The Plant Journal (2005) **44**, 208–222 doi: 10.1111/j.1365-313X.2005.02527.x

The *Rpi-blb2* gene from *Solanum bulbocastanum* is an *Mi-1* gene homolog conferring broad-spectrum late blight resistance in potato

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Received 27 May 2005; revised 12 July 2005; accepted 14 July 2005.

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Summary

The necessity to develop potato and tomato crops that possess durable resistance against the oomycete pathogen *Phytophthora infestans* is increasing as more virulent, crop-specialized and pesticide resistant strains of the pathogen are rapidly emerging. Here, we describe the positional cloning of the *Solanum bulbocastanum*-derived *Rpi-blb2* gene, which even when present in a potato background confers broad-spectrum late blight resistance. The *Rpi-blb2* locus was initially mapped in several tetraploid backcross populations, derived from highly resistant complex interspecific hybrids designated ABPT (an acronym of the four *Solanum* species involved: *S. acaule*, *S. bulbocastanum*, *S. phureja* and *S. tuberosum*), to the same region on chromosome 6 as the *Mi-1* gene from tomato, which confers resistance to nematodes, aphids and white flies. Due to suppression of recombination in the tetraploid material, fine mapping was carried out in a diploid intraspecific *S. bulbocastanum* F1 population. Bacterial artificial chromosome (BAC) libraries, generated from a diploid ABPT-derived clone and from the resistant *S. bulbocastanum* parent clone, were screened with markers linked to resistance in order to generate a physical map of the *Rpi-blb2* locus. Molecular analyses of both ABPT- and *S. bulbocastanum*–derived BAC clones spanning the *Rpi-blb2* locus showed it to harbor at least 15 *Mi-1* gene homologs (MiGHs). Of these, five were genetically determined to be candidates for *Rpi-blb2*. Complementation analyses showed that one ABPT- and one *S. bulbocastanum*-derived MiGH were able to complement the susceptible phenotype in both *S. tuberosum* and tomato. Sequence analyses of both genes showed them to be identical. The Rpi-blb2 protein shares 82% sequence identity to the Mi-1 protein. Significant expansion of the *Rpi-blb2* locus compared to the *Mi-1* locus indicates that intrachromosomal recombination or unequal crossing over has played an important role in the evolution of the *Rpi-blb2* locus. The contrasting evolutionary dynamics of the *Rpi-blb2/Mi-1* loci in the two related genomes may reflect the opposite evolutionary potentials of the interacting pathogens.

Keywords: ***Rpi-blb2*, disease resistance, *Phytophthora infestans*, *Solanum bulbocastanum*, potato, late blight.**

Introduction

The oomycete *Phytophthora infestans*, the causal agent of late blight, remains the most important pathogen in major potato producing regions of the world. It has a potential to devastate crops in a couple of weeks if meteorological conditions are conducive to the onset and spread of an epidemic. To a lesser extent, late blight is also problematic in tomato cultivation (Fry and Goodwin, 1997). Disease management is currently based on the application of fungicides. Frequent seasonal spraying with fungicides imposes high input costs to the farmer, is detrimental to the environment and imposes a pressure on the pathogen for developing resistance to the active ingredients of the crop protectants applied. During the 20th century, breeding of new potato cultivars with high levels of durable resistance to

P. infestans has been considered an alternative to the use of fungicides. Breeders have introgressed from the wild potato species *Solanum demissum* the dominant resistance (*R*) genes *R1*, *R2*, *R3*, *R4* and *R10* up to cultivar level, but races of the pathogen that were able to overcome these genes emerged within a few years after market introduction (Turkensteen, 1993). By the end of the 1950s, most breeders had switched to the use of sources of germplasm with partial/quantitative resistance (Hawkes, 1978). The underlying paradigm was that this type of resistance is *R* gene independent and assumed to be of polygenic nature, which could make it more durable than resistance based on monogenic inherited *R* genes (Turkensteen, 1993). However, partial resistance is day-length dependent and is strongly correlated with late maturity under long-day conditions (Howard, 1970). Also, erosion of general resistance has been reported in case studies (Flier *et al.*, 2003). Genetic studies using molecular markers have shown that general resistance is often based on two or three major quantitative trait loci (QTL), the genome positions of which often correspond to loci harboring *R* gene clusters (Gebhardt and Valkonen, 2001; Grube *et al.*, 2000). The goal of some breeders now is to identify additional major *R* genes conferring broad-spectrum resistance to late blight and to combine these in modern potato varieties. In contrast to introgression breeding, isolation of such genes from *Solanum* species and their stable transformation as *cis* genes into existing potato or tomato varieties is by far the fastest means of exploiting potentially durable late blight resistance present in the *Solanum* gene pool.

Identification of numerous functional *R* genes from model and crop species has revealed that the majority of these genes encode cytoplasmic proteins with nucleotide binding site (NBS) and leucine-rich repeat (LRR) domains and that they often belong to complex loci comprised of arrays of related genes (reviewed in Martin *et al.*, 2003). This structure–function relationship has led to the development of homology-based approaches aimed at the identification of structurally related sequences, termed *R* gene homologs (RGHs), from many host species including *Solanaceae* (Leister *et al.*, 1996; van der Linden *et al.*, 2004; Pan *et al.*, 2000). In a comparative study of genomic organization of *R* genes and RGHs in three solanaceous crop genera, tomato, potato and pepper, Grube *et al.* (2000) observed significant conservation of *R* gene loci, despite limited positional correspondence of phenotypically defined genes conferring resistance to related or identical pathogens. This suggests that the chromosomal locations of *R* gene clusters may be quite broadly conserved through speciation and that comparative genomics can be instrumental for the rapid identification of genes similar to those already mapped in related genera.

Seven functional *R* genes from potato have currently been cloned and all belong to the NBS-LRR class of plant *R* genes (Ballvora *et al.*, 2002; Bendahmane *et al.*, 1999, 2000; Huang *et al.*, 2005; Paal *et al.*, 2004; Song *et al.*, 2003; van der Vossen *et al.*, 2000, 2003). Three of these confer resistance to late blight. The *Solanum demissum*-derived genes *R1* and *R3a* on chromosomes 5 and 11, respectively, confer race-specific resistance whereas the identical genes *Rpi-blb1* and *RB*, further referred to as *Rpi-blb1*, on chromosome 8 from the wild potato species *Solanum bulbocastanum* confer high levels of resistance to a range of *P. infestans* isolates with complex race structures (Helgeson *et al.*, 1998; Song *et al.*, 2003; van der Vossen *et al.*, 2003). *Solanum bulbocastanum* from Mexico and Guatemala is a diploid tuber bearing species that has long been known for its high levels of resistance to late blight (Niederhauser and Mills, 1953). Transfer of its resistance to the gene pool of cultivated potato has been successful by carrying out a tedious and time-consuming breeding scheme involving ploidy manipulations and a series of bridge crosses. The resulting *P. infestans* resistant interspecific hybrids were designated ABPT, an acronym of the four *Solanum* species involved: *S. acaule, S. bulbocastanum, Solanum phureja* and *Solanum tuberosum* (Figure S1) (Hermsen and Ramanna, 1973). Over 20 years of resistance phenotyping of ABPT-derived germplasm under diverse conditions suggested the presence of broad-spectrum late blight resistance, prompting us to analyze its genetic basis. Here, we describe the genetic mapping and cloning of a second late blight *R* gene derived from *S. bulbocastanum*. The *Rpi-blb2* gene was initially mapped in several ABPT-derived tetraploid backcross populations to the same region on chromosome 6 as the *Mi-1* gene from tomato. Subsequently, *Mi-1* gene homolog (MiGH) specific markers were developed to aid in the fine mapping of *Rpi-blb2* in a diploid intraspecific *S. bulbocastanum* F1 population and in the identification and isolation of candidate genes on bacterial artificial chromosome (BAC) clones spanning the locus. The *Rpi-blb2* gene is able to complement the susceptible phenotype in both cultivated potato and tomato. The Rpi-blb2 protein shares 82% sequence identity with the Mi-1 protein, which in tomato confers resistance to three species of root knot nematodes (*Meloidogyne* spp.) as well as to the potato aphid *Macrosiphum euphorbiae* (Milligan *et al.*, 1998; Rossi *et al.*, 1998; Vos *et al.*, 1998) and to both B and Q biotypes of whitefly *Bemisia tabaci* (Nombela *et al.*, 2003).

Results

Evaluation of resistance in ABPT-derived backcross clones and mapping populations

Late blight resistance in the ABPT-derived BC2 clone ARF 87-601 was initially evaluated in a field trial in the Toluca area in Mexico in 1991. A plot of clone ARF 87-601 with seven

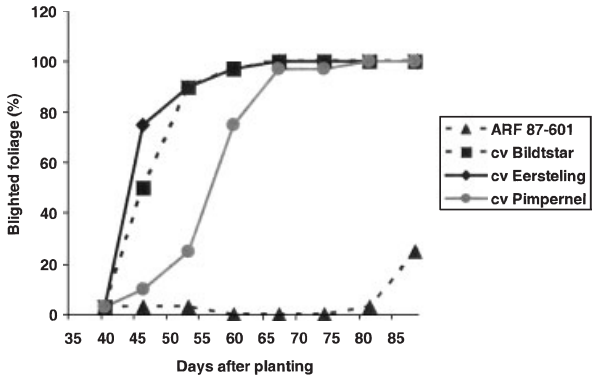


Figure 1. Disease progress curves for clone ARF 87-601 and susceptible control cultivars (cv) Bildtstar, Eersteling and the partial resistant control cultivar Pimpernel in a field test for foliar resistance to late blight in Toluca Valley, Mexico.
At eight time points after planting, the percentage blighted foliage due to a natural late blight infection was scored on the 1 to 9 International Potato Center (CIP) scale (Estrada-Ramos *et al.*, 1983).

plants was evaluated in comparison to plots with control cultivars Bildtstar, Eersteling and Pimpernel, which according to the ratings for resistance to late blight in the Dutch national list of recommended potato cultivars of 1988, scored 3, 3 and 8 respectively on a scale from 3 to 8 of increasing resistance. Cultivar Pimpernel is considered as a source of partial resistance (Colon *et al.*, 1985). About forty days after planting, natural infection by *P. infestans* established. The development of late blight in the foliage was subsequently monitored eight times during the growing season (Figure 1). At seventy four days after planting, foliage of the control cultivars was completely or nearly completely blighted, whereas clone ARF 87-601 showed no visible symptoms.

For the development of mapping populations, clones ARF 87-601, ARF 87-507 and ARF 87-801 were subsequently used for further backcrossing with late blight susceptible cultivars and breeding clones of *S. tuberosum*. This resulted in one tetraploid BC3 population (ARG 95-15), two tetraploid BC4 populations (ARG 95-3 and ARP 96-11) and a diploid BC4 population (DP1; Figure S2). Clear segregation for late blight resistance in ABPT-derived progeny and mapping populations was observed during successive years of field testing in the Netherlands, suggesting the presence of a dominant resistance allele at a single locus. Typically, resistant clones showed no or hardly any sporulating lesions, whereas susceptible clones showed completely blighted foliage. Notably, detached leaf assays with ABPT-derived progeny were found to be less accurate for phenotyping than screening under field conditions. Approximately 17% of the resistant haplotype plants and 3% of the susceptible haplotype plants were miscored as susceptible or resistant, respectively, in the detached leaf assays.

Mapping of the Rpi-blb2 locus to the Mi-1 gene cluster on chromosome 6

To identify Amplified Fragment Length Polymorphism (AFLP) markers linked to resistance, a bulked segregant analysis (BSA; Michelmore *et al.*, 1991) was carried out with 160 primer combinations on two resistant and two susceptible DNA pools, each containing genomic DNA of eight resistant or susceptible ARG 95-15 progeny plants, respectively. This resulted in the identification of 58 AFLP markers potentially linked to resistance. When a number of these markers were tested on progeny plants of ARG 95-3, linkage to resistance was also observed in this population, suggesting that in both populations the resistance was determined by the same locus, which we designated *Rpi-blb2*.

To determine the position of the *Rpi-blb2* locus on the genetic map of potato, two cosegregating AFLP markers, E40M58 and E46M52, were developed into cleaved amplified polymorphic sequence (CAPS) markers. Cleaved amplified polymorphic sequence marker E40M58 was subsequently tested on 46 progeny plants of the reference mapping population CxE (van Eck *et al.*, 1995). By applying JOINMAP linkage analyses (Stam, 1993), we mapped E40M58 8 cM distal to the chromosome 6 specific marker GP79 (Gebhardt *et al.*, 1991), positioning *Rpi-blb2* on the short arm of chromosome 6. This was further confirmed through the conversion of the chromosome 6 specific Restriction Fragment Length Polymorphism (RFLP) markers CT119 and CT216 into CAPS markers, which cosegregated with resistance in all four mapping populations.

Subsequently, for recombinant screening purposes, the cloned AFLP markers E46M52 and E40M58 were extended by thermal asymmetric interlaced (TAIL) Polymerase Chain Reaction (PCR) on genomic DNA of AR 91-1263 and ARD 1197-16, respectively, and converted into the sequence SCAR markers E46M52e and E40M58e. E46M52e was polymorphic in all the mapping populations, whereas E40M58e was amplified only in ARG 95-3 and DP1. As the parental clones of the different mapping populations were derived from different ABPT clones, we extended the AFLP fragment a second time but now from AR 91-1292, the resistant parental clone of ARP 96-11, resulting in E40M58e2. Sequence alignment of the two extended fragments showed that only the first 37 bp of the extended fragments were identical. When both extended markers were tested on genomic DNA derived from *S. bulbocastanum* (BGRC 8005 and 8006), only E40M58e amplified a fragment, indicating that part of the sequence of E40M58e2 was not derived from *S. bulbocastanum*. This observation suggested that E40M58e was located on the border of the *S. bulbocastanum* introgression fragment in clone AR 91-1292 and that the position of the *Rpi-blb2* locus was proximal to marker E40M58e.

In an attempt to identify functionally relevant markers linked to resistance, an RGH-fingerprinting technique was

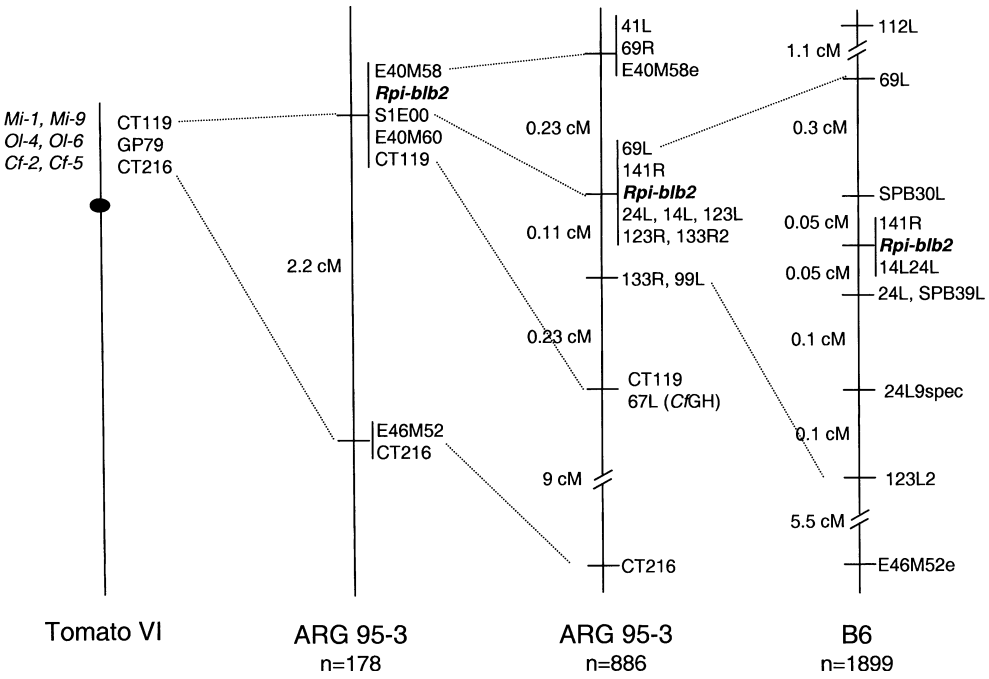


Figure 2. Genetic linkage maps of the *Rpi-blb2* locus generated in two mapping populations, ARG95-3 and B6.
Long vertical lines represent either chromosome 6 of tomato or short stretches of the distal part of chromosome 6 of potato harboring the *Rpi-blb2* locus. Indicated to the left is a schematic representation of tomato chromosome 6, showing the relative positions of resistance loci and markers identified on the short arm of this chromosome. Numbers left of the remaining vertical lines indicate genetic distances (cM). Relative positions of mapped loci are indicated by horizontal lines. Names of the mapped loci are indicated to the right of the horizontal lines. Vertical lines connected to the right side of the horizontal lines group loci that cosegregate. The size of each mapping population is indicated at the bottom of each map.

applied. Using the P- loop based primer S1 from Leister *et al.* (1996) in combination with the Eco00 AFLP primer, an RGH specific marker, S1E00 was identified that cosegregated with resistance and markers E40M58e and CT119 in the ARG 95-3 mapping population (Figure 2). Cloning and sequencing of the 75-bp-long S1E00 fragment did not reveal homology to St3313, an RGH sequence previously mapped to the short arm of chromosome 6 in potato (Leister *et al.*, 1996), but rather to the *Mi-1* gene from tomato.

Physical mapping of the ABPT-derived Rpi-blb2 locus

The diploid clone ARD 1197-16, heterozygous for the *Rpi-blb2* locus, was used as source DNA for the construction of a BAC library (hereafter, referred to as the 1197-16 BAC library), consisting of 104.832 clones with an average insert size of 100 kb (approximately 12 genome equivalents). In order to build a BAC contig across the *Rpi-blb2* locus, the 1197-16 BAC library was initially screened with markers E40M58e, S1E00 and CT119, which are all linked in coupling phase to resistance. E40M58e identified BAC clones 69 and 141, S1E00 identified BAC clones 14, 24, 123 and 133 and CT119 identified BAC clone 67. Sequencing of the left (L) and right (R) borders of these BAC clones led to the development of markers 69R, 69L, 141R, 24L, 14L, 24R, 123L, 123R, 133R and 67L, all of which were linked in coupling phase to resistance. Screening of the identified BAC clones with these BAC-end specific markers indicated that the S1E00 positive BAC clones in fact did not all overlap with each other, implying that S1E00 was a repetitive sequence. This together with the finding that the sequences of 24L and 123R were highly homologous to various regions of the *Mi-1* gene indicated that the *Rpi-blb2* locus harbored a cluster of Mi-GHs. Subsequent screening of the 1197-16 BAC library with the BAC-end specific markers 69R, 24L, 24R, 123L and 133R identified BAC clones 36, 211, 242, 191 and 99 (Figure 3), leading to the development of markers 36L and 99L. Finally, the BAC contig comprising BAC clones 141, 69 and 36 was distally extended with BAC clones 41 and 112, leading to the development of markers 41L and 112L (Figure 3).

Fine mapping of the ABPT-derived *Rpi-blb2* locus was carried out on 2283 ARG 95-3 and 568 ARP 96-11 clones, which were tested in a field trial for resistance to late blight. Finally, only 886 ARG 95-3 and 170 ARP 96-11 clones with a clear resistant phenotype were selected for recombinant screening with SCAR marker CT216 and CAPS marker 41L or 36L, respectively. In total, 85 (9.6 cM) and 22 (12.9 cM) recombinants were obtained in mapping populations ARG 95-3 and ARP 96-11, respectively, three of which positioned the *Rpi-blb2* locus between markers E40M58e and 133R (Figure 2). Interestingly, the left (L) BAC-end sequence of BAC clone 67, which was identified with marker CT119 and shares

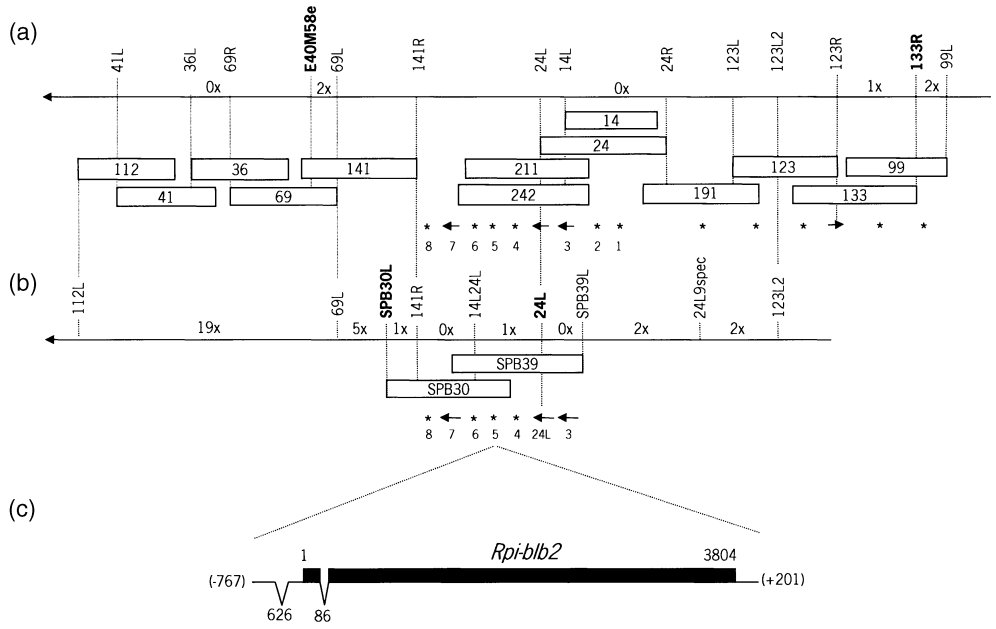


Figure 3. Genetic and physical strategy used to clone the *Rpi-blb2* gene.
High-resolution genetic and physical maps of the *Rpi-blb2* locus in (a) the ABPT-derived plant material and (b) the *S. bulbocastanum*-derived material. Vertical spotted lines indicate the relative positions of markers linked to resistance. Numbers above the horizontal lines are the numbers of recombinants identified between the flanking markers in 886 ARG95-3 and 1899 B6 progeny plants, respectively. Rectangles represent bacterial artificial chromosome (BAC) clones. Relative positions of *Mi-1* gene homologs (MiGHs) are indicated by asterisks and horizontal arrows, the latter indicating the relative orientations there where known.
(c) Schematic representation of the *Rpi-blb2* gene structure. Horizontal lines represent exons. Black rectangles represent open reading frames. Lines angled downwards indicate the positions of introns. Numbers between parentheses indicate the length of the 5′- and 3′- untranslated regions (UTRs) of the putative *Rpi-blb2* transcript.

significant DNA sequence homology (94%) to *Cf-5* from tomato (Dixon *et al.*, 1998), was mapped 0.33 and 1.76 cM proximal to *Rpi-blb2* in ARG 95-3 and ARP 96-11, respectively.

To estimate the number of MiGHs present in the above defined *Rpi-blb2* interval, BAC clones spanning the interval were screened by Southern analysis using markers 123R, 14L, or 24L as probes. These probes share high homology to different regions of the *Mi-1* gene. The results indicated that the BAC contig comprising BAC clones 211, 24, 191, 123 and 133 contained at least approximately 12 MiGHs. Additional MiGH specific markers were developed by cloning and sequencing PCR fragments generated from BAC clones 24 and 123 with the primer combination 14LR and 24LF. Based on the alignment of these sequences, a set of primers was designed, univ14L and univ24L (Table 1), with the aim to amplify the corresponding region of as many as possible MiGHs within the *Rpi-blb2* interval. This universal primer set was subsequently used to develop the MiGH specific SCAR/CAPS markers 14L24L and 24L9spec (Figure 2).

Genetic and physical mapping of Rpi-blb2 *in a* Solanum bulbocastanum *intraspecific mapping population*

To delimit the *Rpi-blb2* locus further, the intraspecific *S. bulbocastanum* mapping population B6 was developed (Figure S3). Repeated detached leaf assays on 47 progeny plants showed a clear 1:1 segregation of susceptibility:resistance in this population. Screening of these plants with markers 112L and E46M52e indicated that the resistance locus was located in the same region as the ABPT-derived *Rpi-blb2* locus. Subsequently, 1899 progeny plants were subjected to a recombinant screen. A total of 138 112L/E40M52e recombinant plants were identified (7.26 cM) and screened with all the available markers within the interval, positioning *Rpi-blb2* between markers 69L and 24L (Figure 2).

As the generated ABPT-derived BAC contig spanning the *Rp-blb2* locus still contained a gap between 141R and the 24L positive BAC clones (Figure 3), a second BAC library was made using high molecular weight DNA of the resistant *S. bulbocastanum* parental clone of mapping population B6. Approximately 100 000 clones with an average insert size of 100 kb were generated and stored as 50 bacterial pools containing approximately 2000 colonies. Screening of this pooled BAC library with markers 141L and 24L led to the isolation of the two overlapping BAC clones SPB30 and SPB39, which spanned the 141R–24L marker interval. Bacterial artificial chromosome end sequences of both BAC clones were used to develop the markers SPB30L and SPB39L (Figure 3). Screening of the recombinants within the 69L–24L interval delimited *Rpi-blb2* between SPB30L and 24L (Figures 2 and 3).

Table 1 Overview of markers used for mapping *Rpiblb2*

Marker	Orientation of the primer	Sequence	Annealing temp (°C)	Restriction enzyme
E46M52	F	TTGTGGTTATCGATGAGAAT	56.5	SCAR (b)
	R	GAAACAACAGCAGGATAGTGAG		
E46M52e	F	TTGTGGTTATCGATGAGAAT	61	SCAR (a,b); MboI (c)
	R	GAAACAACAGCAGGATAGTGAG		
E40M58	F	GAATTCAGCACAAATACCAA	50	DdeI (a)
	R	TTAACGTTTACTATCACGAG		
E40M58e	F	GTAGAAACAGCAGCCTCATAAGC	55	SCAR (a)
	R	TTCTGCCTAATTGCCCTGTG		
S1E00	F	GGGGTTGGGAAGACAACGACAC	50	
	R	AATTCCAAGATACAGTCAAATAC		
41L	F	AGGCAGGATTAACAGTAGAAG	58	TaqI (a)
	R	CATGCTTTTAGGAAGAAGCTC		
36L	F	TTGAGACAAAGCAGCTCCAC	59	ApoI (a,b)
	R	ACGTTTCTCACACCTACAGG		
69L	F	TGATGGCACGTTTGATCGTG	61	TaqI (a,b); HpaII (c)
	R	TAAGATCCAAACCAGCCACC		
69R	F	CCTTATCACACATGTGGCTAC	58	RsaI (a,b); ApoI (c)
	R	ATTGAAACGGAGGAAGTACAAC		
141R	F	TTCTTCATATGGCAGACCAAC	60	RsaI (a,b); DdeI (c)
	R	CTACTCTGCTGACATGCAGG		
24L	F	GAGATTCTCAAAGGTGTCTTCC	60	SCAR (a,b,c)
	R	AACCTGTGCTTTCCCATTCG		
24R	F	CTTTCACAAGCGTCACTTTGG	58	SCAR (a,b)
	R	TAAAAAGAATCAACAGGGCAAC		
14L	F	ACGACTGCTCAAAGTTGGCC	58	SCAR (a,b,c)
	R	CCAAGAAGCCAGTTGAGAGC		
123L	F	GTAGATTACACTATGGATATGG	60	SCAR (a,b)
	R	CAGTTAGCAGCAATGTCAGC		
123L2	F	CATTCAACTAGGCCAAAAGTGG	59	SCAR (a,b); DraI (c)
	R	CCAGGTAGGTGTTTTCTTCC		
123R	F	GTTCTAAGTCAGATGCCACC	62	SCAR (a,b)
	R	AAGTGCTCCAACACGAGCC		
133R	F	TGAGTTCTCTTACCCTGCG	60	SCAR (a,b)
	R	GGATATCCAGCATCAATGCC		
133R2	F	GGTGAGCCTCCTTGCATTCC	60	SCAR (a,b)
	R	CCTGAGGGAAGATGTCACG		
99L	F	CCTAGTTTAGAGTGAGTAGAC	58	SCAR (a,b)
	R	GTGATATATTGCTCAAGGATCC		
67L	F	GATTAGTGTAGATCTTAGCTTG	62	MboI (a,b)
	R	AAATCTCTCTCACAATTATCCC		
112L	F	CTATTGACTGAACCTGCTGAG	56	HaeIII (a); HinfI (c)
	R	TGAAGTCATTTAGTCCACAGC		
CT216	F	CGTAGTCCATCTGAAGCTCC	65	SCAR (a,b)
	R	TCTTCTTCTGCTAGTCGTCG		
CT119	F	ACTATTCTCACGTAAGGGGACAC	60	HindIII (a,b)
	R	GTGTACATGTATGAAACTCTAGC		
14L24L	F	AGAAAGCTCACCAGTGGACC	60	CfoI (c)
	R	ATTTATGGCTGCAGAGGACC		
SPB30L	F	CAAGTTACGGCAACCAAGAG	57	HpaII (c)
	R	CTTTGACACAGTGTTAGAATGC		
SPB39L	F	CGTGATCTAGGAGTTACGAC	52	SCAR (c)
	R	CTTATTTTAAATACAAGACATCTGG		
24L9spec	F	AGAAAGCTCACCAGTGGACC	56	HhaI (c)
	R	CAGAGGAAAGTCAACCAACG		

F, forward primer; R, reverse primers; a, ARG95-3; b, ARP96-11; c, B6.

Complementation analyses

For complementation purposes, all MiGHs present on BAC clones SPB30, SPB39, 242, 211 and 24 were subcloned as approximately 10 kb genomic DNA fragments into the binary vector pBINPLUS (van Engelen *et al.*, 1995). MiGH harboring subclones were identified by screening subclone libraries of each BAC clone with the primer combination 14L24LF/R. Based on the restriction pattern of the 14L24L fragments digested with the enzymes RsaI, TaqI, AluI, DpnII or MseI, eight different MiGHs were identified. Subclones harboring the 24L specific MiGH were not detected with the universal primers 14L24LF/R. These were selected using the 24L specific primers 24LF and 24LR, bringing the total number of identified MiGHs on these BAC clones to nine (Figure 3). Subclones with MiGHs from the SP30L–24L interval were transferred to the susceptible potato cultivars Impala and Kondor through *Agrobacterium* mediated transformation using strain UIA143 (Farrand *et al.*, 1989) or AGL0 (Lazo *et al.*, 1991). Primary transformants harboring the transgenes of interest were tested for resistance to *P. infestans* in detached leaf assays using the complex isolates IPO655-2A and IPO82001 (Table 2). Genetic constructs harboring MiGH5, derived either from the ABPT material or from *S. bulbocastanum*, were able to complement the susceptible phenotype in both cultivars. In total, 34 out of 36 MiGH5 containing primary transformants were resistant whereas all other MiGH containing primary transformants were susceptible to *P. infestans* (Table 2; Figure 4). A selection of primary transformants containing MiGH5 was analyzed for copy number by Southern analysis, identifying four single copy integrations in cultivar Impala and six in cultivar Kondor. Of these, nine were resistant. MiGH5 was therefore designated the *Rpi-blb2* gene.

Primary transformants of cultivar Moneymaker harboring the *Rpi-blb2* gene construct were also produced and tested with the potato-derived isolates IPO82001 and IPO655-2A. The disease resistance assay revealed that *Rpi-blb2* is also able to complement the susceptible tomato phenotype (Table 2; Figure 4).

Rpi-blb2 gene structure and putative amino acid sequence

The inserts of two MiGH5 containing binary subclones, from BAC clone 211 and SPB39 from the ABPT and *S. bulbocastanum* sources respectively were sequenced by a primer walk strategy. The complete sequences of the inserts of clones 211-F/C12 and SPB39-20 consisted of 7967 and 9949 nucleotides (nt), respectively. The sequence of clone 211F/C12 was identical to the corresponding sequence within clone SPB39-20. The size and structure of the gene was determined by comparing the genomic sequence with 5′ and 3′ rapid amplification of cDNA ends (RACE) products. The *Rpi-blb2* gene contains 5′ and 3′ untranslated regions (UTRs) of 767 and 201 nt, respectively, and two introns at similar positions as those in *Mi-1*. Intron 1 is 626 nt long and is positioned within the 5′ UTR ending 32 nt upstream of the ATG start codon. Intron 2 is 86 nt long starting 43 nt downstream of the ATG start codon of the gene. The coding sequence of the *Rpi-blb2* transcript is 3804 nt.

The deduced open reading frame of the *Rpi-blb2* gene encodes a predicted polypeptide of 1267 amino acids with an estimated molecular weight of 146 kDa (Figure 5). The N-terminal half of the Rpi-blb2 protein contains three potential coiled-coil (CC) domains (amino acids 24–62, 326–374 and 413–434) and six conserved motifs indicative of an NBS motif (van der Biezen and Jones, 1998). The C-terminal half of Rpi-blb2 comprises a series of 13 irregular LRRs that can be aligned according to the consensus sequence hxxhxxLxxLxLxxC/N/Sx(x)LxxLPxx observed in other cytoplasmic R proteins, whereby h can be L, I, M, V or F, and x any amino acid residue.

The *Rpi-blb2* coding sequence shares 89.7% homology with *Mi-1* from tomato. At the amino acid level Rpi-blb2 shares 82% sequence identity with Mi-1.1 and 81% with Mi-1.2. Through ClustalW alignment of the deduced amino acid sequences of Rpi-blb2, Mi-1.1 and Mi-1.2, 197 amino acid residues were identified that are unique to Rpi-blb2 (Figure 5). Of these, 12 are encoded by three insertions; a single amino acid insertion at position 317, a three amino acid insertion comprising residues 33–35 and an eight amino acid insertion comprising residues 95–102 (Figure 5). Of the remaining 185 amino acids unique to Rpi-blb2, 111 (60%) are non-conservative substitutions. This means that the relevant amino acid does not belong to the same group as the corresponding amino acid in Mi-1.1 or Mi-1.2 (Figure 5), whereby the amino acid groups are classified based on ionic charges. When comparing the ratio of non-conserved versus conserved substitutions in the different domains of Rpi-blb2 (CC domain, NBS domain and LRR domain; Figure 5), we observed that the non-conservative nature of the substitution was highest in the LRR domain (42/61; 69%) and lowest in the NBS-domain (25/52; 48%). Within the LRR domain, these types of substitutions are centered on the xxLxLxxxx motif, which is predicted to encode the putative solvent exposed residues of the repeats (Figure 5). This fits the model that the LRR region of R proteins mainly defines resistance specificity. However, the relatively high non-conservative substitution rate in the CC domain (44/72; 61%) together with the concentration of insertions within this region also suggest an active role for this region. Correct intramolecular interactions between N- and C-terminal domains of the Mi-1 protein are essential for proper functioning of the protein (Hwang and Williamson, 2003; Hwang *et al.*, 2000). Highlighted in Figure 5 are 26 amino acids in the LRR region of the Mi-1 protein that are crucial for correct functioning of the protein. At 23 positions, alteration of the Mi-1.2 amino acid to that of Mi-1.1 resulted in the loss of both nematode resistance

Table 2 Complementation of late blight susceptibility in potato and tomato

			cv Impala		cv Kondor		Tomato cv Moneymaker	
BAC-library	Source BAC	Genotype[a]	MiGH-containing plants/transformants	R plants/MiGH-containing plants	MiGH-containing plants/transformants	R plants/MiGH-containing plants	MiGH-containing plants/transformants	R plants/MiGH-containing plants
ARD 1197-16	24	R_0 (MiGH1)	12/15[b]	0/12				
			8/10[c]	0/8				
	24	R_0 (MiGH2)	8/11[b]	0/8				
			5/6[c]	0/5				
	24	R_0 (MiGH3)	11/13[b]	0/11				
			5/7[c]	0/5				
	211	R_0 (MiGH4)	5/7[b]	0/5	10/12[b]	0/10		
	242	R_0 (MiGH4)	5/7[b]	0/5	8/8[b]	0/8		
	211	R_0 (MiGH5)	5/7[b]	4/5	12/13[b]	12-dec		
	211	R_0 (MiGH6)						
	211	R_0 (MiGH-24L)						
BIb 2002	SPB39	R_0(MiGH4)	5/6[b]	0/5	3/3[b]	0/3		
	SPB39	R_0 (MiGH5)	11/15[b]	11/11	8/8[b]	7′/8	24/25[b]	22/24
	SPB39	R_0 (MiGH6)	3/3[b]	0/3	6/6[b]	0/6		
	SPB30	R_0 (MiGH7)	3/4[b]	0/3	9/9[b]	0/9		
	SPB30	R_0 (MiGH8)	1/1[b]	0/1				
	SPB39	R_0 (24L)						
		Ro (pBINPLUS)	3/3	0/3	8/10	0/8		

[a]R_0 genotypes are primary transformants obtained from transformation of the susceptible potato cultivars Impala or Kondor with T-DNA constructs containing the Rpi-blb2 gene candidates RGC1 to RGC8 and RGC24L or an empty pBINPLUS vector. *Agrobacterium tumefaciens* strains UIA143[b] or AGL0[c] were used for transformation of the *Phytophthora infestans* susceptible potato cultivars Impala and Kondor or susceptible tomato cultivar Moneymaker.

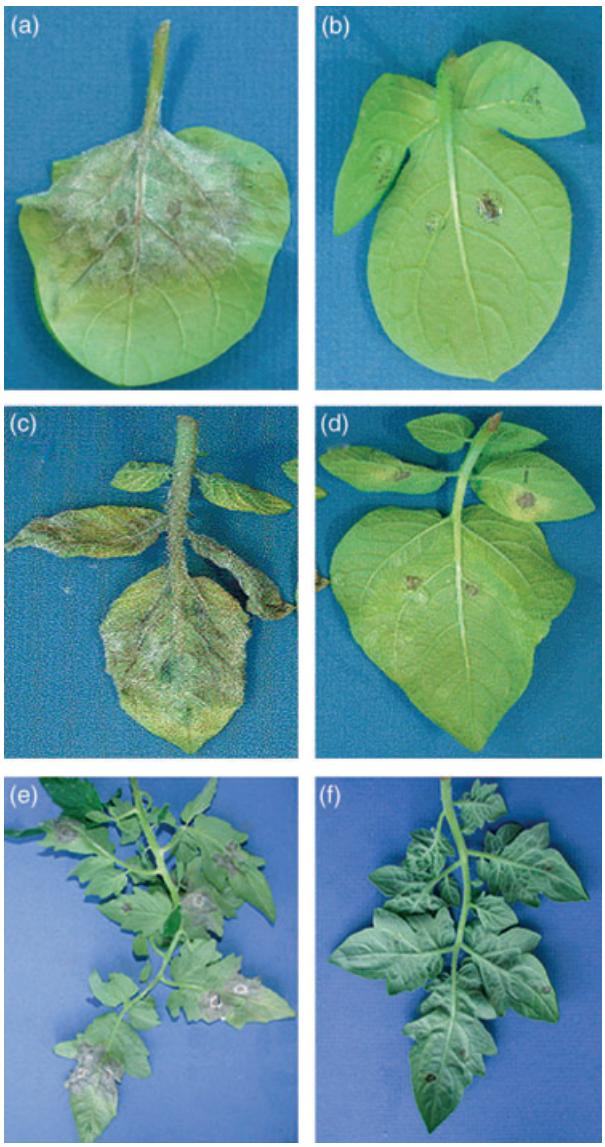


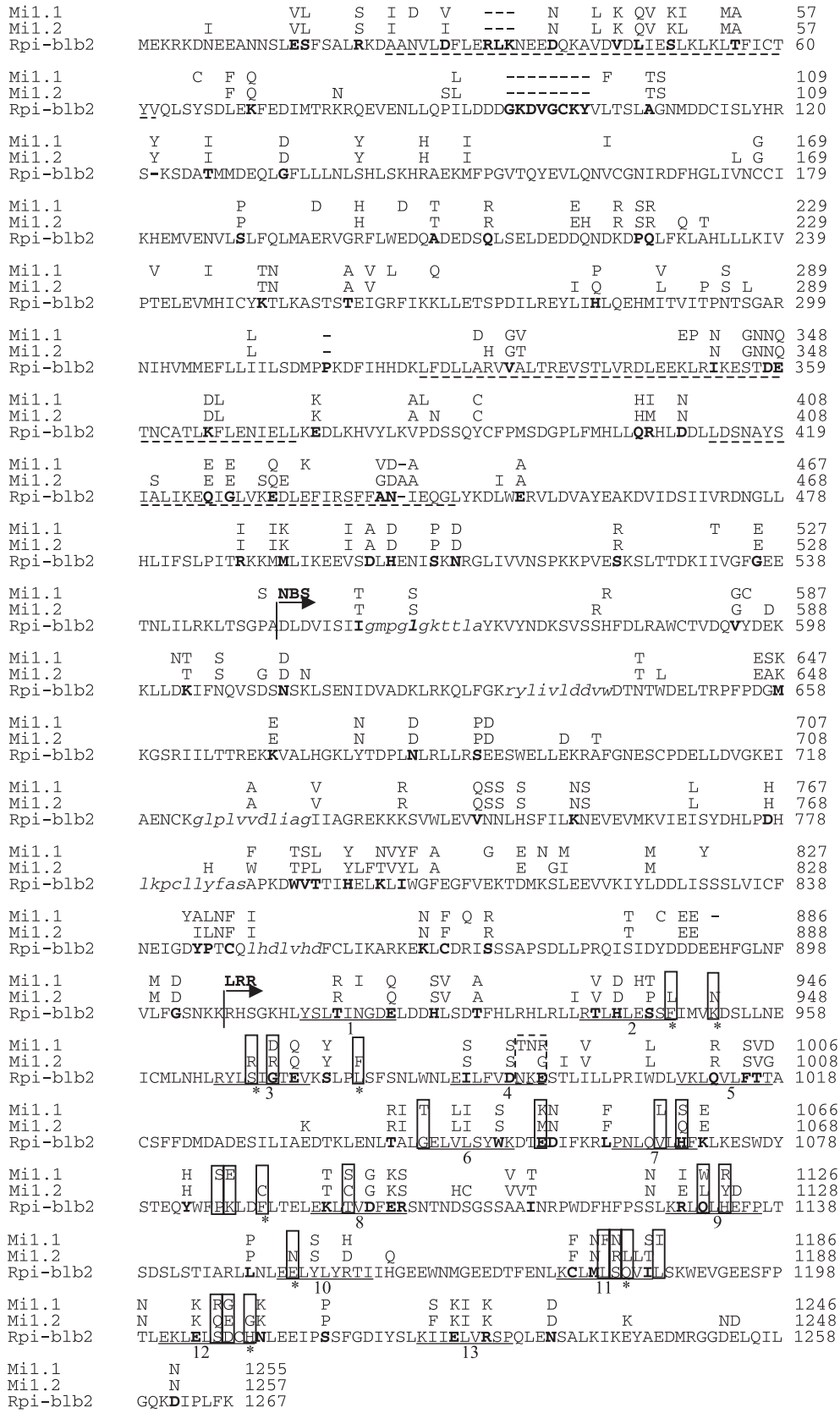
Figure 4. Genetic complementation for late blight susceptibility.
Typical disease phenotypes 6 days after inoculation with a sporangiospore suspension of *Phytophthora infestans* isolate 655-2A. Indicated are leaves derived from: (a) cv Kondor; (b) a primary transformant of cv Kondor harboring the MiGH5 (*Rpi*-blb2) from bacterial artificial chromosome (BAC) SPB39; (c) cv. Impala; (d) a primary transformant of cv Impala harboring MiGH5 (*Rpi*-blb2) from BAC 211; (e) tomato cv. Moneymaker; (f) a primary transformant of tomato cv Moneymaker harboring *Rpi-blb2*.
Panels (a), (c) and (e) depict typical susceptible responses with extensive sporulating lesions of *P. infestans*.
Panels (b), (d) and (f) depict typical resistance reactions observed at the sites of inoculation on transgenic plants harboring *Rpi-blb2*.

mediated by *Mi-1*.2 and the ability of the *Mi-DS3* chimeric gene construct to cause cell death in a transient expression assay on *Nicotiana* benthamiana leaves (Hwang and Williamson, 2003). Their data suggest a role of these amino acids in recognition of signaling molecules or interacting proteins, or changes in protein folding, stability or localization. Interestingly, Rpi-blb2 carries the Mi-1.1 specific amino acid at eight of these positions (Figure 5), suggesting therefore a different function for these amino acids in the Rpi-blb2 protein context. Moreover in the former study, a cluster of three amino acids was identified that appeared to be crucial for nematode recognition and not for cell death (Figure 5). Interestingly, Rpi-blb2 harbors two of the three Mi-1.2 specific amino acids at the corresponding position.

Discussion

Here, we report on the cloning of the late blight resistance gene *Rpi-blb2* from the wild potato species *S. bulbocastanum* and demonstrate that this gene is orthologous to the tomato *Mi-1* gene from tomato. The *Rpi-blb2* locus was initially mapped in several tetraploid BC populations, derived from highly resistant complex interspecific hybrids designated ABPT, to the distal end of the short arm of chromosome 6 using a standard marker BSA approach (Michelmore *et al.*, 1991). However, fine mapping in these BC populations was hampered by low recombination frequencies at the *Rpi-blb2* locus and the fact that only marker alleles linked in coupling phase to resistance could be used. To circumvent these intrinsic problems of genetics in tetraploid potato, a diploid intraspecific *S. bulbocastanum* mapping population was developed in which late blight resistance cosegregated with *Rpi-blb2* locus specific markers previously developed in the BC populations. Higher recombination frequencies in the diploid *S. bulbocastanum* resistant parent together with the additional informative nature of marker alleles linked in repulsion phase to resistance enabled the genetic mapping of BAC-end markers and thus the efficient tiling of BAC clones across the *Rpi-blb2* locus. Instrumental in the cloning process of *Rpi-blb2* was also the syntenous nature of *Rpi-blb2* to the *Mi-1* locus, revealing the probable nature of the gene and thus prompting the development of candidate gene specific markers. By switching to the wild species level and through comparative genomics, problems usually encountered during the positional cloning of an *R* gene in potato were successfully circumvented.

Recently, comparative analyses of the *S. demissum*-derived complex locus *R3*, conferring race-specific resistance to *P. infestans* with the corresponding complex locus *I2* from tomato, conferring resistance to the fungus *Fusarium oxysporum* f. sp. *lycopersici*, revealed a significant expansion of the *R3* locus compared with the *I2* locus (Huang *et al.*, 2005). It was argued that the contrasting evolutionary fates of the two syntenic loci reflect the opposite evolutionary potential of the interacting pathogens (McDonald and Linde, 2002). The similar chromosomal positions of the *Rpi-blb2* and *Mi-1* gene clusters in potato and tomato warrants the comparative analyses of these two loci. *Mi-1* originates from the wild tomato species *Lycopersicon peruvianum* and has been introgressed into many tomato lines. In nematode-


Mi1.1
Mi1.2
Rpi-blb2
NBS
LRR
1
2
3
4
5
6
7
8
9
10
11
12
13

resistant tomato, *Mi-1* and six homologs are grouped into two clusters separated by 300 kb (Vos *et al.*, 1998). *Mi-1* resides in the proximal cluster with two additional MiGHs. Recently, it was shown that the *Mi-1* locus from susceptible tomato carried the same number and distribution of MiGHs as the resistant locus (Seah *et al.*, 2004). The similarity in structure and organization of the *Mi-1* loci in *L. peruvianum* and *Lycopersicon esculentum* could suggest that the locus has not evolved much since the divergence of *L. esculentum* and *L. peruvianum*. In contrast, the *Mi-1* gene cluster in *S. bulbocastanum*, which harbors *Rpi-blb2*, seems to have expanded significantly since speciation of *Solanum* and *Lycopersicon*. Although the two-cluster structure is roughly conserved, the number of homologs per cluster has significantly increased. The distal cluster contains *Rpi-blb2* with eight additional MiGHs, whereas the proximal cluster contains at least six MiGHs (Figure 3). The distal cluster harboring *Rpi-blb2* is inverted compared with the proximal cluster, the proximal cluster having the same orientation as the *Mi-1* harboring cluster in tomato (Figure 3). Although the orientation of the distal cluster in tomato is unknown, the conservation of the two-cluster structure in both species suggests that the original duplication event occurred in the ancestral progenitor species of *Solanum* and *Lycopersicon*. As we also have preliminary data that indicate that the locus in *S. tuberosum* is also expanded compared with *L. peruvianum* and *L. esculentum*, we conclude that intrachromosomal recombination or unequal crossing over has played an important role in the evolution of the *Rpi-blb2* locus in potato. Intergenic and intragenic recombination at *R* gene loci has been described extensively and is thought to be a major mechanism for generating novel resistance specificities (reviewed in Hulbert *et al.*, 2001).

The short arm of chromosome 6 can be defined as a hot spot for resistance. In tomato, other dominant resistance genes that reside in the same region as *Mi-1* are *Mi-9*, conferring heat-stable resistance to root-knot nematodes (Ammiraju *et al.*, 2003), *Ol-4* and *Ol-6* for resistance to *Oidium neolycopersici* (Bai *et al.*, 2005), and the cloned *Cf-2* and *Cf*-5 genes for resistance to *Cladosporium fulvum* (Dixon *et al.*, 1996, 1998). In addition, quantitative resistance traits *Ty-1* for resistance to tomato yellow leaf curl virus (Zamir *et al.*, 1994) and *Bw-5* for resistance to *Ralstonia solanacearum* (Thoquet *et al.*, 1996) also reside in this region. In potato, in addition to *Rpi-blb2*, QTLs for resistance to *P. infestans* and *Erwinia carotovora* have been described in this region (Gebhardt and Valkonen, 2001). Clearly, the *Mi-1* locus in tomato and potato is not only agronomically of interest but also scientifically. Further comparison of the structure of this locus in the *Solanum* and *Lycopersicon* accessions harboring the above described dominant or quantitative resistance traits is likely a rich source of information on the genetic mechanisms associated with the evolution of *R* genes, thereby opening up an important experimental system for comparative studies, with potential economic benefits.

The most straightforward prediction of the gene-for-gene model (Flor, 1971) is that R proteins recognize single pathogen avirulence (Avr) or effector proteins and that recognition involves a direct binding between the two proteins. However, more recent findings are consistent with the so-called guard model for R-Avr interactions. This model predicts that R proteins detect modifications of host proteins targeted by effectors, rather than the effectors themselves (van der Biezen and Jones, 1998; Dangl and Jones, 2001). In light of this model, host genes identified through mutational screens as being required for *R* gene function or proteins that interact with effector proteins in Y2H screens and/or co-immunoprecipitation studies, are possible candidates for virulence targets (Mackey *et al.*, 2002; Rooney *et al.*, 2005; Shao *et al.*, 2003). In case of the Mi-1 protein, mutations at the *Rme* locus of tomato have been shown to suppress Mi-1 function both in the nematode and potato aphid resistance (de Ilarduya *et al.*, 2001). Classic receptor-ligand models would predict that all pathogens recognized by the Mi-1 protein have evolved conserved ligands. However, it seems more likely that Mi-1 recognizes the modification of a conserved host factor, possibly the Rme protein, which is targeted and modified by several unrelated effectors. The finding that Rpi-blb2 and Mi-1 are highly homologous (81% amino acid sequence identity) and that the corresponding genes have a common ancestral origin could suggest that they function through the same signaling pathways. The question that now arises is whether both proteins have evolved to guard the same or similar virulence targets or that the *P. infestans* effector(s) recognized by *Rpi-blb2* is (are) somehow related to those from root-knot nematodes, potato aphids and white flies. The observation that the Rpi-blb2 protein in fact contains one-third of the Mi-1.1 specific amino acid residues that when present in an Mi-1.2 context lead to loss of function (Hwang and Williamson, 2003) may suggest that neither is the case. Further insight into the above questions awaits functional analyses of *Rpi-blb2* in an *rme* background and/or the cloning

Figure 5. Alignment of the deduced protein products encoded by *Rpi-blb2*, *Mi-1.1* and *Mi-1.2*.
The complete amino acid sequence of Rpi-blb2 is shown and amino acid residues from Mi-1.1 and Mi-1.2 that differ from the corresponding residue in Rpi-blb2. Dashes indicate gaps inserted to maintain optimal alignment. The positions of putative coiled-coil (CC) domains are underlined with a dashed line. The N-terminal boundaries of the NBS and LRR region are indicated. Conserved motifs in the NBS domain are indicated in lower-case italics. The regions of the leucine-rich repeats (LRRs) that correspond to the β-strand/β-turn motif xxLxLxxxx are underlined. Rpi-blb2 specific amino acid residues comprising a non-conservative amino acid substitution between Rpi-blb2 and Mi-1.1 and Mi1.2 are highlighted in bold. Amino acid residues in the LRR region of the Mi-1 protein that are crucial for correct functioning of the protein are boxed (Hwang and Williamson, 2003). Boxed amino acid positions where Rpi-blb2 carries the Mi-1.1 specific amino acid are indicated with an asterisk.

of the effectors from the relevant pathogens. Recently, the first *Avr* gene from *P. infestans*, *Avr3a*, was identified using association genetics (Armstrong *et al.*, 2005). *Avr3a* is part of an ancestral oomycete locus and encodes a protein that is recognized in the host cytoplasm, where it triggers *R3a*-dependent cell death.

Like *Rpi-blb1*, *Rpi-blb2* confers broad-spectrum resistance to *P. infestans*. In more than 20 years of late blight resistance breeding involving annual screenings on inoculated fields in the Netherlands, potato clones harboring the *Rpi-blb2* gene showed no or hardly any sporulating lesions. The same observation was performed when such clones were tested under organic farming conditions at multiple sites in regions of the Netherlands where more virulent and aggressive isolates are being found (Flier *et al.*, 2003). The few sporulating lesions that were found only appeared at the end of the growing season at crop maturity. This phenomenon was also observed for the *Rpi-blb1* gene containing plant material (Helgeson *et al.*, 1998). Interestingly, detached leaf assays with ABPT-derived progeny were found to be less accurate for phenotyping than screening under field conditions. Although the apparent partial resistance phenotype observed under detached leaf assay conditions suggests a resistance mechanism similar to what can be observed in clones with enhanced levels of quantitative resistance, under field conditions, the *Rpi-blb2* based resistance is complete and behaves as a dominant trait. It remains to be seen if the use of these broad-spectrum late blight *R* genes is durable under conditions of large-scale agricultural production. Anyway, the cloning of these genes opens the way to efficient gene pyramiding or polyculture strategies (Niederhauser *et al.*, 1996), although durability prediction criteria need to be developed that will allow for the combination of genes that have the greatest potential for conferring durable resistance. For this purpose, it is essential to better understand how late blight *R* gene loci have evolved in the *Solanum* gene pool, how they are combined in natural *Solanum* habitats, the resistance mechanisms by which they confer resistance and, most important of all, to understand the biological function of the effectors that they recognize. Only then can we start thinking of durably exploiting *R* genes in the battle against late blight.

Experimental procedures

Plant material and development of mapping populations

The complex interspecific hybrid clones designated ABPT were made by Hermsen and co-workers (Figure S1; Hermsen and Ramanna, 1973). The *Phytophthora infestans* resistant clones ARF 87-507, ARF 87-801 and ARF 87-601 represent offspring from a second backcross (BC2) with the complex interspecific ABPT clones 55 or 60 and were used to develop the tetraploid mapping populations ARG 95-15 (BC3), ARG 95-3 and ARP 96-11 (BC4) and the diploid mapping population DP1 (Figure S2). Diploid population DP1 was obtained by crossing the resistant clone ARD 1197-16, with the susceptible diploid clone ARD 93-2090. The resistant diploid clone ARD 1197-16 was induced from AR 92-1197. This tetraploid clone AR 92-1197 was derived from a BC3 offspring derived from clone ARF 87-601.

The diploid *S. bulbocastanum* mapping population, designated B6, was developed by crossing a *P. infestans* resistant clone Blb 2002 with a susceptible clone Blb 48-5 (Figure S3). Results from reciprocal crosses of population B6 were combined. The resistant parental clone of population B6 was obtained from a cross between *S. bulbocastanum* clone Blb 93-D26-3 (accession numbers BGRC 8002, CGN 17690 and Pi 275187) as the female parent and *S. bulbocastanum* clone Blb 93-60-10 (accession numbers BGRC 8006 and Pi 275194) as the male parent. The susceptible parental clone of population B6 was obtained from a cross between *S. bulbocastanum* clones from accession numbers BGRC 8005 (CGN 17692 and PI 275193) and BGRC 8006.

Disease assays

Two different *P. infestans* isolates were obtained from Plant Research International B.V. (Wageningen, the Netherlands). Race structures and mating types were as follows: IPO82001, race structure 1.2.3.4.5.6.7.10.11, mating type A2; IPO655-2A. race structure 1.2.3.4.5.6.7.8.9.10.11, mating type A1 (Flier *et al.*, 2003).

Glasshouse-grown seedling tubers or field-grown seed potatoes were planted at trial sites in Marknesse, the Netherlands, from 1985 to 2002 and in the Toluca area of Mexico in 1991. For individual clones, plots were planted consisting of one to ten tubers. Approximately eight weeks after planting, the field at Marknesse was inoculated with a sporangiospore solution of *P. infestans* isolate IPO82001 and disease scores were collected 3 to 6 weeks after inoculation. Clones that were free or nearly free from late blight were classified as having a resistant phenotype, whereas clones with complete or nearly complete blighted foliage were classified as susceptible. Clones with intermediate reactions to late blight were classified as having an unknown phenotype. At the field trial in Mexico, natural infection had to occur. Once this natural infection by *P. infestans* was established, the percentage of blighted foliage of plants on each plot was scored 8 times on a 1–9 scale. Estimated percentages of blighted foliage from 1 to 9 were: 0, 3, 10, 25, 50, 75, 90, 97 and 100 (Estrada-Ramos *et al.*, 1983).

Detached leaf assays were carried out as described previously (van der Vossen *et al.*, 2003).

Plant DNA marker screening

Genomic DNA extractions and PCR analyses were carried out as described previously (van der Vossen *et al.*, 2003). An overview of the markers including primer sequences, annealing temperature and restriction enzymes if appropriate is given in Table 1.

AFLP fingerprinting and cloning and elongation of AFLP fragments

Template preparation and AFLP fingerprinting were performed essentially as described in Vos *et al.* (1995). Cloning of specific AFLP fragments was performed as described in Brugmans *et al.* (2003). Elongation of the sequence of an AFLP fragment was performed by TAIL PCR according to Liu and Whittier (1995). Essentially, elongation of AFLP fragments was performed using two or three nested specific primers (sp) in combination with an arbitrary degenerate

(AD) primer. The first PCR was performed with primers sp1 and an AD, the second with sp2 and an AD and the third with sp3 and an AD. The elongated fragments were cloned in pGEM-T (Promega, Leiden, the Netherlands) and sequenced.

R gene homolog fingerprinting

Template preparation was essentially performed as described in Vos *et al.* (1995). However, the second amplification step was carried out with the P-loop based primer S1 from Leister *et al.* (1996) in combination with the *Eco*RI + 0 AFLP primer. A 10-μl reaction mixture [0.5 μl ^{33}P-labelled S1 primer (10 ng μl^{-1}); 0.5 μl *Eco*R1 + 0 primer (10 ng μl^{-1}); 0.8 μl dNTPs (5 mM); 2 μl 10xGoldstar™ PCR buffer (Eurogentec, Seraing, Belgium); 1.2 μl $MgCl_2$ (25 mM); 0.06 μl Goldstar™ DNA polymerase (5 U μl^{-1}; Eurogentec); 14.94 μl MQ water] was added to a 10 μl diluted template (20× diluted in MQ water) and a PCR reaction performed using the following cycle profile: 45 sec DNA denaturation at 94°C, 45 sec primer annealing at 49°C and 2 min elongation step at 72°C (35 cycles). Prior to the cycling, the template DNA was denatured for 2 min at 94°C and the PCR was finalized by applying an extra 5 min elongation step at 72°C. The labelled PCR products were separated on a 6% polyacrylamide gel and the individual bands visualized by autoradiography according to standard procedures.

Bacterial artificial chromosome library construction and screening

Resistant clones ARD 1197-16 and Blb2002, both heterozygous for the *Rpi-blb2* locus, were used as source DNA for the construction of the ABPT-derived and *S. bulbocastanum*-specific BAC libraries, respectively. High molecular weight DNA preparation and BAC library construction were carried out as described in Rouppe van der Voort *et al.* (1999). Marker screening of the BAC library harboring the individually stored BAC clones was carried out as described in Rouppe van der Voort *et al.* (1999). The BAC library stored as superpools was screened as described in van der Vossen *et al.* (2003). Names of BAC clones isolated from the superpools carry the prefix SP (e.g. SPB39).

Subcloning of candidate genes and transformation to potato and tomato

Candidate MiGHs were subcloned from BAC clones 24, 211, 242, SPB30 and SPB39 as described previously (van der Vossen *et al.*, 2003). Binary plasmids harboring the candidate genes were transformed to *Agrobacterium tumefaciens* strains AGL0 (Lazo *et al.*, 1991) or UIA143 (Farrand *et al.*, 1989), the latter containing the helper plasmid pCH32 (Hamilton *et al.*, 1996). Overnight cultures of the transformed *A. tumefaciens* strains were used to transform potato tuber discs (cvs Impala and Kondor) or tomato leaf discs according to standard protocols (Fillati *et al.*, 1987; Hoekema *et al.*, 1989; van der Vossen *et al.*, 2003).

Rapid amplification of cDNA ends

Rapid amplification of cDNA ends was carried out using the GeneRacer™ kit (Invitrogen™, Groningen, the Netherlands). 5′ rapid amplification of cDNA ends was carried out on cDNA synthesized with primer GSP4 (CTCAGCCATCAGTTGAAACAGAGA). Subsequently, primer GSP6 (GAGAGAGATTCAAGAGGAGGAAGC) was used in combination with the GeneRacer™ 5′ primer and the final amplification was carried out with GSP6 in combination with the GeneRacer™ 5′ nested primer. 3′ rapid amplification of cDNA ends was carried out with the nested primers GSP1 (GTGCTTCATTCAAACTCAAGGAG) and GSP2 (CTGAAC TAGAAAAACTCACTGTAGA) in combination with the GeneRacer 3′ primer. The final amplification was carried out with GSP3 (GTTTGAAAAGATTGCAATTGCATG) in combination with GeneRacer nested 3′ primer. Both 5′ and 3′ RACE amplification steps were carried out using Accuprime (Invitrogen) instead of the Taq polymerase supplied by the GeneRacer™ kit.

Acknowledgements

We thank Pim Lindhout, Rients Niks and Valerie Williamson for constructive comments regarding this manuscript.

Supplementary Material

The following supplementary material is available for this article online:

Figure S1. Schematic representation of the development of the complex interspecific hybrid clones designated as ABPT (an acronym of the four species involved: A, *Solanum acaule*; B, *Solanum bulbocastanum*; P, *Solanum phureja*; T, *Solanum tuberosum*).
2x, diploid (2n = 2x = 24); 3x, triploid; 4x, tetraploid; 6x, hexaploid; cv, cultivar.

Figure S2. Schematic representation of the development of the *Solanum tuberosum* mapping populations derived from ABPT (an acronym of the four species involved: *Solanum acaule*, *Solanum bulbocastanum*, *Solanum phureja*, *Solanum tuberosum*) clones 55 or 60.
(a) ARG 95-3 and DP1.
(b) ARG 95-15.
(c) ARP 96-11.
2x, diploid (2n = 2x = 24); 4x, tetraploid; cv, cultivar; BC, backcross. Codes in italics indicate mapping populations.

Figure S3. Schematic representation of the development of the diploid, intraspecific mapping population B6 of *Solanum bulbocastanum*.

This material is available as part of the online article from http://www.blackwell-synergy.com

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