

Tagging QTLs for late blight resistance and plant maturity from diploid wild relatives in a cultivated potato (*Solanum tuberosum*) background

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Abstract *Phytophthora infestans* causes an economically important disease of potato called late blight. The epidemic is controlled chemically but resistant potatoes can become an environment-friendly and financially justified alternative solution. The use of diploid *Solanum tuberosum* derived from European tetraploid cultivars enabled the introgression of novel genes encoding foliage resistance and tuber resistance from other species into the modern cultivated potato gene pool. This study evaluated the resistance of the obtained hybrids, its quality, expression in leaflets and tubers and its relation to the length of vegetation period. We also identified genetic loci involved in late blight resistance and the length of vegetation period. A family of 156 individuals segregating for resistance to late blight was assessed by three laboratory methods: detached leaflet, tuber slice and whole tuber test, repeatedly over 5 years. Length of vegetation period was estimated by a field test over 2 years. The phenotypic distributions of all traits were close to normal. Using sequence-specific PCR markers of known chromosomal position on the potato genetic map, six quantitative trait loci (QTLs) for resistance

and length of vegetation period were identified. The most significant and robust QTL were located on chromosomes III (explaining 17.3% of variance observed in whole tuber tests), IV (15.5% of variance observed in slice tests), X (15.6% of variance observed in leaflet tests) and V (19.9% of variance observed in length of vegetation period). Genetic characterization of these novel resistance sources can be valuable for potato breeders and the knowledge that the most prominent QTLs for resistance and vegetation period length do not overlap in this material is promising with respect to breeding early potatoes resistant to *P. infestans*.

Keywords Introgression · Foliage resistance · *Phytophthora infestans* · Tuber resistance

Introduction

Late blight of potato and tomato is caused by the oomycete *Phytophthora infestans* (Mont.) de Bary. This economically very important disease attacks both foliage and tubers of potato and demands constant chemical protection of the crop, which is expensive as well as harmful for the environment. Potatoes resistant to late blight can be an alternative solution to chemical control. However, resistance of commercial value should be highly effective, durable and not linked with unwanted traits such as long vegetative period. So far, this has not been found within the gene pool of cultivated potato, where a linkage between the resistance and a long vegetation period is well documented (Świeżyński 1990). Most of the late blight resistance present within potato cultivars originates from *Solanum demissum*, and their lateness used to be attributed to the linkage drag (Umaerus and Umaerus 1994). Both R genes and QTLs for late blight resistance have been introduced from *S. demissum*,

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and although theoretically the *R* genes are considered as easier to separate from long vegetation period than QTL, there are still examples of linkage between the *R* genes and maturity type. The best-known example is the *R1* gene, which is located within the QTL for vegetation period length on chromosome V. Moreover, it was shown in two independent studies (Gebhardt et al. 2004, Beketova et al. 2006) that cultivars possessing *R1* show significantly longer vegetation period than the control group of cultivars. According to Świeżyński (1990), long vegetation period and horizontal resistance to late blight are manifestations of the same physiological phenomenon. This thesis is supported by Allefs et al. (2005) postulating that late blight resistance is always either “maturity related” or “*R* gene based”. It is still unclear whether the relationship between long vegetation period and late blight resistance results from genuine genetic linkage between functionally unrelated genes or from the pleiotropic effect of a gene influencing both traits (Visker et al. 2003, 2005). The most prominent quantitative trait locus (QTL) for plant maturity has been identified on potato chromosome V, which overlaps with QTL for late blight resistance (Collins et al. 1999; Oberhagemann et al. 1999; Bormann et al. 2004; Bradshaw et al. 2004; Visker et al. 2003, 2005).

Although *R* gene-based resistance to *P. infestans* proved to be non-durable (Niederhauser et al. 1954; Malcolmson 1969, Rudkiewicz 1985), field resistance is believed to be more stable and therefore it seems to be more useful for breeders (Landeo et al. 1995). However, different breeding strategies are in use, among them the search for new, more durable *R* genes (van der Vossen et al. 2005), building *R* gene pyramids and combining *R* gene-based and horizontal resistances, which is well supported by the hypotheses that those types of resistance are closely related or even inseparable.

Another issue in breeding potatoes resistant to late blight is tuber resistance that becomes even more important in organic farming where unprotected and blighted foliage is an inoculum source for the tubers. Only some genotypes of potato are resistant to *P. infestans* both in foliage and in tubers, some are resistant in foliage and susceptible in tubers and a few behave in the opposite way (Świeżyński and Zimnoch-Guzowska 2001). Most probably these resistance genes which are constitutively expressed provide the resistance of all plant organs simultaneously; however, some of the resistance genes are expressed either in specific tissue or plant development phase only. Stewart et al. (1994) tested several potato progenies and noted a strong correlation between tuber resistance to *P. infestans* and foliage resistance. Mapping of these two resistance components indicated the overlap of QTLs for foliage and tuber resistance with the most important QTL for plant maturity located on chromosome V (Collins et al. 1999; Oberhagemann et al.

1999; Bradshaw et al. 2004). The effect of *R* genes in tubers has been investigated for the genes *R1*, *R2* and *R3* from *S. demissum* showing the efficacy of *R1* both in foliage and in tubers in contrast to *R2* and *R3*, which have been efficient only in foliage (Roer and Toxopeus 1961). Recently, the functionality of *R1* both in foliage and in tubers, and foliage specificity of *R3a* have been confirmed, and for another investigated *R* gene *Rpi-abpt* foliage specificity has been shown (Park et al. 2005). A similar study was performed for *Rpi-phul* of *S. phureja*, which was shown to be highly effective both in foliage and in tubers (Śliwka et al. 2006). Tuber resistance assessment is very important while introducing a novel source of resistance to *P. infestans* into a breeding program because the relation of potato tuber and foliage resistance is a genotype-dependent trait.

In the present study two wild diploid species of potato, series *Tuberosa*, *S. verrucosum* Schlecht. and *S. microdontum* Bitt. were exploited as main sources of leaflet resistance to late blight, whereas sources of tuber resistance remained unidentified. The origin of resistance was defined on the basis of pedigree resistance analysis which is shown in ESM S1. Both species have long been described as resistant to *P. infestans* (Rudkiewicz 1985; Hawkes 1990). *S. microdontum* is believed to be a source of horizontal resistance, because it comes from regions where *P. infestans* immigrated only in twentieth century, which suggests a possible lack of *R* gene evolution (Sandbrink et al. 2000). Inheritance of late blight resistance originating from *S. microdontum* has been investigated, as well as the chromosomal localization of the underlying QTLs and three major QTLs for foliage resistance have been identified on chromosomes IV, V and X (Sandbrink et al. 2000). A major QTL for late blight resistance, explaining more than 60% of phenotypic variance has been also identified in another study on *S. microdontum* (Bisognin et al. 2005). Late blight resistance of *S. verrucosum* has not been characterized that precisely so far, although it was used in diploid potato breeding (Świeżyński et al. 1991). The goal of this study was to assess new resistance to *P. infestans* in terms of its expression in foliage and tubers, its effectiveness in five consecutive years, its relation to plant maturity and its inheritance including the identification of regions in the potato genome linked with the resistance. A number of studies mapping late blight resistance and maturity in potato are presently available and genome regions important for these traits as well as sequence-specific PCR markers covering these regions are defined and published. We aimed to apply such chosen markers to tag the important chromosome regions and to check if they also play a significant role in our material. This should enable us to answer the question if our material introduces new quality of resistance without producing a dense genetic linkage map.

Materials and methods

Plant material

The diploid potato mapping population 98-21 ($2n = 2x = 24$) was obtained by crossing the clones DG 83-1520 (P1) and DG 84-195 (P2). Both parental clones contributed to resistance of the progeny and both were complex interspecific hybrids. Detailed pedigrees are shown in ESM S1. Based on the pedigree, the maternal clone DG 83–1520 theoretically consisted of 37.6% *S. tuberosum* (several dH *tbr*), 28.1% *S. chacoense* (GLKS 66.51/6/6; PK 133), 12.5% *S. verrucosum* (PI 195170), 12.5% *S. microdontum* (PI 265575), 6.2% *S. gourlayi* (INTA. 7356) and 3.1% *S. yungasense* (GLKS 67.107/3R), whereas the paternal clone DG 84–195 theoretically consisted of 81.3% *S. tuberosum* (several dH *tbr*), 15.6% *S. chacoense* (GLKS 66.51/6/6; CPC 3785) and 3.1% *S. yungasense* (GLKS 67.107/3R). The accessions of *S. verrucosum* and *S. microdontum* were used in IHAR's breeding programs as donors of resistance to *P. infestans*; thus we can assume that they are main sources of resistance, especially foliage resistance in mapping population 98-21. The accessions of *S. chacoense*, *S. gourlayi* and *S. yungasense* were used in these programs as sources of high starch content or resistance to potato viruses but not of late blight resistance. Tuber resistance was inherited from DG 84-195 and it might have originated from one of the wild species contributing to this clone but we cannot exclude that higher resistance of this clone in tuber tests resulted from favorable gene combination. Besides in the pedigree of DG 84-195, there are two cultivars of *S. tuberosum* possessing the *R1* gene from *S. demissum*: Apta and Aquila (<http://www.eucablight.org>); however, this gene should not be effective against contemporary Polish *P. infestans* isolates.

The unselected population consisted of 156 individuals. Four tetraploid Polish cultivars were used as resistance standards: Irys (susceptible in foliage and in tubers), Sokół (susceptible in foliage and moderately resistant in tubers), Bzura (resistant in foliage and susceptible in tubers) and Meduza (resistant in leaflets and moderately resistant in tubers). Additionally, we used two diploid potato hybrids DG 94-15 with resistant foliage and DG 94-668 with resistant tubers (Śliwka et al. 2006).

P. infestans isolate

The isolate MP 324 from pathogen collection of the Plant Breeding and Acclimatization Institute was applied to all resistance tests. The isolate collected in 1997 in Poland was of A1 mating type, highly aggressive, metalaxyl resistant and of complex race (1.2.3.4.5.6.7.8.10.11). Its virulence was confirmed on 11 differentials, each possessing a single *R* gene (*R1–R11*) from *S. demissum* (Black's differential set

obtained from Scottish Agricultural Science Agency, Edinburgh, UK), each time parallel to detached leaflet tests. Before each resistance test, the isolate was multiplied at least twice on susceptible potato tissue.

Late blight resistance assessment

Three laboratory methods of late blight resistance assessment were applied: detached leaflet test, tuber slice test and whole tuber test, performed according to Zarzycka (2001a, b). The parental clones and standard cultivars were tested together with the population. The assessment of resistance in population 98-21 was done in five consecutive years, 1999–2003. Three or five leaflets, slices or tubers per genotype were tested in two replications on one or two dates each year. A droplet of 50 sporangia/μl suspension, prepared as described by Zarzycka (2001a), was used as an inoculum in leaflet and slice tests. Lateral leaflets were collected from the middle parts of plants and placed on plastic trays on the wet wood wool, upside down and trays were covered with glass. After 24 h inoculation, the leaflets were turned over. Double slices, each 10 mm thick, were cut out from the middle part of the tuber, inoculated between the two slices and placed in glass-covered plastic trays. In the whole tuber test, the rose ends of tubers were wounded with 16 pins distributed over an area of $12 \times 12 \text{ mm}^2$ and ca 2 mm deep into the tuber flesh. Whole tubers were sprayed with inoculum. After 6 days (leaflets and slices) or 14 days (whole tubers) of incubation in conditions supportive for disease development (high relative humidity, 16°C and in case of leaflets—constant light of about 1,600 lx), the resistance was scored in a 1–9 scale, where 9 was the most resistant.

Length of vegetation period assessment

Vegetation period length of the population 98-21, the parental clones and standard cultivars, was evaluated in the field in 2003 and 2004. In 2003, clones were planted on 28th of April and harvested on 18th of September, while in 2004 planting was on 6th of May and harvesting was on 23rd of September. Plots of each genotype (seven plants of each genotype) were evaluated once a week for senescent plants, starting from the beginning of August (49 days to harvest in 2003 and 54 days to harvest in 2004). The vegetation period of the plants that died before harvest were evaluated directly in days from planting. The plants, which were still alive at the end of season, were assessed on a 1–5 scale, where 5 indicated a green, flowering plant, and 1 a completely dry plant. The scale was converted into days of vegetation period by adding 7 days for each grade above 1, which simulated vegetation prolonged beyond the harvest date (Ratuszniak and Komorowska-Jędrzyś 1995).

DNA isolation, PCR, polymorphism of amplicons

Genomic DNA was extracted from 1 g fresh, young leaves of greenhouse-grown plants with the DNeasy Plant Maxi kit (Qiagen, Hilden, Germany). All 56 sequence-specific DNA markers were amplified under conditions described by Rickert et al. (2003). Information on type of PCR-markers, primer sequences, annealing temperatures and restriction enzymes used for CAPS (cleaved amplified polymorphic sequences) markers are summarized in Table 1. The amplified DNA fragments were separated on agarose gels stained with ethidium bromide or by SSCP (single strand conformational polymorphism) analysis (Plomion et al. 1999; Slabaugh et al. 1997; Bormann et al. 2004). For SSCP, 4 μ l of restriction digest was mixed with 8 μ l of denaturing solution (97% deionized formamide, 0.01 M NaOH, 0.05% xylene cyanol, 0.05% bromophenol blue), denatured by heating to 94°C for 4 min and put immediately on ice. Samples were then separated on 0.25 \times mutation detection enhancement (MDE) gels (BMA Bio Whittaker Molecular Applications, Rockland, ME, USA) in 53.4 mM Tris–borate, 1.2 EDTA buffer, pH 8.0 and 5% glycerol with addition of 0.06% N, N, N', N'-tetramethylethylenediamine (TEMED) and 0.05% ammonium persulfate. Gels were run at 1.5 W for approximately 17 h at room temperature and silver stained (Bormann et al. 2004).

Statistical and linkage analyses

The results of the assessments of late blight resistance and vegetation period length were analyzed using analysis of variance, which enabled estimation of the influence of the genotype, date, year and their interactions, the date was nested within a year. Mean resistance was calculated from scores of 3–5 leaflets, slices or tubers per replication and that was used as a single data point in ANOVA shown in ESM S2. Fitness to the normal distribution of the phenotypic data was checked by the Kolmogorov–Smirnov test. Data sets deviating from normal distribution were transformed (x^2 , \sqrt{x} , arcsin x). The relationship between components of resistance and length of vegetation period was evaluated by linear Pearson's correlation coefficients based on means over all the years. Marker-trait linkages were estimated by the Student's t test. Only those marker/trait linkages with $P < 0.001$ for at least 1 year were considered significant.

Fitness of allele segregation to the expected 1:1 ratio was checked by the χ^2 test. All statistical analyses were performed using computer programs SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA), GENSTAT (International Ltd: Hemel Hempstead, UK) and STATISTICA for Windows (Stat Soft, Inc., Tulsa, OK, USA). Marker alleles were scored as present or absent and assembled into linkage

groups using the software package MAPRF (E. Ritter, NEIKER, 01080, Vitoria, Spain). In the output of MAPRF, there is a LOD for every pairwise linkage. In our set of marker data, recombination frequencies up to 30% = 0.3 have LOD values >3 (the likelihood of linkage is 1,000 times larger than the H_0 hypothesis of no linkage). In other words, linkages up to 0.3 can be accepted for being real. Linkages larger than 0.3 were taken as putative: LOD < 3 . The principles, on which MAPRF is built, are described by Ritter et al. (1990).

Results

Late blight resistance and vegetation period length assessments

Parent DG 83-1520 (P1) was more resistant than the standard potato cultivars in leaflet tests (mean score 7.1), although it was susceptible in slice and whole tuber tests. The diploid clone DG 94-15 was the most resistant in leaflet tests (mean score 8.0). Parent DG 84-195 (P2) was more resistant than all standards in whole tuber tests (mean score 7.1), but in slice tests cv. Meduza (mean score 6.1), diploid clones DG 94-668 (mean score 7.5) and DG 94-15 (mean score 7.5) were better than P2 (mean score 5.3). P2 was susceptible in leaflet tests. Both parental clones were late maturing, with a mean length of vegetation period longer than 140 days.

Distributions of the 5-year mean (1999–2003) results of leaflet, slice and tuber tests (Fig. 1), as well as distributions of results from single years (not shown), did not deviate significantly from normality, with the exception of the leaflet test in 2000 and the slice test in 2002. These data were transformed to obtain normal distributions. In leaflet and slice tests, the mean grades in the population 98-21 ranged from 1.0–9.0, whereas in the whole tuber tests the range was narrowed to 2.2–9.0. The distribution of mean results of vegetation period length deviated significantly from normality (Fig. 2). Analysis of variance showed significant effects of genotype, date and year of testing and interactions between these factors. Genotype had the largest influence on resistance, explaining 37.0% of the variance in leaflet tests, 54.0% in slice tests and 25.2% in whole tuber tests. The effect of the year of testing was strongest in tuber tests (20.3% of variance explained), slightly weaker in leaflet tests (18.7%) and weakest in slice tests (5.0%). Genotype \times year interactions explained additional 16.8, 24.2 and 19.2% of the variance in leaflet, whole tuber and slice tests, respectively. For details on ANOVA see ESM S2. The data sets of vegetation period length from 2003 and 2004 were significantly correlated ($P < 0.001$, $r = 0.562$). Significant correlations were also found between mean leaflet and slice resistance ($P < 0.001$, $r = 0.480$), slice and

Table 1 Sequence-specific DNA markers scored in population 98-21

Linkage group	Marker	Reference or primer sequence 5'→3'	T_a (°C)	PCR product size (bp)	Type of polymorphism ^a	Restriction enzyme	
I	BA114i24 t3	F: ctt ctt ttg aac aac gca aac, R: cta aca caa tgt act tgc agg tg	56	400	SCAR	–	
	BA157f6 t3	F: aat cat gtt gtt tgt cca aga cc, R: tgc att aag ggt tag ata cc	56	400	SSCP	<i>MseI</i>	
	CP132	F: gag tta gcc ttg cag tgt c, R: ctt ctg act tca tca gga tc	56	250	SSCP	–	
	GP205	F: gta tat gaa aac tgc cta agc tag, R: gct tca atg atc tgt tta aat att cc	56	300	SSCP	<i>MseI</i>	
	GP22	F: acg gga tga agc cga atc c, R: att gac agt aga tet taa gcc c	55	1,500	SCAR	–	
	GP23	Oberhagemann et al. (1999)	52	600	SSCP	<i>MseI</i>	
	GP26	F: aca aga cta gac cga atg cg, R: gtc aag cct tgc agc tgt g	56	600	SSCP	<i>MseI</i>	
	GP321	F: gca caa aac aca cca aca cca, R: tca cct tgt tat ctc cta tgc	56	800	SSCP	<i>MseI</i>	
	GP86 ^a	F: tgc tgc tgc tga tac tca g, R: gat atc agt gat gcc tca tc	56	700	SSCP	<i>MseI</i>	
	SUDND1	Pajerowska et al. (2005)	63.5	900	SSCP	<i>AluI</i>	
II	StPAD4	Pajerowska et al. (2005)	62	600	SSCP	<i>MseI</i>	
	BA202k7 t3 ^a	F: gaa att tgg tcc ctc tat atc cga a, R: cca egt ttc tta taa gtc gtc gta	56	700	SCAR	–	
	GP25 ^a	F: tta tag act att ggc cc, R: gtt gat atc ctt cca ctt g	55	600	SCAR	–	
	GPI ^a	F: gtc tgc tat cgg tcc gat c, R: tgc aat agg gct caa gcc g	58	700	SSCP	<i>MseI</i>	
	GP295 ^a	F: ata tgg ttt aag gct cgg att ag, R: agc gga cag tct gca tat ttc	56	600	SSCP	<i>MseI</i>	
	GPI80	Oberhagemann et al. (1999)	55	400	CAPS	<i>MseI, AluI</i>	
	BA106c14 t7 ^a	Paal et al. (2004)	56	500	SSCP	<i>MseI</i>	
	MBF3 ^a	Oberhagemann et al. (1999)	52	2,000	CAPS	<i>HinfI</i>	
	BA1m23 t3 ^a	Bormann et al. (2004)	56	500	SSCP	<i>MseI</i>	
	C17	F: tac cct acc aat acc gac, R: gct tgt tct gtt cgt act g	56	1,000	SSCP	<i>MseI</i>	
V	CP113	Niewöhner et al. (1995)	47.4	900	ASA	–	
	BA47f2 t7 ^a	A: aca aca cga cga ttt gct atc ag, B: ttc aag atg gtc atg tga tga gg	56	600	CAPS	<i>MseI, AluI</i>	
	GP186	F: tcc tta cat ceg cac tta cg, R: gca gct gga tag tca acg c	53	1,000	CAPS	<i>TaqI</i>	
	StNPR1 ^a	Pajerowska et al. (2005)	63.5	1450	CAPS	<i>HpaII</i>	
	GP78	F: gca tat cac caa cga ttg ct, R: cag gag gct caa aac ttt ca	57	1,000	CAPS	<i>TaqI</i>	
	GP76	Oberhagemann et al. (1999)	52	800	SSCP	<i>HinfI</i>	
	CP18 ^a	F: gca atg gct caa aca atg ttg, R: acc aat cat agc aac acg gc	56	400	SSCP	<i>MseI</i>	
	StSGT1	Pajerowska et al. (2005)	62	1,250	CAPS	<i>TaqI</i>	
	GP219 ^a	F: tgc ctg gtt cac taa gag ctg, R: tga atg act gag aac tat tga g	55	1,000	CAPS	<i>RsaI</i>	
	CP51	F: ccc tga ata ega gtt gct g, R: gaa cta aca cat cct ccc tc	59	800	SCAR	–	
VII	Gro1-2-P40	Bormann et al. (2004)	58	500	ASA	–	
	BA9i23 t7	F: gag aac gtc ctg ctt tgc caa c, R: tca aga gta tgg gta tag tat ttg	56	300	SSCP	<i>MseI</i>	
	CP52	F: tct aaa ggt gtc aaa ggt gc, R: cga gag gca gat gtt gat g	56	300	SSCP	<i>MseI</i>	
	57i3 ^a	Bormann et al. (2004)	58	800	ASA	–	
	VIII						

Table 1 continued

Linkage group	Marker	Reference or primer sequence 5' → 3'	T _a (°C)	PCR product size (bp)	Type of polymorphism ^a	Restriction enzyme
IX	GP94	F: atg tat cac aat cac att ctt gct c, R: tgt aaa acc aac aag tag tgt tgc	56	300	SSCP	–
	B7	F: ggg cct agt tca atg tac, R: agt tgc tat ggt tgt tcc	54	600	SSCP	<i>MseI</i>
	GP267	F: cca cca aga caa gcc tag, R: cag ttg tga ata gga tgc	56	550	SSCP	<i>DpnII, MseI</i>
	GP39	Marczewski et al. (2006)	51	950	SSCP	<i>MseI</i>
	CP44	F: ggt ggg acc tga gaa gac aag c, R: att acc aga tgc aga gat gct ca	56	300	SSCP	<i>MseI</i>
	GPI73b	F: tgg aag aac atg agc atc ag, R: agt tgg tat tgc tcc tct tg	53	550	SSCP	<i>MseI</i>
	BA113a17 t3	F: ctt tca ctg etc aat gat ttg cag, R: tgc aat gag tat ttg tet ttg cag a	56	300	SSCP	<i>MseI</i>
	TG63 ^a	F: ggt ttc cca atg tgt gac, R: cgg att tac cta gag tgc	56	400	SCAR	–
	BA66k2 t3	F: ctt cat tca tac tat atg ttc aat gg, R: ttc aat ceg agc tct tgg aat tg	56	300	SSCP	–
	CP72 ^a	F: caa gaa acc tca aat gat caa g, R: tca tca gta aag att tgg aac c	60	500	SSCP	<i>AluI</i>
XI	GP287	F: tca ttc cca aga cac tca tgc, R: act caa cca cca tat acc acc	62	700	SSCP	<i>MseI</i>
	TPT ^a	F: cct tct ctc tca ctg cca atg, R: ctc acc aag caa tat acc acc	56	1100	SSCP	<i>MseI</i>
	BA228d23 t3	F: gtg atc tgg aat ctt tga cat ctg, R: aaa agc ttt taa tat ttg ggc cgc	56	500	SSCP	<i>HinfI</i>
	BA88b3 t7	F: ctt cag gat aca agc ttt caa tct g, R: att ttg aga agt tga aca tcc etc a	56	300	SCAR	–
	GPI25	Oberhagemann et al. (1999)	57	1,000	SCAR	–
	GP38	Marczewski et al. (2006)	56	700	SSCP	<i>MseI</i>
	GP34	Bendahmane et al. (1997)	52	850	CAPS	<i>DdeI, HinfI</i>
	BA31n11 t3	F: att aca tgc tgc tac att cca cg, R: atg ctt gta agc ttg tga gta aca	56	300	CAPS	<i>RsaI</i>
	E22	Bormann et al. (2004)	56	600	CAPS	<i>TaqI</i>
	GP229	F: agt ctt ggc atc aaa tat ttg, R: att atc ttc aca gca gta gag	56	600	SSCP	<i>MseI</i>
III?	S1d11 ^a	F: gtt gta att tct tea gca aaa tta gc, R: ttc acg aat egg act aaa aga ttg	50	1,000	SSCP	<i>MseI</i>
	P1g7 ^a	F: ggt gcg tta ggt ggt gat g, R: agt ctc caa caa cat cgt cc	56	500	SSCP	–

SSCP single strand conformation polymorphism, CAPS cleaved amplified polymorphic sequence, SCAR sequence characterized amplified region, ASA allele specific amplification

^a Distorted segregation ratio

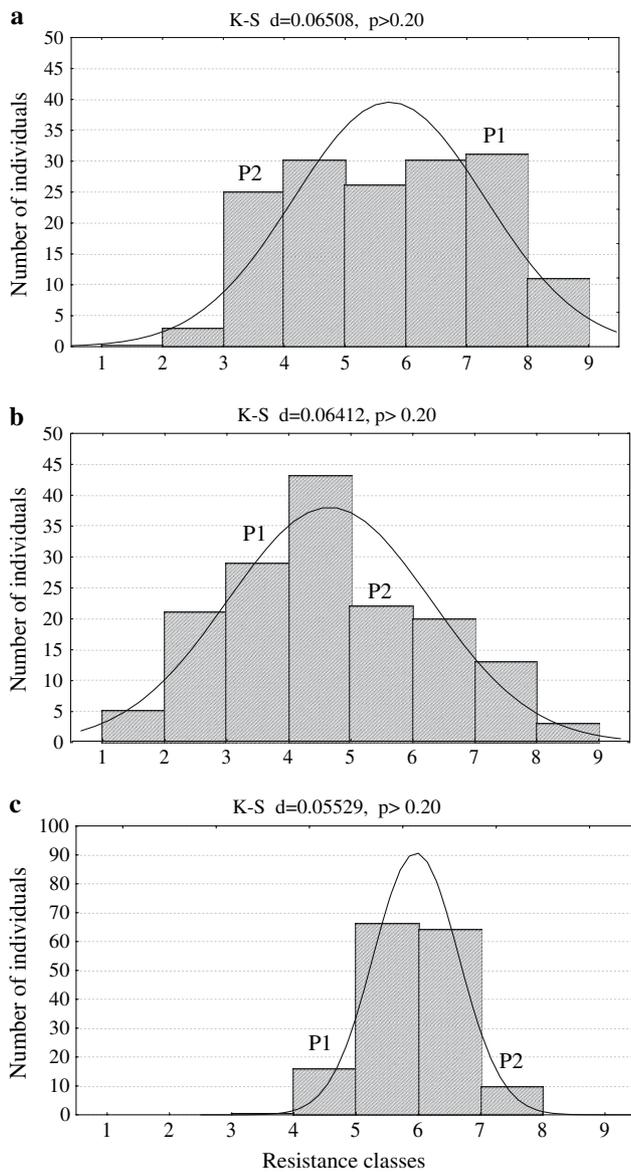


Fig. 1 Distributions of mean (1999–2003) leaflet (a), slice (b) and tuber (c) resistance to *P. infestans* assessed in 1–9 scale, where 9 means the most resistant (x -axis) in the population 98-21 and their fitness to the normal curve. *K-S* Kolmogorov–Smirnov test, d coefficient calculated for this test, p probability, the *line* indicates the normal curve. Resistance levels of parental clones is marked: P1 DG 83-1520, P2 DG 84-195

tuber resistance ($P < 0.001$, $r = 0.282$) and between mean tuber resistance and mean length of vegetation period ($P < 0.001$, $r = 0.284$). Correlations between the remaining trait combinations were not significant.

Molecular linkage map

Of the 56 DNA markers tested, 11 markers showed direct length polymorphism (SCAR or ASA), 12 were polymorphic after restriction digestion (CAPS), and SSCP analysis

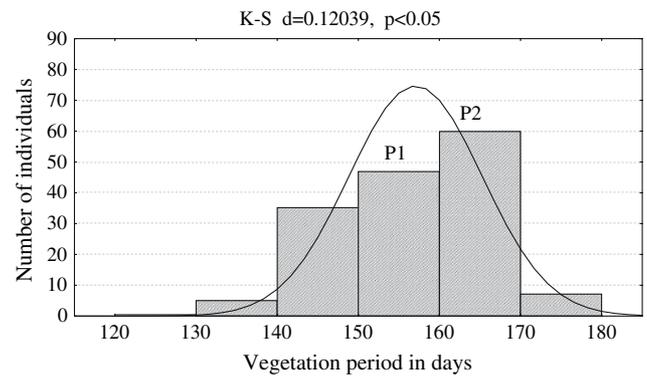


Fig. 2 Distribution of mean (2003–2004) vegetation period length (in days) in the population 98-21 and its fitness to the normal curve. *K-S* Kolmogorov–Smirnov test, d coefficient calculated for this test, p probability, the *line* indicates the normal curve. Vegetation period length of the parental clones is marked: P1 DG 83-1520, P2 DG 84-195

revealed 33 polymorphic marker alleles. Out of the 56 markers, 17 were co-dominant, which means 34 segregating alleles. The remaining 39 markers were inherited either from P1 or from P2, giving 73 segregating alleles in total. Among the 73 segregating marker alleles, 35 were inherited from P1 and 38 from P2. The marker alleles were scored in population 98-21 and assembled into separate linkage groups for P1 and P2. The chromosomes corresponding to the linkage groups were identified based on markers of known chromosomal position. Parental linkage groups corresponding to the same chromosome were joined by shared markers, with the exception of chromosome VII, where no shared marker allele was found. On chromosome VIII, only one marker allele was scored. The locations of the markers (Fig. 3) were mostly as expected from previous maps (<http://gabi.rzpd.de/projects/Pomamo/>; Rickert et al. 2003; Pajerowska et al. 2005), with the exception of ten markers (GP22, GP321, GP173, S1d11, BA157f6 t3, BA202k7 t3, BA1m23 t3, BA9i23 t7, BA31n11 t7, StNPR1), which mapped in population 98-21 to positions other than expected. This was most likely due to duplicated loci. Depending on whether the hybridization-based RFLP assay or PCR-based assays were used to score the marker, different copies of the markers were detected and mapped. Total map length for P1 was 509 cM and for P2 was 447 cM, with the average distance between markers 12.7 cM. Segregation of 19 alleles was distorted from 1:1 ratio according to the χ^2 test, ten out of them were grouped in three regions on chromosomes III, IV and X, whereas the remaining nine were located on chromosomes II, V, VI, VII and XII (Table 1).

Quantitative trait loci

In analysis of variance, the date of testing had little or no effect on the results. Marker-trait linkages were calculated

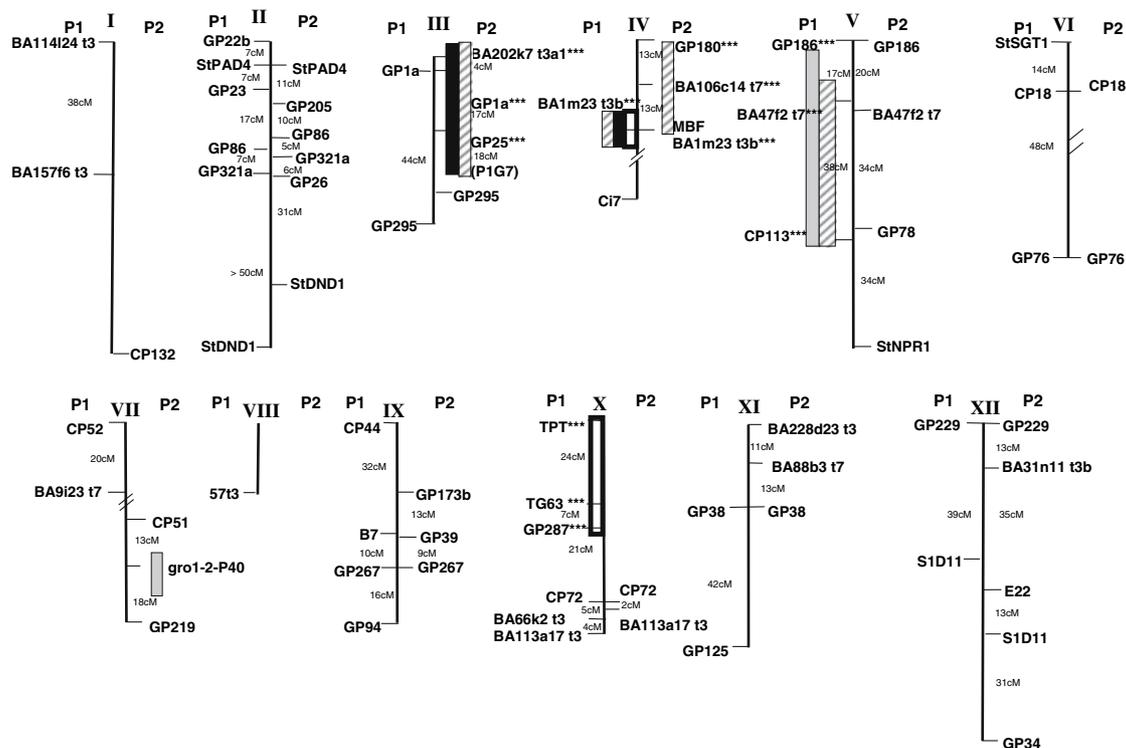


Fig. 3 Linkage and QTL map of the population 98-21 parents (P1—DG 83-1520, P2—DG 84-195). QTLs for resistance to *P. infestans* in leaflets (white rectangles), slices (black), and tubers (stripes), and

QTLs for the vegetation period length (grey). Marker trait linkages, *t* test probabilities: *** $P < 0.001$. Diagonal lines indicate putative linkages

therefore using the mean results of each year and the means over all test years (1999–2003 in case of late blight resistance and 2003–2004 for vegetation period length, Table 2). Fifteen marker alleles were significantly linked with the investigated traits forming 6 QTLs located on 5 chromosomes of potato (Fig. 3), when QTLs on chromosome IV were treated as two separate ones. Most of the QTLs influenced more than one trait. The association of marker alleles with increased or decreased resistance or length of vegetation period is indicated by arrows in Table 2. Significant marker-trait linkages were usually revealed only in some years of testing. The reason could be the use of leaflets and tubers from field grown plants which differed in their physiological state depending on the year and its weather and soil conditions. Possibly, various genetic factors and resistance mechanism were activated in consecutive years of testing depending on plants' state. The length of vegetation period was linked to marker alleles GP186 (P1) and BA47f2 t7 (P1) in both the years (Table 2). The most prominent QTL for leaflet resistance to *P. infestans* was inherited from P1 and was identified on chromosome X linked to markers TPT, GP287 and TG63. Marker allele TPT (P1) explained from 15.5% (2002) to 22.0% (2003) of the variance in detached leaflet tests depending on the year of testing and 15.6% of the variance on average,

although it did not show significant linkage to the resistance in 2000 and 2001. Resistance revealed by the tuber slice tests was influenced mostly by a QTL on chromosome IV, inherited from P1, which explained on average 15.5% of the variance in slice tests. Percentage of variance in slice resistance explained by this QTL varied from 9.0% in 2001 to 20.0% in 2000, with no significant linkage in 2002. QTL on chromosome IV had also some effects on leaflet resistance (in 2000 13.4% of variance explained) and whole tuber resistance (11.7% in 2002) (Table 2). The strongest QTL for whole tuber resistance originating from P2 was found on chromosome III. It explained on average 17.3% of the variance. In 1999, 2001 and 2002 the effect of this QTL on tuber resistance was not significant. It explained 24.3 and 8.7% of variance in 2000 and 2003, respectively. The QTL on chromosome III influenced also slice resistance (on average 8.1% of variance explained) but had no effect either on leaflet resistance to *P. infestans* or length of vegetation period (Table 2). Other QTL influencing whole tuber resistance was located on chromosome V, linked to marker alleles BA47f2 t7 (P1) and CP113 (P1). Marker allele BA47f2 t7 (P1) explained on average 10.8% of variance in tuber resistance to late blight and 19.9% of variance in vegetation period length. This was the strongest QTL for vegetation period length and it was significant in both 2003 (24.7% of

variance explained) and 2004 (11.0% of variance explained) (Table 2). Later maturity was linked to higher resistance, because the same marker alleles were associated both with higher resistance and longer vegetation period (Table 2).

Discussion

In this study we characterized a novel source of resistance to late blight in diploid potatoes with complex pedigrees.

The expression of blight resistance depends on the inoculated tissue (Świeżyński and Zimnoch-Guzowska 2001). In other studies, where tuber blight resistance was mapped, whole undamaged tubers were inoculated, i.e. the periderm (Collins et al. 1999; Oberhagemann et al. 1999; Bradshaw et al. 2004). Park et al. (2005) compared two different slice tests and a test on whole wounded tubers and, because no differences were found, they chose the tuber test which was then used for evaluating the effects of some *R* genes in tubers.

Leaflet resistance as well as slice and whole tuber resistance were analyzed in population 98-21. The tuber skin and the layer just below the tuber skin (inoculated in whole tuber tests) form two different, important barriers for the late blight infection (Toxopeus 1958, 1961). However, cortex and medulla (inoculated in slice tests) can also be involved in resistance mechanism (Flier et al. 2001). Three resistance components: leaflet, slice and whole tuber resistance showed normal distribution in population 98-21 (Fig. 1) and strong influence of the environment on those traits and therefore their quantitative character. Genetic analysis of slice resistance to *P. infestans* was done for the first time in our study. The markers used for QTL tagging were selected based on the assumption that most of the QTLs for the evaluated traits were already located on the potato genetic map in different mapping populations (Gebhardt 2004). The growing number of potato PCR markers with known chromosomal position recently published (Bormann et al. 2004; Rickert et al. 2003) and available in databases (<http://www.gabi.rzpd.de> and <http://www.ncbi.nih.gov>) allowed us to choose DNA markers from most targeted regions of the potato genome. Ten markers mapped in population 98-21 to positions other than expected. Markers GP22, GP173 and S1d11 were originally mapped in different mapping populations as RFLP bands, but in the present study they were applied as sequence-specific PCR markers. Taking into account that potato is a highly polymorphic heterozygous species and that the sequence duplications are common, we assumed that PCR primers, although designed on the basis of sequence of the RFLP bands, were more compatible with different loci in our genotypes. GP321 was mapped originally

as an RFLP secondary band, whereas the main band, located differently, was not polymorphic (C. Gebhardt, personal communication). Most probably, in our study the PCR amplified this main band and polymorphism was detected within the fragment. Markers with names starting with BA...were BAC ends, of BAC clones hybridizing to RGL probes and mapped as RFLP (Rickert et al. 2003). The BAC ends were random and, quite likely, multi-copy sequences, which could have been the reason for their different mapping positions in our study, when compared to the original one (Rickert et al. 2003). An example of sequence similarity affecting PCR detection of particular locus in potato is described by Marczewski et al. (2006), where the PCR fragment linked to the *Gm* gene located on chromosome IX showed 94 and 84% identity to sequences from chromosome XII and 87% identity to sequence from chromosome VIII. In such case differently located primers can quite likely amplify different loci.

The applied approach enabled tagging 6 QTLs. We used the expression 'QTL'; however, our study did not allow defining what genes are underlying the resistance in population 98-21. They may be polygenes as well as *R* genes. In fact, some researchers speculate that major genes are just very effective alleles at QTLs and that the borders between the two traditionally distinguished types of resistance can be blurred (Gebhardt and Valkonen 2001). Also, *R* genes overcome by pathogen can still have some residual effect, enhancing the quantitative resistance of the plant (Stewart and Bradshaw 2001; Stewart et al. 2003). QTLs for the resistance components and length of vegetation period in our study only partially overlapped (Fig. 3), which was a result corresponding well with low-correlation coefficient between the results of phenotypic assessments.

In previous studies (Leonards-Schippers et al. 1994; Collins et al. 1999; Oberhagemann et al. 1999; Sandbrink et al. 2000; Ghislain et al. 2001; Visker et al. 2003, 2005; Bormann et al. 2004; Bradshaw et al. 2004; Costanzo et al. 2005) the most frequently detected QTL for foliage and whole tuber resistance is located on chromosome V, in the neighborhood of the *R1* gene. In population 98-21, this region harboured the strongest QTL for length of vegetation period, which also influenced leaflet and whole tuber resistance to late blight. The strongest and most robust QTLs for leaflet, slice and whole tuber resistance found in population 98-21 were located on chromosomes X, IV and III, respectively. The QTL for leaflet resistance on chromosome X is in a similar region as described by Sandbrink et al. (2000) as harbouring QTL for late blight resistance originated from *S. microdontum*. The QTL for tuber resistance on chromosome III corresponds with QTLs for foliage resistance to late blight detected in other genetic materials (Leonards-Schippers et al. 1994; Collins et al. 1999; Oberhagemann et al. 1999; Costanzo et al. 2005).

The QTL for slice resistance found on chromosome IV influenced also leaflet and whole tuber resistance and most probably it corresponds to a QTL from *S. microdontum* described by Sandbrink et al. (2000). Leonards-Schippers et al. (1994), Oberhagemann et al. (1999) and Collins et al. (1999) detected QTLs for foliage resistance to late blight and plant maturity in similar locations. Bormann et al. (2004) described QTLs for plant maturity and for maturity-corrected resistance in this region, whereas Bradshaw et al. (2004) detected on chromosome IV a QTL for foliage resistance to *P. infestans* with no effect on maturity.

In population 98-21, the most significant and robust QTLs for resistance to *P. infestans* did not overlap with the strongest QTL for the length of vegetation period, which was a promising result with regard to selection of resistant and early potatoes originating from this source of resistance. Taking into account that our material was generally late we cannot be conclusive, but we can hypothesize that crossing it with early genotypes and selecting, possibly with markers, plants with certain genotypes at QTLs on chromosomes III, IV and X combined with negative selection for the QTL on chromosome V, could result in obtaining early potatoes resistant to late blight in next generations. Still, probably not all genetic factors involved in resistance to late blight and maturity of population 98-21 have been revealed in the present study due to the limited genome coverage. Analysis of marker-trait linkages with lowered stringency of significance, which detected 15 QTLs on all 12 chromosomes, can be an indication for that. The material was generally late maturing which could also influence mapping QTLs for maturity. Weak correlations between different resistance components noted for this source should be taken into consideration, when breeding potatoes resistant both in foliage and in tubers.

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