Theor Appl Genet (2003) 106:317–325
DOI 10.1007/s00122-002-1021-2

M. H. P. W. Visker · L. C. P. Keizer · H. J. Van Eck
E. Jacobsen · L. T. Colon · P. C. Struik

Can the QTL for late blight resistance on potato chromosome 5 be attributed to foliage maturity type?

Received: 2 April 2002 / Accepted: 8 May 2002 / Published online: 22 August 2002

Abstract We investigated the association between late blight resistance and foliage maturity type in potato by means of molecular markers. Two QTLs were detected for foliage resistance against *Phytophthora infestans* (on chromosomes 3 and 5) and one for foliage maturity type (on chromosome 5). The QTL for resistance to late blight and the QTL for foliage maturity type on chromosome 5 appeared to be mapped on indistinguishable positions. We were interested whether this genetic linkage was due to closely linked but different genes, or due to one (or more) gene(s) with pleiotropic effects. We therefore developed an approach to detect QTLs, in which resistance to late blight was adjusted for foliage maturity type. This analysis revealed the same two QTLs for resistance against *P. infestans*, but the effect of the locus on chromosome 5 was reduced to only half the original effect. This is a strong indication that the two indistinguishable QTLs for foliage maturity type and for late blight resistance on chromosome 5 may actually be one gene with a pleiotropic effect on both traits. However, there was still a significant effect on resistance against *P. infestans* on the locus on chromosome 5 after adjusting for foliage maturity type. Therefore we cannot rule out the presence of two closely linked QTLs on chromosome 5: one with a pleiotropic effect on both late blight resistance and foliage maturity type, and another with merely an effect on resistance. In addition, the two QTLs for resistance to late blight showed an important epistatic interaction, suggesting that QTLs for resistance affect each other's expression.

Keywords Earliness · Epistasis · *Phytophthora infestans* · Pleiotropy · QTL mapping

Communicated by G. Wenzel

M.H.P.W. Visker (✉) · L.C.P. Keizer · L.T. Colon
Plant Research International, P.O. Box 16, 6700 AA, Wageningen, The Netherlands
e-mail: M.Visker@plant.wag-ur.nl
Tel.: +31-317-477023, Fax: +31-317-418094

H.J. van Eck · E. Jacobsen
Laboratory of Plant Breeding, Department of Plant Sciences, Wageningen University, P.O. Box 386, 6700 AJ, Wageningen, The Netherlands

P.C. Struik
Crop and Weed Ecology, Department of Plant Sciences, Wageningen University, P.O. Box 430, 6700 AK, Wageningen, The Netherlands

Introduction

Late blight, caused by *Phytophthora infestans*, is the most devastating potato (*Solanum tuberosum*) disease worldwide. Late blight epidemics may cause serious losses and resistance is essential to allow potato production in low external input agriculture. Large amounts of fungicides are applied to avert late blight epidemics in high external input agriculture, which is environmentally unfriendly, time-consuming and costly, and durable resistance against *P. infestans* would be a welcome alternative (Ghislain et al. 1997).

Eleven different resistance genes have been identified in *Solanum demissum* and several have been introduced into modern potato varieties (Ross 1986). Compatible races of *P. infestans* have already arisen for all these *R* genes and the abundance of (highly) complex races of the pathogen (Turkensteen et al. 1996) has diminished the value of these 11 *R* genes for resistance breeding. Therefore, potato breeders presently focus on race-nonspecific resistance against *P. infestans* that is not based on one of the 11 known *R* genes and is expected to be more durable (Wastie 1991; Vleeshouwers 2000).

Recently, resistance breeding faces an additional new challenge: the old population of *P. infestans* (the US-1 clonal lineage with only the A1 mating type) has been replaced by a new population (a mixture of clonal lineages with both A1 and A2 mating types) in most potato-growing areas of the world (Spielman et al. 1991; Fry et al. 1993). This current population of the pathogen is more aggressive and genetically more variable, and is challenging the established concept of durability (Flier 2001).

Besides the difficulty of dealing with a highly versatile and aggressive polycyclic leaf pathogen, breeding for durable resistance against *P. infestans* is also complicated by the strong association between race-non-specific resistance and late foliage maturity. Toxopeus already described this association in 1958 (Toxopeus 1958), and numerous authors have confirmed this first report (e.g. Umaerus et al. 1983; Swiezynski 1990). So far, potato breeders have not been successful in combining durable resistance against *P. infestans* with early maturing foliage (Anonymous 2001). These observations suggest that late blight resistance and foliage maturity type are either controlled by closely linked genes or that the loss of resistance during foliage maturation is due to the physiological process of ageing, presumably controlled by the same (pleiotropic) gene(s) (Colon et al. 1995). The hypothesis of the role of physiological changes during foliage maturation is supported by the influence of photoperiod on resistance to late blight: resistance is lost when short days induce early maturity (Colon 1994).

In the last decade, molecular marker technology has served as a valuable tool to study the genetic background of the association of late blight resistance with late foliage maturity in more detail. A range of QTLs for race-non-specific resistance against *P. infestans* has been found in several diploid potato populations (Leonards-Schippers et al. 1994; Van Eck and Jacobsen 1996; Collins et al. 1999; Oberhagemann et al. 1999; Ewing et al. 2000; Sandbrink et al. 2000; Ghislain et al. 2001) and one tetraploid population (Meyer et al. 1998). The diploid populations originated from crosses between *S. tuberosum* and different wild *Solanum* species (*Solanum berthaultii, Solanum kurtzianum, Solanum microdontum, Solanum phureja, Solanum spegazzinii, Solanum stenotomum, Solanum tarijense, Solanum vernei*). All potato chromosomes appear to harbour loci that can contribute to race-non-specific resistance against *P. infestans* (Gebhardt and Valkonen 2001), but common to most populations is the major QTL on chromosome 5 near marker GP21 (Leonards-Schippers et al. 1994; Van Eck and Jacobsen 1996; Collins et al. 1999; Oberhagemann et al. 1999).

Some potato populations were also investigated for QTLs for foliage maturity type. Whereas QTLs for foliage maturity type were less frequent, all of them coincided with QTLs for resistance to late blight (Van Eck and Jacobsen 1996; Collins et al. 1999; Oberhagemann et al. 1999; Ewing et al. 2000). Indeed, common in most populations is the major QTL for foliage maturity type on chromosome 5 near marker GP21 (Van Eck and Jacobsen 1996; Collins et al. 1999; Oberhagemann et al. 1999).

The concurrence of QTLs for late blight resistance and foliage maturity type supports the previous suggestion that these traits are genetically linked. A detailed analysis of these loci in which both traits are considered simultaneously might reveal whether this linkage is due to closely linked but different genes, or due to one (or more) gene(s) with pleiotropic effects. Elucidation of the nature of the relationship between late blight resistance and foliage maturity type will be of great value for future resistance breeding in early maturing potatoes.

This paper describes the detailed analysis of QTLs for race-non-specific resistance against *P. infestans* and for foliage maturity type in a diploid potato population derived from crosses between *S. tuberosum* and wild relatives. The analysis focuses on the locus on chromosome 5, since previous research suggested that this locus is the most important for both late blight resistance and foliage maturity type (Van Eck and Jacobsen 1996; Collins et al. 1999; Oberhagemann et al. 1999).

Materials and methods

Plant material

The potato population consisted of 67 genotypes derived from a cross between two diploid clones: USW5337.3 (coded C: *S. phureja* × *S. tuberosum*) (Hanneman and Peloquin 1967) and 77.2102.37 [coded E: USW5337.3 × (*S. vernei* × *S. tuberosum*)] (Jacobsen 1980). Clone C is late maturing and susceptible to late blight, clone E is mid-early and moderately susceptible to late blight. This population was also used in previous genetic studies (e.g. Jacobs et al. 1995; Van Eck 1995).

Evaluations of late blight resistance and foliage maturity type

Resistance against *P. infestans* and foliage maturity type were evaluated in separate field trials. No fungicides were applied to the crops for assessments of late blight resistance, whereas those for foliage maturity type assessments were fungicide protected.

Field-tests for late blight resistance

The population was evaluated for foliage resistance to late blight in field trials in 3 consecutive years: 1995, 1996 and 1997. In 1995 only 35 individuals of the population were evaluated, whereas all 67 individuals were tested in the 2 successive years. The trials were located on sandy soil near Wageningen (the Netherlands) and consisted of three randomized complete blocks. Each genotype was present in a plot of two plants in each block; these two-plant plots were treated as single experimental units. Inoculations were performed with a spore suspension of race 1.2.3.4.5.6.7.10.11 of *P. infestans* (IPO82001); the composition of this complex race of the pathogen was confirmed by an *R* gene differential-set of potato varieties that was part of the field trial in 1996. Plants were inoculated approximately 8 weeks after emergence, plants and soil were thoroughly wetted prior to inoculation and the spore suspension was applied late in the evening using a tractor-driven sprayer. Subsequently, overhead irrigation was applied to sustain the development of the epidemic (for details see Colon and Budding 1988). Disease assessments were made at weekly intervals over a period of 5 weeks after inoculation. The data collected were used to calculate the normalized or relative area under the disease progress curve (AUDPC) (Shaner and Finney 1977; Fry 1987). Normalized AUDPC-values range between 0 and 1 and reflect both disease development and disease severity, resulting in high values for susceptible genotypes and low values for resistant genotypes.

Field-tests for foliage maturity type

All 67 individuals of the population were evaluated for foliage maturity type in field trials in the years 1991 and 1994. The trials

were located on clay soil in Wageningen (the Netherlands) and both consisted of three randomized complete blocks. Each genotype was present in a plot of four plants in each block; these four-hill plots were treated as single experimental units. Assessments of foliage maturity type were based on visual classification of senescence of the foliage, using an ordinal scale ranging from 2 (late maturing) to 9 (early maturing) (Anonymous 2001). Evaluations were performed once each year, at the stage when the standard variety Bintje (mid-early; score 6.5) was just starting to senesce (for details see Van Eck 1995).

Data analysis

Averages for AUDPC and foliage maturity type were estimated with the residual maximum-likelihood (REML) method (Patterson and Thompson 1971) of GenStat 5 (GenStat 2000). Averages per year were calculated with the factors year, year × replication, genotype and year × genotype as fixed, and the factor year × replication × genotype as random. Multiple year averages were calculated with the factors year, year × replication and genotype as fixed, and the factors year × genotype and year × replication × genotype as random.

The frequency distribution of phenotypic classes of foliage resistance to late blight was tested for skewness according to Sokal and Rohlf (1969).

To estimate the genetic part of the total observed phenotypic variation, broad-sense heritabilities of late blight resistance and foliage maturity type were calculated: the respective genotype variance component was divided by the respective sum of all variance components (Hanson 1963). Variance components were estimated with REML in GenStat 5, using the factors year and year × replication as fixed, and the factors genotype, year × genotype and year × replication × genotype as random.

Construction of genetic maps

The initial genetic map of the population consisted of RFLP, isozyme and morphological markers (Jacobs et al. 1995) and was expanded with AFLP markers (Van Eck et al. 1995). Improvement of this map was necessary to perform a reliable QTL analysis with the Interval/MQM-mapping procedure. Interval/MQM-mapping considers flanking markers when estimating QTL effects on a given map position (Lander and Botstein 1989) and consequently relies on the quality of the genetic map: estimations are biased when the considered flanking markers are not the right ones.

Two separate maps were made: one for the female parent (C) and one for the male parent (E). Optimization was done mainly by removing markers and individuals with many missing values (Jansen et al. 2001). Linkage analysis for the construction of the maps was performed with JoinMap 3.0 (Van Ooijen and Voorrips 2001).

QTL analysis

QTLs for resistance against *P. infestans* and for foliage maturity type were identified using three different methods. In Method 1, the significance of individual markers for each trait was tested by analysis of variance (ANOVA) in GenStat 5. In Method 2, the analysis was done with the Kruskall-Wallis procedure of MapQTL 4.0 (Van Ooijen et al. 2000). Methods 1 and 2 do not take the map-position of the individual markers into account and are thus insensitive to map quality. In Method 3, the Interval/MQM-mapping procedure of MapQTL 4.0 was used, which does consider the position of the markers on the genetic map (Lander and Botstein 1989) and also includes the presence of other QTLs for the same trait (Jansen 1994). This Interval/MQM-mapping procedure is the most powerful, but also the most sensitive to error, since it depends on a good marker order. Therefore, Methods 1 and 2 were used to verify the Interval/MQM-mapping results.

The presence of interaction between two QTLs for the same trait was estimated with REML in GenStat 5. Calculations were made with the fixed factors QTL/marker 1, QTL/marker 2 and QTL/marker 1 × QTL/marker 2.

The percentages of variation accounted for, as provided by MapQTL 4.0, could not be used due to the presence of interaction between QTLs for the same trait. The MapQTL 4.0 estimations of variation accounted for are based on a simple, additive genetic model and thus not valid when epistatic effects are involved. Therefore, the percentage of variation that could be accounted for by a single locus or by an interaction was based on variance components that were estimated with REML in GenStat 5, using all factors [QTL/marker(s), interaction(s)] as random (Alonso-Blanco et al. 1998). The experimental design enabled data analyses which ensured that the total amount of variation that could be accounted for was mostly genetic. Because of missing values the data were not orthogonal and consequently the percentages of variation accounted for do not add up. However, these approximate estimations are relevant for comparison with other results.

QTL analysis of associated traits

The effect of the association between late blight resistance and foliage maturity type on the detection of QTLs for these two traits was studied in more detail. This detailed QTL analysis was performed with three different tests to correct resistance to late blight for foliage maturity type (estimated multiple year average).

In Test 1, foliage maturity type was added as a covariate to ANOVA in GenStat 5; the significance of individual markers for late blight resistance was therefore directly adjusted for foliage maturity type. This was different from Tests 2 and 3 in which the phenotypes for resistance against *P. infestans* (AUDPC-values) were first corrected for foliage maturity type, and then new QTL-analyses were performed. AUDPC-values were re-calculated with foliage maturity type added as a covariate to REML in GenStat 5, and then used as new input for the QTL analyses with both the Kruskall-Wallis (Test 2) and the Interval/MQM-mapping (Test 3) procedures of MapQTL 4.0.

Results

Phenotypes

Foliage resistance to late blight

The C × E population displayed a rather normal distribution for late blight resistance (slightly, but significantly skewed to the left; $p < 0.0005$) and transgressive segregation (Fig. 1). Resistance to late blight was not caused by one of the 11 known *R* genes: guaranteed by the use of a complex race of the pathogen and the presence of symptoms on all 67 individuals, excluding absolute resistance due to *R8* or *R9* (inoculum not virulent to *R8* and *R9*). The 3 year average AUDPC was 0.55 for the susceptible female parent (C) and 0.50 for the moderately susceptible male parent (E). Even though differences between years were significant, this did not result in the detection of dissimilar QTLs in different years.

Broad-sense heritability for late blight resistance was estimated at 0.54, indicating that about half of the observed variation was genetic and thus selectable.

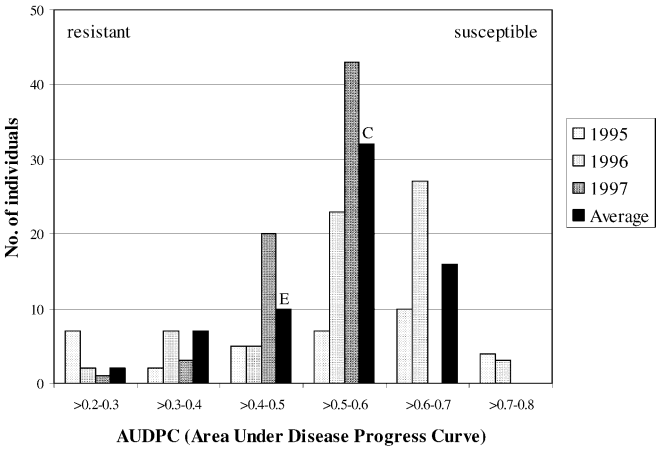

Fig. 1 Frequency distribution of phenotypic classes of foliage resistance to late blight. Data of population C × E (USW5337.3 × 77.2102.37), collected in 3 years (1995: 35 individuals, 1996 and 1997: 67 individuals) and expressed in normalized AUDPC-values. Presented are the data of each year separately, the estimated 3 year average, and the parental values (indicated by "C" and "E")

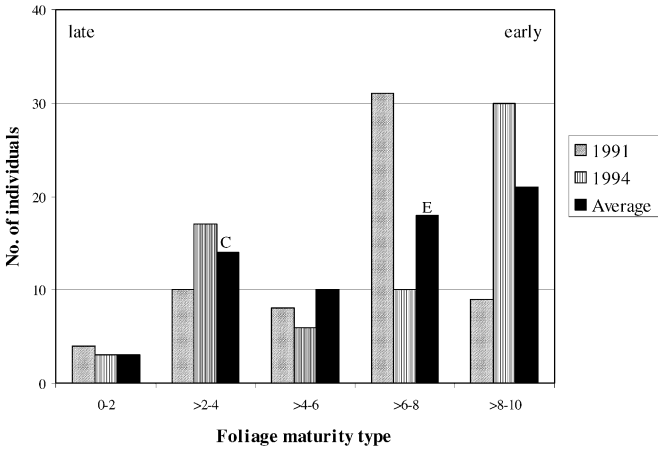

Fig. 2 Frequency distribution of phenotypic classes of foliage maturity type. Data of population C × E (USW5337.3 × 77.2102.37), collected in 2 years (1991 and 1994: 67 individuals). Presented are data of each year separately, the estimated 2 year average, and the parental values (indicated by "C" and "E")

Foliage maturity type

The C × E population displayed a bimodal distribution for foliage maturity type with peaks near each of the parental values (Fig. 2), suggesting the presence of a major gene or major QTL for foliage maturity type. The 2 year average for foliage maturity type was 3.9 for the late female parent (C) and 7.0 for the mid-early male parent (E). Differences between years were significant but this did not result in the detection of dissimilar QTLs in different years.

Broad-sense heritability for foliage maturity type was estimated at 0.79, indicating that most of the observed variation was genetic and thus selectable.

Genetic map

Two separate maps were made: one for the female parent (C) and one for the male parent (E). This study concentrated on chromosome 5 of the male parent, for which the map was composed of 13 markers and had a length of 36 cM (see Fig. 6).

The initial map of chromosome 5 of parent E was 71 cM (Van Eck et al. 1995), the reduction in map length (of 35 cM) was mainly caused by discarding markers with many missing values that originally led to problematic map construction and overestimated genetic distances. The map of the remaining 13 markers was very similar to the analogous part of the original map, with only minor changes in marker order and map distances.

QTL detection

Differences in late blight resistance and in foliage maturity type segregated with alleles from both parents, but the contrasts between the alleles of the female parent (C) were negligible compared to the contrasts between the alleles of the male parent (E). Therefore, only results of parent E are presented.

The results of the two single-marker analyses (Methods 1 and 2) confirmed the results of the Interval/MQM-mapping procedure (Method 3), which proved that the genetic map was of good quality and that the use of Interval/MQM-mapping was warranted. Only the results of the Interval/MQM analysis are presented, since this method is the most powerful.

QTLs for foliage resistance to late blight

QTL analyses were performed for all four data sets of foliage resistance to late blight: i.e. for three individual years (1995, 1996 and 1997) and the estimated multiple year average. Although the phenotypic differences between years were significant, the QTL analyses for all four data sets yielded very similar results and therefore only the results of the multiple year average data are presented. The significance threshold was empirically determined at Lod 2.7 ($p < 0.01$; 10,000 permutations) with the permutation test of MapQTL.

The analysis revealed two QTLs for resistance against *P. infestans*: one on chromosome 3 (lod-value: 7.6), and one on chromosome 5 (lod-value: 11.1). The first locus accounted for about 25%, and the second locus for about 41% of the total variation for late blight resistance in this population. For the locus on chromosome 3, individuals with the Y-allele for the nearest marker (ATG/CTA-178) were more resistant to late blight than individuals with the X-allele: the presence of the Y-allele caused an average reduction in the normalized AUDPC-value of 0.043 ($p < 0.001$). For the locus on chromosome 5, individuals

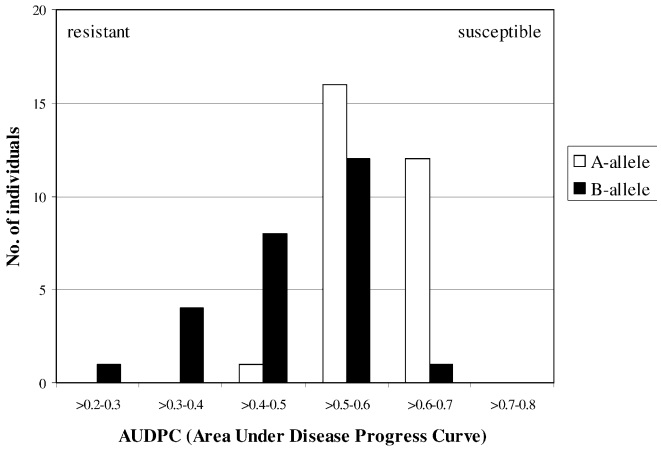

Fig. 3 Distribution of the two alleles (A and B) of marker GP21 on chromosome 5 of parent E, overlaying the frequency distribution of phenotypic classes of foliage resistance to late blight. Multiple year average data of population C × E (USW5337.3 × 77.2102.37), expressed in normalized AUDPC-values

with the B-allele for the nearest marker (GP21) were more resistant to late blight than individuals with the A-allele: the presence of the B-allele caused an average reduction in the normalized AUDPC-value of 0.061 ($p < 0.001$). This is illustrated for RFLP marker GP21 on chromosome 5 that had the strongest linkage with resistance against *P. infestans* (Fig. 3). All (but one) individuals that were susceptible in spite of the presence of the favourable B-allele for marker GP21 on chromosome 5 had the unfavourable X-allele for marker ATG/CTA-178 on chromosome 3 (see Fig. 4).

There was a significant interaction between the two QTLs for resistance to late blight: the presence of both the Y-allele on chromosome 3 and the B-allele on chromosome 5 caused an additional reduction in the normalized AUDPC-value of 0.083 ($p = 0.002$). The result of this interaction is illustrated in Fig. 4 and Table 1: the combined effect of both alleles is far more than the sum of the effects of the two individual alleles. The interaction accounted approximately for another 15% of the total variation for late blight resistance in this population.

QTL for foliage maturity type

QTL analyses were performed for all three data sets of foliage maturity type: namely, two individual years (1991 and 1994) and the estimated multiple year average. Although the phenotypic differences between years were significant, the QTL analyses for all three data sets yielded very similar results and therefore only the results of the multiple year average data are presented. The significance threshold was determined at Lod 2.3 ($p < 0.01$; 10,000 permutations).

The analysis revealed only one QTL for foliage maturity type, which was located near RFLP marker GP21 on chromosome 5 (lod-value: 17.9). This locus accounted for about 84% of the total variation for foliage maturity type in this population. Individuals with the B-allele for marker GP21 matured later than individuals with the A-allele for this marker: the presence of the B-allele caused an average reduction in foliage maturity type of 4 ($p < 0.001$) (Fig. 5).

Detailed analysis of linked QTLs for late blight resistance and foliage maturity type on chromosome 5

The most important QTL for resistance against *P. infestans* was found on chromosome 5 and could not be distinguished from the (only) major QTL for foliage maturity type: both QTLs were most closely linked to the same

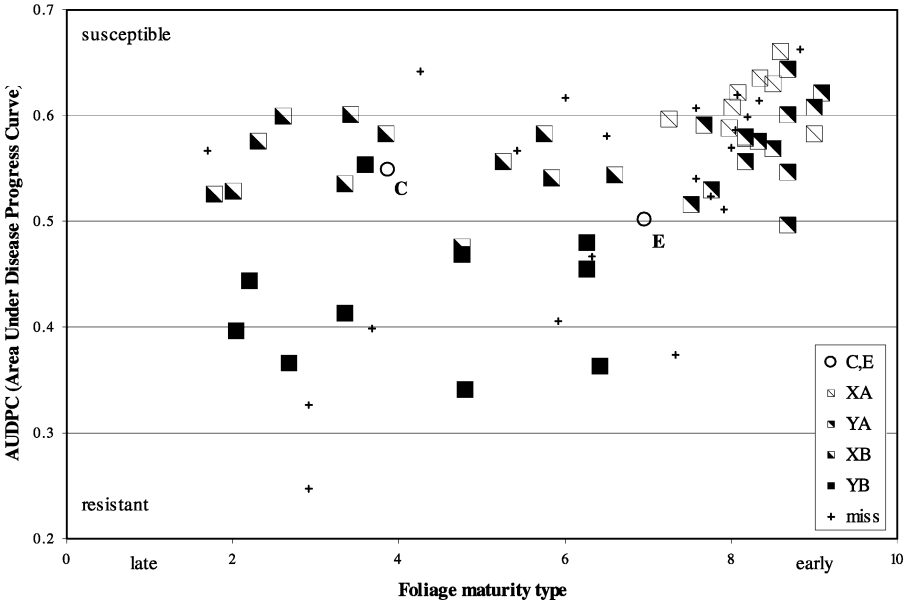

Fig. 4 Phenotypic correlation between late blight resistance and foliage maturity type, combined with genotypes of marker loci near QTLs involved in these traits. X/Y indicate alleles of marker ATG/CTA-178 on chromosome 3 and A/B indicate alleles of marker GP21 on chromosome 5, both of parent E. Multiple year average data for both late blight resistance (LSD: 0.12) and foliage maturity type (LSD: 1.99) of population C × E (USW5337.3 × 77.2102.37); late blight resistance expressed in normalized AUDPC-values (parental values indicated by "C" and "E", "miss" = missing data for either one of the two markers)

Table 1 Average reduction of normalized AUDPC-values in relation to the allele combinations of marker ATG/CTA-178 (X/Y) on chromosome 3, and marker GP21 (A/B) on chromosome 5. Estimations based on multiple year average data of population C × E (USW5337.3 × 77.2102.37)

GP21 (chromosome 5)	ATG/CTA-178 (chromosome 3)	
Allele	X	Y
A	0	0.043
B	0.061	0.187

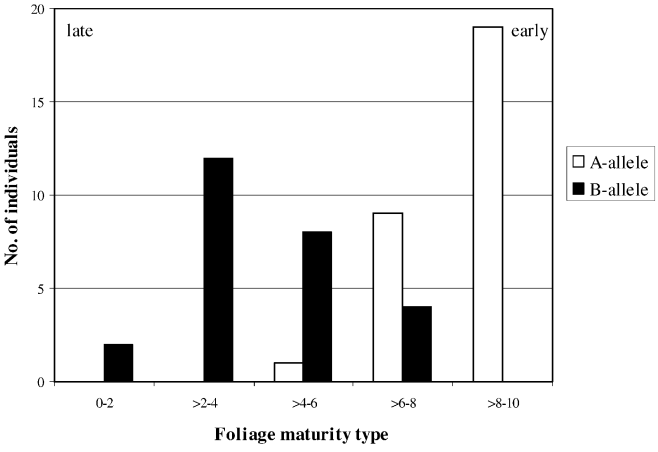

Fig. 5 Distribution of the two alleles (A and B) of marker GP21 on chromosome 5 of parent E, overlaying the frequency distribution of phenotypic classes of foliage maturity type. Multiple year average data of population C × E (USW5337.3 × 77.2102.37)

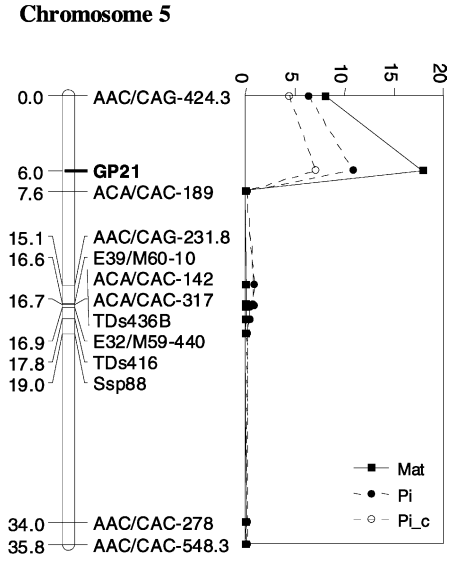

Fig. 6 Lod-values for foliage maturity type (Mat), late blight resistance (Pi) and late blight resistance corrected for foliage maturity type (Pi_c) plotted on the map of chromosome 5 of the male parent (E) of population C × E (USW5337.3 × 77.2102.37). Lod-values are estimated with the MQM procedure of MapQTL and based on multiple year average data for both traits

genetic marker GP21. These results confirmed our previous assumption of genetic linkage of late blight resistance and foliage maturity type on chromosome 5 and justified our attempt to study this linkage in more detail in this population.

The results of all three tests of QTL analysis of associated traits were very similar and therefore only the results of the Interval/MQM analysis are presented. Detailed QTL analyses were performed for all four data sets of foliage resistance to late blight: namely, 3 individual years (1995, 1996 and 1997) and the estimated multiple year average; multiple year average data of foliage maturity type were used as a covariate. The QTL analyses for all four data sets yielded very similar results and therefore only the results of the multiple year average data are presented.

The analysis revealed two QTLs for resistance against *P. infestans*, both on the same positions as in the previous analysis in which foliage maturity type was not used as a covariate: one on chromosome 3 and one on chromosome 5. The significance and the effect of the locus on chromosome 3 were almost identical to what resulted from the analysis without foliage maturity type as a covariate: the presence of the Y-allele for marker ATG/CTA-178 (lod-value: 8.1) caused an average reduction in the normalized AUDPC-value of 0.046 ($p < 0.001$). However, the significance and the effect of the locus on chromosome 5 were only half of what was found in the analysis without foliage maturity type as a covariate: the presence of the B-allele for marker GP21 (lod-value: 7.4) caused an average reduction in the normalized AUDPC-value of 0.031 ($p < 0.001$). Figure 6 illustrates the reduction in significance of the QTL for late blight resistance on chromosome 5 that is caused by the use of foliage maturity type as a covariate in the analysis.

The interaction between the two QTLs for resistance against *P. infestans* had the same effect and significance as in the previous analysis in which foliage maturity type was not used as a covariate: the presence of both the Y-allele on chromosome 3 and the B-allele on chromosome 5 caused an additional reduction in the normalized AUDPC-value of 0.084 ($p = 0.002$).

Discussion

Van Eck and Jacobsen (1996) were the first to detect the close linkage of a QTL for late blight resistance and a QTL for foliage maturity type on potato chromosome 5; they hypothesized that a single gene with a pleiotropic effect might be involved. To test their hypothesis, we used the same diploid potato population and extended the phenotypic data, improved the genetic map, and made use of more powerful statistical tools to detect QTLs.

Our enhanced analyses confirmed the position of the two QTLs on chromosome 5: the QTL for resistance to late blight could not be distinguished from the QTL for

foliage maturity type (only 1 cM apart). These results imply that, in spite of the improvements, we were still unable to separate the QTLs for the two traits and thus that these analyses were insufficient to reject the hypothesis of a single gene with a pleiotropic effect on both late blight resistance and foliage maturity type.

We then developed a new approach to detect QTLs, in which both late blight resistance and foliage maturity type were considered simultaneously. This test examined whether there was still variation left that could be accounted for by a QTL for resistance against *P. infestans*, after the variation for foliage maturity type had already been accounted for. This approach was implemented in a QTL analysis for late blight resistance in which foliage maturity type was used as a covariate.

Resistance to late blight

We detected two QTLs for foliage resistance to late blight: one on chromosome 3 and another on chromosome 5. The location of these QTLs was in accordance with previous research in other populations (Leonards-Schippers et al. 1994; Collins et al. 1999; Oberhagemann et al. 1999; Ghislain et al. 2001), although one population had only the QTL on chromosome 3 in common (Ewing et al. 2000). The interval near marker GP21 on chromosome 5 is also known to harbour the (recently cloned) *R1* gene for race-specific resistance against *P. infestans* (Leonards-Schippers et al. 1992; Ballvora et al. 2002). The QTL for late blight resistance that we detected on chromosome 5 was not race-specific (the disease test with a complex race of *P. infestans*, virulence for *R1* was included) but is most likely part of the cluster of genes involved in resistance, which is present on chromosome 5 linked to marker GP21 (Gebhardt and Valkonen 2001).

Both QTLs for resistance against *P. infestans* were highly significant ($p < 0.001$) and their additive effect accounted for about 66% of the total variation for late blight resistance; an additional (approximately) 15% of variation was accounted for by the epistatic interaction of these two loci ($p = 0.002$). The total of about 81% of the variation accounted for in race-non-specific resistance against *P. infestans* is rather high, but not uncommon for disease resistance (Young 1996). The locus that we detected on chromosome 3 accounted for about 25% of the total variation for resistance against *P. infestans*, which is similar to what was found by Ewing and co-workers for the same locus (Ewing et al. 2000). The locus that we found on chromosome 5 accounted for about 41% of the total variation for late blight resistance, which is similar to the results of Collins and co-workers for this locus (Collins et al. 1999).

The total of only two QTLs that we detected for late blight resistance was fairly low, but not unusual (Meyer et al. 1998; Ewing et al. 2000; Sandbrink et al. 2000). The two loci accounted for a rather large proportion of variation for resistance against *P. infestans* (approximately 81%), which implies that very likely other loci were not heterozygous in the parents of this population.

The frequency distribution of phenotypic classes of foliage resistance to late blight was slightly, but significantly, skewed towards resistance. This was not the result of our method of evaluation, since the residuals were distributed normally (data not shown) and therefore the data did not require transformation. Skewness caused by distorted segregation due to gamete or zygote selection (Kreike and Stiekema 1997) was not supported by the segregation of molecular markers near the two QTLs for resistance against *P. infestans* that we detected on chromosomes 3 and 5. Distorted segregation of molecular markers was found in other populations near these loci on chromosome 3 (Leonards-Schippers et al. 1994) and chromosome 5 (Collins et al. 1999). A more plausible explanation for the skewness in our population appears to be the epistatic effect of the two QTLs for resistance to late blight: all individuals in the left tail of the distribution have both the favourable Y-allele for the locus on chromosome 3 and the favourable B-allele for the locus on chromosome 5 (Fig. 4) and are thus more resistant than expected based on additive effects only (Table 1). Epistasis of QTLs for late blight resistance was also found by Ewing and co-workers, which also resulted in a higher level of resistance (Ewing et al. 2000).

Foliage maturity type

We detected only one major QTL for foliage maturity type in this population. This QTL was located on chromosome 5, it was highly significant ($p < 0.001$) and accounted for about 84% of the total variation for foliage maturity type in this population.

The QTL for foliage maturity type was previously identified as the *El* locus by Van Eck (1995). The location and the magnitude of the effect of the QTL for foliage maturity type are also in accordance with previous results from other populations. These other populations had more than one QTL for foliage maturity type, but the locus on chromosome 5 was clearly the most important one (Collins et al. 1999; Oberhagemann et al. 1999).

Genetic linkage of late blight resistance and foliage maturity type on chromosome 5

The most important QTL for resistance against *P. infestans* was located very close to the QTL for foliage maturity type on chromosome 5 (their respective highest lod-values were only 1 cM apart; Fig. 6). Both traits had the strongest linkage with the same genetic marker GP21. Resistance to late blight was in coupling phase with marker GP21, while early foliage maturity was in repulsion phase. This implies a negative association between the two traits, as the B-allele for marker GP21 favours resistance to late blight whereas the A-allele for GP21 favours early foliage maturity.

This outcome confirmed the results of previous research (Van Eck and Jacobsen 1996; Collins et al. 1999; Oberhagemann et al. 1999) that late blight resistance and foliage maturity type are genetically linked on chromosome 5. The results also explain the virtual non-existence of potato varieties that are both early maturing and resistant against *P. infestans* (Anonymous 2001). The combination of a major QTL effect (approximately 84% of variation accounted for) and a high heritability (0.79) for foliage maturity type enables simple phenotypic selection for favourable early genotypes. Because of the strong genetic linkage of late blight resistance and foliage maturity type on chromosome 5, this selection for early foliage maturity will inevitably result in selection against resistance to late blight. The negative association between late blight resistance and early foliage maturity is not only restricted to the locus on chromosome 5: all QTLs for foliage maturity type that were found in other populations were, without exception, associated with loci for late blight resistance. This association always resulted in a loss of resistance to late blight when foliage maturity became earlier (Collins et al. 1999; Oberhagemann et al. 1999; Ewing et al. 2000). As a consequence, the resistance against *P. infestans* in early maturing genotypes can only result from QTLs for resistance that are not linked with QTLs for foliage maturity type, like the one on chromosome 3 in this research.

Resistance against *P. infestans* in early maturing genotypes is also hampered by the epistatic interaction of the two QTLs for late blight resistance in this population. All early maturing individuals have the A-allele for marker GP21 on chromosome 5 and are susceptible to late blight; the role of the locus on chromosome 3 is consistent, but completely subordinate. Only for the late maturing individuals that all have the B-allele for GP21 on chromosome 5, the effect of the locus on chromosome 3 becomes relevant (Fig. 4). Apparently, the locus on chromosome 5 influences the expression of the other QTL for resistance to late blight in this population. Consequently, early foliage maturity not only has a direct negative association with late blight resistance on chromosome 5, but also an indirect negative association with resistance on chromosome 3. This phenomenon has not been reported before for late blight resistance and foliage maturity type in potato, but might generally play an important role in the association of the two traits. We will check the relevance of this new phenomenon in different, larger potato populations of other genetic constitutions, to estimate the impact on resistance breeding in early maturing potatoes.

Detailed analysis of linked QTLs for late blight resistance and foliage maturity type on chromosome 5

The detailed analysis of the locus on chromosome 5 revealed the same loci for resistance to late blight as in the analysis in which foliage maturity type was not used as a covariate. However, the effect on late blight resistance of the locus on chromosome 5 was only half of that found in the analysis without the correction for foliage maturity type (–0.031 instead of –0.061, $p < 0.001$); the effects of the locus on chromosome 3 and of the interaction had not changed considerably. Obviously, the correction for foliage maturity type only affects the QTL for late blight resistance that is closely linked to the QTL for foliage maturity type. The decrease in significance of the locus on chromosome 5, which is caused by the use of foliage maturity type as a covariate, illustrates that we were able to use foliage maturity type to account for part of the variation for resistance to late blight on this locus. This is a strong indication that the two indistinguishable QTLs for foliage maturity type and for resistance against *P. infestans* on chromosome 5 are actually just one gene with a pleiotropic effect on both traits.

However, there is still a significant effect on late blight resistance of the locus on chromosome 5 after the correction for foliage maturity type. This effect may be due to our rather straightforward approach, or because there is actually still variation left for resistance to late blight after correction for foliage maturity type. We cannot rule out the presence of two closely linked QTLs on chromosome 5: one with a pleiotropic effect on both late blight resistance and foliage maturity type, and another with an effect on resistance against *P. infestans* only. This hypothesis of two QTLs on chromosome 5 is not unlikely, considering the cluster of resistance genes in this region (Gebhardt and Valkonen 2001), and we will verify this by using new potato populations with more individuals derived from different parents.

Acknowledgements The authors are grateful to Dirk Budding, Caroline Celis Gamboa, Wilbert Flier, Hans van Os, Johan van Ooijen, Roeland Voorrips and Eric van de Weg for their contribution to the research; and to Kees van Loon, Eric van de Weg and the members of the users' committee of project WBI.3871 of the Dutch Technology Foundation (STW) for suggestions on the manuscript. The first author's participation in this research was made possible by a grant from STW (project WBI.3871).

References

Alonso-Blanco C, El-Assal SE-D, Coupland G, Koornneef M (1998) Analysis of natural allelic variation at flowering time loci in the Landsberg *erecta* and Cape Verde Islands ecotypes of *Arabidopsis thaliana*. Genetics 149:749–764

Anonymous (2001) 76th list of varieties of field crops. Plant Research International, Wageningen, The Netherlands, pp 185–229

Ballvora A, Ercolano MR, Weiss J, Merksem K, Bormann C, Salamini F, Gebhardt C (2002) The *R1* gene for potato resistance to late blight (*Phytophthora infestans*) belongs to the Leucine Zipper/NBS/LRR class of plant resistance genes. In: Plant, Animal and Microbe Genomes X. Int Plant and Animal Genome Conf, San Diego, pp 70

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 (1994) Resistance to *Phytophthora infestans* in *Solanum tuberosum* and wild *Solanum* species. PhD thesis, Wageningen Agricultural University, Wageningen, The Netherlands

Colon LT, Budding DJ (1988) Resistance to late blight (*Phytophthora infestans*) in ten wild *Solanum* species. Euphytica S:77–86

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

Ewing EE, Simko 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

Flier WG (2001) Variation in *Phytophthora infestans*, sources and implications. PhD thesis, Wageningen University, Wageningen, The Netherlands

Fry WE (1987) Quantification of general resistance of potato cultivars and fungicide effects for integrated control of potato late blight. Phytophathology 68:1650–1655

Fry WE, Goodwin SB, Dyer AT, Matuszak JM, Drenth A, Tooley PW, Sujkowski LS, Koh YJ, Cohen BA, Spielman LJ, Deahl KL, Inglis DA, Sandlan KP (1993) Historical and recent migrations of *Phytophthora infestans*: chronology, pathways, and implications. Plant Dis 77:653–661

Gebhardt C, Valkonen JPT (2001) Organization of genes controlling disease resistance in the potato genome. Annu Rev Phytopathol 39:79–102

GenStat (2000) GenStat for Windows. Release 4.2, 5th edn. VSN International Limited, Oxford

Ghislain M, Nelson R, Walker T (1997) Resistance to potato late blight: a global research priority. Biotechnol Dev Monitor 31:14–16

Ghislain M, Trognitz B, Herrera Ma del R, Solis J, Casallo G, Vásquez C, Hurtado O, Castillo R, Portal L, Orrillo M (2001) Genetic loci associated with field resistance to late blight in offspring of *Solanum phureja* and *S. tuberosum* grown under short-day conditions. Theor Appl Genet 103:433–442

Hanneman RE, Peloquin SJ (1967) Crossability of 24-chromosome potato hybrids with 48-chromosome cultivars. Eur Potato J 10:62–73

Hanson WD (1963) Heritability. In: Hanson WD, Robinson HF (eds) Statistical genetics and plant breeding. NAS-NRC, Washington, pp 125–140

Jacobs JME, Van Eck HJ, Arens P, Verkerk-Bakker B, Te Lintel Hekkert B, 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

Jacobsen E (1980) Increase of diplandroid formation and seed set in $4x \times 2x$ crosses in potatoes by genetical manipulation of dihaploids and some theoretical consequences. Z Pflanzenzüchtg 85:110–121

Jansen J, De Jong AG, Van Ooijen JW (2001) Constructing dense genetic linkage maps. Theor Appl Genet 102:1113–1122

Jansen RC (1994) Controlling the type I and type II errors in mapping quantitative trait loci. Genetics 138:871–881

Kreike CM, Stiekema WJ (1997) Reduced recombination and distorted segregation in a *Solanum tuberosum* ($2x$) × *S. spegazzinii* ($2x$) hybrid. Genome 40:180–187

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

Leonards-Schippers C, Gieffers W, Salamini F, Gebhardt C (1992) The *R1* gene conferring race-specific resistance to *Phytophthora infestans* in potato is located on potato chromosome V. Mol Gen Genet 233:278–283

Leonards-Schippers C, Gieffers W, Schäfer-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

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

Patterson HD, Thompson R (1971) Recovery of inter-block information when block sizes are unequal. Biometrika 58:545–554

Ross H (1986) Potato breeding: problems and perspectives. J Plant Breed, Suppl 13

Sandbrink JM, Colon LT, Wolters PJCC, Stiekema WJ (2000) Two related genotypes of *Solanum microdontum* carry different segregating alleles for field resistance to *Phytophthora infestans*. Mol Breed 6:215–225

Shaner G, Finney RE (1977) The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67:1051–1056

Sokal RR, Rohlf FJ (1969) Biometry: the principles and practice of statistics in biological research. Freeman, New York

Spielman LJ, Drenth A, Davidse LC, Sujkowski LJ, Gu W, Tooley PW, Fry WE (1991) A second world-wide migration and population displacement of *Phytophthora infestans*? Plant Pathol 40:422–430

Swiezynski KM (1990) Resistance to *Phytophthora infestans* in potato cultivars and its relation to maturity. Genetica Pol 31: 99–106

Toxopeus HJ (1958) Some notes on the relations between field resistance to *Phytophthora infestans* in leaves and tubers and ripening time in *Solanum tuberosum* subsp. *tuberosum*. Euphytica 7:123–130

Turkensteen LJ, Flier WG, Van Baarlen P (1996) Evidence for sexual reproduction of *Phytophthora infestans* in an allotment garden complex in the Netherlands. In: Struik PC, Hoogendoorn J, Kouwenhoven JK, Mastenbroek LJ, Turkensteen LJ, Veerman A, Vos J (eds) Abstracts of Conference Papers, Posters and Demonstrations of the 13th Triennial conference of the EAPR. European Association for Potato Research, Wageningen, pp 356–357

Umaerus V, Umaerus M, Erjefält L, Nilsson BA (1983) Control of *Phytophthora* by host resistance: problems and progress. In: Erwin DC, Bartnicki-Garcia S, Tsao PH (eds) *Phytophtora*, its biology, taxonomy, ecology and pathology. The American Phytopathological Society, St. Paul, Minnesota, pp 315–326

Van Eck HJ (1995) Localisation of morphological traits on the genetic map of potato using RFLPs and isozyme markers. PhD thesis, Wageningen Agricultural University, Wageningen, The Netherlands

Van Eck HJ, Jacobsen E (1996) Application of molecular markers in the genetic analysis of quantitative traits. In: Struik PC, Hoogendoorn J, Kouwenhoven JK, Mastenbroek LJ, Turkensteen LJ, Veerman A, Vos J (eds) Abstracts of Conference Papers, Posters and Demonstrations of the 13th Triennial conference of the EAPR. European Association for Potato Research, Wageningen, pp 130–131

Van Eck HJ, Rouppe van der Voort J, 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

Van Ooijen JW, Voorrips RE (2001) JoinMap Version 3.0: software for the calculation of genetic linkage maps. Plant Research International, Wageningen, The Netherlands

Van Ooijen JW, Boer MP, Jansen RC, Maliepaard C (2000) MapQTL Version 4.0: software for the calculation of QTL positions on genetic maps. Plant Research International, Wageningen, The Netherlands

Vleeshouwers VGAA (2000) Molecular and cellular biology of resistance to *Phytophthora infestans* in *Solanum* species. PhD thesis, Wageningen University, Wageningen, The Netherlands

Wastie RL (1991) Breeding for resistance. In: Ingram DS, Williams PH (eds) Advances in Plant Pathology, vol. 7, *Phytophthora infestans*: the cause of Late Blight of Potato. London Academic Press, pp 193–224

Young ND (1996) QTL mapping and quantitative disease resistance in plants. Annu Rev Phytopathol 34:479–501