Tagging Quantitative Trait Loci for Maturity-Corrected Late Blight Resistance in Tetraploid Potato with PCR-Based Candidate Gene Markers

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Late blight caused by the oomycete *Phytophthora infestans* is the economically most important and destructive disease in potato cultivation. Quantitative resistance to late blight available in tetraploid cultivars is correlated with late maturity in temperate climates, which is an undesirable characteristic. A total of 30 DNA-based markers known to be linked to loci for pathogen resistance in diploid potato were selected and tested as polymerase chain reaction-based markers for linkage with quantitative trait loci (QTL) for late blight resistance and plant maturity in two half-sib families of tetraploid potatoes. Most markers originated from within or were physically closely linked to candidate genes for quantitative resistance factors. The families were repeatedly evaluated in the field for quantitative resistance to late blight and maturity. Resistance was corrected for the maturity effect. Nine of eleven different map segments tagged by the markers harbored QTL affecting maturity-corrected resistance. Interactions were found between unlinked resistance QTL, providing testable strategies for marker-assisted selection in tetraploid potato. Based on the linkage observed between QTL for resistance and plant maturity and based on the genetic interactions observed between candidate genes tagging resistance QTL, we discuss models for the molecular basis of quantitative resistance and maturity.

Additional keywords: late blight, marker-assisted selection (MAS).

The most important disease in potato cultivation worldwide is late blight, caused by the oomycete *Phytophthora infestans*. If not controlled, late blight epidemics can lead to complete yield loss. The first outbreak of late blight in the 19th century led to one of the most destructive famines in Europe (Salaman 1985). Since then, resistance to late blight has been an important character in variety development. At the beginning of the 20th century, single genes for race-specific resistance to late blight (*R* genes) were discovered in the wild hexaploid potato species *Solanum demissum*. The introgression of *R* genes from *S. demissum* into tetraploid potato cultivars resulted in nondurable resistance to late blight, because new races of *P. infestans* soon overcame this monogenic resistance (Ross 1986). Quantitative resistance to late blight is

mostly race-nonspecific, controlled by more than one gene and, therefore, more durable (Wastie 1991). Genotypes with high levels of field resistance to late blight often have other, undesirable agronomic characteristics. In particular, such cultivars usually are late maturing under the long-day conditions of middle and northern Europe (Wastie 1991). The well-known correlation between field resistance to late blight and late maturity can have two reasons. Either both characters are pleiotropic effects of the same gene or genes or the genes that control resistance and plant maturity are different but closely linked (Colon et al. 1995). To break the correlation between resistance and late maturity, resistance factors that do not affect maturity or are separated by recombination from the genes controlling maturity must be identified.

QTL (quantitative trait loci) for late blight resistance have been mapped in a number of experimental populations of diploid potatoes, using DNA-based markers (Collins et al. 1999; Ewing et al. 2000; Ghislain et al. 2001; Leonards-Schippers et al. 1994; Oberhagemann et al. 1999; Sandbrink et al. 2000; Trognitz et al. 2002; Visker et al. 2003) and in one tetraploid population (Meyer et al. 1998). In these experiments, QTL for late blight resistance were identified on most of the 12 potato chromosomes (equal to one complement). Based on common restriction fragment length polymorphism (RFLP) markers that anchor together the homologous linkage groups of different mapping populations, some QTL were located in the same map segments in different genetic backgrounds (Oberhagemann et al. 1999; Simko 2002). The most persistent and most prominent QTL for late blight resistance is located on chromosome V in a map segment tagged by RFLP markers GP21 and GP179 (Leonards-Schippers et al. 1994; Oberhagemann et al. 1999; Simko 2002). Linked to the same RFLP markers is a major QTL for plant maturity that is not separable from the QTL for late blight resistance by linkage analysis (Collins et al. 1999; Oberhagemann et al. 1999; Visker et al. 2003). Effects on plant maturity were also linked to some but not all QTL for late blight resistance detected on other chromosomes. QTL alleles that increase foliage resistance to late blight delay plant maturity and vice versa, thereby explaining the observed phenotypic correlation between resistance to late blight and late maturity (Collins et al. 1999; Oberhagemann et al. 1999; Visker et al. 2003).

The molecular basis of the QTL for late blight resistance and maturity is not known. It was proposed, based on the observation of close linkage between a major QTL for late blight resistance and the *R1* gene for race specific resistance to late blight on chromosome V, that the QTL for late blight resistance and *R1* are related at the molecular level (Leonards-

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Schippers et al. 1992, 1994). Recently, the *R1* gene was shown to be a member of the large family of plant resistance genes that have in common a putative nucleotide-binding (NB) domain and a leucine-rich-repeat (LRR) domain (Ballvora et al. 2002). Additional cases of colocalization of resistance QTL, *R* loci, and genes with sequence similarity to cloned plant *R* genes for pathogen resistance (resistance-gene-like [RGL] genes) were observed in the potato genome (Gebhardt and Valkonen 2001). Linkage between QTL for late blight resistance and pathogenesis-related (PR) genes has also been observed (Leonards-Schippers et al. 1994; Trognitz et al. 2002). Genes known or suspected to have a functional role in the expression of a phenotypic trait that colocalize with QTL for the same trait are considered as candidates for controlling the QTL (Pflieger et al. 2001). RGL and PR genes are therefore, besides unknown genes, known candidates for controlling quantitative resistance to late blight.

Here, we describe the tagging of QTL for late blight resistance and plant maturity in two tetraploid *S. tuberosum* breeding populations, based on genotyping with polymerase chain reaction (PCR)-based markers that were selected using the following criterion. The markers map to segments of the potato molecular genetic maps that have previously been shown in diploid mapping populations to contain factors controlling quantitative or qualitative resistance, or both, to pathogens.

Some of the markers originated from within or were physically tightly linked to RGL or PR candidate genes. Quantitative late blight resistance was evaluated in the field and was corrected for the influence of plant maturity, in order to identify QTL for late blight resistance having no effect on plant maturity. Models are discussed for the molecular basis of resistance QTL and for marker-assisted selection (MAS) of genotypes with increased resistance to late blight that is not compromised by late maturity.

RESULTS

Field evaluation of late blight resistance and plant maturity.

Based on field evaluation of plant maturity in 1999, the plants of the Escort and Nikita family were ordered into an early maturing group (maturity scores from 1 to 3) and a late maturing group (maturity scores from 4 to 8). To ensure similar infection pressure on plants at a similar developmental stage, infection of the late maturing group was started one to two weeks later than infection of the early maturing group. Both families were evaluated in the field for late blight resistance in years 2000 and 2001. The Nikita family was evaluated a third time in 2002. In 2002, the infection level of *P. infestans* was higher than in the previous years. AUDPC (area under dis-

Fig. 1. Regression curves of maturity (mean values over all test years) against **A** and **C**, area under disease progress curve (AUDPC) and **B** and **D**, maturity-corrected resistance (MCR) (mean values over all test years). Data of the Escort population are shown in **A** and **B**, and that of the Nikita population are shown in **C** and **D**.

ease progress curve) was strongly correlated ($P \leq 0.01$) with maturity in both families, with higher resistance (lower AUDPC values) being correlated with later maturity (Fig. 1A and C). Based on the means over all test years, the Escort family (Fig. 1A) was, on average, more resistant to late blight than the Nikita family (Fig. 1C). This was due to the higher infection level observed in the Nikita family in year 2002. The Escort family showed less variability for maturity (scores between 1.5 and 6.5) when compared with the Nikita family (scores between 1 and 8). Very late maturing genotypes (score 9) were absent from both families due to preselection applied against very late genotypes. Maturity-corrected resistance (MCR) was no longer correlated ($P > 0.200$) with maturity (Fig. 1B and D).

Analysis of variance of the average AUDPC and MCR data over two (Escort family) and three (Nikita family) years of testing resulted in significant genotype-by-genotype and genotype-by-year variation ($P \leq 0.01$), whereas error variance was not significant. The mean-based heritability (h^2) of AUDPC was 0.86 and 0.90 for the Escort and Nikita families, respectively. The heritability of MCR was lower, with $h^2 = 0.76$ for the Escort family and $h^2 = 0.87$ for the Nikita family.

Genotypic analysis.

PCR with 30 specific primer pairs (Table 1) resulted in clear amplicons in the two families. A total of 22 amplicons (73%) were polymorphic in members of the Escort and Nikita families, either directly (six sequence characterized amplified region [SCAR] or allele-specific oligonucleotide [ASO] markers) or after restriction enzyme digestion and single strand conforma-

tion polymorphism (SSCP) analysis (16 cleaved amplified polymorphic sequence [CAPS] or SSCP markers; Table 1). Three polymorphic SSCP markers (GP180, BA228D23t3, and BA1M23t3) were not scorable in the genotypes analyzed, due to insufficient fragment separation. The remaining 19 informative PCR-based markers (63%) generated 60 segregating fragments, some of which were redundant or highly correlated with each other ($>90\%$), indicating close linkage in coupling phase between them. Counting such highly correlated fragments as one allele reduced the number of different marker alleles to fifty. The 50 marker alleles tagged 11 different map segments, one each on potato chromosomes II to IX and on chromosome XII and two on chromosome XI (Fig. 2).

Segregation of the marker alleles was tested for goodness of fit to one of four genetic models using the χ^2 test. When the marker allele was present in both parents, the observed segregation ratio was tested for goodness of fit to the ratios 3:1 (simplex by simplex, presence vs. absence) or 11:1 (duplex by simplex). When the marker allele was present in only one parent, the models 1:1 (simplex by nulliplex) and 5:1 (duplex by nulliplex, presence vs. absence) were tested. Table 2 shows the results for the 40 marker alleles that had significant trait effects (Tables 3 to 7). Of the marker alleles, 70% fit one of the four genetic models tested. The segregation ratios of the remaining 30% were distorted.

PCR-based markers with effects on late blight resistance and maturity.

When testing each marker allele individually, 21 of 50 marker alleles (43%) tagging five and seven different map seg-

Table 1. Markers genotyped by polymerase chain reaction (PCR) in populations Escort $\times$ Leyla and Nikita $\times$ Leyla

LG	Marker	Primer sequence 5'→3', reference, accession number	T _a (°C)	PCR product size (bp)	Type of polymorphism
I	CP108	ttt agc tgc ata caa gat gc (f), ccc aag gcc tag gcc c (r)	52	450	— ³
II	GP23	Oberhagemann et al. 1999	52	600/1200	SSCP/MseI
III	GP25	Oberhagemann et al. 1999	52	600	CAPS/TaqI, SSCP/TaqI
IV	P450 (–SSCP2)	cat caa caa caa ctg tat ggg cta tgg (f), aaa gat cgt cct tac tag ccg cag (r), accession no. U48434	56	560	SSCP/AluI
V	GP180	Oberhagemann et al. 1999	55	400	SSCP/MseI ²
	STM3016	Milbourne et al. 1998	53	110/150	SCAR
	CosA	Gebhardt et al. 2003	55	210/250	SCAR
	R1	Ballvora et al. 2002	55	1400	ASO
	GP179	Meksem et al. 1995	55	500/570	SCAR
VI	BA121o1t7	cat aaa gtc ttg cag agt tct caa (f), gga ttg gga gca ccg ggt tgt (r)	55	233	SSCP
	P450 (–SSCP1)	Same as P450(–SSCP2) on LG IV			SSCP/AluI
	CP113	Niewöhner et al. 1995	45	900	—
VII	BA1m23t3	acc ctc cta ttt agt att gtt cag (f), gga aaa gat ata tag tgg ggt aca (r)	55	270	SSCP ²
	GP76	Oberhagemann et al. 1999	52	800	CAPS/RsaI
VIII	gro1-2-P40	agt ata aca ggg ata tat gct c (f), tag gca tcc att tgt cga aaa g (r)	58	500	ASO
	gro1-11-P41	acc tga cta atg aag aag aag c (f), tca aag cag tag cac cca aac (r)	58	600	—
IX	RC5t7	aga cca atg atg gag atg tat acg (f), cac acc tgg taa gga ttt aca tca (r)	56	650	—
	RC5t3	gac cgt gtg cac gcg gag gag (f), tga att cgc ata tta atg gtt agc (r)	56	900	CAPS/RsaI
	RC1t3	gct tca cca tga aac ggg tct aca t (f), cct ttc gac tta cat gac cat tac g (r)	58	800	—
	573	cac agc ttg ata ttg tgg ttc tgc a (f), tga atc ctg agc aag caa gtt cag (r)	56	500	—
	57t3	caa tcc gtt gga gtt tag acg atc (f), gaa gag gag aac ctt ggt gga tgg (r)	58	800	SSCP/AluI
XI	Prp1	Oberhagemann et al. 1999, Taylor et al. 1990	55	200/600	SCAR
XII	BA228d23t3	tcg tag gta aat ttt caa ggt cc (f), ctc ttc tcg gaa tga ttt aca ag (r)	54	90	SSCP ²
	GP125	Oberhagemann et al. 1999	57	1000	SSCP/MseI
	NL25	tat tgt taa tcg tta ctc cct c (f), aga gtc gtt tta ccg act cc (r)	60	200/1000	SSCP/MseI
	NL27	Marczewski et al. 2001	62	1200	SSCP/MseI
XII	683	tga tgg tgc tgg tag agg ttc ttg (f), aag acc gat tgc ctg aag cat tag (r)	58	600	SSCP/AluI
	E22	gat ggt agt ggc cga ggc aaa tg (f), tcc agc agg aca tgt aaa caa act (r)	56	400/600	—
	319	gga cac gaa cct aca att gca gat g (f), ggt aat gtc aaa agt ggc agc atg agt (r)	56	400	—
	GP34	Bendahmane et al. 1997	52	850	SSCP/MseI
	Rx1	ggg aga att ctc tgt ggc atg g (f), tat agc aag aat gag cat gca gg (r)	56	771	SSCP/MseI

³ PCR product was not polymorphic after restriction enzyme digestion and single strand conformation polymorphism (SSCP) analysis in either the Escort or in the Nikita family.

² SSCP were not scorable in Escort and Nikita families.

Fig. 2. Genomic positions of 30 polymerase chain reaction (PCR)-based markers and quantitative trait loci (QTL) for maturity, area under disease progress curve (AUDPC), and maturity-corrected resistance (MCR) detected in the tetraploid Escort and Nikita families. The 12 potato chromosomes are represented as schematic linkage groups with anchor restriction fragment length polymorphism and microsatellite loci at approximate positions on the left and the PCR markers tested on the right. PCR markers that did not segregate or were not scorable are shown in parentheses. Bars to the left and right of the linkage groups indicate a significant effect ($P \leq 0.05$) of PCR marker alleles in the map segment on maturity (white bars), AUDPC (grey bars), and MCR (black bars), separately or in combination, in families Nikita and Escort, respectively. Map segments where QTL for late blight resistance have been located previously in diploid mapping populations are indicated to the right by numbers in brackets. 1 = Leonards-Schippers et al. 1994, 2 = Oberhagemann et al. 1999, 3 = Collins et al. 1999, 4 = Ewing et al. 2000, 5 = Trognitz et al. 2002, and 6 = Visker et al. 2003. Positions of major genes for late blight resistance are shown on the left. *R1* = Leonards-Schippers et al. 1992, Ballvora et al. 2002; *R3*, *R6*, *R7* = El-Kharbotly et al. 1994, 1996; *R2* = Li et al. 1998; *R_{per}* = Ewing et al. 2000; *RB* = Naess et al. 2000, Song et al. 2003; and *Rpi1* = Kuhl et al. 2001. Single genes or major QTL for resistance to other pathogens that are located in the map segments tagged by the PCR markers are: nematode resistance genes *Gro1* (Barone et al. 1990), *H1* (Gebhardt et al. 1993; Pineda et al. 1993), *GroVI* (Jacobs et al. 1996), *Gpa2*, *Grp1*, *Gpa5* (Roupe van der Voort et al. 1997, 1998, 2000), *Gpa* (Kreike et al. 1994), *Gpa4* (Bradshaw et al. 1998), and *R_{Mcl}* (Brown et al. 1996); virus resistance genes *Rx1*, *Rx2* (Ritter et al. 1991; Bendahmane et al. 1999, 2000), *Nb* (De Jong et al. 1997), *Ry* (Brigneti et al. 1997; Hämmäläinen et al. 1997), *Na* (Hämmäläinen et al. 2000), and *PLRV.1* (Marczewski et al. 2001); and wart resistance gene *Sen1* (Hehl et al. 1999). Pathogenesis-related loci tagged by the PCR markers were *Prp1* (Leonards-Schippers et al. 1994), *Osm8*, *Osm11*, and *PR-5* (Castillo-Ruiz 2002; Trognitz et al. 2002).

ments in the Escort and Nikita families, respectively, were reproducibly linked to QTL, separately or in combination, for maturity, AUDPC, and MCR (Tables 3 and 4, Fig. 2). Seventeen marker alleles showed persistent effects in the same direction but of variable size over two or three years of testing, the average trait value over all years, or both. None of the marker alleles gave consistent results in both populations. However, markers tagging map segments V_p , IX_{sd} , and XI_{ld} showed effects in both families, although the significant marker alleles only partially overlapped and effects varied across populations and years. No major effects were detected when the markers were tested individually. Fewer significant marker alleles and smaller effects were found in the Escort family as compared with the Nikita family. The amount of the variance explained by a single marker allele ranged from 4.4 to 11.6% in the Escort family and from 4.1 to 17.4% in the Nikita family. Of 15 significant marker alleles in the Nikita family, 11 were linked to higher susceptibility, earlier maturity, or both. In the Escort family, five marker alleles were linked to higher susceptibility, earlier maturity, or both and six were linked to higher resistance, later maturity, or both. All three parents contributed positive (more resistant,

earlier maturing) and negative (more susceptible, later maturing) trait alleles. Interestingly, marker allele GP179-570 was linked to opposite effects in the two families. In Escort, GP179-570 was linked to higher susceptibility and in Nikita to higher resistance.

Effects on maturity were found in three of five map segments of the Escort family and in all seven map segments of the Nikita family. The largest and most consistent effects on maturity were observed in segments V_p and XI_{ld} of the Escort family (Table 3) and in segments V_p , XI_p , and XI_{ld} of the Nikita family (Table 4). In the Escort family, effects on AUDPC were detected only in segment V_p (Table 3), whereas in the Nikita family, effects on AUDPC were linked to markers in six segments, V_p , $VIII_{ld}$, IX_{sd} , XI_p , XI_{ld} , and XII_{ld} (Table 4). Marker alleles were linked to MCR in the Escort family in four segments, III_p , V_p , IX_{sd} , and XI_{ld} , and in all segments in the Nikita family except segment IV_{sd} . The most persistent effects on MCR (average trait values over two or three years) were associated with markers tagging segments V_p , IX_{sd} , and XI_{ld} in both families and, in addition, with markers tagging segments $VIII_{ld}$, XI_p , and XII_{ld} in the Nikita family. The largest effects on MCR were detected by marker

Table 2. Observed segregation, genetic model and origin of marker alleles, which detected effects on either late blight resistance, plant maturity, or both either individually, in interaction tests, or both

MS ^x	Marker allele	Escort × Leyla				Nikita × Leyla			
		Allele segregation ratio			Origin of allele	Allele segregation ratio			Origin of allele
		Present	Absent	Best model		Present	Absent	Best model	
II_{sd}	GP23-SSCP1	47	36	1:1	Leyla	86	9	11:1	Nikita, Leyla
	GP23-SSCP2	49	34	3:1 ^{distorted}	Escort, Leyla	62	33	3:1 ^{distorted}	Nikita, Leyla
	GP23-SSCP4	41	42	1:1	Leyla	49	46	1:1	Leyla
	GP23-SSCP5	65	18	5:1	Escort	95	0	— ^y	Nikita
III_p	GP25-SSCP1	70	14	5:1	Leyla	78	17	5:1	Leyla
	GP25-SSCP2	36	48	1:1 ^{distorted}	Escort	51	44	1:1	Nikita
	GP25-Taql-550	37	43	1:1	Escort	—	—	—	Absent
IV_{sd}	P450-SSCP2	64	20	3:1	Escort, Leyla	77	18	3:1	Nikita, Leyla
	STM3016-151	—	—	—	Absent	47	48	1:1	Nikita
V_p	CosA-210 ^z	71	13	5:1	Escort	34	61	1:1 ^{distorted}	Nikita
	R1-1400 ^z	73	12	5:1	Escort	33	62	1:1 ^{distorted}	Nikita
	GP179-570	35	49	1:1 ^{distorted}	Escort	42	53	1:1	Nikita
	BA121olt7-SSCP1	57	27	3:1	Escort, Leyla	83	12	3:1 ^{distorted}	Nikita, Leyla
	BA121olt7-SSCP2	70	13	3:1 ^{distorted}	Escort, Leyla	79	16	3:1 ^{distorted}	Nikita, Leyla
	BA121olt7-SSCP3	34	50	1:1 ^{distorted}	Leyla	47	48	3:1 ^{distorted}	Nikita, Leyla
	BA121olt7-SSCP4	72	11	11:1	Escort, Leyla	74	19	3:1	Nikita, Leyla
	BA121olt7-SSCP5b	41	43	1:1	Escort	—	—	—	Absent
	BA121olt7-SSCP6a	—	—	—	Absent	41	52	1:1	Nikita
	BA121olt7-SSCP9	60	23	1:1 ^{distorted}	Leyla	75	20	3:1	Nikita, Leyla
	P450-SSCP1	46	38	1:1	Escort	91	4	— ^y	Nikita
	GP76-Rsa-500	60	24	5:1 ^{distorted}	Escort	—	—	—	Absent
VI_{ld}	GP76-Rsa-600	68	16	3:1	Escort, Leyla	94	0	— ^y	Nikita, Leyla
	gro1-2-P40-500	—	—	—	Absent	75	20	5:1	Nikita
VII_p	57t3-SSCP	32	47	1:1 ^{distorted}	Escort	74	20	5:1	Nikita
	RC5t3-RsaI-280	—	—	—	Absent	51	44	1:1	Nikita
IX_{sd}	Prp1-200	41	43	1:1	Leyla	70	25	3:1	Nikita, Leyla
	683-SSCP1	37	41	1:1	Escort	49	42	1:1	Nikita
XI_p	683-SSCP2	69	9	5:1	Leyla	75	17	5:1	Leyla
	GP125-SSCP1	—	—	—	Absent	72	22	5:1 ^{distorted}	Nikita
XI_{ld}	GP125-SSCP2	80	2	— ^y	Escort, Leyla	51	43	3:1 ^{distorted}	Nikita, Leyla
	GP125-SSCP4	—	—	—	Absent	40	55	1:1	Nikita
	NL25-SSCP3	62	17	3:1	Escort, Leyla	69	24	3:1	Nikita, Leyla
	NL27-SSCP3	63	11	5:1	Leyla	79	14	5:1	Leyla
	NL27-SSCP4b	41	35	1:1	Leyla	17	76	1:1 ^{distorted}	Leyla
	NL27-SSCP5	—	—	—	Absent	45	48	1:1	Nikita
	NL27-SSCP7	36	41	1:1	Escort	72	21	5:1 ^{distorted}	Nikita
	GP34-SSCP2	46	35	1:1	Leyla	85	10	11:1	Nikita, Leyla
XII_{ld}	Rx1-SSCP1	95	0	— ^y	Escort, Leyla	43	41	1:1	Leyla
	Rx1-SSCP2	—	—	—	Absent	32	52	1:1 ^{distorted}	Nikita

^x The map segment (MS) tagged by the markers is indicated by chromosome number and suffixes p, ld, and sd for proximal or distal position on the long and short chromosome arm, respectively (Dong et al. 2000).

^y No genetic model applicable.

^z Marker fragments CosA-210 and R1-1400 are linked in coupling phase.

Prp1-200 (IX_{sd}) in the Escort family and by markers R1-1400 (V_p), GP125-SSCP1 (XI_{ld}), and GP34-SSCP2 (XII_{ld}) in the Nikita family.

All marker alleles were tested for pair-wise interactions, using as trait the all year average of MCR. Of 470 interaction tests in the Escort family, eight (1.7%) were significant at $P \leq 0.01$, including two intralocus interactions (Table 5). Half of those interactions are expected to be chance effects. One interaction test was significant at $P \leq 0.001$ (Table 5). In the Nikita family, 22 of 579 interaction tests (3.8%) were significant at $P \leq 0.01$, with five to six of those expected by chance. Eight interaction tests were significant at $P \leq 0.001$ (Table 6). No intralocus interaction was found in the Nikita family. Testing for interaction identified two additional map segments that were not significant in the single marker tests. These were map segments II_{sd}, tagged by GP23 alleles in the Escort family (Table 5), and map segment VII_p, tagged by the marker gro1-

2-P40 in the Nikita family (Table 6). Markers tagging map segment V_p in both families interacted with unlinked loci on chromosomes III, IV, VII, VIII, IX, XI, and XII. The marker allele gro1-2-P40-500 (VII_p) interacted with five other loci. Single interlocus interactions explained between 9 and 24% of the phenotypic variance. Genotypes having favorable allele combinations (negative MCR class means) were less frequent than genotypes with unfavorable allele combinations (Tables 5 and 6).

Multiple regression analysis with forward selection was performed with all marker alleles for the all-year average of MCR in families Escort and Nikita (Table 7). With exception of GP179-570, the selected marker alleles differed between the families. For the Nikita family, five marker alleles tagging segments V_p, VIII_{ld}, XI_p, and XII_{ld} were selected that together explained 38% of the phenotypic variance. Selection for presence of the marker alleles in segment V_p and absence of the

Table 3. Effects of single marker alleles on maturity, AUDPC (area under disease progress curve), and MCR (maturity-corrected resistance) in population Escort × Leyla. The phenotypic variance explained by the marker (R^2) is shown as percentage

MS ^w	Marker allele	Origin of allele	Maturity				AUDPC			MCR		
			1999	2000	2001	1999-2001	2000	2001	2000-2001	2000	2001	2000-2001
III _p	GP25-SSCP1 ^x	Leyla	— ^y	—	—	—	—	—	—	—	7.5**	—
V _p	CosA-210 ^x	Escort	—	—	—	—	—	7.5**	5.6*	—	4.9*	4.4*
	GP179-570 ^z	Escort	—	—	—	—	5.5*	4.7*	6.5*	—	4.9*	5.7*
	BA121o1t7-SSCP3 ^x	Leyla	—	7.4**	7.2**	7.6**	—	6.8*	—	—	—	—
	BA121o1t7-SSCP5b ^z	Escort	5.3*	6.6*	—	5.1*	7.8**	5.9*	8.8**	—	4.6*	5.2*
	P450-SSCP1 ^x	Escort	7.6**	8.1**	7.2**	9.8**	5.7*	6.8*	7.6**	—	—	—
VI _{ld}	GP76-Rsa-600 ^x	Escort, Leyla	4.8*	—	5.4*	5.7*	—	—	—	—	—	—
IX _{sd}	Prp1-200 ^z	Leyla	—	—	—	—	—	—	—	6.0*	—	7.7**
XI _{ld}	NL25-SSCP3 ^x	Escort, Leyla	—	6.9*	11.6**	7.8**	—	—	—	—	—	—
	NL27-SSCP3 ^z	Leyla	—	—	5.9*	—	—	—	—	—	7.8*	—
	NL27-SSCP4b ^z	Leyla	—	—	—	—	—	—	—	5.2*	—	5.4*

^wThe map segment (MS) tagged by the markers is indicated by chromosome number and suffixes p, ld, and sd for proximal or distal position on the long and short chromosome arm, respectively (Dong et al. 2000).

^x Marker allele present = plant is more resistant and/or later maturing.

^y — indicates $P > 0.05$, * indicates $P \leq 0.05$, and ** indicates $P \leq 0.01$.

^z Marker allele present = plant is more susceptible and/or earlier maturing.

Table 4. Effects of single marker alleles on maturity, AUDPC (area under disease progress curve), and MCR (maturity-corrected resistance) in population Nikita × Leyla. The phenotypic variance explained by the marker (R^2) is shown as percentage.

MS ^w	Marker allele	Origin of allele	Maturity					AUDPC				MCR			
			1999	2000	2001	2002	1999-2002	2000	2001	2002	2000-2002	2000	2001	2002	2000-2002
IV _{sd}	P450-SSCP2 ^x	Nikita, Leyla	4.2*	— ^y	—	—	4.9*	—	—	—	—	—	—	—	—
V _p	R1-1400 ^z	Nikita	—	—	10.9§	17.4§§	7.7**	—	9.1**	7.5*	7.7*	—	11.9§	8.0**	8.7**
	GP179-570 ^z	Nikita	—	—	—	—	—	4.8*	4.2*	—	4.0*	4.4*	—	—	—
	BA121o1t7-SSCP3 ^z	Nikita, Leyla	4.6*	—	—	—	—	—	—	—	—	—	—	—	—
	BA121o1t7-SSCP4 ^x	Nikita, Leyla	4.6*	4.4*	5.8*	5.8*	7.4**	—	—	—	—	—	—	—	—
VIII _{ld}	57T3-SSCP ^x	Nikita	—	—	—	4.9*	—	—	4.2*	5.5*	4.7*	—	—	6.0*	5.5*
IX _{sd}	Prp1-200 ^x	Nikita, Leyla	—	—	4.5*	6.5**	4.1*	—	5.5*	5.6*	4.4*	—	8.7**	6.9**	5.6*
XI _p	683-SSCP1 ^x	Nikita	—	8.0**	7.0**	5.6*	8.6**	4.7*	—	5.4*	6.4*	—	—	—	—
	683-SSCP2 ^x	Leyla	—	—	—	—	—	—	—	—	—	—	6.4*	—	4.1*
XI _{ld}	GP125-SSCP1 ^x	Nikita	—	—	8.3**	12.5§	8.6**	5.4*	8.5**	8.6**	9.8**	—	8.3**	8.5**	8.3**
	GP125-SSCP2 ^z	Nikita, Leyla	—	—	7.5**	5.4*	—	—	—	4.2*	—	—	—	4.1*	—
	NL25-SSCP3 ^x	Nikita, Leyla	—	—	—	—	—	—	4.3*	—	—	—	—	—	—
	NL27-SSCP3 ^x	Leyla	—	—	—	—	—	—	6.4**	—	—	—	4.8*	—	—
	NL27-SSCP5 ^x	Nikita	—	—	8.1**	4.5*	6.2*	4.1*	4.5*	5.8*	6.5**	—	—	4.2*	—
XII _{ld}	GP34-SSCP2 ^x	Nikita, Leyla	—	—	—	4.3*	—	—	4.5*	6.6**	4.5*	—	12.2§	10.1**	10.4**

^wThe map segment (MS) tagged by the markers is indicated by chromosome number and suffixes p, ld, and sd for proximal or distal position on the long and short chromosome arm, respectively (Dong et al. 2000).

^x Marker allele present = plant is more susceptible, earlier maturing, or both.

^y — indicates $P > 0.05$, * indicates $P \leq 0.05$, ** indicates $P \leq 0.01$, § indicates $P \leq 0.001$, and §§ indicates $P \leq 0.0001$.

^z Marker allele present = plant is more resistant, later maturing, or both.

marker alleles in the other segments should improve resistance. Taking into account the segregation ratio of each marker allele (Table 2) and assuming independent segregation of each marker allele, the expected frequency of genotypes that combine the positive alleles of the five markers suggested by the model was 0.0008 or eight in 10,000 plants. Only two markers

in segments V_p and XI_{ld} were selected in the Escort family that together explained 15% of the phenotypic variance. Selection against the GP179-570 and NL27-SSCP4b marker alleles should increase resistance. The expected frequency of genotypes lacking both the GP179-570 and NL27-SSCP4b allele was 0.27 or 27 of 100 plants.

Table 5. Interactions ($P \leq 0.01$) between pairs of marker alleles in population Escort $\times$ Leyla for the two-year average of MCR (maturity-corrected resistance). The phenotypic variance explained by the marker (R^2) is shown as percentage.

				Marker class ^w									
				00		02		10		12			
MS 1 ^x	Marker allele 1	MS 2	Marker allele 2	Mean ^y	n	Mean	n	Mean	n	Mean	n	R ^{2z}	
II _{sd}	GP23-SSCP1	V _p	P450-SSCP1	13.3 a	17	−8.2 b	19	4.1 ab	21	8.0 a	26	13.1**	
	GP23-SSCP1	VI _{ld}	GP76-RsaI-500	−19.0 a	7	7.0 b	29	11.9 b	17	3.1 b	30	14.0***	
	GP23-SSCP2	VI _{ld}	GP76-RsaI-500	−16.4 a	6	7.1 b	28	9.3 b	18	3.1 ab	31	9.2**	
	GP23-SSCP4	II _{sd}	GP23-SSCP5	−14.6 a	7	7.8 b	35	10.2 b	11	2.7 ab	30	9.4**	
V _p	BA121o1t7-SSCP1	IX _{sd}	Prp1-210	13.9 a	14	7.5 ab	13	−7.4 b	30	10.1 a	28	17.5**	
	BA121o1t7-SSCP1	XI _{ld}	NL25-SSCP3	30.4 a	6	4.6 b	19	−0.8 b	11	2.6 b	43	13.6**	
	BA121o1t7-SSCP9	III _p	GP25-TaqI-550	18.8 a	12	−3.2 b	11	0.6 b	31	3.3 ab	24	11.9**	
	BA121o1t7-SSCP9	IX _{sd}	Prp1-210	14.8 a	11	4.9 ab	12	−6.3 b	31	11.8 a	29	17.5**	
	BA121o1t7-SSCP2	V _p	BA121o1t7-SSCP4	−5.1 a	4	22.0 b	9	12.3 ab	7	0.6 a	62	12.9**	

^w Mean MCR and number of individuals n that have neither of the two marker alleles (00), only marker allele 2 (02), only marker allele 1 (10), or both marker alleles (12). Negative means indicate increased resistance.

^x The map segment (MS) tagged by the markers is indicated by chromosome number and suffixes p, ld, and sd for proximal or distal position on the long and short arm, respectively (Dong et al. 2000).

^y Groups of individuals labeled with different small letters (a or b) are different ($P \leq 0.05$) from each other.

^z ** indicates $P \leq 0.01$, *** indicates $P \leq 0.001$.

Table 6. Interactions ($P < 0.01$) between pairs of marker alleles in population Nikita $\times$ Leyla for the three-year average of MCR (maturity-corrected resistance). The phenotypic variance explained by the marker (R^2) is shown as percentage.

MS 1 ^x	Marker allele 1	MS 2	Marker allele 2	Marker class ^w								R ^{2z}
				00		02		10		12		
				Mean ^y	n	Mean	n	Mean	n	Mean	n	
III _p	GP25-SSCP2	V _p	BA121o1t7-SSCP2	12.3 b	5	1.1 ab	39	−9.9 a	11	10.7 b	40	10.2**
	GP25-SSCP2	XI _{ld}	GP125-SSCP2	0.8 ab	21	3.8 ab	23	16.8 a	22	−1.6 b	28	10.5**
IV _{sd}	STM3016-151	V _p	BA121o1t7-SSCP3	−10.3 a	19	8.1 b	29	10.3 b	29	4.7 ab	18	12.3**
	STM3016-151	VII _p	gro1-2-P40-500	−10.3 a	14	5.4 ab	34	21.9 b	6	6.2 ab	41	11.3**
	STM3016-151	XI _{ld}	GP125-SSCP1	−22.9 a	12	9.2 b	35	12.6 b	10	7.0 b	37	24.0§§
	STM3016-151	XI _{ld}	GP125-SSCP4	−8.6 a	27	12.9 b	21	10.4 b	28	4.8 ab	19	15.9**
V _p	R1-1400	IV _{sd}	STM3016-151	9.0 a	32	9.2 a	30	−15.6 b	16	6.4 ab	17	17.6 **
	R1-1400	III _p	GP25-SSCP1	25.0 a	11	5.6 b	51	−14.2 b	6	−2.1 b	27	17.9 **
	R1-1400	VII _p	gro1-2-P40-500	16.2 a	12	7.4 a	50	−25.9 b	8	2.6 a	25	21.5***
	R1-1400	VIII _{ld}	RC5T3-RsaI-280	11.5 a	28	7.2 a	34	−14.7 b	16	5.5 a	17	16.9**
	BA121o1t7-SSCP2	IX _{sd}	Prp1-210	−19.7 a	8	13.8 b	8	3.2 b	17	6.7 b	62	12.8**
	BA121o1t7-SSCP2	XI _{ld}	GP125-SSCP1	−38.1 a	4	10.1 b	11	0.1 b	18	7.7 b	61	19.2**
	BA121o1t7-SSCP2	XI _{ld}	GP125-SSCP4	−19.3 a	9	18.2 b	7	5.1 b	46	7.1 b	33	15.0**
	BA121o1t7-SSCP2	XI _{ld}	NL27-SSCP4b	9.8 a	9	−19.4 b	7	4.1 a	67	17.8 a	10	13.7***
	BA121o1t7-SSCP2	XI _{ld}	NL27-SSCP7	−15.8 a	7	7.0 ab	9	13.6 b	14	4.1 ab	63	9.3**
	BA121o1t7-SSCP2	XII _{ld}	GP34-SSCP2	−45.5 a	4	11.1 b	12	3.8 b	6	6.1 b	73	24.0***
VII _p	BA121o1t7-SSCP6a	VIII _{ld}	RC5T3-RsaI-280	−3.7 a	24	13.7 b	28	9.5 ab	18	−3.2 a	21	13.0***
	gro1-2-P40-500	V _p	BA121o1t7-SSCP3	−20.9 a	10	19.5 b	10	8.2 b	38	3.3 b	37	20.8§§
	gro1-2-P40-500	IV _{sd}	P450-SSCP2	−24.9 a	4	5.4 b	16	10.5 b	14	4.7 b	61	8.9**
	gro1-2-P40-500	VIII _{ld}	57T3-SSCP	−30.8 a	5	11.6 b	14	3.5 b	15	6.4 b	60	16.3**
	gro1-2-P40-500	IX _{sd}	Prp1-210	−22.7 a	8	14.0 b	12	4.6 b	17	6.2 b	58	16.0**
	gro1-2-P40-500	XI _p	683-SSCP1	−14.4 a	10	13.1 b	10	5.8 b	32	5.3 b	39	10.1**
	gro1-2-P40-500	XI _{ld}	GP125-SSCP1	−20.2 a	10	18.9 b	10	4.4 b	12	6.3 b	62	18.0**
	gro1-2-P40-500	XI _{ld}	NL27-SSCP5	−13.9 a	12	19.3 b	8	5.0 b	36	6.4 b	37	13.5**
	Prp1-210	VIII _{ld}	RC5T3-RsaI-280	−17.1 a	14	12.4 b	11	10.8 b	30	5.0 b	40	18.9***
	Prp1-210	XI _{ld}	NL27-SSCP4b	1.7 ab	14	−16.2 a	9	5.4 b	62	23.5 b	8	15.6**
IX _{sd}	Prp1-210	XI _{ld}	NL27-SSCP7	−21.0 a	6	0.3 b	17	13.8 b	15	5.8 b	55	12.6**
	GP34-SSCP2	XI _p	683-SSCP2	—	0	−20.2 a	9	−5.2 a	17	9.8 b	66	20.5§§
	Rx1rl1-SSCP1	V _p	BA121o1t7-SSCP4	−22.5 a	8	8.5 b	33	7.6 ab	8	6.5 b	33	15.0**
XII _{ld}	Rx1rl1-SSCP2	XI _{ld}	GP125-SSCP1	−11.0 a	11	10.9 b	41	8.9 ab	8	2.8 ab	23	13.2**

^w The map segment (MS) tagged by the markers is indicated by chromosome number and suffixes p, ld, and sd for proximal or distal position on the long and short chromosome arm, respectively (Dong et al. 2000).

^x Mean MCR and number of individuals (n) that have neither of the two marker alleles (00), only marker allele 2 (02), only marker allele 1 (10), or both marker alleles (12). Negative means indicate increased resistance.

^y Groups of individuals labeled with different lower case letters (a or b) are different ($P \leq 0.05$) from each other.

^z ** indicates $P \leq 0.01$, *** indicates $P \leq 0.001$, and §§ indicates $P \leq 0.0001$.

DISCUSSION

Tagging QTL for late blight resistance in tetraploid potato by targeted genotyping.

Based on linkage maps constructed with RFLP markers for the 24 chromosomes of diploid potato (Gebhardt et al. 1991, 2003; Leonards-Schippers et al. 1994; Tanksley et al. 1992), the genetic length of one complement of the potato genome (12 chromosomes) is in the order of 1,000 cM. The genetic length of a map that fully covers the eight complements (96 linkage groups) of a cross between heterozygous tetraploid genotypes should then be approximately 8,000 cM. With the liberal assumption that each marker allele can detect effects of QTL located up to 10 cM in distal or proximal direction, a minimum of 400 regularly spaced, polymorphic and single-dose markers are required for a genome-wide, fully informative QTL analysis in a tetraploid mapping population. In the two half-sib families derived from crossing the tetraploid cvs. Escort and Nikita with cv. Leyla, 50 nonrandom marker alleles were tested for linkage with QTL, some having simplex and others having duplex allele dosage. The polymorphic markers tagged 11 map segments and only some of the eight homologues per segment that segregated in the F1 families. When testing 50 marker alleles at random, the probability of tagging one QTL with any of the 50 markers is under the favorable circumstances outlined above, 50/400 or 0.12. The number of QTL segregating in the tetraploid populations is not known. In diploid populations with genome-wide map coverage, no more than 15 QTL for late blight resistance have been detected (Leonards-Schippers et al. 1994; Oberhagemann et al. 1999). With the assumption that 30 QTL segregate in a tetraploid population, three to four of 50 random markers (0.12×30) are expected to detect QTL effects. In the experiment reported, 40 of 50 marker alleles (Table 2), either individually or in pairwise interactions, detected QTL in six and eight map segments of the Escort and Nikita families, respectively. This is far more than expected when using random markers. Targeted genotyping based on 50 marker alleles identified a similar number of QTL as found with a random approach using 337 amplification fragment length polymorphism markers in a different tetraploid cross, which covered approximately 25% of the genome (Meyer et al. 1998). The criterion for marker selection, which was the position of the markers in map segments previously shown to harbor genes for either quantitative or qualitative resistance, or both, to pathogens in diploid potato, was therefore effective.

However, targeted genotyping identified only part of the genetic variation for resistance to late blight and maturity present in the two families. Marker alleles tagging QTL for maturity-corrected resistance explained up to 38% of the phenotypic

variance in the Nikita family but only up to 15% in the Escort family. The observed differences can result from genetic differences between the late blight resistant seed parents, the smaller population size of the Escort family when compared to the Nikita family, or both. Escort might be more homozygous than Nikita at the loci tagged by the markers. This is indicated by the smaller number of segregating marker alleles descending from cv. Escort. Alternatively, most resistance QTL segregating in the Escort family are located in map segments that were not tagged by the tested markers.

QTL for plant maturity and resistance to late blight.

The experiment described in this paper is the first in which QTL for both quantitative resistance to late blight and plant maturity were jointly analyzed in tetraploid breeding materials. After correcting the AUDPC values for maturity, heritable variation for resistance was still detected, indicating that, in the potato genome, factors for resistance to late blight exist that can be separated from the maturity effect. This result is in agreement with a QTL analysis of the same traits in a diploid, unrelated potato population (Visker et al. 2003).

Most map segments having QTL for resistance also harbored QTL for maturity. Why are several, unlinked QTL for resistance to late blight repeatedly linked to QTL for plant maturity? In model one, the genes controlling resistance and maturity are physically linked but structurally and functionally unrelated. The conserved linkage among them results from duplications of the corresponding genomic segments during the evolution of the species. Based on current knowledge on segmental genome duplications in potato (Gebhardt et al. 2003), there is no evidence for duplications that include the reported map segments having both QTL for late blight resistance and maturity. In model two, a large number of unrelated genes control late blight resistance and maturity, which are evenly distributed over the whole potato genome, such that linkage among the QTL is observed by chance alone. This model is not supported by the current knowledge on localization and distribution of QTL for resistance to late blight and maturity on the genetic maps of potato (Collins et al. 1999; Gebhardt and Valkonen 2001; Oberhagemann et al. 1999; Visker et al. 2003). In model three, the effects on resistance to late blight and plant maturity are controlled by pleiotropic alleles of the same gene or by alleles of related, physically linked genes that evolved from common ancestors by local gene duplication and subsequent functional diversification. This model (Collins et al. 1999; Oberhagemann et al. 1999) provides a plausible explanation for the observed linkage between QTL for late blight resistance and maturity and for the variability of QTL effects in the Escort and Nikita families. Marker alleles linked in coupling phase to QTL alleles having

Table 7. Multiple regression analysis with forward selection for MCR (maturity-corrected resistance, mean over years) in populations Escort $\times$ Leyla and Nikita $\times$ Leyla. Phenotypic variance explained by the marker allele (R^2) is shown as percentage

MS ^v	Marker allele	Origin of allele	Escort $\times$ Leyla	Nikita $\times$ Leyla
V _p	R1-1400 ^w	Escort, Nikita	—	7.2***
	GP179-570 ^y	Escort, Nikita	7.8*	3.8*
VIII _{ld}	57T3-SSCP ^z	Escort, Nikita	—	4.9*
XI _p	683-SSCP2 ^z	Leyla	—	7.9**
XI _{ld}	NL27-SSCP4b ^z	Leyla	7.7*	—
XII _{ld}	GP34-SSCP2 ^z	Nikita, Leyla	—	14.3***
			$\Sigma = 15.5$	$\Sigma = 38.1$

^v The map segment (MS) tagged by the markers is indicated by chromosome number and suffixes p, ld, and sd for proximal or distal position on the long and short chromosome arm, respectively (Dong et al. 2000).

^w Marker allele present = plant is more resistant.

^x * indicates $P \leq 0.05$, ** indicates $P \leq 0.01$, and *** indicates $P \leq 0.001$.

^y Presence of the GP179-570 allele increases susceptibility in the Escort family and resistance in the Nikita family.

^z Marker allele present = plant is more susceptible.

a pleiotropic effect on late blight resistance and maturity should show an effect on maturity and AUDPC but not on maturity-corrected resistance. This was indeed observed for few marker alleles, most clearly for P450-SSCP1 in segment V_p of the Escort family and 683-SSCP1 in segment XI_p of the Nikita family. Other marker alleles tagged the same map segments but different subsets of the eight homologous chromosomes. This is indicated by the lack of correlation between marker alleles tagging the same map segment and by the different genetic models that fitted marker segregation (Table 2). Some of these marker alleles showed effects on maturity only, others only on resistance, but most markers detected effects on all three traits. This suggests the presence of multiple QTL alleles with variable trait effects in these map segments. Multiple QTL alleles have been shown for a major QTL controlling tuber shape in a cross between diploid, heterozygous potatoes (Van Eck et al. 1994).

Models for the molecular basis of quantitative resistance to late blight.

R genes and QTL for resistance to various pathogens have been mapped previously to regions IV_{sd}, V_p, VII_p, XI_{id}, and XII_{id} (Fig. 2) (Gebhardt and Valkonen 2001). The same regions of the potato genome contain clustered families of NB-LRR-type genes, some of which have been characterized as functional *R* genes (Ballvora et al. 2002; Bendahmane et al. 1999, 2000; Hehl et al. 1999; Rickert et al. 2003; Paal et al. 2004; Van der Vossen et al. 2000; Vidal et al. 2002). The markers used for tagging the segments were either derived from NB-LRR-type genes or were closely linked to such genes. In particular, the highly significant marker R1-1400 detects specifically the *R1* gene for late blight resistance, which is a member of a clustered family of NB-LRR-type genes on potato chromosome V (Ballvora et al. 2002). This suggests that the *R1* gene family, other NB-LRR-type genes, or both are good candidates for the molecular basis of the resistance QTL in these regions of the potato genome. This model does not rule out the possibility that physically linked but unrelated genes are causal for the observed QTL effects on resistance.

Interlocus interactions were found between marker alleles linked to the NB-LRR-type gene clusters (Tables 5 and 6). Particularly striking were the interactions observed between markers tagging the *R1* locus for late blight resistance on chromosome V and seven other loci on different chromosomes, among others the *Gro1* nematode resistance locus on chromosome VII (Paal et al. 2004). *Gro1* occupies a similar position as the *Rpi1* locus for resistance to late blight derived from the wild potato species *S. pinnatisectum* (Kuhl et al. 2001). If NB-LRR-type gene families are causal for resistance QTL, the observed phenotypic interactions among the loci may indicate physical interaction between two or more NB-LRR-type proteins. Specificity and kinetics of pathogen recognition, signal transduction, and defense response could be determined quantitatively by the subunit composition of such recognition complexes. The qualitative, hypersensitive resistance response would be nothing else than an extremely fast reaction triggered by a recognition complex including an *R* gene (Robertson 1985). Recognition complexes have been proposed to explain the failure of finding in vitro a specific interaction between a single *R* gene having a LRR domain and the corresponding avirulence gene product (McDowell and Woffenden 2003). No interaction between pairs of NB-LRR-type plant resistance genes has been identified so far at the molecular level.

Further candidates for participating in the control of quantitative resistance to late blight are genes functional in the de-

fense response (Leonards-Schippers et al. 1994; Trognitz et al. 2002), such as glutathione S-transferase (Hahn and Strittmatter 1994; Martini et al. 1993; Taylor et al. 1990), encoded at the *Prp1* locus in map segment IX_{sd}, genes for phenylalanine ammonia-lyase (Hahlbrock and Scheel 1989), mapping to the same region as *Prp1* (Gebhardt et al. 1991), and osmotin-like genes located in map segments VIII_{id} and XI_p (Castillo-Ruiz 2002; Liu et al. 1994; Zhu et al. 1995). Markers tagging map segments with PR genes interacted with each other and with map segments having NB-LRR-type genes. Pathogen recognition and defense responses are connected by networks of signal transduction pathways (McDowell and Woffenden 2003). Allelic variation in one part of the network can have indirect, differential effects on the allelic variation affecting other parts, thereby providing a molecular model for the interaction observed between unlinked resistance QTL.

MAS for late blight resistance in tetraploid potato.

Potato breeding in temperate Europe aims at the selection of cultivars with high levels of resistance to late blight that is not compromised by late maturity. The results of our study allow researchers to design testable MAS strategies towards this goal but also show some limitations. Multiple regression and interaction analysis of QTL for maturity-corrected resistance showed that this trait may be improved by MAS in progeny of cvs. Escort, Nikita, and Leyla. In fact, a first MAS experiment based on three unlinked markers tested in hybrids of Escort × Leyla resulted in a group of plant individuals that were significantly more resistant to late blight when having the three favorable marker alleles, compared with a control group that had the unfavorable alleles of the same markers. However, the field resistance observed in these plants in a single season could not yet be corrected for the maturity effect (data not shown). Selection will have to be applied mainly against susceptibility alleles rather than in favor of resistance alleles. The only marker alleles consistently linked to increased MCR were CosA-210 and R1-1400, which both tag a resistance “hot spot” in map segment V_p. Both marker alleles are associated with resistance to late blight in a large collection of potato varieties (Gebhardt et al. 2004). Presence of most other marker alleles was linked to higher susceptibility. This is particularly evident from the interactions between marker alleles, where the most resistant genotype class (negative MCR means) was the one that lacked both marker alleles (00 genotype class). Due to prevalence of susceptibility alleles, the frequency of genotypes combining more than two favorable alleles is very low in the Escort and Nikita families. Large numbers of progeny are needed to find them, but this can now be approached based on the DNA-based markers reported in this study.

With the exception of marker GP179, the marker alleles chosen for MAS of MCR by multiple regression and forward selection were different for the two half-sib families. Marker GP179 is linked to the *R1* locus and to several resistance QTL (Ballvora et al. 2002; Oberhagemann et al. 1999; Rouppe van der Voort et al. 1998, 2000). The marker allele GP179-570, descending from Escort, was, however, linked to increased susceptibility, whereas the same allele when descending from Nikita was linked to increased resistance. This is due either to a crossing over between the resistance QTL and the marker allele present in both parental genotypes or to nonidentity of the 570-base-pair fragment in the two genotypes. The predictive value of this marker is therefore limited to crosses with cvs. Escort and Nikita. Association studies, as performed for CosA-210 and R1-1400, are required for the marker alleles tagging the other map segments, in order to assess their general predictive value for MAS.

MATERIALS AND METHODS

Plant material.

Two tetraploid *S. tuberosum* F1 families were analyzed, which segregated for quantitative resistance to late blight and did not show a hypersensitive resistance phenotype against the *P. infestans* isolates used for field inoculations. They are referred to as the Escort and Nikita family and originated from crossing cv. Leyla as pollen parent with cvs. Escort and Nikita as seed parents. Leyla is a susceptible cultivar, whereas Escort and Nikita both have good field resistance to late blight. Escort contains the race-specific genes *R1*, *R2*, *R3*, and *R10*. Nikita has at least *R1*, based on presence of an *R1*-specific PCR marker (Table 2). A single tuber per genotype was harvested from pot-grown seedlings in 1998 and was propagated in the field in the subsequent years under the same phytosanitary measures as seed tubers. Individuals maturing very late or having a very poor growth habit were eliminated from the families before the field evaluations for late blight resistance, leaving 84 and 95 genotypes for the Escort and Nikita families, respectively.

Field assessment of late blight and maturity.

Field experiments were located at Windeby in northern Germany. The Escort and Nikita families were planted in a randomized block design with two replications and four plants per block. Between each block, an infector row with a semi-susceptible, medium early cultivar (cvs. Secura and Granola in 2000, cv. Satina in 2001 and 2002) was planted (distance between the blocks, 80 cm; distance between the rows, 75 cm). After tuber planting (7 to 9 weeks), every second plant in the infector row was inoculated with a sporangial suspension of a complex isolate of *P. infestans* that was able to overcome the *R1* to *R11* late blight resistance genes (provided by B. Schöber-Butin, Biologische Bundesanstalt, Braunschweig, Germany). Inoculum was sprayed on the adaxial surface of the leaf, and the leaf was covered overnight with a plastic bag to keep it humid. To maintain continuous infection pressure throughout the experiment, infector rows separating blocks with later maturing genotypes were infected two weeks after the first inoculation. *P. infestans* spread from the inoculated plants into the evaluation plots, simulating a natural infection. From 2 weeks after the first inoculation onwards, nine assessments were made in 3- or 4-day intervals, using a 1 to 9 scale (1 = 0% infection, 9 > 90%) (Cruickshank et al. 1982). Average scores per year were calculated from the replications. Late blight resistance was evaluated in years 2000, 2001 (both families), and 2002 (Nikita family only).

The maturity of uninfected plants grown for multiplication was evaluated two to three times within one growing season, using a 1 to 9 scale (1 = very early and 9 = very late maturity). The plants were compared with standard potato varieties with known maturity type. Maturity evaluations were carried out in years 1999, 2000, 2001 (both families), and 2002 (Nikita family only).

Isolation of total genomic DNA.

Freeze-dried leaf tissue (25 mg) was ground to fine powder. Genomic DNA was extracted with the DNeasy plant mini kit (Qiagen, Hilden, Germany), according to the supplier's instructions. Peeled, freeze-dried tubers (0.7 g) were ground to fine powder and were extracted with 20 ml of 100 mM Tris-HCl, pH 9.5, 1.4 M NaCl, 20 mM EDTA, 2% (wt/vol) CTAB, 1% (wt/vol) polyethylenglycerol 6000, and 0.25% (vol/vol) 2-mercaptoethanol, at 74°C for 20 min. The suspension was mixed with 1 volume of chloroform/isoamyl alcohol (24:1 vol/vol) and was centrifuged. The upper aqueous phase was adjusted to pH

7.0 with 1 M HCl and was treated with 10 µg DNase-free RNase per ml. The DNA was purified on Qiagen Tip 100 columns (Qiagen) according to the supplier's instructions.

PCR-based marker analysis.

The Escort and Nikita families were genotyped with 30 PCR-based markers located on all potato linkage groups except linkage group X (Table 1, Fig. 2). Markers R1 (AF447489), Rx1 (AJ011801), BA121o1t7 (Ballvora et al. 2002), gro1-2-P40 (AY196153), NL25 (AJ009719), and NL27 (AJ009720) were derived from NB-LRR-type genes. Markers STM3016, P450, CosA, GP179, GP125, and GP34 are closely linked to NB-LRR-type gene families. Markers RC5t7, RC5t3, RC1t3, 573, 57t3, and 683 tagged osmotin-like PR loci, and markers E22 and 319 tagged a PR-5 homologous locus (Castillo Ruiz 2002). Marker Prp1 originates from a PR glutathione S-transferase gene (Taylor et al. 1990). Primer information is shown in Table 1. PCR was carried out with the following conditions: 50 ng genomic DNA was amplified in a total volume of 25 µl of 20 mM Tris-HCl, pH 8.0, 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM each of dATP, dCTP, dGTP, and dTTP (Carl Roth & Co. KG, Karlsruhe, Germany), 0.3 µM of each primer, and 0.03 U of *Taq* DNA polymerase (Invitrogen, Life Technologies, Karlsruhe, Germany) per µl. The reaction conditions were as follows: 1× at 93°C for 2 min, 40× denaturation at 93°C for 45 s, annealing at *T_a* (specified in Table 1) for 45 s, extension at 72°C for 1 min 30 s, 1× at 72°C for 10 min. PCR products of different length (SCAR markers) or allele specific PCR products (ASO markers) were separated on standard agarose gels or MetaPhor agarose (microsatellite marker STM3016) and were stained with ethidium bromide. Homomorphic PCR products were digested with 4-cutter restriction enzymes (Table 1), were separated on standard agarose gels, and were stained with ethidium bromide (CAPS markers) or were analyzed for SSCP (Sanguinetti et al. 1994; Slabaugh et al. 1997).

For SSCP analysis, PCR products were digested with the restriction enzymes *Mse*I (New England Biolabs GmbH, Frankfurt, Germany), *Alu*I, or *Taq*I (Roche Diagnostics GmbH, Mannheim, Germany) (Table 1). Digests (4 µl) were added to 8 µl of denaturing solution (97% deionized formamid, 0.01 M NaOH, 0.05% xylene cyanol, 0.05% bromophenol blue), heated to 94°C for 4 min and put immediately on ice. Samples (6 µl) were run on 0.25× MDE gels (BMA BioWhittaker Molecular Applications, Rockland, ME, U.S.A.) in 53.4 mM Tris-borate, 1.2 mM EDTA buffer, pH 8.0 (0.6× Tris-borate-EDTA), and 5% glycerol. Gel polymerization was initiated with 0.06% N, N, N', N'-tetramethylethylenediamine and 0.05% ammoniumperoxodisulfate (Merck, Darmstadt, Germany). Gels were run at 1.5 W for 17 h at room temperature. Silver staining was performed as follows: 3 min in fixation solution (10% ethanol and 5% acetic acid), 5 min in staining solution (0.2% (wt/vol) AgNO₃, 10% ethanol, and 5% acetic acid), 2 min in deionized water, 20 min in developing solution (0.75 M NaOH, 0.27% formaldehyde), 5 min in fixation solution, and rinsed with deionized water.

Mapping of SSCP fragments.

The segregation of SSCP fragments P450-SSCP1 and P450-SSCP2 (Table 1) was scored as presence or absence in the diploid mapping population F1840 (Gebhardt et al. 2003). The fragments were mapped relative to the existing RFLP marker database, using the software package MAPRF (Ritter et al. 1990).

Data analysis.

Marker alleles (SCAR, ASO, CAPS, or SSCP fragments) were scored as present or absent. Assuming monogenic inheri-

tance of a single fragment and presence of the fragment being dominant (*A*) over absence (*a*), four allelic states are possible in tetraploid potato: homozygous (AAAA) or heterozygous AAAa (triplex/simplex), AAaa (duplex/duplex), or Aaaa (simplex/triplex). Based on the model of tetrasomic inheritance and absence of double reduction (chromatide segregation), the Mendelian ratios expected in a cross between a heterozygous parent having *A* and a homozygous parent lacking *A* (aaaa) are 1:0 (presence versus absence) for an AAAa parent, 5:1 for an AAaa parent, and 1:1 for an Aaaa parent. When two heterozygous plants, both having *A*, are crossed, the segregation ratios expected are 1:0 when one or both parents are AAAA or AAAa, 35:1 when both parents are AAaa, 11:1 when one parent is AAAa and the other Aaaa, and 3:1 when both parents are Aaaa. The observed segregation ratio of each marker allele was tested for goodness of fit to one of these models by a χ^2 test. The test model was accepted at $P > 0.200$.

The relative AUDPC was calculated from the field assessments, according to Jeger and Viljanen-Rollinson (2001). Missing values were estimated with an analysis of variance using the software PLABSTAT version 2P from July 14, 2001 (copyright by F. H. Utz, University of Hohenheim, Stuttgart, Germany). A regression curve of AUDPC against maturity was computed (Fig. 1) using the statistic software SAS, release 8.02 (SAS Institute Inc., Cary, NC, U.S.A.). For each AUDPC measurement, the vertical distance to the regression curve was then calculated, which resulted in negative values for more resistant plants and positive values for more susceptible plants. The phenotypic data were tested for normal distribution, using the software SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA). AUDPC values for years 2001 and 2002 and the mean over all years in the Nikita population were normal distributed after x^4 transformation. Maturity values were normal distributed only for the mean values over all years but not in the single years.

To test for significant ($P \leq 0.05$) effects of single marker alleles on maturity, AUDPC, or MCR, a *t*-test was performed in the cases of normal distributed data. Otherwise, a Kruskal-Wallis test was used. Statistical analysis was omitted when one of the two genotype classes (fragment present or absent) had less than 10 individuals, in order to avoid false positive significance due to the large size difference between the classes. Markers having small effects (P values between 0.01 and 0.05) were included in Tables 3 and 4 only when the effect was repeatedly observed in two or three test years, in a single year and in the average trait value over all test years, or in both mapping populations. Interactions were analyzed by two-way ANOVA between pairs of marker alleles. The interaction test was not further considered when genotype classes consisted of one to three individuals only. The H_0 hypothesis of no interaction was rejected at $P \leq 0.01$.

All marker alleles were used in a multiple regression analysis with forward selection at the $P \leq 0.05$ significance level (SAS FORWARD). In each calculation step, the program chooses the independent variable (marker allele) that has the largest significant effect on the dependent variable (phenotypic data) and keeps this variable in the regression model. This marker selection is repeated until no independent variable has a new significant effect ($P \leq 0.05$) on the dependent variable.

The χ^2 test, *t*-test, two-way ANOVA, Kruskal-Wallis test, and the multiple regression were computed with the statistic software SAS, release 8.02 (SAS Institute Inc.) (Dufner 1992).

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