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The *R3* Resistance to *Phytophthora infestans* in Potato is Conferred by Two Closely Linked *R* Genes with Distinct Specificities

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The *R3* locus of potato (*Solanum tuberosum* L.) confers full resistance to avirulent isolates of *Phytophthora infestans*, the causal agent of late blight. *R3* resides in the distal part of chromosome 11 and segregates in a potato mapping population, from which a well-saturated amplified fragment length polymorphism map is available. Using a population of 1,748 plants, we constructed a high-resolution genetic map at the *R3* locus. Using the combination of fine mapping and accurate disease testing with specific *P. infestans* isolates, we detected that the *R3* locus is composed of two genes with distinct specificities. The two genes *R3a* and *R3b* are 0.4 cM apart and have both been introgressed from *S. demissum*, the 'donor' species of most characterized race-specific *R* genes to *P. infestans*. A natural recombinant between *R3a* and *R3b* was discovered in one accession of *S. demissum*. The synteny between the *R3* locus and the tomato *I2* locus is discussed.

The central dogma of molecular plant pathology is the gene-for-gene model, which states that, for every dominant resistance (*R*) gene in the plant, there is a corresponding dominant avirulence (*Avr*) gene in the pathogen (Flor 1971). The presence of matching gene pairs controls the outcome of the interaction of many pathosystems (Thompson and Burdon 1992), including the interaction between potato (*Solanum tuberosum* L.) and its oomycete pathogen *Phytophthora infestans* (Black et al. 1953; van der Lee et al. 2001). A corollary of the gene-for-gene model is that receptors encoded by *R* genes can directly or indirectly perceive the products of corresponding *Avr* genes, enabling recognition of the pathogen and subsequent elicitation of an array of plant defense responses that eventually lead to resistance (Dangl and Jones 2001; Keen 1990). In many cases, the induced defense is manifested as a hypersensitive response (HR) that is associated to a programmed cell death at the initial site of infection (Morel and Dangl 1997). Unexceptionally, HR was found to be associated with all forms of resistance to *P. infestans*, including race- or cultivar-specific resistance, partial resistance, and nonhost resistance, indicating that recognition occurs independently of the type of resistance (Kamoun et al. 1999; Vleeshouwers et al. 2000). To understand the mechanisms underlying recognition, it is essential to isolate the interacting gene pairs from both the host and the pathogen. For this purpose, single dominant *R* genes and *Avr* genes are preferred because they are genetically more accessible.

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Late blight, caused by *P. infestans*, is the most devastating disease for potato production worldwide (Fry and Goodwin 1997). Resistance conferred by dominant *R* genes is easier to manipulate than quantitative resistance. A number of *R* genes for late blight resistance have been introgressed from wild relatives into cultivated potato through sexual and somatic hybridization. Some of these have been mapped to the potato genome through linkage to specific DNA markers (Gebhardt and Valkonen 2001). Recently, two of them have been cloned. One is *R1*, a race-specific *R* gene originating from *S. demissum* (Ballvora et al. 2002). Another is *RB* or *Rpi-blb1*, which confers broad-spectrum resistance and is derived from *S. bulbocastanum* (Song et al. 2003; van der Vossen et al. 2003). Both genes are members of the *R* gene class predicted to encode receptors with coiled coil (CC), nucleotide binding site (NBS), and leucine-rich repeat (LRR) domains (Ballvora et al. 2002; Song et al. 2003; van der Vossen et al. 2003).

Previously, *R3* was mapped as a single dominant locus on the distal part of the short arm of potato chromosome 11, closely linked to restriction fragment length polymorphism (RFLP) markers GP250A, GP185, and TG105A (El Kharbotly et al. 1994). The short arm of chromosome 11 in potato is syntenic to the long arm of chromosome 11 in tomato (Dong et al. 2000; Tanksley et al. 1992). Like many other *R* loci, *R3* resides in a hot spot for resistance to various pathogens. A number of major *R* loci and quantitative trait loci (QTL) for resistance from Solanaceae crops have also been anchored in the same region (Grube et al. 2000; Pan et al. 2000), including another two *P. infestans* resistance genes, *R6* and *R7* (El Kharbotly et al. 1996). To date, the tomato *I2* gene that confers resistance to *Fusarium oxysporum* f. sp. *lycopersici* is the only *R* gene cloned from this region (Ori et al. 1997; Simons et al. 1998).

In this report, we describe the construction of a high-resolution genetic map of the *R3* locus, using the saturation from the ultrahigh density potato map with over 10,000 amplified fragment length polymorphism (AFLP) markers and the resolution from a large segregating F1 population. Interestingly, two functional *R* genes instead of one were found at the locus, and they displayed a differential reaction to some well-defined *P. infestans* isolates. The occurrence of two *R* genes at the *R3* locus was confirmed in a natural population of *S. demissum*. The colinearity between tomato and potato in this region of chromosome 11 enabled the development of *R3*-flanking markers from tomato sequences and the association of the locus with the *I2* locus of tomato.

RESULTS

Identification and localization of *R3* in the ultrahigh density map.

R3 was previously mapped on the distal part of chromosome 11 by using a diploid segregating population, J91-6164, which was derived from the cross between Esc.42 (Table 1, *R1r1*, *R3r3*) and 84-1031-29 (1031) (Table 1, *r1r1*, *r3r3*). Esc.42 is a dihaploid derivative from potato cv. Escort (El Kharbotly et al. 1994). SH83-92-488 (SH) (Table 1), the maternal clone of the mapping population of the ultrahigh density map, shares its genetic background with Escort. Pedigree information revealed that both SH and Escort descended from potato cv. Reaal, which carries *R1* and *R3* (Table 1, Mastenbroek 1953).

To verify whether this genetic background includes *R3* so as to enable the utilization of the global coverage with AFLP markers on the ultrahigh density map, we analyzed the resistant clones SH, J91-6164-21 (6164) (Table 1), Reaal, and susceptible clones RH89-039-16 (RH) (Table 1) and 1031 with polymerase chain reaction (PCR) markers GP250, TG105, and STM0025 (Table 2). The clone 6164 is a progeny of the previous mapping population J91-6164 (El Kharbotly et al. 1994). It carries *R3* but not *R1*. RH is the paternal clone of the population of the ultrahigh density map. In J91-6164, GP250 and TG105 mapped as RFLP markers are proximal to *R3* (El Kharbotly et al. 1994). STM0025 is 5 cM distal to GP250 (Milbourne et al. 1998). The band pattern of all three PCR markers suggested that at the *R3* region all three resistant clones carry a genomic fragment that is identical-by-descend (Fig. 1A).

To confirm the presence of *R3* in SH, we characterized SH, 6164, RH, and 1031 for race-specific resistance with a panel of *P. infestans* isolates (Table 3) on both detached leaves and in vitro plantlets. SH and 6164 displayed a typical HR when inoculated with *P. infestans* isolates 89149-9, IPO-0, and H30P04 (Table 3), which were all avirulent to the *R3* differential Mastenbroek *R*3 (Ma*R*3) (Table 1, van der Lee et al. 2001). Both SH and 6164 exhibited full susceptibility to *P. infestans* isolates 89148-27 and 90128 (Table 3), which are both virulent on Ma*R*3. Control clones RH and 1031 were

Table 1. Plant materials used in this study

Clone	Abbreviation	Species	Known *R* Gene	Remark
RH89-039-16	RH	*Solanum tuberosum*	None	Paternal clone of the SH × RH *R3* mapping population
SH83-92-488	SH	*S. tuberosum*	*R3*	Maternal clone of the SH × RH *R3* mapping population
Esc.42	Esc	*S. tuberosum*	*R1, R3*	Paternal clone of the J91-6164 *R3* mapping population[a]; introgression study
84-1031-29	1031	*S. tuberosum*	None	Maternal clone of the J91-6164 *R3* mapping population; introgression study
J91-6164-21	6164	*S. tuberosum*	*R3*	A progeny of J91-6164; introgression study
Reaal	Reaal	*S. tuberosum*	*R1, R3*	Common ancestor of Esc and SH; syn. to 4768-15[b]; introgression study
Mastenbroek *R3*	Ma*R3*	*S. tuberosum*	*R3*	The *R3* differential developed by Mastenbroek; syn. to CEBECO 4642-1[c]
1024-2	1024	*S. tuberosum*	None	Introgression study
CE-10	CE	*S. tuberosum*	None	Introgression study
Katahdin	KA	*S. tuberosum*	None	Introgression study
CGN17810	17810	*S. demissum*	*R1, R2, R3, R4, R7, R8*	One of the *R3* donor accessions; introgression study
CGN18313	18313	*S. demissum*	None[d]	Introgression study

[a] El Kharbotly et al. 1994.
[b] Mastenbroek 1953.
[c] van der Lee et al. 2001.
[d] This study revealed that 18313 carries *R3b*.

Table 2. Polymerase chain reaction (PCR) markers used in this study, listed according to their position on the high-resolution map

Marker	GenBank accession	Bin	Phase[a]	Primer (5'-3')	Temperature (°C)	Fragment size (bp)	Restriction enzyme
St3.3.11	U60083	46	C	GCT AAG CTG GGA TTG TTG TCT TAC TGT GCC ACC CGT TGA G	52	380	a.s.[b]
cLET5E4	AW038480	65/66	C	CCA GGC ATG CTC AAT TTG GAG T TTC CCT GTT TGG ACT ACT TGT GGA	55	310	*Hha*I
St1.1	U60069	65/66	C	GCT CAT TCG ACT TAA AGG TTG TTG GGC AGC TCC CAT ATT TCA CTT CTC	60-52[c]	450	*Hae*III
TG105		65/66	C	TCA CAT GAG CTG GGA GAA AT AAA GGC CTG TTG CTG AGA G	54	650	*Hin*fI
GP250		65/66	C	ACC AGT AGG ACC ACC ACC AAC AAT GAT CGT GAC GGC TCT ACT CTT TTA TGA	60-52[c]	410	*Vsp*I
STM0025		65/66	C	GTT CAT GAT TGT GAA TGC TC ATG ACT CAA CCC CAA ATG	59	170	a.s.
GP185		65/66	C	CTG GTA ATA GTA GTA ATG ATT CTT CGT C TTG TTC AAT GGA GCA CTT GC	54	440	*Bst*UI
TG26		65/66	C	GAG AGG GGA CAC TTT TAT TTA TTC A GAG ATC TTC CCG CCG CTG TG	49	1600	a.s.
CT120		67	R	CGA GGG GGC GAA GGA TT CCA TGA GAT AAA CGA GGA ACC AGT	52	360	*Tsp*509I

[a] Phase to *R3* (C for coupling and R for repulsion).
[b] a.s. = allele specific.
[c] Touchdown PCR with temperature gradually decreasing from 60 to 52°C.

susceptible to all the five isolates (Fig. 1B). This demonstrated that SH carries an *R* gene (genes) with the same specificity as *R3*.

To test whether the *P. infestans* resistance in SH is inherited as a single *R* gene, we examined the segregation for resistance in the mapping population (SH × RH) of the ultrahigh density map. We inoculated the population with *P. infestans* isolate IPO-0. The distinction between the resistant and the susceptible phenotypes was clear, i.e., the resistant individuals showed a dark localized necrosis (HR) at 3 to 4 days postinoculation (dpi), while the susceptible ones gave extensive sporulation at 5 dpi. This suggested the resistance was controlled by one or more major genes. In a total of 108 individuals tested, 46 were resistant and 62 were susceptible, which fitted the Mendelian 1:1 ratio. Although the segregation slightly skewed toward susceptibility, the ratio supported the inheritance of a single dominant *R* gene ($\chi^2 = 2.37$; $p \geq 0.1$).

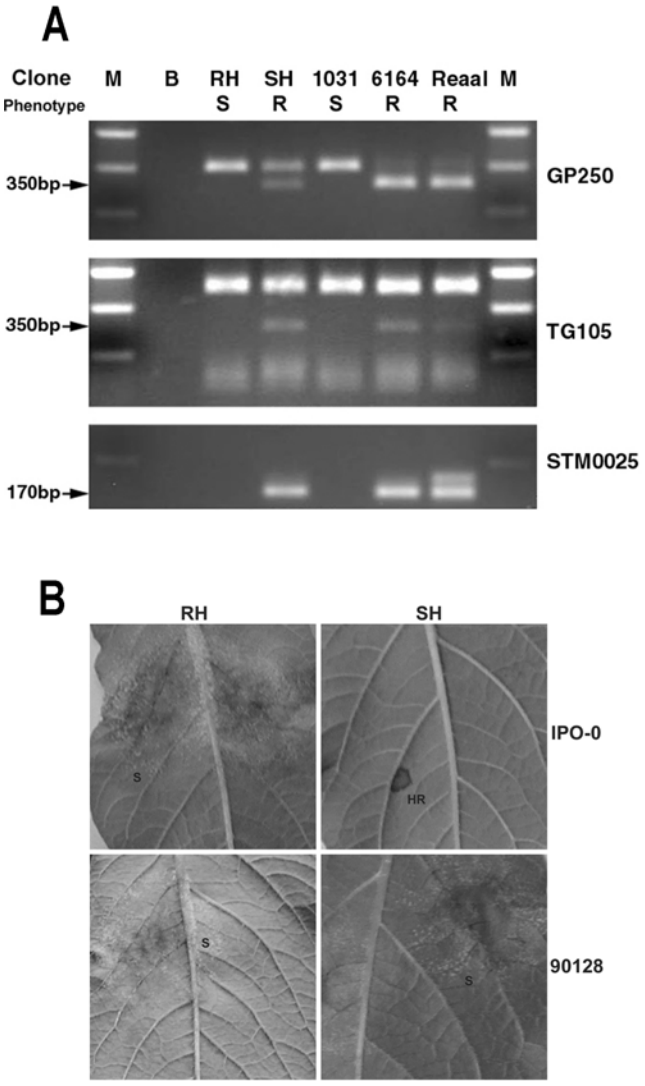

Fig. 1. Molecular and biological evidence for the presence of *R3* in SH83-92-488 (SH). **A,** Analysis of the genomic DNA from RH89-039-16 (RH), SH, 84-1031-29, J91-6164-21, and Reaal with markers GP250 (*Vsp*I), TG105 (*Hin*fI), and STM0025. The phenotype (R = resistant, S = susceptible) of these clones inoculated with *Phytophthora infestans* isolate IPO-0 is indicated. The sizes of polymorphic bands are indicated on the left. DNA size markers are indicated as 'M' and the blank control as 'B'. **B,** A gene-for-gene interaction of potato and *P. infestans*. RH and SH leaves inoculated with isolates IPO-0 (race 0) and 90128 (race 1.3.4.6.7.8.10.11), 5 days postinoculation. A race-specific resistance response occurred on SH leaves upon inoculation with IPO-0 (HR). Extensive sporulation (S) was noted in the other three compatible interactions.

Subsequently, the *R3* locus was mapped to the distal part of the short arm of chromosome 11 on the ultrahigh density AFLP map, confirming the previous map position in J91-6164 (El Kharbotly et al. 1994). Due to the limited population size (n = 130), the more than 10,000 AFLP markers cannot be localized individually. Instead of "centiMorgan" (cM), the genetic distance unit for the ultrahigh density map is "Bin". A Bin is defined as a genetic interval flanked by a recombination event on either side. All markers within a Bin are cosegregating. The length of a Bin is a genetic interval of approximately 0.8 cM ($=1/130$). *R3* was mapped in either Bin SH11B65 (65) or 66. Due to the loss of the diagnostic plant that could separate these two neighboring Bins, we can no longer precisely place *R3* to one of the two Bins. *R3* therefore cosegregates with all the markers mapped in the combined Bin 65/66, i.e., GP250, TG105, STM0025, and 43 cosegregating AFLPs. Bin 67 is the most distal Bin of the short arm of chromosome 11. Based on pedigree information, marker analysis, inoculation results, and genetic mapping, we conclude that SH carries *R3*.

Screening for informative recombination at the *R3* locus.

For high-resolution mapping of *R3*, it was essential to define the order of those cosegregating markers with meiotic crossovers. We applied a flanking marker strategy to screen for recombination events around the *R3* locus (Tanksley et al. 1995). Instead of laborious analysis with AFLP markers available from flanking Bins, we took advantage of the colinearity between tomato and potato at the *R3* region of chromosome 11 (Dong et al. 2000; Tanksley et al. 1992) and the sequences of known RFLP markers from the two species to develop easy-scoring PCR markers.

Based on their position, markers cLET5E4, TG26, and CT120 from tomato and St3.3.11, St1.1, and GP185 from potato were chosen for PCR marker development (Table 2). St3.3.11 was mapped in Bin 46, about 16 cM proximal to the *R3* locus. Like GP250 and TG105, cLET5E4, GP185, St1.1, and TG26 colocalized with *R3* in the ultrahigh density map and then were mapped in Bin 65/66. After testing with 250 additional SH × RH plants, we mapped the marker cLET5E4 proximal to the *R3* locus with three recombinants between the marker and the locus. CT120 was placed in Bin 67 and in repulsion to *R3*. It served as a distal flanking marker. Therefore, the cLET5E4-CT120 interval spans a chromosomal region with the *R3* locus in the center. We used these two markers for recombinant screening of 1,748 SH × RH progeny (Fig. 2). Out of these, we identified 34 recombinants between cLET5E4 and CT120.

Detection of two *R* genes at the *R3* locus.

The resistance phenotype of the 34 recombinants was determined with the *P. infestans* isolates 89148-9, H30P04, and IPO-0, which all have an incompatible interaction with SH. A

Table 3. *Phytophthora infestans* isolates used in this study and their virulence profiles before and after this study

Isolate	Virulence profile		Source
	Before	After	
89148-9	0	0	F. Govers, Wageningen University, The Netherlands
IPO-0	0	3b	W. Flier, Plant Research International, The Netherlands
H30P04	7	3a.7	F. Govers
89148-27	3.7	3a.3b.7	F. Govers
90128	1.3.4.6.7.8.10.11	1.3a.3b.4.6.7.8.10.11	F. Govers

total of 26 recombinants were either resistant or susceptible to all three isolates. Surprisingly, we found the remaining eight recombinants differed in their response to H30P04 and IPO-0. Six of these, SW8536-18, SW8537-033, SW8539-004, SW8540-025, SW8540-054, and SW8540-309, were resistant to *P. infestans* isolate IPO-0 and susceptible to isolate H30P04, while the other two recombinants, SHRHC8-#130 and SW8540-325, were just the opposite, i.e.,, susceptible to isolate IPO-0 and resistant to isolate H30P04. All eight of these recombinants were resistant to 89148-9. The differential reaction to the isolates was confirmed with repeated inoculations using detached leaves and in vitro-grown plantlets (Fig. 3).

The recombinant SHRHC8-#130 is from the mapping population SH × RH of the ultrahigh density map, and it has a cross-over in the cLET5E4-CT120 interval. To exclude the possibility of a second locus that is not within the interval and affects the resistance to *P. infestans* isolate H30P04, we inoculated the mapping population SH × RH with H30P04 as we had previously done with isolate IPO-0. None of the 108 plants tested, but SHRHC08-#130 displayed a differential reaction to these two isolates. Genetic mapping revealed that resistance to isolate H30P04 in the population was inherited as a single *R* gene and was mapped within the cLET5E4-CT120 interval. No additional genetic component outside the interval affected the phenotype.

All the 34 recombinants in the cLET5E4-CT120 interval were subjected to characterization with all molecular markers from Bin 65/66 and Bin 67. The eight recombinants that showed a differential response to *P. infestans* isolates IPO-0 and H30P04 were found to carry a cross-over in the GP250-EATCMAGC_15 interval, which is a subinterval of the cLET5E4-CT120 interval (Fig. 4A and B). The remaining 26 recombinants that were either resistant or susceptible to both isolates did not have a cross-over in the subinterval. The marker pattern of the 34 recombinants indicated that the difference in isolate specificity was due to cross-overs between two closely linked *R* genes with distinct resistance spectra.

The data on isolate specificities, mapping, and marker pattern clearly demonstrated that the phenotype of *R3* resistance in SH is conferred by two closely linked *R* genes. Hereby, we designate them as *R3a* and *R3b*. *R3a* confers resistance to isolate IPO-0 and not to H30P04, and *R3b* vice versa. Both genes confer resistance to isolate 89148-9. Associated with the nomination of *R3a* and *R3b*, we can now designate IPO-0 as race 3b and H30P04 as race 3a.7. The isolate 89148-9 is still race 0 in this scenario (Table 3).

Construction of a high-resolution map at the *R3* locus.

The 34 recombinants identified from the cLET5E4-CT120 interval from the 1,748 SH × RH plants divided the interval into 35 sub-Bins (Fig. 4B). The genetic length of a sub-Bin is equivalent to approximately 0.05 cM (=$^{1}/_{1748}$). The resolution achieved with the high-resolution map of the *R3* locus is 16 times higher than that of the ultrahigh density map (0.05 versus 0.8 cM). A total of 35 molecular markers (27 AFLP markers and eight PCR markers) from the interval were assigned to the sub-Bins. The distribution of cross-overs and markers was nonrandom within the cLET5E4-CT120 interval. Nearly one third of the markers were clustered in sub-Bin 20, while no marker was placed in 22 of the 35 sub-Bins (Fig. 4B).

The *R3a* gene resides in sub-Bin 20, cosegregating with GP250, STM0025, and 10 AFLPs. TG105 and four AFLP markers were the closest proximal markers, separated from *R3a* by two recombination events. GP185, TG26, and EACAMCAG_152.6 were the closest distal markers, separated from *R3a* by three recombination events (Fig. 4B). The order of TG105-GP250-GP185 in SH was well consistent with the previous mapping result (Gebhardt et al. 1994). A total of 20 markers clustered in the TG105-GP185 interval that consists of six sub-Bins.

The *R3b* gene was mapped in sub-Bin 28, 8 sub-Bins (approximately 0.4 cM) distal to *R3a* (Fig. 4B). No marker cosegregates with the gene. The AFLP markers PCAMATA_4 and EATCMAGC_15 are its closest markers, separated by three and two recombinations, respectively. The PCAMATA_4-EATCMAGC_15 interval for *R3b* spans the same genetic distance as the TG105-GP185 interval of *R3a*, i.e., six sub-Bins, but contains much fewer markers (2 versus 20).

A natural recombination between *R3a* and *R3b* in *S. demissum.*

Introgression analysis based on pedigree information can provide additional resolution to determine the order of markers and genes of interest, and thus, it can serve as a supplemental tool to fine mapping based on segregating population (Kanyuka et al. 1999). Reaal is one of the best-characterized clones that carry the *R3* locus introgressed from *S. demissum* (Mastenbroek 1953). There are five generations of crossing and one round of dihaploidization between Reaal and SH (data not shown). The introgression study with markers tightly linked to *R3a* (GP250, STM0025, and TG105) between SH

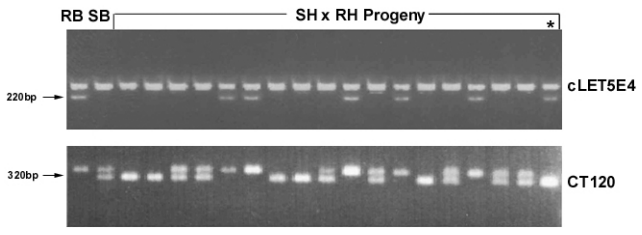

Fig. 2. Screening for recombination events near the *R3* locus in the SH83-92-488 × RH89-039-16 progeny with two flanking markers, cLET5E4 (*Hha*I) and CT120 (*Tsp*509I). The sizes of the diagnostic bands are indicated on the left. RB = resistant bulk consisting of 20 resistant plants; SB = susceptible bulk consisting of 20 susceptible plants. The presence of the 220-bp band of cLET5E4 is associated with the absence of the 320-bp band of CT120 in all progeny except in the recombinants. The right lane (*) shows a recombinant pattern.

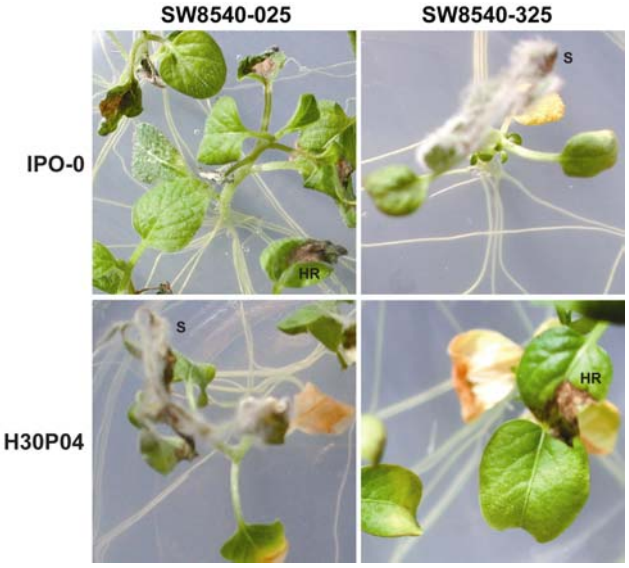

Fig. 3. Phenotype of differential resistance response at 6 days postinoculation with *Phytophthora infestans* isolates IPO-0 and H30P04 on in vitro plants. SW8540-025 is resistant (HR) to IPO-0 and is susceptible to H30P04 as shown by the sporulating lesions (S). SW8540-325 shows the opposite response.

and Reaal suggested that *R3a* in SH descended from Reaal (Fig. 1A). However, these data cannot exclude the possibility that *R3b* in SH was derived from another clone rather than Reaal and *S. demissum*.

To check the origin of *R3b* and to confirm the SH-Reaal link on *R3a*, we conducted a fine comparison of SH, Reaal, two *S. demissum* accessions (17810 and 18313) (Table 1), and four susceptible *S. tuberosum* clones (1024, 1031, CE, and KA) (Table 1) with all 29 coupling phase markers in the cLET5E4-CT120 interval. SH, Reaal, and 17810 showed identical marker patterns in the interval (Fig. 4A and C). Notably, 17810 is synonymous to CPC2127, one of the R3 donor accessions of *S. demissum* (Black 1950). This similarity was not found for any of the four susceptible clones (1024, 1031, CE, and KA). Their marker pattern at the interval was almost identical to that of RH, the susceptible parental line. In the 29 coupling phase markers analyzed, only one marker pattern differed between 1031, CE, KA, and RH, and four differed between 1024 and

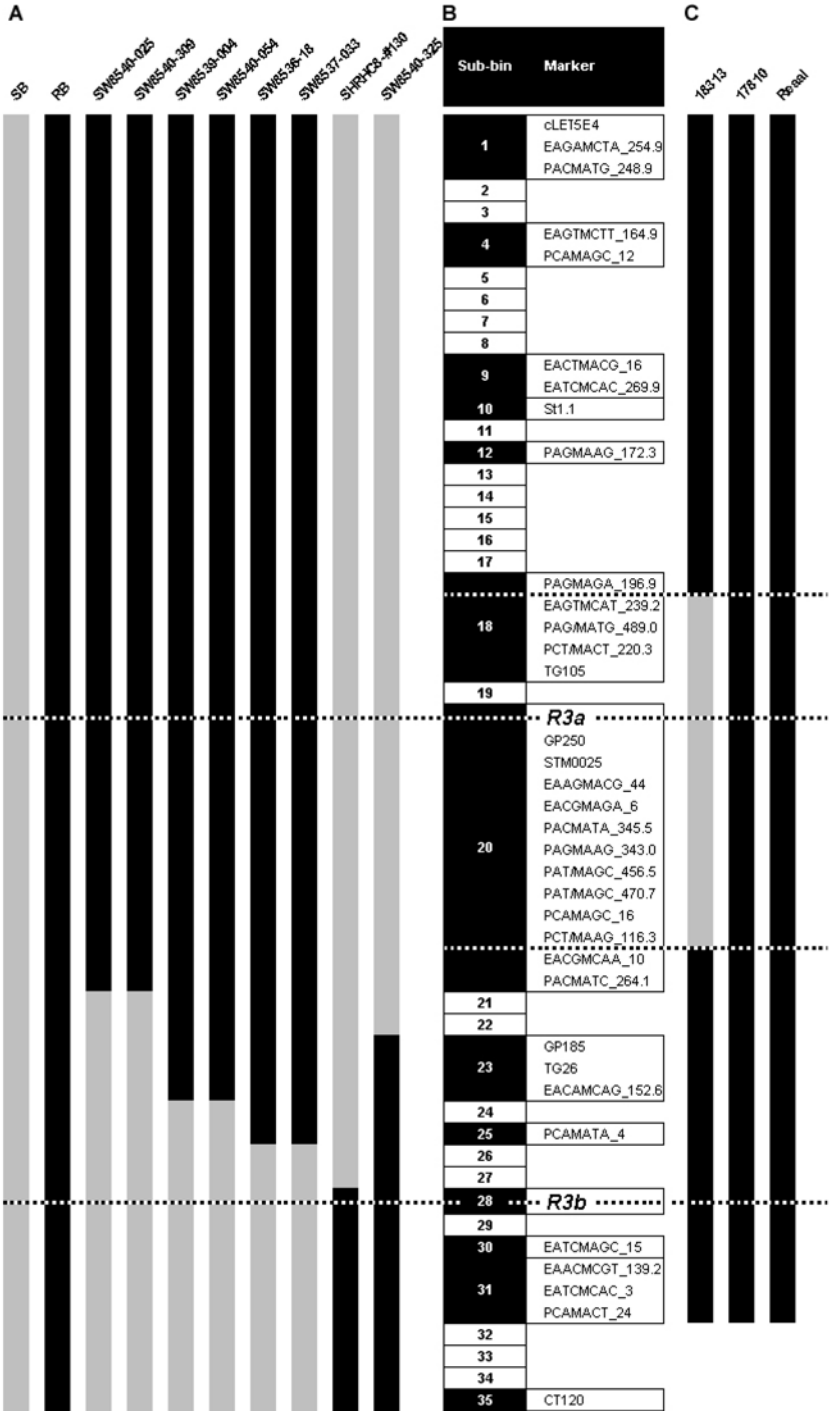

Fig. 4. High-resolution genetic map of *R3a* and *R3b*. **A,** Graphical genotypes of the susceptible bulk (SB, gray bar), the resistant bulk (RB, black bar), and the eight SH83-92-488 × RH89-039-16 progeny that displayed a differential response to H30P04 and IPO-0. The transition of black and gray indicates a cross-over. **B,** The high-resolution map of the *R3* locus. The sub-Bins are numbered from 1 to 35 in a proximal-distal order. The sub-Bins filled with one or more markers, genes, or both are shown with black background and white font. The dotted lines indicate the positions of *R3a* and *R3b* on the genotypes listed in A and C. **C,** Graphical genotypes of Reaal and two *S. demissum* accessions, 18313 and 17810. Reaal and 17810 are identical with RB. Two dotted lines, splitting sub-Bins 18 and 20 indicate the interval of a susceptible "island" at 18313. Marker CT120 was not investigated in the three clones, because it is a repulsion phase marker.

RH. Together with pedigree information, this result strongly supports the notion that the *R3a-R3b*-carrying chromosome region in SH descended from Reaal and originated from *S. demissum*.

Interestingly, marker analysis revealed that another *S. demissum* accession, 18313, contains an "island" of susceptible alleles from sub-Bin 18 to 20, flanked by two blocks of resistance alleles (Fig. 4B and C). The clustering of markers in the island was confirmed by physical mapping (data not shown). The island in 18313 may offer a further resolution for determination of marker order relative to *R3a* because sub-Bin 20 can be divided into two parts based on the result. The AFLP markers EACGMCAA_10 and PACMATC_264.1 were separated from the other markers in sub-Bin 20 (Fig. 4B and C). From the marker pattern, 18313 was predicted to carry *R3b*.

The accession 18313 was subsequently tested for resistance specificity with *P. infestans* isolates 89148-9, IPO-0, and H30P04. It did display a differential reaction to these isolates that was the same as the reaction of the recombinants SHRHC8-#130 and SW8540-325, i.e., resistant to 89148-9 and H30P04 but susceptible to IPO-0. Therefore, we conclude that 18313 does carry *R3b* but not *R3a* and is a natural recombinant of these two *R* genes. The characterization of this accession not only confirmed the presence of the two functionally distinct genes at the *R3* locus but also further delimited *R3a* to the upper part of the otherwise intact sub-Bin 20 (Fig. 4B and C). Thus, the introgression analysis resulted in additional resolution instrumental for fine mapping the *R3* locus.

DISCUSSION

In this report, we describe the construction of a high-resolution genetic map reaching a 0.05-cM resolution at the *R3* locus and the finding that the locus consists of two distinctly functional *R* genes, *R3a* and *R3b*. The high resolution together with accurate disease testing with specific *P. infestans* isolates enabled the detection of two closely linked *R* genes with different resistance spectra. The introgression analysis confirmed that both genes in SH were derived from *S. demissum* and provided a further resolution to narrow down the interval for the *R3a* gene. The resolution obtained from both recombination analysis and introgression study will assist the isolation of both genes using a positional cloning strategy.

For high-resolution genetic mapping, two aspects are essential, marker saturation, i.e., the amount of sequence polymorphisms in the target region, and recombination frequency, i.e., the amount of meiotic cross-overs to resolve the markers. However, these two factors are often negatively correlated. Recombination in general is inhibited by sequence heterogeneity (Borts and Haber 1989; Dooner and Martinez-Ferez 1997; Metzenberg et al. 1991). The frequency of mitotic recombination in yeast can be affected noticeably by less than 1% nucleotide sequence divergence (Datta et al. 1997). Recombination frequency is about eightfold higher in the *Mi* region of a *Lycopersicon peruvianum* cross than a *L. esculentum* cross with *Mi* introgression from *L. peruvianum* (Kaloshian et al. 1998). This is also the case for the *R3* locus in SH, where we found a significant association between sequence heterogeneity, measured by the number of markers per cM and the reduction of recombination frequency using the tomato map as reference (Fulton et al. 2002; Tanksley et al. 1992). The cLET5E4-TG105, TG105-TG26, and TG26-CT120 intervals in SH have 16.5, 80, and 15 markers per cM, respectively. This is correlated to 9, 16, and 4 times less recombination, respectively (r = 0.918). *R3b* resides in a region with higher recombination frequency but less saturation than *R3a*.

Most of the *R* genes that have been characterized at the molecular level belong to complex loci with tightly linked paralogs. Large arrays of similar sequences allow for equal or unequal recombination events, resulting in the formation of new gene family members (Ellis et al. 2000; Hulbert et al. 2001; Michelmore and Meyers 1998). However, the evolutionary fate of new members in complex resistance loci may largely depend on the interaction between plants and their microbial environment. The majority of complex resistance loci carry a functional *R* gene and multiple paralogs with no detectable function, although they are not obvious pseudogenes and appear capable of coding for proteins similar to the functional *R* genes (Hulbert et al. 2001). Only a few complex resistance loci comprise more than one detectable functional *R* gene (Botella et al. 1998; Dixon et al. 1996; Parniske et al. 1997; Takken et al. 1999; van der Vossen et al. 2000; Wang et al. 1998). In contrast to the *R1* and *RB or Rpi-blb1* loci, where only one functional gene for *P. infestans* resistance was found in each locus (Ballvora et al. 2002; Song et al. 2003; van der Vossen et al. 2003), the *R3* locus harbors two *R* genes with distinct resistance specificities to the oomycete pathogen.

Map positions of resistance genes appear to be well-conserved in members of the family Solanaceae (Grube et al. 2000; Pan et al. 2000). The *R3* locus was mapped on the short arm of chromosome 11 in potato (syntenic to the long arm of chromosome 11 in tomato), where potato and tomato show a high macro-colinearity (Tanksley et al. 1992). Up to date, the *SL8* (*I2*) gene family in tomato was the only *R* gene family that has been molecularly characterized on this chromosome

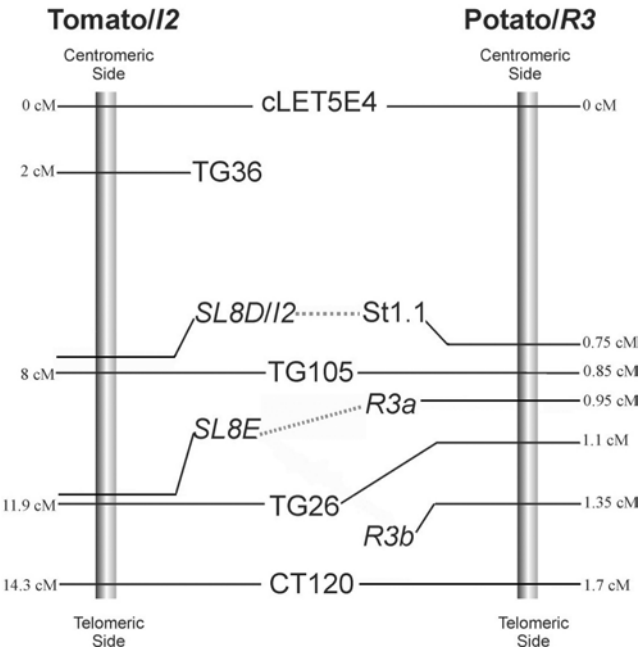

Fig 5. The synteny between the *R3* locus on the short arm of chromosome 11 in potato clone SH83-92-488 and the *I2* locus on the long arm of chromosome 11 of tomato. The left and right solid bars represent the tomato and potato chromosome, respectively. Mapped markers or genes are connected by black lines. On the tomato map, the positions of TG36, TG105, TG26, and CT120 were according to Tanksley and associates (1992), cLET5E4 according to Fulton and associates (2002) and to the Solanaceae Genomics Network, and *SL8D/I2* and *SL8E* according to Ori and associates (1997). The positions of markers and genes on the potato map were determined in this study. For the convenience of comparison, the 'sub-Bin' distances in potato have been changed into the 'cM' distances (1 sub-Bin ≅ 0.05 cM). The dotted line indicated the putative relation between *SL8D* and St1.1 and *SL8E* and *R3a*, respectively.

arm. This gene family distributes to five genomic positions in tomato, and three of these, *SL8C*, *SL8D*, and *SL8E* (in order from the centromere to telomere), were mapped to this chromosome arm (Ori et al. 1997; Simons et al. 1998). The *SL8C* cluster maps within 10 cM of the *Stemphylium* spp. *R* gene *Sm* (Behare et al. 1991). The *SL8D* cluster lies between TG36 and TG105 and contains *I2* and six paralogs (Ori et al. 1997; Simons et al. 1998). The potato marker St1.1 has 91% nucleotide homology with the NBS region of *I2* and localizes between cLET5E4 and TG105. Therefore, St1.1 may be regarded as one of the potato orthologs of the *SL8D* locus (Fig. 5). In tomato, the *SL8E* locus resides between TG105 and TG26 (0.25 cM to TG26) and consists of only one paralog, as indicated by Southern blot analysis (Ori et al. 1997). In this study, *R3a* was also mapped in the TG105-TG26 interval, possibly resembling the *SL8E* locus in tomato. *R3b* is distal to TG26 (Fig. 4B and 5). Moreover, we found several *I2*GAs (tomato *I2* resistance gene analogs) cosegregating with *R3a* and *R3b* (data not shown), but the relationship of these *I2*GAs with *R3a* and *R3b* is yet to be determined. At the *SL8E*-syntenic region of *S. demissum*, *I2*GAs not only grew in number but also duplicated over TG26. It will be interesting to investigate whether this expansion is associated with the capacity to recognize additional elicitors from *P. infestans*.

The genomic region of *R3* is rich in functional diversity for *P. infestans* resistance. Besides *R3*, two additional haplotypes, *R6* and *R7*, confer distinct specificities (El-Kharbotly et al. 1996). The *R3* locus might have passed through multiple rounds of gene duplication and diversifying selection to produce new specificities for *P. infestans* resistance and, thus, could be a good example to study the dynamic evolution of the potato genome in the perspective of coevolution with this (in)famous oomycete pathogen.

MATERIALS AND METHODS

Plant materials.

Plant materials used in the study and their resistance profiles are listed in Table 1. The two *S. demissum* accessions were provided by the Center for Genetic Resources of the Netherlands (Wageningen). All other plant materials were from the Laboratory of Plant Breeding. The population segregating for *R3* was derived from a diploid cross between SH as maternal clone and RH as paternal one. From this cross, 130 progeny have been used for constructing the ultrahigh density map. For high-resolution mapping, 1,618 additional plants from the same cross were used. In total, 1,748 individuals were used to search for recombination events occurring near *R3*. For introgression study, five plants from each *S. demissum* accession were used for inoculation. From each accession, DNA pooled from the five individual plants was subjected to molecular marker analysis.

P. infestans isolates and disease test.

P. infestans isolates used in the study and their virulence profiles are listed in Table 3. Their virulence profiles were confirmed using a standard differential set of potato clones (van der Lee et al. 2001). Inoculum preparation was performed as described previously (Vleeshouwers et al. 1999). Resistance testing was performed on either detached leaves (Vleeshouwers et al. 1999), in vitro plantlets (Huang et al., *unpublished data*), or both. Resistance scoring was performed as described by van der Lee and associates (2001).

DNA isolation.

Genomic DNA from plants grown in greenhouse was isolated according to van der Beek and associates (1992). For DNA extraction of in vitro seedlings, the above method was modified to a high-throughput procedure, using the Retsch machine (Retsch Inc., Haan, Germany) and 96-deep-well Costar microtiter plates (Corning Inc., Corning, NY, U.S.A.).

Molecular markers.

PCR markers were derived from RFLP/SSR markers that have been previously mapped on chromosome 11 of potato (GP185, GP250, St1.1, St3.3.11, and STM0025) and tomato (cLET5E4, CT120, TG26, and TG105) (Gebhardt et al. 1994; Leister et al. 1996; Milbourne et al. 1998; Tanksley et al. 1992). An overview of the markers is given in Table 2. The sequence of GP185 was provided by B. Baker, University of California, Berkeley, CA, U.S.A. Other sequences were obtained from the NCBI database, from the Solanaceae Genomics Network, or from our own sequencing project. Primer pairs were designed with the PrimerSelect module of the DNASTAR package (DNASTAR Inc., Madison, WI, U.S.A.). For PCR analysis, 15-µl reaction mixtures were prepared, containing 20 ng of DNA, 7.5 ng of each primer, 0.1 mM of each dNTP, 0.3 units *Taq* polymerase (Supertaq, Enzyme Technologies Ltd, Cambridge), 10 mM Tris-HCl, pH 9, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, and 0.01% (wt/vol) gelatin. The PCR were performed for 35 cycles, using the following profile: 30 s DNA denaturation at 94ºC, 30 s annealing at melting temperature (Table 2), and 45 s elongation at 72ºC. As a first step in PCR amplification, DNA was denatured for 5 min at 94ºC and finalized by an extra 7-min elongation step at 72ºC. The amplification reactions were performed in a Biometra T-gradient thermocycler (Westburg, Leusden, The Netherlands). Depending on the marker, the PCR product was digested with an appropriate restriction enzyme (Table 2). Subsequently, the (digested) PCR products were analyzed by electrophoresis in agarose gels.

AFLP analysis (Vos et al. 1995) was performed on a Licor sequencer (LI-COR, Lincoln, NB, U.S.A.), using fluorescent-labeled *Eco*RI or *Pst*I primers.

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LITERATURE CITED

Ballvora, A., Ercolano, M. R., Weiss, J., Meksem, K., Bormann, C. A., Oberhagemann, P., Salamini, F., and Gebhardt, C. 2002. The *R1* gene for potato resistance to late blight (*Phytophthora infestans*) belongs to the leucine zipper/NBS/LRR class of plant resistance genes. Plant J. 30:361-371.

Behare, J., Laterrot, H., Sarfatti, M., and Zamir, D. 1991. Restriction fragment length polymorphism mapping of the *Stemphylium* resistance gene in tomato. Mol. Plant-Microbe Interact. 4:489-492.

Black, W. 1950. Inheritance of resistance to blight (*Phytophthora infestans* de Bary) in potatoes: Strain C and its relationships. T. Roy. Soc. Edin. 64:216-228.

Black, W., Mastenbroek, C., Mills, W. R., and Peterson, L. C. 1953. A proposal for an international nomenclature of races of *Phytophthora infestans* and of genes controlling immunity in *Solanum demissum* derivatives. Euphytica 2:173-178.

Borts, R. H., and Haber, J. E. 1989. Length and distribution of meiotic gene conversion tracts and crossovers in *Saccharomyces cerevisiae*. Genetics 123:69-80.

Botella, M. A., Parker, J. E., Frost, L. N., Bittner, E. P. D., Beynon, J. L., Daniels, M. J., Holub, E. B., and Jones, J. D. G. 1998. Three genes of the *Arabidopsis RPP1* complex resistance locus recognize distinct *Peronospora parasitica* avirulence determinants. Plant Cell 10:1847-1860.

Dangl, J. L., and Jones, J. D. 2001. Plant pathogens and integrated defence responses to infection. Nature 411:826-33.

Datta, A., Hendrix, M., Lipsitch, M., and Jinks-Robertson, S. 1997. Dual roles for DNA sequence identity and the mismatch repair system in the regulation of mitotic crossing-over in yeast. Proc. Natl. Acad. Sci. U.S.A. 94:9757-9762.

Dixon, M. S., Jones, D. A., Keddie, J. S., Thomas, C. M., Harrison, K., and Jones, J. D. 1996. The tomato *Cf-2* disease resistance locus comprises two functional genes encoding leucine-rich repeat proteins. Cell 84:451-459.

Dong, F., Song, J., Naess, S. K., Helgeson, J. P., Gebhardt, C., and Jiang, J. 2000. Development and applications of a set of chromosome-specific cytogenetic DNA markers in potato. Theor. Appl. Genet. 101:1001-1007.

Dooner, H. K., and Martinez-Ferez, I. M. 1997. Recombination occurs uniformly within the bronze gene, a meiotic recombination hotspot in the maize genome. Plant Cell 9:1633-1646.

El Kharbotly, A., Leonards-Schippers, C., Huigen, D. J., Jacobsen, E., Pereira, A., Stiekema, W. J., Salamini, F., and Gebhardt, C. 1994. Segregation analysis and RFLP mapping of the *R1* and *R3* alleles conferring race-specific resistance to *Phytophthora infestans* in progeny of dihaploid potato parents. Mol. Gen. Genet. 242:749-754.

El Kharbotly, A., Palomino Sanchez, C., Salamini, F., Jacobsen, E., and Gebhardt, C. 1996. *R6* and *R7* alleles of potato conferring race-specific resistance to *Phytophthora infestans* (Mont.) de Bary identified genetic loci clustering with the *R3* locus on chromosome XI. Theoret. Appl. Genet. 92:880-884.

Ellis, J., Dodds, P., and Pryor, T. 2000. Structure, function and evolution of plant disease resistance genes. Curr. Opin. Plant Biol. 3:278-284.

Flor, H. H. 1971. Current status of the gene-for-gene concept. Ann. Rev. Phytopathol. 78:275-298.

Fry, W. E., and Goodwin, S. B. 1997. Resurgence of the Irish potato famine fungus. Bioscience 47:363-371.

Fulton, T. M., Van Der Hoeven, R., Eannetta, N. T., and Tanksley, S. D. 2002. Identification, analysis, and utilization of conserved ortholog set markers for comparative genomics in higher plants. Plant Cell 14:1457-1467.

Gebhardt, C., Ritter, E., and Salamini, F. 1994. RFLP map of potato. Pages 271-285 in: Advances in Cellular and Molecular Biology. DNA-Based Markers in Plants. R. L. Philips and I. K. Vasil, eds. Kluwer, Boston.

Gebhardt, C., and Valkonen, J. P. 2001. Organization of genes controlling disease resistance in the potato genome. Annu. Rev. Phytopathol. 39:79-102.

Grube, R. C., Radwanski, E. R., and Jahn, M. 2000. Comparative genetics of disease resistance within Solanaceae. Genetics 155:873-887.

Hulbert, S. H., Webb, C. A., Smith, S. M., and Sun, Q. 2001. Resistance gene complexes: Evolution and utilization. Annu. Rev. Phytopathol. 39:285-312.

Kaloshian, I., Yaghoobi, J., Liharska, I., Hontelez, J., Hanson, D., Hogan, P., Jesse, T., Wijbrandi, J., Simons, G., Vos, P., Zabel, P., and Williamson, V. M. 1998. Genetic and physical localization of the root-knot nematode resistance locus Mi in tomato. Mol. Gen. Genet. 257:376-385.

Kamoun, S., Huitema, E., and Vleeshouwers, V. G. A. A. 1999. Resistance to oomycetes: A general role for the hypersensitive response? Trends Plant Sci. 4:196-200.

Kanyuka, K., Bendahmane, A., Rouppe van der Voort, J., van der Vossen, E., and Baulcombe, D. C. 1999. Mapping of intra-locus duplications and introgressed DNA: Aids to map-based cloning of genes from complex genomes illustrated by physical analysis of the *Rx* locus in tetraploid potato. Theor. Appl. Genet. 98:679-689.

Keen, N. T. 1990. Gene-for-gene complementarity in plant-pathogen interactions. Annu. Rev. Genet. 24:447-463.

Leister, D., Ballvora, A., Salamini, F., and Gebhardt, C. 1996. A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. Nat. Genet. 14:421-429.

Mastenbroek, C. 1953. Experiments on the inheritance of blight immunity in potatoes derived from *Solanum demissum* Lindl. Euphytica 2:197-206.

Metzenberg, A. B., Wurzer, G., Huisman, T. H., and Smithies, O. 1991. Homology requirements for unequal crossing over in humans. Genetics 128:143-161.

Michelmore, R. W., and Meyers, B. C. 1998. Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Res. 8:1113-1130.

Milbourne, D., Meyer, R. C., Collins, A. J., Ramsay, L. D., Gebhardt, C., and Waugh, R. 1998. Isolation, characterisation and mapping of simple sequence repeat loci in potato. Mol. Gen. Genet. 259:233-245.

Morel, J. B., and Dangl, J. L. 1997. The hypersensitive response and the induction of cell death in plants. Cell Death Differ. 4:671-683.

Ori, N., Eshed, Y., Paran, I., Presting, G., Aviv, D., Tanksley, S., Zamir, D., and Fluhr, R. 1997. The *I2C* family from the wilt disease resistance locus *I2* belongs to the nucleotide binding, leucine-rich repeat superfamily of plant resistance genes. Plant Cell 9:521-532.

Pan, Q., Liu, Y. S., Budai-Hadrian, O., Sela, M., Carmel-Goren, L., Zamir, D., and Fluhr, R. 2000. Comparative genetics of nucleotide binding site-leucine rich repeat resistance gene homologues in the genomes of two dicotyledons: Tomato and *Arabidopsis*. Genetics 155:309-322.

Parniske, M., Hammond-Kosack, K. E., Golstein, C., Thomas, C. M., Jones, D. A., Harrison, K., Wulff, B. B., and Jones, J. D. 1997. Novel disease resistance specificities result from sequence exchange between tandemly repeated genes at the *Cf-4/9* locus of tomato. Cell 91:821-832.

Simons, G., Groenendijk, J., Wijbrandi, J., Reijans, M., Groenen, J., Diergaarde, P., Van der Lee, T., Bleeker, M., Onstenk, J., de Both, M., Haring, M., Mes, J., Cornelissen, B., Zabeau, M., and Vos, P. 1998. Dissection of the *Fusarium I2* gene cluster in tomato reveals six homologs and one active gene copy. Plant Cell 10:1055-1068.

Song, J., Bradeen, J. M., Naess, S. K., Raasch, J. A., Wielgus, S. M., Haberlach, G. T., Liu, J., Kuang, H., Austin-Phillips, S., Buell, C. R., Helgeson, J. P., and Jiang, J. 2003. Gene *RB* cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. Proc. Natl. Acad. Sci. U.S.A. 100:9128-9133.

Takken, F. L., Thomas, C. M., Joosten, M. H., Golstein, C., Westerink, N., Hille, J., Nijkamp, H. J., De Wit, P. J., and Jones, J. D. 1999. A second gene at the tomato *Cf-4* locus confers resistance to *Cladosporium fulvum* through recognition of a novel avirulence determinant. Plant J. 20:279-288.

Tanksley, S. D., Ganal, M. W., and Martin, G. B. 1995. Chromosome landing: A paradigm for map-based gene cloning in plants with large genomes. Trends Genet. 11:63-68.

Tanksley, S. D., Ganal, M. W., Prince, J. P., de Vicente, M. C., Bonierbale, M. W., Broun, P., Fulton, T. M., Giovannoni, J. J., Grandillo, S., Martin, G. B., and et al. 1992. High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141-1160.

Thompson, J. N., and Burdon, J. J. 1992. Gene-for-gene coevolution between plants and parasites. Nature 360:121-125.

van der Beek, J. G., Verkerk, R., Zabel, P., and Lindhout, P. 1992. Mapping strategy for resistance genes in tomato based on RFLPs between cultivars: *Cf9* (resistance to *Cladosporium fulvum*) on chromosome 1. Theor. Appl. Genet. 84:106-112.

van der Lee, T., Robold, A., Testa, A., van't Klooster, J. W., and Govers, F. 2001. Mapping of avirulence genes in *Phytophthora infestans* with AFLP markers by bulk segregant analysis. Genetics 157:949-956.

van der Vossen, E. A. G., Rouppe van der Voort, J. N. A. M., Kanyuka, K., Bendahmane, A., Sandbrink, H., Baulcombe, D. C., Bakker, J., Stiekema, W. J., and Klein Lankhorst, R. M. 2000. Homologues of a single resistance-gene cluster in potato confer resistance to distinct pathogens: A virus and a nematode. Plant J. 23:567-576.

van der Vossen, E. A. G., Sikkema, A., Hekkert, B. T. L., Gros, J., Stevens, P., Muskens, M., Wouters, D., Pereira, A., Stiekema, W. J., and Allefs, S. 2003. An ancient *R* gene from the wild potato species *Solanum bulbocastanum* confers broad-spectrum resistance to *Phytophthora infestans* in cultivated potato and tomato. Plant J. 36:867-882.

Vleeshouwers, V. G. A. A., van Dooijeweert, W., Govers, F., Kamoun, S., and Colon, L. T. 2000. The hypersensitive response is associated with host and nonhost resistance to *Phytophthora infestans*. Planta 210:853-864.

Vleeshouwers, V. G. A. A., van Dooijeweert, W., Keizer, L. C. P., Sijpkes, L., Govers, F., and Colon, L. T. 1999. A laboratory assay for *Phytophthora infestans* resistance in various *Solanum* species reflects the field situation. Eur. J. Plant Pathol. 105:241-250.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407-14.

Wang, G. L., Ruan, D. L., Song, W. Y., Sideris, S., Chen, L., Pi, L. Y., Zhang, S., Zhang, Z., Fauquet, C., Gaut, B. S., Whalen, M. C., and Ronald, P. C. 1998. *Xa21D* encodes a receptor-like molecule with a leucine-rich repeat domain that determines race-specific recognition and is subject to adaptive evolution. Plant Cell 10:765-79.

AUTHOR-RECOMMENDED INTERNET RESOURCES

European Union project to develop an ultrahigh density (UHD) map of the potato: www.dpw.wageningen-ur.nl/uhd)

The Solanaceae Genomics Network: www.sgn.cornell.edu