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ORIGINAL PAPER

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Late blight resistance linkages in a novel cross of the wild potato species *Solanum paucissectum* (series *Piurana*)

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Abstract The cultivated potato, *Solanum tuberosum*, is affected by a variety of diseases with late blight, caused by *Phytophthora infestans*, being the most severe. Wild potato species have proven to be a continuing source of resistance, sometimes of an extreme type, to this disease. The present study constructs the first late blight linkage map of a member of series *Piurana*, *S. paucissectum*, a tuber-bearing relative of potato, using probes for conserved sequences from potato and tomato. Eight probes mapped to unexpected linkage groups, but syntenic differences with prior maps of potato were not supported by any blocks of rearranged chromosome segments. All 12 linkage groups were resolved and significant associations with late blight resistance were found on chromosomes 10, 11 and 12. A major quantitative trait locus (QTL) on chromosome 11 accounts for more than 25% of the phenotypic variance measured in a field trial. Crossing of *S. paucissectum* with cultivated potato resulted in very few seeds indicating partial reproductive barriers. Differential reactions of accessions of this potential donor species with simple and complex isolates of *P. infestans* suggest that it carries major resistance genes that are not those previously described from the Mexican species, *S. demissum*. However, the additivity of the QTL effects argues for the quantitative nature of resistance in this cross.

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Introduction

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is one of the most important diseases affecting the potato crop (*Solanum tuberosum* L.) worldwide. Late blight destroys the foliage, stems and tubers of growing plants, causing severe losses. The first and most devastating late blight outbreak recorded occurred in Ireland in the 1840s, resulting in famine and migration of more than a million people (Salaman 1985). Control of the disease relies on fungicide application, which increases agricultural inputs and environmental and health risks. Where fungicides are not affordable, total crop loss can result.

Resistance to late blight was incorporated into cultivated potato during the first decades of the twentieth century by repeated backcrossing with the resistant wild species *S. demissum* (Umaerus et al. 1983). These crosses resulted in the incorporation of at least 11 *R* (resistance) genes (Malcolmson and Black 1966; Shaw 1991) into cultivated potato. These genes are classified as *R1* to *R11* and confer race-specific resistance (Mastenbroek 1953; Malcolmson and Black 1966).

The first complete genetic map of potato was reported by Bonierbale et al. (1988). They used tomato RFLP probes to demonstrate a high degree of conserved linkage between potato and tomato. Gebhardt et al. (1989) produced the second potato map through the use of potato probes. These studies and Gebhardt et al. (1991) aligned the two genomes and made both sets of markers useful for common mapping studies. Since then, additional markers have been placed on potato and tomato for high-density maps (Tanksley et al. 1992), and the markers developed by several groups have been used extensively to map quantitative trait loci (QTLs) and genes related to late blight and other diseases (Gebhardt and Valkonen 2001).

Five of the 11 known *R* genes from *S. demissum* have been mapped: *R1* (Leonards-Schippers et al. 1992), *R2* (Li et al. 1998), *R3* (El-Kharbotly et al. 1994; Huang

et al. 2004), *R6* and *R7* (El-Kharbotly et al. 1996). Recently Kuhl et al. (2001) mapped a gene from *S. pinnatisectum* named *Rpi1* to chromosome 7. Their results indicated that *Rpi1* was either not one of the 11 known *R* genes or possibly corresponded to *R9*. Gebhardt and Valkonen (2001) summarized that QTLs and *R* genes related to late blight resistance can be found on every potato chromosome. Two major *R* genes have been cloned and sequenced. The gene *RB* has been mapped to chromosome 8 of *S. bulbocastanum* and cloned using map-based approaches (Naess et al. 2000; Song et al. 2003; Van der Vossen et al. 2003). The *R1* gene, previously mapped to chromosome 5 (above), has been cloned using map-based approaches (Ballvora et al. 2002).

The "European" or "Irish" potato has a narrow genetic base (Mendoza and Haynes 1974; Ross 1986; Glendenning 1987) and wild species have great potential to improve the crop for a range of traits. Researchers are actively screening the wild species for sources of new resistance genes (Van Soest 1984; Jansky 2000; Pérez et al. 2001a), and there is a need to continuously develop new sources to keep pace with the dynamic pathogens like *P. infestans*. Potato (*Solanum* L. section *Petota* Dumort.) has somewhat less than 200 wild species relatives (Spooner and Hijmans 2001) representing diverse genetic pools. Spooner and Sytsma (1992) and Spooner and Castillo (1997) established a molecular phylogenetic framework that distinguished four major clades in potato, in contrast to prior hypotheses that divided sect. *Petota* into as many as 36 or, more recently, 21 series (Hawkes 1990; Spooner and van den Berg 1992). *S. demissum* and most of the sources of late blight resistance that have been mapped (reviewed in Simko 2002; Bonierbale et al. 2003) are members of clade 4; *S. bulbocastanum* (Song et al. 2003; Van der Vossen et al. 2003) is in clade 2; and *S. pinnatisectum* (Kuhl et al. 2001) is in clade 1. This article reports the first genetic map and late blight resistance loci involving species from clade 3, the *Solanum* series *Piurana* clade (Spooner and Castillo 1997).

Materials and methods

Plant material

Solanum chomatophilum Bitter and *S. paucissectum* Ochoa are diploid, outbreeding members of the *Solanum* ser. *Piurana* clade, clade 3 of Spooner and Castillo (1997), hereafter referred to simply as *Piurana*. A 200 offspring BC_1 progeny composed of *S. paucissectum* PI 473489-1 (late blight resistant)/*S. chomatophilum* PI 310991-1 (late blight susceptible)//*S. chomatophilum* PI 310991-1 was generated. We hereafter refer to the F_1 parent (*S. paucissectum* PI 473489-1/*S. chomatophilum* PI 310991-1) of this cross as MP1-8 and the backcross progeny as PCC1. Parental and progeny individuals were germinated from seeds and grown under in vitro conditions for propagation and transfer to the International Potato Center (CIP, Lima, Peru). The recurrent parent *S. chomatophilum* PI 310991-1 was infected with virus and could not be used in subsequent field experiments.

Two accessions of *S. paucissectum* (CIP 762124 and CIP 762126, originally from the Department of Piura in Peru) and two of *S. chomatophilum* (CIP 762611 and CIP 762568, Department of Cajamarca, Peru) were obtained as true seeds from the germplasm collection at CIP. Forty genotypes of each accession were established in vitro. Following the resistance evaluation, selected genotypes were intercrossed and crossed with diploid cultivated potato genotypes to test crossability of the *Piurana* clade (Table 1).

Phytophthora infestans isolates

The parents of PCC1 were previously screened by a detached leaf assay (DLA) in Madison, Wisconsin, USA with *P. infestans* isolate MSU96, which is of the A2 mating type and belongs to the US-8 clonal lineage

Table 1 Crossability of members of the *Piurana* clade with cultivated potato showing attempted and successful intra- and interclade combinations[a]

Cross type	No. of cross combinations	No. of flowers crossed	Total berries	Total seeds	Average seeds/berry
chm × 2*x* cult	131	1,119	614	0	0.0
2*x* cult × chm	189	1,330	228	0	0.0
chm × chm	334	3,034	1,620	234,442	144.7
pcs × 2*x* cult	145	1,452	625	0	0.0
2*x* cult × pcs	67	428	109	55	0.5
pcs × pcs	404	3,845	2,587	275,141	106.4
2*x* cult × 2*x* cult	47	265	193	35,400	183.4

[a]The accessions included *S. chomatophilum* (chm; 21 genotypes of CIP 762661), *S. paucissectum* (pcs; eight genotypes of CIP 762124 and 14 of CIP 762126) and diploid cultivated accessions (2× cult; composed of three diploid breeding lines (2×) and 15 *S. tuberosum* Phureja group genotypes). *S. chomatophilum* was crossed with nine Phureja group clones (three genotypes of PI 225678, two of PI 320362, ASO 862, CHS 625, CCC 81 and CCC 4243) and two diploid breeding lines (HH1-9 and USW 2230). *S. paucissectum* was crossed with Phureja group clones (CIP 703514, CIP 703572, CIP 703800, CIP 703812, CIP 705807, CIP 706784, CIP 706788, ASO 862, CHS 667, three genotypes of PI 225678, one genotype of PI 225706 and two genotypes of PI 320362) and three diploid breeding lines (HH1-9, USW 2230 and PS 3. HH1-9 (CIP 780974) and USW 2230 (CIP 781441) are referenced in Bonierbale et al. (1994) and PS 3 (CIP 591061-19) in Ghislain et al. (2001). Specific genotype codes and decomposed cross list are available from the authors

(Kuhl et al. 2001). Similarly, the pedigree clones contributing to PCC1 and *Piurana* accessions available in the germplasm collection at CIP were assessed using DLA against four isolates from Peru ('PE84006', 'POX-067', 'PCO-002' and 'PCO-093'), since it was not considered prudent to introduce foreign isolates. These isolates are members of the newly migrating pathogen population (Fry et al. 1993) that also predominates in the US, although only the A1 mating type has been reported in Peru (Pérez et al. 2001b). Previous characterization according to Pérez et al. (2001b) demonstrated that 'PE84006' is ''race 0'', i.e., avirulent on all 11 known *S. demissum* major genes and belongs to the US-1 clonal lineage [old population of *P. infestans sensu*, Spielman et al. (1991)]. 'POX-067', 'PCO-002' and 'PCO-093' are ''complex races'' from the EC-1 clonal lineage [new population *sensu*, Spielman et al. (1991)], with avirulences $Avr_{8,9}$, $Avr_{5,9}$ and $Avr_{5,8,9}$, respectively, on the 11 known *S. demissum* major genes. PCO-093 was isolated from a PCC1 genotype in Comas, Peru (11°05′S, 75°02′W, 2,800 m) and the former two from susceptible cultivars in Oxapampa, Peru (10°35′S, 75°24′W, 1,810 m) (POX-067) and Comas (PCO-002). All isolates are resistant to metalaxyl (Pérez et al. 2001b).

The isolates were recovered from storage in liquid nitrogen and increased on tuber slices of potato cv. 'Huayro' at 18°C to promote sporulation. Innoculum was prepared by washing the infected tuber slices with distilled water and diluting the filtered rinsate to 3,000 sporangia/ml as described previously (Pérez et al. 2001b).

Assessment of late blight resistance in PCC1 progeny under high disease pressure in the Andean highlands

A total of 184 genotypes of PCC1, MP1-8 and *S. paucissectum* PI 473489-1 were propagated in vitro to produce 25 plantlets of each. Plantlets were transferred to Jiffy strips (http://www.jiffyproducts.com) in mid-November 2002 and grown in the screenhouse at Huancayo, Peru (11°56′S, 75°15′W, 3,200 m) for 6 weeks and then transplanted to the field at Comas.

Plots of five plants per genotype (experimental unit) were planted at 30 cm within and 90 cm between rows in a randomized complete block design (RCBD) with four blocks. Double rows of the late blight susceptible potato cultivar 'Tomasa' and moderately resistant cultivar 'Perricholi' were established around each block to maintain inoculum throughout the growing season. Four potato cultivar controls ('Chata Blanca', 'Yungay', 'Monserrate' and LBr-40, respectively, susceptible, moderately susceptible, moderately resistant and resistant to late blight) were included to help quantify the resistance levels of the progeny and pedigree clones. The transplants were protected from late blight with a contact fungicide to facilitate establishment under high endemic disease pressure; this has no lasting effect in the field. *P. infestans* isolates at the field site are complex races representative of the EC-1 lineage as demonstrated previously by Pérez et al. (2001b). Percent leaf area infected (PLA) was recorded for each plot beginning 44 days after planting (DAP) and continuing at 5-day intervals until 84 DAP. Area under the disease progress curve (AUDPC) was calculated for each plot as described by Shaner and Finney (1977) using data from nine consecutive evaluations of PLA. AUDPC values underwent standard analysis of variance using SAS/STAT.

Cluster analysis was performed with the FASTCLUS procedure of SAS on the mean readings of PLA of each genotype taken on nine consecutive dates to graphically assess disease progress patterns of the PCC1 progeny with respect to its parents and the experimental controls.

Due to the evidence of non-uniform disease progress during the epidemic, two sets of AUDPC values were derived: 'AUDPC2' represents disease progress observed from the first to the seventh evaluation (74 DAP) and 'AUDPC3' represents disease progress through the whole epidemic (84 DAP). Each variable drew on mean AUDPC values calculated for each genotype and was used independently in QTL analysis.

Assessment of late blight resistance in PCC1 progeny under controlled conditions

The PCC1 mapping progeny was evaluated for late blight resistance under controlled conditions in a screenhouse at La Victoria, Huancayo, Peru. Sporangial suspension (3,000 sporangia/ml) of the complex isolate POX-067 was refrigerated at 5–8°C for 1 h to promote the liberation of zoospores and then applied to 1–4 whole plants of each genotype until runoff using a hand-held sprayer. The female parent 'MP1-8', *S. paucissectum* 'PI 473489-1' and the same controls used in the field trial were included. Visual assessments of PLA were made 3, 5 and 7 days after inoculation and used to calculate AUDPC. This data set is referred to as 'AUDPCLV', for the testing site La Victoria (LV).

Assessment of late blight resistance in *Piurana* germplasm under high disease pressure in the Peruvian highlands

Accessions of *S. paucissectum* (CIP 762124 and CIP 762126) and *S. chomatophilum* (CIP 762611 and CIP 762568) were evaluated in two locations in Peru to assess the levels of late blight resistance available beyond the two genotypes used to generate the PCC1 progeny. In vitro plantlets of 40 genotypes per accession were propagated and acclimated in the screenhouse at Huancayo, for transplanting to field plots in Comas in December 2001, and in Oxapampa, Peru in June 2001. Experiments were established in RCBD of four blocks in each location. Controls, plot size and spacing were as described above. Disease severity was recorded as PLA

per plot beginning at 45 DAP and continuing at 5-day intervals throughout the vegetative period (87 DAP). ANOVA was performed on AUDPC values using SAS/STAT by location, and heterogeneity of variances tested using Bartlett's test before combined ANOVA was realized across locations. Combined ANOVA was performed using SAS/STAT. Broad-sense heritability of late blight resistance was estimated as a ratio of genetic variance (calculated from expected mean squares) to phenotypic variance.

Compatibility tests

Detached leaflets of the genotypes of two of the *Piurana* accessions used in the field experiments and PCC1 parents were inoculated with *P. infestans* isolates PE84006 (race 0), POX-067 and PCO-002 to determine compatibility. PCC1 parents were also tested with isolate PCO-093. Fully developed lateral leaflets were collected from plants of each of the 35 and 36 genotypes, respectively, of the *S. paucissectum* accessions CIP 762124 and CIP 762126 and from the parents of PCC1 (*S. paucissectum* 'PI 473489-1' and *S. chomatophilum* 'PI 310991-1') grown under screenhouse conditions in Huancayo. Six leaflets were inoculated for each genotype–isolate combination. Two leaflets were placed in Petri dishes with 1.5% water agar on the lid and inoculated by placing one 20 µl droplet of sporangial suspension (3,000 sporangia/ml, previously refrigerated at 5–8°C to promote the liberation of zoospores) on the abaxial side of each leaflet. Petri plates with the inoculated leaflets were incubated in a growth chamber for 6 days at 18°C with 12 h light per day. Each genotype was rated as compatible (C) when expanding lesions were observed or incompatible (I) when infection appeared unsuccessful or a hypersensitive response was observed.

Assessment of crossability between the *Piurana* clade and cultivated potato

Within a larger crossing block established in a screenhouse at Huancayo in March–May 2002, a sample of 43 *Piurana* genotypes (21 *S. chomatophilum* and 22 *S. paucissectum* genotypes) ranging from resistant to susceptible to *P. infestans* were planted along with 18 diploid cultivated accessions (three diploid breeding lines and 15 *S. tuberosum* group Phureja genotypes; Table 1). Ten plants of each genotype were established in large pots, and controlled pollinations were made following emasculation of immature pistillate flowers.

DNA isolation

Plant tissue was collected from the leaves of greenhouse-grown plants of PCC1 and their parents, freeze dried, ground in a mortar and pestle and stored in sealed tubes at −20°C. DNA isolations were made from about 7 g of dried tissue following procedures described by Doyle and Doyle (1987). DNA was purified thereafter with CsCl gradients.

Probes, primers and Southern hybridization

To cover all 12 potato chromosomes based on previous published maps (Gebhardt et al. 1989; Tanksley et al. 1992) and information available at the Solanaceae Genome Network (http://www.sgn.cornell.edu), 166 tomato genomic (TG) or cDNA (CD and CT) and potato genomic (GP) probes and nine tomato ESTs were selected. Plasmid replication and isolation followed procedures recommended by QIAprep Miniprep kit (Qiagen, Valencia, CA, USA). Insert DNA was recovered by standard PCR using M13 primers except for markers that did not amplify, and these were recovered by band isolation after digestion with appropriate restriction enzymes from 1% low-melting agarose gels and purified using the QIAEX II Gel Extraction Kit.

Approximately 12.5 µg of genomic DNA of PCC1 and its parents was digested with *Eco*RI, *Eco*RV, *Hin*dIII, *Dra*I and *Xba*I (Promega, Madison, WI, USA). Samples were loaded in a 0.8% agarose gel and subjected to electrophoresis at 30 V for approximately 16 h, stained with ethidium bromide and photographed over ultraviolet light. DNA was transferred by capillarity in S2 buffer (Sambrook et al. 1989) to 'Zetaprobe' GT membranes (Bio Rad, Berkeley, CA, USA) and baked under vacuum at 80°C for 2 h. Southern blot membranes were washed in 0.1× SSC and 0.5% SDS for 30 min at 65°C.

Hybridization buffer (6× SSC, 5× Denhart's solution, 0.5% SDS and 0.1 mg/ml salmon DNA) was incubated with membranes at 65°C for 3 h on a rotational shaker. Approximately 25 ng of probe was radiolabeled with [α-P^{32}]dCTP using the Prime-a-gene kit (Promega). Radiolabeled reactions were passed through Sephadex (Sigma Aldrich) to remove unincorporated radionucleotides (Sambrook et al. 1989). The radiolabeled probe was then added to the hybridization buffer and incubated overnight at 65°C. Membranes were rinsed once with 2× SCC 0.1% SDS, washed twice with 0.1× SCC 0.1% SDS for 10 min at 65°C and finally rinsed with 2× SSC. The membranes were then wrapped in plastic film, exposed to XAR-5 film in X-ray cassettes and placed at −80°C for 2–7 days. Standard film development procedures were followed (Kodak, Rochester, NY, USA).

Linkage analysis

Autoradiograms were scored for segregation of bands originating in *S. paucissectum*. Linkage analysis was performed in Map Manager QTX version b20 (Manly et al. 2001) at $P \leq 1\times10^{-6}$. Linkages were confirmed

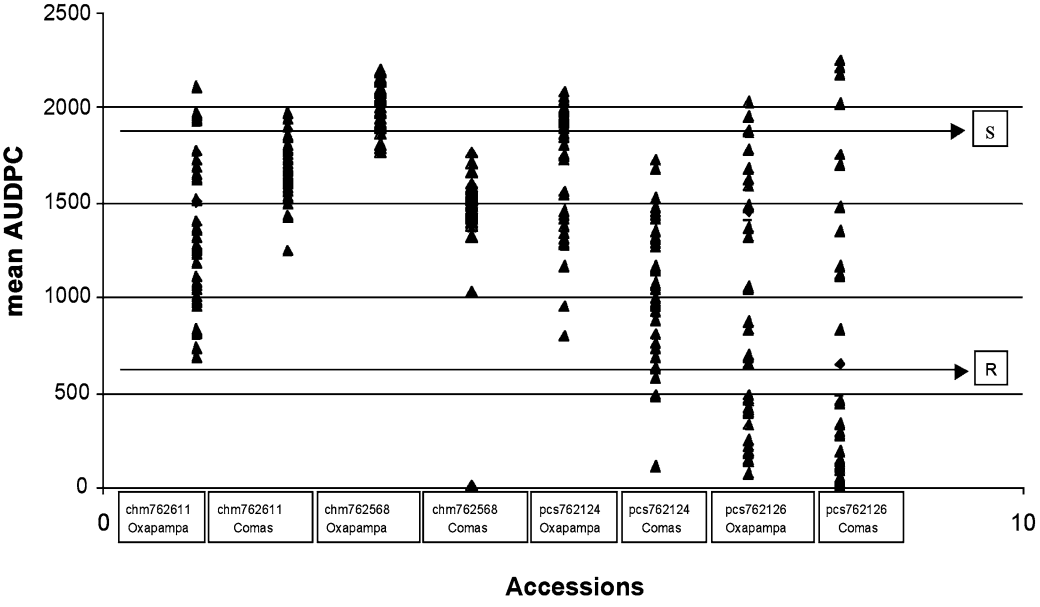

Fig. 1 Summary of mean AUDPC values of nearly 40 genotypes of each of the four heterogeneous accessions of *Solanum* series *Piurana* germplasm for late blight under endemic conditions in two locations in Peru. Each *triangle* represents the mean AUDPC of one genotype grown in four replications in either Comas or Oxapampa. *Lines* indicating "S" and "R" correspond to the mean AUDPC values of the control clones 'Chata Blanca' (susceptible) and LBr-40 (resistant)

using R/QTL (Broman et al. 2003) in R (http://www.R-project.org/) and Mapmaker Version 3.0b (Lander et al. 1987). A marker was considered a member of the framework map if it had a unique position or if an alternative position indicated by the "ripple" command was 500-fold less likely (LOD < 2.7).

Mean AUDPC values of PCC1 genotypes for variables AUDPCLV, AUDPC2 and AUDPC3 were used for QTL analysis. QTLs were analyzed by Composite Interval Mapping (CIM) with the QTL Cartographer computer program 2.0 (http://statgen.ncsu.edu/qtlcart/index.php). The 5 and 1% empirical threshold values were calculated using 1,000 permutations of the original data. The explained variance presented was calculated by the program conditioned on background markers and any other explanatory variables.

Results

Evaluation of *S. paucissectum* and *S. chomatophilum* germplasm accessions under endemic field conditions

Figure 1 summarizes the AUDPC values of two accessions of *S. paucissectum* and two of *S. chomatophilum* exposed to late blight in the field in Comas and Oxapampa, with respect to the average performance of 'Chata Blanca' (S, susceptible) and LBr-40 (R, resistant). All accessions showed quantitative variation in both locations but differ in their phenotypic distributions. Most of the genotypes of *S. chomatophilum* accession CIP 762568 were susceptible or moderately susceptible as compared to the controls in both locations. The second accession of this species, CIP 762611, did present a number of moderately resistant genotypes, but only in Oxapampa. In contrast, the two *S. paucissectum* accessions showed a wider range of phenotypic variation, with CIP 762126 showing the largest proportion of resistant individuals in both locations.

Single ANOVAs performed for each accession by location showed significant differences among individuals, with variances ranging from low for *S. chomatophilum* to higher values for *S. paucissectum* (data not shown). The coefficients of variation were low (CV < 21%) indicating uniform infection and low environment variation. Homogeneity of variances between locations for each accession allowed data combination. Combined ANOVAs showed significant contribution of locations and genotype × location (G × L) interactions ($p < 0.0001$) to genetic variances of all accessions. Despite this, genetic variation was higher than that of location and G × L for *S. paucissectum* accessions, which showed a high broad-sense heritability (CIP 762124, $H^2 = 0.79$; CIP 762126, $H^2 = 0.75$). Broad-sense heritability was distinctly lower for *S. chomatophilum* accessions (CIP 762611, $H^2 = 0.48$; CIP 762568, $H^2 = 0.52$). In this case, the former accession suffered a considerable reduction of its genetic variance after combination across locations.

Compatibility of *Piurana* germplasm and *P. infestans*

Detached leaf assays were performed with distinct combinations of *P. infestans* isolates and *Piurana* genotypes to assess the presence of known or putative novel *R* genes. A race 0 (PE84006) and three complex isolates representing those present in the field test sites (POX-

Table 2 Compatibility patterns detected in DLAs of two *S. paucissectum* accessions and two PCC1 progenitors with three and four isolates of *P. infestans*, respectively

Pattern	*P. infestans* isolates				*S. paucissectum* accessions		Progenitors of PCC1	
	PE84006	POX-067	PCO-093	PCO-002[a]	CIP 762124	CIP 762126	chm PI 310991-1	pcs PI 473489-1
1	C	C	C	nt	17	25		
2	I	C	C	nt	17	4	✔	
3	I	I	C	nt	1			✔
4	I	I	I	nt		1		
5	C	I	I	nt		3		
6	C	I	C	nt		1		
7	C	C	I	nt		2		
8[b]	I	C	C	C			1	
9[c]	I	I	C	I				1
				Total	35	36	1	1

[a]Only the progenitors of PCC1 were tested with PCO-002: nt = no test
[b]Pattern 8 cannot be distinguished from Pattern 2
[c]Pattern 9 cannot be distinguished from Pattern 3

067, Oxapampa; PCO-093 and PCO-002, Comas) were used as inoculum. Inoculation of the genotypes of *S. paucissectum* and *S. chomatophilum* used to develop the mapping population and additional accessions of *S. paucissectum* resulted in differential compatibility patterns (Table 2). Incompatible reactions were detected in the pedigree parents of PCC1 inoculated with race 0 and when the *S. paucissectum* donor was challenged with each of the complex isolates used (Table 2).

The two *S. paucissectum* accessions presented additional evidence for the presence of more than one *R* gene in the donor of resistance to PCC1 (Table 2). Differential isolate–genotype combinations led to a high frequency of genotypes that exhibited compatibility either with race 0 and/or complex races (Patterns 1 and 2). However, there was a small number of genotypes that showed compatibility to race 0 but incompatibility with at least one of the complex races (Patterns 5, 6 and 7) suggesting the presence of *R* genes different from the 11 described for *S. demissum* and of virulence factors in the race 0 isolate other than those from 1 to 11. On the other hand, the presence of an *R9* gene cannot be ruled out since two genotypes showed incompatibility to race 0 and complex races known to lack the avirulance factor *avr9* (Patterns 3 and 4). This might also be true for the donor parent, while the lack of this gene in the recurrent parent is clearly seen by its compatibility with all complex races. Inability of the 'race 0' isolate (PE84006) to infect plants showing the compatibility patterns 2, 3, 4, 8 and 9 likely indicates the presence of unknown *R* genes in the corresponding genotypes as suggested elsewhere by Vleeshouwers et al. (2000).

Field exposure of the PCC1 progeny

ANOVA performed on AUDPC values of 184 PCC1 progeny showed significant differences among progeny clones with a wide range of resistance from 60 to 3,074 AUDPC units under field exposure in Comas. Low coefficient of variation (19%) indicated uniformity in late blight infestation. The distribution of AUDPC values was rather normal appearing to contain more than only a single additive genetic component and skewed toward resistance (Fig. 2). The range of phenotypes could not be separated into two groups, and no improvement of normality was achieved after arcsine transformation was applied. The original *S. paucissectum* donor of resistance and the hybrid female parent MP1-8 means were significantly lower than the progeny mean and close to the most resistant control LBr-40.

Cluster analysis performed on PLA divided the PCC1 progeny into six groups (Fig. 3). Epidemic patterns of most clusters and of the resistant and susceptible controls showed typical logistic disease progress curves with constant rates, except for one group (Cluster 3, $n = 16$) that included the *S. paucissectum* donor, in which a marked deviation was observed due to a sudden increase in the rate of disease progress between the sixth and seventh evaluation, after 64 DAP. Uniform but slower epidemics were shown by Clusters 5 ($n = 34$) and 6 ($n = 34$) that included, respectively, the female parent MP1-8 and the most resistant control LBr-40. On the other hand, Clusters 1 and 2, which included the susceptible controls, were characterized by rapid epidemics, destroying the plants within 4 weeks (Cluster 1 and Chata Blanca) to 6 weeks (Cluster 2 and Yungay). Finally, Cluster 4 and the moderately resistant control Monserrate, though also supporting a fast epidemic, retained nearly 20% healthy foliage until the end of the assay.

Screenhouse evaluation of the PCC1 progeny

The PCC1 progeny was evaluated for resistance against the complex race POX-067 ($Avr_{8,9}$) under screenhouse conditions. Susceptible genotypes reached 80% PLA in 7 days, representing good disease development despite the short time frame which is reflected in low AUDPC values for this assay. Distribution of AUDPC values was skewed toward susceptibility due to a small number of individuals such that each fell into a range of classes

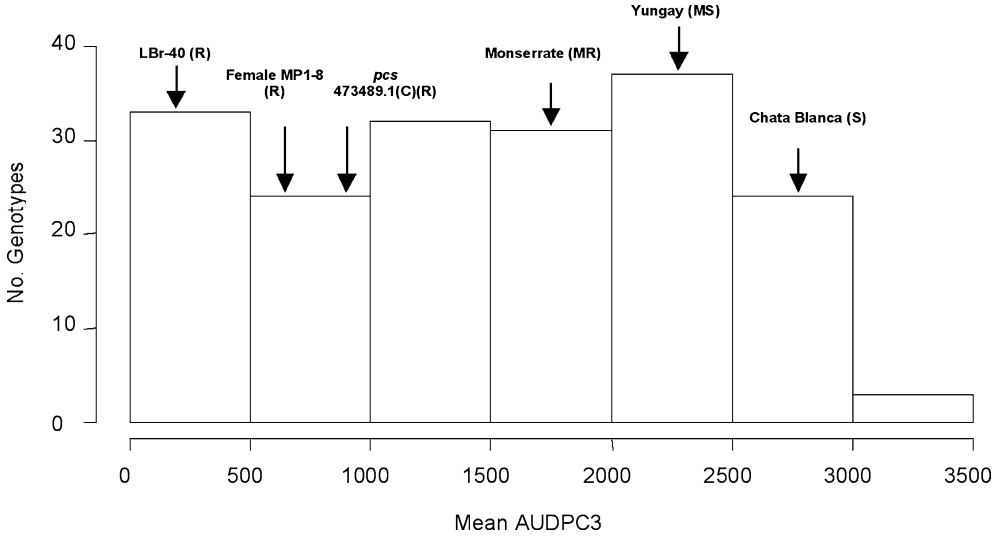

Fig. 2 Frequency distribution of final mean AUDPC values (AUDPC3; 84 DAP) of 184 PCC1 genotypes under endemic late blight pressure in Comas, Peru, 2002. Mean AUDPCs of control and parental clones relative to the progeny individuals are indicated by *arrows*. Numbers on the *x*-axis are upper limits. *MR* moderately resistant, *MS* moderately susceptible

from moderately resistant to susceptible (Fig. 4). Nearly three quarters of the progeny showed AUDPC values close to those of the resistant progenitors, or as low as, or lower than the most resistant control LBr-40, and four did not show any infection at the macroscopic level. In agreement with the incompatibility observed in DLA of *S. paucissectum* PI 473489-1, this skewed segregation pattern suggested the presence of an additional strong underlying resistance component beside the action of minor genes.

Linkage mapping

Out of 166 probes, 86 (52%) showed polymorphism with at least one of the five tested restriction enzymes. A total of 87 segregating bands were scored, as TG18 had two loci (A and B). Eight markers, TG422 (expected: chromosome 6, mapped: chromosome 10), TG162 (6, 4), CD34 (10, 1), GP125 (11, 8), CD18 (11, 10), TG17 (1, 8), cLEW-11-L14 (EST for TG581, 6, 12), cLED-29-N16 (EST for TG474, 1, 12), mapped to unexpected linkage groups based on the published linkage maps of Bonierbale et al. (1988, 1994), Gebhardt et al. (1991) or Solanaceae Genome Network (http://www.sgn.cornell.edu), the latter four showing complex banding patterns typical of multiple copy sequences. Significant distortions ($P \leq 0.01$) from the expected 1:1 monogenic ratio were observed in 16% of the loci. Twelve linkage groups were resolved corresponding to the 12 potato chromosomes (Fig. 5). The total distance covered is 994 cM (Kosambi units). Two probes (TG135 from chromosome 3 and TG421 from chromosome 9) failed to link to any of the groups. The short arm of chromosome 3 and the long arm of chromosome 9 could not be resolved, despite surveys with six and seven probes, respectively, to target these regions that showed no RFLP polymorphism.

QTL analyses were performed for two data sets on AUDPC mean values obtained in the field trial: AUDPC2 (disease progress observed up to 74 DAP) and AUDPC3 (whole disease progress up to the last evaluation date at 84 DAP) and on mean AUDPC values calculated from the screenhouse assessment (AUDPCLV). Composite Interval Mapping revealed three QTLs (Fig. 6) for late blight resistance that exceeded the

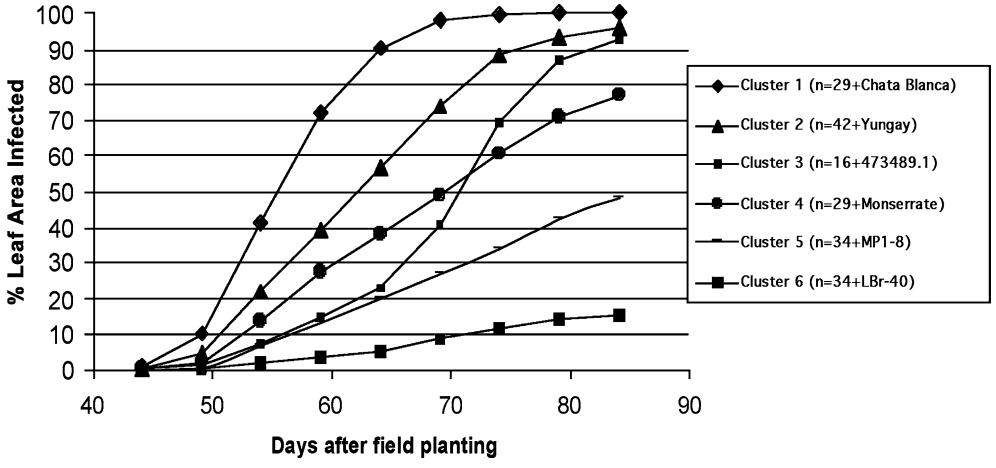

Fig. 3 FASTCLUS procedure of SAS illustrates patterns of disease progress of PCC1, the two pedigree parents MP1-8 and *S. chomatophilum* PI 310991-1 and four unrelated controls in terms of the mean disease readings on each evaluation date in the field at Comas. The *x*-axis depicts days after transplanting (disease reading dates) and the *y*-axis shows percent leaf area infected. *N* is the number of PCC1 genotypes in each cluster, and control or parental clones are indicated by their names

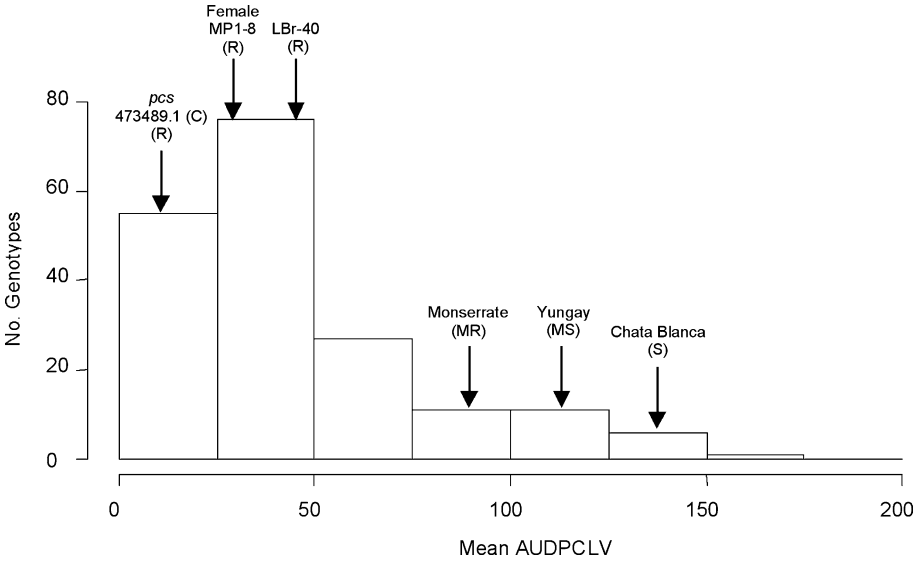

Fig. 4 Frequency distribution of mean AUDPC values (AUDPCLV) for late blight of 187 PCC1 genotypes evaluated in the screenhouse at Huancayo, following inoculation with a complex isolate POX-067 of *P. infestans*. Numbers on the *x*-axis are upper limits

1% threshold values, on chromosomes 10, 11 and 12 (Table 3). The QTL on chromosome 11 accounted for slightly more than 25% of the variance conditioned on other background markers, under field conditions (AUDPC2 and AUDPC3), and was consistent under screenhouse conditions, albeit explaining a lower percent of the variance ($r^2 = 9\%$ AUDPCLV). The additive genotypic value of this QTL reached 1 phenotypic standard deviation, which is considered a large effect for a QTL, but not large enough to qualify it as a major gene (classical Mendelian mutant) (Falconer and Mackay 1996). The QTL on chromosome 12 was present under field conditions accounting for 7 and 10% of the variation for late blight resistance in AUDPC2 and AUDPC3, respectively, but was not observed under screenhouse conditions. The positive sign of the additive effects of these two QTLs (Table 3) indicate an increase in AUDPC values when substituting the allele of the resistant *S. paucissectum* donor parent for that of the susceptible recurrent parent. Hence, these QTLs are associated with resistance from the *S. paucissectum* donor parent.

In contrast, the negative sign of the QTL effect on chromosome 10 that was observed only in AUDPC2 corresponds to a decrease in AUDPC units from that of the recurrent parent indicating its association with a susceptible allele from *S. paucissectum* (Table 3). For only the screenhouse data, an epistatic interaction was found between chromosome 7 marker TG143 and chromosome 11 marker CT182, with an LOD interaction of 5.1.

Crossability of the *Piurana* clade

Crossing was successful among accessions and genotypes of the same species (*S. chomatophilum* × *S. chomatophilum*; *S. paucissectum* × *S. paucissectum*; *S. tuberosum* group Phureja × *S. tuberosum* group Phureja), indicating acceptable levels of both male and female fertilities. In contrast, efforts to cross the *Piurana* accessions with cultivated potato resulted in no or only very few seeds, whose viability has not yet been tested (Table 1). Crossing more than 1,000 flowers in each direction failed to provide hybrid seeds between cultivated potato (represented by diploid breeding lines and accessions of group Phureja) and *S. chomatophilum*. In the case of *S. paucissectum*, no seed was produced from over 1,400 flowers of 145 cross combinations involving the wild species donor as female and cultivated potato as male, and only 55 seeds resulted from 67 genotypic combinations involving 428 flowers crossed in the reciprocal direction. All successful crosses with cultivated potato involved group Phureja, three genotypes of this group contributing to the 55 seeds that resulted from interclade crosses.

Discussion

Late blight resistance in two species of the *Piurana* clade

Wide genetic variation for resistance to *P. infestans* was found in the PCC1 progeny, derived from the interclade backcross of *S. paucissectum* to *S. chomatophilum*, as well as in the germplasm accessions of these two species. High levels of resistance were shown in the *S. paucissectum* sources while *S. chomatophilum* was generally more susceptible. Despite the contribution of interactions and environmental effects to variation for resistance under endemic disease pressure, selectable genetic variation was high for *S. paucissectum* ($H^2 > 0.7$). This is of great importance when a species is considered as a source breeding for durable resistance. However, assays with specific isolates of the pathogen uncovered incompatibilities that suggested the presence of novel and known major genes in this germplasm. Major genes were also evident in the PCC1 parents,

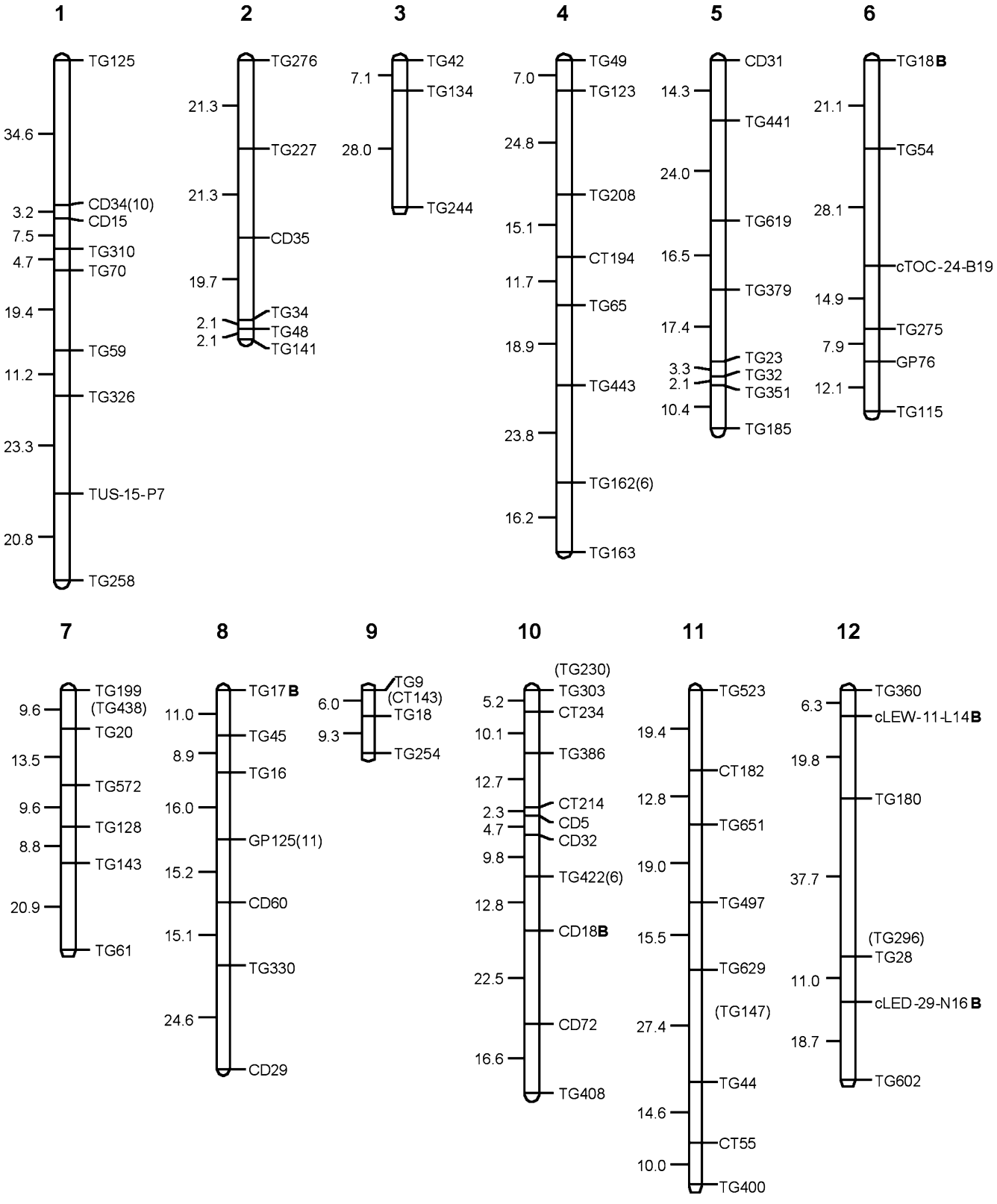

Fig. 5 Molecular linkage map of *S. paucissectum*. Loci with tick marks are ordered with LOD ≥2. Distances are cM (Kosambi units). Marker names with **B** are considered secondary loci, due to multiple copy number or because they have been reported in alternative linkage groups (in *parentheses*) before. Markers in *parentheses* are placed in most likely intervals (1 > LOD < 2) of the framework map. Markers were mapped on a BC1 population of 194 plants from *S. paucissectum*/*S. chomatophilum*//*S. chomatophilum*. EST marker TUS-15-P7 is homologous to TG237; cTOC-24-B19 to TG364; cLEW-11-L14 to TG581l; and cLED-29-N16 to TG474

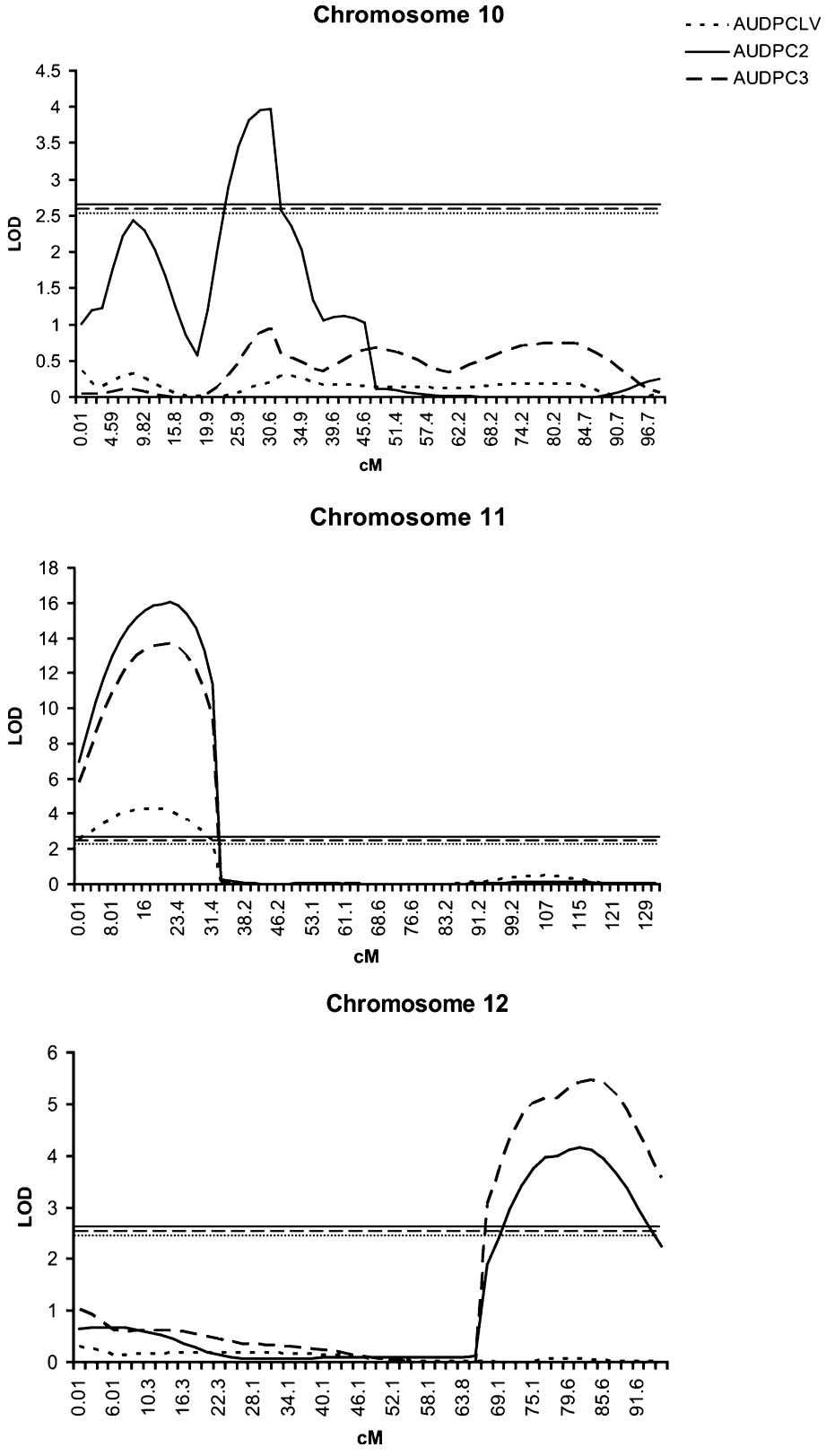

Fig. 6 Quantitative trait locus scans for chromosomes 10, 11 and 12 for three different sets of data named AUDPC2 (until 74 DAP in field), AUDPC3 (until 84 DAP in field) and AUDPCLV (screenhouse data). LOD thresholds for QTL significance at $P \leq 0.05$ are indicated as *horizontal lines*; the legend for the lines and the scans correspond to each other

with one of them suggested to have contributed to the skewed phenotypic distribution of the resistance of the segregating progeny PCC1 (below). Since the *Piurana* members form a distinctive clade (Clade 3) endemic to the Peruvian Andes, novel *R* genes could co-evolve with endemic races of the pathogen. Novel *R* genes have been proposed previously in species endemic to South America (Ewing et al. 2000).

Table 3 Significance and strength of QTLs for late blight resistance detected in field (AUDPC2 and AUDPC3) and screenhouse (AUDPCLV) evaluation of the PCC1 population

Chr	Marker interval	AUDPCLV				AUDPC2				AUDPC3			
		LOD	r^2 (%)	Additive effect	Std dev.	LOD	r^2 (%)	Additive effect	Std dev.	LOD	r^2 (%)	Additive effect	Std dev.
10	TG386–CT214	–	–	–	–	3.97	5.80	−413.93	0.74	–	–	–	–
11	TG523–CT182	4.32	9.70	21.80	0.66	–	–	–		–	–	–	–
	CT182–TG651	–	–	–	–	16.01	28.55	626.143	1.09	13.64	26.30	902.486	1.05
12	CLED29–TG602	–	–	–	–	4.14	7.14	320.866	0.57	5.11	10.18	567.059	0.66

r^2 = variance explained by the QTL conditioned on the background markers and any explanatory variables

Some of the genetic components underlying resistance in this clade were studied through the segregating progeny PCC1. The distribution of resistance observed under endemic field conditions suggested the presence of additional underlying components besides an additive genetic component. Analysis by clustering the segregating genotypes on their respective patterns of disease development showed a group of individuals with a disease progress pattern that was distinctive from the remaining family members (Fig. 3). The distinctive feature of this cluster was a sudden mid-season increase in rate of disease development, following slow development early on, similar to that of the groups with apparent rate-reducing resistance. That the resistance donor, *S. paucissectum* PI 473489-1, also fits this category is consistent with the possible presence of an *R* gene that was matched by a pathogen race arriving or becoming effective late in the season. This could be substantiated by the incompatible reactions shown by this resistance donor when compatibility tests were performed with complex races representative of the pathogen population endemic to the evaluation site (Comas). This putative defeated *R* gene might also be present in most individuals that were clustered in the groups which showed low disease progress rates throughout the season. Field experiments providing evidence for the contribution of defeated *R* genes to field resistance were reported recently by Stewart et al. (2003).

The PCC1 progeny was additionally tested under screenhouse conditions against an isolate representing another endemic area (Oxapampa) of Peru. Segregation for resistance against this isolate allowed the detection of two phenotypic groups, one of them comprising three quarters of the progeny (131 of 187) with levels of resistance as high as or close to those of the resistant *S. paucissectum* donors (Fig. 4). Taking into account that one of these donors (PI 473489-1) was incompatible with this isolate (Table 2), it is possible that two *R* genes were segregating, since the ratio observed between the two groups (3:1) fits this situation and not the 1:1 ratio that would be expected for segregation of an *R* allele at one locus. An incomplete effect or partial resistance (Colon et al. 1995) of at least one of these putative *R* genes might account for the differences in levels of resistance among members of the highly resistant group. *R* genes that can sometimes be weakly expressed have been demonstrated by Mooi (1977). Additional differentiating isolates of the pathogen should be tested to define this situation.

Genetic map of *Solanum paucissectum*

A genomewide sample of 166 RFLP markers was used to develop a framework map of *S. paucissectum* using 187 individuals of the PCC1 progeny. Only 52% (86/166) of the markers were polymorphic in the backcross progeny, reflecting the taxonomic affinity of the mapping parents. While the targeted use of markers was successful in covering the genome at a sufficient density for QTL mapping, we were unable to resolve two large chromosome segments (parts of chromosomes 3 and 9; Fig. 5). These regions might comprise loci associated with fitness in the germplasm sampled, giving rise to fixed alleles. Continued map development will be required to resolve these segments or support this suggestion.

Eight markers (9.3%) mapped to unexpected positions, four (TG17, CD18, cLEW-11-L14 and cLED-29-N16) of which showed complex banding patterns. This compares to 17.2% unexpected map positions found by Kuhl et al. (2001) in the more distantly related clade 1. As no blocks of rearranged segments were found to support syntenic differences, and four of the five paralogous inversions that distinguish potato from tomato are maintained, it appears that linkage order is largely conserved between these representatives of the *Piurana* clade and potato. However, continued map development, particularly to resolve chromosomes 3 and 9, will be required before genome structure can be ruled out as possibly contributing to the low crossability observed here between the *Piurana* clade and *S. tuberosum*. The lack of marker data for these two chromosome arms further impeded our ability to query these regions for their possible contribution to explaining additional trait variation.

Quantitative trait loci for late blight resistance

Three QTLs were identified for late blight resistance under endemic field conditions, on chromosomes 10, 11 and 12. The most prominent (designated *QTLpcs11* for

S. paucissectum and chromosome 11) was linked to the RFLP marker CT182. Although the standard deviation of the effect of *QTLpcs11* is large enough to define it as a major QTL (Falconer and Mackay 1996), it might represent the putative defeated gene mentioned above, which expressed a residual effect against the compatible race, or linked genes for resistance. Contribution of residual effects to field resistance have been demonstrated among several known *R* genes (Stewart et al. 2003).

This large QTL was also detected when the mapping progeny was inoculated with POX-067 under screenhouse conditions (Fig. 6; AUDPCLV), but with a substantially smaller effect than that found under a natural disease epidemic. In this latter case, epistatic action was also detected between effects linked to the QTL-associated marker (CT182) and marker TG143 on chromosome 7. If two *R* genes were segregating in this assay as suggested above, the epistatic effect could have arisen from individuals carrying the two putative *R* genes (*Pcs1*, *Pcs2*) and their respective associated marker alleles *M1*, *M2*. Further, the significantly lower effect shown by this QTL in the screenhouse assay might be accounted for by the small difference between the mean phenotypic value of the highly resistant individuals carrying the associated marker allele on chromosome 11 and that of the group of the highly resistant individuals carrying the other putative *R* gene (but not the marker allele on chromosome 11), together with those more susceptible (1/4 of the progeny), disregarding recombinants. In this latter group, the high AUDPC values of the more susceptible individuals were counteracted by the low values of all the highly resistant individuals not bearing the marker allele on chromosome 11. As mentioned before, testing this progeny with a further range of isolates would be needed to confirm this hypothetical reasoning. This major *QTLpcs11* or putative defeated *R* gene mapped to the opposite chromosome arm from the *R* gene cluster comprised of *R3*, *R6* and *R7* (El-Kharbotly et al. 1994, 1996), but was in the same region as the QTL reported by Leonards-Schippers et al. (1994) segregating from an *S. tuberosum* breeding line of complex pedigree and by Ewing et al. (2000) from the wild species *S. berthaultii*.

The QTL for resistance detected on chromosome 12 was associated with a difference of 300 to nearly 600 AUDPC units as the epidemic progressed, while that on chromosome 10, associated with susceptibility, was not detected at the end of the season (AUDPC2 vs. AUDPC3; Fig. 6). These changes in the magnitude of effects and the relative significance of QTL during an epidemic suggest that quantitative genetic effects can be dynamic (e.g., with respect to the changes in the prevalence of isolates differing in their aggressiveness or virulence) in a single location and season. In the case of the former, the effect increased while that of the latter decreased to the point of being insignificant by the end of the season.

QTLpcs12 (for chromosome 12) mapped, as far as resolution allows, in close proximity to those reported by Ghislain et al. (2001) in a population derived from a dihaploid of an advanced potato line and *S. tuberosum* group Phureja, while *QTLpcs10* was located in the same region as the *R* gene from *S. berthaultii* mapped by Ewing et al. (2000) and on the same arm as a QTL from *S. microdontum* reported by Sandbrink et al. (2000).

QTLpcs11, if it is not a major gene or if its residual effect is stable, and *QTLpcs12*, provided it is proven to be stable across environments, constitute QTLs of great value to late blight. This is due to their potential for significant impact, their effects (> 500 AUDPC units) on epidemic development and reduced fungicide applications as was demonstrated in previous studies on integrated control of late blight (Fry 1986). These QTLs may represent different allelic forms of common QTL or unique ones, due to the distant phylogenetic position of the clade with respect to priorly studied potato species.

Given evidence of location and genotype × location effects on the resistance of *S. chomatophilum* and *S. paucissectum* accessions under endemic conditions and the apparent dynamic picture of QTL described, the PCC1 progeny will be subject to further fields with a view to assessing QTL stability across environments.

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