MPMI Vol. 18, No. 7, 2005, pp. 722–729. DOI: 10.1094/MPMI-18-0722. © 2005 The American Phytopathological Society

The Late Blight Resistance Locus *Rpi-blb3* from *Solanum bulbocastanum* Belongs to a Major Late Blight *R* Gene Cluster on Chromosome 4 of Potato

Tae-Ho Park,[1] Jack Gros,[2] Anne Sikkema,[2] Vivianne G. A. A. Vleeshouwers,[1] Marielle Muskens,[2] Sjefke Allefs,[2] Evert Jacobsen,[1] Richard G. F. Visser,[1] and Edwin A. G. van der Vossen[3]

[1]Graduate School Experimental Plant Sciences, Laboratory of Plant Breeding, Department of Plant Sciences, Wageningen University, Binnenhaven 5, 6709 PD, Wageningen, The Netherlands; [2]Agrico Research, P.O. Box 40, 8300 AA, Emmeloord, The Netherlands; [3]Plant Research International BV, P.O. Box 16, 6700 AA, Wageningen, The Netherlands

Submitted 25 January 2005. Accepted 11 March 2005.

Late blight, caused by *Phytophthora infestans*, is one of the most devastating diseases in cultivated potato. Breeding of new potato cultivars with high levels of resistance to *P. infestans* is considered the most durable strategy for future potato cultivation. In this study, we report the identification of a new late-blight resistance (*R*) locus from the wild potato species *Solanum bulbocastanum*. Using several different approaches, a high-resolution genetic map of the new locus was generated, delimiting *Rpi-blb3* to a 0.93 cM interval on chromosome 4. One amplification fragment length polymorphism marker was identified that cosegregated in 1,396 progeny plants of an intraspecific mapping population with *Rpi-blb3*. For comparative genomics purposes, markers linked to *Rpi-blb3* were tested in mapping populations used to map the three other late-blight *R* loci *Rpi-abpt*, *R2*, and *R2-like* also to chromosome 4. Marker order and allelic conservation suggest that *Rpi-blb3, Rpi-abpt*, *R2*, and *R2-like* reside in the same *R* gene cluster on chromosome 4 and likely belong to the same gene family. Our findings provide novel insights in the evolution of *R* gene clusters conferring late-blight resistance in *Solanum* spp.

Additional keywords: BAC library, bulked segregant analysis, chromosome walking, synteny.

Late blight, caused by the oomycete *Phytophthora infestans*, is one of the most serious diseases in worldwide potato production. It was responsible for the Irish potato famine of the mid-19th century, resulting in the death of one million people. Although a lot of effort has been invested in controlling the pathogen, chemical control of *P. infestans* is still the main crop management strategy, but environmental safety is becoming more important and the pathogen is sometimes able to evolve chemical resistance (Goodwin et al. 1996). Therefore, introduction of resistance into modern potato varieties is the most durable strategy to control the disease.

In the last century, *Solanum demissum*, which is a hexaploid Mexican species, was extensively used in breeding for late-blight resistance in potato. Initially, a series of 11 *R* genes derived from *S. demissum* was described (Malcolmson and Black 1966; Mastenbroek 1953). Of these, *R1*, *R2*, *R3a/b*, *R6*, and *R7* have been localized on the genetic maps of potato (El-Kharbotly et al. 1994, 1996; Ewing et al. 2000; Huang et al. 2004; Leonards-Schippers et al. 1992; Li et al. 1998). However, these *R* genes confer race-specific resistance and those that were introgressed into potato varieties, mainly *R1*, *R2*, *R3*, *R4*, and *R10*, were quickly overcome by the pathogen (Wastie 1991). Hence, new sources for resistance are required, and currently, several other wild *Solanum* species have been reported as being potential sources of resistance (Jansky 2000). Of these, *S. microdontum* (Sandbrink et al. 2000) and *S. phureja* (Ghislain et al. 2001), conferring quantitative resistance, and *S. berthaultii* (Ewing et al. 2000) and *S. pinnatisectum* (Kuhl et al. 2001), conferring monogenic resistance, have been genetically characterized.

In addition, *S. bulbocastanum*, a self-incompatible diploid species from Mexico, is thought to be one of the most promising sources for late-blight resistance (Niederhausen and Mills 1953), despite its sexual incompatibility with *S. tuberosum* (Hermsen and De Boer 1971). Introduction of *S. bulbocastanum*-derived resistance has been achieved through interspecific bridge crosses between *S. bulbocastanum*, *S. acaule*, *S. phureja*, and *S. tuberosum* (Hermsen and Ramanna 1973), resulting in so-called ABPT material that is widely used for potato late-blight breeding. Additionally, Helgeson and associates (1998) generated somatic hybrids between *S. bulbocastanum* and cultivated potato. The somatic hybrids led to fertile plants that retained resistance and could be used for breeding. Molecular cloning of the genes responsible for resistance and subsequent introduction of the genes into potato varieties is a third method that circumvents many of the problems encountered in the previous two strategies.

To date, two *R* genes from *S. bulbocastanum* have been cloned, the allelic genes *RB* and *Rpi-blb1* on chromosome 8 (Song et al. 2003; van der Vossen et al. 2003) and *Rpi-blb2* on chromosome 6 (E. van der Vossen, *unpublished data*). *RB* was mapped using BC_2 populations derived from somatic hybrids (Helgeson et al. 1998; Naess et al. 2000), whereas *Rpi-blb1* was cloned using an intraspecific *S. bulbocastanum* F1 population (van der Vossen et al. 2003). *Rpi-blb2* was mapped both in an intraspecific F1 population and in tetraploid BC_2 and BC_3 populations derived from interspecific ABPT hybrids (E. van der Vossen, *unpublished data*).

Corresponding author: E. A. G. van der Vossen; Telephone: +31 317 485047; Fax +31 317 418094; E-mail: edwin.vandervossen@wur.nl

In this study, we have identified a third locus in *S. bulbocastanum*. The *Rpi-blb3* locus was mapped to an *R* gene hotspot on chromosome 4 in an intraspecific *S. bulbocastanum* BC_1 population. Furthermore, we show that a marker cosegregating with *Rpi-blb3* also cosegregates or is closely linked with late-blight resistance in three other mapping populations harboring the *Rpi-abpt*, *R2-like* and *R2* loci (Li et al. 1998; Park et al. 2005a and b).

RESULTS

Linkage analysis.

Segregation of late-blight resistance was initially analyzed in 40 BC_1 progeny plants derived from the *S. bulbocastanum* intraspecific BC_1 mapping population Blb00-20, in detached leaf assays with the complex *P. infestans* isolate IPO-655-2A. A clear 1:1 segregation ratio for late-blight resistance was observed, suggesting the presence of a dominant resistance allele at a single locus. To localize the *R* locus, we selected 10 clearly resistant and 10 clearly susceptible BC_1 genotypes as a core population and tested randomly selected markers of different chromosomes on these plants. The chromosome 4 marker GP180 (Tanksley et al. 1992) was found to be genetically linked to the resistance phenotype, indicating that the *R* locus was different from *Rpi-blb1* or *Rpi-blb2* (van der Vossen et al. 2003; E. van der Vossen, *unpublished data*). This novel locus was therefore designated *Rpi-blb3*. Further marker development showed the GP180 flanking cleaved amplified polymorphic sequence (CAPS) marker TG506R and the sequence-characterized amplified region (SCAR) marker CT229 (Table 1) to be proximal and distal to the *Rpi-blb3* locus, respectively (Fig. 1).

Table 1. Polymerase chain reaction (PCR)-based markers, primers, annealing temperature, and restriction enzymes used for the genetic mapping of *Rpi-blb3* in this study

Marker	Type	PCR primer (5′ to 3′)	Tm[a]	Enzyme[b]
Markers used for recombinant selection				
CT229	SCAR	F: TTGTGAGTGGTGAACTACGGGC R: CGGCAATGGTTATGGGAACG	65	a.s.
TG506R	CAPS	F: ATGCCAGCAGTCCAGTTTCC R: TTCCTTCCTGAAGTCAACCC	54	*Hha*I
Markers for high-resolution map				
cLPT5B19	CAPS	F: CCTTCATCAGATCTGGTCG R: CAGCTGTCACAATTGCCAAC	55	*Alu*I
AF411807L	CAPS	F: CGTTCCATGACATGTTCAAGC R: GCTCCAGAATCAATCTGAGG	56	*Dde*I
RGH1	CAPS	F: F: GGSAAGACCACTCTTGCAAG R: GGTTTTTAAGCTGCTAATGTTG	50	*Hpy*CH4IV
RGH2	SCAR	F: GGSAAGACCACTCTTGCAAG R: TGGTYATAATYACTCTGCTGC	50	a.s.
RGH3	CAPS	F: ATGRCTGATGCMTTTRTGTC R: CCYAAGTASAGAAAACACTGC	50	*Hae*III
AF411807R	CAPS	F: GCTAAGTTGTCTGAGGTTAG R: TGTTCTGGCTCTTCACAATC	48 to 54	*Alu*I & *Hae*III
TG370F	CAPS	F: TCGAAGCTCTGTTTCTGCTC R: CCCATGTTATGCCATTCAGC	59	*Hpa*II
T1430	CAPS	F: TCCTTTGTCATGGATATGCC R: CTGTGTAAAAGTAGAGCCAC	55	*Hpy*CH4IV
T1261	CAPS	F: CAATTCCCGTGAATCCTTCG R: GTACCAATAGCCCAAACAGG	55	*Hpy*CH4IV
TG339R	SCAR	F: ACTCTTTCGGCCTACAAGTC R: ATCCTTGTAGGACTCCTCTC	58	a.s.
P1433	CAPS	F: CACAGAACCATCAATGGCG R: ACCACTGAGAAATTGAGAGC	56	*Dpn*II
Markers for marker-saturated map				
B57R	CAPS	F: CCGTTTATGGTTCTTACCGC R: GTAGGGCATGAAGCATGATG	58	*Alu*I
54I8L	CAPS	F: GGTGTCTTGAGTATTGTCG R: CCACTTTTTCCTTTGCCTG	58	*Taq*I
54I8R	CAPS	F: GCTTCTTAATCTTAACAAAATA R: CATATACTGTTTTAAATGTCAC	52	*Sau*96I
139K15L	CAPS	F: GGTAAGAAAGAAGAGGAGAT R: CCTTTCCTACTGTCTTTCT	58	*Dde*I
154D15R	CAPS	F: CGAGGACGAGTGACATTTT R: CCATTTTGACCCATTTTCTC	58	*Aci*I
B10L	CAPS	F: TCAGAGGCATGACACCTGTG R: GTTGACCCAGTTGATTAAGTC	58	*Taq*I
20D11R	CAPS	F: AGCATCCGGAGGCAAATC R: TAGGCTTAACTGTCAAATGG	56	*Hinf*I
B175L	CAPS	F: GAAATGTGAACTACTCCAGG R: TCATTTGCCAATCCCTCACC	57	*Mse*I
28I12L	CAPS	F: AGCATCCGGAGGCAAATC R: TAGGCTTAACTGTCAAATGG	56	*Hpy*CH4IV
Marker cosegregating with *Rpi-blb3*				
Th21	SCAR	F: ATTCAAAATTCTAGTTCCGCC R: AACGGCAAAAAAGCACCAC	56	a.s.

[a] Annealing temperature. In AF411807R, annealing temperature was 48°C for the first 7 cycles and 54°C for the last 30 cycles.
[b] Restriction enzymes that reveal polymorphism between resistant and susceptible linked alleles of the marker. a.s. means allele-specific marker showing polymorphism without digestion.

High-resolution mapping.

To be able to construct a high-resolution genetic map of the *Rpi-blb3* locus, the initial Blb00-20 mapping population was extended to 1,400 genotypes and was screened with markers CT229 and TG506R. A total of 334 CT229/TG506R recombinants were identified and phenotyped for late-blight resistance, resulting in 183 resistant and 133 susceptible recombinants. The remaining 18 genotypes gave unclear phenotypes.

In an attempt to develop additional informative markers in the *Rpi-blb3* interval, several existing restriction fragment length polymorphism (RFLP) markers from the CT229-TG506R interval in tomato were targeted for polymerase chain reaction (PCR) marker development in the Blb00-20 mapping population (Table 1). Markers for which specific polymorphisms were identified between the parental genotypes of Blb00-20 were tested on the set of CT229-TG506R recombinants, delimiting the *Rpi-blb3* locus to a 4.5 cM genetic interval between markers TG370F and T1430 (Fig. 1). Interestingly, markers AF411807L and AF411807R, which were based on the bacterial artificial chromosome (BAC)-end sequences of the tomato BAC clone AF411807L (van der Hoeven et al. 2002), harboring several *R* gene analogs (RGA), were mapped distal to TG370F. In an attempt to identify RGA-specific markers within the *Rpi-blb3* interval, primers were designed on conserved regions of the RGA sequences on the tomato BAC and were used to develop RGA-specific CAPS markers in Blb00-20. Unfortunately, all the identified RGA markers also mapped distal to TG370F (Fig. 1).

To estimate the cM/kb ratio within the *Rpi-blb3* interval, the *S. bulbocastanum*-derived BAC library Blb8005-8, which was developed for the cloning of the *Rpi-blb1* gene (van der Vossen et al. 2003), was screened with the two closest markers TG370F and T1430. Both right and left end sequences of the selected BAC clones were used to develop PCR markers and to start a chromosome walk to the *Rpi-blb3* locus (Table 1). In this way the genetic interval harboring the *Rpi-blb3* gene was reduced to 2.6 cM (36 recombinants) between B10L and B175L (Fig. 2). Interestingly, several of the BAC end sequences, including B10L, were highly homologous to the RGA sequences present on the tomato BAC sequence AF411807 (van der Hoeven et al. 2002), indicating that the RGA cluster that was mapped distal to TG370F, extended into the *Rpi-blb3* interval.

As the physical map extended towards the *Rpi-blb3* locus, the efficiency of the chromosome walk was reduced. In an attempt to circumvent this problem, we decided to carry out a bulked segregant analysis (BSA) (Michelmore et al. 1991), in order to identify amplification fragment length polymorphism (AFLP) markers that closely flanked or fully cosegregated with resistance. Resistant and susceptible bulks were composed according to the graphical genotypes depicted in Figure 3A. Each bulk consisted of eight DNA samples derived from genotypes that had a recombination event within the relevant interval. In total, 256 *Eco*+3/*Mse*+3 and 256 *Pst*+2/*Mse*+3 primer combinations were tested on the bulks, 80 of which produced candidate markers putatively linked to resistance. Linkage to *Rpi-blb3* was confirmed by screening the individuals used to compose the bulks (Fig. 3B). Subsequently, 36 primer combinations were used to screen 63 recombinants from the TG370F

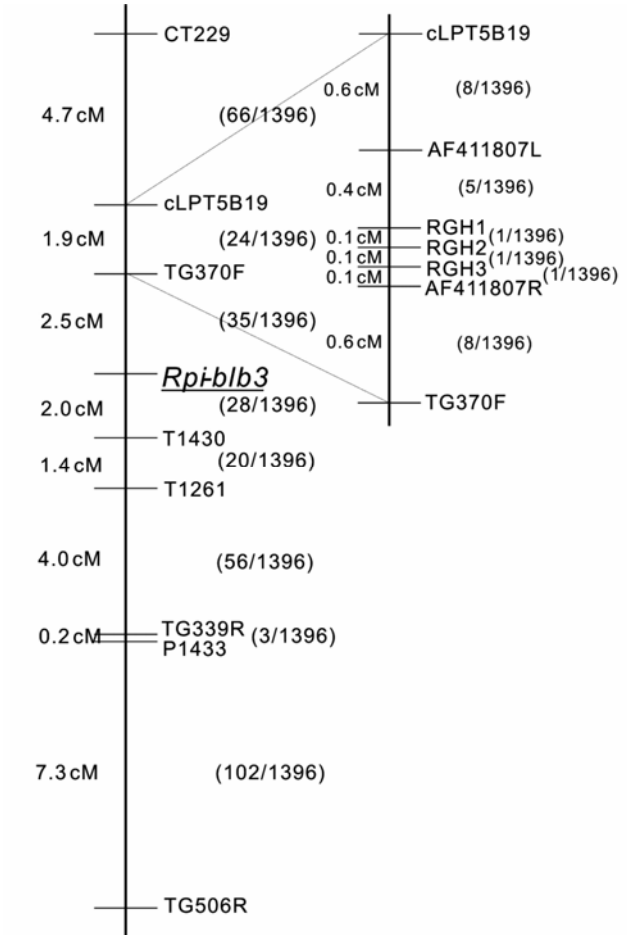

Fig. 1. High-resolution genetic linkage map of the *Rpi-blb3* locus on chromosome 4. Genetic distances and marker names are indicated on the left and right of the map, respectively. The number of recombinants is shown in brackets. The magnification shows the map between cLPT5B19 and TG370F where bacterial artificial chromosome–end sequence markers and resistance gene homolog markers from tomato were localized.

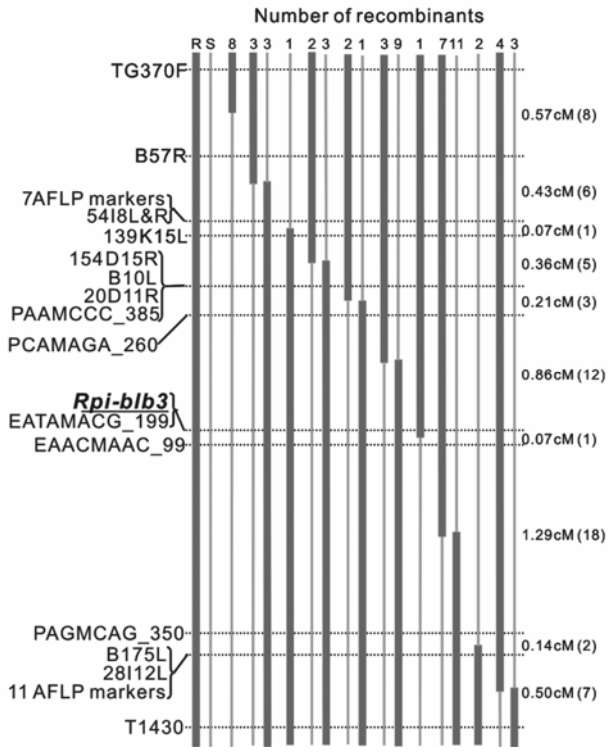

Fig. 2. Graphical genotypes of recombinants that define the *Rpi-blb3* interval. The presence and absence of the markers or resistance allele is indicated by solid bars and lines, respectively. The numbers of recombinants belonging to certain classes are presented at the top, and markers are shown on the left. Map distances between markers and the number of recombinants (brackets) are shown on the right.

and T1430 region, resulting in the identification of 23 informative AFLP markers. One of these markers, EATAMACG_199, fully cosegregated with resistance, and the interval harboring *Rpi-blb3* was reduced to 0.93 cM between markers PCAMAGA_260 and EAACMAAC_99 (Fig. 2). The cosegregating AFLP marker was successfully converted into SCAR marker Th21 (Table 1; Fig. 4).

Comparative genomics at the *Rpi-blb3* locus.

To study the syntenic relationship between the *Rpi-blb3* locus and the three late-blight *R* loci *Rpi-abpt* (Park et al. 2005a), *R2* (Li et al. 1998), and *R2-like* (Park et al. 2005b) that were mapped in different genetic backgrounds to the same genetic interval on chromosome 4 (Fig. 5), we screened each mapping population with the cosegregating AFLP markers developed in the other mapping studies. Interestingly, several markers segregated in more than one mapping population, facilitating the alignment of the four genetic maps (Fig. 5). AFLP marker EATA/MACG_199, which cosegregates with *Rpi-blb3* in 1,396 BC_1 genotypes, also cosegregated with resistance in the *R2* and *R2-like* backgrounds and was separated from *Rpi-abpt* by only one recombination event. These data suggest that *Rpi-blb3*, *Rpi-abpt*, *R2*, and *R2-like* are members of the same *R* gene cluster on chromosome 4 and may be allelic.

DISCUSSION

In this study, we have identified and genetically characterized a new late-blight *R* locus in *S. bulbocastanum*. Initially, CAPS markers developed from chromosome-specific RFLP markers (Meksem et al. 1995; Tanksley et al. 1992) were used to determine the chromosomal position of a dominant late-blight *R* locus that segregated in an intraspecific *S. bulbocastanum* BC_1 population. Linkage to the chromosome 4-specific markers CT229 and TG506 indicated that the *R* locus was different from *Rpi-blb1* and *Rpi-blb2*, located on chromosomes 8 and 6, respectively (van der Vossen et al. 2003; E. van der Vossen, *unpublished data*). This new locus was therefore designated *Rpi-blb3*. A recombinant analysis of 1,396 BC_1 progeny plants with CT229 and TG506 identified a total of 334 recombinants, which were subsequently used to genetically fine-map the *Rpi-blb3* locus, a prerequisite for future positional cloning of the gene.

Marker saturation of any genetic interval can be achieved by several methods (Meksem et al. 1995), four of which were used in this study in an attempt to saturate the *Rpi-blb3* interval: i) CAPS marker development from published RFLP markers (Table 1), ii) the RGA candidate gene approach (Table 1B), iii) BAC chromosome walking (Table 1C), and iv) BSA. Initially, several PCR-based markers were successfully converted from RFLP markers and were genetically mapped between CT229 and TG506 (Fig. 1). The marker order was consistent with that of previously published tomato and potato maps (Tanksley et al. 1992).

The majority of *R* genes characterized to date belong to the nucleotide binding site-leucine rich repeat (NBS-LRR) class (Dangl and Jones 2001). This class of genes contains several conserved motifs within the NBS that can be targeted for candidate gene marker development, resulting in the amplification of RGA. As *R* genes are often part of complex loci (Hulbert et al. 2001), RGA-specific marker strategies often lead to *R* gene cluster landing. This approach has successfully been used in wheat (Yan et al. 2003), barley (Madsen et al. 2003), and sugar beet (Hunger et al. 2003), but also in potato (Leister et al. 1996; Paal et al. 2004). In this study, we specifically targeted RGA sequences from the tomato BAC clone AF411807 (van der Hoeven et al. 2002) for RGA-specific marker development at the *Rpi-blb3* locus. This BAC clone was identified through BLAST analysis of a cloned AFLP fragment that was linked to the syntenous late-blight *R* locus *Rpi-abpt* (Park et al. 2005a). Unfortunately, this did not lead to the identification of a cosegregating RGA-specific marker, suggesting either that *Rpi-blb3* does not belong to the same NBS-LRR gene family as the RGAs on the tomato BAC or that the degree of RGA polymorphism at the *Rpi-blb3* locus is reduced.

Subsequently, a transgenotype BAC chromosome walk was initiated. The most closely linked CAPS markers were used to screen an existing *S. bulbocastanum*-derived BAC library that was previously used to clone *Rpi-blb1* (van der Vossen et al. 2003). Although this BAC library did not contain *Rpi-blb3*, we expected the allelic variation within *S. bulbocastanum* to be lower than that between *S. bulbocastanum* and *S. tuberosum*, of which there are also BAC libraries available (Ballvora et al. 2002; Paal et al. 2004; Rouppe van der Voort et al. 1999). Several positive BAC clones were indeed identified, the BAC end sequences of which were used to develop markers more closely linked to *Rpi-blb3*. However, progress was very slow, mainly due to the fact that the level of polymorphism decreased as we approached the *Rpi-blb3* gene. In addition, the different origin of the BAC library did not facilitate the mapping of repetitive sequences. Although some BAC markers were polymorphic in the BC_1 mapping population, often we were unable to verify the BAC origin of the polymorphic allele, as the BAC-specific allele was not present in the parental genotypes of the BC_1 mapping population.

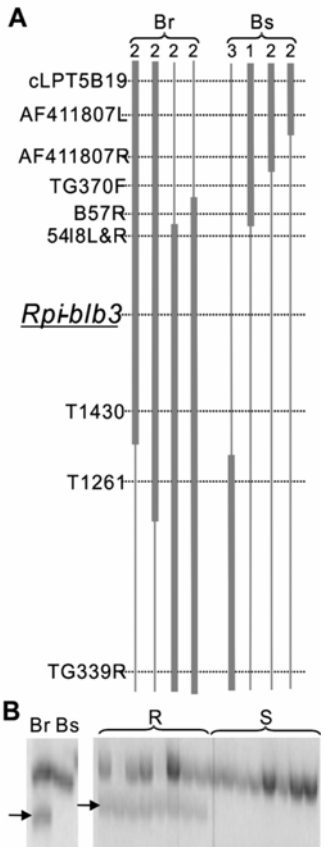

Fig. 3. Bulked segregant analysis (BSA). **A,** Graphical genotypes of individuals composing the resistant bulk (Br) or the susceptible bulk (Bs). The presence and absence of marker alleles is indicated by solid bars and lines, respectively. The number of different genotypes for each bulk is shown at the top. **B,** Gel images of an example of an amplification fragment length polymorphism marker identified by the BSA method. Eight resistant genotypes (R) and eight susceptible genotypes (S) were checked individually. The marker band is indicated by the arrow.

BSA (Michelmore et al. 1991) in combination with the AFLP technique (Vos et al. 1995) is a powerful tool for marker enrichment in a given region of a plant genome (Ballvora et al. 1995; Meksem et al. 1995; Thomas et al. 1995). To make the BSA more efficient and to focus on the *Rpi-blb3* interval, we designed the resistant and susceptible bulks based on informative recombinants from the *Rpi-blb3* interval (Fig. 3). This resulted in the identification of 28 AFLP markers within the B57R-T1430 interval (Fig. 2) and the mapping of the *Rpi-blb3* locus to a 0.93 cM interval that is flanked by the AFLP markers PCAMAGA_260 and EAACMAAC_99. In addition, AFLP marker EATAMACG_199 was identified to cosegregate with the *Rpi-blb3* locus. Although AFLP analysis is a powerful technique, the markers are costly and technologically demanding. Conversion to simple PCR-based markers is, therefore, necessary for use in genotype screening and progeny selection. In this respect, we succeeded in converting the cosegregating AFLP marker EATAMACG_199 into SCAR marker Th21 (Table 1; Fig. 4), which can be used for breeding applications and for BAC landing.

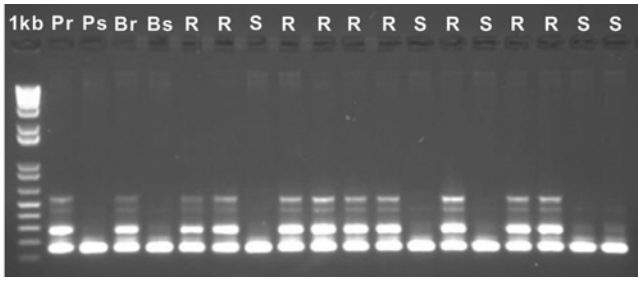

Fig. 4. Polymerase chain reaction marker (Th21) developed from the sequence of the amplification fragment length polymorphism marker (EATAMACG_199) cosegregating with the *Rpi-blb3* locus. A marker-size ladder (1 kb) is indicated. Pr, Ps, Br, Bs, R, and S indicate the resistant parent, susceptible parent, resistant bulk, susceptible bulk, resistant offspring, and susceptible offspring, respectively.

R genes and RGAs have been shown to be clustered in the genomes of many different species (Meyers et al. 1998; Michelmore and Meyers 1998), including members of the family Solanaceae (Grube et al. 2000). At least five *R* genes against diverse pathogens have been mapped in different genetic backgrounds to the GP21-GP179 interval on chromosome 5: *Gpa* and *Grp1* conferring resistance to potato cyst nematodes (Kreike et al. 1994; Rouppe van der Voort et al. 1998), *Nb* and *Rx2* conferring resistance to *Potato virus X* (de Jong et al. 1997; Ritter et al. 1991), and *R1* conferring resistance to *P. infestans* (Leonards-Schippers et al. 1992). *Gro1.3*, conferring resistance to cyst nematodes (Kreike at al. 1993), and *R3*, *R6*, and *R7*, conferring resistance to *P. infestans* (El-Kharbotly et al. 1994, 1996), cluster on the short arm of chromosome 11. In the current study, we have identified a major cluster on chromosome 4, to which several *R* genes conferring resistance to *P. infestans* were mapped. Marker order and allelic conservation were observed, facilitating the alignment of the genetic maps of the different *R* genes, *Rpi-blb3* (this study), *Rpi-abpt* (Park et al. 2005a), *R2-like* (Park et al. 2005b), and *R2* (Li et al. 1998), with AFLP marker EATA/MACG_199 (Fig. 5). Clearly, the short arm of chromosome 4 is also a hotspot for resistance, harboring several distinct *R* gene clusters with resistance specificities to different pathogens. The recently cloned root knot nematode *R* gene *Hero* (Ganal et al. 1995) is part of an extensive *R* gene cluster that is located distal to *Rpi-blb3*. The cyst nematode *R* locus *Gpa4* (Bradshaw et al. 1998), the virus *R* locus Ny_{tbr} (Celebi-Toprak et al. 2002), and quantitative trait loci for resistance to late blight (Leonards-Schippers et al. 1994; Oberhagemann et al. 1999; Sandbrink et al. 2000) and Erwinia carotovora subsp. atroseptica (Zimnoch-Guzowska et al. 2000) are also located on the short arm of chromosome 4.

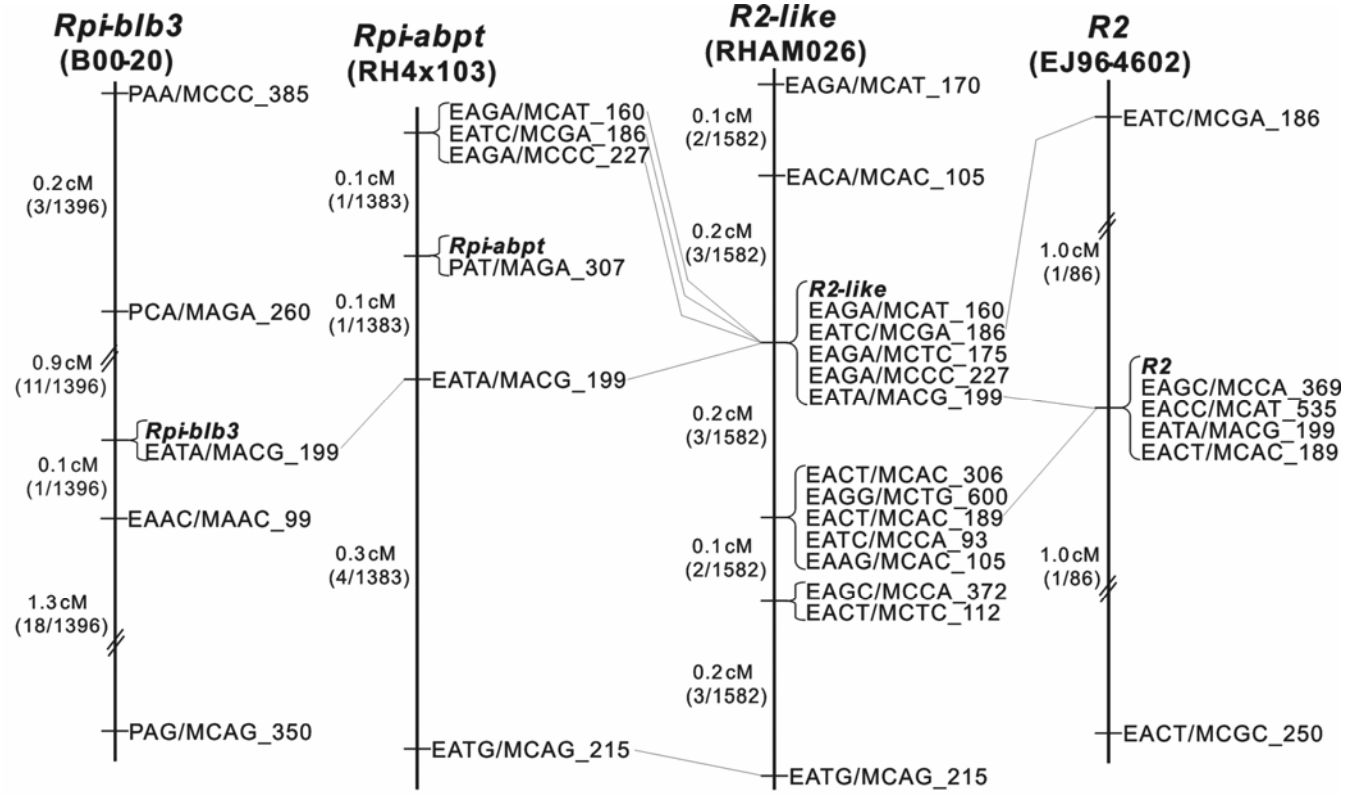

Fig. 5. Integrated genetic linkage map of the different late-blight resistance loci *Rpi-blb3*, *Rpi-abpt* (Park et al. 2005a), *R2-like* (Park et al. 2005b), and *R2* (Li et al. 1998) on chromosome 4.

Rpi-abpt and *Rpi-blb3* confer resistance to *P. infestans* isolates with complex race specificities (Park et al. 2005a). Although *Rpi-blb3* was tested with only two isolates, it displays a different resistance reaction to one of the two tested isolates, as compared with *Rpi-abpt. Rpi-blb3* confers a partial resistance to isolate IPO-82001 (data not shown), whereas *Rpi-abpt* confers complete resistance to this isolate (Park et al. 2005a). Also, the wild species accessions and the geographical regions from which the above two genes originate are different from each other. *Rpi-blb3* is derived from *S. bulbosatanum* subsp. *dolichophyllum* (CGN17688), whereas *Rpi-abpt* is derived from *S. bulbocastanum* subsp. *bulbocastanum* (CGN17693 or CGN21306). These findings suggest that they are distinct genes. On the other hand, the *R2-like* locus displays the same specificity as the *R2* locus from *S. demissum* (Li et al. 1998). The introgression fragment harboring the *R2-like* locus could be derived from either *S. edinense*, *S. tuberosum* subsp. *andigena*, or *S. vernei*, which are all present in the pedigree of the mapping population (Park et al. 2005b). Interestingly, *S. edinense* is a natural hybrid between *S. tuberosum* and *S. demissum,* suggesting that *R2* and the *R2-like* loci may, in fact, have originated from the same gene pool. Alternatively, syntenic loci in different wild *Solanum* species may harbor genes that display the same resistance specificity without sharing a common origin.

Recently, comparative genomics between potato and tomato facilitated the isolation of the late-blight *R* genes *R3a* and *Rpi-blb2* from potato, as these genes mapped to regions of the potato genome that were syntenic to the *I2* and *Mi* loci in tomato, respectively (Huang et al. 2005; E. van der Vossen, *unpublished data*). The synteny of markers and sequences at the late-blight *R* gene hotspot on chromosome 4 will facilitate the cloning of the genes *Rpi-blb3*, *Rpi-abpt*, *R2*, and *R2-like*. Recently, Huang (2005) demonstrated that eight (*R3*, and *R5* through *R11*) of the eleven classical late-blight resistance specificities characterized so far (Black et al. 1953) are either allelic versions or duplicated members of the same *R* gene (super) cluster on the distal region of the short arm of chromosome 11. Furthermore, it was demonstrated that the *R3* specificity is not based on a single *R* gene but on the joint effect of two *R* genes, currently named *R3a* and *R3b* (Huang et al. 2004). These findings provide novel insights in the evolution of *R* gene clusters conferring late-blight resistance in *Solanum* spp. The prevailing hypotheses on the evolution of *R* gene clusters include unequal crossover, which can result in expanding numbers of paralogs with novel specificities (Hulbert et al. 2001). These observations are mainly based on studies in selfing organisms (Anderson et al. 1997; Ellis et al. 1999; Parker et al. 1997; Parniske et al. 1997). The genus *Solanum* harbors both outbreeding and polyploid species. In the latter case, allelic variation is higher. Not only can additional paralogs acquire novel specificities, but also, the homeologous chromosomes of the different genomes within the disomic pairing hexaploid *S. demissum* or the heterozygosity in outbreeders can accommodate additional *R* gene-specificities. Our data and that of Huang (2005) demonstrate that this, indeed, has occurred in *Solanum* spp. in both the chromosome 4 and chromosome 11 *R* gene clusters.

MATERIALS AND METHODS

Plant material.

The interspecific *S. bulbocastanum* mapping population Blb00-20 was developed by crossing a *P. infestans*-resistant clone Blb99-256-3 with the susceptible clone Blb48-5. The resistant parental clone Blb99-256-3 was obtained from a cross between a resistant clone from accession BGRC7999 and the susceptible clone Blb48-5. The susceptible parental clone Blb48-5 was derived from a cross between two susceptible clones from the *S. bulbocastanum* accessions BGRC8005 and BGRC8006.

Resistance assay.

Detached leaf assays were used to determine the resistance phenotypes of BC_1 progeny plants. Two complex isolates, IPO-655-2A (race 1.3.4.7.8.10.11) and IPO-82001 (race 1.2.4.5.10.11), which were provided by W. Flier of Plant Research International, Wageningen, The Netherlands, were used for the resistance assays. Both are A2 type, and they originate from The Netherlands and Belgium, respectively. Inoculum preparation and inoculation were performed as described by Vleeshouwers and associates (1999). Six days after inoculation, plant phenotypes were determined. Leaves showing no symptoms or a localized necrosis at the point of inoculation were scored as resistant and those with clear sporulating lesions as susceptible.

DNA isolation and marker development.

Genomic DNA was isolated from young leaf tissue according to Bendahmane and associates (1997). PCR markers were created using primers designed on specific tomato or potato genomic DNA sequences, BAC end sequences, or cloned AFLP fragments. An overview of the PCR-based markers used to construct the high-resolution map is presented in Table 1.

AFLP analysis was performed as described by Vos and associates (1995). Primary templates were prepared using the restriction enzyme *Eco*RI/*Mse*I and *Pst*I/*Mse*I combinations. BSA (Michelmore et al. 1991) was carried out by performing AFLP analysis on resistant and susceptible bulks comprising secondary template of eight resistant and susceptible genotypes. The bulks were screened with 256 *Eco*+3/*Mse*+3 and 256 *Pst*+2/*Mse*+3 primer combinations.

Marker names include original marker names from public databases, the original accession number of a tomato BAC clone followed by L (for left) or R (for right) and RGH (resistance gene homolog). Microtiter plate, row, and column numbers of the potato BAC library 8005-8 (van der Vossen et al. 2003) were used for BAC end sequence markers. AFLP markers were named by acronyms of enzyme combinations (*Eco*RI/*Mse*I and *Pst*I/*Mse*I) used to prepare the template DNA followed by three or two selective nucleotides and the size of each marker, as described in reference autoradiograms created by Keygene NV, Wageningen, The Netherlands.

Map construction.

Genetic linkage maps were constructed according to recombination frequencies between marker loci and or the *Rpi-blb3* locus. Joinmap software was used to determine linkage groups and to select the *Rpi-blb3*-linked markers (Stam 1993). Map distances were recalculated following marker order determination using Record (van Os et al. 2000).

LITERATURE CITED

Anderson, P. A., Lawrence, G. J., Morrish, B. C., Ayliffe, M. A., Finnegan, E. J., and Ellis, J. G. 1997. Inactivation of the flax rust resistance gene *M* associated with loss of a repeated unit within the leucine-rich repeat coding region. Plant Cell 9:641-651.

Ballvora, A., Hesselbach, J., Niewóhner, J., Leister, D., Salamini, F., and Gebhardt, C. 1995. Marker enrichment and high-resolution map of the segment of potato chromosome VII harbouring the nematode resistance gene *Gro1*. Mol. Gen. Genet. 249:82-90.

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

Bendahmane, A., Kanyuke, K., and Baulcombe, D. C. 1997. High-resolution genetical and physical mapping of the *Rx* gene for extreme resistance to potato virus X in tetraploid potato. Theor. Appl. Genet. 95:153-162.

Black, W., Mastenbroek, C., Mills, W. R., and Peterson, L. C. 1953. A proposal for an international nomenclature of races of *Phytophthora infestans* and of genes controlling immunity in *Solanum demissum* derivatives. Euphytica 2:173-240.

Bradshaw, J. E., Hackett, C. A., Meyer, R. C., Milbourne, D., McNicol, J. W., Phillips, M. S., and Waugh, R. 1998. Identification of AFLP and SSR markers associated with quantitative resistance to *Globodera pallida* (Stone) in tetraploid potato (*Solanum tuberosum* subsp. *tuberosum*) with a view to marker-assisted selection. Theor. Appl. Genet. 97:202-210.

Celebi-Toprak, F., Slack, S. A., and Jahn, M. M. 2002. A new gene, Ny_{tbr}, for hypersensitivity to Potato virus Y from *Solanum tuberosum* maps to chromosome IV. Theor. Appl. Genet. 104:669-674.

Dangl, J. L., and Jones, J. D. G. 2001. Plant pathogens and integrated defence responses to infection. Nature 411:826-833.

de Jong, W., Forsyth, A., Leister, D., Gebhardt, C., and Baulcombe, D. C. 1997. A potato hypersensitive resistance gene against potato virus X maps to a resistance gene cluster on chromosome 5. Theor. Appl. Genet. 95:246-252.

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

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

Ellis, J. G., Lawrence, G. J., Luck, J. E., and Dodds, P. N. 1999. Identification of regions in alleles of the flax rust resistance gene *L* that determine differences in gene-for-gene specificity. Plant Cell 11:495-506.

Ewing, E. E., Simko, I., Smart, C. D., Bonierbale, M. W., Mizubuti, E. S. G., May, G. D., and Fry, W. E. 2000. Genetic mapping from field of qualitative and quantitative resistance to *Phytophthora infestans* in a population derived from *Solanum tuberosum* and *Solanum berthaultii*. Mol. Breeding 6:25-36.

Ganal, M. W., Simon, R., Brommonschenkel, S., Arndt, M., Phillips, M. S., Tanksley, S. D., and Kumar, A. 1995. Genetic mapping of a wide spectrum nematode resistance gene (*Hero*) against *Globodera rostochiensis* in tomato. Mol. Plant-Microbe Interact. 8:886-891

Ghislain, M., Trognitz, B., Gerrera, Ma. Del R., Solis, J., Casallo, G., Vásquez, C., Hurtade, O., Castillo, R., Portal, L., and Orrillo, M. 2001. Genetic loci associated with field resistance to late blight in offspring of *Solanum phureja* and *S. tubersosum* grown under short-day conditions. Theor. Appl. Genet. 103:433-442.

Goodwin, S. B., Sujkowski, L. S., and Fry, W. E. 1996. Widespread distribution and probable origin of resistance to metalaxyl in clonal genotypes of *Phytophthora infestans* in the United States and western Canada. Phytopathology 86:793-800.

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

Helgeson, J. P., Pohlman, J. D., Austin, S., Haberlach, G. T., Wielgus, S. M., Ronis, D., Zambolim, L., Tooley, P., McGrath, J. M., James, R. V., and Stevenson, W. R. 1998. Somatic hybrids between *Solanum bulbocastanum* and potato: A new source of resistance to late blight. Theor Appl Genet 96:738-742.

Hermsen, J. G. T., and De Boer, A. J. E. 1971. The effect of colchicine treatment on *Solanum acaule* and *S. bulbocastanum*: A complete analysis of ploidy chimerasin *S. bulbocastanum*. Euphytica 20:171-180.

Hermsen, J. G. T. H., and Ramanna, M. S. 1973. Double-bridge hybrids of *Solanum bulbocastanum* and cultivars of *Solanum tuberosum*. Euphytica 22:457-466.

Huang, S. 2005. The discovery and characterization of the major late blight resistance complex in potato: Genomic structure, functional diversity and implications. Ph.D. thesis, Wageningen University. The Netherlands.

Huang, S., Vleeshouwers, V. G. A. A., Werij, J. S., Hutten, R. C. B., van Eck, H. J., Visser, R. G. F., and Jacobsen, E. 2004. The *R3* resistance to *Phytophthora infestans* in potato is conferred by two closely linked *R* genes with distinct specificities. Mol. Plant-Microbe Interact. 17:428-435.

Huang, S., van der Vossen, E., Kuang, H., Vleeshouwers, V., Zhang, N., Borm, T. J. A., van Eck, H. J., Baker, B., Jacobsen, E., and Visser, R. G. F. 2005. Comparative genomics enabled the isolation of the *R3a* late blight resistance gene in potato. Plant J. in press.

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

Hunger, S., Gaspero, G. D., Möhring, S., Bellin, D., Schäfer-pregl, R., Borchardt, D. C., Durel, C. E., Werber, M., Weisshaar, B., Salamini, F., and Schneider, K. 2003. Isolation and linkage analysis of expressed disease-resistance gene analogues of suger beet (*Beta vulgaris* L.). Genome 46:70-82.

Jansky, S. 2001. Breeding for disease resistance in potato. P. Breeding Rev. 19:69-155.

Kreike, C. M., De Koning, J. R. A., Vinke, J. H., Van Ooijen, J. W., Gebhardt, C., and Stiekema, W. J. 1993. Mapping of loci involved in quantitatively inherited resistance to the potato cyst-nematode *Globodera rostochiensis* pathotype *Ro1*. Theor. Appl. Genet. 87:464-470.

Kreike, C. M., De Koning, J. R. A., Vinke, J. H., van Ooijen, J. W., and Stiekema, W. J. 1994. Quantitatively-inherited resistance to *Globodera pallida* is dominated by one major locus in *Solanum spegazzinii*. Theor. Appl. Genet. 88:764-769.

Kuhl, J. C., Hanneman, R.E., Jr., and Havey, M. J. 2001. Characterization and mapping of *Rpi1*, a late-blight resistance locus from diploid (1EBN) Mexican *Solanum pinnatisectum*. Mol. Genet. Genomics 265:977-985.

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

Leonards-Schippers, C., Gieffers, W., Salamini, F., and Gebhardt, C. 1992. The *R1* gene conferring race-specific resistance to *Phytophthora infestans* in potato is located on potato chromosome V. Mol. Gen. Genet. 233:278-283.

Leonards-Schippers, C., Gieffers, W., Schafer-Pregl, R., Ritter, E., Knapp, S. J., Salamini, F., and Gebhardt, C. 1994. Quantitative resistance to *Phytophthora infestans* in potato: A case study for QTL mapping in an allogamous plant species. Genetics 137:67-77.

Li, X., Van Eck, H. J., Rouppe van der Voort, J. N. A. M., Huigen, D. J., Stam, P., and Jacobsen, E. 1998. Autotetraploids and genetic mapping using common AFLP markers: The *R2* allele conferring resistance to *Phytophthora infestans* mapped on potato chromosome 4. Theor. Appl. Genet. 96:1121-1128.

Madsen, L. H., Collins, N. C., Rakwalska, M., Backes, G., Sandal, N., Krusell, L., Jensen, J., Waterman, E. H., Jahoor, A., Ayliffe, M., Pryor, A. J., Langridge, P., Schulze-Lefert, P., and Stougaard, J. 2003. Barley disease resistance gene analogs of the NBS-LRR class: Identification and mapping. Mol. Gen. Genomics 269:150-161.

Malcolmson, J. F., and Black, W. 1966. New *R* genes in *Solanum demissum* Lindl. And their complementary races of *Phytophthora infestans* (Mont.) de Bary. Euphytica 15:199-203.

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

Meksem, K., Leister, D., Peleman, J., Salamini, F., and Gebhardt, C. 1995. A high-resolution map of the vicinity of the *R1* locus on chromosome V of potato based on RFLP and AFLP markers. Mol. Gen. Genet. 249:74-81.

Meyers, B. C., Chin, D. B., Shen, K. A., Sivaramakrishnam, S., Lavelle, D. O., Zhang, Z., and Michelmore, R. W. 1998. The major resistance gene cluster in lettuce is highly duplicated and spans several megabases. Plant Cell 10:1817-1832.

Michelmore, R. W., Paran, I., and Kesseli, R. V. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. U.S.A. 88:9828-9832.

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

Naess, S. K., Bradeen, J. M., Wielgus, S. M., Haberlach, G. T., McGrath, J. M., and Helgeson, J. P. 2000. Resistance to late blight in *Solanum bulbocastanum* is mapped to chromosome 8. Theor. Appl. Genet. 101:697-704.

Niederhausen, J. S., and Mills, W. R. 1953. Resistance of *Solanum* species to *Phytophthora infestans* in Mexico. Phytopathology 43:456-457.

Oberhagemann, P., Chatot-Balandras, C., Schafer-Pregl, R., Wegener, D., Palomino, C., Salamini, F., Bonnel, E., and Gebhardt, C. 1999. A genetic analysis of quantitative resistance to late blight in potato: Towards marker-assisted selection. Mol. Breeding 5:399-415.

Paal, J., Henselewski, H., Muth, J., Meksem, K., Menendez, C. M., Salamini, F., Ballvora, A., and Gebhardt, C. 2004. Molecular cloning of the potato *Gro1-4* gene conferring resistance to pathotype Ro1 of the root cyst nematode *Globodera rostochiensis*, based on a candidate gene approach. Plant J. 38:285-297.

Park, T. H., Vleeshouwers, V. G. A. A., Hutten, R. C. B., van Eck, H. J., van der Vossen, E., Jacobsen, E., and Visser, R. G. F. 2005a. High-resolution mapping and analysis of the resistance locus *Rpi-abpt* against *Phytophthora infestans* in potato. Mol. Breeding, in press.

Park, T. H., Vleeshouwers, V. G. A. A., Huigen, D., van der Vossen, E. A.G., van Eck, H. J., and Visser, R. G. F. 2005b. Characterization and high-resolution mapping of a late blight resistance locus similar to R2. Theor. Appl. Genet., in press

Parker, J. E., Coleman, M. J., Szabò, V., Frost, L. N., Schmidt, R., van der Biezen, E. A., Moores, T., Dean, C., Daniels, M. J., and Jones, J. D. G. 1997. The Arabidopsis downy mildew resistance gene *RPP5* shares similarity to the toll and interleukin-1 receptors with *N* and *L6*. Plant Cell 9:879-894.

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

Ritter, E., Debener, T., Barone, A., Salamini, F., and Gebhardt, C. 1991. RFLP mapping on potato chromosomes of two genes controlling extreme resistance to potato virus X (PVX). Mol. Gen. Genet. 227:81-85.

Rouppe van der Voort, J., Lindeman, W., Folkertsma, R., Hutten, R., Overmars, H., van der Vossen, E., Jacobsen, E., and Bakker, J. 1998. A QTL for broad-spectrum resistance to cyst nematode species (*Globodera* spp.) maps to a resistance gene cluster in potato. Theor. Appl. Genet. 96:654-661.

Rouppe van der Voort, J., Kanyuka, K., Van der Vossen, E., Bendahmane, A., Mooijman, P., Klein-Lankhorst, R., Stiekema, W., Baulcombe, D., and Bakker, J. 1999. Tight physical linkage of the nematode resistance gene *Gpa2* and the virus resistance gene *Rx* on a single segment introgressed from the wild species *Solanum tuberosum* subsp. *andigena* CPC 1673 into cultivated potato. Mol. Plant-Microbe Interact. 12:197-206.

Sandbrink, J. M., Colon, L. T., Wolters, P. J. C. C., and Stiekema, W. J. 2000. Two related genotypes of *Solanum microdontum* carry different segregating alleles for field resistance to *Phytophthora infestans*. Mol. Breeding 6:215-225.

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

Stam, P. 1993. Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J. 3:739-744.

Stall, R. E., and Walter, J. M. 1965. Selection and inheritance of resistance in tomato to isolates of races 1 and 2 of the *Fusarium* wilt organism. Phytopathology 55:1213-1215.

Tanksley, S. D., Ganal, M. W., and Prince, J. P. 1992. High-density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141-1160.

Thomas, E. M., Vos, P., Zabeau, M., Jones, D. A., Norcott, K. A., Chadwick, B. P., and Jones, J. D. G. 1995. Identification of amplified restriction fragment polymorphism (AFLP) markers tightly linked to the tomato *Cfg* gene for resistance to *Cladosporium fulvum*. Plant J. 8:785-794.

van der Hoeven, R., Ronning, C., Giovannoni, J., Martin, G., and Tanksley, S. 2002. Deductions about the number, organization, and evolution of genes in the tomato genome based on analysis of a large expressed sequence tag collection and selective genomic sequencing. Plant Cell 14:1441-1456.

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

van Os, H., Buntjer, J., Stam, P. And Van Eck, H. J. 2000. Evaluation of two algorithms for the construction of genetic linkage maps. Abstr. Page 56 in: International Conference of Plant Animal Genome Research, VIII, 2000. International Plant and Animal Genome, San Diego, CA, U.S.A.

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

Vos, P., Hogers, R., Bleeker, M, Reijans, M, van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407-4414.

Wastie, R. L. 1991. Breeding for resistance. Pages 193-224 in: Advances in Plant Pathology. Vol. 7. D. S. Ingram and P. H. Williams, eds. Academic Press, London.

Yan, G. P., Chen, X. M., Line, R. F., and Wellings, C. R. 2003. Resistance gene-analog polymorphism markers co-segregating with the *YR5* gene for resistance to wheat stripe rust. Theor. Appl. Genet. 106:636-643.

Zimnoch-Guzowska, E., Marczewski, W., Lebecka, R. Flis, B., Schäfer-Pregl, R., Salamini, F., and Gebharbt, C. 2000. QTL analysis of new sources of resistance to *Erwinia carotovora* ssp. *atroseptica* in potato done by AFLP, RFLP, and resistance-gene-like markers. Crop Sci. 40:1156-11.

AUTHOR-RECOMMENDED INTERNET RESOURCES

Wageningen University Center for Genetic Resources website: www.cgn.wageningen-ur.nl/pgr/

Cornell University sol genomics network chromosome maps: www.sgn.cornell.edu/cgi-bin/mapviewer/mapviewerHome.pl