Theor Appl Genet (2010) 121:1553–1567
DOI 10.1007/s00122-010-1410-x

ORIGINAL PAPER

Quantitative resistance to late blight from *Solanum berthaultii* cosegregates with $R_{Pi\text{-}ber}$: insights in stability through isolates and environment

Gilda Rauscher · Ivan Simko · Hilary Mayton · Merideth Bonierbale · Christine D. Smart · Niklaus J. Grünwald · Andrew Greenland · William E. Fry

Received: 22 April 2009 / Accepted: 13 July 2010 / Published online: 6 August 2010

Abstract Genetic resistance is a valuable tool in the fight against late blight of potatoes but little is known about the stability and specificity of quantitative resistance including the effect of defeated major resistance genes. In the present study we investigated the effect of different isolates of *Phytophthora infestans* on the mode of action of $R_{Pi\text{-}ber}$, an R-gene originating from *Solanum berthaultii*. The experiments were conducted on progenies derived from two reciprocal inter-specific backcrosses of *Solanum tuberosum* and *S. berthaultii*. The plant–pathogen interaction was tested in diverse environments including field, greenhouse and growth chamber conditions. The $R_{Pi\text{-}ber}$ gene provided complete resistance against a US8 isolate of *P. infestans* in all trials. When isolates compatible with $R_{Pi\text{-}ber}$ were used for inoculation, a smaller, but significant resistance effect was consistently detected in the same map position as the R-gene. This indicates that this R-gene provides a residual resistance effect, and/or that additional resistance loci are located in this genomic region of chromosome X. Additional quantitative resistance loci (QRL) were identified in the analyzed progenies. While some of the QRL (such as those near TG130 on chromosome III) were effective against several isolates of the pathogen, others were isolate specific. With a single exception, the *S. berthaultii* alleles were associated with a decrease in disease severity. Resistance loci reported in the present study co-locate with previously reported R-genes and QRL to *P. infestans* and other pathogens.

Communicated by R. Visser.

G. Rauscher · H. Mayton · C. D. Smart · W. E. Fry (✉)
Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY, USA
e-mail: wef1@cornell.edu

C. D. Smart
Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Geneva, NY, USA

I. Simko
Department of Horticulture, Cornell University, Ithaca, NY, USA

M. Bonierbale
Centro Internacional de la Papa, Lima, Peru

G. Rauscher · I. Simko
Crop Improvement and Protection Research Unit, USDA-ARS, Salinas, CA, USA

N. J. Grünwald
Horticultural Crops Research Unit, USDA-ARS, Corvallis, OR, USA

A. Greenland
Syngenta, Jealott's Hill, Bracknell, UK

Present Address:
A. Greenland
NIAB, Cambridge, UK

Introduction

Phytophthora infestans, the causal agent of late blight is the most aggressive and costly pathogen affecting potato production worldwide. It is managed through the use of fungicides, implementation of proper farming practices, and deployment of genetic resistance. The total cost of fungicide applications and lost revenue due to the disease has been estimated at more than $500 per hectare in the US (Guenthner et al. 2001). However, the development of resistance to fungicides and worldwide migrations of more aggressive and (in some locations) sexually reproducing

populations (Fry 2008) have worsened the incidence and devastation of the disease (Fry and Goodwin 1997). For this reason, the deployment of durable genetic resistance in potato varieties has become a high priority in breeding programs (Simko 2002).

Two kinds of resistance against late blight have been described in potato: qualitative resistance mediated by R-genes and quantitative resistance usually due to the effect of multiple genes of minor effects. Late blight R-gene mediated resistance provides immunity through a hypersensitive reaction and is thought to be monogenic, specific and of short durability due to the high genetic variability of the pathogen (Umaerus and Umaerus 1994). Over 30 R-genes have been described to date in at least 10 different species of *Solanum* (for a review see Hein et al. 2009) including *S. berthaultii* (Ewing et al. 2000), and several have been or are being cloned (Hein et al. 2009).

Quantitative resistance is characterized by a slower development of the disease, in part caused by reduced infection efficiency, smaller lesions, lower sporulation capacity and longer latent period (Wastie 1991). It has been described as being effective against a broad range of pathogen races (Young 1996), and hence is considered to be race non-specific (Wastie 1991). It is thought to be caused by an array of different genes with minor effects and may be more durable than R-gene mediated resistance (Wastie 1991).

Previous studies have suggested that R-genes may have a residual effect, adding to quantitative resistance once they have been overcome by (i.e., matched by compatible isolates of) the pathogen (Tan et al. 2008; Young 1996). Tan et al. describe this phenomenon as a "defeated" R-gene. Such effects have been reported for different pathosystems such as *Venturia inequalis*, on apple (Durel et al. 2000); *Xanthomonas oryzae* pv. *oryzae*, on rice (Talukder et al. 2004); wheat powdery mildew (Chantret et al. 1999); and potato late blight (Stewart et al. 2003). Co-localization of qualitative and quantitative resistance has been described for bean anthracnose (Geffroy et al. 2000), melon powdery and downy mildew (Perchepied et al. 2005a) and tomato powdery mildew (Bai et al. 2003). However, in other plants, such as poplar, there are contradictory reports about the durable effect of defeated R-genes (Dowkiw and Bastien 2007; Woo and Newcombe 2003).

Detection of genes involved in quantitative resistance is typically performed through the analysis of correlation between phenotypic observations and molecular markers in a segregating population (Tanksley 1993). The advantage of quantitative resistance loci (QRL) mapping for the study of resistance is that multiple loci with important phenotypic effects can be identified throughout the genome and epistatic interactions among these loci can be characterized (Kover and Caicedo 2001). Once identified, the stability of the effects of specific QRL can be studied under different environmental conditions, offering a better understanding of the complex genetic and environmental interactions that contribute to resistance in the field.

Quantitative resistance has been studied extensively in many different pathosystems with emphasis on the specificity and nature of the genes involved (Young 1996). In potato, several QRL studies have been published regarding resistance to *P. infestans* (Simko 2002). Some of the QRL for late blight coincide with positions of known R-genes (Stewart et al. 2003) or R-gene clusters (Gebhardt and Valkonen 2001) and many are associated with defense-related (DR) genes, such as those involved in the phenylpropanoid pathway (Trognitz et al. 2002).

Despite extensive studies of resistance genes many questions concerning QRL remain. For example, it is not yet certain that all QRL are stable across different pathogen genotypes. Additionally, little is understood about the relationship between QRL and genes known to provide major resistance or genes related to other traits of agronomical importance. These topics are beginning to receive considerable attention (Collins et al. 1999; Ewing et al. 2000; Ghislain et al. 2001). While significant progress has been made in our understanding of quantitative resistance in potato, there are few studies that have attempted to characterize the phenotypic effects of QRL in reciprocal mapping progenies tested with different isolates under different environmental conditions. In addition, little is known about the role of defeated R-genes in late blight resistance.

This study investigates the role of QRL in potatoes effective against *P. infestans*. Our approach was to use mapped reciprocal backcross progenies from *S. tuberosum* and *S. berthaultii* (Bonierbale et al. 1994) that have been previously analyzed in studies of host resistance and agronomic traits including both major gene resistance and QRL (Ewing et al. 2000; Rauscher et al. 2006). Here we address three specific questions: (1) Does the locus containing the R-gene $R_{Pi\text{-}ber}$ continue to contribute resistance as a QRL once matched by a compatible isolate? (2) Are the same QRL involved in resistance to different isolates of *P. infestans*? (3) Are QRL stable across different environments?

Materials and methods

Plant material

Two backcross populations were available for this study. The BCT (backcross to *S. tuberosum*) and BCB (backcross to *S. berthaultii*) progenies consisted of 158 and 155 individuals, respectively (Bonierbale et al. 1994). For the

Table 1 Characteristics of *Phytophthora infestans* isolates used in this study

ID	Isolate	Origin	Crop	MT	Race	GPI
US8	US940480	Steuben Co, NY (USA)	Potato	A2	*R1–R7* and *R9–R11*	100/111/122
US11	US990025	Sutter Co, CA (USA)	Tomato	A1	*R1*, *R4–7*	100/100/111
US11	US980066	Tompkins Co, NY (USA)	Tomato	A1	*R1–R7* and *R11*, $R_{Pi\text{-}ber}$	100/100/111
Mx5	Mx990005	Toluca Valley (Mexico)	Potato/BCB progeny	A1	*R1–R11*, $R_{Pi\text{-}ber}$	86/100
Mx3	Mx010003	Toluca Valley (Mexico)	Potato/BCB progeny	A1	*R1–R11*, $R_{Pi\text{-}ber}$	100/111/122
Mx4	Mx010004	Toluca Valley (Mexico)	Potato/BCB progeny	A2	*R1–R11*, $R_{Pi\text{-}ber}$	100/100

ID number either refers to the clonal lineage or reference code to which the isolate belongs, *US8* and *US11* are clonal lineages, *isolate* number refers to the identification number within the Fry Lab collection, *origin* refers to the geographical area where the isolate was first collected, *crop* is the crop or progeny from which the isolate was obtained, *BCB* (*S. tuberosum* × *S. berthaultii*) × *S. berthaultii* progeny, *MT* mating type, *race* R-gene compatibility, *GPI* identifies glucose-6-phosphate-isomerase genotype

BCT progeny, in vitro plants were obtained and maintained at 15°C until planted in 1999. The BCB progeny was used and stored as tubers. Both progenies were tested for major potato viruses and other pathogens and found to be healthy. Subsequently, plants were kept as tubers for all further uses.

Differential set

To determine the pathotypes of the *P. infestans* isolates, we screened them with a series of differential *S. tuberosum* plants, each one containing one known resistance gene. For details on this method of race determination see Rauscher et al. (2006). The differential set was originally obtained from the Potato Germplasm Introduction Station in Sturgeon Bay, Wisconsin. Passport data for each accession are available at http://www.ars-grin.gov (USDA, ARS 1999). The cultivars Norchip or Katahdin were used as susceptible controls, and served to indicate the success of inoculations.

Phytophthora infestans isolates

We used isolates collected in two different countries. The isolates US940480 (clonal lineage US8) and US980066 and US980025 (both clonal lineage US11) were collected in the United States. Isolates Mx3 (MX010003), Mx4 (MX010004) and Mx5 (MX990005) were collected from natural infections on the BCB progeny in the Toluca Valley, Mexico. For easier identification throughout this report, we will refer to the isolates as US8, US11-25, US11-66, Mx3, Mx4 and Mx5 (Table 1). Race determination was done as previously described (Rauscher et al. 2006). Briefly, each isolate was grown in Rye B medium and sporangia were harvested after a week; zoospores were released by incubating the inoculum at 4°C for 1 h. Detached leaflets of 5- to 6-week-old plants carrying each one of the known R-genes (differential set), as well as the hybrid parent M-200-30 (heterozygous for $R_{Pi\text{-}ber}$) were spot inoculated with fifty microliters of a 50,000 zoospore/ml suspension. The leaflets were incubated in the top of water agar plates (15 g/l) used as moist chambers for 7 days at 15°C. Compatible reactions were determined by the presence of sporulating lesions, each test was repeated at least five times. Isolates US8, and US11-25 were incompatible with $R_{Pi\text{-}ber}$, whereas the other isolates were compatible.

For all assays, the isolates were grown on Rye B medium (Caten and Jinks 1968) in a moist chamber at 15°C to encourage sporulation and then transferred to new media every other week. Before the inoculations, they were transferred onto potato leaves of the cv. Katahdin and grown in moist chambers at 15°C for 7 days. For long-term storage, all isolates were preserved in oil (Wernham 1946).

DNA extraction

DNA was obtained and purified as described previously (Rauscher et al. 2006) using a CTAB extraction protocol. After purification the DNA was visualized in a 0.8% agarose gel to verify its integrity. Concentration was measured using a DyNA QUANT 200 fluorometer (Hoefer Scientific instruments, San Francisco, CA).

Plant genotyping

Genotyping with RFLP markers have been previously published for both populations (Bonierbale et al. 1988). Because the BCT and BCB progenies had been stored and multiplied since their original development, it was important to confirm the identity of each individual by characterizing its map-based genotype. This was accomplished through a combination of different markers, including Multiplex Allele-Specific Polymorphism mapping (MASP-Map, Rauscher et al. 2006) and cleaved amplified polymorphic sequences (CAPS, Konieczny and Ausubel 1993). The procedure for genotype confirmation was previously

Table 2 Phenotypic analyses of resistance (by year and isolate) to *Phytophthora infestans*

Year	Isolate	ID	# Trials	*N*	Conditions	Max AUDPC	Average rAUDPC (%)
BCT							
1997[a]	US940480	US8	1	152	Field[c]	1,507	32.6
1998[a]	US940480	US8	2	62, 49[b]	Field[c]	1,760, 1,834	72.8, 66.3
1999	US940480	US8	1	342	Field[c]	2,750	35.36
2000[d]	US990025 US980066	US11	2	129	Field[c]	2,016, 1,978	25.39, 25.6
2001	US980066	US11	1	106	Field[c]	2,043	23.47
2002	Mx990005	Mx5	1	107	Greenhouse	470	43.04
BCB							
1999	Natural infection		1	103	Field, Mexico	1,230	9.6
2000	Mx990005	Mx5	2	113, 116	Greenhouse	1,325, 440	9.30, 13.52
	US980066	US11	1	122	Field[c]	588	4.75
2001	Mx990005	Mx5	2	107, 106	Greenhouse	1,376, 2,003	19.81, 19.26
2002	Mx010004	Mx4	1	106	Greenhouse	326	18.17
	Mx010003	Mx3	2	112, 122	Growth chamber	50.0, 60.0	12.28, 14.55

Year shows year of experiment, *isolate* and *ID* indicates isolate reference numbers (as in Table 1), *# trials* number of trials, *N* number of tested genotypes, *conditions* identifies where was the trial performed, *max AUDPC* the maximum AUDPC score obtained in each trial, *average rAUDPC* the mean of relative AUDPC as percentage (see text for details)

[a] Results published by Ewing et al. (2000), used for comparison purposes

[b] in 1998 and 1999 only the susceptible portion (plants lacking $R_{Pi\text{-}ber}$) of the BCT progeny was inoculated

[c] Field experiments for the BCT progeny were conducted in Freeville, NY

[d] During the 2000 field season, the plants were inoculated twice, first with US990025 and later with US980066, the results shown are the effect of both inoculations at the end of the field season

described for the BCT progeny (Rauscher et al. 2006). The primer sequences for the MASP-map BCB marker CT214 were CT214F (forward 5′ GAA CGC GAA AGA GTG CTG ATA G3′), CT214TF1 (specific for the *S. tuberosum* allele, 5′GAT TCC AAC ATT CAC AAG GGC3′) and CT214R2 (reverse primer, 5′ CCC GCT GCC TAT GGA GAG T3′). The TG63 probe was converted into a CAPS marker as previously described (Rauscher et al. 2006). The primers used for amplification were TG63F1 (5′ CCC AGA GTC CCC CTT CCT ATT 3′) and TG63R (5′ CGA GAT GTT GAA TTT GCG TAA GA 3′), and for identification of the *S. tuberosum* allele in the BCB progeny PCR products were digested with *Dra*I (Gibco, Carlsbad, CA). Based on the marker analysis a few mislabeled individuals in each progeny (six in BCT and nine in BCB) were discarded from the QRL study. Through this procedure we also determined the missing TG63 genotype for five BCB individuals, and one individual for CT214.

Phenotypic characterization of progenies

Several different experiments were conducted using both BCT and BCB progenies under different conditions (including field, greenhouse, and growth chamber) during different seasons and with different isolates (Table 2).

BCT assessment

The field experiments were carried out in Freeville, NY, USA. The planting conditions, potato production practices and inoculations were done as previously described (Rauscher et al. 2006). Inoculations were carried out by applying a suspension (10 ml) containing 150 sporangia/ml of the *P. infestans* isolate to each plant after irrigating the plants for 2 h (equivalent to ca 0.2 cm of rainfall). The inoculum was applied with a handheld sprayer. Sporangia had been obtained from sporulating lesions on leaflets of potato cv. Atlantic. For each experiment the cv. Atlantic was included as a positive control because of its high general susceptibility to late blight. A summary of the field inoculations is shown in Table 2.

In every case, the 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 2 or 3 days using the methods and guidelines previously described (Ewing et al. 2000).

For the greenhouse experiment, cuttings of the BCT progeny were taken from greenhouse grown plants. They were transplanted into vermiculite in Speedling trays (Speedling, Sun City, FL) and placed onto a bench and misted regularly until roots were well developed (about

2 weeks). The rooted cuttings were planted into soil in 4-in. diameter plastic pots and placed on greenhouse benches with a 12-h photoperiod. After 8 weeks the plantlets were moved into a greenhouse (winter 2000) and inoculated 2 days later. Inoculation took place with 10 ml of a sporangial suspension of 6,000 sporangia/ml of the isolate Mx5 grown on potato cv. Katahdin and M200-30 (hybrid parent of both progenies) leaflets. Permission was obtained from the Animal and Plant Health Inspection Service (APHIS) for conducting greenhouse assays with Mexican isolates (compatible with the BCB progeny) during the winter in Ithaca, NY. The sporangia of *P. infestans* do not survive winter conditions in upstate NY and there is no susceptible host tissue outdoors during the winter. All greenhouse materials were autoclaved after use. Evenly spaced cold-mist humidifiers were used overnight to keep relative humidity at 100% and the temperature was kept at 15°C (±3). Disease severity was assessed daily for 7 days, starting 2 days after inoculation.

BCB evaluation

The BCB was evaluated in the field, greenhouse and growth chamber. In 1999, we conducted a field trial, in the Toluca Valley (Mexico) in order to select for compatible isolates. Tubers were planted in two plant plots following standard horticultural practices for the area (Grünwald et al. 2002). *Phytophthora infestans* is ubiquitous in the Toluca valley and its genetic diversity is extremely high, therefore natural infection was allowed; scoring took place weekly from 14 July to 1 October. Upon disease development, some compatible genotypes of *P. infestans* were isolated from these plants into pure culture. After the experiment in Mexico, we used these isolates in greenhouse and growth chamber experiments under containment conditions at Ithaca NY. The greenhouse experiments were conducted in the winter 2000 and in winter 2001 with strains isolated from Toluca (Mexico) as described above. There were two trials each winter. In the winter 2000, inoculation was with compatible isolate, Mx5. Disease was assessed at least twice a week, and the AUDPC (area under the disease progress curve) was calculated for each individual in the progeny. The same procedure was followed during the winter of 2001–2002, except that inoculation was done with compatible isolate, Mx4. Growth chamber experiments were conducted in 2002. Cuttings from the BCB progeny were rooted and planted in soil. Six-week-old plants were placed in growth chambers and kept at 100% relative humidity, 12 h photoperiod and 15–18°C. The plants were inoculated with compatible isolate Mx3, and disease severity was scored 10 and 13 days after inoculation. The field experiment in 2000 was conducted as described (above) for the BCT progeny, using US11-66, a NY isolate of *P. infestans.*

Disease severity was measured as percentage of tissue affected. For epidemics that occurred over time, the area under the disease progress curve (AUDPC) was calculated as described by Shaner and Finney (1977). Absolute values were then transformed into relative AUDPC (rAUDPC) as described by Fry (1978), which allowed for comparison of disease severity among different inoculations. When two simultaneous replications of a single experiment were highly correlated, the phenotypic observations were averaged prior to data analysis. Growth chamber experiments with Mx3 were evaluated only once and therefore AUDPC score cannot be calculated. In this case percent disease was transformed into relative value by dividing observed values for each individual by the maximum value detected for the progeny and multiplying by 100. The rAUDPC data (or relative percent of disease for Mx3) for each individual and each inoculation were then input to the mapping software as "trait" data.

Data analysis

RFLP mapping data for both progenies were obtained directly from the Solgenes database (http://www.sgn.cornell.edu/cview/map.pl?map_id=3). When possible, markers common to both progenies were used to allow better comparison of results. The genotypic information was processed with MapManager QTX version 0.30 (Manly et al. 2001) to organize markers into chromosomes for each backcross progeny. New markers developed for chromosome X (Rauscher et al. 2006) were added to the molecular map using the same software. Due to the heterozygosity of the parents, segregation of alleles from both parents can be observed for some markers. Therefore, we have developed maps with 24 chromosomes to accommodate this segregation. In MapManager the arbitrary-cross option was selected, which takes into account heterozygosity in at least some loci of one of the parents and thus is closest to the actual segregation observed in the two mapping populations. We also allowed for distortion in segregation of alleles, which was observed in these mapping populations (Bonierbale et al. 1994). Map data were then exported into QGene software for QTL mapping.

QGene version 4.3.2 (Joehanes and Nelson 2008) was used to locate QRL and perform permutation tests to determine QRL significance thresholds for the LOD (logarithm of the odds) scores. Quantitative trait loci were identified using the composite and multiple-interval mapping procedures (CIM, MIM). In order to create the most parsimonious model, stepwise cofactor selection was performed for each trait. When more than one replication was performed in a single or multiple environments with the

Table 3 QRL to late blight in the BCT and BCB progenies

Chr	Prog	Allele[a]	Position	Add effect[a,b]	LOD[c]	%Var	Isolate
I	BCB	Br	TG71–TG310	−6.8 to −25.9	1.9[d] to 5.8[e]	15	Field99, Mx3, Mx5
II	BCB	T	TG141A–GP102	2.3 to 4.5	2.2[f]	12	Mx5
III	BCT[g]	B	TG130–TG74	−6.5	3.3[e]	34	US8-98
III	BCT[g]	Tr	TG135–TG130	−5.6 to −11.4	2.0[d] to 3.6[e]	26 to 31	US8, US11, Mx5
V	BCB	T	T101–CD127	4.6 to 6.1	2.6[f] to 3.8[e]	6 to 27	Mx5-Mx3
V	BCT	Tr	TG441–TG379	−5.9	1.9[d]	17	US11-01
VI	BCT[g]	B	CT119–TG231	−5.8	2.1[f]	22	Mx5
VII	BCT	B	TG20B–TG572	−4.0 to −5.8	2.1[f]	20	US8
IX	BCT	B	TG404–TG421	8.6	2.6[d]	9	US11-00
X	BCT	B	$R_{Pi\text{-}ber}$	−4.3 to −43.1	2.8[e] to 43.9[e]	13 to 93	US8, US11, Mx5
XII	BCB	T	TG296	3.6 to 5.4	1.9[d] to 2.6[f]	10 to 12	Mx3

Main effect QRL found in both progenies are organized by chromosomes

Chr chromosome, *Prog* backcross progeny, *allele* the origin of the allele modifying the phenotype related to the effect, *position* markers surrounding the QTL interval, *add effect* identifies size of additive effect of the QRL (positive and negative values reflect the parental origin of the allele, recurrent and donor parent, respectively), *LOD* LOD scores for QRL found in the correspondent interval, *%Var* the percent of phenotypic variation explained by a QTL in that position (R^2), *isolate* isolate for which QRL was identified. The two digits following the isolate name correspond to the last two digits of year the QRL identified was significant, if no year is specified, the QRL was found in all repetitions with that particular isolate

[a] Presence of the stated allele (B, *S. berthaultii* allele; Br, *S. berthaultii* recurrent allele; T, *S. tuberosum* allele; Tr, *S. tuberosum* recurrent allele) corresponds to the resistance if the additive effect is negative or susceptibility if the effect is positive

[b] Estimates from composite interval mapping (CIM)

[c] Only QRL significant at genome-wise $p < 0.1$ are shown

Significance shown as [d] $\alpha = 0.1$, [e] $\alpha = 0.01$, [f] $\alpha = 0.05$, [g] shows instances where the QRL were found on analyses of the susceptible subset

same isolate, significance of environmental effect on QRL (QRL × E) was tested with QGene. In order to further analyze quantitative resistance in BCT a susceptible subset was created by eliminating all individuals carrying $R_{Pi\text{-}ber}$.

The significance threshold for QRL was determined empirically by a permutation test for each trait, with 1,000 iterations (Churchill and Doerge 1994). Genome-wide significance levels were calculated for each QRL and categorized as suggestive ($\alpha = 0.1$), significant ($\alpha = 0.05$) or highly significant ($\alpha = 0.01$). When QRL were detected on alleles that segregate from both parents, two-way ANOVA was carried out with GraphPad Prism version 5.02 (GraphPad Software, San Diego, CA) to check for intra-locus interaction. Given the strong influence of $R_{Pi\text{-}ber}$, the test was performed on the susceptible subset only.

Results

P. infestans isolate characterization

The six isolates of *P. infestans* used for this study have complex virulence and aggressive pathogenicity. Collection and phenotypic data, including race phenotype (compatibility with R-genes) of the isolates are summarized in Table 1.

Quantitative resistance

The QRL thresholds determined by permutation analysis (Churchill and Doerge 1994) correspond well with those suggested by van Ooijen (1999). Genome-wide significance levels were determined independently for each population. For the BCT population average thresholds were LOD 1.92 at $\alpha = 0.1$; LOD 2.24 at $\alpha = 0.05$, and LOD 2.92 at $\alpha = 0.01$. For the BCB population the respective values were 1.79, 2.06, and 2.70. Significances reported in Table 3 are based on the specific result for each trial.

Resistance in BCT progeny

The BCT progeny was tested in the field in 1997, 1998, 1999, 2000 and 2001 and in the greenhouse in the winter of 2001/2002. The results for the 1997 and 1998 seasons have already been published (Ewing et al. 2000) but are included in Tables 2 and 3 for comparative purposes. A distribution of relative resistance and summary of the results are presented in Fig. 1 and Table 2. Since results of replicates from the same experiment were similar (correlation from 0.76 to 0.92), averages of the replicates were used for QRL mapping. Correlation between experiments with the same isolate in different years was also high ($r = 0.80$ for US8

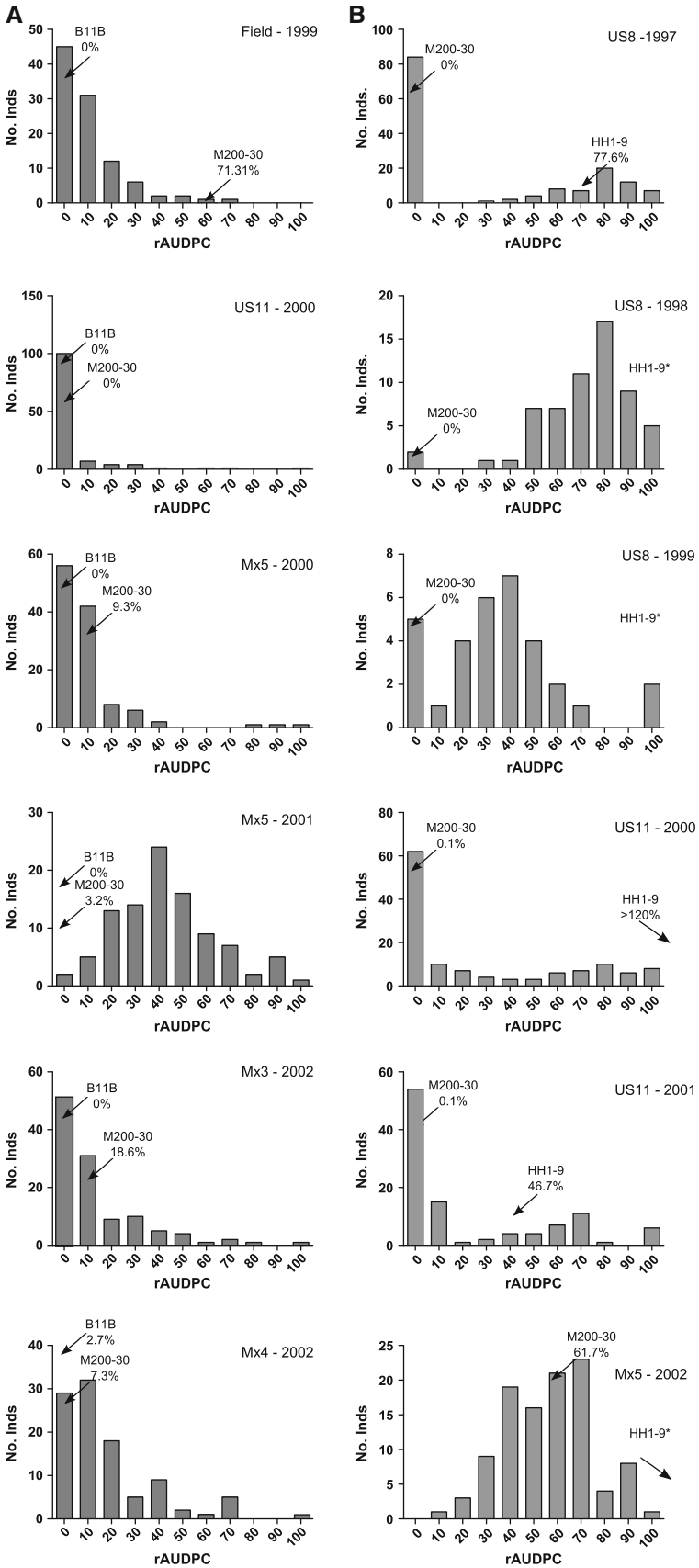


Fig. 1 Frequency distribution of rAUDPC score for individual trials. Each graph shows the distribution of individuals in 10% increments and the bin location of both immediate parents M200-30 and B11B for BCB and M200-30 for HH1-9 for BCT. *Left column* (**a**) illustrates distributions for the BCB trials, while *right column* (**b**) shows data for the BCT trials

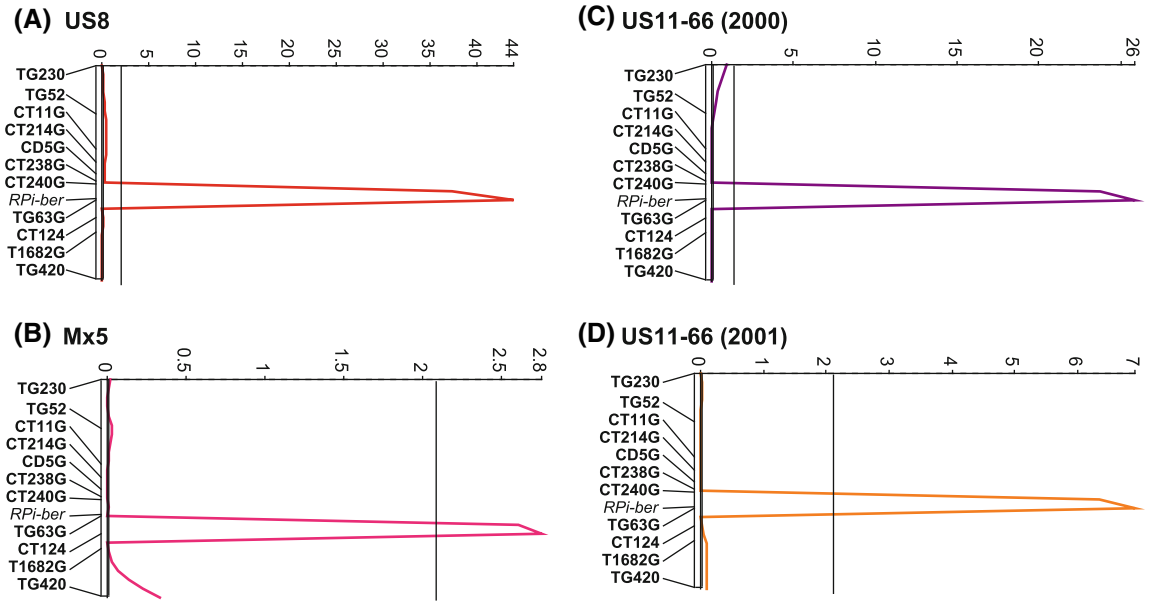


Fig. 2 Multiple interval mapping for QRL on chromosome X. LOD score for a QRL located around $R_{Pi\text{-}ber}$ on interactions with **a** US8 (US940480), **b** US11 (US990025 and US980066) in 2000, **c** US 11 (US980066) in 2001, and **d** Mx5 (Mx990005). The markers are indicated on the *left* of the chromosome; the *X*-axis shows LOD scores. The *black line* to the right of the *Y*-axis indicates the significance threshold ($\alpha = 0.05$) for each trial. Note the difference in scale for each graph

and $r = 0.66$ for US11). Disease symptoms developed rapidly when the Mx5 isolate was used in the greenhouse trial and the most susceptible clones died in just a week.

Effect of $R_{Pi\text{-}ber}$ in BCT

For all isolates (US8, US11-25, US11-66 and Mx5) the most significant QRL was observed at or around $R_{Pi\text{-}ber}$ on chromosome X (Fig. 2). After inoculation in 2000 we detected that isolate US11-25 had low aggressiveness and was incompatible with $R_{Pi\text{-}ber}$. Subsequently, US11-66 isolate [highly aggressive and compatible (causing large sporulating lesions in detached leaflet assays and also in field trials) with $R_{Pi\text{-}ber}$] was used to inoculate plants. Therefore, the phenotypic data in 2000 reflect the combined effect of both isolates. The effect of $R_{Pi\text{-}ber}$ in this experiment was large and highly significant, with LOD of 25.8. The clones carrying the *S. berthaultii* allele developed on average only 35% as much disease as those plants homozygous for the *S. tuberosum* allele. However, 38% of the plants carrying $R_{Pi\text{-}ber}$ developed some kind of symptoms and 52% of those showed rAUDPC scores between 5 and 27%, thus indicating that the major gene effect of $R_{Pi\text{-}ber}$ was much reduced against isolate US11-66. In 2001, when only US11-66 was used for inoculation, the effect of $R_{Pi\text{-}ber}$ was smaller, as expected with a compatible isolate, but still highly significant (Fig. 2) with LOD of 7.00 and a decrease in rAUDPC of 36.27%. The multiple-trait multiple-interval mapping (QRL × E) approach used on the same data confirmed the strong main effect of this QRL (LOD of 11.1). When the most aggressive isolate (Mx5) was used in the greenhouse test, a QRL was found between $R_{Pi\text{-}ber}$ and CT124 (Fig. 2) with a significant LOD = 2.83, and a decrease of 14% rAUDPC.

QRL in BCT

In order to detect other QRL in the BCT progeny, we controlled for the effect of $R_{Pi\text{-}ber}$ by selecting it as a cofactor, and then searched for other QRL of lesser effect through composite and multiple-interval mapping. If a QRL was detected in the same map location in more than one experiment (i.e., different environments or different isolates), it is referred to as a locus with "broad-spectrum" effect (Table 3). A QRL specific to a given isolate (but in different environments), or detected in only one isolate/environment combination, is referred to as a locus with "specific" effect (Table 3; Fig. 2).

The second largest QRL effect (after that at the $R_{Pi\text{-}ber}$ region) was found on chromosome III, close to the marker TG135. This broad-spectrum resistance locus was detected in different environments and against all isolates of the pathogen. The same QRL was previously identified by Ewing et al. (2000; Fig. 3) with the US8 lineage. This QTL explained about 16% of the variation with the US8 isolate, and was also observed with US11 in 2000, although in this year the effect did not reach significance threshold (LOD 1.5). The effect of this QRL varies depending on the

combination of alleles present at the locus and the pathogen isolate. Intralocus interactions were not significant when assessed by two-way ANOVA, but the presence of the recurrent *S. tuberosum* allele (Tr) had a highly significant effect in every trial ($p \leq 0.004$). For all tests with US8, the presence of the *S. berthaultii* allele (B) and the Tr allele showed highly significant effects, accounting for 23–42% of the total variance on the susceptible subset. In contrast, when US11 was used for inoculation, lower disease development was correlated with the presence of the recurrent tuberosum allele (Tr), and the presence of the B allele did not significantly influence the results (Fig. 4).

Another QRL was detected on chromosome V, linked to CD31. In this locus a significant effect of the Tr allele was observed for tests with US11 ($p < 0.05$), while the effect of the B allele, or intralocus interactions were not detected. Putative minor QRL ($R^2 < 0.1$) with broad-spectrum effects were found on chromosomes VII (TG20b) and XI (TG44). Both of these loci were consistently detected with US8 and US11 isolates, but did not reach significance (LOD 1.4–1.6, data not shown).

Some QRL appear to have isolate-specific effects. For example, in the BCT progeny a QRL linked to marker TG71 on chromosome I was previously detected with the US8 isolate (Ewing et al. 2000). This locus was not detected with other isolates used in the current study. Other QRL with minor effects found in specific environments are listed in Table 3. With only one exception the presence of the *S. berthaultii* allele in heterozygous condition was correlated with lower disease rating. The only exception is a small but significant QRL found between TG404 and TG421 on chromosome XI with US11 in 2000. In this locus the presence of the B allele was correlated with a small increase in rAUDPC (about 9%) and therefore higher susceptibility (Table 3).

Resistance in BCB progeny

In general, the BCB progeny were highly resistant to *P. infestans*. This high resistance was observed in all experiments (field, greenhouse and growth chamber) (Table 3). However, there was transgressive segregation towards susceptibility since some progeny developed more disease than either one of the parental clones (Fig. 1). In most experiments with the BCB progeny, the results were skewed toward resistance.

QRL in BCB

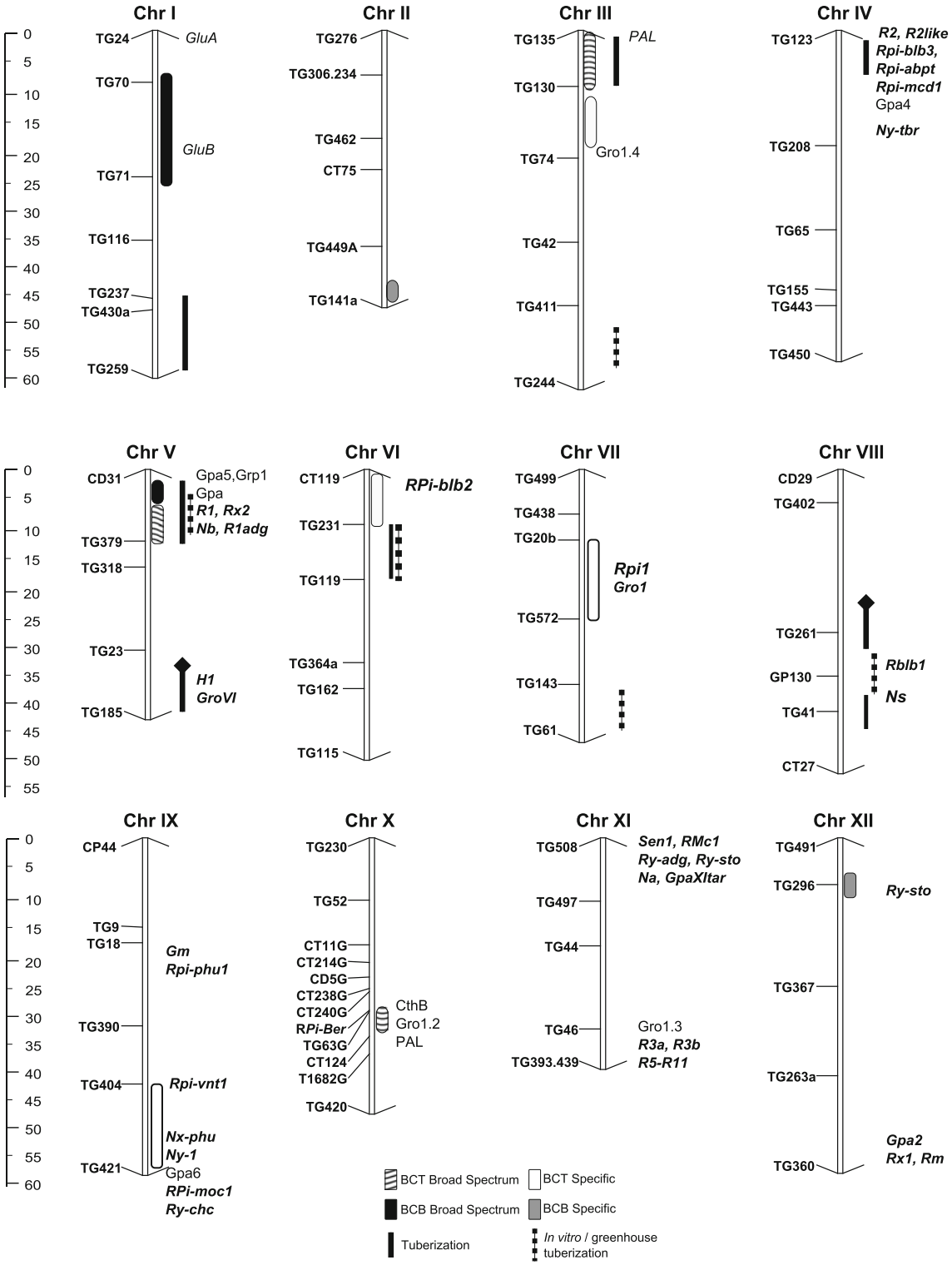
The high degree of resistance in the BCB progeny dramatically decreased the range of phenotypes, and therefore reduced the opportunity to detect significant QRL. In some cases, such as the first trial of the experiment in 2000, no significant QRL were found when the probability for a type I error (probability of false positive linkage) was set to $\alpha = 0.05$. Despite this high level of resistance, we did identify two broad-effect QRL in some experiments, but they had different effects. For the QRL located between markers TG71–TG310 on chromosome I, the presence of the recurrent *S. berthaultii* allele (Br) was associated with resistance. While, for the QRL between markers T101 and CD127 on chromosome V the presence of the *S. tuberosum* allele was associated with susceptibility. We also detected isolate-specific QRL in the BCB. These were located on chromosomes II, and XII (Table 3). In both cases, the presence of the *S. tuberosum* allele was associated with increased susceptibility.

Discussion

$R_{Pi\text{-}ber}$ effect on quantitative resistance

A possible residual effect of an R-gene in the presence of a compatible isolate has been suggested more than 30 years ago. Nelson (1978) then reviewed the work by many researchers reporting the "ghost" effect of such major genes in different pathosystems. Furthermore, the author proposed pyramiding all *S. demissum* R-genes in the same variety of *S. tuberosum* to attain a level of resistance similar to that found in *S. demissum* (Nelson 1978). Thus, the idea of a defeated R-gene having a residual effect is not new. However, it is now easier to test this hypothesis, given the availability of molecular maps and our current knowledge of the chromosomal location of R-genes (Tan et al. 2008; Trognitz et al. 2002).

In this study we show that a region on chromosome X flanking $R_{Pi\text{-}ber}$, provides quantitative resistance to late blight in the presence of compatible isolates. The resistance effect in this region was observed in experiments performed both in the field and in the greenhouse (Fig. 2). The finding is consistent with previous reports that tested the same plant–pathogen interaction. Stewart et al. (2003) found a significant contribution to resistance from *R1*, *R10* and *R11* when plants were inoculated with a compatible isolate. Delayed onset of disease has been reported in plants carrying *R2* (Pilet et al. 2005). As with our data, both studies report that the magnitude of the effect changed in different years. Other mapping studies report coincidence of R-gene location and QRL for late blight. For example, QRL coincided with *R1* in diverse populations (Leonards-Schippers et al. 1994; Oberhagemann et al. 1999; Simko 2002). Similarly, *R2* and the cluster of R-genes on chromosome XI correspond with QRL for late blight resistance (Oberhagemann et al. 1999). In *S. microdontum*, Tan et al. (2008) show a positional correlation between $R_{Pi\text{-}mcd1}$ and quantitative resistance.



Co-localization of R-genes with QRL have also been reported in other pathosystems (Bai et al. 2003; Geffroy et al. 2000; Perchepied et al. 2005b). These co-localizations are consistent with the hypothesis that specific and broad-spectrum resistance may be controlled by the same alleles (Meksem et al. 1995), and that qualitative phenotypes could result from extreme allelic variants of QTLs (Robertson 1985).

Alternatively, our observations may be explained by the effects of other resistance-related genes tightly linked to the R-gene. In our situation, *PAL*, *ChtB* and a regulatory gene involved in the expression of flower color (*F*, van Eck

◀ **Fig. 3** Location of QRL to late blight and other pathogens on the potato molecular linkage map: Potato chromosomes showing positions of QRL to late blight and other resistances, defense-related genes and tuberization (maturity). Chromosome size is shown by the *bar in the left* (in cM), framework markers from the BCT progeny distributed at an average of 10 cM are shown at *left*. The *length of the bars* show the QRL interval. Relative positions of resistance and defense-related genes mapped in potato are shown on the far right of each chromosome. Proper chromosome alignment was done by visual inspection and alignment of previously published maps: Tanksley et al. (1992), Gebhardt et al. (1991) with the makers cited by the specific reference for each gene. Ny_{tbr}: Celebi-Toprak et al. 2002; $R_{Pi\text{-}mcd1}$: Tan et al. 2008; Ry_{adg} *PLRV*: Velásquez et al. 2007; *Ns*: Marczewski et al. 2002; *Rpi-vnt1*: Pel et al. 2009; *RPi-moc1*: Smilde et al. 2005; *Gm:* Marczewski et al. 2006; Ry_{chc}: Sato et al. 2006; *Ny-1*: Szajko et al. 2008; $R_{Pi\text{-}ber}$: Ewing et al. 2000; Rauscher et al. 2006; $GpaXI^{l}_{tar}$:Tan et al. 2009; *Rm:* Marczewski et al. 2006; Ry_{sto}: Song et al. 2005. Specific references for genes: *R2*, *R2*-like, *RPi-abpt*, *RPi-blb3,* R1, *RPi-blb2*, *Rpi1*, *RPi-blb1*, *Rpi-phu1,* R3 (*R3*a, *R3*b), *R6*, *R7*, *R5*–*R11* can be found in Park et al. (2009). The R-genes: *Rx2*, *Nb*, *H1*, *GroVI*, *GroI*, Nx_{phu}, *Sen1*, *Rmc1*, Ry_{adg}, Ry_{sto}, Na_{adj}, *Rx1*, *Gpa2* and the QRL: Gro1.4, Gpa4, Gpa5, Grp1, Gpa, Gpa6, Gro1.2, Gro1.3 are referenced in Gebhardt and Valkonen. (2001). Map position for tuberization was determined by van den Berg et al. (1996) and Simko et al. (1999) mapped in BCT/BCB; the *bars with a diamond on top* indicate areas where early tuberization is correlated with the ST allele. Only QTLs with LOD >2.5 are illustrated

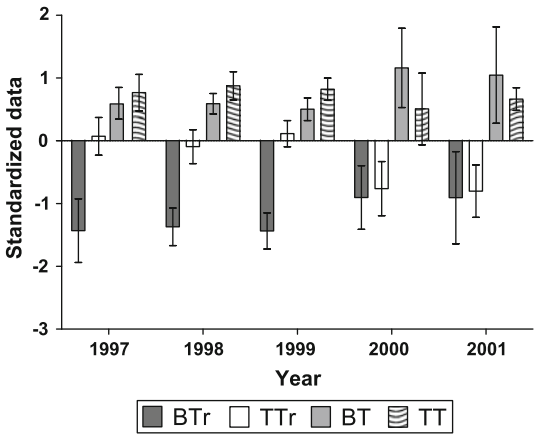


Fig. 4 Effect of allele combination at TG135. Standardized data show the effect of the presence of the *S. berthaultii* (*B*), *S. tuberosum* (*T*) or *S. tuberosum* recurrent (*Tr*) alleles for all trials with US8 (1997–1999) and US11 (2000–2001). The *bars* show the standard error for each year

et al. 1993) are located in close vicinity to $R_{Pi\text{-}ber}$ and have been suggested as having a role in resistance (Büchter et al. 1997; Trognitz et al. 2002). Additionally, there could be a clustered family or allelic variants at the same locus (Ballvora et al. 2002).

Specificity of QRL

We found QRL of diverse specificity in both progenies. The locus of $R_{Pi\text{-}ber}$ was by far the most important and provided some level of resistance to the compatible US11 and Mexican isolates. Additionally, QRL linked to TG71 on chromosome I, to TG135 on chromosome III, to T101-CD127 on chromosome V and to TG296–TG367 on chromosome XII were effective against more than one isolate. The effect was usually the same, with resistance being associated with the presence of the B allele.

In general, however, most QRL in the BCB and BCT were specific to particular strains of *P. infestans*. Of the 11 significant QRL detected, 7 were isolate specific. Other studies in potato have reported similar results with respect to specificity. Leonards-Schippers et al. (1994) found 11 QRL, of which several were isolate specific. Oberhagemann et al. (1999) show stability of QTL across environments and testing methods but show several QRL with an isolate-specific effect when testing races *R0*, *R1* and *R1–11* in a single population. Analysis of results from several independent studies indicates that the proportion of congruent QRL detected in different trials, but on the same mapping population inoculated with the same isolate of *P. infestans* is only about 0.52 (Simko 2002). In general it seems that some QRL of large effect are detectable in different environments and effective against diverse isolates. Other QRL (typically of small effect) may be detected only in some environments and sometimes against a subset of isolates. This situation has also been observed for pathosystems involving fungi (Arru et al. 2003; Ballini et al. 2008; Calenge et al. 2004; Marcel et al. 2008) and virus (Caranta et al. 1997).

There was diversity in the stability of QRL over environments. The most stable QRL across environments was at the locus of $R_{Pi\text{-}ber}$. In the BCT progeny, this locus provided resistance in both field and greenhouse conditions. Other QRL with stability across environments included those at TG135 on chromosome III and at T101 on chromosome V. However, for other QRL we found the effect to be significant to only one of the experiments or replicates. Variation in the localization and significance of QRL can be due to differences in environmental conditions that affect the development of late blight epidemics in different replicates (Harrison and Lowe 1994; Simko 2002). Even for those major QRL a difference in the magnitude of effect was found in different years when using the same isolate. For example, when the plants were inoculated with US11, the QRL located at $R_{Pi\text{-}ber}$ was associated with 65% less disease in 2000; but with 36% less disease in 2001 (Table 3; Fig. 3). Isolate and day-length effects have been previously reported on QRL for late blight on the BCT progeny (Mihovilovich et al. 2010). These observations are common to the pathosystem (Stewart et al. 2003), and confirm the effect of environmental factors in expression of QRL effect (Mackay 2001).

With only one exception, QRL were specific to just one progeny. The exception was the QRL at TG441–TG379 on chromosome V. In the BCB the T allele was associated

with increased susceptibility, whereas in the BCT the Tr allele was correlated with increased resistance.

Association of late blight QRL with other traits

Resistance to other pathogens

Most of the late blight QRL reported here coincide with positions where resistance genes or QRL for *P. infestans* as well as QRL for other pathogens have been reported (Fig. 3) (Park et al. 2009; Simko 2002). However, the BCB-specific QRL at TG141A is reported here for the first time. Previous reports have identified positional associations between resistance to *P. infestans* and resistance to *Globodera* spp in both potato and tomato (Gebhardt and Valkonen 2001; Grube et al. 2000). Several late blight QRL that we identified in the present study also occur in locations which contain *Globodera* resistance (Fig. 3). These findings are consistent with the hypothesis that the genetic locations of resistance genes are conserved through speciation in the Solanaceae (Grube et al. 2000).

Maturity

A major concern regarding the QRL for late blight is undesirable linkage between resistance and late maturity. Previous studies of the two mapping populations have identified several QTL for tuberization (Ewing et al. 2004; Simko et al. 1999; van den Berg et al. 1996), which is considered to be a good indication of plant maturity (Lorenzen and Ewing 1990). Only two areas, near markers TG135–TG130 on chromosome III and CD31 on chromosome V were found to be related to both traits (Fig. 3). The presence of the Tr allele at both locations is related to late tuberization and resistance to late blight.

The correlation of late blight resistance with late maturity has been studied extensively (Collins et al. 1999; Śliwka et al. 2007; Visker et al. 2003), but unfortunately, it is still unclear if the association of the two effects is due to pleiotropy as suggested by Visker et al. (2003) or due to different effects of closely linked genes (Bormann et al. 2004).

Pigmentation and plant defense genes

Anthocyanin biosynthesis has been correlated with plant defense in several pathosystems (Harborne 1986; Huang and Backhouse 2005; Johnson et al. 2008). This may be because the anthocyanin biosynthetic pathway is also involved in producing defense compounds. Additionally, such genes have been shown to activate upon potato infection with *P. infestans* (Tian et al. 2006). In concert with these associations, we found QRL for resistance in areas where genes for anthocyanin production or regulation are located. The largest effect is found around TG63, where $R_{Pi\text{-}ber}$ tuber epidermal color loci (I_{ep}, I_{co}) and flower color (F) are also located (van Eck et al. 1993). Additionally, we found a smaller QRL on chromosome XI near locus TG30, where a locus involved in blue-purple anthocyanin biosynthesis was detected (P; Jung et al. 2005; van Eck et al. 1994).

Implications for breeding

The co-localization of field and R-gene resistance suggests that Nelson's concluding advice (1978) is valuable. He said: "go back young man and gather up your weary and defeated resistance genes of the past, take your currently successful genes, find some new ones if you can and build yourself a genetic pyramid". Clearly more research is needed to determine if there is a residual effect of an R-gene against compatible isolates. However, if QRL and R-genes are co-located, there could still be benefit from combining these alleles in a cultivar.

Acknowledgments This study was supported by Syngenta. We want to thank John Cianchietti, Kevin Myers, Barbara Esposito, Phoi Tran, and Karen Moravec for their help on the field experiments. Clare Nelson provided valuable insight on data analyses. We also wish to thank Jason Rauscher for critical reading and suggestions on the manuscript. Thanks also to Hector Lozoya-Saldaña and PICTI-PAPA for supporting field research in Mexico.

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