Euphytica (2009) 170:77–86
DOI 10.1007/s10681-009-9986-4

Utilization of SSR and cDNA markers for screening known QTLs for late blight (*Phytophthora infestans*) resistance in potato

Enrique Ritter · Jose Ignacio Ruiz de Galarreta · Mónica Hernandez · Giovanna Plata · Leire Barandalla · Raquel Lopez · Isbene Sanchez · Julio Gabriel

Received: 28 November 2008 / Accepted: 18 June 2009 / Published online: 9 July 2009

Abstract All published QTLs and genes for *Phytophthora* resistance were projected onto the bins of a high-density reference map of potato. Further, a transcriptome map containing around 700 cDNA-AFLP (TDF) markers was anchored to this map. We have analysed cDNA markers which are co-located with these resistance QTLs by cloning, sequencing and by performing homology searches in public sequence databases. Several interesting homologies were detected with typical resistance and stress response genes. On the other hand, we have screened all known QTL locations on the 12 potato chromosomes via linked SSR markers for the presence of a selectable QTL for *Phytophthora* resistance in four genetic backgrounds. Progenies descended from different *Solanum* wild species as resistance sources. Leaf and tuber resistances were analysed. In all case studies, several selectable QTLs were detected which descended from either parent. Tuber and leaf QTLs varied from progeny to progeny and between leaves and tubers. In none of the progenies were resistance levels of leaf and tuber blight correlated.

Keywords Transcriptome mapping · cDNA-AFLP · Late blight · *Solanum*

E. Ritter · J. I. Ruiz de Galarreta (✉) · M. Hernandez · L. Barandalla · R. Lopez · I. Sanchez
NEIKER, Instituto Vasco de Investigación y Desarrollo Agrario, Aptdo. 46, Vitoria, Spain
e-mail: jiruiz@neiker.net

L. Barandalla
e-mail: lbarandalla@neiker.net

G. Plata · J. Gabriel
PROINPA, Fundación Promoción e Investigación de Productos Andinos, P.O. Box 4285, Cochabamba, Bolivia

Introduction

Molecular markers are useful to construct linkage maps and to localize monogenic and polygenic traits, allowing the efficient introgression and selection of individuals with specific characteristics already using seedlings of a breeding programme. In potato, a large amount of genomic resources are being established within the frame of international projects including a potato genome sequencing project (Ritter et al. 2005). A high density reference map of potato is available, which contains 10.000 AFLP markers and is cross-referenced by numerous SSR and RFLP to other maps (UHD map; Van Os et al. 2006). In order to improve the quality of data and to identify singletons due to scoring errors, the "bin" concept was applied for constructing this map, which uses recombination patterns of the whole progeny rather than counting absolute numbers of recombination events (Van Os et al. 2005).

Recently, a complete transcriptome map of constitutively expressed genes of the potato genome has been constructed using cDNA-AFLP (Ritter et al. 2008). TDFs (transcript derived fragments) were

anchored to the bins of the UHD map. Numerous QTL analyses have been performed in potato considering resistance and quality traits. We have projected these published QTLs onto the UHD reference map and analyzed co-located TDFs.

Generally, when starting a new QTL study, reduced satellite maps are produced which also involve markers which are present in other maps in order to align maps and to compare results. Single copy markers, which map to identical genome locations in different genetic backgrounds such as SSR or EST markers, are used for this purpose. However, this approach is laborious and time consuming. In order to reduce efforts when analysing a trait in a new genetic background, the analyses could be restricted to the screening of all known QTL positions via linked SSR markers for the presence of a selectable QTL. We have screened in this way four progenies descending from different resistance sources for selectable QTLs for *Phytophthora* resistance in leaves and tubers. We name this process QTA (quantitative trait allele) genotyping.

Materials and methods

Plant material

For QTL screening with SSR markers 81–95 genotypes from the progenies D: *can310956.8* × *gon703354*, E: *buk210042.5* × *phu81*, G: *jam27521.48* × *gon703354* and N: *H88-31/34 (tbr)* × *rap636* were used. These involve different *Phytophthora* resistance sources, descending from *Solanum canasense (can)*, *S. bukakowskii (buk)*, *S. jamesii (jam)* and *S. raphanifolium (rap)*. In the following, we refer to parents and progenies using the corresponding parental abbreviations.

Bio-assays

For producing *Phytophthora* infections, leaflets from young plants of the progeny genotypes mentioned above were inoculated with spores of local isolate NE293. Infection levels were evaluated in each genotype according to Trognitz et al. (2001). Potato tubers were inoculated with the same isolate of *P. infestans* following the methodology of Flier et al. (2001). For resistance screening, the used laboratory tests represents a good alternative for field tests, since ranking of resistance levels was similar under laboratory and field conditions (Vleeshouwers et al. 1999).

Molecular methods

Bands corresponding to transcripts co-located with published QTLs for *Phytophthora* resistance were isolated, cloned and sequenced applying standard methodology (Sambrook et al. 1989). Sequence homology searches were performed in public sequence databases via NCBI using BLAST search algorithms.

SSR analyses were performed according to Milbourne et al. (1998) in parents and progeny genotypes using appropriate PCR conditions. SSR primers were labelled with either fluorescent infrared dyes IRD800 or IRD700 (LI-COR, Lincoln, Nebraska, USA). Amplification products were denatured and separated on 6% denaturing polyacrylamide (19:1) gels. They were visualised on a LI-COR 4200-S1 DNA Sequencer and Fragment Analysis System as described by the manufacturer.

Data analysis

All published QTLs, SSR and other markers were projected onto the bins of the paternal maps of the UHD mapping population as the reference. Sinus projections were used, based on common marker intervals in different maps, as described by Ritter et al. (1990).

Information about parents (SH, RH) and progeny genotypes of the UHD mapping population (SH83-92-488 × RH89-039-16) described by Van Os et al. (2006) were considered for these projections. This map is cross referenced by numerous markers to other potato and tomato maps. For the projection of QTLs and resistance genes, numerous publications (see Table 1) and relevant databases (SolGenes, http://ukcrop.net/perl/ace/search/SolGenes; GABI-Pomamo, https://gabi.rzpd.de/projects/Pomamo) dealing with QTL studies in potato were consulted. The corresponding maps (QTL maps) and flanking markers were used for the projections in each case. The SSR markers from the linkage map of Milbourne et al. (1998) were

Table 1 List of SSR and cDNA-AFLP markers which are linked or co-located with published *P. infestans* QTLs and resistance genes on the potato reference map

	SH	RH
CHR I		
Stm1029	30	13
Stm2020a	32	20
GA/AC_315		27
GA/AT_342	38	
Pi-1[(1)]	39	29
Stm2030	46	35
CHR II		
Stm5011	0	0
Pi-2a[(1)]	7	7
AG/CT_129	8	
CC/TA_115		8
Stm0038	10	10
CC/GG_395	17	
Pi-2b[(1)]	18	19
GA/AC_143		19
Stm1064(2)		73
CC/GC_238		73
Pi-2c[(1)]	90	75
CHR III		
Stm 1054		6
PI-3b[(4)]	6	5
AG/CC_133		6
Stm 0040		23
FB-1[(2)], Pi-3a[(1)]	35	30
CC/CC_423		32
Stm 1025		41
Pi-3c[(1)]	53	55
CG/AC_218	54	
Pi-3d[(3)(5)]	61	58
CHR IV		
Pi-4a1[(1) (2)]	1	1
AC/AG_133		2
Pi-4b[(1) (2)]	20	23
AC/AG_264	21	
Stm 3016		23
R2, Pi-4a2[(6)]	28	33
AT/TC_124	28	
Stm 1050		35
CHR V		
Stm1041		2
PiFTve-5a[(2)]	12	13
GP21		13

Table 1 continued

	SH	RH
AC/GG_124		13
CT/GC_337	14	
Pi-5a, R1[(3)]	15	16
GP 179		19
PiFTve-5c[(2)]	19	20
AG/AC_84		21
CC/AT_122	28	
PiFTve-5b[(2)]	30	30
Stm0013		39
AC/TG_245		55
Pi-5b[(1)]	56	56
Stm1020		63
CHR VI		
CT/CC_136	2	
Pi-6a[(1)]	3	6
AC/AC_185	3	
Stm 0019		9
Stm 1100		59
FB-6[(2)]	52	59
Pi-6b[(1)(3)]	61	68
CT/AT_243	61	
Stm 1056		83
CHR VII		
CC/GC_200		28
Pi-7a[(1)]	34	30
Stm 1065		36
Stm 1003	72	67
GA/GG_264		68
Pi-7b[(1)]	75	69
AC/TA_92	*76*	
Stm 0052		83
CHR VIII		
CC/TT_410	11	
E-5[(2)]	12	21
STM1056		21
Rblc[(7)]	35	56
E-6[(2)]	37	58
STM1024		58
STM1005		92
GA/CC_352	65	
Pi-8[(1) (5)]	67	95
CHR IX		
Stm 1102	0	0
Stm 1051	4	4

Table 1 continued

	SH	RH
AC/AG_70		10
Pi-9[(1) (3)]	11	11
Stm 3012		13
CC/TC_285		14
CHR X		
Stm 0051		45
CT/AA_611		48
Rber[(4)]	34	49
CHR XI		
AG/CG_500	7	
Pi-11[(1) (3)]	7	10
CC/TC_239		10
Stm1009	19	19
Stm0037	22	22
RP-11[(8)], Pi-19[(1)]	66	85
CHR XII		
Stm0007	18	25
Stm0030	22	30
Stm2028	70	50
Pi-12[(9) (1)]	71	51
GA/TA_263		51
CT/CG_463	72	

CHR = chromosome; SH, RH = bin numbers in the parental UHD maps (Van Os et al. 2006). The shown cDNA-AFLP markers always have Ase/Taq adaptors and are indicated by their nucleotide extensions for the specific amplifications, followed by their base pair values

QTL analyses performed by (1) Oberhagemann et al. (1999); (2) Collins et al. (1999); (3) Leonards-Schippers et al. (1994); (4) Ewing et al. (2000); (5) Trognitz et al. (2002); (6) Li et al. (1998); (7) Naess et al. (2000); (8) El-Kharbotly et al. (1994), (1996); (9) Ghislain et al. (2001)

used for SSR projections. Markers flanking a QTL were projected directly, if present in the reference map. Alternatively, consecutive projection steps were performed using the particular QTL map and others such as the maps of Caromel et al. (2003) and the tomato map of Tanksley et al. (1992). Details are described by Sanchez (2006). For QTL analysis, the detected SSR amplification products were scored for presence or absence. *T*-Tests were performed to analyse average resistance level differences in the genotypes belonging to the marker classes (presence vs. absence) of each SSR allele.

Results

Co-location analyses between published QTLs and TDF markers on the UHD map

For detecting co-location between QTLs and TDFs, a total of 249 published QTLs from 48 publications and different databases were considered (Sanchez 2006). Published QTLs were projected as described in materials and methods onto 184 loci due to co-location of QTLs from different studies. Details can be seen at http://www.neiker.net/neiker.PGR and in Sanchez (2006). These loci involve 144 resistance QTLs against different pathogens and 76 loci for quality and other traits. Also 34 *Phytophthora* QTLs and genes were projected to 30 different locations on all 12 potato chromosomes. The projected *Phytophthora* QTLs and their locations on the bins of the parental UHD maps are shown in Table 1. The authors of the corresponding studies are also indicated in this table.

Each QTL locus and TDF marker has a specific bin assignment in the parental linkage maps of the UHD population. For co-location analyses, we have scanned the position data of all TDFs and projected QTLs considering distances of ± two bins between them. (And if not available, up to three bins). In this way, we detected 57 TDFs which were linked to *Phytophthora* QTLs (Sanchez 2006). Some representative TDF markers and their bin assignments are also shown in Table 1.

We isolated, cloned and sequenced several of these co-located TDFs and performed homology searches. The results are shown in Table 2. Some interesting homologies with typical known resistance and stress response genes were detected. Among them figure LRR and NBS proteins, kinases, chitinases and peroxidases.

QTA genotyping in different genetic backgrounds

Table 3 presents the observed characteristics of leaf and tuber resistance variability against *P. infestans* in the four analysed progenies. Average leaf and tuber resistance levels were similar in progenies D, E and G, while progeny N was found to be more susceptible on average, particularly for tuber resistance. Large variations in resistance levels were observed within the progenies from all resistance sources and

Table 2 Examples for detected homologies of TDF markers which are co-located with published QTLs for *Phytophthora* resistance

Code	TDF	Homologywith genes	Accession number	E-value	PI-QTL	CHR
T2	AseGA/TaqAC_315	Catalytical hydrolase. ATPase, cation transporter	NP850072	2 E-30	Pi-1	1
T3	AseGA/TaqAT_342	LRR protein LRP (tomato)	NP191196	1 E-36	Pi1	1
T4	AseCC/TaqTA_115	SNF2 protein, trancriptional regulation	NP192575	2 E-15	Pi-2a	2
T5	AseGA/TaqAC_143	Unknown			Pi-2b	2
T6	AseCC/TaqGC_238	Class III Chitinase	AAD27874	2 E-24	Pi-2c	2
T7	AseGA/TaqTA_364	Unknown			Pi-3d	3
T13	AseAC/TaqGG_124	POD Peroxidase, oxidoreductase	AJ880395	3 E-22	PiFTve5a	5
T15	AseAC/TaqTG_245	Arginin decarboxylase	AB181854	6 E-79	Pi-5b	5
T17	AseAC/TaqAC_185	Peroxidase, oxidoreductase	AJ880395	4 E-27	Pi-6a	6
T21	AseGA/TaqGG_264	Cytochrome P450 monooxigenase CYP97B2p	AAB94586	1 E-23	Pi-7b	7
T27	AseCC/TaqTC_285	SPA2, Serine/Threonine protein kinase	NP192849	3 E-19	Pi-9	9
T33	AseGA/TaqTA_263	NBS protein (P-loop motif [AMHEWGKS])	CAB37451	5 E-15	Pi-12	12

PI-QTL = Co-located QTL for *P. infestans* resistance from Table 1; CHR = chromosome

Table 3 Characteristics of variation of leaf and tuber resistance against *P. infestans* in progenies from four different genetic backgrounds

	Progeny D	Progeny E	Progeny G	Progeny N
Leaf infections				
Mean	58.5	65.9	52.7	97.9
Min	0.0	0.0	0.0	0.0
Max	177.8	173.3	176.9	188.3
CV	88.2	92.3	95.9	66.5
Tuber infections				
Mean	6.8	12.4	10.8	17.2
Min	0.0	1.4	1.0	5.7
Max	23.0	33.0	72.5	75.5
CV	89.6	63.0	112.3	54.3

Leaf resistances are indicated as average AUDPC values (3 evaluation dates), while tuber resistances are expressed as percentage of infected tissues 3 days after infection

CV = coefficient of variation (%)

coefficients of variations ranged from 54 to 112% (Table 3). Correlation coefficients between leaf and tuber infection levels in the progenies were always low, ranging from −0.069 to 0.328, and in no case significant.

Co-location analyses between SSR markers from the map of Milbourne et al. (1998) and published QTL positions for *Phytophthora* resistance revealed 33 linked SSR markers located on all 12 potato chromosomes (Table 1) which were used for QTA genotyping.

Table 4 shows the detailed results of SSR screening for leaf resistance QTLs in progeny E. In this table, the different SSRs from different chromosomes which were analysed and their potentially associated published QTLs are shown. The detected segregating alleles are indicated and their descent. For each allele marker class (presence vs. absence) the average resistance values, the difference between these means and the significance of this difference according to the *t*-Test is presented. As can be seen in Table 4, we have detected four selectable QTLs in progeny E which are located on chromosomes III, V, VI and X. Only one on chromosome III descended from the resistance source P1 (*buk*). Many SSR markers were not polymorphic in this progeny, preventing the evaluation of the corresponding QTLs.

In the same way, the other progenies were also screened for leaf as well as for tuber resistance QTLs. However, in progeny E not enough tubers for tuber resistance screenings were available. Table 5 summarizes and compares the results of QTA genotyping for leaf and tuber blight in different mapping populations. Only significant markers are indicated. With respect to leaf blight, we detected in progeny G (*jam* × *gon*) three selectable QTLs on chromosomes III, VI and VII according to the significance values. Significant QT allele effects were observed on chromosome VI for parent P2 (*gon*) while this was the case for the other parent (*jam*) at the other two locations. In progeny D (*can* × *phu*), only one QTL was detetcted on chromosome VIII which descend

Table 4 Results of QTA genotyping for leaf blight in progeny E (*buk* × *phu*)

CHR	SSR	Potentially associated QTL	DE	Fr	V1	V0	Dif	Prob
I	STM1029	Pi-1	***Non segregating***					
I	STM2020a	Pi-1	***Non segregating***					
I	STM2030	Pi-1	***Non segregating***					
II	STM5011	Pi2a	***Non segregating***					
II	STM0038	Pi2b	C	1	72,42	104,23	−31,81	10,8
			P1	2	93,13	63,09	30,04	8,5
II	STM1064	Pi2c	***Non segregating***					
III	STM1054	Pi3b	***Non segregating***					
III	STM0040	FB-1, Pi-3a	C	1	67,9	88,2	−20,3	32,9
			P1	**2**	**97,19**	**51,70**	**45,49**	**1,0**
III	STM1025	Pi3c,d	***Non segregating***					
IV	STM3016	Pi-4a1,4b	***Non segregating***					
IV	STM1050	R2,Pi-4a2	***Non segregating***					
V	STM1041	PiFTve-5a, Pi5a, R1, PiFTve-5c	P1	1	87,8	72,2	15,6	37,4
V	STM0013	PiFTve-5b	P1	1	74,4	79,1	−4,7	79,2
			C	4	76,7	78,4	−1,7	93,9
			C	2	88,9	58,7	30,2	8,9
			P2	**3**	**61,5**	**97,0**	**−35,5**	**4,0**
V	STM1020	Pi-5b	***Non segregating***					
VI	STM0019	Pi-6a	**P2**	**1**	**103,1**	**62,7**	**40,4**	**1,8**
			P2	**2**	**62,7**	**103,1**	**−40,4**	**1,8**
			P1	3	66,6	96,6	−30,0	8,3
			P1	4	96,6	66,6	30,0	8,3
VI	STM1100	FB-6	P1	1	66,8	83,5	−16,7	36,6
			P1	2	88,9	63,8	25,1	18,0
			P2	3	66,7	81,4	−14,7	44,6
VI	STM1056a	Pi-6b	***Non segregating***					
VII	STM1065	Pi-7a	***Non segregating***					
VII	STM1003	Pi7b	C	1	81,0	88,2	−7,2	77,9
			P2	2	82,8	81,5	1,3	94,5
VII	STM0052	Pi7b	P2	1	83,7	76,6	7,1	68,9
VIII	STM1056b	E-5	***Non segregating***					
VIII	STM1024	Rblc, E-6	C	1	83,3	76,8	6,5	71,4
			P2	2	80,7	80,5	0,2	99,2
VIII	STM1005	Pi-8	P2	1	84,5	76,1	8,4	64,5
			P2	2	80,2	81,0	−0,8	96,8
IX	STM1102		***Non segregating***					
IX	STM1051		***Non segregating***					
IX	STM3012	Pi-9	***Non segregating***					
X	STM0051	Rber	**C**	**1**	**69,6**	**115,1**	**−45,5**	**3,1**
			P2	**3**	**107,5**	**63,7**	**43,8**	**1,4**
			P1	2	83,8	74,3	9,5	59,2
XI	STM1009	Pi-11	***Non segregating***					
XI	STM0037	Pi-11	***Non segregating***					
XI	STM0025	RP-11,Pi-19	***Non segregating***					

Table 4 continued

CHR	SSR	Potentially associated QTL	DE	Fr	V1	V0	Dif	Prob
XII	STM0007	Pi-12	***Non segregating***					
XII	STM0030	Pi-12	***Non segregating***					
XII	STM2028	Pi-12	***Non segregating***					

CHR = chromosome; DE = descent of a fragment [P1 = specific fragment for parent 1, P2 = specific fragment of parent 2, C = fragment common to both parents]; Fr = fragment number; V1, V0 = average AUDPC values for genotypes where a particular marker is present or absent, respectively; Dif = difference V1 − V0; Prob = probability for the existence of the QTL [%]. Markers for significant QTLs are displayed in bold

from parent P2 (*phu*). In progeny N (*H88-31/34* × *rap*), three selectable QTLs were detected which are located on chromosomes V, VI and VIII. Two of them on chromosomes V and VIII descend from the resistance source (*rap*).

With respect to tuber blight, four selectable QTLs were revealed in progeny G. They were located on chromosomes III, V, VI, and X. The locus on chromosome III showed significant effects for alleles from both parents. Only the locus on chromosome V descended from the resistance source (*jam*). We detected in progeny D two QTLs on chromosomes VII and VIII both descending from *S. phureja*. In family N, only one QTL for tuber blight was detected which descended from parent P1 and was located on chromosome VI.

As can be also seen in this table, several QTLs are common to two different genetic backgrounds, although they may descend from different parents. However, no QTL is common to 3 or 4 populations. In some cases, QTLs are also common for leaf and tuber resistance in the same population, while many of them are specific for the different plant organs as reflected in the low correlation coefficients between these characters.

All evaluated SSR markers on chromosomes I, IX and XII did not reveal segregating amplification products in any progeny. This also occurred in many cases for SSR markers from chromosome XI. Therefore, it was not possible to evaluate the corresponding QTL locations.

Discussion

The projection of QTLs and cDNA-AFLP fragments onto the UHD map allowed a co-location analysis between published QTLs for *Phytophthora* resistance and cDNAs. TDF markers generally have a concrete biological meaning since they are derived from mRNA. Therefore, a particular cDNA which is co-located (or closely linked) to a published QTL could represent a potential candidate gene explaining this particular QTL. For example, we found for example one TDF (T27; Table 2) which was collocated with resistance QTL Pi-9 and showed high homology with a Serine/Threonine kinase. Such kinases are known to trigger cascades of defense reactions after pathogen attack (Martin et al. 1993; Yamamizo et al. 2006).

Most resistance genes have characteristic domains such as LRR (leucine rich repeats) or NBS (nucleotide binding sites) (Leister et al. 1996; Meyers et al. 1999). These were also found in co-located TDFs T3 and T33. Peroxidases (T13, T17) are known to be involved in response reactions after P. infestans infections (Polkowska-Kowalczyk and Maciejewska 2001). Using this methodology, we also re-detected by chance a cytochrome P450 gene (T21) which had been previously associated with a QTL for *P. infestans* resistance by Trognitz et al. (2002).

Although the probability that these TDFs could explain co-localized QTLs seems low based on the resolution of the map, we found in several cases homologies with known resistance genes. Considering that families of resistance genes are frequently organized in clusters (Gebhardt and Valkonen 2001), the chance of finding a target gene of interest was higher in this case. However, other linked genes could also be actually responsible for the QTL effect. Therefore, it is necessary to perform a complementation assay or silencing experiments in order to verify the function. If the candidate gene should represent a false positive, then it can be used at least as an allele-specific marker in marker-assisted breeding.

Published QTLs for *P. infestans* resistance have been reported for all 12 potato chromosomes. Part of

Table 5 Summary of QTA genotyping for *Phytphthora* resistance in leaves and tubers in four different genetic backgrounds. Only the significant markers are indicated

Progeny			E (*buk* × *phu*)			G (*jam* × *gon*)						D (*can* × *phu*)						N (*H88-31/34* × *rap*)					
Resistance in			Leaves			Leaves			Tubers			Leaves			Tubers			Leaves			Tubers		
CHR	SSR	QTL	DE	Fr	Prob	DE	Fr	Prob	DE	Fr	Prob	DE	Fr	Prob	DE	Fr	Prob	DE	Fr	Prob	DE	Fr	Prob
III	STM0040	FB-1, Pi-3a	P1	2	1.0																		
III	STM1025	Pi3c,d				P1	1	4.5	P1	1	2.0												
									P2	2	4.7												
V	STM1041	PiFTve-5a, Pi5a, R1, PiFTve-5c																P2	3	3.5			
V	STM0013	PiFTve-5b	P2	3	4.0				P1	3	4.3												
VI	STM0019	Pi-6a	P2	1	1.8				P2	3	4.6												
			P2	2	1.8																		
VI	STM1100	FB-6				P2	1	0.3										P1	2	4.7	P1	2	4.9
VII	STM1003	Pi7b				P1	1	4.4															
															P2	1	5.0						
VIII	STM1024	Rblc, E-6																P2	2	4.9			
VIII	STM1005	Pi-8										P2	2	4.7	P2	1	4.8						
X	STM0051	Rber	C	1	3.1																		
			P2	3	1.4				P2	2	4.1												

CHR = chromosome; DE = descent; Fr = fragment number, Prob = probability for the existence of the QTL [%]

them have been validated by several authors. The SSR map established by Milbourne et al. (1998) allowed us to project linked SSR markers for all published quantitative trait loci. Projection of SSR markers and known QTLs permit the reduction of efforts when analysing traits in a new genetic background. All known positions can be screened for the presence of a QTL by analysing only closely linked SSR markers in a new population.

Sufficient variations in resistance levels were obtained within the progenies of all resistance sources allowing efficient QTL analyses. We have screened these published QTL positions for the presence of QT allele differences in four different progenies and detected selectable QTLs for leaf and tuber blight in all experiments. In this way, we have established a "genotypic fingerprint" of each parent, indicating selectable QTL positions and the corresponding SSR markers for marker-assisted selection (MAS). These markers can be applied within potato breeding programmes for all crosses which involve the corresponding parental genotypes as resistance sources.

Two basic requirements are necessary for detecting a QTL with this type of analysis. The flanking marker must segregate and the alleles at a QTL must show measurable differences in their effects. It is important to realise that differences between individual QT alleles in one parent in combination with the alleles from the other parent (including possible interactions) are evaluated in a mixture of genotypes. If, for example, the allelic configuration at a QTL in the parents of a cross is: q1/q2 × q3/q4, then in QTL analysis we evaluate the average difference of the trait values from the progeny genotypes [q1q3 + q1q4] − [q2q3 + q2q4]. That means, that if the alleles q1 and q2 do not have a measurable difference in their effects, even if they are different in their sequences, then we will not detect the QTL. Furthermore, dominant effects of QTL alleles from the other parent might prevent the detection. However, the gene influencing the trait will probably be present at a QTL in each progeny, but we can not determine the effect of its alleles.

With respect to the second requirement of a segregating SSR marker linked to a QTL, we have seen that in many cases such a marker was not available, preventing the evaluation of QTLs at many genomic locations. Thus it will be necessary to increase the number of available SSR markers in potato in order to have alternative screening possibilities. The physical map and the genome sequence which is currently established in potato will allow the rapid obtainment of such markers and even candidate genes for QTLs.

Acknowledgments Part of this work was financed within the frame of EU projects QLK5-CT02-01849 (Apophys), FOOD-CT-2005-513959 (Bioexploit) and INIA project RTA2008-00045-C02-01.

References

Caromel B, Mugniery D, Lefebvre V, Andrzejewski S, Ellisseche D, Kerlan MC, Rousselle P, Rousselle-Bourgeois F (2003) Mapping QTLs for resistance against *Globodera pallida* (Stone) Pa2/3 in a diploid potato progeny originating from *Solanum spegazzinii*. Theor Appl Genet 106: 1517–1523

Collins A, Milbourne D, Ramsay L, Meyer R, Chatot-Balandras C, Oberhagemann P, De Jong W, Gebhardt C, Bonnel E, Waugh R (1999) QTL for field resistance to late blight in potato are strongly correlated with maturity and vigour. Mol Breed 5:387–398

El-Kharbotly A, Leonards-Schippers C, Huigen DJ, Jacobsen E, Pereira A, Stiekema WJ, Salamini F, Gebhardt C (1994) Segregation analysis and RFLP mapping of the *R1* and *R3* alleles conferring race-specific resistance to *Phytophthora infestans* in progeny of dihaploid potato parents. Mol Gen Genet 242:749–754

El-Kharbotly A, Palomino-Sánchez C, Salamini F (1996) *R6* and *R7* alleles of potato conferring race-specific resistance to *Phytophthora infestans* (Mont.) de Bary identified genetic loci clustering with the *R3* locus on chromosome XI. Theor Appl Genet 92:880–884

Ewing EE, Simko I, Smart CD, Bonier-bale MW, Mizubuti ESG (2000) Genetic mapping from field tests of quantitative and qualitative resistance to *Phytophthora infestans* in a population derived from *Solanum tuberosum* and *Solanum berthaultii*. Mol Breed 6:25–36

Flier WG, Turkensteen LJ, van den Bosch TBM, Vereijken PFG, Mulder A (2001) Differential interaction of Phytophthora infestans on tubers of potato cultivars with different levels of blight resistance. Plant Pathol 75:133–136

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

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

Leister D, Ballvora A, Salamini F, Gebhardt C (1996) A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. Nat Genet 14:421–429

Leonards-Schippers C, Gieffers W, Schäfer-Pregl R, Ritter E, Knapp SJ, Salamini F, Gebhardt C (1994) Quantitative resistance to *Phytophthora infestans* in potato: a case study for QTL mapping in an allogamous plant species. Genetics 137:67–77

Li X, van Eck HJ, Rouppe van der Voort JNAM, Huigen DJ, Stam P, Jacobsen E (1998) Autotetraploids and genetic mapping using common AFLP markers: the *R2* allele conferring resistance to *Phytophthora infestans* mapped on potato chromosome 4. Theor Appl Genet 96:1121–1128

Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spivey R, Wu T, Earle ED, Tanksley SD (1993) Map based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262:1432–1436

Meyers BC, Dickerman AW, Michelmore RW, SivaramakrishnanS Sobral BW, Young ND (1999) Plant disease resistance genesencode members of an ancient and diverse protein family withinthe nucleotide binding superfamily. Plant J 20:317–332

Milbourne D, Meyer RC, Collins AJ, Ramsay LD, Gebhardt C, Waugh R (1998) Isolation, characterisation and mapping of simple sequence repeat loci in potato. Mol Gen Genet 259:233–245

Naess SK, Bradeen JM, Wielgus SM, Haberlach GT, McGrath JM, Helgeson JP (2000) Resistance to late blight in *Solanum bulbocastanum* is mapped to chromosome 8. Theor Appl Genet 101:697–704

Oberhagemann P, Chalot-Balandras C, Bonnel E, Schäfer-Pregl R, Wegener D, Palomino C, Salamini F, Gebhardt C (1999) A genetic analysis of quantitative resistance to late blight in potato: Towards marker assisted selection. Mol Breeding 5:399–415

Polkowska-Kowalczyk L, Maciejewska U (2001) The oxidative processes induced in cell suspensions of *Solanum* species by culture filtrate of *Phytophthora infestans*. Z Naturforsch 56:235–244

Ritter E, Gebhardt C, Salamini F (1990) Estimation of recombination frequencies and construction of RFLP linkage maps in plants from crosses between heterozygous parents. Genetics 224:645–654

Ritter E, Lucca F, Sánchez I, Ruiz de Galarreta JI (2005) Genomic resources in potato and possibilities for exploitation. In: Haverkort AJ, Struik PC et al (eds) Potato in progress; science meets practice. Wageningen Academic Publishers, The Netherlands, pp 55–65

Ritter E, Ruiz de Galarreta JI, van Eck HJ, Sánchez I (2008) Construction of a potato transcriptome map based on the cDNA-AFLP technique. Theor Appl Genet 116:1003–1013

Sambrook J, Fritsch EF, Maniatis T (1989) MolecularCloning. A laboratory manual. Cold SpringHarbor Laboratory Press, New York

Sanchez I (2006) Construcción de un mapa genético funcional y detección de genes de resistencia frente a factores bióticos en patata (*Solanum tuberosum* L.). Universidad del País Vasco, PhD thesis, pp 204

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Young ND (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Trognitz BR, Orrillo M, Portal L, Román C, Ramón P, Perez S, Chacón G (2001) Evaluation and analysis of reduction of late blight disease in a diploid potato progeny. Plant Pathol 50:281–291

Trognitz F, Manosalva P, Gysin R, Niño-Liu D, Simon R, Herrera Ma del R, Trognitz B, Ghislain M, Nelson R (2002) Plant defense genes associated with quantitative resistance to potato late blight in *Solanum phureja* × dihaploid *S. tuberosum* hybrids. Mol Plant-Microbe Interact 15:587–597

Van Os H, Stam P, Visser RGV, van Eck HJ (2005) SMOOTH: a statistical method for successful removal of genotyping errors from high-density genetic linkage data. Theor Appl Genet 112:187–194

Van Os H, Andrzejewski S, Bakker E, Barrena I, Bryan GJ, Caromel B, Ghareeb B, Isidore E, de Jong W, van Koert P, Lefebvre V, Milbourne D, Ritter E, Rouppe van der Voort JNAM, Rousselle-Bourgeois F, van Vliet J, Waugh R, Visser RGV, Bakker J, van Eck HJ (2006) Construction of a 10,000 marker ultra-dense genetic recombination map of potato: providing a framework for accelerated gene isolation and a genomewide physical map. Genetics 173:1075–1087

Vleeshouwers VGAA, van Dooijeweert W, Keizer LCP, Sijpkes L, Govers F, Colon LT (1999) A laboratory assay for *Phytophthora infestans* resistance in various solanum species reflects the field situation. Eur J Plant Pathol 105:241–250

Yamamizo C, Kuchimura K, Kobayashi A, Katou S, Kawakita K, Jones JDG, Doke N, Yoshioka H (2006) Rewiring mitogen activated protein kinase cascade by positive feedback confers potato blight resistance. Plant Physiol 140:681–692