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Rpi-vnt1.1, a *Tm-2²* Homolog from *Solanum venturii*, Confers Resistance to Potato Late Blight

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Despite the efforts of breeders and the extensive use of fungicide control measures, late blight still remains a major threat to potato cultivation worldwide. The introduction of genetic resistance into cultivated potato is considered a valuable method to achieve durable resistance to late blight. Here, we report the identification and cloning of *Rpi-vnt1.1*, a previously uncharacterized late-blight resistance gene from *Solanum venturii*. The gene was identified by a classical genetic and physical mapping approach and encodes a coiled-coil nucleotide-binding leucine-rich repeat protein with high similarity to *Tm-2²* from *S. lycopersicum* which confers resistance against *Tomato mosaic virus*. Transgenic potato and tomato plants carrying *Rpi-vnt1.1* were shown to be resistant to *Phytophthora infestans*. Of 11 *P. infestans* isolates tested, only isolate EC1 from Ecuador was able to overcome *Rpi-vnt1.1* and cause disease on the inoculated plants. Alleles of *Rpi-vnt1.1* (*Rpi-vnt1.2* and *Rpi-vnt1.3*) that differed by only a few nucleotides were found in other late-blight-resistant accessions of *S. venturii*. The late blight resistance gene *Rpi-phu1* from *S. phureja* is shown here to be identical to *Rpi-vnt1.1,* suggesting either that this strong resistance gene has been maintained since a common ancestor, due to selection pressure for blight resistance, or that genetic exchange between *S. venturii* and *S. phureja* has occurred at some time.

Potato (*Solanum tuberosum* L.) is the fourth most important crop and the most important noncereal food crop in the world (Lang 2001). The major biotic factor which limits potato crop yields is late blight, caused by the oomycete pathogen *Phytophthora infestans* (Mont.) de Bary. Famous as the cause of the Great Irish Potato Famine of the mid-nineteenth century (Large 1940), this devastating disease can result in complete loss of crop yield unless effective control measures are applied (Świeżyński and Zimnoch-Guzowska 2001). Fungicide treatment is currently the most common method for controlling late blight. However, sprays can be required every 4 days during severe epidemics and the high cost of fungicide application is a major burden to growers, especially in developing countries. Moreover, because fungicide application can have an impact on health and the environment, the use of many chemicals is becoming restricted. In addition, the pathogen evolves quickly and insensitivity or tolerance to commonly used fungicides can arise (Day and Shattock 1997; Goodwin et al. 1996). Therefore, the introduction of genetic resistance into cultivated potato is considered to be a valuable method to achieve durable resistance to late blight.

Two main types of resistance to late blight have been described in potato: field resistance and resistance (*R*) gene–mediated resistance (Umaerus and Umaerus 1994). Field resistance (also referred to as general or quantitative resistance) is frequently attributed to major quantitative trait loci (QTL) and a few minor QTL and generally results in partial resistance. Field resistance is considered by some to be more durable than resistance conferred by single *R* genes (Turkensteen 1993). However, partial resistance is also strongly correlated with maturity type and, thus, makes resistance breeding more difficult (Wastie 1991). Also, the genetic positions of QTL often correspond to regions of *R* gene clusters (Gebhardt and Valkonen 2001; Grube et al. 2000), raising the possibility that field resistance is due to the action of multiple weak *R* genes which are also susceptible to defeat by the evolving pathogen. Specific resistance is based on major dominant *R* genes. In early breeding programs during the first half of the twentieth century, 11 *R* genes (*R1* to *R11*) were identified in *S. demissum*, a wild species originating from Mexico. The *S. demissum* genes *R1, R3*, and *R10* have been heavily relied on for blight resistance in major breeding programs within Europe since their introgression. As a result, the *R* genes introgressed from *S. demissum* to cultivated potato lines have been overcome as new pathogen strains evolve that are virulent on the previously resistant hosts (Fry and Goodwin 1997; Umaerus and Umaerus 1994; Wastie 1991). This ability of *P. infestans* to rapidly overcome *R* genes limits the durability of any single *R* gene. Although some of the *S. demissum* genes such as *R5, R8*, and *R9* have not been used in European cultivars, isolates of *P. infestans* which overcome these genes are known, albeit rare. However, it is possible that, by deploying multiple *R* genes as a mixture in an otherwise genetically uniform crop, the ability of *P. infestans* to overcome these genes may be impaired (Jones 2001; Pink and Puddephat 1999).

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Nucleotide sequence data for the *Solanum venturii* late blight resistance genes *Rpi-vnt1.1, Rpi-vnt1.2*, and *Rpi-vnt1.3* are available in the GenBank database under accession numbers FJ423044, FJ423045, and FJ423046, respectively.

Recent efforts to identify late-blight resistance have focused on major *R* genes conferring broad-spectrum resistance derived from diverse wild *Solanum* spp. In addition to *S. demissum*, other wild *Solanum* spp. such as *S. acaule*, *S. chacoense*, *S. berthaultii*, *S. brevidens*, *S. bulbocastanum*, *S. microdontum*, *S. sparsipilum*, *S. spegazzinii*, *S., stoloniferum*, *S. sucrense*, *S. toralapanum*, *S. vernei*, and *S. verrucosum* have been reported as new sources for resistance to late blight (Hawkes 1990; Jansky 2000).

Much progress has been made in the identification, mapping, and cloning of *R* genes against late blight. Of the *S. demissum R* genes, *R3*, *R6*, and *R7* have been mapped to a complex locus on chromosome XI (El-Kharbotly et al. 1994, 1996; Huang et al. 2004). The *R3* locus transpired to contain two distinct, closely linked genes, *R3a* and *R3b* (Huang et al. 2004, 2005). *R5, R8, R9, R10*, and *R11* appear to be allelic versions of the *R3* locus (Huang 2005) although *R10* is reported to behave more like a QTL than a dominant *R* gene (Bradshaw et al. 2006). *R1* has been mapped to chromosome V (El-Kharbotly et al. 1994; Leonards-Schippers et al. 1992) and *R2* to chromosome IV (Li et al. 1998). Of these, *R1* and *R3a* have been cloned (Ballvora et al. 2002; Huang et al. 2005). Of the resistances identified in wild *Solanum* spp., three *R* genes, *RB*/*Rpi-blb1*, *Rpi-blb2*, and *Rpi-blb3* from *S. bulbocastanum*, have been mapped to chromosomes VIII, VI, and IV, respectively (Naess et al. 2000; Park et al. 2005a; van der Vossen et al. 2003, 2005). *Rpi-abpt*, probably from *S. bulbocastanum*, has been localized on chromosome IV (Park et al. 2005c). *Rpi1* from *S. pinnatisectum* on chromosome VII (Kuhl et al. 2001), *Rpi-mcq1* (formerly *Rpi-moc1*) from *S. mochiquense* (Smilde et al. 2005), and *Rpi-phu1* from *S. phureja* on chromosome IX (Śliwka et al. 2006) have also been identified. Thus far, *Rpi-blb1* (*RB*) and *Rpi-blb2* are the only late-blight *R* genes reported to have been successfully cloned from wild *Solanum* spp. (Song et al. 2003; van der Vossen et al. 2003, 2005).

Here, we report the identification and cloning of the chromosome IX *Rpi* gene *Rpi-vnt1.1*, a previously uncharacterized late-blight resistance gene from *S. venturii*, an Argentinean wild species with an endosperm balance number (EBN) of 2. The gene was identified by a classical genetic and physical mapping approach and encodes a coiled-coil nucleotide-binding leucine-rich repeat (CC-NB-LRR) protein with high similarity to *Tm-2*^{2} from *S. lycopersicum* (formerly *Lycopersicon esculentum*) which confers resistance against *Tomato mosaic virus* (ToMV).

RESULTS

Variation for resistance to *P. infestans* in Centre for Genetics Resources accessions.

Accessions of *S. venturii* and *S. okadae* were obtained from the Centre for Genetics Resources in Wageningen, the Netherlands (CGN) (Table 1). The *S. venturii* accessions were originally listed as *S. okadae* in the CGN database but have recently been reclassified based on work using amplified fragment length polymorphism (AFLP) markers to study the validity of species labels in *Solanum* section Petota (Jacobs 2008; Jacobs et al. 2008). Species classification of accession CGN18279 is at present unclear because conflicting positions in the species dendrogram were obtained for this accession (Jacobs 2008). Screening of the accessions using *P. infestans* isolates 98.170.3 and 90128 in detached leaf assays showed phenotypic variation for resistance in four of the *S. venturii* accessions, *S. okadae* accession CGN18129, and the unclassified accession CGN18279 (Table 1). Resistance was evident as a complete lack of sporulation on leaf tissue whereas extensive mycelial growth was evident on leaves of sensitive individuals from 5 to 6 days postinoculation. Sensitive leaves often turned completely black by 7 days postinoculation. All individuals tested from the remaining accessions were sensitive to isolates 90128 and 98.170.3, despite CGN data indicating that at least three of these accessions were moderately or very resistant to *P. infestans*.

Development of *S. venturii* mapping populations.

Resistant individuals from five of the *S. venturii* accessions were crossed with sensitive individuals from either the same or different accessions (Table 2). In each of the crosses, resistance to *P. infestans* segregated 1:1 in the resulting progeny, indicating the presence of potentially five *Rpi* genes in the resistant heterozygous parents (Table 2).

Mapping *Rpi* genes in *S. venturii.*

Bulked segregant analysis (BSA) (Michelmore et al. 1991) was carried out on DNA pools comprising DNA from each of 10 resistant and susceptible progeny from the populations Svnt014 (*Rpi-vnt1.1*), Svnt013 (*Rpi-vnt1.2*), and Svnt040 (*Rpi-vnt1.3*) to find AFLP markers linked to these resistance loci. In total, 72 primer combinations were tested for each population and potentially linked markers were confirmed by checking for cosegregation of the marker with the resistance gene in the individual progeny that made up the bulks. A number of linked

Table 1. Reaction to *Phytophthora infestans* of *Solanum okadae* and *Solanum venturii* accessions used in this study

		Reference data		Fine screening[a]			
Accession[b]	Species[c]	Phenotype[d]	Source	R	MR	MS	S
CGN17998	*S. venturii*	Very resistant	CGN	2	…	…	7
CGN17999	*S. venturii*	Resistant	CGN	3	…	…	7
CGN18108	*S. venturii*	Very resistant	CGN	8	…	3	…
CGN18109	*S. venturii*	Very resistant	CGN	…	…	…	10
CGN22703	*S. venturii*	Very sensitive	CGN	4	1	…	4
CGN18269	*S. venturii*	Sensitive	CGN	…	…	…	10
CGN18279	Unclassified	Very resistant	CGN	4	…	…	5
CGN18129	*S. okadae*	Sensitive	CGN	…	2	2	6
CGN20599	*S. okadae*	Sensitive	CGN	…	…	…	10
CGN18157	*S. okadae*	Moderately resistant	CGN	…	…	…	10
CGN22709	*S. okadae*	Very sensitive	CGN	…	…	…	8
BGRC27158	*S. okadae*[e]	Moderately resistant	CGN	…	…	…	1

[a] Number of plants showing resistant (R), moderately resistant (MR), moderately sensitive (MS), or sensitive (S) phenotypes.
[b] CGN = Centre for Genetic Resources in the Netherlands; BGRC, Braunschweig Genetic Resources Center.
[c] Species designation according to Jacobs (2008).
[d] Blight resistance phenotype according to CGN data.
[e] Was not included in the study of Jacobs (2008); hence, original classification given.

AFLP markers were placed on the *Rpi-vnt1.1, Rpi-vnt1.2*, and *Rpi-vnt1.3* linkage maps (Fig. 1), including SvntM2.9L (*Rpi-vnt1.1*); Svnt13M5.17, P12M44_103, P13M42_228, and P17M33_472 (*Rpi-vnt1.2*); and M6.44 (*Rpi-vnt1.3*). Of these, SvntM2.9L (6 centimorgans [cM] from *Rpi-vnt1.1*), Svnt13M5.17 (6.5 cM from *Rpi-vnt1.2*), and M6.44 (23 cM from *Rpi-vnt1.3*) were successfully converted into polymerase chain reaction (PCR)-based markers.

The *Rpi-vnt1.1* marker SvntM2.9L was also found to be polymorphic (by *Alu*I digestion) between *S. lycopersicum* (formerly *L. esculentum*) and *S. pennellii* (formerly *L. pennellii*), the parents of the *L. pennellii* introgression lines (Eshed and Zamir 1994). Screening of the individual introgression lines (IL) showed that this marker was located in IL 9.2, indicating that the marker could be on either arm of chromosome IX. The polymorphism was not present in IL 9.1, which overlaps IL 9.2 substantially, suggesting that the marker was situated proximal to the centromere on either chromosome arm. Further evidence for the location of the *Rpi-vnt* locus on chromosome IX came from the finding that polymorphisms in three chromosome IX simple-sequence repeat (SSR) markers (Stm0010, Stm 1051, and Stm 3012) (Milbourne et al. 1998) were linked to *Rpi-vnt1.3* (Fig. 1).

Additional chromosome IX markers were developed by designing PCR primers from known chromosome IX restriction fragment length polymorphism marker sequences within the SGN database, sequencing the PCR products amplified from both resistant and sensitive parental DNA and identifying single nucleotide polymorphisms (SNP) that could be used to develop cleaved amplified polymorphic sequence (CAPS) markers (Table 3). In this way, *Rpi-vnt1.1* was mapped to a 6.0-cM region of chromosome IX, delimited by markers C2_At4g02680 and TG186. *Rpi-vnt1.2* and *Rpi-vnt1.3* also mapped to the same location as *Rpi-vnt1.1* (Fig. 1). Although the markers TG551, TG35, T1421, C2_At3g63190, and C2_At4g02680 are bridging markers between the maps of at least two of the *Rpi* genes, the SNP present between the parents of the respective populations were not identical (as illustrated by the restriction enzymes used to reveal the polymorphisms) (Table 3), suggesting that each of the genes resides on a distinct haplotype. The markers TG551, TG35, and T1421 were also found to be polymorphic in the *Rpi-vnt1.4* and *Rpi-vnt1.5* populations. How-

Table 2. Crosses within *Solanum venturii* and late-blight-resistant (R) and -sensitive (S) segregants in their progenies

	Population parents[a]		Segregating progeny[b]		
Population	**Female**	**Male**	**R**	**S**	***Rpi* gene identified**
Svnt014	A618, CGN18108, R	A613, CGN18108, S	26	24	*Rpi-vnt1.1*
Svnt012	A622, CGN18279, S	A618, CGN18108, R	18	23	*Rpi-vnt1.1*
Svnt013	A624, CGN18279, R	A613, CGN18108, S	18	25	*Rpi-vnt1.2*
Svnt040	A606, CGN17998, R	A628, CGN18279, S	25	23	*Rpi-vnt1.3*
Svnt241	D986, BGRC08237, R	B419, Svnt012, S	24	26	*Rpi-vnt1.4*
Svnt184	D403, CGN17999, R	D401, CGN17999, S	24	21	*Rpi-vnt1.5*

[a] Plant identifier number followed by its accession number and reaction to *Phytophthora infestans* inoculation. CGN, Centre for Genetic Resources in the Netherlands; BGRC, Braunschweig Genetic Resources Center.
[b] Number of plants showing resistant (R) or sensitive (S) phenotypes.

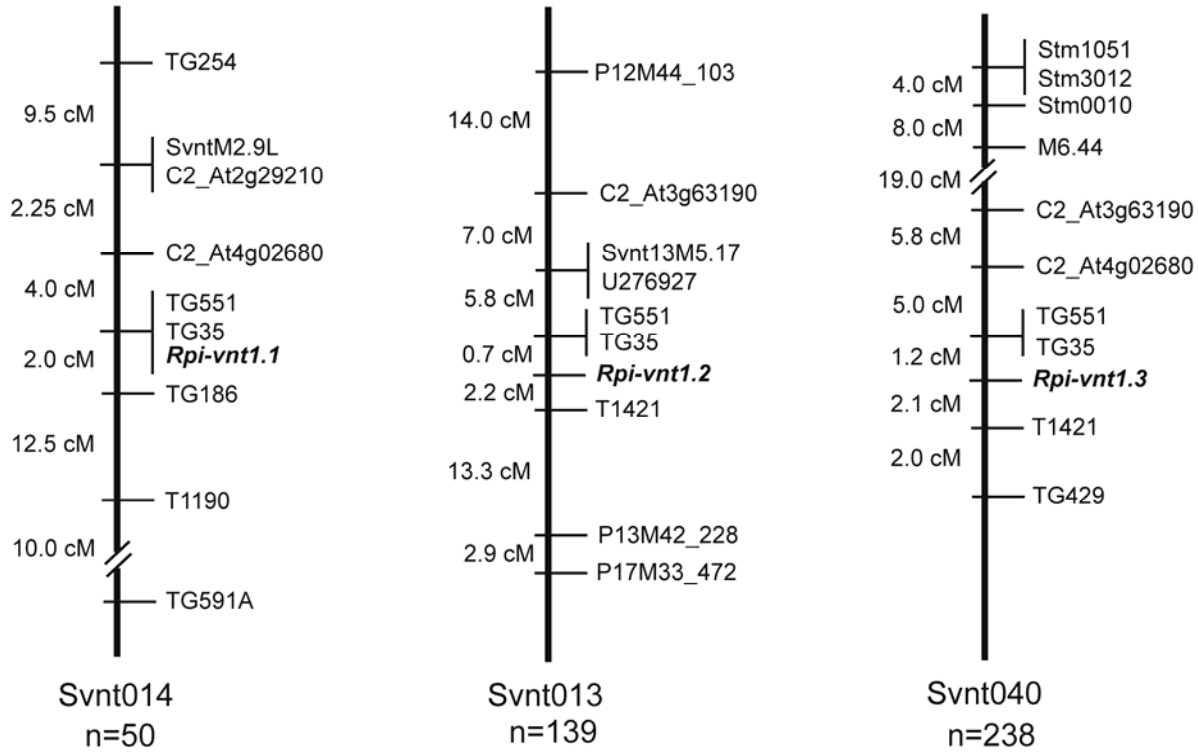

Fig. 1. Genetic linkage maps for *Rpi-vnt1.1, Rpi-vnt1.2*, and *Rpi-vnt1.3* on chromosome IX of *Solanum venturii*. Marker names and genetic distance in centimorgans (cM) are indicated on the right and left of each map, respectively. For each map, the number of individuals on which the mapping is based is given by *n*.

ever, the SNP present in the markers were identical to those of the *Rpi-vnt1.1* haplotype and, thus, we assumed that the haplotype present in *S. venturii* accessions BGRC08237 and CGN17999 was identical to that of CGN18108, from which *Rpi-vnt1.1* is derived.

Concurrent work by Pel and associates (2009) used nucleotide-binding site (NBS) profiling (van der Linden et al. 2004) to identify a candidate NBS marker (NBS3a) with homology to the ToMV *R* gene *Tm-2*^{2} (Lanfermeijer et al. 2003) which was closely linked to *Rpi-vnt1.1* in a population derived from the same *S. venturii* accession (CGN18108). From the sequence of this marker, we designed allele-specific PCR primers (vntNBSHae-F/R) (Table 3) and showed that this marker (vntNBSHae) cosegregated with *Rpi-vnt1.1* and *Rpi-vnt1.2* and mapped 1.2 cM distal to *Rpi-vnt1.3* (Fig. 1).

BAC library screening and contig construction.

The *S. venturii* plant K39 which was used to construct the BAC (bacterial artificial chromosome) library is a transheterozygote carrying both *Rpi-vnt1.1*, originally from the resistant parent plant A618 (accession CGN18108), and *Rpi-vnt1.2* from the resistant plant A624 (accession CGN18279). The *S. venturii* K39 BAC library consisted of 105,216 clones stored in 274-by-384-well microtiter plates. Insert size ranged from

Table 3. Polymerase chain reaction-based markers used for mapping of *Rpi-vnt1.1*, *Rpi-vnt1.2*, and *Rpi-vnt1.3*

			Type of marker[a]		
Marker	**Primer sequence (5′–3′)**	**Temperature (°C)**	***Rpi-vnt1.1***	***Rpi-vnt1.2***	***Rpi-vnt1.3***
vntNBSHae	F: CTTACTTTCCCTTCCTCATCCTCAC R: TGAAGTCATCTTCCAGACCGATG	60	a.s.	a.s.	*Mae*III
vnt1Long	F: AGTTATACACCCTACATTCTACTCG R: CTTTGAAAAGAGGCTTCATACTCCC	60	a.s.	a.s.	a.s.
vnt1ORF	F: GGG**CTCGAG**CGAAATACCAGCTAACAAAAGATG[b] R: GGC**GGATCC**TAGTACCTGTGATATTCTCAACTTTGC[c]	60	a.s.	a.s.	a.s.
TG254	F: AGTGCACCAAGGGTGTGAC R: AAGTGCATGCCTGTAATGGC	60	…	…	…
At2g38025	F: ATGGGCGCTGCATGTTTCGTG R: ACACCTTTGTTGAAAGCCATCCC	55	…	…	*Tsp509*I (R)
Stm1051	F: TCCCCTTGGCATTTTCTTCTCC R: TTTAGGGTGGGGTGAGGTTGG	55	…	…	SSR
Stm3012	F: CAACTCAAACCAGAAGGCAAA R: GAGAAATGGGCACAAAAAACA	55	…	…	SSR
Stm0010	F: TCCTTATATGGAGCAAGCA R: CCAGTAGATAAGTCATCCCA	50	…	…	SSR (R)
M6.44	F: ATTGAAAGAATACACAAACATC R: ATTCATGTTCAGATCGTTTAC	55	…	…	*Dde*I
At3g63190	F: TTGGTGCAGCCGTATGACAAATCC R: TCCATCATTATTTGGCGTCATACC	55	…	*Eco*RI	*Tsp509*I
SvntM2.9L	F: ACAAACCTATGTTAGCCTCCCACAC R: GGCATCAAGCCAATGTCGTAAAG	60	*Dde*I	…	…
At2g29210	F: AGCAGGACACTCGATTCTCTAATAAGC R: TGCACTAAGTAGTAATGCCCAAAGCTC	55	*Nco*I	…	…
Svnt13M5.17	F: CTGAGGTGCAGCCAATAAC R: CCAGTGAGAAACAGCTTCTC	55	…	a.s.	…
U276927	F: GATGGGCAACGATGTTGTTG R: GCATTAGTACAGCGTCTTGGC	60	…	*Hpy188*I	…
At4g02680	F: GTGAAGAAGGTCTACAGAAAGCAG R: GGGCATTAATGTAGCAATCAGC	55	*Mse*I	…	*Nhe*I
TG551	F: CATATCCTGGAGGTGTTATGAATGC R: AACTTTCAGATATGCTCTGCAGTGG	60	*Mwo*I	*Taq*I	*Taq*I
TG35	F: CACGGAGACTAAGATTCAGG R: TAAAGGTGATGCTGATGGGG	55	*Hha*I	*Alu*I	*Tsp509*I
T1421	F: CATCAATTGATGCCTTTGGACC R: CTGCATCAGCTTCTTCCTCTGC	60	…	*Bsl*I	*Rsa*I
TG186	F: AATCGTGCAGTTTCAGCATAAGCG R: TGCTTCCAGTTCCGTGGGATTC	60	*Dra*I (R)	…	…
TG429	F: CATATGGTGACGCCTACAG R: GGAGACATTGTCACAAGG	55	…	…	*Mse*I
T1190	F: GTTCGCGTTCTCGTTACTGG R: GTTGCATGGTTGACATCAGG	55	a.s.	…	…
TG591A	F: CTGCAAATCTACTCGTGCAAG R: CTCGTGGATTGAGAAATCCC	60	a.s.	…	…
266I9F	F: GTATGTTTGAGTTAGTCTTCC R: TATAATAGGTGTTCTTGGGG	55	…	*Hin*fI	…
266I9R	F: AAGGTGTTGGGAGTTTTTAG R: TATCTTCCTCATTTTGGTGC	55	*Hin*dIII	*Hin*dIII	…
185L21R	F: GATTGAGACAATGCTAGTCC R: AGAAGCAGTCAATAGTGATTG	55	*Bsl*I	*Rsa*I	…
148P20R	F: AAGATTCTTTTTCCTCCTTAG R: AAAGATGAAGTAGAGTTTTGG	58	*Hpy*CH4IV	…	…

[a] Restriction enzymes indicate that marker is a cleaved amplified polymorphic sequence marker, a.s. indicates allele-specific markers, R indicates that marker is linked in repulsion phase, SSR indicates that marker is a simple-sequence repeat marker, and … indicates that the marker was either not polymorphic or not tested for that *Rpi* gene.

[b] Bold type indicates *Xho*I site included in 5′ region for cloning purposes.

[c] Bold type indicates *Bam*HI site included in 5′ region for cloning purposes.

60 to 165 kb with an average of 103.5 kb, based on pulsed-field gel analysis of *Not*I-digested DNA from randomly selected clones. The haploid genome size of *S. venturii* is estimated to be approximately 1,000 Mb; therefore, the library was predicted to represent a coverage of approximately 11 genome equivalents.

PCR markers TG551, TG35, and vntNBSHae linked to *Rpi-vnt1.1* and *Rpi-vnt1.2* were used to screen the *S. venturii* K39 BAC library. BAC clones identified using these markers were end sequenced and a contig was constructed using a BAC-end PCR strategy (Fig. 2). Marker vntNBSHae is an allele-specific marker linked to both *Rpi-vnt1.1* and *Rpi-vnt1.2* and, consequently, it was not possible to assign haplotype to the BAC clones identified using this marker. However, using many of the BAC end markers and the CAPS markers TG551 and TG35, BAC clones from the *Rpi-vnt1.1* and *Rpi-vnt1.2* haplotypes can be distinguished by restriction digestion (Table 3). Two of the clones from the K39 library (K39_272N11 with *Rpi-vnt1.2* haplotype and K39_256M23 with *Rpi-vnt1.1* haplotype) had BAC end sequences which were highly similar to each other and to the ToMV *R* gene *Tm-2*^{2} (Lanfermeijer et al. 2003) and were identical to the vntNBSHae marker.

As a parallel contig construction approach, BAC clones containing sequences homologous to the vntNBSHae marker were identified by hybridization of the vntNBSHae probe to the pooled BAC DNA (274 pools of 384 clones each). In all, 67 pools were identified as containing BAC clones with homologous sequences. Clones from the positive pools were double-spotted at high density onto nylon membranes and hybridized with the vntNBSHae probe to identify individual BAC clones from the pools. In total, 85 BAC clones were identified. DNA from identified BACs was subjected to BAC SNaPshot fingerprinting (Luo et al. 2003), along with an additional 10 selected clones which were positive for the TG551 or TG35 markers. Analysis of the SNaPshot fingerprinting patterns using FPC v4.7 (Soderlund et al. 2000) showed that the BAC clones formed nine distinct contigs containing between 1 to 22 clones. From the contigs generated, one contig contained the BAC clones identified by both PCR-based screening using the linked markers TG551, TG35, and vntNBSHae and hybridization using the vntNBSHae marker as a probe (Fig. 2).

High-resolution mapping and cloning of *Rpi-vnt1.1, Rpi-vnt1.2*, and *Rpi-vnt1.3.*

PCR primers were designed from BAC end sequences and used to amplify products from the parental genotypes of *Rpi-vnt1.1* and *Rpi-vnt1.2.* PCR products were sequenced and analyzed for the presence of SNP that allowed use of the PCR products as CAPS markers in the respective populations. In all, 60 recombinants between the closest flanking markers SvntM2.9L and TG186 were selected from the total *Rpi-vnt1.1* population of 1,213 individuals and successfully converted BAC end markers were used to construct a higher-resolution genetic map for *Rpi-vnt1.1*. Similarly, 46 recombinants (between TG551 and T1421) from the *Rpi-vnt1.2* population of 1,706 individuals were used to construct a higher-resolution *Rpi-vnt1.2* genetic map. The positions of these markers in relation to marker vntNBSHae, which co-segregates with both *Rpi-vnt1.1* and *Rpi-vnt1.2*, are shown in Figure 2.

Marker analysis indicated that *Rpi-vnt1.1* was located within a genetic interval of 0.33 cM delimited by the CAPS marker TG35 and the BAC end marker 185L21R (Fig. 2). By reference to the physical map constructed from PCR and SNaPshot fingerprinting analysis of BAC clones from the K39 library (Fig. 2), *Rpi-vnt1.1* was predicted to be present on a physical region covered by the BAC clones K39_148P20, K39_266I9, and K39_185L21 (Fig. 2). Low-stringency Southern blotting of these clones revealed that a single fragment homologous to the vntNBSHae marker was present in both K39_266I9 and K39_185L21, indicating that there was potentially a single CC-NB-LRR gene located in the overlap region between these two clones. BAC clones K39_266I9 and K39_148P20 were sequenced and one candidate open reading frame (ORF) was

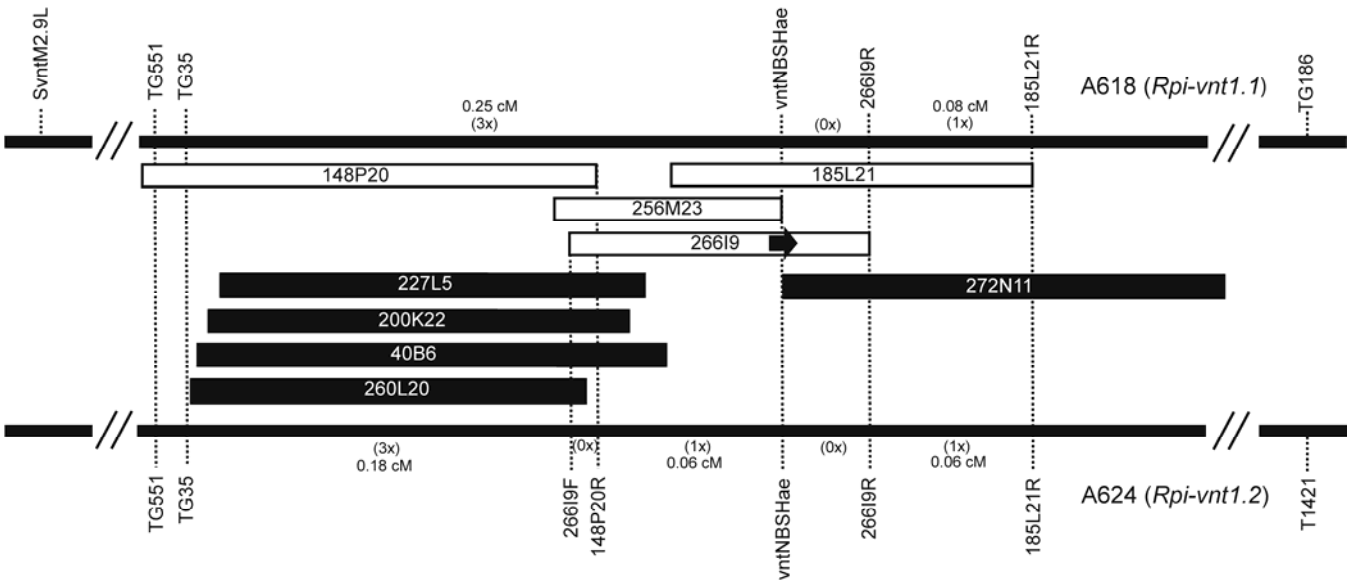

Fig. 2. High-resolution genetic mapping and physical mapping of *Rpi-vnt1.1* and *Rpi-vnt1.2* using the K39 BAC (bacterial artificial chromosome) library. Diagram shows the alignment of BAC clones from both the *Rpi-vnt1.1* and *Rpi-vnt1.2* haplotypes around the marker vntNBSHae, which cosegregates with resistance. BAC clone 148P20 was identified using markers TG551 and TG35; clones 185L21, 256M23, 266I9, and 272N11 were identified using marker vntNBSHae as an allele-specific polymerase chain reaction marker and also as a hybridization probe; clones 227L5, 200K22, and 40B6 were identified using the BAC end markers 266I9F and 148P20R; and clone 260L20 was identified using the BAC end marker 266I9F. Open bars represent BAC clones from the *Rpi-vnt1.1* haplotype and black bars represent BAC clones from the *Rpi-vnt1.2* haplotype. The black arrow within BAC clone 266I9 illustrates the position of *Rpi-vnt1.1*. Vertical dotted lines indicate the positions of markers linked to resistance. Genetic distances are given in centimorgans (cM) and the numbers in parentheses represent the number of recombinants between markers. Markers SvntM2.9L, TG186, and T1421 are included to aid orientation of the physical map with the genetic map in Figure 1.

identified for *Rpi-vnt1.1* on K39_266I9. No candidate *R* genes were present on BAC clone K39_148P20.

Interestingly, two individuals from the preselected *Rpi-vnt1.1* recombinants were resistant despite genotyping results suggesting that they should be susceptible. The mapping data for these two genotypes suggests that there may be a second, closely linked *R* gene located proximal to marker TG35.

High-resolution mapping indicated that *Rpi-vnt1.2* was located within a genetic interval of 0.12 cM delimited by the BAC end markers 266I9F and 185L21R (Fig. 2). By reference to the physical map constructed from PCR and fingerprinting analysis of BAC clones from the K39 library, *Rpi-vnt1.2* was predicted to be present on the same physical region as that identified for *Rpi-vnt1.1*. However, other than the BAC clone K39_272N11, one end of which contained a partial *Tm-2*[2] homolog which was identical to the corresponding region in *Rpi-vnt1.1*, clones from the *Rpi-vnt1.2* haplotype which covered the same physical region as *Rpi-vnt1.1* could not be identified within the library (Fig. 2). As an alternative approach, primers designed to amplify the complete *Rpi-vnt1.1* ORF (vnt1ORF-F/R) (Table 3) were used to genotype the five recombinant individuals between markers TG551 and T1421 from the *Rpi-vnt1.2* population. The resulting PCR product cosegregated with resistance in the three resistant individuals from these recombinants. The PCR product amplified from plant A624, the resistant parent plant of the *Rpi-vnt1.2* population, was cloned into pGEM-T Easy (Promega Corp., Madison, WI. U.S.A.) and was sequenced.

Rpi-vnt1.3 also mapped to the same genetic location as *Rpi-vnt1.1* and *Rpi-vnt1.2*; therefore, rather than initiate high-resolution mapping, the PCR primers designed against *Rpi-vnt1.1* were employed to amplify *Rpi-vnt1.3* candidates. The resulting PCR products were cloned into pGEM-T Easy (Promega Corp.) and sequenced. To confirm that the sequenced PCR product corresponded to *Rpi-vnt1.3,* seven recombinants between markers TG35 and T1421 (Fig. 1) were assessed by PCR. The candidate PCR product was only amplified from the resistant recombinants.

Fig. 3. Alignment of the deduced protein sequences of *Rpi-vnt1.1, Rpi-vnt1.2, Rpi-vnt1.3*, and *Tm-2*[2] (AAQ10736). The complete amino acid sequence of Rpi-vnt1.1 is shown and dots indicate identical residues in the other two proteins. Where residues from Rpi-vnt1.2 and Tm-2[2] differ from Rpi-vnt1.1, the residues in these proteins are given. Amino acid differences between Rpi-vnt1.1, Rpi-vnt1.2, and Rpi-vnt1.3 are indicated in bold type. Predicted coiled-coil domains are underlined and the first and fourth hydrophobic residues of each heptad repeat are double underlined. Conserved motifs within the nucleotide-binding apoptosis, *R* gene products, CED-4 domain are indicated in lowercase italics. Putative leucine-rich repeats are indicated above the sequence line.

Analysis of *Rpi-vnt1.1, Rpi-vnt1.2*, and *Rpi-vnt1.3*.

The *Rpi-vnt1.1* ORF is 2,673 bp long and translates into a protein sequence of 891 amino acids with a calculated molecular weight of 102 kDa and a pI of 8.05. The *Rpi-vnt1.2* ORF comprises 2,715 bp and translates into a protein sequence of 905 amino acids with a calculated molecular weight of 103.6 kDa and a pI of 8.16. The sequence of *Rpi-vnt1.2* differs from *Rpi-vnt1.1* by an insertion of 42 nucleotides in the 5′ end of the gene (Fig. 3). The resulting additional 14 amino acids present in the corresponding region of *Rpi-vnt1.2* do not affect any of the predicted CC domains. There are also three SNP between *Rpi-vnt1.1* and *Rpi-vnt1.2*; A1501T, T1767C, and G2117A (Fig. 3). These nucleotide differences result in two amino acid differences between *Rpi-vnt1.1* and *Rpi-vnt1.2* (Fig. 3). The difference at position 501 is at the end of the NB-ARC (apoptosis, *R* gene products, CED-4) domain, just prior to the LRR region, and results in the change of an asparagine in *Rpi-vnt1.1* to a tyrosine in *Rpi-vnt1.2*. This amino acid change does not affect any of the characterized NB-ARC domains. At position 706, within the ninth LRR, an arginine in *Rpi-vnt1.1* becomes a lysine in *Rpi-vnt1.2*; both of these residues are positively charged polar amino acids and, hence, this can be considered a synonymous change.

The Rpi-vnt1.1 proteins contain all the features characteristic of the CC-NB-LRR class of resistance proteins. Within the first 215 (229 for Rpi-vnt1.2) amino acids of the N-terminal part of the protein were four regions each with three predicted heptad repeat motifs typical of CC domains (Fig. 3). All NB-ARC domains (van der Biezen and Jones 1998) are present in the amino acid sequence from 216 to 505 (230 to 519 in Rpi-vnt1.2). Following the NB-ARC domain is a region consisting of a series of 15 irregular LRR motifs that could be aligned according to the consensus sequence LxxLxxLxxLxLxxC/N/

Sx (x)LxxLPxx (where L can be L, I, M, V, Y, or F and x is any amino acid) (McHale et al. 2006).

Rpi-vnt1.1 and *Rpi-vnt1.2* share 80.9 and 79.7%, identity, respectively, with *Tm-2*^{2} at the nucleic acid level. At the amino acid level, this translates to 72.1 and 71.1% identity, respectively. As expected, given its role in recognition specificity, the percentage of similarity was lowest in the LRR domain where *Rpi-vnt1.1/1.2* and *Tm-2*^{2} share only 57.5% similarity. In contrast, the sequence similarity across the CC and NB-ARC domains of *Rpi-vnt1.1/1.2* and *Tm-2*^{2} is 81.8 and 79.7%, respectively; notably, within the conserved domains of the NB-ARC region, *Tm-2*^{2} and *Rpi-vnt1.1* are identical.

The primers vnt1long-F and vnt1long-R (Table 3) were used to amplify *Rpi-vnt1.1* homologous sequences from the parental material containing *Rpi-vnt1.3*. Resulting PCR products were cloned into pGEM-T and sequenced. The sequences obtained were identical to *Rpi-vnt1.2* with the exception of a single SNP in the 5′ insertion region, relative to *Rpi-vnt1.1*, which resulted in a phenylalanine to serine amino acid substitution (Fig. 3).

It was not possible to amplify full-length *Rpi-vnt1.1* paralogues from the susceptible *S. venturii* parent A613. This observation, together with the fact that the vntNBSHae marker could only be amplified from resistant genotypes, could be taken to suggest that, at least in the susceptible parent used in this study, the susceptible phenotype is caused by an absence of *Rpi-vnt1.1* rather than a nonfunctional copy. An alternative explanation could be that the insertion of a transposable element into the susceptible allele has rendered it recalcitrant to PCR amplification or that sequence at the sites against which primers were designed is sufficiently different so as to prevent successful amplification.

Complementation analysis.

Potato cv. Desiree was transformed with plasmid pSLJ21152 which contained *Rpi-vnt1.1* under the control of its native promoter and terminator. In total, 37 *S. tuberosum* cv. Desiree plants capable of growth on kanamycin were selected as putative *Rpi-vnt1.1* transformants. Following transfer to the glasshouse, leaves from 29 plants were excised and used in a detached leaf assay with *P. infestans* isolates 90128 and BPC2006 3928A (superblight, blue 13) to determine whether the transgene conferred blight resistance (Fig. 4). Of the 29 transformants tested, 24 were confirmed as being resistant and did not show any signs of blight infection. Some plants exhibited signs of a hypersensitive response localized to the inoculation site. The remaining five plants were susceptible to both isolates, as was the control (nontransformed Desiree). The phenotype of the transgenic plants correlated exactly with amplification of the *Rpi-vnt1.1* ORF by PCR; all plants from which *Rpi-vnt1.1* could be amplified were confirmed as resistant. Detached leaves of selected transgenic lines of potato cv. Desiree carrying *Rpi-vnt1.1* were inoculated with a range of *P. infestans* isolates to determine the range of isolates against which *Rpi-vnt1.1* confers resistance (Table 4). Of the 11 isolates tested, only isolate EC1 from Ecuador was able to overcome *Rpi-vnt1.1* and cause disease on the inoculated plants. The resistant parent from which *Rpi-vnt1.1* was isolated was also shown to be susceptible to isolate EC1, demonstrating that the specificity of *Rpi-vnt1.1* was retained in the transgenic plants and that the resistance phenotype was not due to constitutive activation of defense pathways by the transgene.

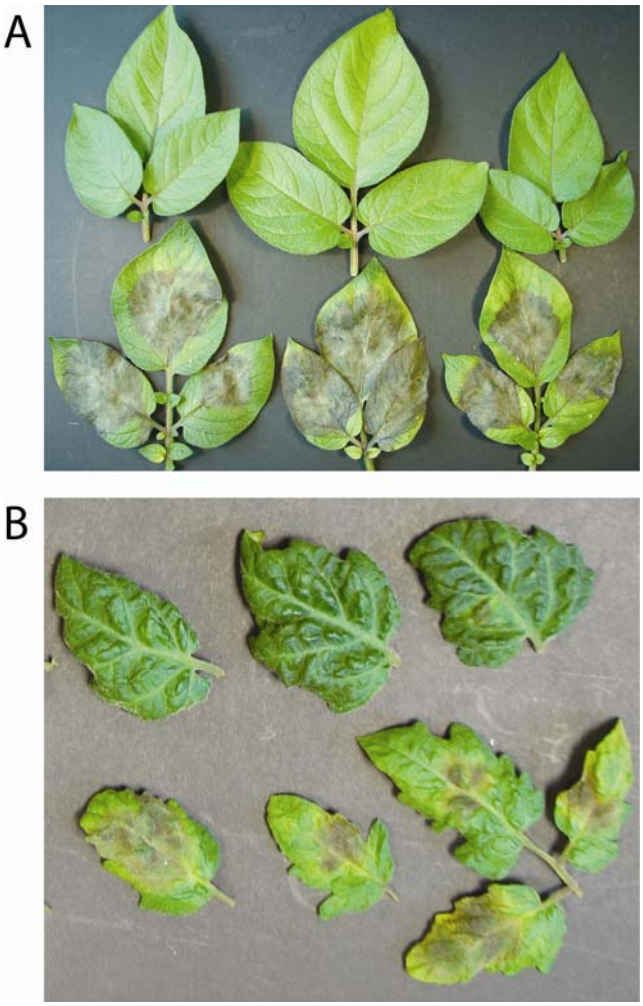

Fig. 4. *Rpi-vnt1.1* confers resistance to *Phytophthora infestans* upon transformation into susceptible potato and tomato cultivars. Detached leaves from plants of **A,** *Solanum tuberosum* cv. Desiree containing (top) or lacking (bottom) *Rpi-vnt1.1* and **B,** *S. lycopersicum* cv. Moneymaker containing (top) or lacking (bottom) *Rpi-vnt1.1* were inoculated at five to six points with 10-µl droplets of zoospore suspensions of *P. infestans* isolate 90128 and incubated at 16°C, 16 h of light, and 8 h of darkness. Photographs were taken 6 days postinoculation.

Table 4. Response of transgenic potato plants containing *Rpi-vnt1.1* against a range of *Phytophthora infestans* isolates

Isolate	Country of origin	Race	*Rpi-vnt1.1* phenotype
90128	The Netherlands	1.3.4.7.8.9.10.11	Resistant
IPO-0	Unknown	Unknown	Resistant
IPO-Complex	Belgium	1.2.3.4.6.7.10.11	Resistant
BPC2006 3928A (superblight, blue 13)	United Kingdom	Unknown	Resistant
Hica	United Kingdom	Unknown	Resistant
MP717	Poland	1.2.3.4.5.6.7.9.10.11	Resistant
MP778	Poland	1.3.4.5.6.7.9.10.11	Resistant
MP674	Poland	1.2.3.4.5.6.7.10.11	Resistant
MP622	Poland	1.3.4.7.8.10.11	Resistant
MP650	Poland	1.2.3.4.5.7.8.10.11	Resistant
EC1	Ecuador	2.4.10.11	Susceptible

Transgenic tomato cv. Moneymaker plants carrying *Rpi-vnt1.1* were also shown to be resistant to *P. infestans* isolate 90128 (Fig. 4).

Rpi-vnt1.1 is also present in *S. venturii* accession CGN18000 and cosegregates with resistance in plants containing *Rpi-phu1*.

We identified an *Rpi* gene in a segregating population derived from a resistant individual of the *Solanum* accession CGN18000 which also showed close linkage between the identified gene (originally named *Rpi-nrs1*) and marker TG551 and cosegregated with marker vntNBSHae, indicating that it was located in the same region as *Rpi-vnt1.1* (data not shown). The same genetic location for this gene was also determined by Pel and associates (2009). Although listed in the CGN collection as *S. neorossii,* AFLP fingerprinting work showed that accession CGN18000 clustered with accessions of *S. venturii* and not *S. neorossii* (Jacobs 2008; Jacobs et al. 2008). Sequencing of a PCR product amplified using primers that amplify full-length *Rpi-vnt1.1* from resistant CGN18000 material showed that a sequence identical to *Rpi-vnt1.3* (i.e., with a single SNP in the 5′ region compared with *Rpi-vnt1.2*) was present in this material and cosegregated with resistance in the CGN1800 mapping population. Resistant CGN18000 material also showed the same response as *Rpi-vnt1.1* to the panel of *P. infestans* isolates. Interestingly, genotypes from the CGN18000 mapping population were identified which were found not to contain *Rpi-vnt1.3* by PCR yet were resistant in detached leaf assays, suggesting the presence of a second, linked gene reflecting the situation with *Rpi-vnt1.1*.

Rpi-phu1 from *S. phureja* was also reported to map to this region (Śliwka et al. 2006). The *Rpi-vnt1.1* marker vntNBSHae-F/R cosegregated with resistance in a population of 148 diploid *S. tuberosum* plants segregating for *Rpi-phu1*. Full-length *Rpi-vnt1.1* paralogues were amplified from DNA of three resistant genotypes containing *Rpi-phu1*. In each case, a single PCR product was obtained and sequencing showed this to be identical to *Rpi-vnt1.1*. Resistant plant material containing *Rpi-phu1* was also shown to be resistant to the *P. infestans* isolates used in this study, with the exception of EC1.

DISCUSSION

Despite the efforts of breeders and the extensive use of fungicide control measures, late blight still remains a major threat to potato cultivation worldwide. The spread of new isolates, including the A2 mating type, from Mexico to Europe from 1984 onward (Goodwin and Drenth 1997) has resulted in a European late blight population which is capable of sexual reproduction and, consequently, has the potential for strains of the pathogen to evolve and overcome *R* genes present within current cultivated potato germplasms which were introgressed from *S. demissum* in the 1930s (Black et al. 1953; Malcolmson and Black 1966). Consequently, there is a need to source new *R* genes and extend the repertoire available. South American wild *Solanum* spp. are a valuable source of *R* genes which have potentially novel recognition specificities and, thus, may prove to be valuable tools in the fight against late blight (Bradshaw and Ramsay 2005). Here, we have identified, mapped, and cloned such a gene from *S. venturii* and shown that this gene can be used to transfer resistance to the susceptible cv. Desiree.

Rpi-vnt1.1 was cloned using a genetic mapping and positional cloning approach. BSA (Michelmore et al. 1991) together with AFLP (Vos et al. 1995) were proven to be a powerful combination of tools for efficiently mapping *Rpi-vnt1.1* and *Rpi-vnt1.2*. In addition, the tomato introgression lines (Eshed and Zamir 1994) were invaluable in allowing the rapid location of the chromosomal positions of these genes, as was found previously for *Rpi-mcq1* (Smilde et al. 2005). *Rpi-vnt1.1* is located on the long arm of chromosome IX and shows highest similarity to *Tm-2²* from *S. lycopersicum*, which confers resistance against ToMV and is located in a centromeric position on the long arm of chromosome IX (Lanfermeijer et al. 2003).

Both *Rpi-vnt1.1* and *Tm-2²* belong to the largest family of *R* genes, which contain a characteristic CC domain in addition to the canonical NB-ARC and LRR domain. The majority of *R* genes cloned from potato are members of this CC-NB-LRR family and all *Rpi* genes cloned thus far are of this class. A member of the toll/interleukin 1 receptor (TIR)-NB-LRR family of *R* genes (characterized by the presence of a TIR domain) has been shown to be responsible for resistance against the nematode *Globodera rostochiensis* in potato (Paal et al. 2004); however, whether or not members of the TIR-NB-LRR family confer resistance against *P. infestans* remains to be seen.

Mapping results from two recombinants within the *Rpi-vnt1.1* locus indicated that a second closely linked *Rpi* gene may be located proximal to *Rpi-vnt1.1* in *S. venturii* accession CGN18108. This gene also confers resistance against *P. infestans* isolate 90128; however, whether this gene has exactly the same spectrum of recognition specificity as *Rpi-vnt1.1* remains to be seen. It was not possible to amplify either the marker vntNBSHae or the full-length *Rpi-vnt1.1* ORF from these genotypes, which suggests that the gene is somewhat different from *Rpi-vnt1.1* at the sequence level. Mapping using populations derived from these genotypes is required to investigate this gene further.

Although no other *R* genes were found within the 185 kb that comprised the BAC clones sequenced during the search for *Rpi-vnt1.1*, the presence of another closely linked *R* gene or cluster of *R* genes would not be unprecedented. All of the *Rpi* genes cloned to date are members of complex loci composed of *R* gene clusters. In the case of *Rpi-blb1*, *Rpi-blb2, R1*, and *R3a,* the other *R* genes present in the clusters did not necessarily confer late-blight resistance, although this possibility could not be ruled out (Ballvora et al. 2002; Huang et al. 2005; Song et al. 2003; van der Vossen et al. 2003, 2005). However, *R2, Rpi-blb3*, and *Rpi-abpt* (all of which confer resistance against a different spectrum of races of *P. infestans*) are found together in a single complex locus on chromosome IV (Park et al. 2005a).

Although no paralogues are found in the immediate vicinity of *Rpi-vnt1.1,* chromosome IX harbors a number of *Tm-2²* paralogues; in addition to *Rpi-vnt1.1*, a contig of BAC clones generated by SNaPshot fingerprinting of BACs from the K39 BAC library also contains paralogous sequences and maps to a region on the distal part of chromosome IX, in the region of marker TG591B (which is itself a *Tm-2²* homolog). This contig contains an estimated three or four *Rpi-vnt1.1* paralogues according to Southern blotting results (data not shown). *Rpi-mcq1,* which has recently been shown to be a *Tm-2²* homolog with high similarity to *Rpi-vnt1.1*, also maps to this locus (Z. Chu, *unpublished data*). Thus, it appears that there is a family of *Rpi-vnt1.1*/ *Tm-2²* paralogues distributed along the length of the long arm of chromosome IX. Additionally, screening of the *S. venturii* BAC library revealed the presence of at least six other contigs containing paralogues. The location of these on the genetic map is currently unknown.

A number of other resistance genes against pests and pathogens of potato and tomato are also found on chromosome IX. *Gm,* a gene which confers resistance against *Potato virus M* (Marczewski et al. 2006), has recently been mapped to a region similar to that occupied by *Rpi-vnt1.1* but it is presently not sufficiently mapped to determine whether it occupies the same locus in *S. gourlayi*, the donor of *Gm.* Proximal to *Rpi-vnt1.1* and the aforementioned *Tm-2²* are *Fr1*, which confers

resistance against *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato (Vakalounakis et al. 1997), and *Ve1*, which confers resistance against *Verticillium dahliae* in both potato and tomato (Kawchuk et al. 2001). Distal to *Rpi-vnt1.1* are *Sw-5*, which confers resistance against *Tomato spotted wilt virus* (Brommonschenkel and Tanksley 1997; Brommonschenkel et al. 2000); *Nx*, conferring resistance against *Potato virus X* (Tommiska et al. 1998) in *S. phureja*; and a QTL for resistance against the nematode *G. pallida* in potato, designated *Gpa6* (Rouppe van der Voort et al. 2000). *Rpi-mcq1* (formerly *Rpi-moc1*) (Smilde et al. 2005) lies distal to *Rpi-vnt1.1* at the end of the short arm of chromosome IX, in a region similar to that to which *Ny-1,* conferring resistance to *Potato virus Y*, has also been mapped (Szajko et al. 2008).

The late blight resistance gene *Rpi-phu1* (Śliwka et al. 2006) was mapped within a segregating diploid mapping population derived from complex *Solanum* hybrids of *S. tuberosum* with other wild species, including late-blight-resistant clones of *S. phureja* and *S. stenotomum.* This gene is shown here to be identical to *Rpi-vnt1.1*. Genotyping shows that *Rpi-vnt1.1* cosegregates with resistance in segregating progeny, and the nucleotide sequence of an ORF amplified using the *Rpi-vnt1.1*-specific primers is identical to *Rpi-vnt1.1.* This level of conservation is surprising, although recent reports that highly conserved and putatively functional *Rpi-blb1* homologs have been found in *S. stoloniferum, S. polytrichon*, and *S. papita* (Vleeshouwers et al. 2008; Wang et al. 2008) and also in *S. verrucosum* (Liu and Halterman 2006) suggest that some *Rpi* genes belong to ancient loci that are conserved through the selection pressure imposed by *P. infestans,* or that genetic exchange (presumably infrequently) can occur between *Solanum* spp. The fact that *Rpi-vnt1.1* and *Rpi-phu1* are identical suggests that the divergence event that led to the two species is relatively recent. This sequence conservation could also be a result of the fact that no other *Rpi-vnt1.1* paralogues are found within the immediate vicinity of *Rpi-vnt1.1*, thereby reducing the opportunity for sequence exchange which is thought to contribute to the evolution of *R* genes and appears to have been the mechanism by which *Rpi-blb1* arose by recombination between two paralogues (*RGA1-blb* and *RGA3-blb*) that reside in the same locus (van der Vossen et al. 2003).

The studies of Jacobs (2008) were not able to assign a species label to the accession CGN18279 due to its occupying conflicting positions within species dendrograms. However, the presence of an *Rpi* gene almost identical to *Rpi-vnt1.1* and the ability of plants from this accession to cross easily with *S. venturii* accessions indicate that CGN18279 is probably *S. venturii*.

It was not possible to identify a BAC clone containing *Rpi-vnt1.2*. This may simply be due to the distribution of BAC clones in the library or it may be that neighboring sequences in the *Rpi-vnt1.2* haplotype cause instabilities within the BAC vector that do not allow it to be propagated in *Escherichia coli.* Due to the lack of a genomic clone of *Rpi-vnt1.2*, we have not been able to demonstrate that this gene confers resistance against *P. infestans* as we have for *Rpi-vnt1.1*. However, a number of lines of evidence lead us to postulate that *Rpi-vnt1.2* is responsible for the resistant phenotype of accession CGN18279. First, mapping data present conclusive evidence that *Rpi-vnt1.2* resides in the same genetic location as *Rpi-vnt1.1.* Second, the resistance proteins differ by only an insertion of 14 amino acids within the CC domain and a further 2 amino acids substitutions within the remainder of the protein; given that only one of these residues is in the LRR region which governs *R* gene specificity (Parniske et al. 1997) and that the substitution is synonymous with respect to physical properties, we expect that this gene is also functional and provides late-blight resistance with the same specificity as *Rpi-vnt1.1*. We also show here that *Rpi-vnt1.3*, the amino acid sequence of which differs from *Rpi-vnt1.2* at a single amino acid residue, maps to an identical region in the resistant *S. venturii* accession CGN18000. Pel and associates (2009) have shown by both *Agrobacterium*-mediated transient assay in *Nicotiana benthamiana* and stable transformation of potato cv. Desiree that this gene is functional and confers resistance against the same spectrum of *P. infestans* isolates as does *Rpi-vnt1.1.*

For a resistance gene to be of use in controlling late blight, it should be demonstrated that the resistance has an element of durability. Although we cannot determine durability of the *Rpi-vnt1.1* gene until it is deployed widely, information from tests against a wide range of isolates in Poland have shown that, between 1999 and 2008, only one isolate (identified in 2008) capable of overcoming *Rpi-phu1* was found (J. Śliwka, *unpublished data*). Importantly, *Rpi-vnt1.1* confers resistance against an aggressive isolate (BPC2006 3928A) of genotype 13, known colloquially as "superblight" or "blue 13", which is prevalent throughout the United Kingdom and is capable of overcoming the durable resistance present in the commercially valuable cv. Stirling and which has been mapped as a major QTL to the R2 locus on chromosome IV (Hein et al. 2007).

To introduce *Rpi* genes into cultivars requires immense efforts by breeders. Potato lines are highly heterozygous out-breeding species and introgression of genes from wild *Solanum* relatives would require recurrent backcrosses to the cultivated parent, which would result in inbreeding depression due to the selfing of the recurrent parent (Bradshaw and Ramsay 2005). Although this can be avoided by using different parents for the backcrossing, doing so would result in the loss of important agronomic and nutritional traits that have been selected at the cost of time and effort. Therefore, the use of genetic modification technologies to transfer single *Rpi* genes into cultivars with combinations of traits that have taken considerable time and financial resources to develop is attractive. Recently, transgenic crops containing the *RB* (*Rpi-blb1*) gene from *S. bulbocastanum* were shown to be highly resistant to *P. infestans* and had no negative effects on tuber size or yield, indicating that the expression of *Rpi* transgenes does not have a fitness cost or cause undesirable physiological traits (Halterman et al. 2008). We propose that the use of genetically uniform lines containing mixtures of *R* genes or, alternatively, mixtures of lines containing single *R* genes isolated from novel *Solanum* spp. is a viable strategy for improving the resistance of current potato cultivars and may provide a means to ensure that valuable *R* genes are not broken.

MATERIALS AND METHODS

Solanum spp. growing conditions.

Seed of *Solanum* accessions (Table 1) was obtained from CGN. Seed was surface sterilized in 70% ethanol for 1 min, disinfected with 1.5% hypochlorite for 5 min, rinsed three times in sterile distilled water, and placed on solid Murashige and Skoog (MS) medium (2% agarose) containing 3% sucrose for germination. Germinated seedlings were transferred to glasshouse facilities and treated regularly with fungicides and pesticides to control thrips, aphids, spider mites, powdery mildew, and early blight (*Alternaria solani*).

P. infestans strains, inoculation, and pathogenicity scoring.

P. infestans isolate 98.170.3 (race 1.3.4.10.11) (Smilde et al. 2005) was provided by D. Shaw at Bangor University, U.K. Isolates 90128, IPO-complex, IPO-0, and EC1 (Table 4) were provided by E. van der Vossen at Plant Research International, Wageningen, The Netherlands. The BPC2006 3928A (superblight, blue 13) isolate was provided by P. Birch, SCRI, Dundee, U.K. and is an isolate currently virulent on a large number of

commercially grown potato cultivars in the United Kingdom and Europe. Isolates MP324 (Śliwka et al. 2006), MP717, MP778, MP674, MP622, MP618, and MP650 were obtained from IHAR, Poland.

The isolates were maintained at 18°C on Rye B agar (Caten and Jinks 1968). Fresh sporangia were produced in a 2-week cycle by subculturing to fresh plates. Periodically, the ability of isolates to infect host material was confirmed on detached leaves of a suitable, sensitive plant. Mature, fresh sporangia were harvested after 10 days of growth on Rye B medium by flooding the plate with sterile deionized water and allowing the harvested spore suspension to stand for 20 min in a fresh petri dish. After this time, most sporangia are stuck to the plastic surfaces of the dish. Water from the original suspension was replaced by fresh cold water and the sporangia resuspended and incubated at 4°C for 1 to 4 h to induce zoospore release.

A detached leaf assay was used to screen for resistance to *P. infestans* (modified from Vleeshouwers et al. 1999). Two leaves per plant were detached and placed onto moistened tissue paper in a 25-by-25-cm assay plate (Nunc, Rochester, NY, U.S.A.) (six genotypes per plate). Leaves were inoculated with 10-µl droplets of a zoospore suspension (50,000 zoospores ml^{-1}). Inoculated leaves were incubated for 7 to 12 days under controlled environmental conditions (16°C, 16 h of light and 8 h of dark) before scoring phenotypes. Plants with leaves showing sporulating lesions were scored as susceptible; plants with leaves showing no visible symptoms or limited necrosis in the absence of sporulation were scored as resistant. For unclear phenotypes, at least three independent inoculations were carried out. For clear phenotypes (either both leaves resistant or both sensitive), two independent rounds of inoculations were considered sufficient.

DNA isolation and sequencing.

DNA was isolated from plant material using the protocol of Park and associates (2005b). BAC clone DNA was isolated using the Qiagen Midi Prep Kit (Qiagen, Hilden, Germany). BAC ends and PCR products were sequenced using the ABI PRISM Big Dye (v. 3.1) Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems, Foster City, CA, U.S.A.) according to the manufacturer's instructions.

AFLP and SSR analysis and PCR-based mapping.

AFLP was performed essentially as described by Thomas and associates (1995) and Vos and associates (1995) on *Pst*I/*Mse*I-digested template DNA using a preamplification step with *Pst*I+0 and *Mse*I+1 primers and a selective amplification step using *Pst*I+2 and *Mse*I+3 primers. Informative AFLP bands were cut from the gel and rehydrated in TE (10 mM Tris-HCl, pH 8.0, and 0.1 mM EDTA). The gel slices were then transferred to fresh TE and crushed, and the debris removed by centrifugation at 14,000 × *g* for 1 min. For cloning, AFLP fragments were first reamplified by PCR using 2 µl of the supernatant and the same primers as for the original amplification and cloned into pGEM-T Easy (Promega Corp.).

SSR PCR reactions were done in 25-µl reaction volumes containing 20 mM Tris-HCl (pH 8.4); 50 mM KCl; 2.5 mM $MgCl_2$; 0.4 mM each dCTP, dTTP, and dGTP; 0.012 mM nonlabeled dATP; 370 kBq (γ-^{33}P)dATP (Amersham Biosciences, Bucks., U.K.); 0.4 µM each primer; 1 U of *Taq* DNA polymerase (Invitrogen, Carlsbad, CA, U.S.A.); and 100 ng of template DNA. Thermal cycling conditions consisted of an initial denaturation step at 94°C for 4 min, followed by a primer annealing step (either 50 or 55°C depending upon the primer pair used) (Table 3) for 2 min, and an extension step at 72°C for 90s. Subsequent cycles were as follows: 29 cycles of 94°C for 1 min, primer annealing temperature for 2 min, 72°C for 90 s, followed by a final extension step of 72°C for 5 min. Amplification products were denatured by the addition of an equal amount of stop solution (95% formamide containing bromophenol blue and xylene cyanol) and heated to 98°C for 10 min. The reaction (2 to 5 µl) was run on 6% denaturing polyacrylamide gels containing 6 M urea at 100 W for 2 to 4 h. Gels were dried and exposed to X-ray film as for AFLP reactions.

Conventional PCR assays were done in 15-µl reaction volumes containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM each dNTP, 0.4 µM each primer, 0.5 U of *Taq* polymerase, and 10 to 100 ng of template DNA. Thermal cycling conditions typically consisted of an initial denaturation step of 94°C for 2 min followed by 35 cycles of 94°C for 15 s, primer annealing temperature (Table 3) for 30 s, 72°C for 1 min/kb of amplified product, followed by a final extension step of 72°C for 10 min. For sequencing, primers and dNTPs were removed from PCR products by incubation with 0.1 U μl^{-1} exonuclease I and 0.1 U μl^{-1} shrimp alkaline phosphatase at 37°C for 30 min followed by incubation at 80°C for 20 min to denature the enzymes. Long-range PCR reactions were done using Phusion High Fidelity DNA polymerase (Finnzymes Oy, Espoo, Finland) according to the manufacturer's protocol.

Construction of an *S. venturii* BAC library containing *Rpi-vnt1.1* and *Rpi-vnt1.2*.

The *S. venturii* plant K39 which was used to construct the BAC library is a transheterozygote carrying both *Rpi-vnt1.1*, originally from the resistant parent plant A618 (accession CGN18108), and *Rpi-vnt1.2*, from the resistant plant A624 (accession CGN18279).

Plant material was grown on MS medium without sucrose in vitro and young leaf tissue was harvested and stored at –80°C. Frozen leaf tissue (20 g) was used to prepare DNA plugs containing high molecular weight DNA using a method slightly modified from Liu and Whittier (1994) and Chalhoub and associates (2004). The DNA plugs were prepared in 0.7% InCert agarose (Biozym, Oldendorf, Germany), washed in lysis buffer solution (1% sodium lauryl sarcosine, proteinase K at 0.2 mg/ml, and sodium diethyldithiocarbamate at 3.8 mg/ml dissolved in 0.5 M EDTA, pH 8.5) and stored at 4°C in 0.5 M EDTA until required. The stored plugs were soaked in TE buffer, chopped into small pieces, and partially digested with 5 units of *Hin*dIII for 1 h to generate DNA fragments of 50 to 300 kb.

Triple size selection was used to improve the size and uniformity of the inserts as described by Chalhoub and associates (2004). The first size selection was performed on 1% Seakem LE agarose (Biozym) using clamped homogeneous electric field pulsed-field gel electrophoresis (Bio-Rad, Hercules, CA, U.S.A.) at 1 to 40 s, 120°C, 16 h, and 200 V in 0.25× Tris-borate-EDTA buffer directly followed by the second size selection in the same gel at 4 to 5 s, 120°C, 6 h, and 180 V in the same buffer. Agarose gel slices containing partially digested DNA between 100 and 200 kb were excised and divided into two. For the third size selection, the excised gel slices were separately run on 1% Sea Plaque GTG low-melting point agarose (Biozym) at 3 to 4.5 s, 120°C, 14 h, and 180 V. Size-selected DNA fragments were excised from the gel and stored at 4°C in 0.5 M EDTA (pH 8). DNA was recovered in 40 µl 1× Tris-acetate-EDTA buffer by electroelution using a Bio-Rad Electro-elution system (Bio-Rad).

The total eluted DNA was ligated in a 100-µl reaction with 10 ng pIndigoBAC-5 vector (EpiCentre Biotechnologies, Madison, WI, U.S.A.) and 800 U of T4 DNA ligase (New England Biolabs, Ipswich, MA, U.S.A.). The ligation was dialyzed against 0.5× TE buffer for 3 h using Millipore membrane (Millipore, Billerica, MA, U.S.A.). Dialyzed ligation (3 µl) was used to

transform DH10β electrocompetent cells (Invitrogen, Paisley, U.K.) by electroporation. The BAC library was picked into 274 384-well microtiter plates (Genetix Ltd., Dorset, U.K.).

Transformation of *S. tuberosum* cv. Desiree with *Rpi-vnt1.1*.

A 4.3-kb fragment carrying the *Rpi-vnt1.1* promoter, ORF, and terminator was amplified by PCR using the primers vnt1long-F and vnt1long-R (Table 3) from the BAC clone K39_266I9. This fragment was cloned into pGEM-T Easy (Promega Corp.) and sequenced to confirm that no mistakes had been introduced during PCR. The resulting plasmid was digested with *Eco*RI and the fragment containing the original gene cloned into the *Eco*RI site of pBin19. The resulting plasmid was named pSLJ21152. Plasmid pSLJ21152 was introduced into *Agrobacterium tumefaciens* AGL1.

Transformation of potato cv. Desiree was carried out as described by Kumar and associates (1996). Transformation of tomato cv. Moneymaker was carried out as described by Fillatti et al. (1987). Transgenic plants capable of growth on kanamycin were weaned out of tissue culture into sterile peat blocks before being transplanted to the glasshouse. PCR using primers vnt1ORF-F and vnt1ORF-R, which amplify the full-length *Rpi-vnt1.1* ORF (Table 3), was used to determine whether the kanamycin-resistant plants also harbored the *Rpi-vnt1.1* transgene.

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AUTHOR-RECOMMENDED INTERNET RESOURCES

Centre for Genetic Resources in the Netherlands: www.cgn.wageningen-ur.nl

Sol Genomics Network: www.sgn.cornell.edu