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High-Resolution Mapping of Two Broad-Spectrum Late Blight Resistance Genes from Two Wild Species of the *Solanum circaeifolium* Group

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Abstract High levels of resistance to *Phytophthora infestans* in *Solanum* are predominantly based on the gene-for-gene interaction. Identification of hitherto unknown *R* genes is essential for future pyramiding approaches. This could be achieved either through classic introgression breeding or through cisgenesis and could lead to sustainable control of late blight. Here, we report on the mapping of *Rpi-cap1* and *Rpi-qum1*, two late blight *R* genes identified in the wild species *Solanum capsicibaccatum* and *Solanum circaeifolium* ssp. *quimense*, respectively, to very similar positions on the long arm of chromosome 11. Despite the difficulties encountered for marker development, a high-resolution genetic map with cleaved amplified polymorphic sequence markers was constructed. Furthermore, an *R* gene cluster-directed profiling approach led to the development of markers that closely

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linked to or co-segregated with the *Rpi-cap1* gene. Both *R* genes are hypothesized to be homologous to the *N* gene, a toll-interleukin1 receptor–nucleotide-binding site–leucine-rich repeat domain type of *R* gene to tobacco mosaic virus from tobacco. To confirm this hypothesis, cloning of *Rpi-cap1* and *Rpi-qum1* should be pursued. Cloning would also be instrumental to facilitate the introduction of these valuable *R* genes into potato crops using cisgenic- and marker-assisted breeding approaches.

Keywords Chromosome 11 · Late blight resistance · *N* resistance gene cluster · *Phytophthora infestans* · *Quimense* · *Solanum capsicibaccatum* · *Solanum circaeifolium* spp.

Introduction

The gene-for-gene hypothesis was first proposed by Harold Flor in the 1940s. It is the most studied model explaining plant resistance to pathogens. According to this model, which was later reviewed by the same author (Flor 1971), for each resistance (*R*) gene of the plant, there is a corresponding avirulence gene in the pathogen. In the past decade, it was found that avirulence is determined by specific pathogen-derived effector proteins (Hogenhout et al. 2009). Recognition of a specific effector by the R protein will lead to the activation of plant defenses and stop pathogen growth, resulting in a hypersensitive response (HR). The most common class of *R* genes encodes proteins with two characteristic domains: a nucleotide-binding site (NBS) and a leucine-rich repeat domain (LRR) (Chisholm et al. 2006; Rairdan and Moffett 2007). Roughly, this class of *R* genes can be divided into two groups, based on the domain present in the N terminus: R proteins with a toll-interleukin1 receptor (TIR) domain in their N terminus and proteins that do not contain a TIR domain. In plant genomes, these genes tend to occur in clusters containing pseudogenes and functional genes (Michelmore and Meyers 1998). In their review, these authors describe a theory for the evolution of *R* genes and propose that the presence of complex *R* gene clusters can explain how plants generate and maintain large numbers of resistance specificities against ever-changing pathogen populations.

The oomycete *Phytophthora infestans* (Mont.) de Bary, causing late blight in potato, is the most devastating pathogen for potato (*Solanum tuberosum*) production. Currently, the disease is mainly controlled by chemicals which involve high costs and raises environmental concerns. Past experiences indicate that an effectively durable control, relying on single *R* genes, is not feasible because of the quick breakdown of the *R* gene by the rapidly evolving pathogen (McDonald and Linde 2002). Pyramiding several *R* genes in a single genotype might be an option to more durably fight late blight (Pink 2002; McDowell and Woffenden 2003). A practical way to pyramid several *R* genes in potato cultivars is through cisgenesis (Schouten et al. 2006; Jacobsen and Schouten 2007; Haverkort et al. 2008). This approach requires the availability of a large number of diverse *R* genes with complementary spectra of resistance. Resistance spectrum and effector recognition are important aspects to take into account in selecting the most promising *R* genes for pyramiding to reach durable resistance.

The genomic region on the distal end of the long arm of chromosome 11 in potato has been characterized as a resistance "hotspot" in a review by Gebhardt and Valkonen (2001), harboring two *R* genes giving resistance to viruses, one to a nematode and one to a fungus, and two QTL, one giving resistance to potato late blight and one to *Erwinia* soft rot. Later, one more gene was added to that list: *Y-1* occurring in *S. tuberosum* subsp. *andigena* and conferring cell death upon infection with *potato virus Y* (PVY). One *R* gene is mapped in the syntenic tomato genomic region, and that is the gene *I* conferring resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Sarfatti et al. 1991). The PVY gene was cloned and is homologous to the *N* gene from tobacco (Vidal et al. 2002). This *R* gene-rich genomic region on chromosome 11 appears to be syntenic to the tobacco genome segment containing the *N* gene (Whitham et al. 1994). The tobacco *N* gene was one of the first *R* genes to be cloned; it confers resistance to tobacco mosaic virus, and it encodes a TIR–NB–LRR type of protein. The syntenic *R* gene cluster in potato contains sequences homologous to the *N* gene, as was demonstrated by homology in cDNA clones (Hehl et al. 1999), *N*-like specific PCR products, (Hämäläinen et al. 1998; Leister et al. 1996), and BAC-end sequences (Zhang and Gassmann 2007). This *R* gene-rich region apparently contains a large number of *N*-like sequences, suggesting that the *R* genes present in this region must be homologous to the *N* gene. Therefore, we refer to this region as the *N* cluster.

In a recent study, Jacobs et al. (2010) mapped *Rpi-cap1*, a gene from the diploid wild *Solanum* species *Solanum capsicibaccatum* Cardenas giving resistance to *P. infestans*, on chromosome 11. A repulsion phase marker had homology to the *N* gene. This *R* gene is a candidate for genetic fine mapping and cloning as the *Rpi-cap1*-containing genotype displays high levels of resistance to late blight in the field and has a broad resistance spectrum. Genotypes of *S. circaeifolium* ssp. *quimense* Hawkes & Hjerting display similar levels of broad-spectrum resistance, and the late blight *R* gene occurring in the latter species will be referred to as *Rpi-qum1*. Both species phylogenetically locate to the diploid series *Circaeifolia*, endemic in Bolivia (Hawkes and Hjerting 1989). The aim of this study was to fine map the genes *Rpi-cap1* and *Rpi-qum1*. Large mapping populations were developed and closely linked cleaved amplified polymorphic sequence (CAPS) markers were identified in both populations. Also, a cluster-directed profiling (CDP) approach combined with bulked segregant analysis (BSA) was pursued for *Rpi-cap1* and led to the identification of a marker co-segregating with *Rpi-cap1*.

Materials and Methods

Plant Material and Mapping Population

Accessions of *S. capsicibaccatum*, *S. circaeifolium* ssp. *quimense*, and *S. circaeifolium* ssp. *circaeifolium* (Table 1) were provided by the Centre of Genetic Resources in Wageningen, The Netherlands. The two resistant genotypes *S. capsicibaccatum* (cap) 536-1 and *S. circaeifolium* ssp. *quimense* (qum) 567-1 were selected and were crossed with susceptible plants of *S. circaeifolium* ssp. *circaeifolium* (crc) 564-3 and crc564-4 to generate intra-specific F1 mapping populations for *Rpi-cap1* and *Rpi-qum1*, respectively (Table 2).

Table 1 List of accessions tested from the wild *Solanum* section *Circaeifolia* species *S. capsicibaccatum* (cap) and *S. circaeifolium* ssp. *circaeifolium* (crc) and ssp. *quimense* (qum)

Species	Accession	Gene bank	Number of genotypes tested	Number of resistant genotypes
cap	535	CGN18254	5	0
cap	538	CGN18265	4	0
cap	335	CGN18268	9	0
cap	261	BGRC35377	5	0
cap	534	CGN18291	5	0
cap	334	CGN18297	5	1
cap	**536**	**CGN22388**	**2**	**1**
cap	566	CGN22767	5	0
crc	564	CGN18133	5	0
crc	563	BGRC27058	5	5
qum	340	CGN18127	3	1
qum	341	CGN18128	9	8
qum	**567**	**CGN18158**	**3**	**3**
qum	565	CGN20643	5	5

Individual genotypes were tested in detached leaf assay for their resistance to *P. infestans* isolate 90128. The accessions from which the resistant genotypes of this study originated are indicated in bold

CGN Centre for Genetic Resources, The Netherlands (http://www.cgn.wur.nl/uk/); *BGRC* Braunschweig Genetic Resource Center

Phytophthora and Disease Test

The *P. infestans* isolates used in this study which were kindly provided by Prof. Francine Govers and Dr. Geert Kessel from Wageningen University & Research Centre and Prof. Paul Birch from the James Hutton Institute in Scotland (Table S1). *P. infestans* isolate 90128 was used to screen for resistant genotypes from the accessions *S. capsicibaccatum* and *S. circaeifolium* ssp. *quimense*. The F1 populations were inoculated with the same isolate to test for the segregation of resistance. The segregants were also tested with four other isolates VK98014, IPO-4282, NL01096, and EC1. The resistance spectrum of the parents was characterized using inoculation with 21 isolates (Table S1).

Table 2 Description of the *Rpi-cap1* and *Rpi-qum1* F1 populations

Gene	R parent	S parent	Population size	*R*	*S*	Chi-square (χ^2)
Rpi-cap1	cap536-1	crc564-3	108	64	44	<0.05
Rpi-qum1	qum567-1	crc564-4	50	25	25	<0.05

Segregation of resistance in the detached leaf assay is indicated as: R for resistant and S for susceptible F1 plants. Chi-square test assumed a 1:1 segregation if the resistance is based on one *R* gene in heterozygous state in the R parent

Screening the *Solanum* series *Circaeifolia* accessions for resistance to *P. infestans* isolate 90128 was performed using an in vitro inoculation assay (Huang et al. 2005a). Further resistance screens of selected genotypes were performed according to the detached leaf assay (DLA) protocol (Fig. 1), as described by Vleeshouwers et al. (1999). Depending on their size, the leaves of 5-week-old plants were inoculated with 6 to 10 drops of inoculum containing 50,000 zoospores/ml. After 6 days of inoculation, the leaves were scored as resistant (HR, small necrotic lesions) or as susceptible (large lesions with sporangiophores).

DNA Isolation

Young leaf tissue was collected from greenhouse grown plants and samples were frozen in liquid nitrogen. The frozen material was homogenized with the Retsch machine (Retsch Inc., Hannover, Germany). Genomic DNA was isolated using either of two different protocols. DNA that needs long storage, like that from the mapping population and the recombinants, was isolated using the cetyltrimethylammonium bromide (CTAB) protocol described by Huang et al. (2005b). DNA that does not require long time storage, like DNA for the screening for recombination events in the population, was isolated using the NaOH protocol described by Wang et al. (1993).

Marker Development and Map Construction

Two different approaches were followed to identify additional PCR markers on the long arm of chromosome 11. First, primers deriving from known markers on the long arm of chromosome 11 were screened for CAPS polymorphisms using a set of 24 frequently cutting restriction enzymes. In a second approach, primers were designed on the basis of RH89-039-16 (RH) BAC sequences mapped on the long arm of chromosome 11 in the potato genome sequencing project (http://www.potatogenome.net/). These primers were first tested for amplification on the parents. Potential allele-specific markers were selected that amplified only a fragment in the resistant parent. If a fragment was produced for both parents, the PCR product was screened with a selection of

Fig. 1 Detached leaf assay on 19 F1 individuals of *Rpi-cap1* population, 6 days after inoculation with *Phytophthora infestans* (isolate 90128). The resistant phenotypes stand out as green leaves, the susceptible phenotypes are recognized from their sporulating, brownish, and wrinkled leaves

restriction enzymes for the identification of a CAPS polymorphism. Successively, polymorphisms occurring between the parents were tested for segregation in the F1 population. Next, a possible association with the resistance phenotype was assessed. Genetic maps were constructed using the recombination frequency between the markers and between the markers and the resistant/susceptible phenotype.

Recombinant Screening

Seeds from the F1 crosses were sown in 96-well format plant trays in order to facilitate the screen. Two weeks after germination, the second true leaf was harvested and DNA was isolated by following the NaOH protocol. The markers Nl27 and T079, flanking *Rpi-cap1*, and the markers Nl27, T081, and ADG2, flanking *Rpi-qum1*, were applied on the freshly isolated DNA. Eight hundred forty-eight individuals for *Rpi-cap1* and 349 individuals for *Rpi-qum1* were screened with these markers. The F1 individuals that showed a recombination between the flanking markers were transplanted to pots. We later isolated DNA using the CTAB method to confirm the recombination. All recombinant plants were tested for resistance phenotypes, using the detached leaf assay and isolate 90128. The recombinants were maintained in in vitro cultures and their phenotype was retested in DLA.

N-Like Profiling

The *N*-like profiling is based on the NBS profiling protocol as described by van der Linden et al. (2004). We designed a total of eight degenerate primers on *N*-like specific sequences, in the TIR, NBS, and LRR domains. Genomic DNA was digested using five different restriction enzymes generating blunt ends. Adaptors were ligated to the restriction ends and a PCR was performed using the *N*-like primers in combination with a fluorescently labeled adaptor primer. Fragments were size-separated and gel images were generated using a LI-COR DNA sequencer (Lincoln, Nebraska, USA). A BSA approach was carried out with bulks of 10 resistant and 10 susceptible non-recombinant plants. Also, 10 resistant and 10 susceptible recombinant plants were bulked. The identified bulk-specific markers were verified in the F1 individuals of the bulks and were successively tested on the complete population for validation and mapping.

Results

Resistance in *S. capsicibaccatum* and *S. circaeifolium* ssp. *quimense* Accessions

To identify resistant genotypes to be used as parents for crossing with susceptible plants, several accessions of the wild species *S. capsicibaccatum* and of both *S. circaeifolium* subspecies were screened for resistance to *P. infestans* isolate 90128. At least two plants per accession were tested (Table 1). For *S. capsicibaccatum*, 40 plants from eight accessions were tested. Two genotypes showed resistance to *P. infestans*, one was from accession 536 and the other one was from accession 334. For *S. circaeifolium*, 30 plants from six accessions were tested and the majority of these,

22, were resistant. It seems, therefore, that resistance to *P. infestans* isolate 90128 is the rule in S. *circaeifolium* and the exception in *S. capsicibaccatum*.

Population Development and Spectrum of Late Blight Resistance

The resistant genotypes cap536-1 and qum567-1 from *S. capsicibaccatum* and *S. circaeifolium* ssp. *quimense*, respectively, were crossed with the susceptible *S. circaeifolium* ssp. *circaeifolium* crc564-3 and crc564-4, respectively. A detached leaf assay performed on the F1 plants allowed an unambiguous classification of resistant and susceptible phenotypes (Fig. 1). The segregation of the resistance in the two populations followed a 1:1 ratio ($p<0.05$) which is consistent with the presence of single dominant *R* genes *Rpi-cap1* and *Rpi-qum1* in the resistant parental plants cap536-1 and qum567-1, respectively (Table 2). The resistance spectra of the resistant parental genotypes cap536-1 and qum567-1 were determined by challenging them with 21 isolates of different complexity and geographic provenance (Table 3 and S1). Both genotypes were resistant to all 21 isolates tested, indicating that they have a

Table 3 Resistance phenotypes observed after inoculations with *Phytophthora infestans* isolates on the parents of the F1 populations (cap536-1, qum567-1, crc564-3, and crc564-4), and 10 resistant and 10 susceptible F1 genotypes from the *Rpi-cap1* population in detached leaf assays

P.i. isolate	cap536-1 *Rpi-cap1*	crc564-3 *rpi-cap1*	10 R F1 *Rpi-cap1*	10 S F1 *rpi-cap1*	qum567-1 *Rpi-qum1*	crc564-4 *rpi-qum1*
VK98014	R	S	R	S	R	S
IPO428-2	R	S	R	S	R	S
NL01096	R	S	R	S	R	S
EC1	R	S	R	S	R	S
90128	R	S	Nd	Nd	R	S
NL00228	R	S	Nd	Nd	R	S
F95573	R	S	Nd	Nd	R	S
H30P04	R	R	Nd	Nd	R	R
USA618	R	R	Nd	Nd	R	R
IPO-C	R	R	Nd	Nd	R	R
PIC99183	R	R	Nd	Nd	R	R
PIC99177	R	R	Nd	Nd	R	R
PIC99189	R	R	Nd	Nd	R	R
IPO-0	R	Nd	Nd	Nd	R	Nd
UK7824	R	Nd	Nd	Nd	R	Nd
88133	R	Nd	Nd	Nd	R	Nd
91011	R	Nd	Nd	Nd	R	Nd
88069	R	Nd	Nd	Nd	R	Nd
NL05-194	R	Nd	Nd	Nd	R	Nd
CA-65		R	Nd	Nd	Nd	R
3128-A	R	Nd	Nd	Nd	R	Nd

R resistant, *S* susceptible, *Nd* not determined

similar broad resistance spectrum. Genotypes crc564-3 and crc564-4, used for the crosses that were susceptible to isolate 90128, showed resistance to several other isolates (Table 3), which suggests that additional *R* genes are present in *S. circaeifolium*. As a consequence, to test the segregation of *Rpi-cap1* and *Rpi-qum1* in the two F1 populations, only the isolates that were virulent on the two *S. circaeifolium* ssp. *circaeifolium* genotypes (crc564-3 and crc564-4) could be used.

Mapping and Fine Mapping of *Rpi-cap1*

In a previous study by Jacobs et al. (2010) on the same F1 population of cap563-1 × crc564-3, *Rpi-cap1* was shown to be located in the *N* cluster at the distal end of the short arm of chromosome 11. They identified the map position by NBS profiling and could link the resistance phenotype to the CAPS marker Cp58 with one recombinant out of 20 F1 individuals. To confirm the map position and to construct a more robust genetic map, we phenotyped 108 F1 individuals of the *Rpi-cap1* population for resistance to *P. infestans* isolate 90128. The linkage of *Rpi-cap1* with CAPS marker Cp58 was confirmed. Only two recombinants were found in a set of 91 individuals. Markers previously mapped in the *N* cluster in *S. tuberosum* were also tested. Six out of seven primer combinations tested gave amplification on the resistant genotypes, and only two resulted in polymorphism. These were markers Nl27 (Marczewski et al. 2001), derived from the NL-27 cDNA, and M33 (Brigneti et al. 1997). In parallel, markers were developed using sequence information available from the RH physical map on chromosome 11 (Bakker et al. 2011). Primers, designed on these BAC sequences, were applied to the DNA of cap536-1 and crc564-3. Out of 17 primer pairs giving PCR amplification in RH, only five gave an amplification product on the cap536-1 and qum567-1 genotypes, and only two resulted in CAPS markers: T179 and T081. Table 4 summarizes the markers developed for the *Rpi-cap1* population.

Table 4 PCR markers for the F1 mapping populations of *Rpi-cap1* (cap) and *Rpi-qum1* (qum), with primer sequences based on BAC sequences located on the long arm of chromosome 11 from the reference genotype RH or references for primers already published, annealing temperature in °C (TM), and restriction enzyme to identify a polymorphism for each population

Marker	Sequence[a] or reference	TM	Enzyme	
			cap	qum
Nl27	Marczewski et al. 2001	55	a.s.	XapI
M33	Brigneti et al. 1997	62	AluI	–
ADG2	Hämäläinen et al. 1998	55	–	PsuI
T081	F GTTGGGCAGGTACTCAATGG	55	MseI	HpyF3I
	R ATTCAGGACGGGTCATTAGG			
T179	F CTAGCTCTGTCCCCGTCCAC	55	AluI	–
	R CCGTGTTTACACCTAACTCAACC			
Tir300F-Hinc	NTAGTRAAGAYATGGAATGC	55	HincI	–
Nbs15F-BspL	ATGCATGAYTTRATWVAAGABATGGG	55	BspLI	–

[a] Sequences of primers designed in this study

In total, five markers (Cp58, Nl27, M33, T179, and T081) were developed that could be used to construct the genetic map of the *Rpi-cap1* region based on a population of 108 individuals. The markers Cp58 and Nl27 were on one side, T081 and T179 were on the other side of the gene, and M33 was co-segregating with *Rpi-cap1* (data not shown). This allowed to pursue a genetic fine mapping approach since the flanking markers could be used for a recombinant screen. Eight hundred forty-eight individuals were screened with the flanking markers Cp58, Nl27, and T179. The sequence-characterized amplified region (SCAR) marker Nl27 was multiplexed with the M33 primer pair as internal control to test for DNA quality and false-negative amplification. In total, 792 plants out of 848 could be scored for these three markers. Nine 96-well plates were screened and each plate is referred to as a series. In the first two series of screening, more recombinants were identified between the marker Nl27 and *Rpi-cap1*, than between Cp58 and *Rpi-cap1*, suggesting that Nl27 was further away from the gene than Cp58. So, in the last seven series, only Nl27 and T179 were used. In total, 85 recombinants were detected, phenotyped, and re-genotyped after CTAB DNA isolation. Out of the 85 recombinants, only 14 F1 individuals were confirmed as true recombinants. False recombinants were mainly due to the SCAR marker Nl27. Later, also, the remaining markers M33 and T081 were tested on the newly identified recombinants. The recombinant population (n= 792), combined with the initial F1 population (n=108), represents a total population of 900 individuals. The genetic map of this *Rpi-cap1* population is presented in Fig. 2. The closest flanking markers to *Rpi-cap1* are the markers Nl27 and M33, both 0.1 cM (one recombinant) away on either side of the gene. In the population of 108 individuals, the distance between *Rpi-cap1* and Nl27 appeared larger because of false positives. The Cp58 marker mapped 0.9 cM (eight recombinants) distal to Nl27.

High-Resolution Mapping of *Rpi-qum1*

Similarly as for *Rpi-cap1*, the mapping position of *Rpi-qum1* was hypothesized to be located on chromosome 11 because of a linked NBS profile marker with homology to *N*-like sequences. We characterized 53 individuals of the *Rpi-qum1* mapping population with *P. infestans* isolate 90128. To confirm the presumed mapping position, the markers linked to *Rpi-cap1* population were also tested on the *Rpi-qum1* population. Unfortunately, Cp58 and M33 were not polymorphic in the *Rpi-qum1* population. Only Nl27 was polymorphic and segregated in the *Rpi-qum1* population. Therefore, an additional known marker ADG2 (Hämäläinen et al. 1998) was tested and was found to be polymorphic and segregating in the *Rpi-qum1* population. Unfortunately, ADG2 was not polymorphic in the *Rpi-cap1* population. The primers designed on the RH sequences did not give any PCR products, like it was the case in the *Rpi-cap1* population. A segregating polymorphism was found only for marker T081. The marker Nl27 was linked to *Rpi-qum1* with one recombinant, T081 was on the other side of the *Rpi-qum1* with two recombinants, and ADG2 was distal to T081 and the gene with four additional recombinants, in a population of 53 individuals (data not shown). It is therefore confirmed that *Rpi-qum1* maps to a syntenic locus as *Rpi-cap1*. In order to achieve fine mapping, a recombinant screen was performed with the markers Nl27, T081, and ADG2 on 349 individuals of the *Rpi-qum1* population. In total, 306 plants could be scored with at least two markers and 21 recombinants were initially

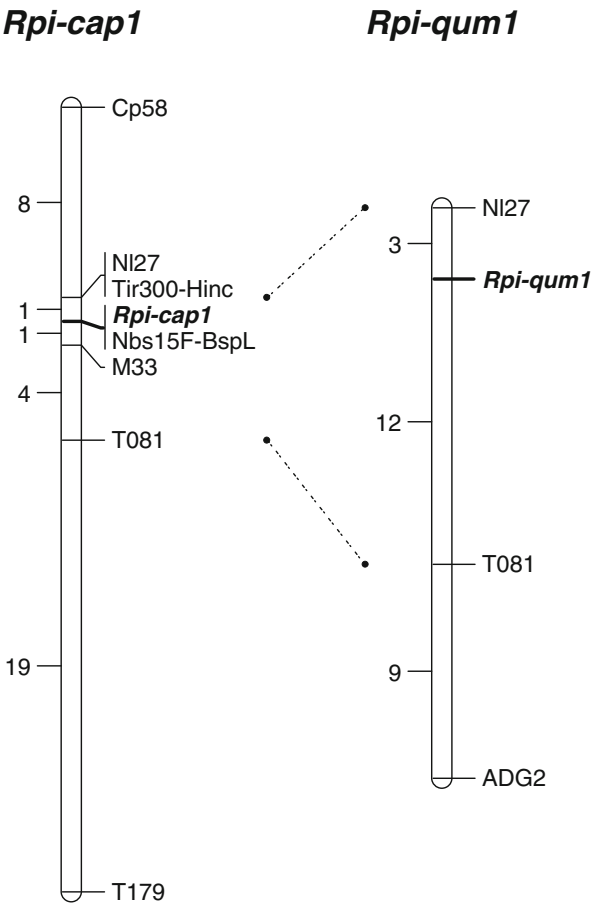

Fig. 2 The genetic maps of *Rpi-cap1* and *Rpi-qum1* on chromosome 11. The *numbers* on the left indicate the number of recombinants out of 900 individuals for *Rpi-cap1* and 306 for *Rpi-qum1*. Most markers are CAPS markers except Nbs15F-BspL and Tir300-Hinc that are *N*-like profiling markers and Nl27 in the *Rpi-cap1* population which is an allele-specific marker. The *dotted lines* connect the markers present in both populations

found between the markers Nl27 and ADG2. Recombinants were re-genotyped and 16 could be confirmed. The 16 confirmed recombinants were maintained in in vitro culture and their resistance phenotype was tested at least twice by DLA. The genetic map of *Rpi-qum1* resulting from that analysis is given in Fig. 2. In the population of 360 individuals, the markers Nl27 and ADG2 were 6.7 cM away from each other (24 recombinants). The closest flanking markers were Nl27 and T081, which were located 0.8 cM (three recombinants) distal and 2.5 cM (12 recombinants) proximal to *Rpi-qum1*, respectively.

Development of Co-Segregating *N*-Like Profiling Marker

Since *Rpi-cap1* and *Rpi-qum1* are presumed to locate in an *N*-like gene cluster, we developed markers on the basis of *N*-gene-specific sequences. We performed this study on *Rpi-cap1* as we here had at our disposal the larger of the two populations. The *N*-like profiling approach was done by following the NBS profiling protocol (van

der Linden et al. 2004) and substituting the NBS degenerate primers for degenerate *N*-like primers (Verzaux et al. 2011). From the 40 primer enzyme combinations tested, 21 markers were identified on the bulks and 10 were confirmed on the individuals of the bulks as linked with the resistance phenotype. Linked markers were identified on both sides of the gene, indicating that the *N* cluster is extending on both sides of the gene. Only the two closest linked markers, Tir300F-Hinc and Nbs15F-BspL, were added to the genetic map (Fig. 2). Marker Tir300-Hinc had one recombinant with *Rpi-cap1* and was co-segregating with marker Nl27. Marker Nbs15F-BspL co-segregated with the resistance in 900 individuals (Fig. 3). This confirmed that *N* homologous sequences are located near *Rpi-cap1* as well as to *Rpi-qum1* and shows that the resistance genes *Rpi-cap1* and *Rpi-qum1* are located in an *N*-like gene cluster, suggesting that *Rpi-cap1* and *Rpi-qum1* are homologous to *N* as well.

Discussion

Resistance to *P. infestans* in the studied genotypes of wild Bolivian accessions of *Solanum* species *S. capsicibaccatum* and *S. circaeifolium* ssp. *quimense* is monogenic and is conferred by dominant genes designated *Rpi-cap1* and *Rpi-qum1*, respectively. Both genes are located in the *N* cluster on chromosome 11. Segregating F1 populations, generated by crossing the selected resistant genotypes with susceptible genotypes, was characterized and CAPS markers from the *N* cluster were developed to construct a genetic map of the region where both *R* genes were located. Next step was to screen for individuals with recombination events between flanking markers to fine map the gene more precisely. The plants with the closest recombination events to *Rpi-cap1* were used to develop an *N*-like-specific marker fully co-segregating with the resistance. This marker can be very useful for future cloning of *Rpi-cap1*. To date, the first functional potato *R* gene located on the long arm of chromosome 11 in the *N* cluster has yet to be cloned (Hein et al. 2009). RH BAC sequences and potato genome sequences (http://www.potatogenome.net) revealed that many *R* gene homologs are present in the *N* cluster, and since gene sequence homologies vary considerably, an allele mining approach as described by Pel et al. (2009) is not feasible. A map-based cloning approach is probably the most appropriate strategy to follow and should be successful as the phenotypic resistance contrast between genotypes is large even though the levels of polymorphism and heterozygosity are relatively low. *Rpi-*

Fig. 3 An *N*-like profiling marker co-segregating with the resistance in 900 F1 individuals from the *Rpi-cap1* population. It was generated with primer Nbs15F in combination with the enzyme BspLI. The phenotype is indicated with *R* for resistant and *S* for susceptible. *On the left*: Screen of the bulks of 10 resistant and susceptible F1 plants, respectively. *From left to right*: the parents, bulks with non-recombinant F1 plants, bulks with F1 plants recombined between the closest markers to the gene. *On the right*: Individual F1 plants including the ones with recombination events close to *Rpi-cap1*. The fourth F1 genotype had a susceptible phenotype and did not contain the marker band of the right size; however, a fragment was observed that was one nucleotide longer

cap1 and *Rpi-qum1* have a very wide resistance spectrum as they are resistant to all 21 isolates tested so far and are thus very interesting genes to include in breeding programs.

In the tested accessions of *S. capsicibaccatum*, the occurrence of resistance to late blight isolate 90128 is an exception, whereas in those of *S. circaeifolium* ssp. *quimense*, it is more the rule. These observations suggest that the occurrence of the resistance in *S. circaeifolium* ssp. *quimense* is well established. In addition to *Rpi-cap1* and *Rpi-qum1*, additional resistance was identified in *S. circaeifolium* ssp. *circaeifolium*. Altogether, these data suggest a strong disease pressure in the locations where *S. circaeifolium* accessions were collected, especially for *S. circaeifolium* ssp. *quimense*. Plants containing *R* genes have a selective advantage in regions favorable for late blight infections and the gene is more easily maintained then in regions where late blight is less frequently occurring.

The wild diploid tuber-bearing species *S. capsicibaccatum* and *S. circaeifolium* ssp. *quimense* are not closely related to *S. tuberosum*. However, hybridization with diploid genotypes of *S. tuberosum* is possible resulting in F1 individuals with different ploidy levels (Louwes et al. 1992). Therefore, in this study, the resistant genotypes were crossed with susceptible genotypes from the series *Circaeifolia*. Marker development was hampered because of a large difference between the *S. tuberosum* sequences used to design the primers and the genomic DNA of the series *Circaeifolia* on which these primers were applied. Crosses, although inter-specific, are within the series *Circaeifolia*, which in this case implied a relatively low level of polymorphism. So, from the large number of primer combinations designed on the *S. tuberosum* RH sequences, the relatively few primer combinations that gave amplification products on the wild species were often not polymorphic. The small phylogenetic distance within the series *Circaeifolia* renders the development of markers to be difficult because of the absence of a sufficient amount of polymorphism. The large phylogenetic distance between these wild species and *S. tuberosum* limits the application of reference genome sequence information in the latter species to speed up the cloning process of *R* genes from the series *Circaeifolia*.

Limitations in marker development would make the chromosome walking step of the map-based cloning approach quite challenging. So, the development of a marker co-segregating with the resistance in a large population is very helpful and could be used for BAC landing. The *Rpi-cap1* population was more suitable for that analysis than the *Rpi-qum1* population as the available population was larger. *N*-like profiling is a CDP approach, derived from NBS profiling, used to identify markers closely linked to *R* genes in the *N* cluster on chromosome 11. *N*-like profiling was also successfully applied to map a late blight *R* gene from *Solanum avilesii* (*Rpi-avl1*; Verzaux et al. 2011) and from *Solanum edinense* (*Rpi-edn3*; Verzaux 2010). Variants of the CDP technique were recently used to specifically tag *R* gene homologs from Tm-2^2, Sw5, Hero, and *R2* gene clusters, for the mapping of the *R8* late blight resistance gene from *Solanum demissum* (Jo et al. 2011). The current study clearly shows that CDP profiling is not only suitable for mapping but also for fine mapping of *R* genes. Because of the presence of multiple copies of *R* gene homologs in *R* gene clusters, high marker saturation in the genomic region of interest is obtained and can efficiently be pursued. In a follow-up research, closely linked or co-segregating CDP markers could be used for BAC landing and BAC walking purposes. It remains,

however, to be shown how effective this works. Upon screening of a BAC library, different target complexities will be encountered as compared to profiling of a full genome, which might result in amplification of different set of polymorphic bands. Our own unpublished results show that this problem can be overcome by appropriate dilutions of the restriction ligation reactions, prior to PCR amplification. Overall, it is concluded that CDP can be very useful to speed up the genetic and physical mapping and BAC walking.

The very similar mapping positions of *Rpi-cap1* and *Rpi-qum1*, the small phylogenetic distance between the two species, their resistance spectrum, and their common geographic origin suggest that they could be the same gene or alleles of the same gene. *Rpi-avl1* from the wild species *S. avilesii* from the series *Tuberosa* also maps on chromosome 11 in the same *N*-like cluster (Verzaux et al. 2011). A resistance gene from *S. edinense* (*Rpi-edn3*), which shows a similar recognition specificity as *R4* from *S. demissum*, also maps on chromosome 11 in the *N* cluster (Verzaux 2011). Both *R4 and Rpi-avl1* have a different, non-overlapping, isolate resistance spectrum than *Rpi-cap1* and *Rpi-qum1*. It shows that *Rpi* genes mapped in the same cluster can have different specificities as also has been observed in the *R3* cluster with the *S. demissum R3a* and *R3b* genes (Huang et al. 2005a, b; Li et al. 2011) and in the *R2* cluster with the *R2* family and *Rpi-mcd1* (Lokossou et al. 2009).

Two major genes conferring resistance to *P. infestans* from wild *Solanum* species can now be added to the *N* cluster, a hotspot for resistance to many pathogens on chromosome 11 (Gebhardt and Valkonen 2001). Because of their map position in the *N* cluster, *Rpi-cap1* and *Rpi-qum1* can be hypothesized to belong to the TIR–NBS–LRR class, similar to the *N* gene. They would be the first major genes conferring resistance to *P. infestans* from that class, as all cloned *Rpi* genes so far belong to the CC–NBS–LRR class (Hein et al. 2009). The *R* genes present in *S. capsicibaccatum* and *S. circaeifolium* ssp. *quimense* appear to be very useful for resistance breeding programs applying a cisgenic approach (Jacobsen and Schouten 2007; Schouten and Jacobsen 2008). Cisgenes are defined as natural genes with their own expression elements that originate from the same plant species or from crossable species. The series *Circaeifolia* was suggested to be a rather primitive, geographically, and phylogenetically isolated series (Hawkes and Hjerting 1989) and was reported to only rarely intercross with species from other series (Louwes et al. 1992). However, successful crosses with other series, *Tuberosa* in particular, have been described: the species *S. capsicibaccatum* and *S. circaeifolium* ssp. *quimense* could both successfully be crossed with *Solanum lignicaule* from the series *Tuberosa* and with di(ha) ploid clones from *S. tuberosum* resulting in true hybrids (Chavez et al. 1988). However, many rounds of backcrosses are anticipated before the resistance traits can be introgressed into a potato cultivar. Therefore, after cloning, *Rpi-cap1* and *Rpi-qum1* would qualify as cisgenes that are preferably introduced into selected potato cultivars by genetic modification. Co-introduction with other *Rpi* genes to obtain a compilation of *R* genes might make it nearly impossible for *P. infestans* to overcome the resistance.

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