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A genetic analysis of quantitative resistance to late blight in potato: towards marker-assisted selection

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Abstract

Late blight caused by the oomycete *Phytophthora infestans* is the most important fungal disease in potato cultivation worldwide. Resistance to late blight is controlled by a few major genes (*R* genes) which can be easily overcome by new races of *P. infestans* and/or by an unknown number of genes expressing a quantitative type of resistance which may be more durable. Quantitative resistance of foliage to late blight was evaluated in five F_1 hybrid families originating from crosses among seven different diploid potato clones. Tuber resistance was evaluated in four of the families. Two of the families were scored for both foliage maturity and vigour. The five families were genotyped with DNA-based markers and tested for linkage with the traits analysed. QTL (quantitative trait locus) analysis identified at least twelve segments on ten chromosomes of potato having genes that affect reproducibly foliage resistance. Two of those segments also have major *R* genes for resistance to late blight. The segments are tagged by 21 markers that can be analyzed based on PCR (polymerase chain reaction) with specific oligonucleotide primers. One QTL was detected for tuber resistance and one for foliage vigour. Two QTLs were mapped for foliage maturity. Major QTL effects on foliage and tuber resistance to late blight and on foliage maturity and vigour were all linked with marker *GP179* on linkage group V of potato. Plants having alleles at this QTL, which increased foliage resistance, exhibited decreased tuber resistance, later maturity and more vigour.

Introduction

Since its first outbreak in Europe in the middle of the 19th century, late blight caused by the oomycete *Phytophthora infestans* remains the most important fungal disease in potato cultivation. Both foliage and tubers are infected, and, when not controlled, late blight can lead to complete loss of the crop yield. In current potato cultivation, late blight is controlled worldwide by the application of fungicides. This causes environmental concern and increases selection pressure towards the evolution of resistant fungal isolates [4]. Therefore, much effort has been devoted to breed resistant cultivars [37]. Initial success, though transient and limited, was obtained from dominant alleles (*R* genes) introgressed into potato cultivars from the wild species *Solanum demissum* which conferred race-specific resistance to *P. infestans*. However, this monogenic type of resistance was soon overcome by new races of the fungus [29]. Phenotypic selection for polygenic or horizontal resistance to late blight, assumed to be more durable and race non-specific, remains difficult [37]. In addition, late maturity, which is an undesirable trait, is frequently associated with high levels of foliage resistance. Moreover, the relationship between late blight resistance of foliage and tubers is unclear: some studies suggested a correlation between resistance of foliage and tubers whereas others did not [37].

The advent of DNA-based markers that are phenotypically neutral and available in unlimited numbers

provides new technology for addressing the long-standing problem of selecting genetic resistance to late blight. Markers have been used to localize genetic factors controlling qualitative and quantitative expression of resistance to late blight on the molecular maps of potato [19, 20, 8, 9, 21]. Mapping experiments are one prerequisite for the design of marker-assisted selection strategies combining favourable alleles for late blight resistance and/or selecting against unfavourable alleles. Marker-assisted selection in plant breeding is now becoming more economically feasible since diagnostic marker assays based on PCR [30] can be developed which require only minute quantities of plant DNA and no nucleic acid hybridization techniques.

Few segregating populations of potato, thus far, have been analysed for linkage between markers and quantitative trait loci (QTLs) affecting resistance to late blight. The first QTL mapping experiment carried out in a diploid population revealed eleven chromosome segments on nine potato chromosomes having putative QTLs [20]. Resistance was quantified using a bioassay where leaf discs were inoculated with specific races of *P. infestans*.

In this paper we address the following questions: (1) whether QTLs previously identified in one mapping population [20] can be also detected in independent genetic materials, (2) whether QTLs for foliage resistance detected under field conditions overlap with QTLs for resistance that were detected based on laboratory bioassays, and (3) whether genetic relationships can be identified between QTLs for foliage and tuber resistance to late blight and between QTLs for foliage resistance and maturity. Based on the results, we provide information for PCR-based analysis of a set of marker loci tagging QTLs for late blight resistance in several breeding lines of potato.

Materials and methods

Plant material

Five hybrid families (2×) were analysed that were derived by crossing heterozygous potato clones of diverse origin including dihaploids (DH) of *Solanum tuberosum* ssp. *tuberosum* and wild *Solanum* species such as *S. chacoense*, *S. kurtzianum*, *S. stenotomum* and *S. vernei*. No known major *R* genes were present in this material. The five families are referred to as populations GA, GB, GC, GDE and K31.

GDE population (n = number of clones = 109): clone G87D2.4.1 [(DH Flora × PI 458.388) × (DH Dani × PI 230.468)] was crossed as a female parent with clone I88.55.6 {[DH (Belle de Fontenay × Katadhin) × PI 238.141] × [DH Jose × (PI 195.304 × WRF 380)]} as a male parent. The parental lines are referred to as G87 and I88. G87 is moderately resistant to late blight (average score 6.0 for foliage and 7 for tuber resistance), whereas clone I88 is rather susceptible (average score 3.3 for foliage and 6 for tuber resistance).

GB population ($n = 29$): G87D2.4.1 (G87), the same female clone as in population GDE, was crossed with clone I86.102.1 {[DH (Belle de Fontenay × Katadhin) × PI 558.146] × (DH Maris Page × PI473.305)}, referred to as I86, as a male parent. Foliage of I86 is rather susceptible to late blight in the field (average score 3.7). I86 is rated as highly tuber resistant (score 9).

GC population ($n = 80$): G87D2.4.1 (G87) was crossed as a female parent with a diploid clone of unknown origin, referred to as PX, as a male parent. Foliage and tubers of PX were not evaluated for resistance to late blight.

GA population ($n = 70$): clone G88D2.20.2 [(Bulk 2x × PI 320.286) × (DH BF15 × PI 225.682.13)] was crossed as a female parent with PX, the same clone used as a pollinator in population GC, as the male parent. Clone G88D2.20.2, referred to as G88, was moderately resistant to late blight (average score 6.2 for foliage resistance, tuber resistance not evaluated).

K31 population ($n = 113$): The 2× clone H80.577/1 was crossed as a female parent with 2× clone H80.576/16 as a male parent. Pedigrees are not available for these clones which have been previously characterized with RFLP markers (clones 3 and 38 in Gebhardt *et al.* [13]) and are reported as having high and intermediate levels of foliage resistance to late blight (unpublished data). Clone H80.577/1 is referred to as P3 and clone H80.576/16 as P38.

Assessment of late blight resistance

Field assessment of foliage resistance

Evaluations were conducted as described in Dowley *et al.* [7]. Test plots were located in Brittany, France. In mid April, one or two tubers per clone were planted next to an infector row planted with the susceptible cv. Bintje. A set of fourteen commercial cultivars was included among the test plots as standards for resistance and susceptibility (1st early: Bintje, Escort, Jaerla, Pentland Dell, Sirtema; 2nd early: Arran Banner, Desiree, Lori, Spartaan; maincrop: K. Vandel, Kerpondy, Maritta, Rector, Saturna). After emergence,

Table 1. Selective genotyping of populations GDE, GB, GC and GA.

Map segments	Markers	GDE		GB		GC		GA	
		G87	I88	G87	I86	G87	PX	G88	PX
III_a	*GP25, CP6*	√[4]	√	√	√	√	√	√	√
III_b	*St4cl*	√	√	na[1]	na	na	na	na	na
IV	*GP180, MBF*	√	√	√	np[2]	√	np	√	np
V	*GP179, GP21, SPUD237, StPto, CP113*	√	√	√	√	√	√	np	√
VI_a	*GP79*	√	√	na	na	√	√	np	√
VI_b	*GP76, WUN2*	√	√	√	√	√	√	√	√
IX	*prp1*	√	√	√	np	co[3]	co	np	√
XI_a	*GP125*	√	np	√	np	√	np	√	np
XI_b	*GP250*	√	√	√	√	√	√	√	√
XII	*GP34, IPM4*	√	np	√	np	√	√	np	√

[1]na = not analysed. [2]np = not polymorphic.
[3]co = common allele.
[4]√ = map segments where an allele of at least one marker tagging the segment segregated.

when plants reached 20 cm height (20 June 1995, 28 June 1996, 13 June 1997), infector rows were sprayed with a sporangial suspension (ca. 5000 sporangia and zoospores/ml) of a *P. infestans* isolate obtained from plants infected by local and naturally occurring epidemics in the preceding year. The race structure was representative, therefore, of the local conditions in Brittany. The isolates in the three test years carried the virulence genes 1, 3, 4, 7, and 11 as determined by inoculating *R*-gene differential hosts. After inoculation, three to four assessments were made in weekly intervals for every clone, using a 1 to 9 scale ($1 = >$ 90%, and $8 = <$ 10% necrotic leaf tissue). A weighted sum of the three more representative consecutive assessments in one year was used to obtain an overall rating for each clone.

Laboratory assessment of tuber resistance

Tubers were collected from an isolated multiplication field that was chemically protected against late blight. The same *P. infestans* isolate as sprayed in the field was used to inoculate ten whole undamaged tubers per clone. Tubers were sprayed with a sporangial suspension of *P. infestans* (1 to 3×10^4 zoospores and sporangia/ml) and incubated at 19 °C in a dark chamber maintained at maximum humidity. After two weeks each tuber sample was cut and assessed for blight using a scale from 1 (more than 8 blighted tubers) to 9 (no blighted tubers). Ten standard cultivars were included in each test, among them BF15 and Roseval for high susceptibility (score 2 to 3) and Jaerla and Saturna for high resistance (score 8).

Leaf disc bioassay

This assay was performed on population K31 as described in Leonards-Schippers *et al.* [20]. Plants used for cutting the leaf discs were grown in pots either in a controlled environment chamber (16 h light, 6480 lux, 20 °C) or in a climatized greenhouse under natural daylength conditions (night 8 h at 16 °C, day 16 h at 21 °C) between March and July. Leaf discs cut from leaves of the third and fourth acropetal node from plants that were between eight and fourteen weeks old were spray inoculated with inoculum (4×10^4 zoospores/ml) prepared from three week old fungal mycelium. Infected leaf discs were visually evaluated after four to nine days for percentage of infected leaf disc area. Results of eight leaf discs per genotype per experiment were averaged. Three *P. infestans* races were tested. In 1995, race 1 was provided by W. Gieffers (MPI for Breeding Research, Köln, Germany). In 1996, race 0 was provided by F. Govers (Wageningen Agricultural University, Netherlands) and the complex race (1.2.3.4.7.10.11, A1 mating type) was provided by B. Schöber-Butin (BBA Braunschweig, Germany).

Assessment of foliage maturity and vigour

In a multiplication field that was chemically protected against late blight, each genotype was assessed for maturity and vigour of the foliage in comparison with standard cultivars, at 90 to 100 days of field growth. The highest score 9 was attributed to cultivars like Eersteling whose haulm reaches maximum development at 90 days and then starts senescing. The lowest score (3 or less) was attributed to late maturing cul-

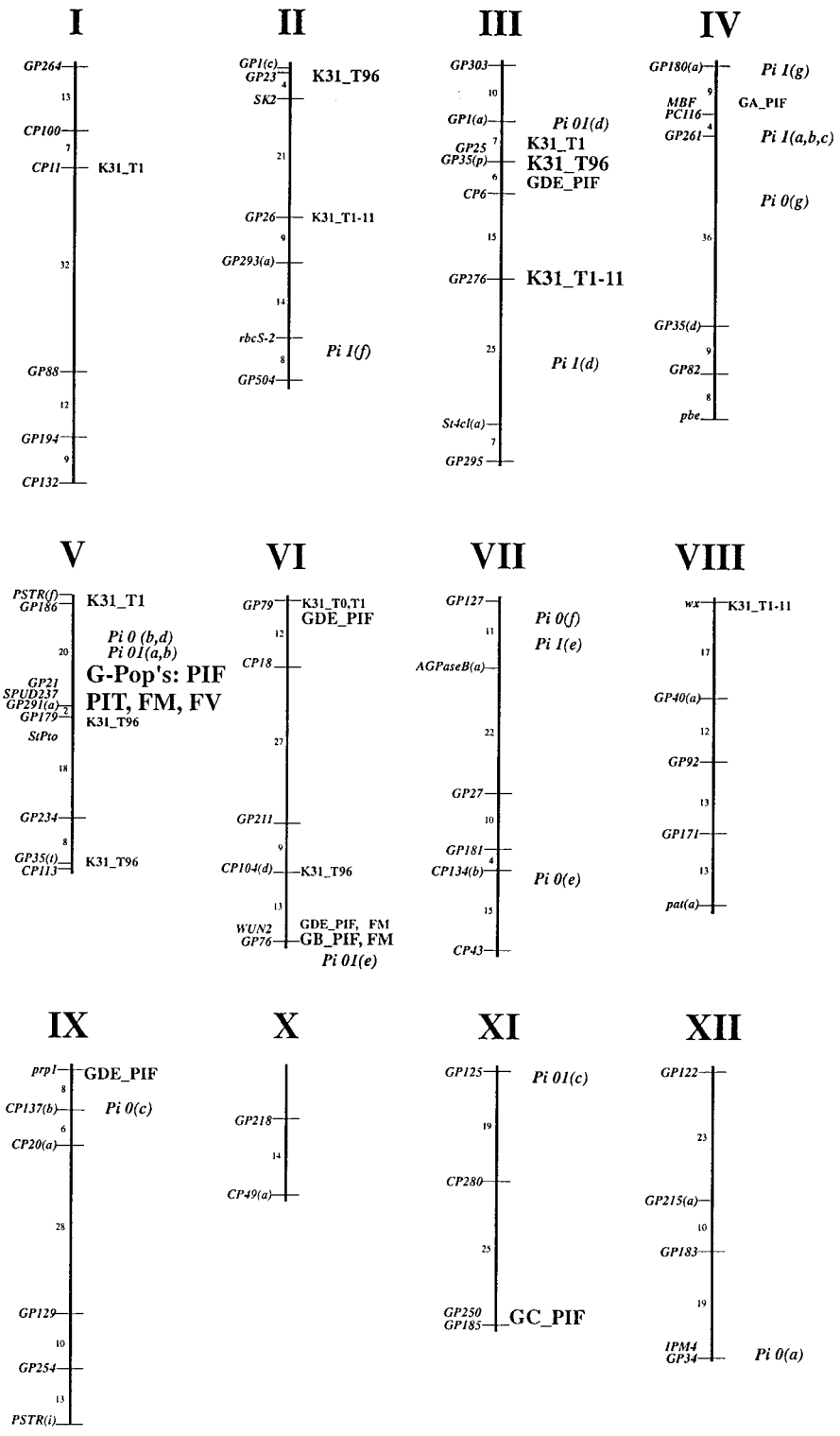
I
GP264
13
CP100
7
CP11
K31_T1
32
GP88
12
GP194
9
CP132
II
GP1(c)
GP23
4
SK2
K31_T96
21
GP26
K31_T1-11
9
GP293(a)
14
rbcS-2
Pi 1(f)
8
GP504
III
GP303
10
GP1(a)
Pi 01(d)
GP25
7
K31_T1
GP35(p)
K31_T96
6
CP6
GDE_PIF
15
GP276
K31_T1-11
25
Pi 1(d)
St4cl(a)
7
GP295
IV
GP180(a)
Pi 1(g)
9
MBF
PC116
GA_PIF
4
GP261
Pi 1(a,b,c)
Pi 0(g)
36
GP35(d)
9
GP82
8
pbe
V
PSTR(f)
GP186
K31_T1
Pi 0 (b,d)
Pi 01(a,b)
20
GP21
SPUD237
GP291(a)
2
GP179
G-Pop's: PIF
PIT, FM, FV
K31_T96
StPto
18
GP234
8
GP35(t)
CP113
K31_T96
VI
GP79
K31_T0,T1
GDE_PIF
12
CP18
27
GP211
9
CP104(d)
K31_T96
13
WUN2
GP76
GDE_PIF, FM
GB_PIF, FM
Pi 01(e)
VII
GP127
11
Pi 0(f)
Pi 1(e)
AGPaseB(a)
22
GP27
10
GP181
4
CP134(b)
Pi 0(e)
15
CP43
VIII
wx
K31_T1-11
17
GP40(a)
12
GP92
13
GP171
13
pat(a)
IX
prp1
GDE_PIF
8
CP137(b)
Pi 0(c)
6
CP20(a)
28
GP129
10
GP254
13
PSTR(i)
X
GP218
14
CP49(a)
XI
GP125
Pi 01(c)
19
CP280
25
GP250
GP185
GC_PIF
XII
GP122
23
GP215(a)
10
GP183
19
IPM4
GP34
Pi 0(a)

Figure 1. Core RFLP map of population K31 including random genomic markers (*GP*), random cDNA markers (*CP*), subtelomeric repeat markers (*PSTR*, [16]) and functional gene markers (*SK2*, *rbcS-2*, *St4cl*, *PC116*, *pbe*, *AGPaseB*, *wx*, *pat*, *prp1*, for further details see [15] and [32]). Small letters in parenthesis after a marker name indicate that the marker probe detected more than one locus. Mean distances (cM) between marker loci were calculated from the distances between the markers in the two parental maps [32] and are indicated on the left of the linkage groups. Markers *GP25*, *MBF*, *GP21*, *SPUD237*, *WUN2*, *GP250* and *IPM4* used for genotyping populations GDE, GB, GC and GA but not used for genotyping population K31 are included at their approximate map positions on linkage groups III, IV, V, XI and XII. Positions of QTL effects on resistance to late blight, foliage maturity and vigour are indicated on the right of the linkage groups. GA_PIF, GB_PIF, GC_PIF and GDE_PIF: effects on foliage resistance in populations GA, GB, GC and GDE, respectively. G-Pop's: PIF, PIT: Major effects on foliage (PIF) and tuber (PIT) resistance linked to markers *GP21*, *SPUD237* and *GP179* on linkage group V in populations GA, GB, GC and GDE. FM and FV indicate effects on foliage maturity and foliage vigour, respectively, in populations GB and GDE. Only those effects are included in the figure that were detected based on average scores over two or three years of testing (see also Table 2). K31_T0, K31_T1, K31_T1-11 and K31_T96: effects on resistance to specific races of *P. infestans*, detected in population K31 based on combined data sets T0, T1, T1-11 and T96, respectively (see Table 4), and using the leaf disc bioassay. Different font sizes indicate significance of the effects: small: $P < 0.05$, medium: $P < 0.01$, large: $P < 0.001$. *Pi 0*(*a* to *g*), *Pi 1*(*a* to *g*) and *Pi 01*(*a* to *e*): approximate location of QTLs identified by Leonards-Schippers *et al.*[20].

tivars whose haulm was still growing at the time of evaluation. Cultivar Bintje usually has a score of 6. Foliage vigour was visually scored, integrating in a single score various aspects of haulm habits such as total leaf area, stem number, stem height and stem diameter. A score of 1 was given to very weak plants, while a score of 9 was given to the best field grown plants when compared to other diploids. Diploids are known to be on average less vigourous than tetraploids. The tetraploid cultivar Bintje scores 7 on average when compared to other tetraploid cultivars.

Isolation of total genomic DNA

Freeze dried leaf material was ground to a fine powder (0.3 g) and extracted with 20 ml of 100 mM Tris-HCl, pH 9.5, 1.4 M NaCl, 20 mM EDTA, 2% (w/v) CTAB, 1% (w/v) polyethylenreglycol 6000 and 0.25% (v/v) 2-mercaptoethanol, for 20 min at 74 °C. The suspension was mixed with 1 volume of chloroform/isoamyl alcohol (24:1 v/v). After separation of organic and aqueous phase by centrifugation, the aqueous phase was adjusted to pH 7.0 with 1 M HCl and treated with 400 μg/ml DNAse-free RNAse. DNA was purified by affinity chromatography on Qiagen Tip 100 columns (Qiagen, Hilden, Germany) according to the supplier's instructions.

Markers

Plants of populations GDE, GB, GC and GA were genotyped with the following markers: *GP25*, *CP6* and *St4cl* (*S. tuberosum* 4-coumarate CoA ligase, [12]) map to linkage group III; *GP25* and *CP6* are linked at a 15 cM distance and occupy a central position on linkage group III. *St4cl* maps to a distal position on the same linkage group. *GP180* and *MBF* [11] tag a segment of ca. 20 map units on linkage group IV. *GP21*, *SPUD237* and *GP179* map to a 5 cM interval on linkage group V [25, 6] whereas *StPto* (potato homologue of the tomato gene for resistance to *Pseudomonas syringae*) and *CP113* occupy more distal positions on linkage group V [24, 18, 27]. *GP76*, which is tightly linked to *WUN2* (wound-induced, [22]), and *GP79* tag different distal segments of linkage group VI. Marker *prp1* (pathogenesis-related glutathione *S*-transferase, [35]) occupies a distal position on linkage group IX. *GP125* and *GP250* tag different distal segments of linkage group XI. The tightly linked markers *GP34* and *IPM4* [2] map to a distal region on linkage group XII. Map positions of the markers are shown in Figure 1. Most markers and genes used in this study have been described in Gebhardt *et al*. [15].

Segregation analysis using 95 RFLP markers [32] was used to construct linkage maps of the twelve chromosomes of population K31.

RFLP analysis

Markers *GP25*, *CP6*, *GP180*, *GP21*, *GP179*, *StPto*, *GP79*, *WUN2*, *GP250* and *GP34* were genotyped by RFLP analysis. Gel-purified insert DNA of marker plasmids was labelled [10] with [α-^{32}P]dCTP (Amersham-Buchler, Braunschweig, Germany) and hybridized to nylon filters (Amersham Hybond N) carrying 4 to 5 μg of restricted total genomic DNA of the parents (except PX) and F_1 progeny as described in Gebhardt *et al.* [13]. The restriction enzymes *Taq*I or *Rsa*I (Boehringer, Mannheim, Germany) were used.

PCR-based analysis

Markers *St4cl*, *MBF*, *SPUD237*, *GP179*, *CP113*, *GP76*, *prp1*, *GP125* and *IPM4* were genotyped by PCR-based analysis. Specific oligonucleotides were designed based on marker sequences or, in the case

Table 2. Effects on foliage resistance (PIF) and tuber resistance (PIT) to late blight, on foliage maturity (FM) and vigour (FV) in populations GDE, GB, GC and GA.

Linkage group	Marker	GDE-pop (G87 × I88) $n = 109$					GB-pop (G87 × I86)[1] $n = 29$			GC-pop (G87 × PX) $n = 80$			GA-pop (G88 × PX) $n = 70$		
		allele origin	PIF 95/96/97	PIT 96/97	FM	FV	allele origin	PIF 95/96/97	FM	Allele origin	PIF 95/96	PIT 96	allele origin	PIF 95/96	PIT 96
III_a	*GP25*	G87	**[4]	–	–	–		–	–		–	–		np[3]	np
	CP6	I88	*[4]	–	–	–		–	–		–	–		–	–
IV	*MBF*		–[4]	–	–	–		–	–		–	–	G88	*	–
V	*GP179*	G87	****[4]	***	****	****	G87	*	–	G87	****	–	G88	np	np
		I88	****	**	****	****	I86	***	***	PX	****	**	PX	****	*
VI_a	*GP79*	G87	**	–	–	–	–	na[2]	na		–	–		–	–
VI_b	*WUN2*	G87	**	–	**	–	G87	*	**		–	–		–	–
	GP76		–	–	–	–	G87	**	***		–	–		–	–
IX	*prp1*	I88	**	–	–	–		–	–		–	–		–	–
XI_b	*GP250*		–	–	–	–		–	–	G87	***	–		–	–

[1]No significant effects on PIT and foliage vigour were detected in the GB population.
[2]na = not analysed.
[3]np = not polymorphic.
[4]$-P > 0.05$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$

of the *St4cl*, *MBF* and *prp1* genes, based on sequence information [1, 11, 35]. Primer sequences for several locus-specific markers were available from the literature [25, 27, 6, 2]. Allele-specific oligonucleotides (ASOs) for markers *GP76* and *GP125* were designed based on DNA-sequence differences between marker alleles, which had been amplified using as template DNA of potato lines P40 and P49. Lines P40 and P49 are the 2× parents of the cross analysed for resistance to *P. infestans* by Leonards-Schippers *et al.* [20]. PCR conditions were as follows: 80 ng of total genomic DNA of potato were amplified by PCR [30] in 50 μl total volume of 20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dGTP and dTTP and 0.27 μM primer, using 1 unit of *Taq* DNA Polymerase (Life Technologies, Eggenstein, Germany). The reaction conditions were: 1×93 °C for 2 min, 40× denaturation at 93 °C for 30 s, annealing at T_a of various temperatures (as specified in Table 6) for 30 s, extension at 72 °C for 1 min. PCR products with different length (SCAR markers, sequence-characterized amplified region) or showing presence/absence polymorphism (ASO markers) were separated on agarose gels. Homomorphic PCR products were size separated after digestion with one of the 4 bp cutter restriction enzymes *Taq*I, *Rsa*I, *Alu*I, *Hha*I, *Nla*III, *Mse*I, *Mnl*I, *Mbo*I, *Hin*P1I and *Hpa*II (CAPS markers, cleaved amplified polymorphic sequence).

QTL analysis

Marker alleles (RFLP fragments or PCR products) segregating in the hybrid progeny and originating from either the female, the male or both parents (common fragments) were scored as present or absent. In populations GC and GA, the mode of inheritance of marker alleles present in the G87 and G88 parent, respectively (either specific for G87 and G88, or common to both parents) could not be identified because the PX parent was not available for genotyping. A hypothesis on the mode of inheritance was made in such cases based on the χ^2 test for deviation from the segregation ratios 1:1 (presence versus absence) expected for parent-specific fragments and 3:1 (presence versus absence) for common fragments. This method of inferring the mode of inheritance based on segregation ratios may result in a false hypothesis when segregation ratios are highly distorted.

In F_1 progeny of non-inbred diploid parents as used in this study, up to four alleles segregate at each genetic locus. Let the female parent have alleles *a* and *b* and the male parent alleles *c* and *d*. In F_1, four genotype classes, with allele combinations *ac*, *ad*, *bc* and *bd*, segregate in the expected ratio of 1:1:1:1. The four genotype classes can be distinguished by scoring at least two polymorphic DNA marker fragments for presence or absence, one (*a* or *b*) of the female parent and another one (*c* or *d*) of the male parent. For a number of markers only one parental marker allele was scorable, either from the female or the male parent, resulting in only two distinguishable genotype classes in F_1 (fragment present or absent). All segregating marker alleles were, therefore, first tested individually for a significant difference ($P < 0.05$) between phenotypic means of two marker genotype classes defined by presence or absence of an RFLP fragment or PCR product. In a fully informative subset of marker loci (one scorable allele in each parent), four marker genotype classes were subsequently tested for a significant difference ($P < 0.05$) between means.

The phenotypic data evaluated in populations GA, GB, GC and GDE were in a ranking order (scores from 1 to 9). The non-parametric Mann-Whitney U-test [23] was used, therefore, to test for a difference between means of two marker genotype classes. At RFLP locus *GP179* in population GDE, four marker alleles were distinguished, *a* and *b* in parent G87 versus *c* and *d* in parent I88. Kruskal-Wallis 1-way ANOVA using SPSS software [33] was performed to test for differences between means of F_1 marker genotype classes *ac*, *ad*, *bc* and *bd*. In population K31, where phenotypic data were continuously distributed, the two sample *t*-test was used for comparing means of two marker genotype classes. Programs written with SAS software [31] performed the *t*-test automatically on all 288 RFLP fragments scored in the K31 population [32]. Four marker genotype classes were distinguished in F_1 at 48 RFLP marker loci distributed on the twelve chromosomes of population K31. In few map segments, where fully informative single marker loci were not available, two marker loci were used instead of one to distinguish four genotype classes (Figure 1). The two marker loci were positioned opposite each other on the male and female parental maps and were known to be closely linked based on previous mapping studies [14, 15]. Analysis of variance (GLM procedure in SAS) was performed to test for differences between means.

Results

Phenotypic analysis of resistance to late blight

Populations GDE and GB were evaluated in the field for foliage resistance in 1995, 1996 and 1997, and in the laboratory for tuber resistance in 1996 and 1997. Populations GC and GA were evaluated for foliage resistance twice in the field, in 1995 and 1996, and once for tuber resistance in 1996. In 1995 and 1996, phenotypic distributions were obtained for foliage resistance that ranged from score 2 to 8. In 1997, a severe natural late blight epidemic occurred parallel to the artificial inoculation which resulted in overall reduced resistance levels, with scores ranging from 2 to 6. Scores for tuber resistance varied between 2 and 9. The plants of populations GDE and GB were also scored in the field in two years for foliage maturity and vigour. Scores for foliage maturity ranged from 3 to 7 and for foliage vigour from 4 to 8. Phenotypic distributions for the traits analysed in population GDE are shown in the accompanying paper of Collins *et al*. [5].

Population K31 was evaluated in 1995 and 1996 six times for quantitative resistance to defined races of *P. infestans* using the leaf disc bioassay. The K31 plants were assayed twice each with race 1 (data sets T1a and T1b in 1995), race 0 (data sets T0a, T0b in 1996) and the complex race 1–11 (data sets T1-11a, T1-11b in 1996). Four additional data sets were derived by calculating the average percent infection of the combined test data obtained with the same race (data sets T1, T0, T1-11) and with all races tested in 1996 (T96). Phenotypic variability of resistance levels was normally distributed (Kolmogorov-Smirnov coefficient >0.2) after arcsin data transformation in six of the ten data sets. Population means varied between tests from 29.0% to 57.0% infected leaf disc area. No evidence was found that the average resistance level obtained in a test were influenced by the race used. Resistance levels of the parental lines varied, depending on the test, between 2.5 and 77.5% for P3 and between 8.0% and 71.0% for P38.

Mapping QTL for foliage and tuber resistance, foliage maturity and vigour in populations GDE, GB, GC and GA

Plants of populations GDE, GB, GC and GA were genotyped with 18 markers of known position on linkage groups III, IV, V, VI, IX, XI and XII of the potato molecular map (Table 1). The markers were selected because they tagged ten different linkage group segments (III_a, III_b, IV, V, VI_a, VI_b, IX, XI_a, XI_b, XII) shown to have QTL for late blight resistance in independent mapping experiments ([20] and K31 population, see below) and/or major *R* genes for late blight resistance [19, 8, 9]. Map positions of the markers are indicated in Figure 1. Marker loci were analysed by PCR and/or RFLP-based assays. A map segment was considered analysed for presence or absence of effects on late blight resistance in a given population when at least one maternal and one paternal allele at the marker loci tagging the segment were segregating and scorable. Table 1 gives an account of the completeness of the analysis carried out for the ten selected map segments in populations GDE, GB, GC and GA, based on the 18 selected markers. The most complete analysis was achieved in the GDE population where eight of ten linkage group segments were fully analysed in both parents. In populations GA, GB and GC, three (GA), four (GB) and six (GC) map segments were analysed for both parents.

Marker genotype classes were tested for significant differences between means using the scoring data of individual years and the average scores over all test years for foliage resistance (PIF), tuber resistance (PIT), foliage maturity (FM) and vigour (FV). Table 2 shows the results based on the average scores after combining the data of all test years. Highly significant and consistent effects on all four traits were detected in population GDE with the linked markers *GP21*, *SPUD237*, *GP179* and *StPto* (shown only for *GP179* in Table 2) which all tag the same map segment on linkage group V (Figure 1). *P* values for markers *GP21* and *StPto* bordering this map segment were lower than for *GP179,* indicating that the internal markers *SPUD237* and *GP179* were the ones most closely linked to the QTL located in the segment. Figure 2 shows the segregation of the PCR products obtained with *GP179* specific primers in population GDE, when F_1 plants were ordered according to increasing foliage resistance based on the scores of 1995. A PCR product of 570 bp descending from parent G87 was associated with increased foliage resistance (scores 6 and 7) whereas a PCR product of 700 bp descending from parent I88 was associated with decreased foliage resistance (scores 2 to 5).

When population GDE was genotyped with the same marker *GP179* based on RFLP analysis, distinction of four parental alleles was possible, *a* and *b* originating from parent G87 and *c* and *d* originating

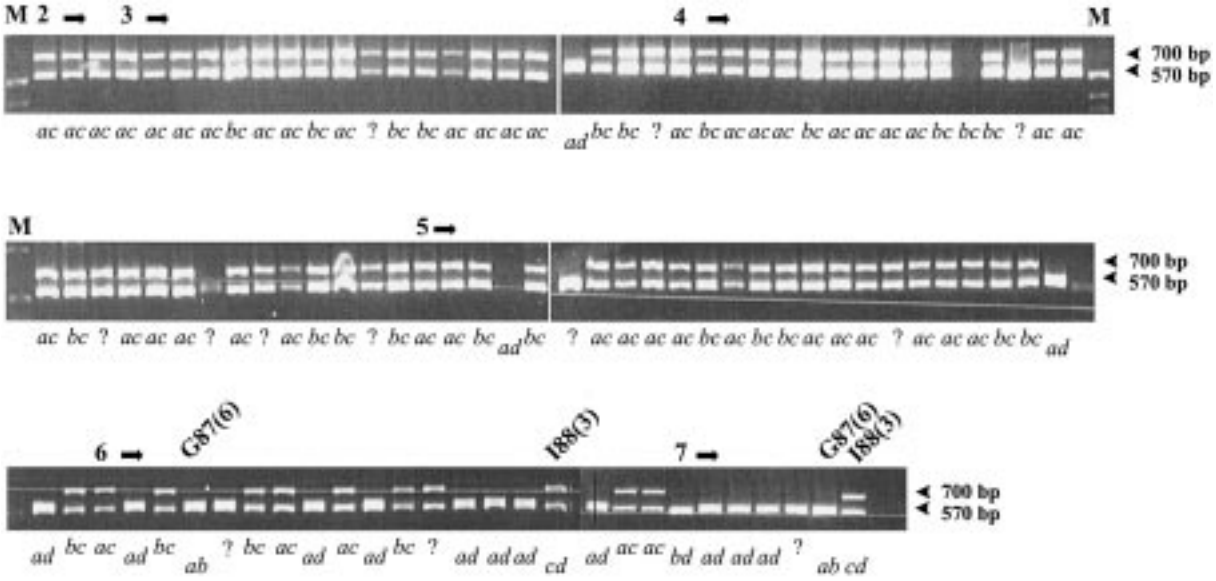

Figure 2. PCR-based genotyping of the GDE population with marker *GP179*. Plants were arranged according to field scores in 1995 for foliage resistance to late blight. The most susceptible plants (score 2) are on the top left, the most resistant plants (score 7) are on the bottom right. G87, female parent; I88, male parent; M, size marker. Marker allele combinations *ac*, *ad*, *bc* and *bd* in F_1 plants originating from cross G87 (*ab*) × I88 (*cd*) were determined by RFLP analysis of marker *GP179* and are shown underneath the corresponding PCR pattern. The 570 bp PCR product was linked with increased foliage resistance ($P < 0.0001$), decreased tuber resistance ($P < 0.001$), later maturity ($P < 0.0001$) and more vigour ($P < 0.0001$). The 700 bp PCR product was linked with increased foliage susceptibility ($P < 0.0001$), increased tuber resistance ($P < 0.01$) earlier maturity ($P < 0.0001$) and less vigour ($P < 0.0001$).

Table 3. Means (and standard deviations) of genotype classes in population GDE at marker locus *GP179* for foliage (PIF) and tuber (PIT) resistance to late blight, for foliage maturity (FM) and vigour (FV).

	Genotype classes				*P*
	ac	*bc*	*ad*	*bd*	
n	47	27	14	1	****[1,3]
PIF	4.0 (0.8)	3.8 (0.6)	5.5 (0.6)	5.3	****[2,3]
PIT	5.6 (2.0)	5.5 (2.1)	3.8 (1.6)	6.0	*[2,3]
FM	5.7 (0.7)	5.6 (0.7)	3.8 (0.7)	4.0	****[2,3]
FV	6.0 (0.8)	5.7 (0.8)	6.6 (0.5)	7.0	**[2,3]

[1]Determined by χ^2-test for deviation from 1:1:1:1 segregation.
[2]Determined by Kruskal-Wallis 1-way ANOVA.
[3]* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, **** $P < 0.0001$.

from parent I88. This allowed the full classification of all plants into one of four genotype classes *ac*, *bc*, *ad* and *bd*, which were expected to segregate in equal proportions. Segregation was, however, highly distorted, favouring the *ac* genotype class. Only one of 89 plants analysed with *GP179* was in the *bd* genotype class (Table 3). Means and standard deviations for foliage and tuber resistance to late blight, foliage maturity and vigour were calculated for genotype classes *ac*, *bc* and *ad* as shown in Table 3. The majority of plants in the *ac* or *bc* genotype class was, when compared with plants in the *ad* genotype class, on average less resistant to *P. infestans* in the field, earlier maturing (higher score for foliage maturity) and less vigourous (lower score for foliage vigour). The same plants had, on average, higher levels of tuber resistance. Alleles *c* and *d* descending from the more susceptible parent I88 dominated the resistance levels observed in the GDE population (Table 3).

In addition to population GDE a major QTL for foliage resistance was also linked to the same marker *GP179* in populations GB, GC and GA, for tuber resistance in populations GC and GA and for foliage maturity in population GB. QTL alleles descending from parents G87, I86, I88 and PX contributed to these effects. Parent G88 (GA population) was not polymorphic for all markers tested in this map segment (Table 2). As it was the case in the GDE population, alleles of the more susceptible parents I86 and PX of populations GB, GC and GA dominated resistance levels (data not shown).

A less prominent effect on foliage resistance as well as maturity was present in map segment VI_b tagged by markers *GP76* and *WUN2* in populations

Table 4. Effects on quantitative resistance to *P. infestans* races 1, 0 and 1-11 in population K31 based on individual tests (T1a, T1b, T0a, T0b, T1-11a, T1-11b) and combined data sets (T1, T0, T1-11, T96).

Linkage group	Marker locus	1995 T1a	1995 T1b	1995 T1	1996 T0a	1996 T0b	1996 T0	1996 T1-11a	1996 T1-11b	1996 T1-11	1996 T96
I	*GP264*	–	–	–	–	–	–	–	11.4*	–	–[1]
	CP100	–	–	–	–	9.6*	–	–	–	–	–
	CP11	13.0*[2]	–	10.6*	–	–	–	–	–	–	–
II	*GP23/GP1(c)*	–	–	–	8.7*	–	10.3**	10.2*	20.7**	11.1*	13.5**[1]
	GP26	–	–	–	–	–	–	10.2*	–	11.1*	–
III	*GP35(p)*	–	14.9**	12.9**	13.3**	–	11.3*	16.9**	11.1*	19.1***	16.3***[1]
	CP6	–	–	–	–	–	–	–	14.4**	17.2***	9.8*[1]
	GP276	–	–	–	–	–	–	11.8*	10.6*	17.1***	–
IV	*PC116/GP261*	–	9.0*	–	–	–	–	–	–	–	–
V	*GP186/PSTR(f)*	–	10.9*	15.1**	–	–	–	–	15.8**	–	–
	GP179/GP291(a)	–	–	–	–	13.8*	–	–	–	–	8.7*
	GP35(t)/CP113	–	–	–	9.1*	12.3*	12.2**	–	–	–	9.1*
VI	*GP79*	17.8**	–	10.6*	–	13.5**	8.3*	–	–	–	–
	CP104(d)	–	14.6**	–	–	9.7*	8.8*	–	–	13.4**	10.5*
VII	*CP134(b)*	–	–	–	–	–	–	11.6*	–	–	–
	CP43	–	11.2*	–	–	–	–	–	–	–	–
VIII	*WX*	–	–	–	–	9.4*	–	13.7**	–	9.3*	–
IX	*CP137(b)*	12.2*	–	–	–	–	–	–	–	–	–
	GP129	–	–	–	–	–	–	17.1*	–	–	–
X	*CP49(a)*	–	14.7**	–	–	–	–	–	–	–	–
XII	*GP34*	–	–	–	–	11.9*	–	–	–	–	–

[1] – $P > 0.05$; * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$.
[2] Effects are expressed as the amount of variance explained at the marker locus (R^2) and as level of significance P.

GDE and GB. The effect was inherited from the G87 parent (Table 2, Figure 1). The effects on foliage resistance and maturity detected with markers *GP76* and/or *WUN2* were similarly correlated to each other as the effects on linkage group V. Increased foliage resistance was accompanied by later maturity.

Further effects on foliage resistance were detected in map segments III_a (population GDE, both parents), IV (population GA, parent G88), VI_a (population GDE, parent G87), IX (population GDE, parent I88) and XI_b (population GC, parent G87)(Figure 1). Interestingly, the QTL linked to marker *GP250* tagging map segment XI_b which originated from parent G87 was detected in population GC but not in populations GDE and GB, although all populations share parent G87. The same QTL alleles of G87 were present, therefore, in the three populations. Population size of GB was too small for detecting an effect with *GP250*. In population GDE, however, this observation may indicate that QTL alleles of G87 interact differently with QTL alleles of I88 and PX in populations GDE and GC, respectively.

Mapping QTL for resistance to P. infestans *in population K31*

RFLP linkage maps covering the twelve potato chromosomes have been constructed in population K31 [32]. Figure 1 represents a backbone map of this population based on a subset of RFLP loci. Marker genotype classes were tested for significant difference between means based on the ten sets of data derived from the leaf disc bioassay. Marker loci linked to putative QTL for resistance to *P. infestans* and the amount of variance explained at the marker locus (R^2) for a given data set are shown in Table 4. Effects were detected on most linkage groups. The most consistent and race independent effects were, however, present on linkage groups II, III, V and VI. For example, the QTL linked to *GP35(p)* on linkage group III was detected with all three races of the fungus and in eight of ten data sets. On linkage groups I and VIII, effects were revealed when analysing the combined data sets T1 and T1-11, respectively. On linkage groups I, IV, VII, IX, X and XII, effects were sporadically detected in only one of ten data sets (Table 4). Considering

the large number of tests performed, some of those sporadic effects may have occurred by chance alone. The map positions of the QTL effects detected based on the combined data sets T1, T0, T1-11 and T96 are shown in Figure 1. For comparative reasons, the QTLs for resistance to *P. infestans* (*Pi* loci) as identified by Leonards-Schippers *et al.* [20] have also been included at approximate map positions in Figure 1.

A more detailed analysis of QTL effects linked to marker loci on linkage groups II, III, V and VI of population K31 is shown in Table 5. Marker genotype classes *ac*, *ad*, *bc* and *bd* present in the F_1 were distinguished based on alleles *a* and *b* originating from the female parent P3 and alleles *c* and *d* originating from the male parent P38. Class means were tested for significant differences by GLM. Comparison between average percent infection of marker genotype classes revealed allele combinations that increased resistance when compared to others that decreased resistance. Both parents contributed favourable and unfavourable alleles. At marker loci *GP23/GP1(c)*, *GP35(p)* and *GP186/PSTR(f)* only one of four allele combinations resulted in lower average percent infection when compared to the other three allele combinations. Vice versa, at marker locus *CP104(d)*, only allele combination *ac* was less favourable for resistance when compared to the other three. At marker locus *GP276*, the *b* allele was favourable for resistance versus the *a* allele when tested with the complex fungal race. The most resistant plant with allele *b* was, however, the only individual in the *bd* genotype class. The *bc* genotype class with 45 plants was overrepresented indicating strong selection acting against plants with the allele combination *bd*.

Markers tagging putative QTL for resistance to late blight

Table 6 lists 21 RFLP markers and functional genes that are landmarks for most of the genomic regions having QTLs for resistance to late blight. Markers and genes can be amplified by PCR using the information on specific oligonucleotides–including allele-specific oligonucleotides for markers *GP76* and *GP125* – and gene sequences in column 3 of Table 6. Figure 3 shows examples of the PCR products obtained in ten dihaploid potato breeding lines when using PCR-based marker assays. Polymorphic PCR products were obtained directly for markers *GP179* and *prp1* (SCAR, sequence-characterized amplified region), and after restriction with *Rsa*I for *GP76* (CAPS, cleaved amplified polymorphic sequence). Allele specific amplification is shown for marker *GP125* (ASO, allele-specific oligonucleotide).

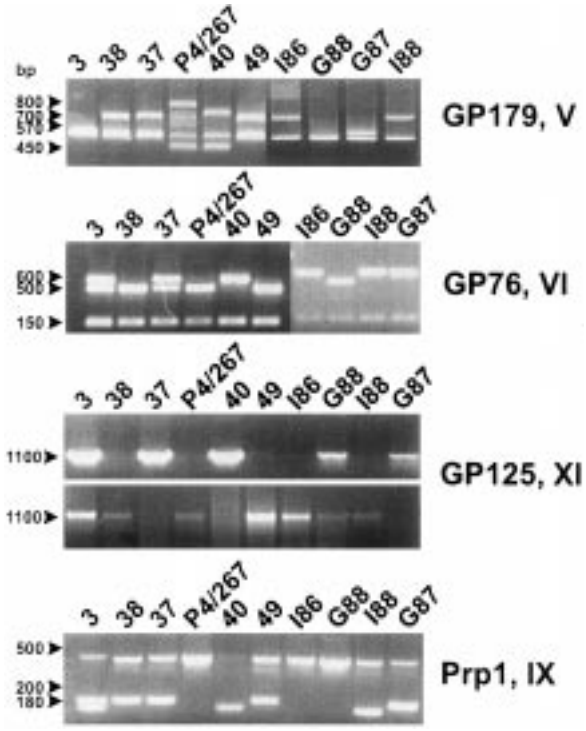

Figure 3. PCR-based analysis of markers *GP179*, *GP76*, *GP125* and *prp1* in ten diploid potato genotypes. Amplification with primers for *GP179* and *prp1* resulted in polymorphic PCR products, *GP76* was polymorphic after restriction digestion with *Rsa*I, and allele specific oligonucleotides were used to amplify *GP125*. Fragment sizes are indicated on the left.

Discussion

Stability of QTL for late blight resistance across different mapping populations and evaluation methods

Populations GDE, GB, GC and GA were evaluated for resistance to late blight in the field using as inoculum complex isolates of *P. infestans*, whereas population K31 and the population analysed by Leonards-Schippers *et al.* [20] were evaluated with a leaf disc assay and specific races of *P. infestans*. Despite the fact that different genetic materials, different evaluation methods and different isolates of the fungus have been used in these experiments, QTLs for foliage resistance to late blight were repeatedly detected in similar regions of the potato genome. These QTLs

Table 5. Means of percent infection (no of plants) of genotype classes at marker loci linked to QTL for resistance to *P. infestans* in population K31.

Linkage group	Marker locus	Data set	Genotype class				P^1
			ac	*ad*	*bc*	*bd*	
II	*GP23/GP1(c)*	T96	36.1 (29)	30.5 (19)	24.9 (22)	38.4 (21)	**
	GP26	T1-11	37.5 (16)	32.7 (36)	28.4 (20)	25.7 (16)	*
III	*GP35(p)*	T96	36.2 (30)	23.0 (23)	37.4 (24)	31.0 (18)	***
	GP276	T1-11	37.4 (26)	32.3 (18)	27.3 (45)	6.9 (1)	***
V	*GP186/PSTR(f)*	T1	40.7 (16)	51.8 (27)	46.2 (18)	32.0 (27)	**
	GP179/GP291(a)	T96	28.8 (14)	32.3 (30)	28.3 (18)	38.6 (19)	*
	GP35(t)/CP113	T96	29.2 (24)	27.9 (26)	38.1 (12)	36.6 (31)	*
VI	*GP79*	T1	52.1 (27)	37.6 (28)	43.5 (18)	36.3 (21)	*
	CP104(d)	T96	40.3 (23)	30.9 (25)	28.3 (24)	30.3 (26)	*

[1]Obtained with the GLM procedure; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

are located on linkage groups III, IV, V, VI and IX (Figure 1). None of the QTLs were, however, uniformly detected in all populations. Localization of a QTL at a similar position on the molecular maps of different mapping populations suggests that the same functional gene or gene family are causal for the QTL effects observed. Multiple alleles present at the same loci in different genetic materials may be responsible for the genotypic variability of QTL effects. The genetic resolution of our mapping experiments was limited, however, by population size, marker density and precision of the phenotypic analysis. It cannot be excluded that functionally different but closely linked genes are responsible for a QTL effect detected at a similar position on different maps.

Only a limited portion of the genome was tested in populations GDE, GB, GC and GA with markers from 10 map segments on 7 potato chromosomes (Table 1). Markers from 7 segments on 6 chromosomes tagged a QTL for foliage resistance to late blight in at least one population (Table 2). The success rate was 70%, therefore, for the markers chosen. A map covering all twelve potato chromosomes with AFLP and SSR markers and including segregation data of the markers used in our study has been constructed in population GDE [5]. In addition to the QTLs tagged by RFLP or CAPS markers in map segments III_a, V, VI_a, VI_b and IX (Table 2), QTLs for foliage resistance to late blight were identified on linkage groups I, II, IV, VII, VIII, XI and XII of the GDE map. Linkage groups I, II, VII, VIII and XII were not analysed in our study of population GDE with the 18 selected markers. With the exception of the QTLs on linkage groups I and XII, the additional QTL positions correlated well with QTLs detected in populations K31, GA, GB, GC and in a previously analysed population [20].

The most prominent QTL in terms of reproducibility and size of effect was linked to RFLP marker *GP179* on chromosome V (Table 2). This QTL caused the major effect on resistance to late blight found in populations GDE, GB, GC and GA and one of two major effects in the population analysed by Leonards-Schippers *et al.* [20]. In population K31, effects were spread over the whole linkage group V, without a clear maximum at tightly linked marker loci *GP179* or *GP291(a)* (Figure 1). The most consistent effect on late blight resistance in population K31, was identified on linkage group III in the segment flanked by markers *CP6* and *GP25*. The same markers also tagged a QTL in the GDE population (Table 2).

Genes with effects on late blight resistance were also detected on linkage group V in QTL mapping experiments using several hybrid populations derived from interspecific crosses between *S. microdontum* and *S. tuberosum* (Hans Sandbrink, CPRO-DLO, Wageningen, Netherlands, personal communication). The QTL was linked to marker *CP113* which tags a distal map segment on linkage group V (Figure 1). A QTL effect (K31_T96) was detected with the same marker in population K31. Lack of marker polymorphism prevented QTL analysis in other segments of linkage group V and, therefore, further comparisons between the mapping experiments.

QTL for foliage and tuber resistance to late blight

This paper and the accompanying paper of Collins *et al.* [5] are the first reports on mapping QTL for tuber resistance to late blight. One major QTL for tuber

Table 6. Markers tagging QTL for resistance to late blight in potato

Linkage group	Marker	PCR primer sequences $5' \rightarrow 3'$ DNA sequence: reference (GenBank accession no)	Orientation	T_a (°C)
II	*GP23*	CAT AGC AGA CAA TGT TGA GC	f	52
		TCA TTA ATT TCC CAG TAA G	r	
	SK2	Wemmer *et al.* [38] (X67693)		
III	*GP25*	TTG GCC CTT GGT CAT TCA	f	52
		CTT GTC ACA CGC GTA GGC	r	
	GP276	CAC CCA ATT CCC GAC AAG C	f	55
		TTG ACG CTG AGG CAT CAA AC	r	
	St4cl	GGG GAT CCA ACA GAA GGA TAC	f	65
		ATC GCC AGC ACA GGA CCG GCT	r	
		Becker-Andre *et al.* [1]		
IV	*GP180*	TCC ACC CCT GGA TTC AAG AA	f	55
		TCA AAC TCC TTC ACA AAG CA	r	
	MBF3	GAA CTG CTG TAT TTG ATA CG	f	55
		GAT CTA AAT CCA TAA TTT TAC TAG	r	
		Feltkamp *et al.* [11] (S73826)		
V	*GP21, GP179*	Meksem *et al.* [25]		
	SPUD237	de Jong *et al.* [6]		
	StPto	Leister *et al.* [18]		
	CP113	Niewöhner *et al.* [27]		
VI	*GP79*	GTC TTT AGG GAT ATG GAT TAC	f	52
		CCT TTA CTT GTA ATT ATG CAT C	r	
	GP76	ATG AAG CAA CAC TGA TGC AA	f	52
		TTC TCC AAT GAA CGC AAA CT	r	
	GP76-L40	ATC TTC AGA GTT CAG TTC AAG G	f	57
	GP76-L49	ATC TTC AGA GTT CAG GTC AAA A	f	55
	GP76-L40/49	TAA CCA CCA CC ATT TAT GTA TTC	r	57
VIII	*wx*	Van der Leij [36] (X58453)		
IX	*prp1*	GTG ACA TGA GCA CAT AAG TC	f	55
		GCA ACT TCA CTT CTG CCA TC	r	
		Taylor *et al.* [35]		
XI	*GP125*	AGC AGC TCT GAT GGC AAT GC	f	57
		GAG CCT AGC TGC CCA GCT TC	r	
	GP125-L40	TCA CTT AGT ATG TGC TAA AAT G	f	52
		CTG CCC AGC TTC TTA CGA T	r	
	GP125-L49	TCT CTT AGC ATG TGC AAG ATT	f	53
		CTG CCC AGC TTC TTC CGA G	r	
	GP250	AGT TCA ACA CCA GTA GGA C	f	51
		GAC ATC AAG TTA CCT ATG AC	r	
XII	*GP34, CP60, IPM4*	Bendahmane *et al.* [2]		

resistance was detected with the highest significance linked to marker *GP179* on linkage group V in populations GDE, GC and GA (Table 2). Full map coveridge of the GDE population with additional markers revealed only two additional, sporadic effects upon tuber resistance on other linkage groups [5].

The major QTL for tuber resistance was closely linked to the major QTL for foliage resistance. This notion is based on detection of both QTLs with the same group of tightly linked markers *GP21*, *SPUD237* and *GP179* on chromosome V. The observation of tight linkage between the two QTLs in three popu-

lations is in accordance with the hypothesis that the effects on foliage and tuber resistance are controlled by the same or a group of related genetic factor(s) located on potato chromosome V.

The effects on foliage and tuber resistance detected in populations GDE, GC and GA were negatively correlated. This was shown using marker *GP179*. RFLP analysis of this marker in population GDE made possible the distinction of four allele combinations *ac*, *ad*, *bc* and *bd* segregating in the F_1 which originated from non-inbred parents with allele combinations *ab* and *cd*, respectively. The QTL allele combinations favouring foliage resistance were unfavourable to tuber resistance and *vice versa* (Table 3). Reports in the literature on wether or not there is a relationship between foliage and tuber resistance to late blight are conflicting [37]. The different results may have been the consequence of the presence of multiple QTL alleles and QTL allele combinations in the genetic materials analysed by different researchers.

The question arises as to how expression of the same genetic factor(s) can result in increased resistance to late blight in leaves and decreased resistance in tubers or *vice versa*. One possible explanation is a differential expression in leaves and tubers of multiple alleles and allele combinations present at the locus on chromosome V resulting in a differential effect on late blight resistance in the two tissues.

QTL for late blight resistance, foliage maturity and vigour

A major QTL for foliage maturity was linked to marker *GP179* on linkage group V and a second one was linked to *GP76* and/or *WUN2* on linkage group VI in both populations GDE and GB. One QTL for foliage vigour was detected with marker *GP179* in population GDE (Table 2). Additional QTLs for both foliage maturity (earliness) and vigour were identified in other regions of the potato genome in population GDE, where a map of all chromosomes has been constructed by Collins *et al.* [5]. Those regions have not been tagged by the markers used in our study.

QTLs for foliage maturity and vigour tagged by *GP179* were closely linked to the major QTLs for late blight resistance on linkage group V. The QTL for foliage maturity in linkage group segment VI_b was also linked to a QTL for foliage resistance. Higher foliage resistance was correlated with later maturity at both loci in both populations and later maturity was positively correlated with higher foliage vigour. The same correlation between effects on foliage resistance and maturity has been found at a QTL in similar position on linkage group V in an independent QTL mapping experiment in diploid potato (Leontine Colon, CPRO-DLO, and Herman van Eck, Wageningen Agricultural University, Netherlands, personal communication). This close association between QTLs for resistance to late blight and foliage maturity again suggests that both traits may be controlled by the same gene or a group of related gene(s). The correlation found between foliage resistance to late blight and late maturity at two independent QTLs is in complete agreement with the well known phenomenon that most potato cultivars with high levels of field resistance to late blight are late-maturing. The difficulty of breeding early maturing cultivars with high levels of field resistance to late blight may indicate that, by phenotypic selection, resistance alleles at the major QTL on chromosome V have been primarily fixed. Identification and marker-assisted selection of favourable alleles at QTLs other than the ones associated with late maturity is now a possible alternative based on QTL mapping experiments in different genetic materials.

Segregation distortion towards susceptibility and dominance of susceptibility alleles

In population GDE, strong segregation distortion was observed at the marker locus *GP179* linked to the major QTL for resistance to late blight on chromosome V. The two marker genotype classes *ad* and *bd* linked to favourable alleles for foliage resistance, represented only 1/6 instead of 1/2 of the population (Table 3, Figure 2). In population K31, the average resistance levels of marker genotype classes were moderate (Table 5), with the exception of one highly resistant transgressive segregant. This was the only plant with allele combination *bd* at marker locus *GP276* on linkage group III. Moreover, at several marker loci linked to QTL in population K31, only one of the four genotype classes was characterized by increased resistance (Table 5), suggesting that the alleles favouring resistance were recessive. Similar observations have been made previously [20].

Our findings are consistent with the observations that transgressive segregation towards susceptibility is frequent in potato progeny segregating for resistance to late blight and that absolute resistance levels decrease during the backcrossing process to susceptible parents [29]. Distortion of segregation ratios is frequently observed in potato [14] and could result

from linkage with balanced lethal factors [3]. Markers designed to detect specific QTL allele combinations may assist efficient selection and evaluation of large numbers of new seedling genotypes.

Linkage between QTL and major loci for late blight resistance, QTL for foliage maturity and vigour

The *R1* gene conferring race specific resistance to *P. infestans* is located in the interval between markers *GP21* and *GP179* on linkage group V at ca. 1 cM distance from *GP179* [19, 25]. The *R1* locus and the QTLs for resistance to late blight, foliage maturity and vigour all tagged by marker *GP179* are, therefore, linked. The genes controlling these traits at the molecular level may be just physically linked but otherwise functionally unrelated. The limited resolution of the genetic analysis does not allow a distinction between this explanation for the observed linkage and the alternative one: There is the intriguing possibility that the same gene or a cluster of related genes may control resistance to late blight as well as foliage maturity and vigour. It has been suggested previously that the qualitative, race specific resistance and the quantitative resistance to *P. infestans* linked to *GP179* are controlled by allelic variants of the same gene or of a cluster of related genes [20]. This was based on Robertson's proposal that qualitative mutant phenotypes are extreme allelic variants at a quantitative trait locus [28]. In the case that resistance to late blight, foliage maturity and vigour are indeed pleiotropic effects of the same gene(s), the question arises as to what the link may be between the different phenotypes? The hypersensitive resistance response (HR) is triggered by *R1* upon infection with a specific race of *P. infestans* and manifests itself after a few days as necrotic lesions caused by rapid, localized cell death. Foliage maturity, on the other hand, is determined by developmental stages and senescence of leaves, a phenomenon which is also related to cell death but occurs at a much slower rate over several weeks. In this sense, the *R1* gene might have evolved from mutations in genes that normally control the plant's life cycle. Interaction of the mutant allele with a pathogen elicitor that mimics a signal molecule involved in that developmental process might trigger extreme acceleration of the process, a reaction manifesting itself as local cell death. Mutants are known in maize and other plants which mimic disease lesions or HR without pathogen infection, and the phenotype of these disease lesion mimics is influenced by light, temperature, developmental state and genetic background [17], all factors affecting also a plant's life cycle.

A second case of linkage between qualitative and quantitative resistance of foliage to late blight was discovered on linkage group segment XI_b tagged by marker *GP250* which is closely linked with the *R3*, *R6* and *R7* alleles for race specific resistance to *P. infestans* [8, 9]. The same marker also detected a QTL for foliage resistance in the GC population.

The *R2* gene has been localized on linkage group IV in a map segment bordered by markers *TG123* and *TG22* [21]. Both markers have also been used in the QTL mapping experiment of Leonards-Schippers *et al.* [20]. In this experiment, *TG123* but not *TG22* was linked to a major QTL for resistance to *P. infestans*. Markers *GP180* and *MBF* tagging linkage group IV in our current study detected a QTL with minor effect in population GA. The comparison of different maps based on reference markers suggests a linkage between the *R2* gene and QTLs for resistance to late blight on potato chromosome IV.

Marker assisted selection for quantitative resistance to late blight

Based on QTL mapping experiments in six hybrid families originating from nine different diploid parental lines [20, this paper] we propose the following segments of the potato genome as the best candidate regions for having genes controlling resistance to late blight (see Figure 1 for orientation): top of chromosome II, centre and bottom part of chromosome III, top half of chromosome IV, the whole of chromosome V, both distal regions of chromosomes VI and XI, the top of chromosomes VIII and IX and the bottom part of chromosome XII. Chromosome specific landmarks for these regions are 21 RFLP markers and genes (Table 6) which were linked to QTL for late blight resistance in one or more of the populations analysed. The markers can be used as PCR-based diagnostic assays for selecting specific QTL alleles or QTL allele combinations in crosses among the nine diploid parental lines.

The same markers may be used for characterization of unrelated germplasm, for example new sources of resistance to late blight. Whether or not favourable QTL alleles for late blight resistance are present in unrelated germplasm in any of the genomic regions identified in this and other mapping studies, will have to be verified in each single case. PCR-based marker assays will also have to be adjusted to be informative

in new germplasm. Many more RFLP markers have been mapped to the selected regions in the potato and the colinear tomato genome [15, 34] that are potentially useful and may be converted into PCR-based marker assays. Moreover, microsatellites have also been mapped to most of the map segments indicated above [5, 26] which provide a convenient and highly polymorphic type of PCR-based markers.

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