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High-resolution mapping and analysis of the resistance locus *Rpi-abpt* against *Phytophthora infestans* in potato

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Abstract

Introduction of more durable resistance against *Phytophthora infestans* causing late blight into the cultivated potato is of importance for sustainable agriculture. We identified a new monogenically inherited resistance locus that is localized on chromosome 4. The resistance is derived from an ABPT clone, which is originally a complex quadruple hybrid in which *Solanum acaule*, *S. bulbocastanum*, *S. phureja* and *S. tuberosum* were involved. Resistance data of the original resistant accessions of the wild species and analysis of mobility of AFLP markers linked to the resistance locus suggest that the resistance locus is originating from *S. bulbocastanum*. A population of 1383 genotypes was screened with two AFLP markers flanking the *Rpi-abpt* locus and 98 recombinants were identified. An accurate high-resolution map was constructed and the *Rpi-abpt* locus was localized in a 0.5 cM interval. One AFLP marker was found to co-segregate with the *Rpi-abpt* locus. Its DNA sequence was highly similar with sequences found on a tomato BAC containing several resistance gene analogues on chromosome 4 and its translated protein sequence appeared to be homologous to several disease resistance related proteins. The results indicated that the *Rpi-abpt* gene is a member of an *R* gene cluster.

Introduction

Late blight caused by *Phytophthora infestans* is one of the most serious threats to potato cultivation. Controlling late blight usually involves the application of a lot of chemicals. Nevertheless, restrictions on chemical control, environmental safety and the development of more virulent forms of the pathogen that are resistant to these chemicals indicate that the most promising approach to achieve late blight resistance is the introduction of resistance genes from wild *Solanum* species into potato cultivars (Kato et al. 1997).

In the last century, 11 resistance genes against *Phytophthora infestans* were introgressed from the wild *Solanum* species *S. demissum* into potato (Black et al. 1953; Malcolmson and Black 1966). However, these resistance genes confer race-specific resistance controlled by single resistance factors and these genes are not durable (Mastenbroek 1953;

Malcolmson and Black 1966). In the meantime, not only asexual but also sexual forms (A1 and A2) of *P. infestans* have been introduced into Europe, enabling the pathogen to overcome chemicals and resistance genes. Therefore, new sources are required for the creation of durable and race-non-specific resistance. Instead of the genes from *S. demissum*, resistance loci and QTLs originating from wild species such as *S. berthaultii*, *S. pinnatisectum* and *S. microdontum* have been localized on chromosomes 10, 7 and 4, respectively, as new sources of resistance (Ewing et al. 2000; Kuhl et al. 2001; Sandbrink et al. 2000). *S. bulbocastanum* has also been considered as another new source of resistance to *P. infestans* (Niederhausen and Mills 1953). *S. bulbocastanum* is a self-incompatible diploid wild species from Mexico which cannot be crossed to the common potato *S. tuberosum* (Hermsen and De Bore 1971). Recently two resistance genes *Rpi-blb1*/*RB* and *Rpi-blb2* derived from *S. bulbocastanum* were cloned (Song et al. 2003; van der Vossen et al. 2003, 2004). The *RB* gene was localized on chromosome 8 (Naess et al. 2000). Because of the inability of direct sexual crosses between *S. tuberosum* and *S. bulbocastanum*, BC_2 populations derived from somatic hybrids were used (Helgeson et al. 1998). Due to the same reason, the *Rpi-blb1* gene was cloned using an intraspecific hybrid between resistant and susceptible *S. bulbocastanum* genotypes (van der Vossen et al. 2003). To clone *Rpi-blb2*, which is located on chromosome 6, both an intraspecific hybrid and a complex interspecific hybrid, which was designated ABPT, were used (van der Vossen et al. 2004). ABPT is a quadruple species hybrid created through bridge crosses using *S. acaule* (A), *S. bulbocastanum* (B), *S. phureja* (P) and *S. tuberosum* (T) (Figure 1a; Hermsen and Ramanna 1973).

Map-based cloning is one of the most challenging methods for gene cloning in plant species with a relatively large genome (Lukowitz et al. 2000). In addition to *Rpi-blb1*/*RB* and *Rpi-blb2*, the *S. demissum* derived *R1* (Ballvora et al. 2002) and *R3a* (Huang 2005) have also been isolated using the map-based cloning approach. The first essential step for map-based gene cloning is to determine the accurate position of the target gene.

In this paper, we identify another new resistance gene named *Rpi-abpt* in ABPT material, which differs from that used by van der Vossen et al. (2004). We constructed a high-resolution map of the *Rpi-abpt* locus and analyzed this region by investigating the sequence of closely linked markers. We also unraveled the predicted wild species origin of the resistance gene.

Materials and methods

Plant materials

The pedigree of the mapping population RH4X-103 is shown in Figure 1b. The tetraploid resistant female parent 707TG11-1 was derived from a quadruple hybrid ABPT clone (Hermsen and Ramanna 1973). The diploid susceptible male parent RH89-039-16, producing unreduced 2*n* pollen, is one of the advanced clones widely used for mapping research at the Laboratory of Plant Breeding and the Laboratory of Nematology, Wageningen University (Rouppe van der Voort et al. 1997a, b, 1998, 1999; Huang et al. 2004; van Os et al. personal communication).

Seeds of RH4X-103 progeny were sterilized in three different steps: 70% alcohol for 1 min, 1.5% sodium hypochlorite for 5 min and three times rinsing with sterilized water. Plants were generated from seeds sown *in vitro*. When the size of the *in vitro* plants was approximately 5 cm, they were multiplied for DNA isolation, resistance assay and genotype maintenance. Recombinants were maintained *in vitro* and transferred to the greenhouse for generation of leaf material for the detached leaf assays.

Accession of *S. acaule* CGN17843 and CGN20620, *S. bulbocastanum* CGN21306 and CG N17693, *S. phureja* CGN17667 and CGN18301, *S. demissum* CGN17810 were obtained from the Center of Genetic Resources in The Netherlands (CGN) and maintained *in vitro*. Potato clone Ceb44-158-4, which has *S. demissum* in its pedigree and *S. tuberosum* cultivars Titana, Alcmaria, Van Gogh, Agria, Bintje and Nicola were planted in the greenhouse, and leaves were collected for DNA isolation.

Resistance assay

The mapping population of 233 genotypes was subjected to a detached leaf assay in four replications using leaf material from the maintenance field. Fully expanded and healthy leaflets were collected and incubated in moist trays. Each leaflet was inoculated

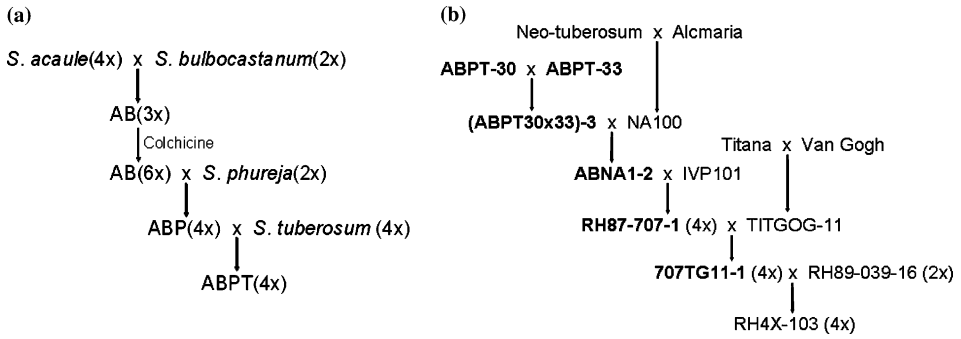

Figure 1. Plant materials used for creation of the tetraploid mapping population RH4X-103. (a) Pedigree of the tetraploid ABPT breeding clone. A, B, P and T indicate *S. acaule*, *S. bulbocastanum*, *S. phureja* and *S. tuberosum*, respectively. (b) Pedigree of RH4X-103 derived from two quadruple hybrid ABPTs. The genotypes carrying resistance against *P. infestans* are indicated in bold.

with a 10 µl droplet of inoculum (Vleeshouwers et al. 1999). Leaves of plants were evaluated 3 and 5 days after inoculation.

P. infestans isolates 90128 (Race 1, 3, 4, 7, 8 and 11), which was kindly provided by Drs. F. Govers of Wageningen University, Wageningen, the Netherlands, USA618 (Race 1, 2, 3, 6, 7 and 11) by W. Fry of Cornell University, Ithaca, USA and IPO-82001 (Race 1, 2, 4, 5, 10 and 11) by W. Flier of Plant Research International, Wageningen, The Netherlands were used to determine the resistance. The isolates were cultured on rye sucrose agar medium (Caten and Jinks 1968) in the dark at 15 °C for 2 weeks. When mycelium was growing on the medium, it was rinsed with 5 ml ice-cold water for the release of the zoospores from sporangia. After incubating the suspension at 4 °C for 3 h, it was re-suspended to make an inoculum concentration of 5×10^5 zoospores ml^{-1}.

DNA isolation and AFLP analysis

DNA isolation was performed using a Retch protocol. Fresh leaf tissue was ground with two steel balls, in nuclear lysis buffer (0.2 M Tris–HCl, 0.05 M EDTA, 2 M NaCl, 2% CTAB, with an end pH of 7.5), DNA extraction buffer (100 mM Tris–HCl, 5 mM EDTA, 0.35 M Sorbitol, 20 mM NaBisulfite, with an end pH of 7.5) and 5% sarcosyl in 96 wells Costar plates (Corning Inc., Corning, NY, USA) using a Retsch machine (Retsch Inc., Haan, Germany). The ground leaf tissue was incubated at 65 °C for an hour followed by adding ice-cold chloroform: isoamyl alcohol (24:1). After centrifugation, the supernatant was transferred to new tubes. Adding isopropanol and another centrifugation allowed the precipitated DNA to be extracted. After the DNA pellet was dried, the DNA was dissolved in $T_{0.1}$E-buffer (+0.5 µg RNAse).

AFLP analysis was performed as described by Vos et al. (1995). Primary template DNA of 233 genotypes was prepared using the restriction enzymes *Eco*RI/*Mse*I and *Pst*I/*Mse*I combinations, ligated with adaptors fitting to the *Eco*RI, *Mse*I and *Pst*I sites, and diluted 10 times prior to the selective pre-amplification with single nucleotide extended primers. For the selective amplification, 11 E + 3/M + 3 primer combinations were selected based on their appearance in the Ultra High Density (UHD) Map (http://www.dpw.wau.nl/uhd/). Extra primer combinations were selected according to the results of a bulked segregant analysis (BSA) with 204 primer combinations as described by Michelmore et al. (1991) and six primer combinations from the *R2* map (Li et al. 1998).

In addition to this, AFLP marker analysis was performed to detect the wild species origin of the resistance in five different *Solanum* species as described by Li et al. (1998). The mobility of the AFLP markers, which are linked to the resistance locus, was compared.

Map construction

The AFLP markers inherited from the female parent were scored. Band intensity was discarded to use AFLP markers as dominant markers and only simplex-inherited markers [single-dose restriction fragments, (SDRFs); Wu et al. 1992]

with a simplex trait allele for the detection of linkage in the coupling phase were included in the data analysis. The linkage group containing the resistance locus was determined by JoinMap 2.0 (Stam 1993) and marker order by Record (van Os et al. 2000). The map distance was calculated based on the frequency of the recombination between AFLP markers.

AFLP marker and BAC clone analysis

Blasting of the sequences was performed on the web site http://www.ncbi.nlm.nih.gov/BLAST/. Genescan (http://bioweb.pasteur.fr/seqanal/interfaces/genscan.html; Burge and Karlin 1997) and Genemark (http://www.ebi.ac.uk/genemark/; Lukashin and Borodovsky 1998) were used to analyze the sequences of some BAC clones. ClustalX (Jeanmougin et al. 1998) was used to align sequences. For physical alignment of the resistance gene, the BAC library constructed from the wild species *S. bulbocastanum*, BGRC8005–8 (van der Vossen et al. 2003) was used and BAC-end sequences were performed at Greenomics (Wageningen, The Netherlands).

Marker nomenclature

AFLP markers were named with acronyms of enzyme combinations *Eco*RI/*Mse*I and *Pst*I/*Mse*I, which were used to prepare the template DNA, followed by three selective nucleotides and the size of each marker as described in reference autoradiograms created by Keygene NV, Wageningen, The Netherlands. Developed PCR markers, which were based on the sequence of a tomato BAC clone, were named with the original accession number followed by a defined part of the sequence, for instance right (R), left (L) and resistance gene analogue (RGA). Markers from potato BAC-end sequences were named with the plate, row and column number of the BAC library.

Results

Localization of the resistance locus

ABPT-derived clones were routinely inoculated with *P. infestans* during the breeding process and resistance was specifically noted in ABPT-30, ABPT-33, (ABPT30 × 33)-3, ABNA1–2, RH87-707-1 and 707TG11-1 (Figure 1). A tetraploid mapping population of 233 genotypes, which was generated by a cross between 707TG11-1 and RH89-039-16, was assessed for resistance to *P. infestans* isolate 90128. 707TG11-1 was assigned as resistant, RH89-039-16 as susceptible (Figure 2) and 83 and 90 offspring were unambiguously classified as resistant or susceptible, respectively. This 1:1 segregation ratio indicates a simplex inheritance of the resistance gene. The remaining 60 genotypes were tentatively classified, but excluded from the analysis for localization of the resistance locus because of less accurate observations and insufficient humidity during the incubation period.

To localize the resistance locus on the genetic linkage map of potato, 11 primer combinations

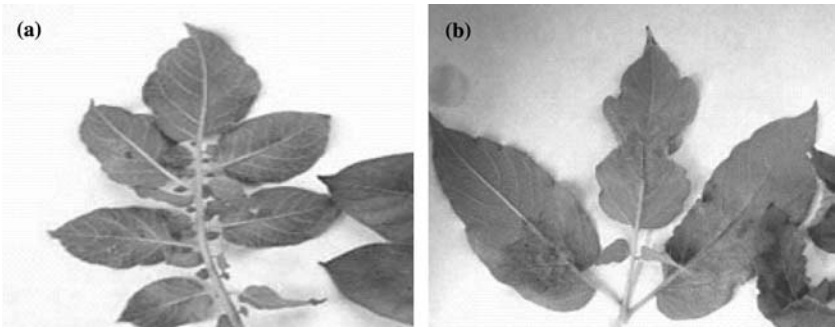

Figure 2. Detached leaf assay on resistant and susceptible parents of RH4X-103, 5 days after inoculation with *P. infestans* isolate 90128. (a) The resistant parent 707TG11-1 shows the hypersensitivity response. (b) The susceptible parent RH89-039-16 is heavily sporulating.

were selected from the UHD map. In total 1187 AFLP bands were observed in 707TG11-1 ranging between 79 and 164 per primer combination. 243 bands (20.5%) of these showed a polymorphism that was scorable with accuracy in the offspring. A subset of 191 marker loci showed a 1:1 segregation (simplex inherited markers) with the χ^2-test ($p < 0.05$). Those markers should be considered as SDRF's. In the first round of the JoinMap analysis, two of the 191 markers, i.e. EAAC/MCAG_101 and PAC/MAAT_498 were grouped with the *Rpi-abpt* locus.

To obtain additional AFLP markers with linkage to the *Rpi-abpt* locus, BSA was applied with 128 *Eco*/*Mse* and 76 *Pst*/*Mse* primer combinations using four samples: resistant parent (Pr), susceptible parent (Ps), resistant bulk (Br) and susceptible bulk (Bs). The bulks were composed of equal amounts of pre-amplified templates of eight resistant and eight susceptible genotypes as determined by the resistance assay. AFLP markers that were present in Pr and Br and absent in Ps and Bs were selected as candidate markers with putative linkage to the *Rpi-abpt* locus. Three and five markers from *Eco*/*Mse* and *Pst*/*Mse* primer combinations, respectively, were confirmed progressively to have more definite linkage to the *Rpi-abpt* locus by analyzing the 16 genotypes of Br and Bs individuals. An example is shown in Figure 3. This analysis resulted in an efficiency of determining one marker with linkage to the resistance locus per 25 AFLP primer combinations. In the second round of JoinMap analysis, two AFLP markers from 11 primer combinations and eight AFLP markers from the BSA were grouped with the *Rpi-abpt* locus.

One of the AFLP markers, PAT/MAGA_307, was converted into a CAPS marker Th2 (Foward: AGGATTTCAGTATGTCTCG and Reverse: TCCATTGTTGATTGCCCCT) to determine the chromosomal location of the *Rpi-abpt* locus. In the UHD mapping population, Th2 was localized on chromosome 4. *R2*, one of the *S. demissum*-derived *R* genes against *P. infestans*, was also mapped on chromosome 4 (Li et al. 1998). To confirm the chromosome number, six primer combinations selected from the *R2* map were tested on our mapping population of 233 genotypes and four *R2*-linked markers were grouped with the *Rpi-abpt* locus in the third round of JoinMap analysis. The marker order was determined by Record software (van Os et al. 2000) and genetic map distances were determined by the recombination frequencies between flanking markers. The linkage map containing 15 loci, i.e. 14 markers and the *Rpi-abpt* locus was constructed. Subsequently the map was merged with the high-resolution map in Figure 4.

Construction of a high-resolution map

From the genetic linkage map of *Rpi-abpt*, two flanking AFLP markers were selected for a large-scale recombinant analysis. The first one is EAGT/MCTT_247 mapping 3.4 cM telomeric from the *Rpi-abpt* locus and the other one is EAGA/MCAC_202 mapping 1.3 cM centromeric from the *Rpi-abpt* locus in the population with 233 genotypes. A total 1383 offspring of the population was screened with the two AFLP markers, and 98 recombinants were selected. This resulted in 7.1 cM genetic distance between the two AFLP markers. The 98 recombinants were screened with the AFLP markers located on and between EAGT/MCTT_247 and EAGA/MCAC_202 and tested for resistance to *P. infestans* isolate 90128.

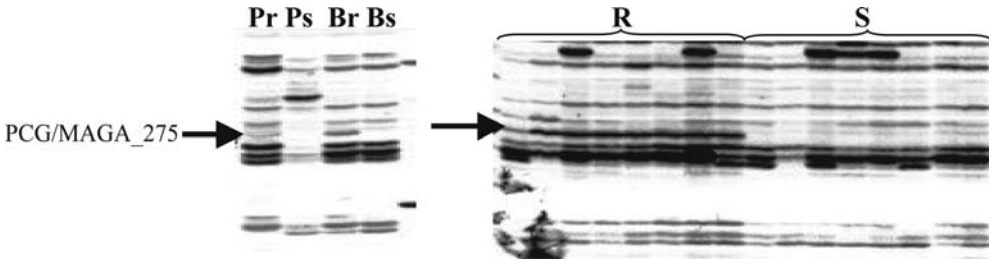

Figure 3. Part of an AFLP fingerprint showing a bulk-specific band associated with resistance using the PCG/MAGA primer combination. Pr, Ps, Br and Bs indicate the resistant parent, susceptible parent, resistant bulk and susceptible bulk, respectively. R and S represent resistant and susceptible individual genotypes of Br and Bs.

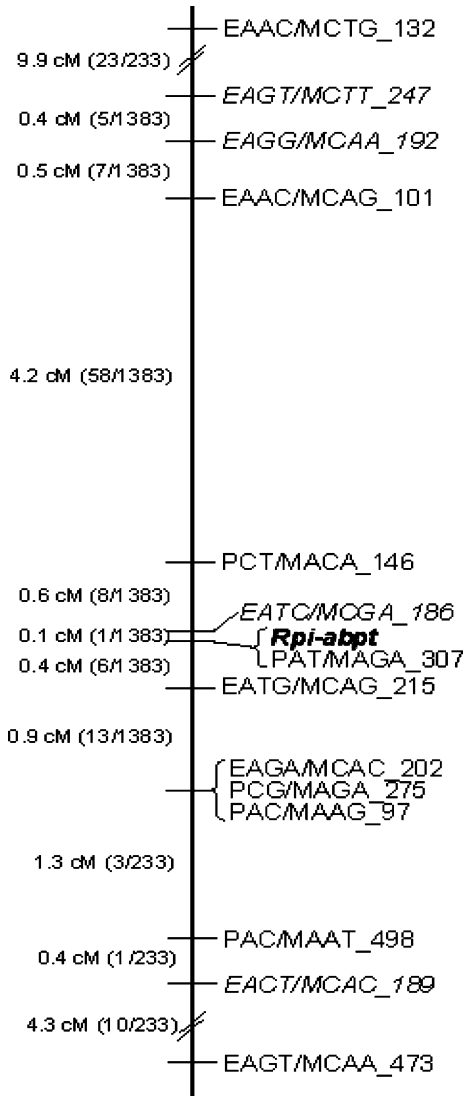

Figure 4. High-resolution map of *Rpi-abpt* on chromosome 4. The constructed map was based on 98 recombinants selected from the extended population of 1383 genotypes between the markers EAGT/MCTT_247 and EAGA/MCAC_202. The rest was based on the original map with 233 genotypes. The AFLP markers, which are also present in the map of *R2*, are indicated in italic. In the population of 86 genotypes, *R2* was co-segregating with EACT/MCAC_189 and was located 1 cM below EATC/MCGA_186 (Li et al. 1998). Genetic distance and the number of recombinants between markers are shown on the left side of the map.

The high-resolution map containing the resistance locus and six AFLP markers was constructed (Figure 4). The closest markers on both sides of the *Rpi-abpt* locus, EATC/MCGA_186 and EATG/MCAG_215, are separated with one recombinant in 0.1 cM and six recombinants in 0.4 cM, respectively. One AFLP marker PAT/MAGA_307 was co-segregating with the *Rpi-abpt* locus.

Analysis of the Rpi-abpt locus

AFLP marker PAT/MAGA_307 co-segregating with the *Rpi-abpt* locus was sequenced. A blast search revealed that the protein sequence of the marker showed a high degree of similarity to several well-known plant resistance proteins that belong to the NBS-LRR class. In addition, the DNA sequence of the marker showed high similarity with sequences of *Lycopersicon esculentum* BAC clone 127E11 (Gene back number: AF411807) derived from the tomato cultivar Heinz 1709 (van der Hoeven et al. 2002). This BAC has been localized between CT229A and TG370 at the short arm of the tomato chromosome 4 with a total length of 95,845 bp. By using Genescan and Genemark, 19 resistance gene analogues (RGA) were predicted in this clone. Three RGAs, i.e. Gene 4, 5 and 7 out of the 19 RGAs are homologous to disease resistance protein *RPP13* against *Peronospora parasitica* (Downy Mildew) in Arabidopsis (Bittner-Eddy et al. 1999). Marker PAT/MAGA_307 is highly similar to the 5′ end of these three RGAs of the tomato BAC clone (Figure 5b). The markers AF411807L and AF411807R of the tomato BAC clone were localized at 1.2 cM and 0.8 cM distance from the *Rpi-abpt* locus, respectively. Based on the consensus sequence of the three RGAs of the tomato BAC clone, a RGA marker was designed (Forward: CCT TTGTATCATTTGCAGTT and Reverse: ACATCCTCCATTCTTTCTT). Analysis of the RGA marker in the mapping population revealed that the RGA marker was also co-segregating with the *Rpi-abpt* locus and PAT/MAGA_307 (Figure 5a).

For physical alignment of resistance gene homologues, we screened the BAC library from *S. bulbocastanum* (van der Vossen et al. 2003) with PAT/MAGA_307 and the RGA marker. Several BACs contained these two markers. BAC clones 139K15 and 54I8 were selected to form a small contig (Figure 5a). The BAC-end sequence marker from 139K15L is also coding for a resistance gene homologue.

Identification of the wild species origin of the resistance

The wild *Solanum* accessions CGN17843, CGN20620, CGN21306, CGN17693, CGN17667 and CGN18301 that were involved in the pedigree of RH4X-103 were inoculated with *P. infestans* isolate 90128, using an *in vitro* inoculation assay (Huang 2005). All genotypes from *S. acaule* and *S. phureja* were heavily sporulating whereas all genotypes from *S. bulbocastanum* showed a hypersensitive response. These results suggest that *S. bulbocastanum* is the most likely donor species of the *Rpi-abpt* resistance.

To identify the wild species origin of the resistance gene *Rpi-abpt* at the molecular level, the 14 AFLP markers linked to the *Rpi-abpt* locus (Figure 4) were examined in the five *Solanum* species used in this study, i.e. *S. acaule, S. bulbocastanum*, *S. phureja*, *S. demissum* and *S. tuberosum* (Figure 6). Four to six genotypes of each accession or species were included in this experiment. PAT/MAGA_307 generated AFLP fragments with the same mobility in *S. bulbocastanum* as in 707TG11–1, but not in the other species. The AFLP markers PCT/MACA_146, PCG/MAGA_275, EAGA/MCAC_202 and PAC/MAAG_97 were identified in the fingerprints of both *S. acaule* and *S. bulbocastanum*. The AFLP markers EAAC/MCTG _132, EAAC/MCAG_101, PAC/MAAT_498 and EAGT/MCAA_473 that were more distant from *Rpi-abpt* were only identified in *S. acaule*, *S. tuberosum* or both. Also within species, variation between genotypes was found, which can be explained by the enormous genetic variation in outbreeding *Solanum* spp. (Hawkes 1990). Marker EATG/MCAG_215 was not identified in any of the *Solanum* species we tested possibly because of the same reason. None of the AFLP markers was detected in *S. phureja*. Two of the four *R2*-linked markers from *S. demissum* were also observed in *S. bulbocastanum* and another in *S. acaule* as well. The pattern of the 14 *Rpi-abpt*-linked AFLP markers distributed over the *Solanum* species revealed that the closest markers are present in *S. bulbocastanum* that supports our previous position that the introgression fragment containing *Rpi-abpt* is likely derived from this species.

The ABPT-derived *Rpi-abpt* is located at a similar position as *R2* in *S. demissum* (Figure 4). As described above, the *P.infestans* isolate 90128, which lacks virulence to *R2*, was unable to infect any plant carrying *Rpi-abpt*. To test whether the *Rpi-abpt* shares the same race-specificity as *R2*, the virulent *P.infestans* isolates USA618 and IPO82001 were inoculated on 55 genotypes of the segregating population RH4X-103. Both isolates showed an identical segregation for resistance as *P.infestans* isolate 90128 (data not shown). We conclude that *Rpi-abpt* functionally differs from *R2*, and thus represents a novel *R* gene to late blight.

Discussion

The mapping population RH4X-103 was derived from ABPT clones in which the wild species *S. acaule, S. bulbocastanum* and *S. phureja* were involved (Hermsen and Ramanna 1973). *S. bulbocastanum* is a well-known source of resistance to *Phytophthora infestans* (Umearus and Umearus 1994; Helgeson et al. 1998; van der Vossen et al. 2003, 2004) but unfortunately is not directly crossable with potato. In this study *S. bulbocastanum* was introgressed via ABPT bridge crosses (Hermsen and Ramanna 1973) and in the ABPT-derived mapping population RH4X-103, monogenic inheritance of *Rpi-abpt* was identified. Inoculation studies with a panel of *P.infestans* isolates revealed that the *Rpi-abpt* is a novel resistance gene with broad-spectrum resistance to all tested isolates. The definite origin of the *R* gene cannot unambiguously be determined, because the original breeding material has not been maintained during the entire process of ABPT breeding. However, several lines of research suggest that the resistance was obtained from *S. bulbocastanum*. First, the *Rpi-abpt* resistance can be traced back in the pedigree to ABPT material, and only *S. bulbocastanum* accessions were identified to contain resistance to late blight. Second, by applying a method previously described by Li et al. (1998), the mobility of AFLP markers linked to *Rpi-abpt* was examined in *S. acaule, S. bulbocastanum*, *S. phureja, S. tuberosum* and *S. demissum*. Except one marker which could not be amplified from any wild species, the six markers that were closest to *Rpi-abpt* showed an identical mobility in *S. bulbocastanum* accessions. The closest co-segregating marker was specific to *S. bulbocastanum*

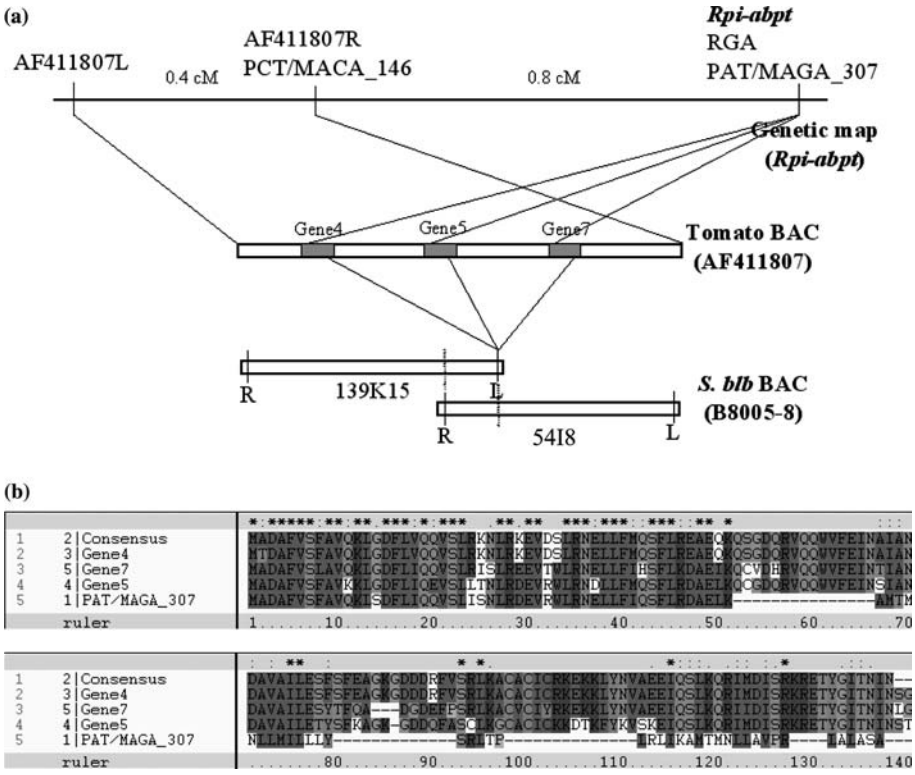

Figure 5. A physical alignment of the resistance gene *Rpi-abpt* and its homologues. (a) Graphical illustration of sequence homology among the ABPT-derived AFLP marker PAT/MAGA_307, tomato derived NBS-LRR sequences of gene4, gene5 and gene7 and *Solanum bulbocastanum* derived BAC-end sequences. GENESCAN predicted NBS-LRR genes in the tomato BAC clone with high homology to the *Rpi-abpt* candidate tagged by PAT/MAGA_307 marker. (b) Sequence alignment among the sequences of resistance gene analogues and PAT/MAGA_307 created by using CLUSTAL-X software.

only. Other markers were less specific and appeared to be conserved also on *S. acaule* or *S. demissum*. Although this indicates that this method is not providing ultimate proof about descent, the introgression fragment is most likely of *S. bulbocastanum* origin.

In the BSA analysis, eight markers linked to the *Rpi-abpt* locus in coupling phase were detected from 204 primer combinations. This observation is similar to other results in tetraploid potato. Li et al. (1998) detected 11 AFLP markers linked to *R2* from 205 primer combinations. BAS in diploid potato is more efficient than in tetraploid potato as Meksem et al. (1995) identified 29 bulk specific loci linked to *R1* from 108 primer combinations. The difference of the results between diploid and tetraploid levels can be explained by the fact that markers with linkage in repulsion cannot be used in tetraploids and that the composed bulks were based on the genotypes with RFLP markers linked to *R1* in the diploid.

Since the molecular isolation of genes based on their map location could be applicable to any gene whose phenotypic effect can be followed in a segregating population (Ballvora et al. 2001), a reliable map position of the *Rpi-abpt* locus was successfully determined using 1383 offspring. The *Rpi-abpt* locus was localized in a 0.5 cM interval between the closest flanking AFLP markers and co-segregated with one AFLP marker. Although the relationship between genetic and physical distances is known to vary considerably in potato (Marano et al. 2002), we expect that the physical distance between AFLP markers at the region of the *Rpi-abpt* locus is relatively small because of the resistance gene homology of the co-segregating AFLP marker and the RGA marker.

Loci	Distance	a	b	p	t	d
EAAC/MCTG_132	0	—			—	
EAGT/MCTT_247	9.9		—			—
EAGG/MCAA_192	10.3					—
EAAC/MCAG_101	10.8	—			—	
PCT/MACA_146	15.0	—	—			
EATC/MCGA_186	15.6		—			—
Rpi-abpt	15.7		.....			
PAT/MAGA_307	15.7		—			
EATG/MCAG_215	16.1					
EAGA/MCAC_202	17.0	—	—			
PCG/MAGA_275	17.0	—	—			
PAC/MAAG_97	17.0	—	—			
PAC/MAAT_498	18.3	—				
EACT/MCAC_189	18.7	—				—
EAGT/MCAA_473	23.0				—	

Figure 6. A representation of mobility of AFLP markers in various *Solanum* species. The symbol, '–', indicates marker presence with the same mobility on the AFLP gels in one or more genotypes within presented *Solanum* species and the dotted line indicates the expectation of the *Rpi-abpt* locus presence. Marker order and genetic distance are the same as those indicated in the high-resolution map. The AFLP markers, which are also present in the map of *R2*, are indicated in italic. 'a', 'b', 'p', 't' and 'd' represent *S. acaule*, *S. bulbocastanum*, *S. phureja*, *S. tuberosum* and *S. demissum*, respectively.

Comparative genomics between potato and tomato can facilitate the cloning of *R* genes (Gebhardt et al. 1991; Tanksley et al. 1992; van der Vossen et al. 2004; Huang 2005). The sequence of the co-segregating AFLP marker is highly similar with the sequence of one of the tomato BAC clones on chromosome 4 (van der Hoeven et al. 2002), which contains 19 predicted genes, some belonging to the NBS-LRR gene family. The translated protein of the sequence of the co-segregating AFLP marker is homologous to the resistance gene protein *RPP13* which belongs to the CC-NBS-LRR class in Arabidopsis (Bittner-Eddy et al. 1999) and is highly similar to RGA open reading frames of the tomato BAC clone. These results suggest that *Rpi-abpt* is a member of a resistance gene cluster. The construction of a BAC library of the resistant parent 707TG11-1 is in progress. The co-segregating and flanking AFLP markers are expected to facilitate the creation of a physical contig to span the *Rpi-abpt* locus in a few BAC walking steps. The results described in this paper predict that the novel resistance gene *Rpi-abpt* will be cloned soon.

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