Theor Appl Genet (2006) 112: 674–687
DOI 10.1007/s00122-005-0171-4

ORIGINAL PAPER

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Characterization and mapping of $R_{Pi\text{-}ber}$, a novel potato late blight resistance gene from *Solanum berthaultii*

Received: 5 January 2005 / Accepted: 30 November 2005 / Published online: 10 January 2006

Abstract *Phytophthora infestans*, the causal agent of late blight, threatens potato production worldwide. An important tool in the management of the disease is the use of resistant varieties. Eleven major resistance genes have been identified and introgressed from *Solanum demissum*. However, new sources of resistance are continually sought. Here, we report the characterization and refined genetic localization of a resistance gene previously identified as *Rber* in a backcross progeny of *Solanum tuberosum* and *Solanum berthaultii*. In order to further characterize *Rber*, we developed a set of *P. infestans* isolates capable of identifying each of the 11 R-genes known to confer resistance to late blight in potato. Our results indicate that *Rber* is a new resistance gene, different from those recognized in *S. demissum*, and therefore, it has been named $\boldsymbol{R_{Pi\text{-}ber}}$ according to the current system of nomenclature. In order to add new molecular markers around $\boldsymbol{R_{Pi\text{-}ber}}$, we used a PCR-based mapping technique, named MASP-map, which located $\boldsymbol{R_{Pi\text{-}ber}}$ in a 3.9 cM interval between markers CT240 and TG63 on potato chromosome X. The location of $\boldsymbol{R_{Pi\text{-}ber}}$ coincides with an area involved in resistance to different pathogens of potato and tomato.

Abbreviations AUDPC: Area under the disease progress curve · BCT: Backcross to *S. tuberosum* progeny · BSA: Bulk segregant analysis · CAPS: Cleaved amplified polymorphic sequences · MASP-map: Multiplex allele-specific PCR mapping · R-gene: Resistance gene · chr: Chromosome · RFLP: Restriction fragment length polymorphisms

Communicated by G. Wenzel

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Introduction

Potato is the fourth most important crop in the world. In 2004, more than 328 million metric tons of potatoes were produced worldwide (FAOSTAT 2005). Potato production is threatened by diverse pathogens, the worst of which is the oomycete *Phytophthora infestans*, the causal agent of late blight. Late blight epidemics can be devastating, sometimes causing total crop losses (Fry and Goodwin 1997). It has been estimated that the management of the disease costs $3.5 billion annually in developing countries alone (GILB 2004).

Management strategies for late blight include the application of fungicides, the use of healthy seed tubers for planting and host resistance. However, the occurrence of isolates resistant to some modern fungicides emphasizes the need for host resistance (Deahl et al. 1993; Goodwin et al. 1996; Grunwald et al. 2001). Additionally, the high cost of fungicide applications, increasing awareness of health and environmental risks and world-wide pressures to minimize the use of chemical sprays (Fry and Goodwin 1997) also make the use of host resistance a priority.

Two types of resistance to late blight have been described in potato. First, general resistance slows the spread of the disease and it is understood to be often polygenic (Leonards-Schippers et al. 1994; Umaerus and Umaerus 1994), and strongly correlated with maturity type (Bormann et al. 2004; Simko 2002) which makes it difficult to breed into new varieties (Wastie 1991). Second, specific resistance confers immunity or near immunity to the plant through a hypersensitive response and is thought to be monogenic. Genes governing such resistance have been termed R-genes, and are thought to produce proteins involved in pathogen recognition and the initiation of defense responses. Eleven resistance genes have been introgressed into the cultivated potato (*Solanum tuberosum*) from its wild relative *Solanum demissum* (Van der Plank 1963; Wastie 1991), and they are "named" *R1*, *R2*,..., *R11*. Of these, only five have been located on the genetic map of potato: *R1* on chromosome V (Leonards-Schippers et al. 1992); *R2* on chromosome IV (Li et al. 1998); and *R3a, R3b* (Huang et al. 2004), *R6*, and *R7* on chromosome XI (El-Kharbotly et al. 1996). However, *R5–R11* have recently been identified as alleles of *R3* (Huang 2005), which would also locate them in chromosome XI. Of the R-genes from *S. demissum*, only *R1*(Ballvora et al. 2002) *and R3a* (Huang et al. 2005) have been cloned and sequenced.

R-genes from other hosts of *P. infestans* have been reported. Three resistance genes against *P. infestans* have been mapped in tomato—a close relative to potato: *Ph-1* on chromosome 7, *Ph-2* on chromosome 10 (Moreau et al. 1998), and *Ph-3* on chromosome 9 (Chunwongse et al. 2002). An *R*-gene from *Solanum bulbocastanum* on chromosome VIII (Helgeson et al. 1998; Naess et al. 2000), named *RB*, has now been cloned (Song et al. 2003). *Rpi* has been found in *S. pinnatisectum* and mapped to chromosome VII (Kuhl et al. 2001). We previously reported an R-gene located chromosome X of *Solanum berthaultii* (Ewing et al. 2000), named *Rber*, since it segregates from the *S. berthaultii* parent in a backcross with *S. tuberosum*.

Unfortunately, *P. infestans* has been shown to rapidly overcome the classic R-genes from *S. demissum* when they were deployed in potato cultivars. The result is that such specific resistance has had short durability (Wastie 1991), and the immunity hoped for in such R-genes has contributed little to practical late blight management. However recent reports (Bormann et al. 2004; Stewart et al. 2003) suggest that there may be some residual disease suppression effect of R-genes even when interacting with a "compatible" isolate. Knowledge of signaling pathways and downstream events may open new approaches to disease suppression, so it is important to learn as much as possible about such genes.

Rber was mapped to a 13 cM interval between the restriction fragment length polymorphism (RFLP) markers CT214b and TG63 in a diploid backcross progeny. However, it was uncertain if *Rber* was a new gene or an allele of a resistance gene previously introgressed from *S. demissum*. The isolate of *P. infestans* used for the identification of *Rber* was a complex race, compatible with (i.e., not recognized by) R-genes: *R1, R2, R3, R4, R5, R6, R7*, and *R10*. Therefore, we inferred that *Rber* might be a new R-gene (Ewing et al. 2000).

The goal of the research reported here was to determine if *Rber* was different from *R8, R9* or *R11*, and to provide a finer map location of this R-gene. We determined the identity of *Rber* using a set of *P. infestans* isolates known as a "tester set", since they allowed potato plants to be screened for the 11 known R-genes and therefore the identification of new R-genes. We also investigated a series of markers from diverse sources to provide a finer map location of this R-gene.

Materials and methods

Plant material

Host differential set

We used a differential set of *S. tuberosum* genotypes, each one containing one known resistance gene (Table 1). This differential set was obtained from the US Department of Agriculture—Potato Germplasm Introduction Station in Sturgeon Bay, WI, USA and had been used previously (Abu-El Samen et al. 2003; Spielman et al. 1989). Historical and collection data are available for each accession (USDA 1999). The cultivars Norchip or Katahdin were used as R-gene-free controls with no known R-genes, and served to indicate the success of inoculations (Mastenbroek 1952). For all experiments, plants were grown and maintained under greenhouse conditions at the Cornell University facilities (Ithaca, NY).

Backcross mapping population

The backcross population was developed by Bonierbale et al. (1994). Briefly, an interspecific progeny was developed by crossing a dihaploid *S. tuberosum* clone (USW-2230 Saco, GS 193 in the GRIN database) (USDA 1999) as female with an individual of the accession PI473331 of *S. berthaultii* from Cochabamba, Bolivia (USDA 1999). One individual from the F1 progeny (M200-30) was then backcrossed with the 2x *S. tuberosum* parent clone HH1-9 to generate the backcross to *S. tuberosum* (BCT) progeny (Bonierbale et al. 1994).

Originally the progeny consisted of 158 diploid individuals, of which 133 were still available for this study. A total of 665 genotypes of the BCT progeny were produced at Centro Internacional de la Papa (CIP–Lima, Peru) and shipped to Cornell University as a true seed to use in this study. They were tested for, and found to be free of, major potato viruses and other pathogens. For both the original and extended progenies, in vitro plants were obtained and maintained at 15°C until planting them in the greenhouse and field in July 1999.

Table 1 Differential set of *Solanum tubersoum*

R-gene	Accession no.	Origin	Reference
R1	Kennebec	Wisconsin, USA	
R2	PI 203905	Limburg, Netherlands	Mastenbroek (1952)
R3	PI 203902		Spielman et al. (1989)
R4	PI 203900	Netherlands	Mastenbroek (1952)
R5	PI 303146	Scotland, UK	Malcomson and Black (1966)
R6	PI 587059	Netherlands	
R7	PI 303148	Scotland, UK	Malcomson and Black (1966)
R8	PI 303149	Scotland, UK	Malcomson and Black (1966)
R9	Hogdson 2573	Wisconsin, USA	Malcomson and Black (1966)
R10	PI 423656	Netherlands	
R11	PI 587060	Netherlands	

The accession numbers correspond to the plant introduction numbers assigned by USDA. Origin stands for the country collecting the material, which subsequently donated it to the Potato Germplasm introduction station (Sturgeon Bay, WI)

Plants were then stored as tubers for future use. For the characterization of *Rber* we used the pedigree clones of the BCT progeny.

P. infestans isolates

A set of 30 isolates of *P. infestans* was characterized for compatibility/incompatibility with each of the 11 known R-genes. The set of *P. infestans* isolates was obtained from the Cornell University *P. infestans* culture collection, and included strains from Ecuador, Kenya, Mexico, Uganda, United States, Peru, South Africa and Poland (Table 2). The strains were chosen on the expectation that phenotypic differences would occur among isolates from the geographically diverse regions. Specific information regarding the isolates is available upon request. For the assays, isolates were first grown on Rye B medium (Caten and Jinks 1968) in a growth chamber at 15°C to encourage sporulation and then transferred to new media every other week.

The isolates were tested on each host differential plant using a detached leaflet assay (Black et al. 1953; Dorrance and Inglis 1997). Each inoculation involved recently expanded detached leaflets from 5-, 6-week-old plants of each host differential. Fifty microliters of a suspension bearing at least 15,000 zoospores/ml were deposited on the abaxial side of the leaflets using Petri dishes with water agar (15 g/l) on the bottom as moist chambers (Tooley et al. 1986). Each inoculated leaflet was incubated for 7 days at 15°C and scored for presence/absence of infection (detected as sporulation from the leaflet) on the seventh day after inoculation (Dorrance and Inglis 1997; Rivera-Peña 1990).

Wastie (1991) and Dorrance et al. (1997) have suggested that small-scale tests, such as detached leaflets, should be interpreted with caution, because the conditions may not reliably reflect the real resistance or susceptibility interaction other than in extreme cases. Therefore, we scored as "compatible" only those interactions in which sporulation was evident. All cases without sporulation, and where hypersensitive response was present, were scored as incompatible. Each test was repeated at least five times and only those strains that showed consistent reactions were chosen to comprise a "tester set" (Table 2). A dichotomous key (Fig. 1) was then built to facilitate the identification of each of the 11 known R-genes from *S. demissum*.

Once the tester set of *P. infestans* isolates was developed, it was used to characterize the pedigree clones parents of the BCT progeny (USW22-30, HH1-9, and M200-30; the B11B clone was used as a representative of *S. berthaultii*), two of which were known to carry the *Rber* gene (M200-30 and B11B). Detached leaflet assays were again used to identify specific reactions against each isolate. The cultivar Norchip was used as a positive control in every inoculation due to its high susceptibility and absence of known R genes to *P. infestans*.

Phenotypic characterization of the BCT progeny

The presence or absence of *Rber* was determined in the extended progeny and reconfirmed in the original BCT progeny. We used isolate US940480 (ATCC# 208834, a member of the US-8 clonal lineage) obtained from the Cornell University *P. infestans* culture collection. We had previously shown that this isolate can distinguish *Rber* from 8 out of the 11 known R-genes (Ewing et al. 2000), and so it was used in both field inoculations and detached leaflet assays. Field inoculations are desirable because they reflect better resistance under agricultural conditions. The original BCT progeny had been previously exposed twice to the US8 isolate (US940480) (Ewing et al. 2000). The US8-compatible individuals from the original progeny and all individuals from the extended progeny were inoculated in the field at Freeville, NY, during the summer of 1999.

For field studies in 1999, plantlets from in vitro cultures were transplanted into Jiffy7-Peat Pellets (Jiffy Products) supports and grown under greenhouse conditions between May 15 and June 15; from June 15 to June 18 they were conditioned to the field environment in cold frames and transplanted to the field in single plant plots during the week of June 18. The spacing between plots was 0.86 m, and the space between rows was 0.9 m. The cultivar Atlantic was included as a positive control because of its high general susceptibility to late blight.

Table 2 Set of *Phytophthora infestans* isolates tested and the correspondent reaction with the R-genes carried by the respective members of the differential host set

Isolate/plant	Country of origin	R0	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11
EC1	Ecuador	C	C	I	C	I	I	I	C	I	I	I	I
KE980002(KE2)	**Kenya**	**C**	**C**	**C**	**I**	**I**	**C**	**C**	**C**	**I**	**I**	**C**	**C**
KE980004	Kenya	C	C	C	C	C	C	C	C	C	I	C	C
KE980008 (KE8)	**Kenya**	**C**	**C**	**C**	**C**	**I**	**C**	**C**	**C**	**I**	**I**	**I**	**I**
KE980012	Kenya	C	C	C	I	I	C	C	C	I	I	C	I
KE980014 (KE14)	**Kenya**	**C**	**C**	**I**	**C**	**I**	**C**	**I**	**C**	**I**	**I**	**C**	**I**
KE980016	Kenya	C	C	I	C	I	C	I	C	I	I	C	C
MX980002	Mexico	C	C	C	I	I	C	C	C	I	I	C	C
MX980005	Mexico	C	C	C	I	I	C	C	C	I	I	C	C
MX980006	Mexico	C	C	C	I	I	C	C	C	I	I	C	C
MX980082	Mexico	C	C	C	I	I	C	C	C	I	I	C	C
MX980083	Mexico	C	C	C	I	C	C	I	I	I	I	C	C
MX980084	Mexico	C	C	C	I	I	C	I	I	I	I	C	C
MX980085 (MX85)	**Mexico**	**C**	**C**	**C**	**I**	**I**	**C**	**I**	**I**	**I**	**I**	**C**	**C**
MX980099	Mexico	C	C	C	I	I	C	C	I	I	I	C	C
MX980100 (MX100)	**Mexico**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**I**	**I**	**C**	**C**
PE970002	Peru	C	C	C	C	C	C	C	C	I	I	C	C
PO149	Poland	C	C	C	I	C	C	C	C	I	I	C	C
SA960001	South Africa	C	C	C	C	I	C	I	I	I	I	I	C
UG980001	Uganda	C	C	C	C	C	C	C	C	V	V	C	I
UG980005 (UG5)	**Uganda**	**C**	**C**	**C**	**I**	**I**	**C**	**C**	**C**	**I**	**I**	**I**	**I**
UG980006	Uganda	C	C	C	I	I	C	C	C	I	I	I	I
UG980008	Uganda	C	C	I	C	I	C	I	C	I	I	C	C
UG980010	Uganda	C	C	C	I	I	C	C	C	I	I	I	C
US980066 - *US11*	USA	C	C	C	C	C	C	C	C	I	I	C	C
US970001 (*US17*)	**USA**	**C**	**I**	**I**	**C**	**C**	**C**	**I**	**C**	**I**	**I**	**I**	**I**
US940480 (*US8*)	**USA**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**I**	**C**	**C**	**C**
US940501 – *US1*	USA	C	I	I	I	I	I	V	I	I	I	I	I
IPO428.2	Netherlands	C	C	I	C	I	C	C	C	I	I	C	C
VK98014	Netherlands	C	C	I	C	I	C	I	C	I	I	I	C

The accession numbers are given by the year of isolation (i.e., 1998) and the consecutive number from the FryLab collection (Cornell University), isolates IPO428.2 and VK98014 were provided by W. Flier (Plant Research International, The Netherlands). Isolates in bold were chosen for the tester set, codes in parenthesis correspond to Fig. 1, italics represent clonal lineages. Reaction to inoculation is recorded as *C* Compatible; *I* Incompatible and *V* Variable

Potato production practices typical of northeastern USA were used to encourage plant growth. Fertilization was at the rate of 167 kg/ha of a 13-13-13 blend of nitrogen, phosphorous and potassium. Weeds were controlled as needed by applications of Lorox DF (lineron, Dupont, Wilmington, DE, USA) and Dual II (metalochlor, Syngenta, Greebsboro, NC, USA) at recommended label rates. Insects were controlled with the

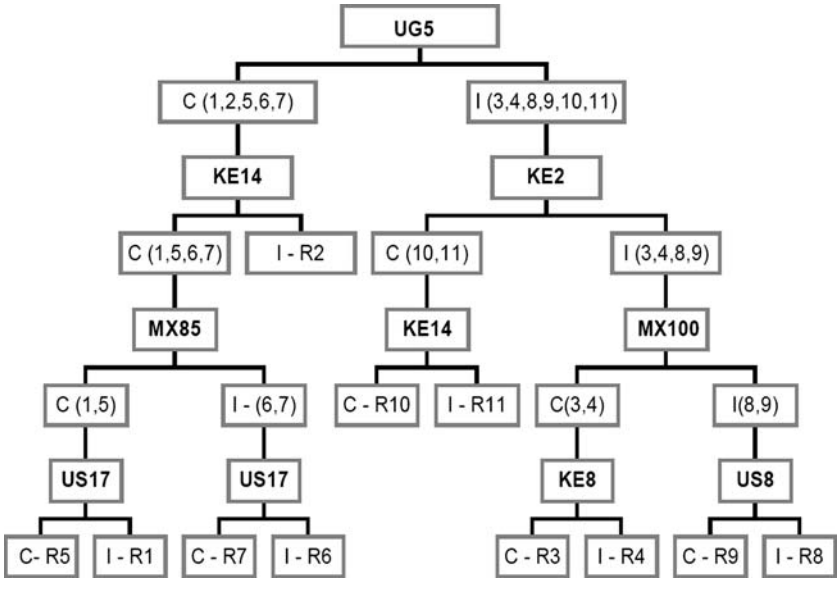

Fig. 1 Flow chart for identification of resistance genes to *Phytophthora infestans* in potato germplasm. The names in *bold* correspond to Table 2. C/I stand for compatible/incompatible reactions, respectively, and the numbers in *parentheses* are the resistance genes that show the corresponding reaction

applications of Provado 3.75 oz/A (imidacloprid, Bayer CropScience, Durham, NC, USA), Sevin XLR 1 qt/A (carbaryl, Bayer CropScience), or Ambush 6.4 oz/A (permethrin, Amvac chemical corp, Newport Beach, CA, USA) when necessary.

Inoculation with *P. infestans* took place during the evening of August 13 by applying a suspension (10 ml) containing 150 sporangia/ml of isolate US940480 to each plant. Immediately prior to inoculation, the entire area had been sprinkler-irrigated to an equivalent of about 0.2 in. of rainfall. The inoculum was applied with a hand-held sprayer. Sporangia had been obtained from sporulating lesions on leaflets of potato cv. Atlantic.

The epidemic developed rapidly and rate of disease development on each progeny genotype was recorded. The amount of foliar disease (as a percentage of the total tissue affected) was evaluated every 3 days between September 5 and October 2 using the methods and guidelines previously described (Ewing et al. 2000). These data were used to calculate the area under the disease progress curve (AUDPC) as described by Shaner et al. (1977). Those plants that showed inconsistent results or that died in the field for reasons other than late blight were subsequently tested using detached leaflets from plants grown in the greenhouse as described before.

DNA extraction

DNA was obtained from each of the individuals in the original and extended BCT progeny. Expanding leaflets were collected from 826 plants growing under greenhouse conditions and were placed in liquid nitrogen. DNA was extracted using a CTAB extraction protocol (Doyle and Doyle 1990). The precipitated DNA was treated with 5 µl of a solution (10 mg/ml) of RNAse A for 30 min at 37°C. It was then visualized in a 0.8% agarose gel in TAE buffer to verify its integrity, and concentration was measured using a Hoefer DyNA QUANT 200 fluorometer (Amersham Biosciences, San Francisco, CA, USA).

Restriction fragment length polymorphism

Restriction fragment length polymorphism probes for chromosome 10 were chosen based on the tomato genetic map and obtained from Dr. Steve Tanksley's lab (Cornell University). To obtain sufficient probe DNA for RFLP hybridization, each cloned probe was amplified by PCR using M13 universal primers in a 25 µl final volume. For the template, we used 1 µl of a liquid culture of *Escherichia coli* transformed with the probe which had been diluted to half concentration in distilled water and incubated in boiling water for 5 min. PCR conditions were: 2.5 mM $MgCl_2$, 0.2 µM of each primer, 200 mM dNTPs, and 1 U of *Taq* Polymerase (Invitrogen Corporation, Carlsbad, CA, USA). Each probe was then amplified as follows: initial denaturation 5 min at 94°C, followed by 30 cycles of 94°C for 60 s, 55°C for 30 s, and 72°C for 90 s, and a final extension at 72°C for 5 min. The amplification products were visualized on 1% agarose gels and cleaned using the Wizard PCR preps DNA purification system (Promega, Madison, WI, USA).

To find polymorphisms in the progeny we performed Southern hybridizations with each tomato probe. DNA from each of the BCT pedigree clones was digested in separate reactions with: *Dra*I, *Eco*RI, *Eco*RV, *Hae*III (all from Invitrogen Corporation), and *Bst*NI (New England Biolabs, Beverly, MA, USA). Digestion and Southern blot procedures were done as described by Sambrook et al. (1989) using Hybond N+ membrane (Amersham Pharmacia Biotech) for capillary transfer. For hybridization buffer we used Hyb-9 Hybridization solution (Gentra Systems, Plymouth, MN, USA).

Probes were labeled with ^{32}Pα-dATP with the Random Primers DNA Labeling system (Invitrogen Corporation) and cleaned with Bio-Spin 30 Tris columns (Bio Rad, Hercules, CA, USA). Hybridization took place for at least 4 h at 65°C. Washes were performed with 2× SSC—0.1% SDS for 15 min, 1× SSC—0.1% SDS for 15 min, and 0.5× SSC—0.1% SDS for 15 min at 65°C. The membranes were then exposed to BioMax MS film (Eastman Kodak Company, Rochester, NY, USA) overnight at −80°C and developed with a Kodak X-Omat automatic developer.

Multiplex allele-specific polymorphism mapping

We used a mapping technique that we named Multiplex Allele-Specific Polymorphism (MASP-map), since it allows the simultaneous amplification of one allele and a control band. It is designed to follow a single allele through a mapping progeny, via a modification of the similar methods such as PASA (Okimoto and Dogson 1996) and tetra-primer ARMS-PCR (Ye et al. 2001). MASP-map allowed us to change RFLP probes into PCR markers. The first step is to design primers from known RFLP probe sequences and use them to amplify and sequence the parents of the cross (Fig. 2). These sequences are then used to find single nucleotide polymorphisms that allow the unique identification of one of the parental alleles. A new allele-specific primer is designed so that the 3′ end anneals to the polymorphic site. In addition a mismatch is added in the penultimate nucleotide on the 3′end of the specific primer to help improve allele specificity.

To screen individuals of the progeny for the parental polymorphism of interest, PCR is carried out with three primers simultaneously; two that amplify both alleles and a third that is specific for the allele with the targeted polymorphism. Thus, individuals lacking the allele of interest amplify only the control band, while in individuals containing the allele of interest, both the specific allele and the control band are amplified.

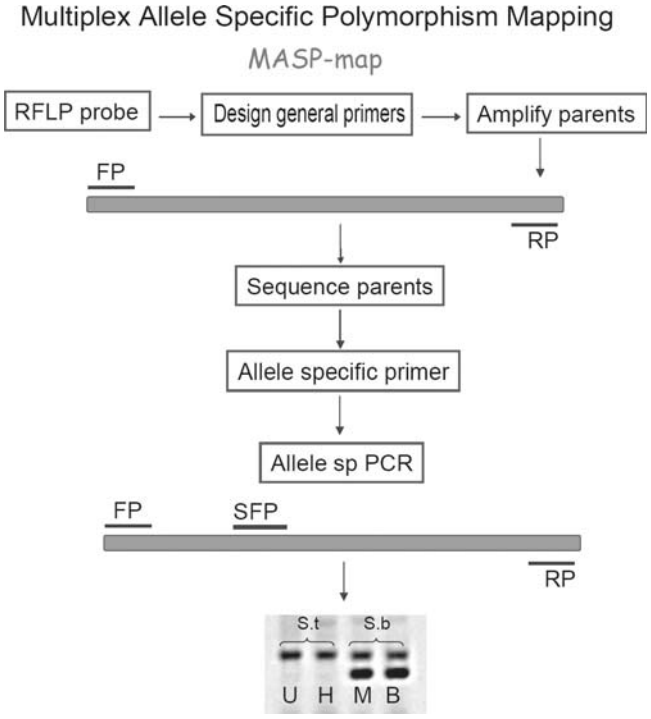

Fig. 2 Diagram of the MASP-map method. See text for explanation of the methodology. FP and RP stand for forward and reverse primer, respectively. SFP stands for specific forward primer. The *last box* shows results of MASP-map: *S.t.* and *S.b.* stand for *Solanum tuberosum* and *Solanum berthaultii,* respectively. UHMB are U: *S. t* parent USW 22-30; H: *S. t.* Recurrent parent HH1-9; M: hybrid parent M200-30; B: *S.b.* representative B11B, respectively

The amplification products are then separated and visualized in agarose gels.

In order to add markers to the interval between CT214 and TG63, the sequences of six tomato RFLP probes were obtained from the Solanaceae Genome Network (http://www.sgn.cornell.edu). From these, forward and reverse primers were designed using Primer Select from the DNASTAR DNA analysis software package (DNASTAR, Inc.), trying to localize them within the 100 initial or terminal base pairs on each probe.

These primers were used to amplify alleles in each individual parent from 50 ng of template DNA in a reaction containing 0.8 U of *Taq* DNA Polymerase (Invitrogen Corporation), 1× PCR buffer (10× stock: 200 mM Tris–HCl pH 8.4, 500 mM KCl), 2.5–3.0 mM $MgCl_2$, 200 nM of forward and reverse primers, 200 nM of dNTPs, and double distilled sterile water. The reactions were carried out in a final volume of 50 µl set on ice to minimize nonspecific amplification. Prior to sequencing, 1 µl of the PCR product was visualized to verify amplification and size of the products on a 1% agarose gel, and the remaining 49 µl were purified using the Wizard PCR PrepDNA purification system (Promega). Amplification products were sequenced by the Cornell Biotechnology Sequencing Facility using only the forward primer for short products (around 500 bp) or both forward and reverse primers for longer products.

The two parental sequences obtained for each probe were compared with Sequencher (version 4.0.5, Gene Codes Corp. MI, USA) to look for single nucleotide polymorphisms that were specific to the *S. berthaultii* parent. Specific primers for *S. berthaultii* polymorphisms were designed from the parental sequences with a T_m about 5°C lower than those of the initial primers (Table 3) and included a mismatch at the penultimate base at the 3′ end of the primer to improve specificity as described by Cha et al. (1992).

When the polymorphism was close to the 5′ end of the fragment a forward primer was designed (5/6 probes, Table 3); when polymorphisms were closer to the 3′ end, a reverse primer was designed (1/6 probes, Table 3). For the sake of clarity, the three primers used will henceforth be referred to as allele-specific (usually forward), general-forward and general-reverse.

To facilitate mapping the region of interest, we screened only individuals with a recombination at any point along chromosome X as first determined by Bonierbale et al. (1988a). Using this method, markers mCT11, mCD5, mCT238, mCT240, and mT1682 were added to the genetic map of chromosome X, the "m" refers to RFLP makers detected by MASP-map.

Tomato marker sequences have been published by Ganal et al. (1998). In order to assess percent similarity between potato and tomato sequences, they were aligned, visually inspected to identify sites with signal from more than one nucleotide (i.e., double peaks), and coded according to standard IUPAC degenerate code. Sequences were then trimmed to the same size using Sequencher version.4.2 (GeneCodes Corp.). Since tomato sequences were obtained from ESTs, for accurate comparison, introns were edited out of potato sequences. However, alignments among potato accessions were done using the whole length of the sequences. To find sequence similarity, the ClustalW utility of the software package MegAlign (Lasergene Corp, Clewley and Arnold 1997) was used. Multiple alignments were performed using the slow/accurate option weighted to account for diploidy.

Finally, sequences were compared to previously reported sequences at GenBank using the BLAST algorithm. ESTs were compared to the dbEST database, which contains GenBank, EMBL, and DDBJ sequences from ESTs. Sequences for the genomic marker TG63 were compared both with the nonredundant and the EST databases.

Cleaved amplified polymorphic sequences (CAPS)

The sequence of tomato probe TG63 was used to design primers to amplify homologous sequences in potato. However, due to multiple insertions and deletions in the sequence, it was not possible to design MASP-map primers. Therefore, we decided to use CAPS to transform this RFLP probe into a PCR marker. After screening the PCR products of all the pedigree

Table 3 Primers used for MASP-map

Name	Sequence 5′ → 3′	T_m (°C)	°C/S	Prim. size (bp)	Diff. (bp)
CT214F	**GAA CGC GAA AGA GTG CTG ATA G**	**57.1**			
CT214BF1	***GAT TCC AAC ATT CAC AAG GGT***	**54.5**	**63/45**	**618/537**	**81**
CT214R2	**CCC GCT GCC TAT GGA GAG T**	**59.7**			
CT238F2	**GGA TAA GGC GGT TCT GTC**	**50.9**			
CT238BF1	***TTC GAT GCC AAT CTC CTA***	**51.0**	**53/30**	**300/225**	**75**
CT238R1	**AAT TTC TCC ATG TTT TTC AG**	**47.6**			
CD5F	**TTG AGG CTA TTG TAC GAG TGT GCG**	**60.6**			
CD5BF1	***TGA GCA ACG TAA TGT GGA AAA***	**54.3**	**57/30**	**464/270**	**194**
CD5R	**AAA GCC TCT TAG GTA CAT TAT GTC G**	**56.2**			
CT11F1	**AGA TTG CTT GTT TGG TGG TT**	**54.3**			
CT11BR1	***TCT CAA AAG GAA TCT TGA CAC AG***	**54.4**	**61/20**	**316/250**	**66**
CT11R1	**TGG AGC AGT CAA CAG AGG**	**54.7**			
CT240F1	**CCA AAG CCC AGG CTG TCA AG**	**59.7**			
CT240BF1	***GGT TCT AAA ATG TCC TCT TAA A***	**49.5**	**55/20**	**890/718**	**172**
CT240R1	**AGT CGG GTG TCA CAA TAA**	**59.9**			
T1682F2	**CGG AAG AAC ATG GAT TTG AAG C**	**55.8**			
T1682BF4	***CAT CTC CCA GCT CAT CAT***	**51.9**	**56/10**	**523/325**	**198**
T1682R2	**CGT CAT TTT CCG ACG AGG ATT T**	**56.9**			

Sequences of primers used to develop and validate the MASP-map technique as well as their melting temperatures and specific amplification conditions. The specific primers are shown in italics; the *Solanum berthaultii* specific nucleotide is underlined and mismatches are noted in bold. The third column shows each one of the primer melting temperatures (T_m). The next column shows the anneal temperature (°C) and the extension time (s) for each PCR reaction. The product sizes (Prim. Size) difference in size (Diff.) between the products of general and allele specific products are illustrated in base pairs (bp)

accessions (USW22-30, HH1-9, M200-30, and B11B) with multiple restriction enzymes, a polymorphism unique to the *S. berthaultii* allele was discovered using *Eco*RV. This polymorphism could then be used as a cleaved amplified polymorphic sequence (CAPS, Konieczny and Ausubel 1993) marker for mapping. For the final screening, 50 ng of genomic DNA were amplified using primers TG63F1 and TG63R (Table 3), and a master mix identical to that described for amplification of RFLP probes, except that the $MgCl_2$ concentration was 3 mM. The PCR conditions for this marker were: initial denaturation 95°C for 2 min; 35 cycles of 95°C for 30 s; 50°C for 30 s; 72°C for 90 s; and final extension at 72°C for 7 min.

The PCR product was digested with 2.5 U of *Eco*RV (Invitrogen Corporation) at 37°C for 3 h and visualized with ethidium bromide on 1.5% agarose gels in 1× TAE buffer. The gels were examined with a FotoAnalyst Investigation Column Mount System (Fotodyne Inc. Hartland, WI, USA).

Simple sequence repeats

Previous studies have shown that SSR marker STM1056 is located close to TG63 in potato (Milbourne et al. 1998), and hence was also screened for progeny that are recombinant on chromosome X. For amplification we followed the procedure and primers described by Milbourne et al. (1998). PCR products were run on a 5.5% Long Ranger Gel (Cambrex Corporation, East Rutherford, NJ, USA) in a SequiGen GT Nucleic Acid Electrophoresis Cell (Bio Rad Inc.). The gels were run in 0.6× TBE buffer at 2,000 V for about 2 h, dried and exposed to Kodak BioMaxMR film (Eastman Kodak Company) overnight at room temperature.

Search for new markers with bulk segregant analysis and resistance gene analogs (RGA)

To increase the chances of finding bands linked to *Rber* using RGA mapping (see subsequently), we used bulk segregant analysis (Michelmore et al. 1991). DNA of selected BCT progeny was bulked in two groups, one with 10 incompatible and the other with 10 compatible individuals. The respective bulks were made based on the genetic data (Bonierbale et al. 1994) and phenotypic (Ewing et al. 2000) information confirmed for the original BCT progeny in chromosome X. The resistant and the susceptible bulks each comprised individuals with recombination events similar in the area of interest between CT240 and TG63, but differing for the rest of the chromosome to minimize the area of interest. The bulks contained the same amount of DNA from each of 10 individuals in such a way that the incompatible group had the *S. berthaultii* allele (segregating from M200-30 and in common with B11B) for the area of interest, regardless of the rest of the chromosome; and the compatible bulk contained alleles from the *S. tuberosum* progenitors in the area between CT240 and TG63 but could differ in the rest of the chromosome.

We used resistance gene analog (RGA) amplification to search for markers linked to *Rber* because previously cloned R-genes to late blight are known to code for nucleotide binding sites (NBS) and leucine-rich repeats (LRR) (Ballvora et al. 2002; Song et al. 2003). Primers designed to amplify resistance gene analogs, including

regions containing NBS, LRR, and protein kinase (Table 4) were obtained. PCR was carried out using 50 ng of DNA and 0.8 U of *Taq* DNA Polymerase (Invitrogen Corporation), 1x PCR buffer (10× stock, 200 mM Tris–HCl pH 8.4, 500 mM KCl), 2.5 mM $MgCl_2$, 100 nm of 33P-γATP labeled forward primer and 200 nM of reverse primer, 200 nM of dNTPs, and bidistilled sterile water in a final volume of 15 µl. The thermocycler (Hybaid Touchdown TD7200, Thermo Electron Corporation, Waltham, MA, USA) was programmed as follows: initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s; annealing of 51–56°C depending on the primer pair for 30 s, extension at 72°C for 20 s, and a final extension step of 72°C for 2 min. Each of the reactions was mixed with 5 µl of formamide loading buffer (80% formamide, 10 mM EDTA, 1 mg/ml bromophenol blue, 1 mg/ml xylene cyanol), denatured for 3 min at 94°C and quickly chilled. RGA products were separated and visualized using the same procedure described for SSRs.

Data analysis

The BCT mapping data set was obtained directly from the Solgenes database (currently available at http://www.grain.jouy.inra.fr/gendatabasemirror.html). The RFLPs, MASP-map markers, RGA, and SSR gels were scored visually for presence or absence of the bands originating in the *S. berthaultii* parent. The genotype for each individual was coded as A (homozygous) or H (heterozygous) for each marker, and these data were combined with the previously published framework markers. The chromosome map was assembled with MapManager QTX, version 0.27 (Manly et al. 2001) using a backcross linkage evaluation with $P = 1\times10^{-5}$ and the Kosambi mapping function. We added the information for the new markers to a different (new) chromosome and used the distribute command. Once the markers were located on chromosome X, we found the best localization for them using the 'ripple' command.

Results

Characterization of *Rber*

The 30 isolates of *P. infestans* we tested were diverse in their interaction (compatible/incompatible) with the differential plants (Table 2). Of these isolates, about half had unique phenotypes; 20% were compatible with R-genes 1, 2, 5, 6, 7, 10, and 11; 10% were compatible with 1, 2, 3, 4, 5, 6, 7, 10, and 11; 6.6% were compatible with 1, 3, 5, 7, 10, and 11; 6.6% with 1, 2, 5, 10, and 11; and 6.6% with 1, 2, 5, 6, and 7. In general, isolates with the same compatibility phenotype came from diverse geographical locations, except for some isolates from Mexico that showed the same compatibility pattern.

We designed a flow chart using the "tester set" of nine isolates (Table 2, Fig. 1) to identify known R-genes. The chart is used as a dichotomous key as follows. An unknown potato genotype is inoculated with each member of the tester set independently and the reactions recorded. The results are then read sequentially following the chart. For example, if a plant is incompatible with the strain UG980005, the next step is to follow the right branch. If it is compatible with the strain KE980002, the left branch should be followed, and so on, until a terminal point is reached, which will show the

Table 4 Resistance gene analogs (RGA) and cleaved amplified polymorphic sequences (CAPS) primers used

Name	Sequence 5′ → 3′	Target	Reference
S1	GGT GGC GTT GGG AAG ACA ACG	P-loop motif and NBS	Leister et al. (1996)
AS1	CAA CGC TAG TGG CAA TCC		
AS2	IAA IGC IAG IGG IAA ICC		
AS3	IAG IGC IAG IGG IAG ICC		
NBS F1	GAA ATG GGN GTN GGN AAR AC	NBS	Yu et al. (1996)
NBS R1	YCT AGT TGT RAY DAT DAY YYT RC		
LM638	GGI GGI GTI GGI AAI ACI AC	P-loop and a transmembrane region	Kanazin et al. (1996)
LM637	ARI GCT ARI GGI ARI CC		
LRR F1	CGC AAC CAC TAG GAG TAA C	Leucine-rich repeats of *RPS2* (F1/R1), *Xa21* (F2/R2) and *Cf9* (F3/R3)	Chen et al. (1998)
LRR R1	ACA CTG GTC CAT GAG GTT		
LRR F2	CCG TTG GAC AGG AAG GAG		
LRR R2	CCC ATA GAC CGG ACT GTT		
LRR F3	TTT TCG TGT TCA ACG ACG		
LRR R3	TAA CGT CTA TCG ACT TCT		
PtoKIN 1	GCA TTG GAA CAA GGT GAA	Protein kinase from tomato *Pto*	Chen et al. (1998)
PtoKIN2	AGG TGG ACC ACC ACG TA		
PtoKIN3	TAG TTC GGA CGT TTA CAT		Zhang et al.(2002)
PtoKIN4	AGT GTC TTG TAG GGT ATC		
TG63F1	CCC AGA GTC CCC CTT CCT ATT	RFLP probe TG63	This study
TG63R	CGA GAT GTT GAA TTT GCG TAA GA		

Resistance gene analog primers used for PCR amplification and search of candidate sequences linked to $\boldsymbol{R_{Pi\text{-}ber}}$. Codes are Y = C/T; R = A/G; D = A/G/T, N = A/C/G/T. See text for details

R-gene that is present. All interactions are recorded and analyzed to determine the number and identification of R-genes present in the potato genotype.

The tester set was applied to three of the pedigree genotypes of the BCT progeny and a representative individual of *S. berthaultii* PI 473331 (Table 5). The *S. tuberosum* 'grand'parent USW-2230 was compatible with all isolates but US17, indicating the presence of *R1*. We also found that the recurrent *S. tuberosum* parent HH1-9 was compatible with all isolates, including US970001, which is only compatible with plants lacking all resistance genes; therefore, no resistance genes were detected in HH1-9. We do not have the original *S. berthaultii* donor (a genotype of PI473331), and therefore could not assess its phenotype. However, the hybrid parent M200-30 (product of a cross between PI473331 and USW22-30) carries *Rber* and is resistant to all isolates in the tester set, suggesting that *Rber* is a new resistance gene. Field tests performed in the Toluca Valley, Mexico allowed the isolation of several strains from plants carring $\boldsymbol{R_{Pi\text{-}ber}}$ allele showing that $\boldsymbol{R_{Pi\text{-}ber}}$ can be overcome by isolates MX990005, MX010003, MX010004, and Mx010008. Testing of these isolates with the differential set also demonstrated their compatibility with *R1–R11* (unpublished results).

Genetic mapping of *Rber*

Phenotypic characterization

We confirmed the phenotype of all individuals in the original BCT progeny evaluated by Ewing et al. (2000) and determined the phenotype of the extended BCT progeny using either field or detached leaflet assays. For plants from the original progeny inoculated in the field with the US8 isolate US940480 during the summer of 1999, the AUDPC score ranged from 0 to 3,652 U. Only those individuals with AUDPC less than 1 were scored as incompatible and thus containing $\boldsymbol{R_{Pi\text{-}ber}}$. The average AUDPC for compatible individuals was 1,580. For the extended population, we tested 336 individuals, of which 180 were incompatible (53.57%). For the combined progeny (original and extended) 52% were resistant (428). For mapping purposes we coded incompatible individuals as carrying the *S. berthaultii* allele ($\boldsymbol{R_{Pi\text{-}ber}}$) in heterozygous condition and the compatible ones as carrying only alleles from the tuberosum pedigree (for purposes of the mapping program these are considered being "homozygous" for the *S. tuberosum* allele, although the pedigree did permit heterozygosity).

MASP-map sequences

Given that MASP-map marker development required the characterization of nucleotide sequences in the pedigree clones of BCT (GenBank acc. AY874391 to AY874411), we thought it would be useful to find the level of similarity among them and with tomato. On average, potato and tomato sequences were over 90% similar. The biggest differences were found in noncoding sequences, e.g., for TG386, where tomato was only 70.1 and 69.2% similar to *S. tuberosum* and *S. berthaultii*, respectively. Regarding *S. tuberosum* and *S. berthaultii*, sequences were over 97% similar on an average. Interestingly the same average similarity was found between the *S. tuberosum* accessions (USW22-30 and HH1-9), most differences were caused by heterozygosity in one of the accessions, especially HH1-9.

Blast searches confirmed previous reports by Ganal et al. (1998) except for CT217, where we found the best homology with an *Avr9/Cf9* elicited protein. Also, mCT11, mCT214, mCT238, and mCT240, each showed homology with sequences from EST libraries made from potato leaves challenged with *P. infestans* (P value $> e^{-111}$). These results, the presence of an R-gene, and QTLs for resistance to other pathogens (Gebhardt and Valkonen 2001) may suggest that this area of chromosome X could be involved in resistance responses common to other pedigrees including tomato.

For the marker TG63, forward and reverse sequences from tomato, obtained from the Solanaceae Genomics Network, were compared to sequences in GenBank. No matches were found when the search used the nonredundant database, but the sequences share homology with one EST from tomato roots (EST303128, $1e^{-85}$) and one from the tomato callus (EST542946, $1e^{-55}$). However, since TG63 is a marker from a total genome library, these findings are inconclusive. We also sequenced the amplification products of all pedigree genotypes from primers TG63F1 and TG63R; however, heterozygosity for insertion/deletions precluded clean direct sequences that could be tested for homologies.

Table 5 Results of testing parents of backcross to *S. tuberosum* (BCT) with tester set. C/I refers to compatible and incompatible reactions, respectively. (Norchip was a positive control)

Isolate	Norchip	USW22-30	HH1-9	M200-30	B11B
KE980002	C	C	C	I	I
KE980008	C	C	C	I	I
KE980014	C	C	C	I	I
MX980085	C	C	C	I	I
MX980100	C	C	C	I	I
UG980005	C	C	C	I	I
US17 (US 970001)	C	I	C	I	I
US8 (US940480)	C	C	C	I	I

Genetic markers

Several new genetic markers were added to chromosome X in order to assess the precise location of $\boldsymbol{R_{Pi\text{-}ber}}$. First, we confirmed the identity of each individual from the original BCT progeny by testing with the flanking markers mCT214 and TG63. Doing this, we were able to assess previously missing genotypes for five individuals for CT214 and six individuals for TG63. There was disagreement between the genotyping results in this study and a previous one (Bonierbale et al. 1994) for three individuals that were originally scored for TG63 as double recombinants. In our study, these individuals were recorded as heterozygous (presence of *berthaultii* alleles), whereas in the previous study they were recorded as homozygous, which caused them to show as double recombinants. For a progeny of $N = 158$ and given the original map distance between the markers TG63–*Rber*–CT214b of 4.8 and 8.2 cM, respectively (Ewing et al. 2000), the number of double recombinants should be about 1(0.62). The probability of the three double recombinants reported is 0.0012 suggesting that the original designation is highly unlikely. Using the revised designations determined in this study we found a new distance between TG63 and $\boldsymbol{R_{Pi\text{-}ber}}$ of 0.6 cM, instead of the 4.8 cM originally reported (Ewing et al. 2000); the corrected distance between CT214 and $\boldsymbol{R_{Pi\text{-}ber}}$ is 5.8 cM.

We added the RFLP markers CT217, CD32, CD72, and CT20, from the tomato genetic map to potato chromosome X. Because of the inversion between tomato and potato chromosomes, we found that only CD32 was located in the area of interest at 0.6 cM from CT214. Marker CT217 is located closer to TG52; CD72 and CT20 are located in a terminal position relative to TG63 (Fig. 3).

Using MASP-map, three new markers (mCD5, mCT238, and mCT240) were placed within the interval between mCT214 and TG63. Markers mCT240 and TG63 are the closest to $\boldsymbol{R_{Pi\text{-}ber}}$ at 3.9 and 0.63 cM, respectively (Fig. 3). Two other markers, mCT11 and mT1682, were added outside the interval which confirmed the area where an inversion took place in potato chromosome X with respect to tomato chromosome 10.

When the entire progeny (original and extended; 826 individuals) was tested with the flanking markers mCT214 and TG63 to identify recombinant individuals in this interval, we found 54 recombinants, very close to the 52 expected for a 6.4 cM distance. There were six additional individuals for which results were inconsistent and from which we did not use data. By adding marker mCT240 to the 54 recombinants, 29 recombination events were found between CT240 and $\boldsymbol{R_{Pi\text{-}ber}}$ (a genetic distance of 3.51 cM) and seven recombinants between $\boldsymbol{R_{Pi\text{-}ber}}$ and TG63 (0.84 cM).

The microsatellite STM1056 resulted in a total of 12 markers, most of which are located on chromosome VIII. The band STM1056-7 showed linkage to markers on chromosome X, with LOD scores between 10.9 for AS1-13 and 26.3 for $\boldsymbol{R_{Pi\text{-}ber}}$. It is most likely located between $\boldsymbol{R_{Pi\text{-}ber}}$ and TG63 but precise localization was not possible.

From all the RGA primers tested we found three bands linked to chromosome X. These were detected with primers S1 × AS1, which were designed to amplify the P-loop motif and the nucleotide binding site

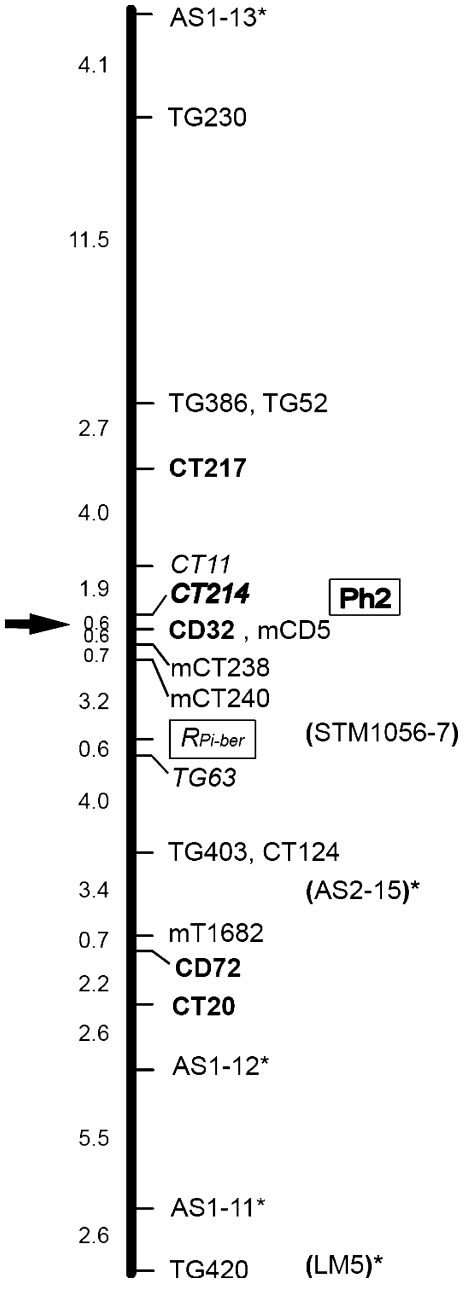

Fig. 3 Localization of $R_{Pi\text{-}ber}$ on chromosome X in the potato map. Previously published framework markers are TG230, TG386, TG52, TG403, CT124, and TG420. RFLP markers are shown in *bold*; MASP-map markers are preceded by "m"; RGA markers have *asterisks* after the name. Approximate locations of RGA and SSR markers are shown in *brackets*, names of cosegregating markers are separated by *commas*. Resistance genes $\boldsymbol{R_{Pi\text{-}ber}}$ and *Ph2* (position inferred from tomato map) are surrounded by *boxes*. Inversion point between tomato and potato is indicated by an *arrow*

common to resistance genes (Leister et al. 1996). They are located at 4.1 cM distal to TG230 (AS1-13), and between markers CT20 and TG420 (AS1-11 and AS1-12). There were two other bands, AS2-15 and LM5, with linkage to the chromosome (LOD 18.3 and 18.6, respectively), but we were not able to locate them confidently between other markers on the chromosome. It is possible that the *S. tuberosum* and *S. berthaultii* alleles do not differ enough to show differential amplification with the RGA primers used; therefore, we were unable to find markers closely linked to ***R*** $_{Pi\text{-}ber}$. Approximate locations are shown in Fig. 3.

Discussion

The objective of this study was to determine the novelty and precise location of the *Rber* resistance gene from *S. berthaultii*. The first step was to design a method that could rule out known R-genes in potato which are responsible for the resistance reaction conferred by the gene. Using a detached leaflet assay, a host differential set and *P. infestans* isolates from diverse geographic regions. We were able to select nine isolates that together differentiate each of the previously characterized resistance genes through their known incompatibility reactions. Using this tester set and the flow diagram illustrated (Fig. 1), we determined that *Rber* is a new previously uncharacterized R-gene against *P. infestans*. In order to avoid confusion with the recently published RB gene and maintain the currently used nomenclature, that describes R-genes as $R_{\text{pathogen}-\text{donor of resistance}}$ (Kuhl et al. 2001; Solomon-Blackburn and Barker 2001) we renamed the gene ***R*** $_{Pi\text{-}ber}$.

The rationale for characterizing ***R*** $_{Pi\text{-}ber}$ as a locus with a novel specificity, is as follows: it is incompatible with UG980005 suggesting that it could only be *R3, R4, R8, R9, R10*, *R11, or new;* it is incompatible with KE980002, ruling out *R10* and *R11*; it is again incompatible with MX980100, eliminating *R3* and *R4*, besides *R3* is located on Chr. XI; incompatibility with US8 rules out *R9*. Finally, M200-30 is incompatible with the R8-compatible isolate Ke980004, allowing us to conclude that ***R*** $_{Pi\text{-}ber}$ is also not *R8*. Besides, new findings by Huang (2005) suggest that *R5–R11* are allelic to *R3*, ruling them out from chromosome X. This leaves only the proposition that ***R*** $_{Pi\text{-}ber}$ is different from previously identified and ''named'' R-genes. Finally, as previously stated, ***R*** $_{Pi\text{-}ber}$ is overcome by several Mexican isolates including Mx990005 and Mx010003. In order to keep the current nomenclature of resistance genes to *P. infestans* in potato, we will refer to *Rber* (Ewing et al. 2000) as ***R*** $_{Pi\text{-}ber}$.

While the development of the R-gene tester set proved to be a useful tool, its widespread application is unfortunately limited. Some avirulent characteristics in a few of the isolates have changed over time. Others have also observed this phenomenon (Abu-El Samen et al. 2003). Thus, we found it necessary to include the appropriate host differential plant as positive controls in all inoculation experiments.

The use of a new genotyping method for this study allowed us to add several specific RFLP markers to the map with relative ease. The MASP-map method provides an efficient alternative to RFLPs for mapping in large populations. It is based on the use of primers that specifically amplify one parental allele and that can be used to screen segregation in a progeny. This system is inexpensive and efficient, needing only one PCR step, three primers per set, the usual PCR reagents, and visualization on agarose gels. Another significant advantage of this method is the quantity of DNA required; only 50 ng of DNA per reaction was used in this study. In contrast, the same study using RFLP methods would have required at least 10 μg of DNA per probe. Possibly its most important advantage is that it provides amplification of a control band in addition to the allele-specific band. The lack of the target allele is visualized by amplification of just the control band; the absence of all amplification products in a sample indicates a failure of the PCR.

The original map placed ***R*** $_{Pi\text{-}ber}$ between markers CT214b and TG63, an area spanning 13 cM. Through confirmation of phenotypes and genotypes at these markers, we have demonstrated this area to be smaller than originally thought (6.4 cM). While the precise map position of ***R*** $_{Pi\text{-}ber}$ is still not known, we were able to add four new markers within the interval and locate ***R*** $_{Pi\text{-}ber}$ between CT240 and TG63, to an area spanning 3.8 cM. Map distances calculated for the original progeny increased slightly when the extended BCT progenies were added, from 3.2 between CT240 and ***R*** $_{Pi\text{-}ber}$ and 0.63 cM between ***R*** $_{Pi\text{-}ber}$ and TG63 to 3.51 and 0.84 cM in the same interval. A possible explanation for this difference is the dissimilar environments in which the crosses were made, as suggested by Paterson (1996). Since the size of the potato genome is close to 1,000 cM (Meksem et al. 1995), which corresponds to 1,000 Mb (haploid genome equivalent, Leister et al. 1997), 1 cM is roughly equivalent to 1 Mb. This means that (on average) there is predicted to be about 3.8 Mb of DNA in the area of interest, and the closest marker TG63 may be only 630 Kb away from ***R*** $_{Pi\text{-}ber}$.

The molecular maps of potato and tomato show a strikingly high level of colinearity (Bonierbale et al. 1988a; Gebhardt et al. 1991; Tanksley et al. 1992). The tomato map, consisting of more than 1,030 markers (Tanksley et al. 1992), has been saturated to a higher degree than the potato map. For this reason, our effort to saturate the area with new markers began by using orthologous probes from tomato. Despite the similar genetic structure and colinearity between potato and tomato, their chromosome X homologues differ by a paracentric inversion (Bonierbale. et al. 1988b), close to ***R*** $_{Pi\text{-}ber}$. We found the break point to be in a 2.7 cM area between markers TG280 and TG408, in agreement with Tanksley et al. (1992). In contrast to previous expectation, however, our results indicate that CT217 in potato

is located closer to TG386 and is not within the inversion (Tanksley et al. 1992).

One question of interest is whether resistance genes found in one species are orthologous to those found in other species. This is particularly interesting because a recent study by Grube et al. (2000) showed that there are clusters of resistance genes that are conserved across the Solanaceae. Not all proposed homologies, however, turn out to be accurate. For example, *R6* was originally thought to be present in both *S. demissum* and *S. stoloniferum* (Schick et al. 1958). Later work showed these to be two different loci (Malcomson and Black 1966). The same seems to be the case with ***R*** ***Pi-ber***. A previous study (Ewing et al. 2000) considered the possibility that ***R*** ***Pi-ber*** was orthologous to *Ph2* on tomato chromosome X (Moreau et al. 1998) due to the map position of both genes. Based on the position of the inversion point, we show here that they are not located at the same map position. *Ph2* is located between markers CT240 and TG233 in tomato, which are upstream of ***R*** ***Pi-ber*** in the potato map, and which therefore occupy different loci in the long arm of Chromosome X (Moreau et al. 1998).

In our study, resistance gene analogs (RGAs) were amplified with primers designed for nucleotide-binding sites, leucine-rich repeats and *Pto*-kinase. We identified five bands located in chromosome X, three of which could be added to the map (Fig. 2). The other two had high LOD scores for linkage to markers in chromosome X, but we were unable to map them confidently due to too many apparent double crossovers. Another RGA sequence was reported by Leister et al. (1996) on the long arm of chromosome X (St 1.2.4), which could be allelic to AS1-11 or AS1-12. These results may be suggestive of the presence of a resistance gene cluster on chromosome X.

Our results provide a more detailed characterization and improved understanding of ***R*** ***Pi-ber***, including a more precise map position on potato chromosome X. Because we converted RFLP markers into MASP-map and CAPS markers, selection in the future for individuals containing ***R*** ***Pi-ber*** will be easier and faster, particularly if the gene is used in breeding programs. In addition, the improved localization of the inversion on chromosome X will make it easier to identify new markers from the always-growing tomato map to saturate the area between mCT240 and TG63. Further steps are needed to clone and characterize ***R*** ***Pi-ber***. These future analyses will provide insights on resistance interactions between potato and *P. infestans*, and perhaps contribute to the management of the disease.

Acknowledgements The present study was supported by Syngenta. We are grateful to Jason Rauscher, for critical reading and helpful suggestions on the manuscript, and to Kevin Myers and Barbara Esposito for their skilled technical assistance. RFLP markers were kindly provided by S. Tanksley.

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