Theor Appl Genet (2001) 103:433–442

M. Ghislain · B. Trognitz · Ma. del R. Herrera
J. Solis · G. Casallo · C. Vásquez · O. Hurtado
R. Castillo · L. Portal · M. Orrillo

Genetic loci associated with field resistance to late blight in offspring of *Solanum phureja* and *S. tuberosum* grown under short-day conditions

Received: 5 July 2000 / Accepted: 17 November 2000

Abstract Field resistance to late blight – a fungal disease caused by *Phytophthora infestans* – has been genetically characterized by analyzing trait-marker association in a *Solanum phureja* (*phu)*×dihaploid *Solanum tuberosum* (dih-*tbr*) population. Trait data were developed at three locations over a 3-year period under natural infection pressure. RAPD (random amplified polymorphic DNA) and AFLP (amplified fragment length polymorphism) markers were used to develop anonymous genetic linkage groups subsequently anchored to potato chromosomes using mapped RFLP (restriction fragment length polymorphism), SSR (single sequence repeats) and AFLP markers. RFLP and SSR markers achieved the most-accurate anchoring. Two genetic maps were obtained, with 987.4 cM for *phu* and 773.7 cM for dih-*tbr*. Trait-marker association was revealed by single-marker and interval mapping analyses. Two important QTLs (quantitative trait loci) were detected on chromosomes VII and XII as a contribution from both parents, totalling up to 16% and 43%, respectively, of the phenotypic variation (PH). One additional QTL was detected on chromosome XI (up to 11% of the PH) as a contribution from the *phu* parent, and three others were detected on chromosome III (up to 13% of the PH), chromosome V (up to 11% of the PH) and chromosome VIII (up to 11% of the PH) as a contribution from the dih-*tbr* parent. Our results reveal new genetic loci of the potato genome that contribute to resistance to late blight. We postulate that some of these loci could be related to plant growth under short-day conditions.

Communicated by F. Salamini

M. Ghislain (✉) · B. Trognitz · Ma. del R. Herrera · J. Solis
G. Casallo · C. Vásquez · O. Hurtado · R. Castillo · L. Portal
M. Orrillo
Department of Crop Improvement and Genetic Resources,
Centro International de la Papa (CIP), PO Box 1558, Lima 12,
Perú
e-mail: m.ghislain@cgiar.org
Tel.: +51-1-349-6017, Fax: +51-1-317-5326

Keywords *Phytophthora infestans* · Potato · Genetic mapping · Quantitative trait loci · Disease resistance

Introduction

Resistance to late blight of potato has been an old breeding objective since the 19th century: in the 1840 s the disease caused tremendous damage to the potato crop in Ireland, resulting in widespread famine and the emigration of about a million people. This disease is caused by the oomycete *Phytophthora infestans*, and the only effective way to control the disease is by fungicide. Race-specific reactions to infection leading to resistance have been characterized from *Solanum demissum*, a Mexican wild potato, by 11 R genes that condition resistance with different kinds of symptoms from invisible to large necrotic areas. This resistance was introgressed into the cultivated potato early last century. However, the oomycete rapidly overcomes such resistance (often called vertical resistance) and nowadays such gene-for-gene resistance is abandoned. Race non-specific resistance to late blight has also been found in potato, and breeding and genetic studies have suggested that it is quantitatively inherited. These two criteria, race non-specificity and polygenic inheritance, have given hope that such resistance would be durable. Today, this idea still stands, as several resistant potato varieties have now been around for decades without their resistance breaking down (Colon et al. 1995a).

Quantitative resistance to late blight was first analyzed by genetic linkage mapping in a cross between non-inbred *Solanum tuberosum* dihaploid parents (Leonards-Schippers et al. 1994). Eleven quantitative trait loci (QTLs) were identified on nine potato chromosome segments. Surprisingly, some of these QTLs were found to be race-specific and in some cases were also located near known race-specific genes or defense genes. This finding led to the hypothesis that race-specific resistance R genes might be extreme allelic variants of QTLs for quantitative resistance (Gebhardt

1994). However, Leonards-Schippers et al. (1994) used a leaf-disk assay of diseased leaves from greenhouse inoculations, which may not be representative of field infections. Recently, three genetic analyses of field resistance to late blight were conducted with artificial inoculation (Collins et al. 1999; Oberhagemann et al. 1999; Ewing et al. 2000). There is now solid evidence to support the contribution to field resistance of several QTLs of these maps. The most-significant, in terms of effect strength and frequency of detection across years and environments, are the one on chromosome V near the RFLP probe GP179 and a QTL on chromosome III. Other QTLs have been identified but with much less contribution to field resistance and are less frequently detected across trials. These studies have also revealed that field resistance assessed as foliage resistance is correlated with late maturity and vigor. A marker-assisted genetic approach has been proposed to determine whether this correlation has a direct relationship with resistance or is due to linkage drag.

Marker-assisted selection for quantitative resistance to late blight needs additional genetic studies before it can be a valid methodology in potato breeding. In particular, different sources of resistance, and other potato species, need to be studied under different environmental conditions. Our work has tackled this essential question. Valuable sources of field resistance to late blight have been characterized in several cultivated South American potato species (Colon et al. 1995b; Trognitz et al. 1997). Quantitative inheritance of this resistance and the high load of unfavourable characteristics in wild and native potatoes, however, slow down the wide use of this type of resistance to develop new potato cultivars. In an attempt to overcome these constraints, at least partially, a molecular marker-based approach has been developed to identify QTLs for selective introgression into cultivated germplasm. We selected the native cultivated potato species, *Solanum phureja*, for the genetic analysis of field resistance to late blight under short-day conditions (De Maine et al. 1993). Several reasons make this species a particularly attractive source of resistance to late blight. The inheritance of resistance to late blight is presumably quantitative – often thought to be highly polygenic – and no race-specific resistance genes, which could mask the quantitative resistance, have been observed in this species. Diploid *S. phureja* gives fertile hybrids when it is crossed as the seed parent with dihaploid (2n=2*x*=24) potato. Many of the hybrids have the capacity to produce unreduced diploid pollen. Therefore, these diploid *tuberosum*×*phureja* hybrids can be used in crosses with tetraploid potato varieties to produce tetraploids that carry the resistance genes of *S. phureja*. *S. phureja* is a cultivated potato that already possesses advanced agronomic properties and favorable tuber characteristics, as well as good culinary quality.

In this paper we report the genetic analysis of field resistance to late blight based on segregation analysis in hybrids between *S. phureja* and *S. tuberosum* subsp. *tuberosum* dihaploid.

Material and methods

PD population and field evaluations

The potato population comprising 246 individuals resulted from a cross between the diploid *S. phureja* (P or *phu*) accession CHS-625 (kindly provided by H. Andrade, Instituto Nacional Autónome de Investigación Agropecuaría) and the *S. tuberosum* dihaploid (D or dih-*tbr*) clone PS-3 (Trognitz et al. 1997). The female parent CHS-625 was selected because of its high level of resistance to late blight and its fertility (Cañizares and Forbes, 1995). The male parent PS-3 is highly susceptible to potato late blight but displays good agronomic performance. This population, referred to as PD, was selected because it segregates for late blight resistance in the field. Both parents are susceptible to avirulent isolates of *P. infestans* (race zero) and thus do not express any R genes conferring race-specificity. The direction of the cross (P×D) was chosen to avoid hybrid male sterility because of incompatibility of the *phu* nuclear genes with *tbr* germplasm (Perez-Ugalde et al. 1964).

The 246 PD progenies are maintained pathogen-free in vitro, and plants or tubers used for disease evaluations were raised in the greenhouse at the location where the evaluations were performed. Foliage late blight resistance was evaluated in four field experiments: at Quito, Ecuador, in 1997, at Huancayo, Peru, in 1997, and at Comas (locality Mariscal Castilla), Peru, in 1998 and 1999. A randomized complete block design was used and each clone was represented by ten plants in a plot within each of four replications. The percent of diseased foliage per plot was evaluated at weekly intervals for 6 or 8 weeks. The data were log(arcsin) transformed to improve the normality of distribution, and an analysis of variance (ANOVA) was carried out for each reading within each experiment for the factor clones. Phenotypic variance based on the plot mean of each clone was used to calculate broad-sense heritability according to Nyquist (1991). The last four disease readings within each experiment had the relative largest broad-sense heritability (H^2>0.50) and, therefore, the average disease for these four readings was chosen as the phenotypic expression of the disease level. The percent of diseased foliage was correlated with the area under the disease progress curve (AUDPC) with r>0.97 and was therefore used directly for further analyses.

DNA extractions and molecular-marker assays

All molecular methods have been recently compiled in a laboratory manual available at the International Potato Center (CIP) or on request by e-mail to mbl@cgiar.org (Ghislain et al. 1999). Only protocols that have been modified from the original publications are reported here.

Random amplified polymorphic DNA (RAPD)

Adapted from Williams et al. (1993), typically RAPD reactions were conducted with 10 ng of DNA in a 1× PCR buffer (10 mM Tris HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton-X), 0.2 mM of each dNTP, 20 ng of RAPD primer and 0.5 U of *Taq* polymerase. Reactions were set up in microplates and processed in an MJ Research model PTC-100 PCR thermocycler with the following cycles: 1 cycle at 94°C for 3 min, 40 cycles at 94°C for 1 min plus 35°C for 1 min plus 72°C for 2 min, and 1 cycle at 72°C for 7 min. Reactions were then loaded onto standard 1.4% agarose gels using Tris-borate buffer. Amplification products were visualized using ethidium bromide fluorescence under UV light.

Amplified fragment length polymorphism (AFLP)

Adapted from Vos et al. (1995), high quality genomic DNA (1 µg) was first restricted in a 3-h reaction at 37°C in 10 mM of Tris H Acetate, pH 7.5, 10 mM of $MgCl_2$, 50 mM potassium acetate,

5 mM of DTT, 5 µg of BSA using ten units of *Eco*RI and four units of *Mse*I. Complete restriction digestion was controlled on 1% agarose-gel electrophoresis. *Eco*A and *Mse*A adaptors (0.1 mM and 1 mM respectively) were then ligated in a 3-h reaction at 37°C in the same buffer used for the restriction digestion plus 2 mM of ATP with 1 unit of T_4 DNA ligase. The reaction solution was then diluted with $T_{10}E_{0.1}$ (end volume 200 µl) and stored at –20°C. Then 5 µl were pre-amplified using *Eco*A1 and *Mse*A1 (30 ng each) in 1×PCR buffer (10 mM Tris HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM Kcl), 0.2 mM of each dNTP, and 0.4 units of *Taq* polymerase. PCR amplifications were performed with 20 amplification cycles at 92°C for 60 s plus 60°C for 30 s plus 72°C for 60 s. The pre-amplification reaction was diluted to 500 µl in $T_{10}E_{0.1}$ and stored at –20°C until use. Selective amplification of restricted fragments was performed using selective primers. The *Eco*RI primer (5 ng) was labeled using γP^{33} ATP (10 µCi/µl of 2,000 Ci/mmol) at 37°C for at least 30 min in 1×T_4 kinase buffer (25 mM Tris HCl, pH 7.5, 10 mM $MgCl_2$, 5 mM DTT, 10 mM spermidine [3 HCl form], and 1 unit of T_4 kinase). The enzyme was denatured at 70°C for 10 min. The labeled primer was added to 5 µl of the pre-amplified reaction to proceed to the amplification with the other selective *Mse* primer (30 ng). Reactions were done in 1×PCR buffer, 0.2 of each dNTP, 0.4 units of *Taq* polymerase with: 1 cycle at 94°C for 30 s plus 65°C for 30 s plus 72°C for 60 s; 12 cycles at 94°C for 30 s plus (65–0.7)°C in each cycle for 30 s plus 72°C for 60 s, 22 cycles at 94°C for 30 s plus 56°C for 30 s plus 72°C for 60 s. Amplification products were separated using standard 6% denatured (7 M urea) polyacrylamide gel-electrophoresis and detected using autoradiographic procedures. AFLP adaptors and primers follow the authors' codes.

Single sequence repeats (SSR) or microsatellites

Adapted from Provan et al. (1996), genomic DNA (10 ng) was amplified using SSR primers (5 pmol each) in 1×reaction buffer [90 mM Tris base, 20 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, adjust to pH 9.0 with HCl], 0.2 mM of each dNTP, and 1 unit of *Taq* polymerase. PCR (polymerase chain reaction) was conducted as follows: 1 cycle at 94°C for 3 min, 30 cycles at 94°C for 1 min plus T_{ann} (determined for each primer pair) for 2 min plus 72°C for 1.5 min, and 1 cycle at 72°C for 5 min. Amplification products were detected using standard polyacrylamide denaturing gel-electrophoresis and silver-stained.

Genetic linkage groups and maps construction

AFLP and RAPD markers, used to construct the PD genetic linkage groups, are dominant markers. Polymorphic DNA markers deriving from each parent at heterozygous loci are expected to display a 1:1 segregation ratio of its marker alleles (presence of the band versus absence of the band) in the PD population. Non-polymorphic bands, segregating in the progeny in a 3:1 ratio, were not considered due to limitations of the software used for linkage analysis. Deviation from the 1:1 expected ratio, referred to as marker skewness, was measured using a goodness of fit χ^2 test with one degree of freedom. Linkages between markers were analyzed separately for each parent. Dominant marker segregation in the offspring of heterozygous parents was given an arbitrary code (1 and 0). Linkage analysis was done using MAPMAKER/EXP v3.0b software. MAPMAKER considers only coupling configurations for calculating recombination frequencies. Hence, each marker was encoded both 1:0 and 0:1. In this way it can be ensured that the appropriate configuration is always found and two sets of identical linkage groups with alternate configurations are obtained. This program does not handle data corresponding to the F_1 segregation of markers from heterozygous parents. However, the observed inheritance is analogous to the one corresponding to an F_2 backcross if: (1) the presence of a band in an offspring is explained by the occurrence of one locus producing the band and one producing a null allele (no band), and (2) the absence of a band in an offspring is explained by two null alleles at the marker locus. Hence, the F_2 backcross model of MAPMAKER/EXP v3.0b is applicable using marker data for each parent. Two genetic linkage maps were produced using a LOD threshold of 5 and a 30-cM recombination distance. Marker order was determined and tested using a LOD threshold of 3 and tested by a permutation of marker order for the five consecutive markers at LOD >3 (ripple 5, 3 command of MAPMAKER/EXP v3.0b).

Anonymous linkage groups were associated and oriented (anchored) to known potato chromosomes by using mapped AFLP, SSR and RFLP markers. Eight AFLP primer combinations have been used to generate AFLP markers anchored to the potato genetic map (Van Eck et al. 1995; Rouppe van der Voort et al. 1997). AFLP markers originating from the same primer combination and with the same gel mobility were assumed to map to identical loci. Comparison with the PD mapping population was done with DNA from five parents of the mapping populations used to develop the AFLP catalog (Rouppe van der Voort et al. 1998). Sixty eight primer pairs of microsatellites were used as anchors for the potato maps (Provan et al. 1996; Milbourne et al. 1998). In addition, two RFLP markers per chromosome were selected to anchor linkage groups to the potato map (Gebhardt et al. 1991; Tanksley et al. 1992).

Analyses of trait-marker association

Trait-marker association was analyzed using the following statistical tests. Single-marker analysis was used to detect associations between marker (genotypic) classes (presence or absence of the band) and their respective phenotypic values (level of late blight resistance). Significance of association was determined with a two-sample Student's *t*-test. This test detects whether the means of the phenotypic values for two genotypic classes differ significantly. The interval mapping method using LOD score (Lander and Botstein 1989) was applied through the use of MAPMAKER/QTL v1.1b software and Qgene software (Nelson 1997). The QTL allele combination was tested by applying the 4-allele model of Schäfer-Pregl et al. (1996).

Results

Phenotypic data of the PD population

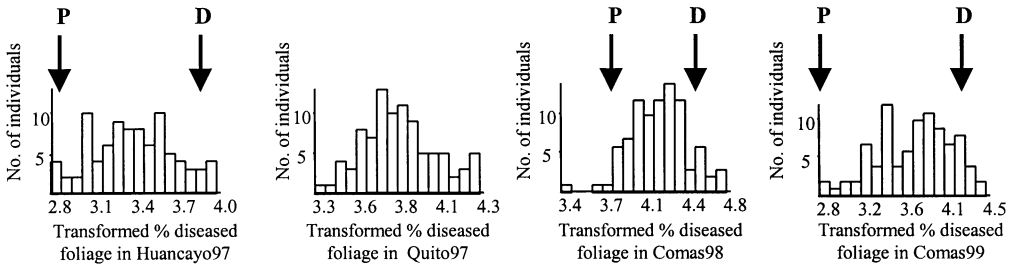
The genetic mapping work was conducted on 92 progenies selected at random from the 246 individuals of the PD population. Field evaluations were developed at three locations during 3 years. This generated four data sets (Fig. 1 A). Good correlations (r=0.37 and 0.75: Fig. 1B) were observed between data sets using transformed values. Each data set was analyzed separately in the QTL analysis.

Genetic linkage maps

RAPD and AFLP markers were scored on 92 hybrids selected at random from the PD population to develop genetic linkage groups for each parent. Several markers displayed a skewed segregation when tested by a χ^2 test at $P<0.01$. These were predominantly derived from the dih-*tbr* parent and were not included in the genetic maps. Both female- and male-derived genetic maps have been constructed (framework markers are shown on Fig. 2). Linkage between markers was declared at a high confi-

Fig. 1A, B Phenotypic classes of field resistance to late blight and correlation between evaluations. **A** frequency distribution of transformed percent of diseased foliage for the 92 hybrids and parents (*P*=*phu* and *D*=dih-*tbr*) for four data sets (parents were not evaluated in Quito 1997); **B** correlation coefficients between the four data sets

A



B

Trial	Correlation coefficients		
Huancayo 97			
Quito 97	0.37		
Comas 98	0.62	0.57	
Comas 99	0.56	0.72	0.75

dence level. Only one marker for each parental map could not be linked significantly to one of the linkage groups out of a total of 240 markers for the *phu* parent and 386 markers for the dih-*tbr* parent. Markers were ordered at high confidence with the exception of one linkage group (chromosome VII) which was ordered at LOD>2.4 for the dih-*tbr* map.

The *phu* linkage map consists of 72 RAPD and 168 AFLP markers assembled into 12 linkage groups summing up 987.4 cM. The map length of the *phu* parent is comparable in size with that of other potato maps (Tanksley et al. 1992, Jacobs et al. 1995). The dih-*tbr* map was constructed from 98 RAPD and 288 AFLP markers in 12 linkage groups totaling 773.7 cM. This map is less complete due in part to higher number of skewed markers, which were not considered in map construction.

Each linkage group (12 for each parent) was anchored with RFLP, SSR and AFLP mapped markers (Fig. 2). This process resulted in the unambiguous identification of all chromosomes and their alignment with respect to the potato genetic map (Tanksley et al. 1992). We were able to identify unambiguously 18 and 38 AFLP anchors to the potato genetic map for the *phu* and dih-*tbr* maps, respectively. These anchors represent 85 and 57% of mapped AFLP markers. Microsatellites with known position on the potato genetic map were used on 44 PD genotypes selected randomly. This provided 12 and 21 SSR anchors for the potato genetic map and the *phu* and dih-*tbr* maps respectively. These anchors represent 92 and 75% of the mapped SSR markers. Selected RFLP were mapped on 51 genotypes selected randomly resulting in the identification of 18 and 21 anchors to the potato genetic map for the *phu* and dih-*tbr* maps respectively. These anchors represent 86 and 84% of the mapped RFLP markers. Of the three classes of markers, AFLP markers presented homoplasic bands (same primer combination and gel mobility, but different loci) more frequently. We found 15 and 18% homoplasic bands for the *phu* and dih-*tbr* maps, respectively. SSR and RFLP markers also revealed new genetic loci, three out of 41 SSRs and one out of 46 RFLPs. A total of 39 mapped markers could not anchor the linkage groups derived from the male parent because of the skewness and distal position of these markers. Such an observation corroborates the difference in map length for the male-derived map with the published potato maps.

Genetic analysis of field resistance

Analysis of trait-marker association was performed for each genetic map independently for the four phenotypic data sets of field resistance to late blight (Huancayo 97; Quito 97; Comas 98; Comas 99) and is illustrated in Fig. 3. Two methods were used in parallel: simple regression for single markers using an *F* test (association was rejected at $P<0.05$) and the interval mapping method for both *phu* and dih-*tbr* maps (QTLs were not considered significant at LOD<2).

Noteworthy was the fact that simple regression analysis, performed with individual trait data, revealed only three chromosomes (VII, XI, XII) derived from the female parent bearing genetic components determining resistance to late blight in at least one of the trait evaluations (significance indicated for each marker in Fig. 3). The interval mapping method (illustrated as a LOD

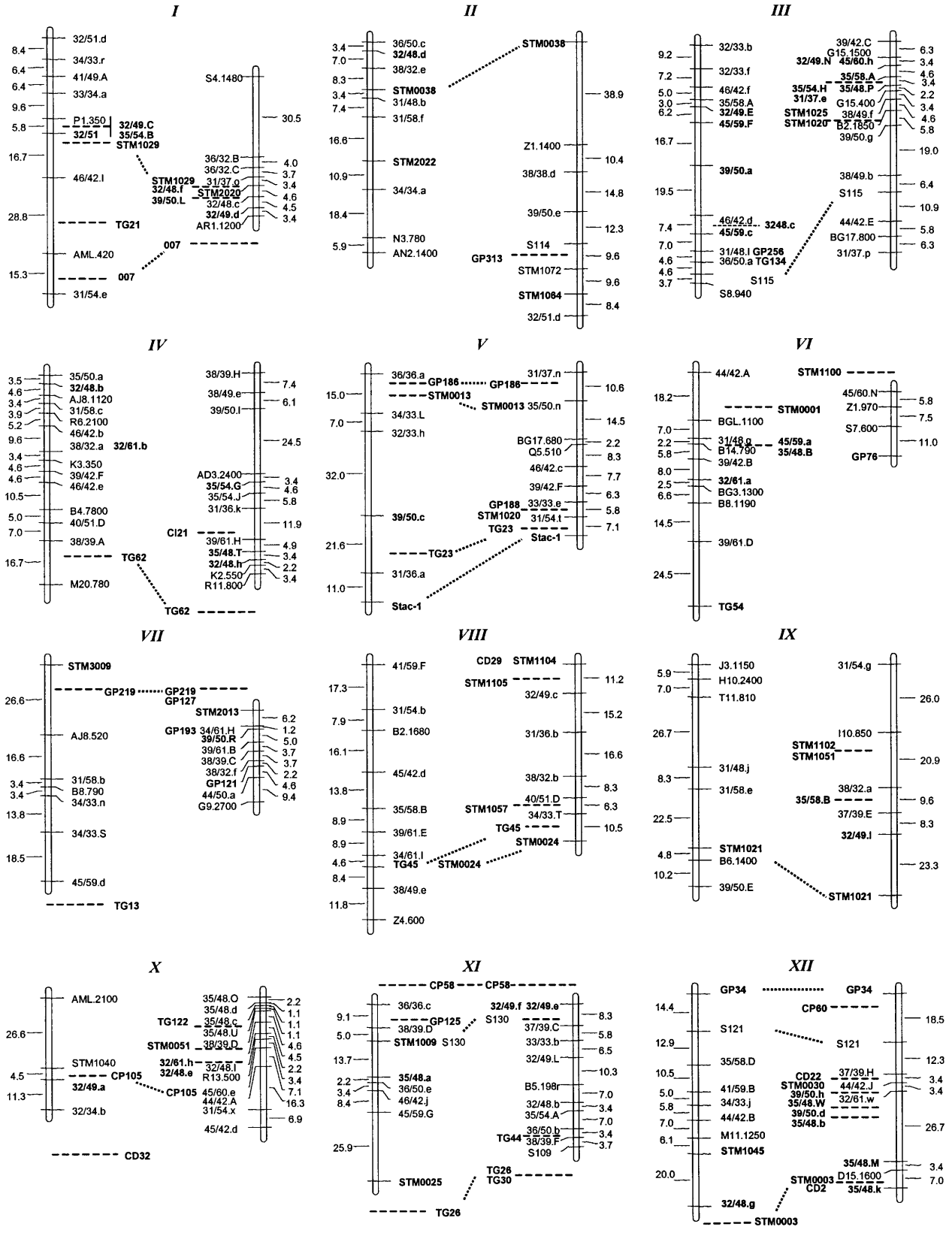


Fig. 2 Genetic linkage maps of the *phu* and dih-*tbr* parents. Anchored markers are indicated in *bold* (redundant AFLP markers have not been indicated). (·····) indicates positions of anchored markers and (– – -) indicates marker bridges between both linkage groups. Genetic distances (cM) are indicated to the left of each linkage group, and markers are on the right side

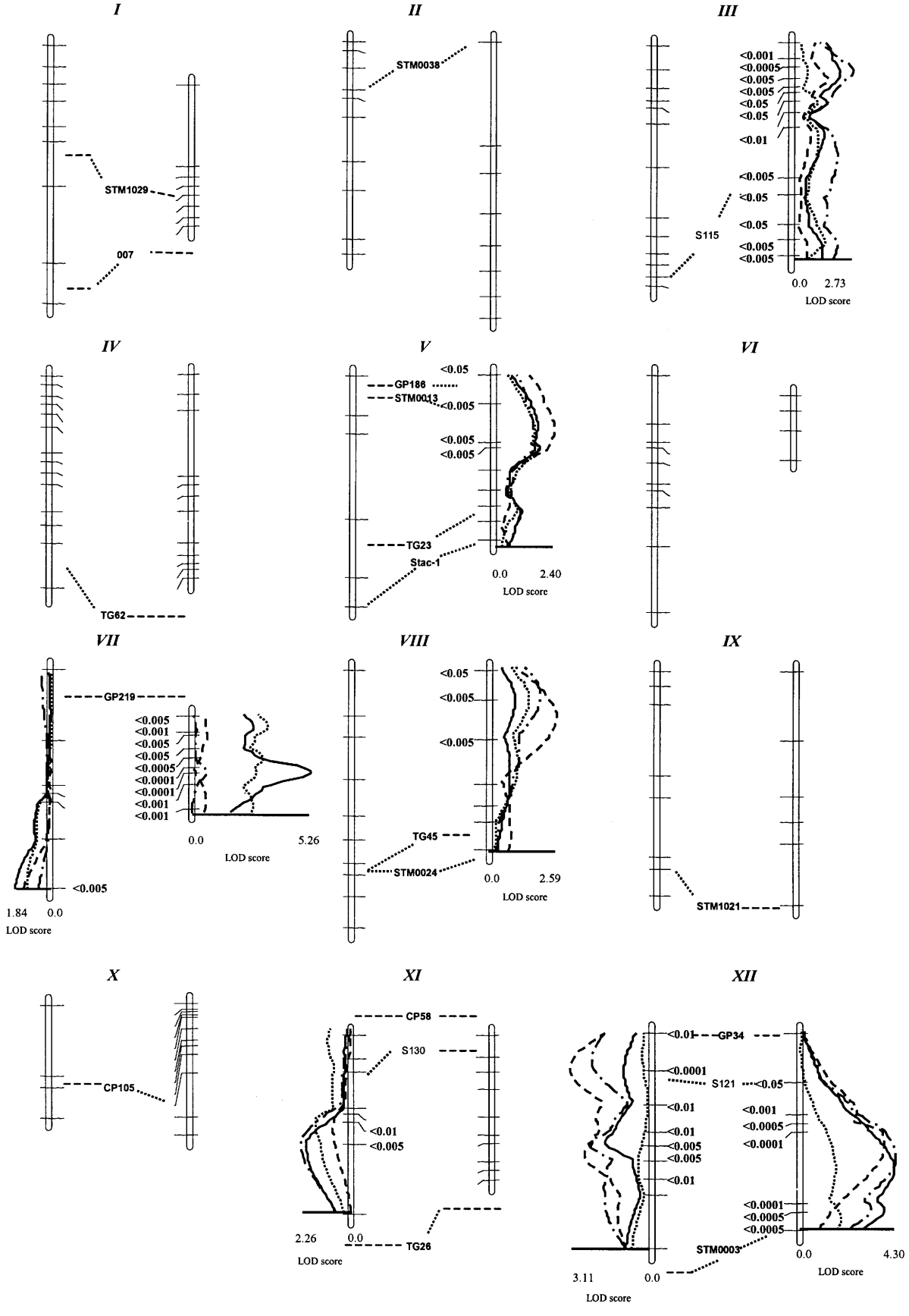

I
STM1029
007
II
STM0038
III
<0.001
<0.0005
<0.005
<0.005
<0.05
<0.05
<0.01
<0.005
<0.05
<0.05
<0.005
<0.005
S115
0.0 2.73
LOD score
IV
TG62
V
<0.05
GP186
STM0013
<0.005
<0.005
<0.005
TG23
Stac-1
0.0 2.40
LOD score
VI
VII
GP219
<0.005
<0.001
<0.005
<0.005
<0.0005
<0.0001
<0.0001
<0.001
<0.001
0.0 5.26
LOD score
<0.005
1.84 0.0
LOD score
VIII
<0.05
<0.005
<0.005
TG45
STM0024
0.0 2.59
LOD score
IX
STM1021
X
CP105
XI
CP58
S130
<0.01
<0.005
2.26 0.0
LOD score
TG26
XII
<0.01
GP34
<0.0001
S121
<0.05
<0.01
<0.001
<0.01
<0.0005
<0.005
<0.0001
<0.005
<0.01
<0.0001
<0.0005
<0.0005
STM0003
0.0 4.30
LOD score
3.11 0.0
LOD score

Table 1 R^2 value (%) of QTLs for four sets of trait data using single-marker analysis (SM) at $P<0.01$ and interval mapping (IM) at LOD>2. The total represents the % of variance explained for the model as obtained by multiple-regression analysis. Q represents the most-significant marker at a QTL locus from the *phu* (P) and dih-*tbr* (D) parents, and the roman number indicates the chromosome assignation

Locus of *Phu* parent	R^2 value (%) for different locations and methods							
	Huancayo 97		Quito 97		Comas 98		Comas 99	
	SM	IM	SM	IM	SM	IM	SM	IM
Q_{PVII}	n.s.[a]	n.s.	n.s.	n.s.	n.s.	n.s.	9.1	n.s.
Q_{PXI}	n.s.	n.s.	10.9	10.7	8.0	n.s.	9.9	n.s.
Q_{PXII}	15.2	15.6	10.3	11.5	n.s.	n.s.	8.4	n.s.
$Q_{PVII+XII}$	15.2		10.3		8.0		16.1	
Q_P Total	19.2		22.7		8.0		21.8	
Locus of dih-*tbr* parent								
Q_{DIII}	n.s.	n.s.	12.7	13.0	n.s.	n.s.	9.9	10.1
Q_{DV}	11.0	14.0	7.5	n.s.	8.5	n.s.	8.8	n.s.
Q_{DVII}	n.s.	n.s.	n.s.	n.s.	16.4	17.6	23.2	23.2
Q_{DVIII}	11.2	13.9	8.6	n.s.	n.s.	n.s.	n.s.	n.s.
Q_{DXII}	17.0	25.1	18.0	26.5	8.2	n.s.	16.0	29.2
$Q_{DVII+XII}$	17.0		18.0		36.7		43.3	
Q_D Total	40.8		47.9		38.5		55.7	

[a] n.s., not significant

score plot in Fig. 3) detected only the QTLs on chromosomes XI and XII repeatedly for two field evaluations. Therefore, the effect on chromosome VII from the female parent remains to be confirmed by additional field-testing.

Using the dihaploid genetic map, simple regression analysis performed with individual trait data revealed at least eight chromosome segments having a QTL effect (I, III, IV, V, VII, VIII, X and XII) for at least one of the trait evaluations, at significance levels ranging between $P<0.01$ and $P<0.0001$. When the interval mapping method was applied, similar results were found with six chromosome (III, V, VII, VIII, X and XII) having a significant QTL effect on field resistance. As the QTL effects on chromosomes I, IV and X were observed only for one set of trait evaluation data, we consider these QTLs less-reliable than the other five detected.

Variance explained by the QTL

Both types of analysis (single-marker and marker-intervals) allow an estimation of the strength of each QTL by the percentage of trait variation (see Table 1). However, the interval mapping method provides a better estimate as it considers the interval between markers rather than the marker itself as the location of the QTL (Lander and Botstein 1989). To estimate the effect of all detected QTLs on phenotypic variance, a multiple regression analysis was conducted considering all markers significantly associated ($P<0.01$) with the trait in the single-marker analysis (Q_P or Q_D total in Table 1).

Both types of analyses, single-marker and interval mapping, reveal a QTL model for field resistance to late blight as follows:

(1) Three QTLs from the *phu* parent contribute up to 23% of the phenotypic variation of the field resistance. The QTL with the largest effect is located on chromosome XII (Q_{PXII}). The second most important and reproducible is the one on chromosome XI (Q_{PXI}). Note also the small QTL on chromosome VII (Q_{PVII}).
(2) Five QTLs from the dih-*tbr* parent contribute together up to 56% of the phenotypic variation for field resistance. The QTL with the biggest effect is located on chromosome XII (Q_{DXII}) followed closely by a QTL on chromosome VII (Q_{DVII}). Three additional QTLs, on chromosomes III, V and VIII, are repeatedly found with moderate effects.

Two QTLs from both parents coincided in being present on the same chromosome: one pair on chromosome VII and one on chromosome XII. These account for up to 16 and 43% of the phenotypic variation for the *phu* and dih-*tbr* parents respectively.

The interaction between alleles from both parents cannot be assessed accurately using dominant markers in an

◀ **Fig. 3** QTL locations on the genetic linkage maps of the *phu* and dih-*tbr* parent: QTLs are represented as LOD-score plots only for chromosomes with an association with field resistance in at least two or more data sets either by single-marker ($P<0.01$) or interval mapping (LOD>2). The lowest *P* value among the four data sets is indicated next to the markers associated with field resistance at a minimum of $P<0.05$. Only bridging markers are repeated from Fig. 2. Data sets are (– – -) Huancayo 97, (– · –) Quito 97, (·····) Comas 98, (——) Comas 99

Table 2 QTLs for field resistance to late blight detected using the four-allele model for the interval between markers S121 and STM0003 of chromosome XII

Trial location	N[b]	P[c]	Mean trait value[a]			
			$Q_{PXII}\ Q_{DXII}$	$Q_{PXII}\ q_{DXII}$	$q_{PXII}\ Q_{DXII}$	$q_{PXII}\ q_{DXII}$
Huancayo 97	74	0.00023	3.26557	3.31774	3.32518	3.67810
Quito 97	77	0.00062	3.75070	3.74997	3.77166	4.05171
Comas 98	75	0.049274	4.11390	4.25819	4.16901	4.38289
Comas 99	76	0.00080	3.48610	3.91191	3.69494	4.02050

[a] For each genotypic class, Q and q represent the QT alleles with and without an effect

[b] Number of genotypes pertaining to each genotypic class

[c] Significance level at which H_0 (no QTL) is rejected

F_1 population because maps cannot be aligned and phases are not known. However, co-dominant markers can serve as bridges to allow lining up of both maternal and paternal maps and testing allelic interaction at the marker locus. We performed such an analysis for chromosome XII, which shows QTL effects from both parents and for which three bridging markers were mapped using the four-allele model of Schäfer-Pregl et al. (1996). QTL effects were detected in the interval between markers S121 and STM0003 (Table 2). The means of the trait value for each genotypic class vary in a consistent manner for the four data sets. Indeed, QTLs from both parents contribute positively to increase the field resistance.

Discussion

We have taken advantage of the non-inbred nature of the potato by using its high heterozygosity to generate genetic maps in the first hybrid generation (H_1). The hybrids fall into four genotypic classes at each locus (Leonards-Schippers et al. 1994). These genotypic classes can be used to study phenotypic variation of the trait if at least one of the parents is heterozygous at this locus. RAPD and AFLP markers are dominant markers that present three expected segregation ratios in an H_1. A monomorphic 1:0 segregation is indicative of a homozygous locus and hence is not informative for map construction. A 1:1 segregation corresponds to a polymorphic and heterozygous locus useful for map construction. And a 3:1 segregation is indicative of a marker that is in a heterozygous state in both parents, but in homozygous and heterozygous states in the progeny which cannot be distinguished for such a segregation ratio. Two genetic maps were constructed, one for each parent, using these anonymous markers, and AFLP, RFLP and SSR markers previously mapped in other genetic mapping studies were used to identify and orient each chromosome. Considering reproducibility, cost, and time, the present work allows us to conclude that a combination of AFLP and SSR markers is most appropriate for efficient genetic map-construction in potato.

A significant correlation was observed among the four data sets collected under three different environments for 3 consecutive years, which is indicative of common genetic components acting in concert across environments.

An initial attempt to apply markers associated to late blight resistance failed to detect associations with resistance on a potato population (data not shown). The markers tested were those flanking QTLs found in the leaf-disk assay for plants grown under different conditions (Castillo 1998). Only markers on chromosome V were associated weakly, but significantly ($P<0.05$), with resistance to late blight in the present PD mapping population. Contrasting with this first disappointing genetic analysis, the QTL analysis revealed three QTLs from the *phu* parent and five from the dih-*tbr* parent. Two QTLs are detected on the same chromosomes (VII and XII), possibly at an homologous location. The most important QTL effect is on chromosome XII, with a large effect on the variation of field resistance to late blight. Most of the QTLs detected in the PD genetic maps locate at different map positions to those found by other groups (Leonards-Schippers et al. 1994; Meyer et al. 1998; Collins et al. 1999; Oberhagemann et al. 1999; Ewing et al. 2000). The coincidence between the main QTL effect in a *Solanum berthaultii* and a dihaploid *S. tuberosum* population (Ewing et al. 2000) and the QTL effect detected on chromosome III, are noteworthy. However, as a general overview of QTL-mediated resistance to late blight, the genetic base of late blight field resistance in the PD population differs in important respects from other genetic studies according to published map locations. We have used one species in common with other genetic mapping (*S. tuberosum* subsp. *tuberosum*) and have generally not found a similar genetic component associated with field resistance. This result can have several interpretations. It could simply be due to homozygosity at the respective loci in genetic material used by the respective groups, but it could also be due to a different response to the late blight disease under different day length conditions. Because potato is a highly heterozygous crop, we feel that the first interpretation is less likely than the second, although we cannot at present rule out the first hypothesis. Hence, we propose that day length difference is responsible for at least some of the genetic-analysis differences observed. Indeed, field resistance to late blight has been recently suggested to be more a physiological response than a strict plant response to a disease (Collins

et al. 1999; Oberhagemann et al. 1999). Earliness and plant and foliage vigor were highly correlated with QTLs for field resistance in this study. Additionally, quantitative resistance to late blight and late maturity (dependent on day length) have been generally observed to be correlated (Colon et al. 1995a).

Another complication arises from the predominant contribution to field resistance from the susceptible parent. This observation is not uncommon in the case of late blight, as has been already reported by Leonards-Schippers et al. (1994). The *phu* and dih-*tbr* QTL maps coincide for four QTLs out of eight. This result highlights the difference between potato species for QTL architecture conferring field resistance to late blight. However, we do not know if this is also true for other potato species.

Future developments that will be needed before marker assisted selection becomes a reality in potato breeding are the development of additional QTL maps from more-diverse sources of germplasm field-tested in various environments. The application of QTL-associated markers from the present study will aid in the selection of genotypes with field resistance to late blight. The validation of this selection process will also require a few rounds of additional field testing.

Acknowledgments The authors are grateful to the following scientists for providing DNA probes and templates: Rhonda Meyer and Robbie Waugh at the Scottish Crop Research Institute (United Kingdom); Christiane Gebhardt at the Max Planck Institute (Germany); Herman van Eck and Jeroen Rouppe van der Voort at Wageningen Agricultural University (Netherlands); and Steve Tanksley at Cornell University (USA). We also wish to thank Jorge Andrade at INIAP Ecuador for providing the original source of plant material used in the present study. Finally, we thank E. Ritter for helpful suggestions and critical reading of the manuscript and for providing help in applying the four-allele QTL model. This work was in compliance with Ecuadorian and Peruvian laws. It was made possible initially through a grant from the UNDP special project program and has been fully developed through a grant from the Bundesministerium für Wirtschaftliche Zusammenarbeit und Entwicklung (BMZ) and the Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) (project no 96.7860.8–001.00).

References

Cañizares CA, Forbes GA (1995) Foliage resistance to *Phytophthora infestans* (Mont.) de Bary in the Ecuadorian national collection of *Solanum phureja* ssp. *phureja* Juz. & Buk. Potato Res 38:3–10

Castillo RR (1998) Analisis molecular de extremos fenotípicos evaluados con respecto a la expresión de resistencia horizontal a *Phytophthora infestans* en papa. Graduate Thesis in Biological Sciences, Universidad Nacional Agraria La Molina, Lima, Peru

Collins A, Milbourne D, Ramsay L, Meyer R, Chatot-Balandras C, Oberhagemann P, De Jong W, Gebhardt C, Bonnel E, Waugh R (1999) QTLs for field resistance to late blight in potato are strongly correlated with maturity and vigour. Mol Breed 5:387–398

Colon LT, Turkensteen LJ, Prummel W (1995a) Durable resistance to late blight (*Phytophthora infestans*) in old potato cultivars. Eur J Plant Pathol 101:387–397

Colon LT, Jansen RC, Budding DJ (1995b) Partial resistance to late blight (*Phytophthora infestans*) in hybrid progenies of four South American *Solanum* species crossed with diploid *S. tuberosum*. Theor Appl Genet 90:691–698.

De Maine MJ, Carrol CP, Stewart HE, Solomon RM, Wastie RL (1993) Disease resistance in *Solanum phureja* and diploid and tetraploid *S. tuberosum*×*S. phureja* hybrids. Potato Res 36:21–28

Ewing EE, ·imko I, Smart CD, Bonierbale MW, Mizubuti ESG, May GD, Fry WE (2000) Genetic mapping from field tests of qualitative and quantitative resistance to *Phytophthora infestans* in a population derived from *Solanum tuberosum* and *Solanum berthaultii*. Mol Breed 6:25–36

Gebhardt C (1994) RFLP mapping in potato of qualitative and quantitative genetic loci conferring resistance to potato pathogens. *Am Potato J* 71:339–345

Gebhardt C, Ritter E, Barone A, Debener T, Walkemeier B, Schachtschabel U, Kaufmann H, Thompson RD, Bonierbale MW, Ganal MW, Tansksley SD, Salamini F (1991) RFLP maps of potato and their alignment with the homoeologous tomato genome. Theor Appl Genet 83:49–57

Ghislain M, Dapeng Z, Herrera Ma del R (Eds) (1999) Molecular biology laboratory protocols: plant genotyping. Crop Improvement and Genetic Resources Department, Training Manual, 2nd edn. International Potato Center (CIP), Lima, Peru

Jacobs JME, Van Eck HJ, Arens P, Verkerk-Bakker B, Hekkert BL, Bastiaanssen HJM, El-Kharbotly A, Pereira A, Jacobsen E, Stiekema WJ (1995) A genetic map of potato (*Solanum tuberosum*) integrating molecular markers, including transposons, and classical markers. Theor Appl Genet 91:289–300

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

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

Meyer RC, Milbourne D, Hackett CA, Bradshaw JE, McNichol JW, Waugh R (1998) Linkage analysis in tetraploid potato and association of markers with quantitative resistance to late blight (*Phytophthora infestans*). Mol Gen Genet 259:150–160

Milbourne D, Meyer RC, Collins AJ, Ramsay LD, Gebhardt C, Waugh R (1998) Isolation, characterization and mapping of simple sequence repeat loci in potato. Mol Gen Genet 259:233–245

Nelson JC (1997) Q-gene: software for marker-based genome analysis and breeding. Mol Breed 3:229–235

Nyquist WE (1991) Estimation of heritability and prediction of selection response in plant populations. Crit Rev Plant Sci 10:235–322

Oberhagemann P, Chatot-Balandras C, Schäfer-Pregl R, Wegener D, Palomino C, Salamini F, Bonnel E, Gebhardt C (1999) A genetic analysis of quantitative resistance to late blight in potato: towards marker-assisted selection. Mol Breed 5:399–415

Perez-Ugalde G, Hougas RW, Peloquin SJ (1964) Fertility of *S. phureja*- haploid *S. tuberosum* F_2 hybrids. Am Potato J 41:256–262

Provan J, Powell W, Waugh R (1996) Microsatellite analysis of relationships within cultivated potato (*Solanum tuberosum*). Theor Appl Genet 92:1078–1084

Rouppe van der Voort JNAM, Van Zandvoort P, Van Eck HJ, Folkertsma RT, Hutten RCB, Draaistra J, Gommers FJ, Jacobsen E, Helder J, Bakker J (1997) Allele specificity of comigrating AFLP markers used to align genetic maps from different potato genotypes. Mol Gen Genet 255:438

Rouppe van der Voort JNAM, Van Eck HJ, Draaistra J, Van Zandvoort PM, Jacobsen E, Bakker J (1998) An online catalogue of AFLP markers covering the potato genome. Mol Breed 4:73–77

Schäfer-Pregl R, Salamini F, Gebhardt C (1996) Models for mapping quantitative trait loci (QTLs) in progeny of non-inbred parents and their behaviour in the presence of distorted segregation ratios. Genet Res 67:43–54

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, Young ND (1992) High-den-

sity molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Trognitz BR, Eslava M, Portal L, Ramón C (1997) Resistance to late blight from diverse wild sources. In: Program report 1995–1996, International Potato Center (CIP), Lima, Peru, pp 127–137

Van Eck HJ, Van der Voort JNAM, Draaistra J, Van Zandvoort P, Van Enckevort E, Segers B, Peleman J, Jacobsen E, Helder J, Bakker J (1995) The inheritance and chromosomal localization of AFLP markers in a non-inbred potato offspring. Mol Breed 1:397–410

Vos P, Hogers R, Bleeker M, Reijans M, vande Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Williams J, Hanafey M, Rafalski A, Tingey S (1993) Genetic analysis using amplified polymorphic DNA markers. Methods Enzymal 218:704–740