi), all associations between the trait value and the markers of chromosome 4 reached significance levels of $P < 0.0001$, except for the most distal marker eATGmCAG_187 ($P < 0.005$). The position of the Kruskal-Wallis-identified QTL peak at 11 dpi is illustrated in Figure 2. With interval mapping using the angular transformed data, the explained variance was 50.3% at a log of the likelihood ratio (LOD) of 34, indicating a QTL with a large effect, located on chromosome 4. From 13 dpi, the test statistic lowered gradually from $K^* = 86$ at 13 dpi to $K^* = 31$ at 29 dpi (end of observation). At 29 dpi, the QTL effect resulted in an explained variance of 5.5% at a LOD of 2.88. Observations later than 11 dpi did not offer the same power to detect this QTL because, at later observations, an increasingly larger part of the population was completely infected.

Two other QTL involved in percent foliage infection were found, namely on chromosome 5 of the maternal and the paternal map. The QTL on maternal chromosome 5 of RH90-038-21 had significant ($p < 0.0001$) association with two AFLP markers and Sti032 marker with a K^* value of 17 increasing gradually to the highest value, $K^* = 32$, at 25 dpi. With interval mapping, the QTL increased gradually to the highest value of LOD 8.85 with an explained variance of 16.2% at 25 dpi. The QTL on paternal chromosome 5 of RH88-025-50 had significant ($P < 0.0001$) association with three AFLP markers and SSR markers STM5148 and Sti032. From 7 dpi, the K^* value of 19 increased gradually to the highest $K^* = 39$ value at the final observation date. With interval mapping, this QTL reached the highest LOD 6.36 at 27 dpi (11.6%).

When observations for maturity type were used as trait value in MapQTL, two K^* peaks were observed near markers eACAmCAC_19 ($K^* = 31$) and Sti032 ($K^* = 41$) on paternal and maternal linkage group 5, respectively. On female and male chromosome 5, the QTL involved in percent infected foliage

Fig. 3. Late blight disease progress curve in percentage of leaf area infected across 23 days postinfection (dpi) of 244 offspring clones of *Solanum microdontum* BC2 population RH94-076.

and maturity type coincided; therefore, we proceeded with QTL analysis using maturity-corrected resistance (MCR) (Bormann et al. 2004; Visker et al. 2004). This trait value represents late blight resistance compensated for the effect of maturity, and was obtained by subtracting maturity values from percent foliage infection, leaving the residuals. MapQTL analysis using the residuals (MCR) resulted in the detection of the same QTL on chromosome 4 with equal significance, but the QTL on chromosome 5 were no longer detected ($P > 0.05$).

In conclusion, among the initial three QTL, we deduced that the large-effect QTL near marker eATGmCAG_235 on chromosome 4 of the resistant parent is a major-effect QTL involved in *Phytophthora* resistance. The smaller-effect QTL, both residing on chromosome 5 of either the susceptible and resistant parent, are QTL involved in plant maturity, and affect late blight infection indirectly.

Qualitative genetic analysis of resistance.

In addition to quantitative trait analysis, an attempt was made to categorize offspring clones in a resistant and susceptible class. The disease progress curves of each offspring genotype are shown in Figure 3. This figure suggests that the most clear-cut classification can be made at 11 dpi. The distribution at 11 dpi is presented in Figure 4. At 11 dpi, the susceptible group had an average foliage infection of 64% whereas the resistant group was on average 7% attacked. On the last day of observation (29 dpi), the susceptible group was 100% infected and the resistant group had an average foliage infection of 88%, using the tentative classification of the offspring at 11 dpi. The 11 dpi classification resulted in a segregation of 150 resistant and 74 susceptible genotypes, which is a significant deviation from a 1:1 ratio ($\chi^2 = 25.8$; $P < 0.0001$). Linkage analysis with this locus representing late blight resistance along with the molecular markers resulted in the construction of a linkage group with a locus named $R_{Pi-mcd1}$ (Fig. 5). The mapping

Fig. 4. Histogram showing the frequency distribution of percent leaf area infected by late blight at 11 days postinfection (dpi), the observation date which gives the most clear-cut division between resistance and susceptibility. The arrow indicates the cut-off value (the average population infection at 11 dpi = 25.7%). Genotypes to the left of the arrow were classified as resistant, genotypes to the right were classified as susceptible. Black = $R_{Pi-mcd1}$ present and white = $R_{Pi-mcd1}$ not present according to flanking markers.

results of $R_{Pi-mcd1}$ allowed validation of the qualitative genetic analysis of late blight resistance, because the expected resistance genotype, based on flanking markers, could be compared with the observed phenotype based on tentative classification. Only three false-resistant phenotypes (clones 59, 187, and 232) and seven false-susceptible phenotypes (clones 20, 21, 84, 135, 140, 264, and 276) among 224 offspring classifications were identified (Fig. 4). All of these false resistant or susceptible classifications belonged to genotypes without recombination event in linkage group 4 and, thus, could not interfere with the position of $R_{Pi-mcd1}$ on the short arm of chromosome 4. At 13 dpi, three additional resistant plants were incorrectly scored as susceptible and one susceptible plant was incorrectly scored as resistant.

Based on the absence or presence of $R_{Pi-mcd1}$ in the offspring, the two average disease progress curves could be calculated. These average curves clearly differ in slope. At 50% loss of leaf tissue, the infection rates were 8.4% (without $R_{Pi-mcd1}$) and 5.1% (with $R_{Pi-mcd1}$) increase of foliage infection per day. The average difference in time to reach the level of 50% leaf infection was 11.3 days between genotypes without $R_{Pi-mcd1}$ compared with genotypes with $R_{Pi-mcd1}$.

Anchoring of $R_{Pi-mcd1}$ and saturation of the chromosome 4 linkage map.

The identity of the linkage group comprising the QTL or $R_{Pi-mcd1}$ locus was first indicated by several AFLP markers which were known chromosome-4-specific markers. This was confirmed when RFLP marker TG339, converted into a cleaved amplified polymorphic sequence (CAPS) marker, mapped six

recombination events (2.8 cM) below $R_{Pi-mcd1}$ (toward the centromere) as is shown in Figure 5.

BSA with 256 primer combinations of PstI+NN/MseI+ANN resulted in five bulk-specific bands, of which two were false-positive bands, two markers pCTmAGC_378.0 and pCTmACG_90 mapped in coupling phase, and marker pCTmACG_310, in repulsion phase, showed complete cosegregation with $R_{Pi-mcd1}$. The second BSA, with 256 primer combinations PstI+NN/MseI+CNN, did not result in additional markers.

Tomato COS marker T0703, converted into a CAPS marker, mapped at the same position as TG339. Tomato RFLP marker CT229, converted into a CAPS CT229, mapped nine recombination events (4.2 cM) distal of $R_{Pi-mcd1}$. By the addition of this marker, marker pGTmACG_310, no longer co-localized with $R_{Pi-mcd1}$; a genotype which previously was thought to have a singleton event, proved to be a true recombinant, with the recombination between marker pGTmACG_310 and $R_{Pi-mcd1}$.

AFLP marker pGTmACG_310 was excised from gel and sequenced, and a Blastn search resulted in strong homology to tomato BAC AF411807 (van der Hoeven et al. 2002), which was assigned to chromosome 4 of tomato between markers CT229 and TG370 (Tanksley et al. 1992). The sequence of marker pGTmACG_310 was aligned with vector NTI to position 95,735 to 95,837 of BAC AF411807, which is close to the R end of this 95,845-bp-long BAC sequence.

The segregation pattern based on the classification using 11 dpi positioned $R_{Pi-mcd1}$ proximal to marker pGTmACG_310 on the graphical genotype map, with one recombination event between marker pGTmACG_310 and $R_{Pi-mcd1}$ at a distance of 0.5

Fig. 5. $R_{Pi-mcd1}$ maps on chromosome 4 to a known cluster of resistance genes and nucleotide-binding site leucine-rich repeat resistance gene candidates. **A**, Genetic map of chromosome 4 of the maternal resistant parent RH90-038-21 showing the location of $R_{Pi-mcd1}$. CEN = centromere location. **B**, Genetic map of a part of chromosome 4 with the location of $R_{Pi-blb3}$ (Park et al. 2005b). **C**, High-resolution map of the chromosome 4 interval distal to $R_{Pi-blb3}$ with the genetic position of CAPS marker AF411807L and AF411807R based on the BAC end sequences, and three resistance (*R*) gene homologs (RGH1-3) of tomato BAC AF4011807. Amplified fragment length polymorphism marker pGTmACG_310 from this study was identified on BAC AF411807 near the position of AF411807R.

cM. *R_{Pi-mcd1}* was positioned at a distance of nine recombination events (4.2 cM) distal to the flanking markers T0703 and TG339. *R_{Pi-mcd1}* appeared to be located on the short arm of chromosome 4. Centromere location was inferred from the clustering of *EcoRI/MseI* markers around the centromere (Park et al. 2007; van Os et al. 2006) and from tomato and AFLP anchor markers.

Validation of CAPS markers.

CAPS markers TG339, CT229, and T0703 were tested for *S. microdontum*-specific polymorphism in a range of germplasm ($n = 96$) comprising DNA of *S. tuberosum*, *S. berthaultii*, *S. microdontum*, *S. bulbocastanum*, *S. tarijense*, *S. fendleri*, *S. hougassi*, and *S. vernei*. None of the marker alleles were exclusively diagnostic for *R_{Pi-mcd1}*. Neither were these markers specific for wild germplasm and absent in cultivated potato. Therefore, these markers, in combinations with these restriction enzymes, which work well for this mapping population, cannot be used for marker-assisted breeding in a wider range of germplasm.

DISCUSSION

In this article, a range of *Phytophthora* assays has been used that are usually not applied in combination on the same material. Although the in vitro and detached-leaf assays did not contribute to the localization of *R_{Pi-mcd1}*, the joint results allowed us to characterize the phenotype of *R_{Pi-mcd1}* as a locus involved in late blight resistance, and to evaluate our results in relation to current models on late blight resistance.

The in vitro assay resulted in inconclusive data, because most of the genotypes of the *S. microdontum* mapping population were sporulating within 7 days although half of the population initially showed a weak HR. With the detached-leaf assay, clones of the mapping population showed inconsistent phenotypes. These results indicated that some form of resistance should be present in this material; however, a qualitative genetic interpretation of the in vitro and detached-leaf assays was not obvious. For a quantitative interpretation, data were subsequently collected from a field assay. When using rAUDPC and percent foliage infection as trait values, three QTL were detected. One major QTL associated with resistance was located on chromosome 4. The two other QTL were detected on the maternal and paternal linkage groups corresponding to potato chromosome 5. However, when the trait values were corrected for maturity, QTL on chromosome 5 could no longer be detected. This indicates that the QTL of chromosome 5 were involved in plant maturity.

The rAUDPC is currently the most accepted trait value to assess resistance levels. In this study however, the best trait value for QTL analysis proved not to be the rAUDPC but percent foliage infection at 9 to 11 dpi. The latter method showed the highest association in K^* values in QTL analysis. The explanation for the lower power of rAUDPC as a trait value in QTL identification can be the effect of inclusion of too late and, thus, indiscriminate observations within the highly informative observation at the beginning.

However, the best approach to localize hereditary factors is not QTL analysis at all but Mendelian classification. Classification of the foliage infection values resulted in accurate localization of *R_{Pi-mcd1}* on chromosome 4. At this genetic position, only 10 false positives or false negatives were inferred using markers flanking *R_{Pi-mcd1}* among 224 offspring. Timing is important, because the observations at 7 to 11 dpi—the early start of infection—resulted in a reliable classification. When later time points were used, additional genotypes resulted in misclassification (based on the flanking markers) as resistant or

susceptible. Classification results in a single genetic position, whereas the QTL peak (Sandbrink et al. 2000) occupies a much larger genetic interval. It cannot be excluded that the resistance QTL on chromosome 4 reported by Sandbrink and associates (2000) is identical to the locus mapped in this study, although a different accession, BGRC 24981, was used.

Marker saturation with BSA resulted in the identification of marker pGTmACG_310 at a distance of 0.4 cM of *R_{Pi-mcd1}*. The DNA sequence of this marker showed strong homology to a part of tomato BAC AF411807, which had been identified in a previous study (Park et al. 2005a). Park and associates identified the same BAC clone with AFLP marker pATmAGA_307 which was tightly linked (<0.1 cM) to another late blight resistance gene, *R_{Pi-abpt}*, on chromosome 4. On this BAC clone, three nucleotide-binding site leucine-rich repeat (NBS-LRR) genes were identified with high homology to AFLP marker pATmAGA_307, which also has an NBS-LRR sequence. Currently, three more resistance genes, *R2*, *R2-like*, and *R_{Pi-blb3}*, have been mapped along with *R_{Pi-abpt}* in this chromosome 4 *R*-gene cluster (Li et al. 1998; Park et al. 2005a, b, and c). Our data indicate that *R_{Pi-mcd1}* is identified as the fifth member of this *R*-gene cluster.

Strong indication that *R_{Pi-mcd1}* belongs to the family of NBS-LRR genes.

In our view, the mapping of *R_{Pi-mcd1}* in a well-known *R*-gene cluster of NBS-LRR genes is the most fascinating result of this study. Theoretically, we cannot exclude that the gene underlying the *R_{Pi-mcd1}* locus does not belong to the family of NBS-LRR genes. However, other projects in our lab (E. van der Vossen, *personal communication*) suggest a limited genetic and physical size of the region on potato chromosome 4, comprising the series of NBS-LRR genes underlying the *R_{Pi-blb3}*, *R2*, *R2-like*, and *R_{Pi-abpt}* loci. Thus, it appears that it is more plausible to accept the hypothesis that *R_{Pi-mcd1}* is a member of an allelic series of *R* genes than to expect an entirely different kind of gene, although supporting information is required to arrive at a final conclusion.

Embarking from the hypothesis that *R_{Pi-mcd1}* is an NBS-LRR gene, we should now discuss the phenotypic effects observed for *R_{Pi-mcd1}* in comparison with the effects associated with NBS-LRR-based *R* genes involved in late blight resistance. To this end, we first need to give an overview of the different models of the potato-late blight interaction, as they have appeared in the scientific literature (Table 1).

Different models of potato-late blight interaction.

The most intriguing distinction between late blight resistance models is based on *R*-gene-based and non-*R*-gene-based resistance. This distinction is of primary importance because, in general, *R*-gene-based resistance is assumed to be race specific and not durable, whereas non-*R*-gene-based resistance should be durable, race-nonspecific, and, thus, valuable (van der Plank 1968). The *R*-gene-based resistance is based on monogenic factors. The first resistance model in Table 1, “non-durable race-specific resistance,” refers to the *S. demissum*-derived *R* genes which were soon defeated once cultivars were grown on a larger scale. Although lumped in one model, there are differences between the effects of these *R* genes. Some *R* genes are known as stronger-effect (*R1* and *R3*) or weaker-effect (*R10*) *R* genes. Furthermore, although defeated, *S. demissum* *R* genes such as *R2* still offer protection in certain growing areas (Pilet et al. 2005), which might be explained by the possibility that a proportion of the *Phytophthora* population participating in the epidemic carried the avirulence factor to *R2*, although increased deployment of cultivars with *R2* is unlikely to make a lasting contribution to late-blight control.

The second resistance model, “broad-spectrum resistance,” refers to *S. bulbocastanum*-derived resistance (Song et al. 2003; van der Vossen et al. 2003, 2005). Because this resistance is based on the usual NBS-LRR-type *R* gene, we assume that broad spectrum only indicates the current absence of a compatible race. There is no reason to assume that the durability of these newly deployed *R* genes would differ from the durability experienced in the 20th century with *S. demissum*-derived resistance. Therefore, we anticipate that our first and second models are not essentially different.

The third model, “residual resistance,” refers to the contribution of defeated *R* genes to the quantitative level of field resistance (Durel et al. 2003; Pedersen and Leath 1988; Stewart et al. 2003). A classical example of a residual effect of defeated *R* genes has been presented for various *Xanthomonas* resistance genes in rice (Li et al. 1999). This term should not be confused with the horizontal resistance observed after vertical resistance has been defeated (van der Plank 1968). Here, the residual effect is ascribed to the defeated *R* gene itself (Durel et al. 2003). Therefore, residual resistance should not be regarded as a different model but as an asset of the normal *R* genes belonging to the first model.

The fourth model, “field resistance in *R*-gene-free cultivars,” was historically pursued in response to the lack of durable *R* genes. Using old potato cultivars, Colon and associates (1995) observed that current resistance ratings correlated well with ratings given between 1929 and 1954. This suggests that old potato cultivars, free from *R* genes, offer a stable and heritable source for resistance. Although Colon and associates (1995) noticed the correlation between plant maturity and late blight resistance, they suggested the presence of durable resistance, acting in addition to the maturity effect (Toxopeus 1958). The stability of resistance to *P. infestans* in old cultivars without *R* genes (Colon et al. 1995; van der Plank 1971) resulted in a new breeding strategy as promoted by Turkensteen (1993) and Landeo and associates (1995). However, stable field resistance as perceived by Van der Plank and Colon and associates in old potato cultivars is, in our opinion, nothing but the effect of plant maturity and, perhaps, the residual effect of indigenous *S. tuberosum* *R* genes. This view was also strongly advocated by Allefs and associates (2005).

The fifth model, “QTL involved in resistance,” is represented by a number of studies that were among the first to fully exploit the molecular genetic tools and QTL analysis. These studies embarked, in general, on the assumptions that resistance is phenotypically quantitative, race nonspecific, and possibly polygenically inherited. The choice of the plant material in these studies was largely influenced by the desire to reidentify not *R* genes but loci involved in partial, field, or horizontal resistance. The major QTL identified by the studies of Leonards-Schippers and associates (1994), Collins and associates (1999), and Oberhagemann and associates (1999)

were always on potato chromosome 5 and coincided with a QTL for maturity type. In our opinion, the major QTL involved in resistance are essentially the same as the loci involved in field resistance of the fourth model (van Eck and Jacobsen 1996). Current methods of data analysis include maturity as co-variable during QTL analysis. Alternatively, resistance data are corrected for plant maturity (MCR), hence leaving the residual variance as trait value (Bormann et al. 2004; Visker et al. 2003). In view of our remarks on the fallacy of field resistance in *R*-gene-free material, we assume that MCR is caused by genes that belong to the NBS-LRR family.

Sifting through these views on the potato–late blight interaction, we construe that, in essence, resistance is determined by two factors only: plant maturity and *R* genes that belong to the NBS-LRR family. The latter factor can be subdivided in stronger- and weaker-effect *R* genes, broad-spectrum and race-specific *R* genes, broken *R* genes with larger or smaller residual effects, and so on. Nevertheless, all these classes are to be ascribed to NBS-LRR genes and the underlying elicitor–receptor model (Allefs et al. 2005; Kamoun et al. 1999).

Evaluation of *R_{Pi-mcd1}* in relation to current models of potato–late blight interaction.

Having described i) the phenotypic effects of *R_{Pi-mcd1}* in different assays, ii) the quantitative and qualitative genetic analysis of the resistance, and iii) its genetic position in an *R*-gene cluster on potato chromosome 4, we now wish to evaluate *R_{Pi-mcd1}* in relation to the models of the potato–late blight interaction described above.

Our quantitative genetic analysis of *R_{Pi-mcd1}* resulted in explaining 50% of the phenotypic variance. This suggests partial resistance, as described in our fourth and fifth models; however, our phenotypic observations disagree with the commonly held views on partial resistance. By definition, partial resistance results in a reduced epidemic build-up of the pathogen, notwithstanding a susceptible, nonhypersensitive infection type (Parlevliet and van Ommeren 1975). Partial resistance due to genes with small effects is thought not to be based on receptor-elicitor recognition (McDonald and Linde 2002). The effect of *R_{Pi-mcd1}* is associated with an HR, followed by sporulation. The HR is not in accordance with the definition of partial resistance but fits more closely with observations described by Vleeshouwers and associates (2000), where the quantitative nature of *P. infestans* resistance is explained by differences in timing of HR induction and differences in percentage of cells displaying HR after infection, possibly due to inadequate or delayed recognition of elicitors by weak-effect *R* genes. Their histological studies also indicated that, at the cellular level, the difference between compatible and incompatible interactions was quantitative rather than qualitative in nature. This may result in phenotypes which have been described by terms such as restricted lesion, a restricted but sporulating lesion, or a

Table 1. Overview of various models used in literature to describe the potato–late blight interaction in qualitative and quantitative genetic terms

Model	Genes	Literature reference
<i>R</i> -gene-based resistance ^a		
1. Nondurable race-specific resistance	<i>R1-R11</i>	Black et al. 1953; Eide et al. 1959; Malcolmson 1969; Malcolmson and Black 1966
2. Broad-spectrum resistance ^b	<i>RB, R_{Pi-blb1}</i>	Song et al. 2003; van der Vossen et al. 2003
3. Residual resistance	<i>R1, R10, R11</i>	Stewart et al. 2003
QTL-based resistance ^c		
4. Field resistance in <i>R</i> -gene-free cultivars	...	Colon et al. 1995; Landeo et al. 1995; Turkensteen 1993
5. QTLs involved in resistance ^d	Various QTLs	Bormann et al. 2004; Collins et al. 1999; Leonards-Schippers et al. 1994; Oberhagemann et al. 1999; Visker et al. 2003

^a Where often monogenic *R*-gene based resistance is implied.

^b Race-nonspecific resistance.

^c QTL = quantitative trait locus; where often a polygenic and non-*R*-gene based resistance is implied.

^d With or without correction for plant maturity.

spreading and sporulating lesion. In particular, the second table of Vleeshouwers and associates (2000) demonstrates that HR is observed during all forms of potato-late blight interaction (susceptible, *R*-gene-based, and nonhost resistance). Hence, HR and successful infection are not a paradox.

Our *S. microdontum*-derived BC2 mapping population was susceptible in the in vitro assay, susceptible in the detached-leaf assay, and showed a delayed infection in a field assay. The in vitro assay is known to be very efficient but also very rigorous and will allow the identification only of *R* genes with strong effects (i.e., which always show full resistance to avirulent *P. infestans* isolates) (Huang et al. 2005). Likewise, the detached-leaf assay is also more suitable for *R* genes with strong effects (Vleeshouwers et al. 1999). From this perspective, *R_{Pi-mcd1}* should be considered as an *R* gene with a weak effect. Thus, the phenotype of *R_{Pi-mcd1}* resembles the incompatible reaction of the *S. demissum* differentials *R10* and *R11* (Huang et al. 2005), supporting our conclusion that *R_{Pi-mcd1}* may belong to the family of NBS-LRR genes.

We have never observed any race-specific interaction for *R_{Pi-mcd1}*. The 1- to 2-week delay in infection in the field has been observed consistently in material from various backcross generations and regardless of the late blight races (*unpublished results*). The material is also used by commercial breeders, who annually confirm the consistent effect of delay of infection against randomly blown-in *P. infestans* isolates. Therefore, *R_{Pi-mcd1}* cannot be regarded as a broken *R* gene (first model). Neither can *R_{Pi-mcd1}* be regarded as a gene involved in residual resistance (third model) because, from a historical perspective, the *R_{Pi-mcd1}* locus never conferred complete resistance (was never unbroken) and all races ultimately sporulate (there is no distinction between compatible and incompatible races).

This effect results in the conclusion that *R_{Pi-mcd1}* combines two features. It is both a weak resistance gene as well as a broad-spectrum (race-nonspecific) resistance gene. Nevertheless, the value of a weak *R* gene in economic terms is considerable. When infection is delayed for 1 week in organic potato cultivation, at a biomass production of 1 ton per hectare per day, the economic value of such a delay exceeds \$1,500 per hectare.

For decades, it has been advocated that breeding for durable late blight resistance should require the screening of potato germplasm for the absence of *R* genes. These nondurable *R* genes only blurred the view on the level of partial, race-nonspecific, field resistance. In our opinion, this is a flawed strategy and may have unnecessarily removed *R* genes from the potato gene pool. Moreover, it ignores the possible residual effect of broken *R* genes, as was demonstrated by Stewart and associates (2003). The *R_{Pi-mcd1}* gene described in this article indicates that partial resistance could well be *R*-gene based. We conclude that NBS-LRR-genes, sometimes in black and white but also in many shades of gray, all possess value in breeding for late blight resistance.

This example of *R_{Pi-mcd1}* illustrates the unwarranted relation between phenotype, experimental approach, and scientific model. Nonabsolute phenotypic differences should not necessarily be addressed by a quantitative genetic approach. Quantitative genetic studies should not necessarily result in QTL and exclude *R* genes from the hypothesis tested. In our opinion, the distinction that *R*-gene-based resistance should always give absolute resistance and that partial resistance is always mediated by QTL should be reevaluated.

MATERIALS AND METHODS

Plant material.

A diploid interspecific mapping population RH94-076 ($n = 224$) was created with a cross between RH90-038-21 and

RH88-025-50. The pedigree of RH94-076 is presented in Figure 1. Because of the susceptible back-cross (BC) parents and the agronomical selection in the BC1 offspring, this BC2 mapping population should be regarded as essentially a susceptible *S. tuberosum* genetic background with less than 12.5% *S. microdontum* introgression. The *S. microdontum* accession BGRC 18302 (CGN21342) was collected by Hjerting, during a Danish expedition (HPR 0293) in 1965 and 1966 to Argentina.

In vitro assay.

In all, 371 newly sown genotypes of RH94-076 were tested in an in vitro assay as described by Huang and associates (2005) with *P. infestans* isolate 90128 (race 1, 3, 4, 6, 7, 8, 10, and 11) provided by F. Govers, Laboratory of Phytopathology, Wageningen University, The Netherlands. Symptoms were recorded at 3, 4, 5, 6, and 7 days after infection. Sporulation or large-scale necrosis was interpreted as susceptible. An HR or lack of symptoms was interpreted as resistant. The susceptible cv. Bintje and the *R_{Pi-abpt}* containing resistant genotype 707TG11-1 (Park et al. 2005a) were used as control plants.

Detached-leaf assay.

Leaves collected from greenhouse plants of 96 genotypes of RH94-076 were tested in four replications with a detached-leaf assay (Vleeshouwers et al. 1999). Parents RH90-038-21 and RH88-025-50 were included as control plants. Complex isolate 90128 (race 1, 3, 4, 6, 7, 8, 10, and 11) was used to determine leaf resistance. Inoculation was performed as described by Vleeshouwers and associates (1999), except that the concentration of the inoculum was adjusted to 5×10^5 zoospores/ml.

Field test 2001.

The field test was performed in 2001 on 224 genotypes of RH94-076. The location of the field was the Meenthoeve, in the area near Wageningen, The Netherlands, on sandy soil. Tubers were planted on 15 May 2001. Experimental design was a randomized block design. Four tubers per genotype were planted. The four plants per genotype were treated as one experimental unit. Inoculation was performed with a spore suspension of a complex *P. infestans* isolate IPO-82001 (race 1, 2, 3, 4, 5, 6, 7, 10, and 11; provided by W. Flier, Plant Research International) as described by Vleeshouwers and associates (1999) on 11 July. Because of a lack of infection, the field was reinoculated with the same isolate on 17 July. Infection levels were scored 12 times, every other day, over a period of 3.5 weeks as the average leaf area covered by lesions (as a percentage of the total leaf area). Observation time points are indicated as days postinfection, taking 17 July as day zero. Values of rAUDPC were calculated as described in Fry (1978).

Maturity type was scored on a fungicide-protected field experiment in the third week of August on an ordinal scale ranging from 2 (green and vigorous, late to very late) to 6 (yellowing and plant is sagging) to 10 (dead, very early plant types).

Classification of foliage infection values into resistant or susceptible phenotypes.

Field test observations from 2001 were converted into a tentative phenotypic classification of descendants with or without resistance factors, taking the observations at 7 to 13 dpi. This classification used the population mean as threshold. Offspring with infection lower or higher than the population mean were classified as resistant or susceptible, respectively.

DNA extraction.

Genomic DNA of the individuals of mapping population RH94-076 and parents RH90-038-21 and RH88-025-50 was isolated from young leaf material as described by Van der

Beek and associates (1992). Tissue was ground with steel balls using a Retsch machine (Retsch Inc., Haan, Germany) in the STE buffer as described, with volumes adapted to 2-ml deep 96-wells Costar plates (Corning Inc., Corning, NY, U.S.A.).

Amplified fragment length polymorphism analysis.

AFLP analysis was performed according to Vos and associates (1995). Twelve primer combinations were used: E+AAC/M+CAC, E+AAC/M+CAG, E+AAC/M+CCA, E+AAC/M+CTG, E+AAT/M+CGA, E+ACA/M+CAA, E+ACA/M+CAC, E+ACA/M+CCT, E+ACA/M+CGT, E+AGA/M+CTC, E+ATG/M+CAG, and E+ATG/M+CTC. AFLP fragments were visualized on radioactive gels and scored visually for absence or presence. AFLP marker names are composed of the restriction enzyme combination followed by the three selective nucleotides of the primers followed by the migration on gel relative to the 10-base ladder (Sequemark, Research Genetics, Huntsville, AL, U.S.A.). Marker names with sizes in tenths of nucleotides refer to markers mapped before in reference populations. These reference markers can be used to assign linkage groups to potato chromosomes. The other markers are unique for this material.

Map construction.

With JoinMap (van Ooijen and Voorrips 2001), 210 AFLP markers were assigned to linkage groups using a LOD threshold of 5.0 for grouping. In a second version of the map, 32 simple-sequence repeat (SSR) markers were included for confirmation of chromosome identity. The entire map was composed of 12 maternal and 12 paternal linkage groups, with 26 markers remaining unassigned. Details on the map and map construction will be published elsewhere (J. Werij, *unpublished data*). The tentative classification of late blight resistance phenotypes, described above, was used as marker data of the *R_{Pi-mcd1}* locus and included in map construction.

Bulked segregant analysis.

Bulked segregant analysis (BSA) (Michelmore et al. 1991) was applied to identify additional markers in the proximity of the locus involved in resistance, with the aim to further saturate the genetic map. Four bulks were composed as follows: Br and Bs, each containing eight nonrecombinant genotypes with a resistant or susceptible phenotype, respectively; and two additional bulks, Brc (n = 5) and Bsc (n = 8), composed to narrow down the target window on chromosome 4, using descendants with crossover events between markers flanking the locus involved in resistance. The crossover events were inferred between AFLP markers eAGAmCTC_155 and eATGmCAG_187.2 with a distal position, as known from our marker catalog. Bulks were analyzed with 256 PstI+NN/MseI+ANN and 256 PstI+NN/MseI+CNN primer combinations. Fluorescently labeled AFLP fragments were visualized on a denaturing polyacrylamide gel using a NEN Global Edition IR2 DNA Analyzer (LI-COR Biosciences, Lincoln, NE, U.S.A.). AFLP primer combinations using *PstI/MseI* template were used, because the chromosomal distribution of *PstI/MseI* compares favorably with *EcoRI/MseI*-derived AFLP markers. The majority of *EcoRI/MseI*-derived AFLPs cluster in centromeric regions as shown by Van Os and associates (2006).

Excision of AFLP fragments.

PstI primer PstI+GT was radioactively labeled with ³³P. AFLP was performed according to (Vos et al. 1995). AFLP bands of marker pGTmACG_310 of eight samples (two of RH90-038-21, two of R88-250-50, plus four susceptible individuals) were excised from gel and dissolved in 50 µl of water. Supernatant was reamplified with P+0 and M+A primers and

purified with a G50 column before sequencing. pGTmACG_310 sequence was Blastn.

CAPS markers.

Further anchoring of the resistance locus was achieved with chromosome 4 specific markers TG339, CT229, and T0703. Polymerase chain reaction (PCR) primers were designed using sequence information from the SOL Genomics Network with Primer3 software (Rozen and Skaletsky 2000). PCR products were digested with 27 restriction enzymes to identify polymorphic sites. The potato version of TG339 was developed using GenBank accession number X87370 (*S. tuberosum* mRNA for 14-3-3 protein) obtained with Blastn using the original tomato TG339 restriction fragment length polymorphism (RFLP) sequence. TG339 primers were F:GCTGAACGCTATGAGGAG ATG and R:TGAGGTTATCACGCAGAAGTTG. The CAPS polymorphism was obtained by digestion with *MnII*. Marker CT229 was amplified with primers F:TTGTGAGTGGTGAA CTACGGGC and R:CGGCAATGGTTATGGGAACG (Park et al. 2005b), and displayed a CAPS polymorphism using restriction enzyme *HpyCH4IV*. The sequence of tomato COS marker T0703 was used for conversion into a CAPS marker. Primers for the T0703 marker were F:CCAGTAAGAACAAGCCG ATT and R:ATCACCAATTACGCGATCTA. A polymorphism was obtained upon restriction with *Bme1390I*.

Chromosome 4 map construction.

The marker order of maternal linkage group 4 including, the added AFLP markers from BSA and CAPS markers TG339, CT229, and T0703, was reassessed with RECORD (van Os et al. 2005) and visually inspected by graphical genotyping the raw data in MS-Excel using the conditional formatting of cells conditional to marker genotype and linkage phase. Marker pairs without obvious recombination events were placed at the same map position. This avoids suggestive marker orders without clear data support. Final map length of the linkage group, including *R_{Pi-mcd1}*, reflects the sum of adjacent recombination frequencies (Stam 1993).

QTL analysis.

QTL analysis was performed with MapQTL 5 (van Ooijen 2004) using a linkage map of 1,179.2 centimorgans (cM) (maternal plus paternal map length). An initial search for QTL was performed with Kruskal-Wallis because no assumptions of normality were made. For interval mapping purposes, the average leaf area covered by lesions (percent) per observation and per genotype was converted to degrees by the variance-stabilizing angular transformation (angle whose sine is the square root of the percentage of foliage infection).

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AUTHOR-RECOMMENDED INTERNET RESOURCES

National Center for Biotechnology Information Blast server:
www.ncbi.nlm.nih.gov/BLAST
 SOL Genomics Network website: www.sgn.cornell.edu
 Wageningen UR Plant Breeding website: www.dpw.wau.nl
 The Wageningen UR Plant Breeding group Ultra dense recombination map of potato website: www.plantbreeding.wur.nl/potatomap