Differential Recognition of *Phytophthora infestans* Races in Potato *R4* Breeding Lines

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ABSTRACT

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Introgression breeding has resulted in several potato lines that are resistant to late blight, a devastating plant disease caused by the oomycete *Phytophthora infestans*. The traditional differential set consists of potato lines with 11 late blight resistance specificities, referred to as *R1* to *R11*. With the exception of the *R4* locus, all the resistance loci in these lines have been genetically mapped or positioned in resistance (*R*) gene clusters. In this study, we show that potato lines that are defined to carry

R4 do not necessarily recognize the same *P. infestans* strains. Field isolates appeared to be avirulent on either the *R4* differential developed by Mastenbroek or the one developed by Black but not on both. Previously, we identified the avirulence gene *PiAvr4*, which is a member of the RXLR effector family. In planta expression of *PiAvr4* revealed that recognition of *PiAvr4* is strictly confined to the Mastenbroek *R4* differential. Segregation of the trait in two independent F1 progenies showed that late blight resistance in this differential is determined by a single dominant gene, now referred to as *R4^{Ma}*.

Additional keywords: gene-for-gene interaction, resistance gene.

The gene-for-gene model, first postulated by Harold H. Flor (16), states that a single resistance (*R*) gene product in the plant is required for recognition of a single protein encoded by a pathogen avirulence (*Avr*) gene. The recognition of such an *Avr* factor results in the activation of plant defenses that culminate in a hypersensitive response (HR), local cell death, and the halt of pathogen growth. Introgression breeding has been used to introduce disease resistance in many crop species. In potato, this has resulted in breeding lines and cultivars that are resistant to different races of the late blight pathogen *Phytophthora infestans* (10,14). Initially, 11 different recognition specificities were identified, all originating from *Solanum demissum* and named *R1* to *R11*. This was the basis for a differential set of potato lines used worldwide to determine the virulence phenotypes occurring in the pathogen population (5,9,27,28). Isolates are defined as virulent when they can infect and colonize a plant genotype carrying a particular *R* gene. These virulent isolates lack the cognate functional *Avr* factor. Isolates that have a functional *Avr* factor and, thus, elicit HR on plants with the cognate *R* gene are defined as avirulent. Virulence variability in *P. infestans* populations is recognized as a major reason for failure of *R* genes in cultivated potato. Therefore, insight into virulence phenotypes is of utmost importance for planning and successfully implementing breeding programs, and this requires standardized methods and standardized *R* gene differentials for phenotyping *P. infestans* populations.

In the last decade, several *R* genes that confer late blight resistance have been mapped in potato, and some have been

cloned (17,32). The *R1* gene was positioned on chromosome V in the same genetic region as the *Potato virus X* (PVX) resistance genes *NB* and *Rx2* (11). *R2* colocalizes with the late blight *R* genes *Rpi-blb3* and *R2-like* on chromosome IV (30). Chromosome XI harbors a region that contains a cluster of late blight *R* genes. Initially, El-Kharbotly et al. (12) mapped a cluster of three *R* genes (*R3*, *R6*, and *R7*) on this chromosome and, later, *R10* and *R11* were mapped in the same cluster (7). Furthermore, Huang (19) showed that *R5*, *R8*, and *R9* also could be located in this cluster as either separate loci or allelic versions of the *R3* locus. This major late blight resistance complex may even carry more *R* genes or allelic versions than currently known. In fact, one new *R* gene has already been identified at this locus (20). When an F1 mapping population that was predicted to show segregation for *R3* was tested with *P. infestans* isolates that were known to be virulent on one of the parents and avirulent on the other, the progeny appeared to give differential responses to these isolates. This suggested the presence of two different *R3* genes and, indeed, high-density linkage mapping of the *R3* region revealed two distinct *R3* genes (20). For both *R3a* and *R3b*, the cognate avirulence loci in *P. infestans* have been identified. One carries the effector gene *Avr3a* (3) and the other encodes a putative transcription factor that governs avirulence on *R3b*, *R10*, and *R11* potato differentials (23). From the original *R1-R11* differential set defined by Black and Mastenbroek (5), three *R* genes have been cloned (*R1*, *R2*, and *R3a*) and all encode coiled-coil nucleotide binding site leucine-rich repeat (NBS-LRR) proteins (4,21,26). Of the 11 *R* loci, the *R* locus in the differential line *R4* is the only one for which the putative genomic position is unknown (13).

Work on the *R3* gene showed that a single potato differential might contain more specificities than originally postulated. As it happens, *R3a* appears to originate from the differential set that was developed in Scotland by Black (cv. Pentland Ace) (28) whereas the *R3b* gene originates from the Cebeco46-42-1 differential in the set that was developed by Mastenbroek in the Nether-

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lands (29). As for *R3*, two independent potato differentials were presented for *R4* (5). The series developed by Mastenbroek contains Cebeco44-31-5 as *R4* differential whereas 1563 c (14) was described as equivalent in the series developed by Black (Fig. 1). Other differentials carrying *R4* include Cebeco46-174-30 (*R1R4*) (Fig. 1) and Cebeco44-14-2 (*R2R4*) and the commercial cvs. Avondale, Epoka, Gelda, Greta, Isola, Pentland Squire, and Tylva. In commercial potato crops, however, *R4*-carrying cultivars have not been used extensively.

Previously, we cloned the *P. infestans* Avr gene *PiAvr4* and demonstrated that introduction of this gene into race 4 *P. infestans* strains confers avirulence on differentials from the Mastenbroek set carrying *R4* (40). Similar to other oomycete Avr genes, *PiAvr4* encodes an RXLR-dEER protein and is a member of a large family that comprises over 550 genes in *P. infestans* and ≈370 in *P. sojae* and *P. ramorum* (22,42). They share the host cell targeting motif RXLR-dEER in the N-terminus but are highly diverse in the C-terminus (6,22). In this study, we compared the *R4* differentials in the Mastenbroek set and the Black set and investigated how they respond to inoculation with *P. infestans* isolates that carry a dominant avirulent allele of *PiAvr4* (and, thus, have an AVR4 phenotype), or isolates that have only virulent alleles (*Piavr4*; avr4 phenotype). Because the isolates were recognized by either the Mastenbroek differential or the Black differential, our data show that potato lines defined to carry *R4* have a differential recognition of *PiAvr4*. Moreover, we show that recognition of *PiAvr4* is strictly confined to potato lines carrying the *R4* locus that was introgressed by Mastenbroek.

MATERIALS AND METHODS

Potato differentials, cultivars, and crosses. Potato differentials and cultivars used in this study are described in Table 1. Information on their pedigrees was downloaded from the potato pedigree database (36). Plantlets were maintained in vitro on Murashige and Skoog (MS) medium with sucrose (MS30) at 30 g/liter and 0.8% agar and grown at 20°C with 16 h of light and 8 h of darkness for 4 weeks. Then, they were transferred to potting soil and maintained in the greenhouse (21°C for 16 h of light and 19°C for 8 h of darkness).

The F1 population 98-4801 was generated in 1998 at the former Laboratory of Plant Breeding of Wageningen University. The female parent is the tetraploid line Cebeco44-31-5, which is the *R4* differential from the Mastenbroek set. The tetraploid cv. Katahdin that lacks any known *R* gene and, thus, is susceptible to late blight was used as the male parent. F1 population ARP05-464 was generated in 2006 from a cross between cv. Isola as female parent and Katahdin as male parent. Isola is described as carrying resistance to race 4 of *P. infestans* (24).

Virulence assays and resistance screening. *P. infestans* isolates used in this study are listed in Tables 2 and 3. In order to determine the virulence phenotype of the isolates, detached-leaf assays were performed. Each isolate was tested in three independent assays and, in each assay, four leaflets of each potato line were inoculated with zoospores that were harvested from 10-day-old *P. infestans* cultures grown on rye sucrose agar medium (8). To obtain zoospores, mycelium was flooded with cold water and

Fig. 1. Pedigrees of potato differentials and cultivars used in this study (underlined). From left to right: BI-R4 (1563 c (14)), cv. Isola, Ma-R4 (Ceb44-31-5), and Ma-R1R4 (Ceb46-174-30). ARP05-464 and 98-4801 (shaded) are segregating F1 populations. *Solanum* spp. and potato lines depicted in bold have the *R4* resistance phenotype. Information was obtained from the potato pedigree database (36).

TABLE 1. Potato differentials and cultivars used in this study

Line, cultivar	Differential	Year	Origin	Parent (♀)	Parent (♂)	Before this study	After this study
Cebeco44-31-5	Ma-R4	1944	The Netherlands	Cebeco43-13-10	JK 38-43	<i>R4</i>	<i>R4</i> ^{Ma}
Isola		1958	Germany	2285/44	MPI_19175	<i>R4</i>	<i>R4</i> ^{Ma}
1563 c (14)	BI-R4	...	United Kingdom	Craig Defiance	1104 a (3)	<i>R4</i>	<i>R4</i> ^{Bl}
Cebeco43-154-5	Ma-R1	1943	The Netherlands	Eigenheimer	Erika	<i>R1</i>	<i>R1</i> ^{Ma}
Cebeco46-174-30	Ma-R1R4	1946	The Netherlands	Cebeco44-56-35	Flava	<i>R1R4</i>	<i>R1 R4</i> ^{Ma}
Katahdin		1932	United States	USDA 40568	USDA 24642	<i>r0</i>	<i>r0</i>
Bintje		1910	The Netherlands	Munstersen	Jaune d'or	<i>r0</i>	<i>r0</i>

placed at 4°C for 3 h, after which the zoospore suspension was collected and the concentration adjusted to 1×10^5 zoospores ml⁻¹. Each of the leaflets, which came from plants that were grown for 4 weeks in potting soil, was inoculated at the abaxial side with 4 drops of 10 µl containing 1,000 zoospores. Infection efficiency and lesion growth rate was monitored for 5 days and measured 3, 4, and 5 days postinoculation. An isolate showing expanding and sporulating lesions on a particular potato line was scored as virulent (i.e., has the *avr4* phenotype) and the interaction as compatible. An isolate that was arrested in growth was scored as avirulent (AVR4 phenotype) and the interaction as incompatible (38).

Binary PVX constructs and in planta expression assays. For in planta expression of *PiAvr4*, we used recombinant PVX particles carrying *PiAvr4* as insert. PVX was propagated in *Nicotiana clevelandii* and subsequently inoculated on potato leaves. Binary PVX constructs carrying recombinant PVX (pGR106-*Avr4*¹⁻²⁸⁷) or wild-type PVX (pGR106) were maintained in *Agrobacterium tumefaciens* strain GV3101 (40). For agroinfection of *N. clevelandii*, *A. tumefaciens* strains carrying the binary constructs were inoculated on the lower leaves of 2-week-old plants by wounding the leaves with a toothpick. After ≈3 weeks, the leaves that showed systemic mosaic symptoms were cut and homogenized in 50 mM potassium phosphate buffer, pH 7.0. Inoculum was prepared by mixing the homogenate containing the PVX particles with carborundum powder. The inoculum was spread on the leaves of potato plantlets that had been grown in potting soil for 1 week after transfer from in vitro culture. Each potato line was inoculated 10 times with either the wild-type PVX or the recombinant PVX expressing *PiAvr4*. The responses were monitored up to 5 days postinoculation.

RESULTS

The potato differentials Ma-R4 and Bl-R4 recognize different *P. infestans* isolates. To determine whether the *R4*

TABLE 2. Compatible (C) and incompatible (I) interactions between *Phytophthora infestans* isolates and potato differentials and cultivars

Isolate	Ma-R4	Isola	Bl-R4	Bintje
NL80029	C	C	C	C
NL88133	I	I	C	C
GER8601	I	I	C	C
US87000	I	I	C	C
NL68308	C	C	C	C
NL85026	I	I	C	C
NL88069	C	C	C	C
NL89094	C	C	C	C
NL89148-27	I	I	C	C
NL89148-9	I	I	C	C
NL91001	I	I	C	C
NL99018	C	C	C	C
PIC99180	C	C	C	C
SC96.21.1.1	C	C	I	C

TABLE 3. Compatible (C) and incompatible (I) interactions between parental isolates and F1 progeny of *Phytophthora infestans* cross 71 and potato differentials and cultivars

Isolate	Ma-R4	Isola	Bl-R4	Bintje	Ma-R1	Ma-R1R4
NL80029	C	C	C	C	I	I
NL88133	I	I	C	C	C	I
D12-2	C	C	C	C	C	C
D12-17	C	C	ND ^a	C	I	I
D12-23	C	C	C	C	C	C
T20-2	I	I	C	C	C	I
T30-2	I	I	C	C	I	I
T30-4	I	I	C	C	I	I
T35-3	C	C	C	C	C	C
RE11-16	I	I	C	C	I	I

^a ND = not determined.

differentials that are included in the sets of Mastenbroek (Cebeco44-31-5; hereafter named Ma-R4) and Black (1563 c [14]; hereafter named Bl-R4) recognize the same *P. infestans* isolates, we performed a series of infection assays on detached leaves. In these assays, the cvs. Isola (*R4*), Bintje (*r0*), and Katahdin (*r0*) were included. We tested 14 *P. infestans* field isolates and found that 7 of these were avirulent on Ma-R4 and Isola (Table 2). The resistance in both Ma-R4 and Isola is derived from *S. demissum* but we were unable to trace whether the *S. demissum* accession in their pedigree is the same (Fig. 1). Surprisingly, the response of Bl-R4 differed from that of Ma-R4. Of the tested isolates, 13 were virulent on Bl-R4 whereas only 1 (i.e., SC96.21.1.1) was avirulent on this differential. Because isolate SC96.21.1.1 is virulent on Isola and Ma-R4 (Fig. 2A), these data show that the determinants of resistance in Bl-R4 differ from those in Ma-R4 and Isola.

Fig. 2. Bioassays demonstrate the different recognition specificities of Ma-R4 and Bl-R4 toward *PiAvr4*. **A**, Interactions of Ma-R4 and Bl-R4 with *Phytophthora infestans* isolates T30-4 containing *PiAvr4* and SC96.21.1.1 lacking a functional *PiAvr4* gene. Photographs were taken 5 days post inoculation. **B**, Responses of Ma-R4 and Bl-R4 after inoculation with wild-type *Potato virus X* (PVX) (pGR106) and recombinant PVX expressing *PiAvr4* (pGR106-*Avr4*¹⁻²⁸⁷). Photographs were taken 5 days postinoculation.

The AVR4 phenotype in cross 71 is confined to Ma-R4. In a previous study, the map position of *PiAvr4* in *P. infestans* was determined by making use of a mapping population that showed a 1:1 segregation of the AVR4 phenotype (37). The mapping population consisted of F1 progeny of cross 71 and the AVR4 phenotyping was performed on Ma-R4. Here, we repeated the AVR4 phenotyping of eight offspring of cross 71 on Ma-R4 and, in addition, determined their phenotype on BI-R4 and Isola. In the cross 71 progeny, the segregation of the AVR4 phenotype on Ma-R4 and Isola was identical, further confirming that Ma-R4 and Isola contain the same *R4* specificity (Table 3). However, the parents of cross 71, NL80029 and NL88133, and all tested progeny appeared to be virulent on BI-R4 (Table 3), thus demonstrating that BI-R4 lacks resistance to the parents and the progeny of cross 71.

***R4* specificity of potato differential Ma-R1R4.** The set developed by Mastenbroek also contains an *R1R4* differential (Cebeco46-174-30, hereafter named Ma-R1R4). This differential, however, has a pedigree different from Ma-R4 (Fig. 1). In order to verify that Ma-R4 and Ma-R1R4 have the same recognition specificity toward *PiAvr4*, infection assays were performed on Ma-R1R4 and on Cebeco43-154-5, the differential that carries *R1* (Ma-R1). Of the 10 isolates tested, only 2 behaved differently on Ma-R1R4 compared with Ma-R4 (Table 3). These two isolates, NL80029 and D12-17, both have an AVR1 phenotype; therefore, it is likely that the presence of *R1* in Ma-R1R4 is responsible for the difference in behavior. These results strongly suggest that Ma-R1R4 and Ma-R4 possess the same *R4* specificity.

In planta expression of *PiAvr4* on *R4* lines results in differential responses. Recently, we have cloned the *Avr* gene *PiAvr4* from isolate T30-4, an F1 progeny of cross 71, and have demonstrated in transient in planta expression assays that this gene encodes an effector that elicits HR on Ma-R4 (40). The observation that Ma-R4 and BI-R4 respond differently toward certain *P. infestans* isolates prompted us to determine their responses toward *PiAvr4* on the two potato differentials. To test this, we expressed *PiAvr4* in planta using PVX as expression vector. Wild-type PVX particles derived from the empty pGR106 vector caused no necrotic response on either Ma-R4 or BI-R4. Inoculation of Ma-R4 with recombinant PVX particles derived from pGR106-*PiAvr4*¹⁻²⁸⁷ caused necrotic lesions on the inoculated leaves 5 days postinoculation. In contrast, no necrosis was observed on inoculated leaves of BI-R4 (Fig. 2B). These results demonstrate that the RXLR effector encoded by *PiAvr4* is not recognized by BI-R4 and confirm its recognition by Ma-R4.

Resistance in Ma-R4 is determined by a single dominant locus. In order to determine the genetic basis of the resistance phenotype in Ma-R4, we tested how late blight resistance segregates in two independent F1 populations, 98-4801 and ARP05-464, that have Ma-R4 and Isola, respectively, as resistant parents (Fig. 1). Two *P. infestans* isolates, IPO-0 and T30-4, were used for inoculation. Both have the AVR4 phenotype and carry a full-length dominant allele of *PiAvr4*. In total, 67 progeny of cross 98-4801 were tested; 34 were resistant and 33 susceptible (Table 4). Of the second cross, ARP05-464, 60 progeny were tested; 28 were resistant and 28 susceptible while, for 4 progeny, the phenotype was difficult to score (Table 4). As a control, we used *avr4*

isolates that have frameshift mutations in *PiAvr4* and, as expected, all parents and progeny were susceptible to these isolates.

The observation that the segregation ratios in both populations did not differ significantly from the expected 1:1 ratio shows that the resistance phenotype is determined by a single dominant locus. To discriminate between the two tentative *R4* genes we refer to the *R* gene in differential Ma-R4 as *R4*^{Ma}.

DISCUSSION

In this study, we demonstrated that two potato differentials that were thought to carry the same late blight resistance locus recognize different strains of *P. infestans*. Several isolates that were avirulent on Ma-R4 appeared to be virulent on BI-R4, whereas one isolate that is avirulent on BI-R4 could infect Ma-R4. This difference in recognition specificity was also demonstrated by the observation that only one of the two differentials, Ma-R4, gave a necrotic response when challenged with the effector protein *PiAvr4*. BI-R4 gave no response and this is in line with the observation that isolates that carry the dominant allele of *PiAvr4* can colonize BI-R4. Analysis of an F1 population with Ma-R4 as the female parent demonstrated that the resistance in Ma-R4 is determined by a single gene (i.e., *R4*^{Ma}) thus pinpointing *PiAvr4* and *R4*^{Ma} as pairs in a gene-for-gene interaction.

In 1953, Black et al. (5) proposed a standardized international nomenclature for resistant potato lines and the corresponding *P. infestans* races. In order to compare their results, Black and Mastenbroek exchanged potato differentials and tested their resistance responses to different *P. infestans* races. The actual isolates, however, were not exchanged and there is no record indicating which isolates were used for these resistance tests. The first differential set that they defined consisted of the differentials *R1*, *R2*, *R3*, and *R4*. Later, seven more were added, which resulted in the *S. demissum* *R1-R11* set (27,28).

To explain why Ma-R4 and BI-R4 are resistant to different *P. infestans* races, we traced the origin of the late blight resistance trait that was introgressed into these potato differentials. In both cases, the resistance originates from *S. demissum* but the accessions used are different. In contrast, Ma-R4 and Ma-R1R4 seem to share a common ancestor (Fig. 1). The *S. demissum* genotypes 29A and 49 were originally imported for potato breeding in Indonesia (29,35). It is likely that genotypes 29A and 49 were two individuals derived from accession 741 but this was not documented. Because Ma-R4 and Ma-R1R4 are both resistant to *P. infestans* isolates that have a dominant *PiAvr4* allele, it is conceivable that *R4*^{Ma} originates from *S. demissum* 741. We were unable to trace the relationship between *S. demissum* 741 and MPI 41969/377, the resistant ancestor of Isola. Our data, however, clearly show that Ma-R4 and Isola have an identical recognition specificity and, therefore, it is likely that the Mastenbroek differentials and MPI 41969/377 carry the same *R4* gene, possibly originating from the same ancestor, or highly homologous *R4* genes (orthologs) from different ancestors.

The observations that there are two different *R4* specificities resemble those of Huang et al. (20) with respect to *R3* recognition specificity. The *R3* differential developed by Mastenbroek (Cebeco46-42-1) and the one developed by Black (cv. Pentland

TABLE 4. Segregation of resistance to *Phytophthora infestans* in F1 progeny of two potato crosses

Cross	Parents		Progeny				<i>P</i> ^c
	♀	♂	Observed ^a	Expected ^a	<i>N</i> ^b	χ^2 ^c	
98-4801	Ceb44-31-5	Katahdin	34:33	1:1	67 (67)	0.015	0.903
ARP05-464	Isola	Katahdin	28:28	1:1	56 (60)	0.000	1.000

^a Ratio resistance to susceptible.

^b Number of progeny categorized; in parentheses, total number of progeny tested.

^c The χ^2 and the corresponding *P* value were calculated to test the probability that the data fit an expected ratio of 1:1 for segregation of a single resistance gene.

Ace) also differ in their specificity toward *P. infestans* races. The *R3a* gene in cv. Pentland Ace is derived from *S. demissum* CPC 2127, the same accession that gave rise to Bl-R4. The *R3b* gene in Cebeco46-42-1 originated from *S. demissum* 49, the genotype that also carries *R4^{Ma}* (19). Despite their different origins, *R3a* and *R3b* map on the same chromosome (X1) and are tightly linked (20). Whether or not *R4^{Ma}* is linked to the resistance locus in Bl-R4 and where *R4^{Ma}* is located have yet to be determined. A previous attempt by El-Kharbotly et al. (13) to map *R4* by analyzing the progeny of a cross with Ma-R4 as resistant parent was unsuccessful. They found an excess of resistant progeny and a deviation from the 1:1 Mendelian segregation, suggesting involvement of a second locus. In contrast, we have found that *R4^{Ma}* segregates as a single dominant locus, indicating that a single gene in Ma-R4 is responsible for recognition of PiAvr4. To avoid the possibility that other gene-for-gene interactions obscure the resistance phenotype conferred by the *R* gene that is targeted, it might be more efficient to screen segregating populations for response to one defined effector, rather than scoring resistance phenotypes by inoculation with pathogen races (41). Because the *PiAvr4* gene has been cloned (40), one can now use in planta expression of *PiAvr4* to monitor segregation of *R4^{Ma}*, and this may accelerate mapping and cloning of the *R4^{Ma}* gene.

Studies describing race structures of *P. infestans* populations have shown that isolates virulent on R4 plants occur more frequently than avirulent ones (15,18,25). However, these studies often do not mention which potato differentials have been used for the bioassays and, therefore, it is impossible to conclude which Avr factor is less prominent, the one recognized by Ma-R4 or the one recognized by Bl-R4. Our findings stress the importance of using well-documented biological materials for research. Just mentioning which pathogen race or *R* differential is used is potentially problematic and, when possible, specific *R* gene and effector alleles should be identified. Screening of our own culture collection revealed several isolates that carry a dominant allele of *PiAvr4* and infection assays confirmed that these isolates are avirulent on Ma-R4 (40). In contrast, we could not easily trace an isolate that was incompatible with Bl-R4; based on literature searches and personal communications, we obtained just one isolate that was collected in Scotland in 1996 (SC96.21.1.1.). This isolate was compatible with Ma-R4. The latter is consistent with the fact that the two *PiAvr4* alleles in SC96.21.1.1 have frameshift mutations that give rise to truncated PiAvr4 proteins (data not shown). Isolates avirulent on both differentials, Ma-R4 and Bl-R4, were not found.

The finding that the R4 differentials have different recognition specificities may also explain contradicting data with respect to inheritance studies of *Avr4*. Al-Kherb et al. (1) analyzed several F1 progenies of *P. infestans* isolates that differed in virulence spectra in an effort to define the genetic determinants of virulence and avirulence. All parental isolates and the majority of the progeny were virulent on Bl-R4. The authors considered the appearance of a few avirulent progeny to be an indication that avirulence is dominant but further evidence for this was lacking. Because they used only Bl-R4 as R4 differential, these analyses were not informative for the *Avr* gene determining avirulence on *R4^{Ma}*. In earlier studies, Spielman et al. (33,34) described F1 populations that segregated for virulence on potato differential PI 203900, which is identical to the Ma-R4 differential Cebeco44-31-5. Data from one cross suggested that virulence toward Ma-R4 was dominant but the fit for this model was very poor. In another cross, avirulence was found to be dominant but there was a divergence from a 1:1 segregation that pointed to the involvement of two genes. In the cross 71 mapping population, that was the basis for the positional cloning of *PiAvr4*, avirulence on Ma-R4 segregated as a single dominant locus (38).

In recent years, several oomycete *Avr* genes have been cloned, mainly by positional cloning. Nearly all these *Avr* genes, includ-

ing *PiAvr4*, encode RXLR-dEER effector proteins (2,3,31,40). Also, *R* proteins that can stop invasion of oomycete pathogens follow a common theme. They all belong to the class of NBS-LRR proteins and reside inside the host cell (4,21,39,43). As yet, we have no clue about the molecular nature of the *R4^{Ma}* gene, or about the *Avr-R* pair that underlies the resistance in Bl-R4 (i.e., the putative *R4^{Bl}* and *PiAvr4^{Bl}*). Given the fact that PiAvr4 is an RXLR-dEER protein, it is very likely that *R4^{Ma}* is an NBS-LRR gene. When anticipating that the cognate *Avr* gene *PiAvr4^{Bl}* that triggers resistance in Bl-R4 is an RXLR-dEER effector, one could use an effector genomics approach to identify this *Avr* gene. *Phytophthora* spp. have hundreds of different RXLR-dEER effectors (22,42). A subset has been cloned in in planta expression vectors, and high-throughput screening for effector activity on a wide range of *Solanum* spp. has already resulted in the identification of novel *Avr-R* pairs (41). Including Bl-R4 in these screenings could lead to the identification of *PiAvr4^{Bl}*.

Because we lack crucial information on the differentials and isolates that were used by Black et al. (5), it is difficult to reconstruct why they could not discriminate between Ma-R4 and Bl-R4. From the pathogen side, we can think of a hypothesis that is based on coregulation of expression of RXLR-dEER genes. Jiang et al. (23) described the cloning of one avirulence locus in *P. infestans* that does not encode an RXLR-dEER effector. Instead, this locus harbors a gene named *Pi3.4* that encodes a protein reminiscent of a transcription factor. This *Pi3.4* locus determines avirulence toward at least three *R* genes, *R3b*, *R10*, and *R11*, and the current hypothesis is that *Pi3.4* regulates transcription of a subset of RXLR-dEER genes, including the ones that trigger recognition in *R3b*, *R10*, and *R11* potato differentials. A similar situation could exist for *PiAvr4* and *PiAvr4^{Bl}*: coregulation of these two effector genes by one transcription factor. To fit this hypothesis, the isolates used by Black and Mastenbroek should have had dominant alleles of the two effector genes that then conferred avirulence on both Ma-R4 and Bl-R4. In the broad set of field isolates that we tested in a previous study (40), this is not the case; the virulent phenotype was always due to frameshift mutations in both alleles, resulting in truncated proteins that are no longer recognized by the cognate *R* protein. However, it cannot be excluded that transcriptional regulation of *PiAvr4* plays an additional role, especially when considering the fact that the positional cloning was partly based on transcriptome markers (40).

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