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# Genetic linkage of QTLs for late blight resistance and foliage maturity type in six related potato progenies

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**Summary**

A set of test crosses of diploid potatoes was used to identify QTLs for foliage resistance against *Phytophthora infestans* and QTLs for foliage maturity type, and to assess their genetic relationship. The most important locus for both traits was found on chromosome 5 near marker GP21: the allele of marker GP21 that is associated with resistance to late blight is also associated with late foliage maturity. An additional QTL with a small effect on foliage maturity type was identified on chromosome 3, and additional QTLs for late blight resistance were found on chromosomes 3 and 10. Another QTL was detected on chromosome 7 when resistance was adjusted for the effect of foliage maturity type. The additional QTLs for resistance against *P. infestans* on chromosomes 3 and 10 seem to be independent of foliage maturity type and are not affected by epistatic effects of the locus on chromosome 5. The effects of the additional QTLs for resistance are small, but early maturing genotypes that necessarily have the allele for susceptibility for late blight on chromosome 5 may benefit from the resistance that is provided by these QTLs on chromosomes 3 and 10.

*Abbreviations:* AUDPC: area under the disease progress curve; QTL: quantitative trait locus

## Introduction

Potato (*Solanum tuberosum*) production throughout the world is threatened by late blight epidemics that are caused by *Phytophthora infestans*. Devastating outbreaks of the disease in North America and Europe in the 1840s initiated breeding for late blight resistance (Wastie, 1991), which at first resulted in introgression of race-specific resistance (R) genes from *S. demissum* (Ross, 1986). Unfortunately, this race-specific approach turned out not to be durable, because compatible races of *P. infestans* appeared rapidly and are now present for (combinations of) all 11 known R genes (Turkensteen, 1993). Race-non-specific foliage resistance to late blight in potato appears to be more durable (Thurston, 1971), though true race-non-specificity is impossible to prove (Johnson, 1979). This type of resistance is characterised by a continuous variation in phenotypic appearance and a polygenic inheritance that complicate breeding considerably (Umaerus, 1970).

The use of molecular markers has provided breeders with more knowledge of the genetic background of race-non-specific foliage resistance against *P. infestans* in potato. Quantitative trait loci (QTLs) for late blight resistance have been identified in several studies (e.g. Leonards-Schippers et al., 1994; Meyer et al., 1998), of which the joint results suggest that all 12 potato

chromosomes may harbour one or more QTLs for this trait (Gebhardt & Valkonen, 2001; Simko, 2002). Some of these studies have also identified QTLs for foliage maturity type, which are fewer in number, but coincide all with QTLs for resistance to late blight (Collins et al., 1999; Oberhagemann et al., 1999; Ewing et al., 2000; Visker et al., 2003). This touches on a further complication in breeding for resistance against *P. infestans*: the association of race-non-specific resistance with late foliage maturity (Toxopeus, 1958). Due to the strong association of these two traits, early maturing potato varieties with satisfactory levels of late blight resistance do not exist (Swiezynski, 1990).

The most important QTL for both traits is located on chromosome 5 near marker GP21: the alleles that provide foliage resistance to late blight also provide late foliage maturity (Collins et al., 1999; Oberhagemann et al., 1999; Visker et al., 2003; Bormann et al., 2004; Bradshaw et al., 2004). However, phenotypic evaluations of race-non-specific resistance against *P. infestans* and of foliage maturity type in a set of test crosses indicated that some selection for resistance without affecting foliage maturity type should be possible (Visker et al., 2004). Therefore, the same set of test crosses was used for a QTL analysis that is described in the present paper. The aim was to identify QTLs for foliage resistance to late blight and QTLs for foliage maturity type, to assess their genetic relationship, and to determine whether (marker assisted) selection for resistance as well as early foliage maturity type is feasible. The most important locus was found on chromosome 5 near marker GP21 with a pleiotropic effect on both traits. Additional QTLs for late blight resistance were detected on chromosomes 3 and 10 that were independent of foliage maturity type.

## Materials and methods

### *Plant material*

Four diploid potato clones were used as parents in a half-diallel set of crosses (for details see Visker et al., 2004). This resulted in six progenies (Table 1), of which progeny 2 and progeny 5 were studied in more detail. Progeny 5 was chosen because it was expected to display the broadest segregation for the two traits of interest, based on the parental phenotypes. This progeny consisted of 227 genotypes derived from a cross between SH82-44-111 (SH; Colon et al., 1995; Sandbrink et al., 2000) and CE51 (CE; Jacobs et al., 1995; Van Eck et al., 1995). Clone SH is early maturing and susceptible to late blight, whereas clone CE is late maturing and resistant. Progeny 2 was chosen because it was derived from the other two parents of the half-diallel set of crosses. Progeny 2 consisted of 300 genotypes derived from a cross between DH84-19-1659 (DH; Plant Research International) and I88.55.6 (I; Collins et al., 1999; Oberhagemann et al., 1999). Clone DH is early maturing and relatively resistant to late blight, while clone I is early maturing and susceptible.

*Table 1.* Scheme of the half-diallel set of crosses with four diploid potato parents: DH84-19-1659 (DH), SH82-44-111 (SH), I88.55.6 (I), and CE51 (CE), comprising six progenies with their selected numbers of offspring

| | Male parent | | | |
|---|---|---|---|---|
| Female parent | DH | SH | I | CE |
| DH | – | 300 (1) | 300 (2) | 300 (3) |
| SH | | – | 300 (4) | 227 (5) |
| I | | | – | 300 (6) |
| CE | | | | – |

*Note.* Progenies are numbered 1 to 6, as indicated between brackets.

### *Phenotypic evaluations*

Plants were field-evaluated for race-non-specific foliage resistance to late blight and for foliage maturity type in separate fields, because assessment of the two traits on the same plants is not feasible. Progeny 2 was evaluated in the year 2000; progeny 5 was evaluated in the years 2000 and 2001. The phenotypic evaluations have been described in detail by Visker et al. (2004), and main features are given below.

The field tests for late blight resistance consisted of three randomised blocks with two plants per plot. Plants were inoculated approximately 8 weeks after emergence by spray application of a spore suspension of race 1.2.3.4.5.6.7.10.11 of *P. infestans* (IPO82001; Flier et al., 2003). Percentages of late blight-affected leaf tissue were assessed over a period of 6 weeks after inoculation at weekly intervals in the year 2000 and twice a week in 2001. These disease ratings were used to calculate the normalised or relative Area Under the Disease Progress Curve (AUDPC; Shaner & Finney, 1977; Fry, 1978). Relative AUDPC values range between 0 and 1, and reflect both onset and rate of disease development, resulting in low values for resistant genotypes and high values for susceptible ones.

The field tests for foliage maturity type consisted of three randomised blocks with three plants per plot. Assessments of foliage maturity type comprised visual classification of a whole syndrome of features representing foliage maturity (sagging of plants, termination of apical growth, and discoloration of leaves). Assessments were made biweekly over a period of several months that started when the first symptoms of senescence were visible and ended at the first ground frost. Assessments of foliage maturity type were recorded on a scale comprising eight classes: 0, 1, 2, 4, 6, 8, 9, 10, ranging from completely unblemished to fully deceased plants. Consecutive assessments were combined and adjusted for the length of the evaluation period (similar to relative AUDPC). This resulted in foliage maturity type values that ranged between 0 and 10, with low values for genotypes with late foliage maturity and high values for the ones with early foliage maturity.

Relative AUDPC values were adjusted for the correlation between resistance against *P. infestans* and foliage maturity type as described by Visker et al. (2004). These adjusted relative AUDPC values represent resistance that is not associated with foliage maturity type.

The phenotypic evaluations of progeny 2 resulted in three sets of trait data: one for late blight resistance (2000), one for foliage maturity type (2000), and one for resistance that was adjusted for foliage maturity type (2000; Visker et al., 2004). The phenotypic evaluations of progeny 5 resulted in seven sets of trait data: three for late blight resistance (2000, 2001, and 2-year average), three for foliage maturity type (2000, 2001, and 2-year average), and one for resistance that was adjusted for foliage maturity type (2000; Visker et al., 2004). Resistance was adjusted for foliage maturity type for all progenies in the year 2000, because of correlation between the two traits in progenies 1, 2, 3, 4, and 6. Resistance was not adjusted for foliage maturity type in the year 2001, because the absence of correlation between the two traits in the only progeny that was tested in this year (progeny 5) made such an adjustment pointless.

*Molecular markers*

Molecular markers were generated for 201 randomly chosen genotypes of progeny 2 and for all 227 genotypes of progeny 5. DNA was extracted from frozen young leaf tissue according to Fulton et al. (1995) or using the DNeasy Plant Mini Kit (Qiagen).

AFLP markers were generated as described by Vos et al. (1995). DNA was digested with the restriction enzyme combination *Eco*RI/*MSE*I, a non-selective pre-amplification step was included, and selective amplification was performed with E and M primers (E: 5′-GACTGCGTACCAATTC, M: 5′-GATGAGTCCTGAGTAA) with three additional selective nucleotides each (E+3 and M+3 primers). Ten primer combinations were used for both progenies: E+AAC/M+CAC, E+AAC/M+CAG, E+AAC/M+CCC, E+AAC/M+CCT, E+ACA/M+CAG, E+ACA/M+CCT, E+ACT/M+ACA, E+ACT/M+CAG, E+ACT/M+CCT, and E+AGA/M+CAT. Eight additional primer combinations were used for progeny 2: E+AAC/M+CAT, E+AAC/M+CGT, E+AAC/M+CTG, E+ACA/M+CGT, E+ACT/M+CAT, E+ACT/M+CGT, E+AGT/M+CTA, and E+ATG/M+CAG. Three other additional primer combinations were used for progeny 5: E+AAA/M+ACG, E+AAC/M+CTA, and E+ATG/M+CTC. All primer combinations that were used for progeny 2 were first applied only to a random subset of 65 of the 201 genotypes. The 11 primer combinations that resulted in the best marker coverage of the genome were subsequently applied to the rest of the 201 genotypes. Nomenclature of each AFLP marker was based on the primer combination used and the electrophoretic mobility of the amplification product (Van Eck et al., 1995) relative to a 30–330 bp AFLP DNA ladder (GibcoBRL/Invitrogen).

Cleaved Amplified Polymorphic Sequence (CAPS) markers of loci GP21 and GP179 were generated as described by Meksem et al. (1995). PCR products of GP21 and GP179 were digested with the restriction enzymes *Taq*I and *Hae*III, respectively, and separated on 1.5% agarose gels.

Simple sequence repeat (SSR) or microsatellite markers were generated as described by Provan et al. (1996). SSR marker names, primer sequences, and annealing temperatures were derived from Milbourne et al. (1998). SSR markers were applied to enable assignment of chromosome numbers to linkage groups and, therefore, generated only for a random subset of 70 of the 201 genotypes of progeny 2, and also for a subset of 70 of the 227 genotypes of progeny 5.

*Genetic linkage maps*

Linkage analysis for construction of the genetic maps was performed with JoinMap 3.0 (Van Ooijen & Voorrips, 2001). Separate genetic maps were made for each of the four parents and homologous linkage

groups were not integrated. For this purpose the marker dataset of progeny 2 was converted into two datasets: one for the female segregation to construct the genetic map of parent DH, and the other for the male segregation to construct the map of parent I. The same conversion was applied to the marker dataset of progeny 5 to construct the genetic maps of parents SH and CE. The dataset of each parent was restricted to genotypes with scores for at least 50% of the markers, and to markers with scores for at least 25% of the genotypes. Markers and genotypes with many missing values were removed because they lead to problematic map construction and overestimated genetic distances (Jansen et al., 2001), and because they result in underestimated significances in QTL analyses.

Maps consisted mainly of dominantly scored AFLP markers. CAPS and SSR markers were scored co-dominantly, and were used to enable assignment of chromosome numbers to linkage groups. Additional information was taken from the UHD Potato map database (http://potatodbase.dpw.wau.nl/UHDdata.html) for assignment of chromosome number and chromosome orientation based on alignment with putatively homologous AFLP markers (Rouppe van der Voort et al., 1997).

*QTL analysis*

QTL analyses of progeny 2 were done with the genetic map of all (non-integrated) linkage groups of both parents DH and I, and the three sets of phenotypic trait data. QTL analyses of progeny 5 were done with the genetic map of all (non-integrated) linkage groups of both parents SH and CE, and the seven sets of phenotypic trait data.

QTLs for all traits were identified with the Kruskall–Wallis and the Interval-mapping procedures of MapQTL 4.0 (Van Ooijen et al., 2002). Significance thresholds for Interval-mapping were determined with the permutation test of MapQTL ($p < 0.05$, 2500 permutations). When QTLs were found at approximately corresponding positions in both parents of a progeny, the presence of interactions between alleles of the marker(s) closest to the QTL was estimated with the residual maximum likelihood (REML) method (Patterson and Thompson, 1971) of GenStat 6 (GenStat, 2002). Calculations were made with the factors female marker, male marker, and female marker × male marker in the fixed part of the statistical analysis. When more than one QTL was found for the same trait, the presence of interactions between the markers closest to the QTLs was also estimated with REML, with the factors marker closest to QTL 1, marker closest to QTL 2, and their interaction as fixed. These analyses also gave estimated effects and predicted means of the alleles of the marker closest to each QTL. The percentages of variation that could be accounted for by a single QTL or by an interaction were based on variance components that were estimated with REML, using all factors [marker(s) closest to QTL(s), interaction(s)] as random (Alonso-Blanco et al., 1998).

## Results

*Progeny 2*

Progeny 2 was field-evaluated for foliage resistance to late blight and for foliage maturity type in the year 2000. The phenotypic distribution for relative AUDPC was skewed towards resistance and showed transgression in both directions. The phenotypic distribution for foliage maturity type was skewed towards early foliage maturity and transgressed also in both directions. There was a positive correlation between the two traits: low relative AUDPC values coincided with low values for foliage maturity type and high relative AUDPC values coincided with high values for foliage maturity type, $r = 0.56$ (Figure 1; Visker et al., 2004).

The genetic map of all (non-integrated) linkage groups of progeny 2 comprised the maps of both parents DH and I. The map of parent DH had 145 markers in 12 linkage groups with a total length of 490 cM. The map of parent I had 122 markers in 15 linkage groups with a total length of 534 cM. All 12 chromosomes were represented in the maps of both parents, except for chromosomes 3 and 4 in the map of parent DH, and chromosome 4 in parent I.

In progeny 2 one QTL was detected for relative AUDPC (on chromosome 5), one for foliage maturity type (also on chromosome 5), and one for adjusted relative AUDPC (on chromosome 7; Table 2). The QTL for relative AUDPC on chromosome 5 was found near marker GP21 in both parents. The effects of the alleles of the two parents were similar and no interaction was found between these alleles (Table 3). The QTL for foliage maturity type on chromosome 5 was found also near marker GP21 in both parents. Also for this trait the effects of the alleles of both parents were similar and no interaction was found between the alleles of the two parents (Table 3). The QTL for adjusted relative AUDPC on chromosome 7 was found in parent I. None

*Table 2.* Molecular markers that are most closely linked with QTLs for relative AUDPC, foliage maturity type, and adjusted relative AUDPC in progeny 2: DH84-19-1659 (DH) × I88.55.6 (I)

| | | | | Parent DH | | | | | | Parent I | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Pred. mean | | | | | | Pred. mean | |
| Trait | Thr. | Chr. | % | Marker | cM | Lod | Effect | A | B | Marker | cM | Lod | Effect | A | B |
| Relative AUDPC 2000 | 4.3 | 5 | 40 | GP21 | 1 | 5.5 | −0.064 | 0.64 | 0.58 | GP21 | 7 | 6.6 | −0.052 | 0.64 | 0.59 |
| Foliage maturity type 2000 | 4.5 | 5 | 51 | GP21 | 1 | 4.6 | −1.10 | 4.7 | 3.6 | GP21 | 7 | 9.2 | −1.08 | 4.7 | 3.6 |
| Adj. relative AUDPC 2000 | 4.0 | 7 | 16 | | | | | | | AAC/CGT-118 | 32 | 4.0 | +0.029 | 0.62 | 0.65 |

*Note.* Phenotypic data were obtained in the year 2000. Provided are lod-thresholds (Thr.), chromosome numbers (Chr.), percentages of variation accounted for (%), chromosome positions (cM), lod-values (Lod), effects, and predicted means (Pred. mean) of the two alleles (A and B) of each marker.

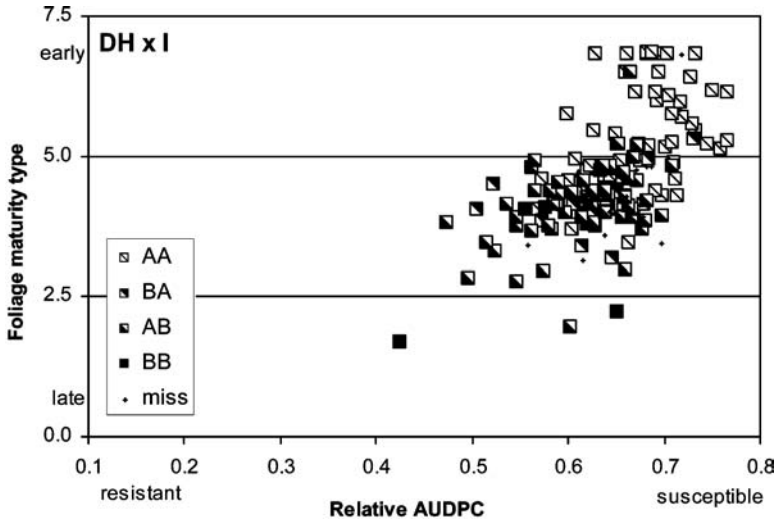


*Figure 1.* Phenotypic correlation between relative AUDPC and foliage maturity type in progeny 2: DH84-19-1659 (DH) × I88.55.6 (I), combined with marker phenotypes AA, BA, AB, and BB of GP21 on chromosome 5 of both parents.

of the non-linked markers had a significant effect on any of the evaluated traits.

The segregation ratio of chromosome 5 of parent DH was significantly skewed, most severely on marker GP21. The A allele of this marker of parent DH was present in 147 genotypes, while the B allele was present in only 35 genotypes. This resulted in a relative excess of genotypes with high values for relative AUDPC (susceptible) and high values for foliage maturity type (early; Figure 1).

*Progeny 5*

Progeny 5 was field-evaluated for foliage resistance to late blight and for foliage maturity type in the years 2000 and 2001. The phenotypic distribution for relative AUDPC was skewed towards resistance and showed transgression in both directions in the year 2000, whereas it was not skewed and transgressed only towards susceptibility in 2001. The phenotypic distribution for foliage maturity type was skewed towards early foliage maturity in the year 2000 but not in 2001, and transgressed towards early foliage maturity in both years. No correlation was found between relative AUDPC and foliage maturity type in either of the two years, as the variation for foliage maturity type was limited (Visker et al., 2004).

The genetic map of all (non-integrated) linkage groups of progeny 5 consisted of the maps of both parents SH and CE. The map of parent SH had 85 markers in 11 linkage groups with a total length of 482 cM. The map of parent CE had 122 markers in 13 linkage

*Table 3.* Effects and predicted means of combinations of A and B alleles of each parent of marker GP21 on chromosome 5 for relative AUDPC and foliage maturity type in progeny 2: DH84-19-1659 (DH) × I88.55.6 (I)

| | | Parent DH | | | |
|---|---|---|---|---|---|
| | | Effect | | Predicted mean | |
| Trait | Parent I | A | B | A | B |
| Relative AUDPC 2000 | A | 0 | −0.062 | 0.67 | 0.61 |
| | B | −0.051 | −0.118 | 0.62 | 0.55 |
| Foliage maturity type 2000 | A | 0 | −1.08 | 5.2 | 4.1 |
| | B | −1.07 | −2.22 | 4.2 | 3.0 |

*Note.* Phenotypic data were obtained in the year 2000.

groups with a total length of 456 cM. All 12 chromosomes were represented in the maps of both parents, except for chromosomes 4 and 10 in the map of parent SH, and chromosome 4 in parent CE. In progeny 5 two QTLs were detected for relative AUDPC (on chromosomes 3 and 10), one for foliage maturity type (on chromosome 3), and two for adjusted relative AUDPC (on chromosomes 3 and 10; Table 4). The QTL for relative AUDPC on chromosome 3 was assumed to be the same in both parents. This was deduced from the relative positions of the QTL on the maps of the two parents, as absolute confirmation was lacking due to the absence of common markers on this chromosome. No interaction was found between the different alleles of this locus. The QTL on chromosome 10 was found in parent CE, and no interaction was detected between this locus and the one on chromosome 3. The QTL for foliage maturity type on chromosome 3 was found in parent SH. The QTLs for adjusted relative AUDPC on chromosomes 3 and 10 were both found in parent CE, and no interaction was detected between the two loci. None of the non-linked markers had a significant effect on any of the evaluated traits.

Not all QTLs were found with the different sets of data for the same trait. The QTL for relative AUDPC on chromosome 10 was not found with the phenotypic data of the year 2001, whereas the QTL for foliage maturity type was not detected with the phenotypic data of the year 2000.

### *Progenies 1, 3, 4, and 6*

The QTLs that were detected in progenies 2 and 5 were verified in progenies 1 (DH×SH), 3 (DH×CE), 4 (SH×I), and 6 (I×CE). For this purpose the

*Table 4.* Molecular markers that are most closely linked with QTLs for relative AUDPC, foliage maturity type, and adjusted relative AUDPC in progeny 5: SH82-44-111 (SH) × CE51 (CE)

| | | | | Parent SH | | | | | | Parent CE | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Pred. mean | | | | | | Pred. mean | |
| Trait | Thr. | Chr. | % | Marker | cM | Lod | Effect | A | B | Marker | cM | Lod | Effect | A | B |
| Rel. AUDPC 2000 | 3.0 | 3 | 20 | ACT/CAG-208 | 0 | 3.7 | +0.038 | 0.56 | 0.60 | ACT/ACA-92.5 | 6 | 6.5 | −0.052 | 0.60 | 0.55 |
| | | 10 | 12 | | | | | | | ACT/CCT-200 | 12 | 3.8 | −0.039 | 0.60 | 0.56 |
| Rel. AUDPC 2001 | 3.0 | 3 | 42 | ACT/CAG-208 | 0 | 3.5 | +0.054 | 0.31 | 0.37 | ACT/ACA-92.5 | 6 | 10.8 | −0.095 | 0.39 | 0.29 |
| Rel. AUDPC mean | 2.9 | 3 | 34 | ACT/CAG-208 | 0 | 3.5 | +0.040 | 0.43 | 0.47 | ACT/ACA-92.5 | 6 | 11.8 | −0.075 | 0.49 | 0.42 |
| | | 10 | 8 | | | | | | | ACT/CCT-200 | 12 | 3.3 | −0.034 | 0.47 | 0.44 |
| Fol. maturity type 2000 | 2.7 | – | | | | | | | | | | | | | |
| Fol. maturity type 2001 | 2.7 | 3 | 20 | ACT/CCT-85 | 39 | 4.9 | −0.31 | 2.8 | 2.5 | | | | | | |
| Fol. maturity type mean | 2.7 | 3 | 10 | ACT/CCT-85 | 39 | 3.1 | −0.23 | 3.1 | 2.9 | | | | | | |
| Adj. rel. AUDPC 2000 | 2.8 | 3 | 17 | | | | | | | ACT/ACA-92.5 | 6 | 5.0 | −0.048 | 0.64 | 0.59 |
| | | 10 | 12 | | | | | | | ACT/CCT-200 | 12 | 3.2 | −0.042 | 0.64 | 0.59 |

*Note.* Phenotypic data were obtained in the years 2000 and 2001. Provided are lod-thresholds (Thr.), chromosome numbers (Chr.), percentages of variation accounted for (%), chromosome positions (cM), lod-values (Lod), effects, and predicted means (Pred. mean) of the two alleles (A and B) of each marker.

*Table 5.* Presence of QTLs in parents DH84-19-1659 (DH) and I88.55.6 (I) as detected in progeny 2 (DH×I), and their verification in progenies 1 (DH×SH) and 3 (DH×CE), and progenies 4 (SH×I) and 6 (I×CE), respectively. Correspondingly, presence of QTLs in parents SH82-44-111 (SH) and CE51 (CE) as detected in progeny 5 (SH×CE), and their verification in progenies 1 (DH×SH) and 4 (SH×I), and progenies 3 (DH×CE) and 6 (I×CE), respectively

| | | Parent | | | |
|---|---|---|---|---|---|
| Trait | QTL presence | DH | I | SH | CE |
| Relative AUDPC | On parental chromosome | *5* | *5* | *3* | *3* *10* |
| | Confirmed in progeny | 1; 3 | 4; 6 | 1 | 3 3; 6 |
| Foliage maturity type | On parental chromosome | *5* | *5* | *3* | |
| | Confirmed in progeny | 1; 3 | 4; 6 | 1; 4 | |
| Adj. relative AUDPC | On parental chromosome | | *7* | | *3* *10* |
| | Confirmed in progeny | | – | | 3 3; 6 |

*Note.* Phenotypic data of progenies 1, 3, 4, and 6 were obtained in the year 2000.

phenotypic evaluations of the year 2000, the two CAPS markers of loci GP21 and GP179, and AFLP markers of four primer combinations (E+AAC/M+CAC, E+AAC/M+CAG, E+AAC/M+CCT, and E+AGA/M+CAT) were available, but genetic linkage maps were not made. Markers that were (closely) linked with one of the QTLs that had been detected in progenies 2 and 5, and that were also available in one or more of the other four progenies were tested for significant effects on late blight resistance and/or foliage maturity type (REML) in these other progenies.

Almost all QTLs that were detected in parents DH or I in progeny 2, or in parents SH or CE in progeny 5 were confirmed in the other two progenies of each of these parents (Table 5). No significant interactions between QTLs were found. Similar QTLs in different progenies of the same parent can have different effects and percentages of variation accounted for, as illustrated for the QTL for relative AUDPC and the QTL for foliage maturity type on chromosome 5 near marker GP21 (Table 6). Skewed segregation of marker GP21 was found for parent DH in progeny 3 and for parent I in progeny 6, and resulted in a relative excess of genotypes with high values for relative AUDPC (susceptible) and high values for foliage maturity type (early).

## Discussion

The association between race-non-specific foliage resistance against *P. infestans* and foliage maturity type in potato was studied in six progenies that were derived from crosses between four unrelated diploid parents. QTL analyses were performed on two contrasting progenies: one that displayed a phenotypic correlation between the two traits (progeny 2) and another without such a correlation (progeny 5), and results were verified in the remaining four progenies.

The most important locus for resistance to late blight and for foliage maturity type in potato has been identified on chromosome 5 in previous crosses (Collins et al., 1999; Oberhagemann et al., 1999; Visker et al., 2003; Bormann et al., 2004; Bradshaw et al., 2004). The same locus was also found in five out of six progenies in the present analysis, of which progeny 2 was studied in more detail. This progeny revealed one QTL with a large effect on late blight resistance on chromosome 5, one QTL with a large effect on foliage maturity type on the same chromosome, and

*Table 6.* Percentages of variation accounted for (%), effects, and predicted means (pred. mean) of combinations of A and B alleles of each parent of marker GP21 on chromosome 5 for relative AUDPC and foliage maturity type in progenies (prog.) 1, 3, 4, and 6 of parents DH84-19-1659 (DH), I88.55.6 (I), SH82-44-111 (SH), and CE51 (CE)

| | | Relative AUDPC | | | | | | | Foliage maturity type | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Female parent | | | Male parent | | | | Female parent | | | Male parent | | |
| | | | | Pred. mean | | | Pred. mean | | | | Pred. mean | | | Pred. mean | |
| Prog. | Cross | % | Effect | A | B | Effect | A | B | % | Effect | A | B | Effect | A | B |
| 1 | DH×SH | 58 | −0.154 | 0.66 | 0.51 | n.s. | – | – | 57 | −1.94 | 5.0 | 3.1 | n.s. | – | – |
| 3 | DH×CE | 70 | −0.180 | 0.59 | 0.41 | n.s. | – | – | 76 | −1.75 | 4.4 | 2.6 | n.s. | – | – |
| 4 | SH×I | 27 | n.s. | – | – | −0.071 | 0.65 | 0.58 | 63 | n.s. | – | – | −1.86 | 4.7 | 2.9 |
| 6 | I×CE | 27 | −0.051 | 0.63 | 0.58 | n.s. | – | – | 80 | −2.25 | 4.3 | 2.0 | n.s. | – | – |

*Note.* Phenotypic data were obtained in the year 2000.

no additional QTLs for either of the two traits. The two QTLs were detected in both parents, and the QTLs could not be distinguished from one another: both were most closely linked with the same molecular marker GP21. The co-location of these QTLs is responsible for the phenotypic correlation between the two traits in this progeny (Visker et al., 2004): the allele of marker GP21 that is associated with resistance against *P. infestans* is also associated with late foliage maturity. The QTL for late blight resistance was no longer detected once resistance was adjusted for the effect of foliage maturity type. When a similar adjustment was applied to a different diploid progeny, the QTL for resistance on chromosome 5 was still evident, but the effect of this locus on resistance to late blight was reduced to only half the original effect (Visker et al., 2003). Comparable results were found in other studies in which a similar approach was applied to tetraploid progenies. Bormann et al. (2004) did find a QTL for late blight resistance on chromosome 5 after adjustment for foliage maturity type, while Bradshaw et al. (2004) did not. The reduction of the effect of the QTL for resistance after adjustment for foliage maturity type suggests that the two indistinguishable QTLs for resistance to late blight and for foliage maturity type on chromosome 5 are actually just one gene with a pleiotropic effect on both traits. However, the (residual) effect on resistance against *P. infestans* after adjustment for foliage maturity type that is found in some progenies can also support the alternative hypothesis that the two traits are controlled by different genes that are closely linked. Because the approach to adjust late blight resistance for foliage maturity type renders different results when applied to different progenies, it does not provide conclusive evidence for the elucidation of the association between the two traits.

The locus on chromosome 5 for resistance to late blight and for foliage maturity type was not detected in progeny 5 in the present study. The absence of segregation for this locus indicates that both parents SH and CE are homozygous for it. Instead, progeny 5 revealed QTLs for resistance to late blight on chromosome 3 of both parents and on chromosome 10 of parent CE, together with a QTL for foliage maturity type on chromosome 3 of parent SH. The QTL for resistance against *P. infestans* on chromosome 3 of parent SH was significant in both years, but no QTL for adjusted relative AUDPC was found (determined only in the year 2000), suggesting that this QTL for resistance is also involved in foliage maturity type. However, the QTL for foliage maturity type on this chromosome was not significant in the year 2000, it was not located at the exact same position as the QTL for relative AUDPC, and no phenotypic correlation was found between late blight resistance and foliage maturity type in any of the two years of evaluation. These results contradict the previous suggestion that the locus on chromosome 3 of parent SH is involved in both traits. In parent CE the QTL for resistance to late blight on chromosome 3 was significant in both years, whereas the QTL on chromosome 10 was only significant in the year 2000. Both QTLs for resistance were not associated with foliage maturity type, because QTLs for adjusted relative AUDPC were found on the same positions with comparable significances and effects. QTLs for resistance against *P. infestans* have been identified on similar regions of chromosome 3 (Leonards-Schippers et al., 1994; Collins et al., 1999; Oberhagemann et al., 1999; Ewing et al., 2000; Ghislain et al., 2001; Visker et al., 2003) and chromosome 10 (Sandbrink et al., 2000; Ghislain et al., 2001) in previous studies of other progenies. Expression of the QTL for resistance to late blight on chromosome 10 of parent CE and of the QTL for foliage maturity type on chromosome 3 of parent SH seems to be affected by environmental (year) influences. Such environmental effects have been found in previous QTL analyses (Collins et al., 1999), and limit the applicability of these loci for practical breeding.

Progenies 2 and 5 did not reveal the same QTLs. This difference may be because actually different QTLs did segregate in these progenies. Alternatively, this difference may be related to the presence or absence of segregation of the locus near marker GP21 on chromosome 5 that can have epistatic effects. The presence on this locus of the allele that is associated with late blight resistance and late foliage maturity was shown previously to be required for the expression and, hence, identification of another QTL for resistance (Visker et al., 2003). In progeny 2 such an epistatic effect of the locus on chromosome 5 in combination with the skewed segregation may have hampered the detection of a QTL for resistance against *P. infestans* on chromosome 7, the presence of which is suggested by the identification of a QTL for adjusted relative AUDPC on this chromosome in parent I. The skewed segregation of GP21 on chromosome 5 in this progeny resulted in only a small number of genotypes with the allele associated with late blight resistance and late foliage maturity (Figure 1). If other QTLs for resistance were expressed only in this small group of genotypes, their detection is unlikely. In progeny 5 all genotypes inherited at least one allele associated with resistance to late blight and late

foliage maturity on the locus near marker GP21, because parent CE is homozygous for this allele (unpublished results). Consequently, expression and detection of other QTLs were not hampered by segregation of this locus.

The lengths of the genetic maps ranged from 456 cM for parent CE to 534 cM for parent I, which is rather short when compared to previous studies of different progenies that presented map lengths of 600–1100 cM (Isidore et al., 2003). This limited marker coverage of the genome is due to the relatively small number of markers that was generated and the predominant use of AFLP markers. These AFLP markers tend to cluster around the centromeres (Van Eck et al., 1995) and result in incomplete coverage of the chromosome telomeres. This limited genome coverage and the lack of assignment of one or two chromosomes in each of the maps impose limitations on the subsequent QTL analyses: QTLs in the parts of the genome that are not covered with genetic markers may not be detected. However, none of the non-linked markers had a significant effect on any of the evaluated traits. Because these non-linked markers probably belong to the non-covered parts of the genome, it is not likely that QTLs of large effects have been missed.

The QTLs that were identified in progenies 2 and 5 were verified in the other progenies of the half-diallel set of crosses. Presence of additional QTLs was not examined. Differences in genetic background do not seem to influence the detection of QTLs, because almost all QTLs that were identified in parents DH or I in progeny 2, or in parents SH or CE in progeny 5 were also found in the other two progenies of each of these parents. However, extrapolation of QTLs to other crosses must take into account differences in effects and percentages of variation accounted for (Table 6). Lack of confirmation of some QTLs was probably due to low significance (chromosome 7, parent I), and/or verification with markers that were not close enough to the QTL. The locus near marker GP21 on chromosome 5 did segregate in all four progenies without any epistatic effects, because significant interactions with other QTLs were not found.

The skewed segregation of marker GP21 in progenies 2, 3, and 6 was not caused by deliberate selection in any of the progenies at any stage during crossing or multiplication. Skewed segregation resulted from parents DH and I, and seems to depend on the specific combination of parents in each cross, because the segregation of marker GP21 was not skewed in all progenies of these parents. Skewed segregation of chromosome 5 of parent I was also found in a previous study (Collins et al., 1999), in which marker GP21 was associated not only with late blight resistance and foliage maturity type, but also with vigour. The skewed segregation of the locus on chromosome 5 is responsible for the distinct, unequal separation into two classes of foliage maturity type with correlating relative AUPDC values in progenies 3 and 6. In both progenies a small group of genotypes had foliage maturity type values that were similar to one parent (CE), whereas a large group had foliage maturity type values that were similar to the other parent (Visker et al., 2004). The skewed segregation of chromosome 5 always results in a relative shortage of late maturing, resistant genotypes. Apparently, these late maturing, resistant genotypes are less viable than early maturing, susceptible genotypes.

QTLs were identified for foliage resistance against *P. infestans* and for foliage maturity type. In accordance with previous results, the most important locus for both traits was found on chromosome 5 near marker GP21 in five out of six related diploid potato progenies: the allele of marker GP21 that is associated with resistance to late blight is also associated with late foliage maturity. The present study demonstrates the existence of additional QTLs for late blight resistance that are independent of foliage maturity type. These QTLs were found on chromosomes 3 and 10, and seem to be independent not only of foliage maturity type but also of the segregation of the locus on chromosome 5. Although the effects of the additional QTLs are small, early maturing genotypes that necessarily have the allele for susceptibility for late blight on chromosome 5 may benefit from the resistance that can be provided by these QTLs on chromosomes 3 and 10. But it is unlikely that the effects of these QTLs are sufficient to obtain satisfactory levels of late blight resistance in such early maturing genotypes, because such genotypes could not be identified in the phenotypic distributions of any of the six progenies that were studied. It is, therefore, important to further screen the *Solanum* gene pool for additional loci for resistance against *P. infestans* that are independent of foliage maturity type.

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