

# Mapping of the *S. demissum* late blight resistance gene *R8* to a new locus on chromosome IX

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**Abstract** The use of resistant varieties is an important tool in the management of late blight, which threatens potato production worldwide. Clone MaR8 from the Mastenbroek differential set has strong resistance to *Phytophthora infestans*, the causal agent of late blight. The F1 progeny of a cross between the susceptible cultivar Concurrent and MaR8 were assessed for late blight resistance in field trials inoculated with an incompatible *P. infestans* isolate. A 1:1 segregation of resistance and susceptibility was observed, indicating that the resistance gene referred to as *R8*, is present in simplex in the tetraploid MaR8 clone. NBS profiling and successive marker sequence comparison to the potato and tomato genome draft sequences, suggested that the *R8* gene is located on the long arm of chromosome IX and not on the short arm of chromosome XI as was suggested previously. Analysis of SSR, CAPS and SCAR markers confirmed that *R8* was on the distal end of the long arm of chromosome IX. *R* gene cluster directed profiling markers CDP<sup>Sw54</sup> and CDP<sup>Sw55</sup> flanked the *R8* gene at the distal end (1 cM). CDP<sup>Tm21-1</sup>, CDP<sup>Tm21-2</sup> and

CDP<sup>Tm22</sup> flanked the *R8* gene on the proximal side (2 cM). An additional co-segregating marker (CDP<sup>Hero3</sup>) was found, which will be useful for marker assisted breeding and map based cloning of *R8*.

## Introduction

*Phytophthora infestans*, causing late blight in potato, is one of the most devastating pathogens and threatens food production worldwide. The use of resistant varieties is considered to be the most sustainable approach for the management of late blight. Today, commercial potato crops are mainly protected by the use of disease-free seeds and frequent fungicide application (Fry 2007; Struik and Wiersema 1999). However, considerable financial and environmental costs are incurred for fungicides and their application (Vleeshouwers et al. 2011). Production of disease-free seeds causes considerable additional costs for the refined infrastructure and regular operation of the facilities. Furthermore, the capacity of the pathogen to develop resistance to modern fungicides (Goodwin et al. 1996; Grünwald et al. 2001) necessitates the development of durably resistant varieties. Therefore, breeders have been extremely interested in creating resistant cultivars ever since the first late blight epidemic in Europe in the 1840s that caused the Irish potato famine. Breeding activities at the beginning of the twentieth century have mainly focused on dominant resistance genes, as the complete resistance they conferred was easy to follow and promised a fast and effective way to protect the crop against late blight. Dominant resistance genes were initially identified in the Mexican species *Solanum demissum* and introgressed by crossing and backcrossing into cultivated potato. Eleven potato resistance (*R*) gene differentials from *S. demissum*

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have been identified (Black et al. 1953; Malcolmson and Black 1966). The *S. demissum* *R* genes from MaR1, -R2, -R3, -R4 and -R10 have been introgressed into potato cultivars. However, their durability proved to be a problem due to the rapid appearance of compatible races of the pathogen after market introduction (Wastie 1991). In recent years, other wild species of the genus *Solanum* are also being considered as possible sources of resistance in addition to *S. demissum*. Introgression of these genes into cultivars sometimes requires interspecific bridge crosses (Hermsen and Ramanna 1973). This approach resulted in the introgression of *Rpi-blb2* from *S. bulbocastanum* into the cultivars Toluca (NL 2006) and Bionica (NL 2008) (Haverkort et al. 2009).

In the last two decades, the chromosomal positions of many *R* genes from *S. demissum* have been determined. Eight *R* genes have been mapped; *R1* on chromosome V (Leonards-Schippers et al. 1992), *R2* on chromosome IV (Li et al. 1998), *R3a* and *R3b* (Huang et al. 2004), *R6* and *R7* (El-Kharbotly et al. 1996) *R10* and *R11* (Bradshaw et al. 2006) on chromosome XI. *R5*, *R8* and *R9* have been suggested to be allelic variants of *R3*, located on chromosome XI (Huang 2005). Furthermore, four *R* genes from *S. demissum* have been cloned, *R1* (Ballvora et al. 2002), *R2* (Lokossou et al. 2009), *R3a* (Huang et al. 2005) and *R3b* (Li et al. 2011). In the Mexican species *S. bulbocastanum*, three *R* genes were identified and cloned, the alleles *RB* (Helgeson et al. 1998; Naess et al. 2000; Song et al. 2003) and *Rpi-blb1* (van der Vossen et al. 2003) on chromosome VIII, *Rpi-blb2* on chromosome VI (van der Vossen et al. 2005) and *Rpi-blb3* on chromosome IV (Park et al. 2005). In the wild species *S. pinnatisectum* a dominant *R* gene, *Rpi1*, was mapped by Kuhl et al. (2001) to chromosome VII. On chromosome IX genes from *S. mochiquense* (*Rpi-moc1*; Smilde et al. 2005), *S. phureja* (*Rpi-phu1*; Sliwka et al. 2006), *S. venturii* (*Rpi-vnt1*; Foster et al. 2009; Pel et al. 2009), *S. dulcamara* (*Rpi-dlc1*; Golas et al. 2010) and *S. caripense* (Trognitz and Trognitz 2004; Nakitandwe et al. 2007) were mapped. In *S. berthaultii* three genes, *Rpi-ber* (Rauscher et al. 2006), *Rpi-ber1* and *Rpi-ber2* (Park et al. 2009), were mapped to the long arm of chromosome X. Besides these resistance genes, there are additional sources of resistance from a wide range of species, which have not been located in the genome yet. *S. microdontum*, *S. paucisectum* and *S. stoloniferum* are considered as important resistance sources (Sandbrink et al. 2000; Villamon et al. 2005; Wang et al. 2008).

Despite the rapid breakdown of *R1*, *R2*, *R3*, *R4* and *R10* in the past, *S. demissum* is still considered a valuable source for both race-specific and race-non-specific resistance (Niederhauser and Mills 1953; Colon et al. 1995). Differentials MaR8 and MaR9 were shown to be durably resistant to several *P. infestans* isolates (Haynes et al.

2002), however, *R8* and *R9* have never been used in breeding (Huang 2005). Haynes et al. (2002) evaluated 22 potato clones including seven late blight differentials for late blight resistance in seven US locations in 1997. The authors found that the area under disease progress curve (AUDPC) of MaR8 was very low. Evaluation of the reaction of potato differentials to over 5,000 *P. infestans* isolates, collected in various parts of the world, showed that the resistances of differentials MaR5, MaR8 and MaR9 were most durable (Swiezynski et al. 2000). Also, *P. infestans* isolates derived from clonal lineage US8, the most common and aggressive genotype of *P. infestans* present in the US (Fry and Goodwin 1997) overcame all known *R* gene differentials except MaR8 and MaR9, both in detached leaf assay and in field trials (Bisognin et al. 2002).

The apparent durability of the *R* gene in MaR8 led us to study the molecular basis of the resistance in this plant. Here, we describe the use of dedicated molecular marker techniques [NBS- and cluster directed profiling (CDP)] and genetic analysis of the resistance to *P. infestans* isolate IPO-C (race 1, 2, 3, 4, 5, 6, 7, 10, 11) in MaR8, and provide evidence that this resistance is located on the distal end of the long arm of chromosome IX.

## Materials and methods

### Plant material and mapping population

MaR8, corresponding to 2424a(5) and PI 303149 (Black et al. 1953; Malcolmson and Black 1966), and cultivar concurrent were maintained and multiplied in the laboratory of plant breeding in vitro. MaR8, as resistant female parent, and the susceptible cultivar Concurrent were crossed to generate an F1 mapping population in the summer of 2008 (population code 3020). Seeds were sown under sterile conditions and 100 plants were maintained in in vitro culture.

### *Phytophthora infestans* isolates and disease testing

*Phytophthora infestans* isolate IPO-C (race 1, 2, 3, 4, 5, 6, 7, 10, 11) was kindly provided by Prof. Francine Govers (Laboratory of Phytopathology, Wageningen University). IPO-C was used in detached leaf assays as described by Vleeshouwers et al. (1999) but also to inoculate field trials. In 2009 and 2010, respectively, four and two in vitro plants per genotype from population 3020 were planted in the beginning of June. Spreader rows and the border rows consisted of the susceptible potato cultivars Bintje and Nicola, which served to support a local late blight epidemic. In the beginning of July, the trial fields were

inoculated. For the inoculum production, a large number of detached leaves of potato cultivar Bintje were inoculated with isolate IPO-C. After 6 days, spores were washed off to prepare a spore suspension in large containers. Zoospore release was induced by incubating the containers at 10°C. After the release of the zoospores, the inoculum was adjusted to a concentration of  $5 \times 10^4$  zoospores/ml. At nightfall, the zoospore suspension was sprayed on the potato field using a tractor using a spraying arm. After 2 weeks severe late blight symptoms were observed in susceptible plants and a clear segregation of resistance and susceptibility was observed in F1 population 3020. Scoring was performed in a qualitative way (resistant or susceptible).

#### DNA isolation and marker analysis

Genomic DNA was isolated as described by Fulton et al. (1995). Young leaf tissue was collected for DNA isolation according to the CTAB protocol with the Retsch machine (RETSCH Inc., Hannover, Germany). Primers used for marker analysis are listed in Table 1. PCR reactions were performed using DreamTaq<sup>TM</sup> polymerase (Fermentas) in a standard PCR program (start: 94°C for 30 s; amplification: 35 cycles of 94°C for 30 s, 55°C for 30 s; 72°C for 1 min; termination: 72°C for 2 min).

NBS profiling was performed as described by Van der Linden et al. (2004), with minor modifications. The restriction enzyme digestion of genomic DNA and the ligation of adapters were made in one incubation step. Restriction enzymes *MseI*, *HaeIII* and *RsaI* were used for restriction ligation reactions and NBS primers NBS1, NBS2, NBS3, NBS5a6 and NBS9 in combination with the adaptor primer were used for the successive PCR reactions. Primers with corresponding names and sequences have been described previously (Van der Linden et al. 2004; Wang et al. 2008; Mantovani et al. 2006; Brugmans et al. 2008). Totally, 15 primer enzyme combinations were used for NBS profiling. For *R* gene CDP, *R2* and *Tm-2<sup>2</sup>* primers (R2LF1, R2LF2, R2LF3, R2LF4, R2LR2, R2LR3, R2LR4, Tm1F, Tm1R, Tm3F, Tm3R, Tm6F, Tm15F, Tm15R, Tm19F, Tm19R, Mcq19F, Mcq21R and Mcq23F) were used as described by Verzaux (2010). HotStarTaq<sup>TM</sup> polymerase (QIAGEN) was used in the first PCR and DreamTaq<sup>TM</sup> polymerase (Fermentas) in a second PCR. For designing *Hero*-CDP-primers, *Hero*-like sequences available from NCBI (<http://www.ncbi.nlm.nih.gov/>) and *S. phureja* DM1-3 516R44 (CIP801092) whole genome assembly scaffolds v3 available from the Potato Genome Sequencing Consortium (PGSC; <http://www.potatogenome.net/index.php/Data>; <http://potatogenomics.plantbiology.msu.edu/>) were collected and aligned. Primers were

**Table 1** Markers used for mapping of *R8* in the F1 population MaR8 × Concurrent

Marker name	Primer name	Primer sequence (5' → 3')*	Polymorphism	References
NBS5a6H	NBS5a6	YYTKRTHGTMITKGATGAYRTITGG	<i>HaeIII</i>	Van der Linden et al. (2004)
NBS1 M	NBS1	GCIARWGTWGTYYTTICCYRAICC	<i>MseI</i>	
CDP <sup>Tm2</sup> 1-1	Tm15R	GTAACAAGTCATGTATGCGAC	<i>MseI</i>	Verzaux (2010)
CDP <sup>Tm2</sup> 1-2	Tm15R	GTAACAAGTCATGTATGCGAC	<i>MseI</i>	Verzaux (2010)
CDP <sup>Tm2</sup> 2	Tm19F	GCCAAATAGTATTGTCAAGCTC	<i>MseI</i>	Verzaux (2010)
CDP <sup>Hero3</sup>	Hero4064F	RRAGATTCAGCCATKGARATTAAGAAA	<i>HaeIII</i>	This study
CDP <sup>Sw54</sup>	Sw55F	AGTCTCCAAACATTCCTGCTTCTC	<i>MseI</i>	Dianese et al. (2010)
CDP <sup>Sw55</sup>	Sw55F	AGTCTCCAAACATTCCTGCTTCTC	<i>HaeIII</i>	Dianese et al. (2010)
Stm1021	STM1021 F	GGAGTCAAAGTTTGCTCACATC	SSR	Collins et al. (1999)
	STM1021 R	CACCCTCAACCCCATATC		
TG328	TG328F	TGAATGGACTGGTGATCTGC	SCAR	This study
	TG328R	TTGGAAAGAATTGGCTTTTGA		
184-81	184-81F	CCACCGTATGCTCCGCCGTC	CAPS, <i>RsaI</i>	This study
	184-81R	GTTCCACTTAGCCTTGCTTGTCTCA		
General primers for profiling				
<i>MseI</i> adaptor	Mse-ad-top	CCCGAAAGTATAGATCCCAT		Van der Linden et al. (2004)
	Mse-ad-bottom	TAATGGGATCTATACTT		
Blunt adaptor	B-ad-top	ACTCGATTCTCAACCCGAAAGTATAGATCCCA		Van der Linden et al. (2004)
	B-ad-bottom	TGGGATCTATACTT		
Adaptor primer		ACTCGATTCTCAACCCGAAAG		Van der Linden et al. (2004)

F forward primer, R reverse primer

\*All markers were produced using a standard PCR program (see “Materials and methods”)

designed on cluster specific conserved domains encoding CC and LRR. A total of six *Hero*-CDP degenerate primers were designed and one produced a marker that was linked to *R8* (Table 1). For *Sw5*-CDP seven specific primers described by Dianese et al. (2010) were used. Like in NBS profiling, the CDP primers were used in combination with a labeled adaptor primer (fluorescent dye IRD700) to enable visualization on a denaturing polyacrylamide gel using a NEN® IR<sup>2</sup> DNA analyser (LI-COR® Biosciences, Lincoln, NE, USA). NBS profiling was carried out first on a set of 10 resistant and 10 susceptible F1 plants, including the parents. If in this first round polymorphic bands between the parents and co-segregation of these bands with resistance in the F1 plants were found, a second round of NBS profiling was carried out on genomic DNA of the remaining F1 progeny. If multiple markers are found with one primer/enzyme combination, numbers behind the dash are consecutive numbers ordered from low to high molecular weight produced by the same primer enzyme combination. For example, marker CDP<sup>Tm2</sup>1-1 and CDP<sup>Tm2</sup>1-2 were produced using primer/enzyme combination Tm15R/*Mse*I.

In order to screen for cleaved amplified polymorphic sequences (CAPS), PCR was done using primers listed in Table S1a and successively the PCR products were digested using the restriction enzymes listed in Table S1b. 5 µl of PCR product were added to a 15 µl restriction enzyme digestion according to the manufacturers' instructions.

#### Isolation and sequence analysis of NBS fragments

Fragments were excised as described in the Odyssey® manual for band extraction (Westburg, The Netherlands) and re-amplified with the specific profiling primer and the adapter primer. PCR products were checked on agarose gels and purified with QIAquick PCR purification spin columns (QIAGEN Benelux, The Netherlands). Fragments were cloned into the pGEM-T Easy vector (Promega, USA) prior to sequencing with M13 primers. Sequencing was carried out with the BigDye Terminator kit and an ABI 3700 automated sequencer from Applied Biosystems (USA). Blast analysis of the sequences was performed using the websites from NCBI, PGCS and SGN (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>; <http://potatogenomics.plantbiology.msu.edu/index.php?p=blast>; <http://solgenomics.net>). ClustalX (Jeanmougin et al. 1998) was used to align sequences.

#### Map construction

Co-segregating, simplex-inherited NBS and CDP markers from the tetraploid female parent (*MaR8*) were scored as

dominant markers (Wu et al. 1992). The marker order was determined by TetraploidMap (Hackett and Luo 2003; <http://www.bioss.ac.uk/knowledge/tetraploidmap/>). The map distance was calculated based on the frequency of the recombination between markers.

Publicly available potato and tomato genetic maps from the SH × RH population (Van Os et al. 2006), SGN ([http://sgn.cornell.edu/cview/map.pl?map\\_id=9&show\\_offsets=1&show\\_ruler=1](http://sgn.cornell.edu/cview/map.pl?map_id=9&show_offsets=1&show_ruler=1)) and GABI (<http://www.gabipd.org/database/>) databases were included for comparison of marker positions and synteny.

## Results

### Segregation of resistance in the mapping population

F1 progeny and the parental clones *MaR8* and *cv* Concurrent were screened for resistance against *P. infestans* isolate IPO-C. The detached leaf assay with leaves from greenhouse grown plants turned out not to be suitable for the F1 population. In contrast to the mother plant *MaR8*, the F1 plants showed no clear resistance. Initial screens indicated some variation in resistance, however, these findings were not reproducible for most of the individuals. In contrast, highly reproducible results were obtained in two field trials performed in Wageningen, The Netherlands, in the summer of 2009 and 2010. *MaR8* plants remained devoid of late blight symptoms, while *cv* Concurrent was completely infected within 2 weeks after inoculation. Among 100 F1 genotypes screened, 52 were resistant, 46 were susceptible and 2 showed intermediate phenotypes. This demonstrates that the resistance in *MaR8* is inherited as a dominant simplex allele ( $\chi^2 = 0.54$ ,  $P > 0.05$ ) at a single locus. The corresponding gene is referred to as *R8* hereafter.

### Identification of *R8* flanking markers

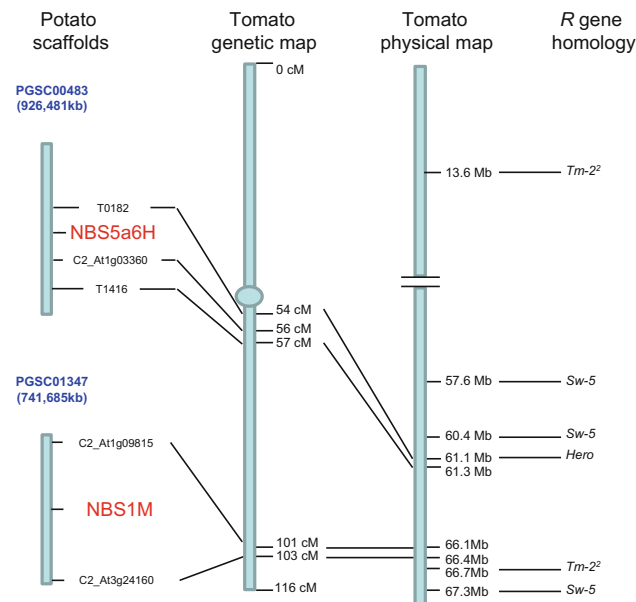
In order to identify markers linked to *R8*, we used NBS profiling since this technique can also give an indication about the *R* gene family of the targeted gene. Initially, NBS profiling experiments were carried out using combinations of the NBS5a6 primer and three enzymes (*Hae*III, *Rsa*I and *Mse*I) on both parents and 10 resistant and 10 susceptible F1 individuals from the mapping population. Marker, NBS5a6H was linked to the resistance phenotype and was found at a frequency of one recombinant in twenty F1 plants. Subsequently, an additional set of NBS primers (NBS1, NBS2, NBS3 and NBS9) was used which resulted in the identification of an additional marker, NBS1M showing linkage to the resistance but without recombinants in twenty F1 plants. The NBS5a6H and NBS1M markers were tested on the complete F1 progeny. 22 additional



recombinants were found between NBS5a6H and *R8*, and three recombinants were identified between NBS1M and *R8*. These recombinants were not overlapping resulting in 26 recombinants between NBS1M and NBS5a6. This showed that the two NBS profiling markers flank the *R8* gene (Fig. 1).

#### Localization of *R8* flanking markers in the genome

The NBS5a6H (361 bp) and NBS1M (301 bp) fragments were cut out of the gel and sequenced (genbank accession numbers: JF317286 and JF317287 respectively). In potato scaffold PGSC0003DMS000000483, a 93% identity match was found for the NBS5a6H sequence. PGSC0003DMS000000483 could be located to chromosome IX using genetic and physical maps of tomato (Fig. 1). NBS1M showed 97% identity to potato scaffold PGSC0003DMS000001347. This scaffold could be linked to the telomeric region at the long arm of chromosome IX using markers C2\_At1g09815 and C2\_At3g24160 (Fig. 1). The proposed inversions between potato and tomato on chromosome IX (Tanksley et al. 1992) did not affect the positioning of the *R8* flanking markers.



**Fig. 1** Positions of NBS profiling markers and *R* gene homologs on chromosome 9. Markers in *large font* indicate the NBS profiling markers that were linked to *R8*. Potato genome sequences, the tomato genome sequences and the marker sequence database from the SGN were searched using the NBS5a6H and NBS1M by BLAST analysis. The bars on the left indicate *S. phureja* scaffolds (PGSC v3). In the middle are the tomato EXPEN 2000 genetic map and the tomato SL2.40 Ch9 physical map (SGN). On the right positions of tomato genome sequences with homology to *R* genes are shown. *Horizontal and diagonal lines* indicate corresponding marker positions in the different maps

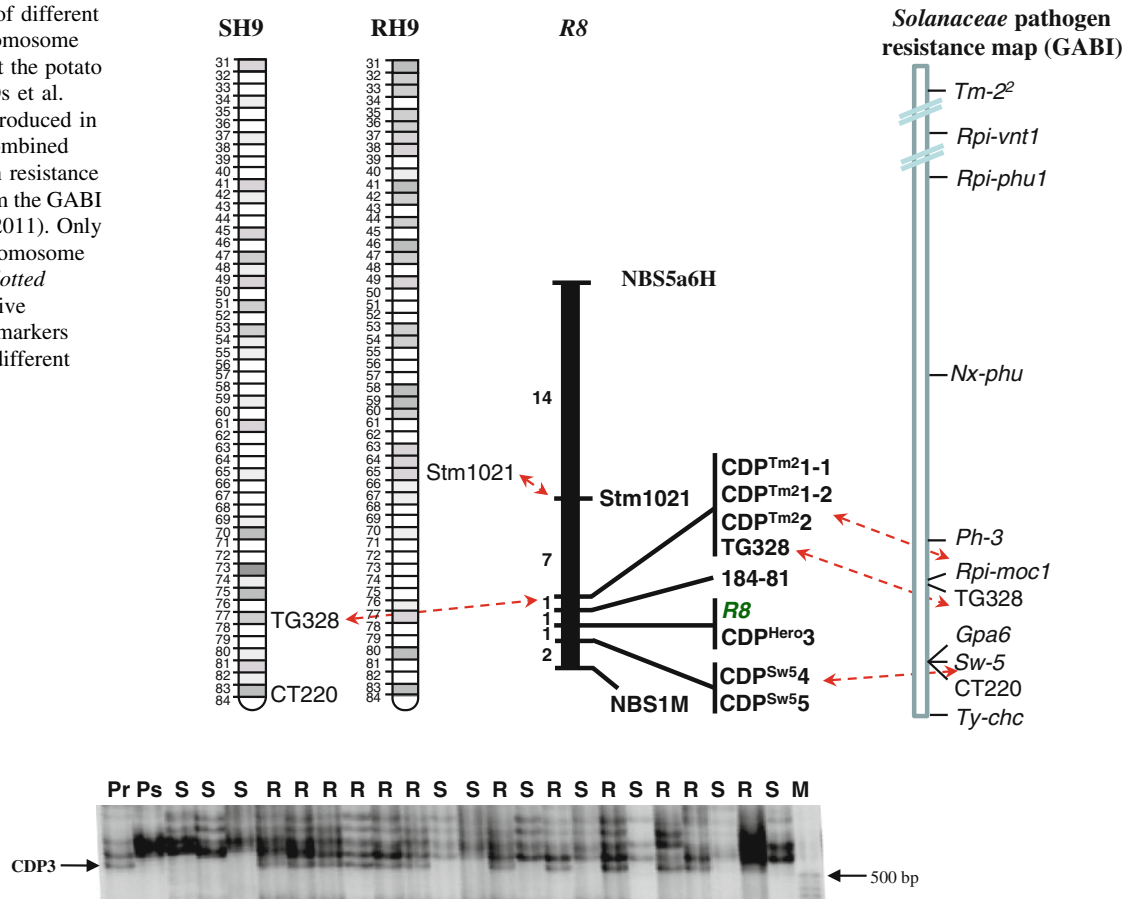
For marker NBS1M, there was no similarity to sequences with known function. The sequence of marker NBS5a6H, however, showed 90% identity to the tomato *Hero* gene (Ernst et al. 2002), which is located on chromosome IV. Apparently, *Hero*-like genes are not only present on chromosome IV but are located in other genomic regions as well (Fig. 1).

#### Localisation of *R8* on chromosome IX

In order to verify that *R8* and its flanking markers were on chromosome IX, more closely linked markers near the *R8* gene were required. Therefore, *R* gene CDP was performed. Two *R* gene clusters known to locate on chromosome IV (*R2* and *Hero*), and two clusters known to locate on chromosome IX (*Tm-2*<sup>2</sup> and *Sw-5*) were targeted for *R* gene-CDP. Using *R2*-CDP no bands linked to the resistance were found among 24 primer/enzyme combinations (data not shown). Three linked markers, CDP<sup>Tm2</sup>1-1 (240 bp), CDP<sup>Tm2</sup>1-2 (345 bp) and CDP<sup>Tm2</sup>2 (120 bp), were identified using *Tm-2*<sup>2</sup> primers out of 36 primer/enzyme combinations. CDP<sup>Tm2</sup>1-1 and CDP<sup>Tm2</sup>1-2 were identified using the same primer enzyme combination (Tm15R/*Mse*I). All *Tm-2*<sup>2</sup>-CDP markers are at 2 cM distance (proximal) from *R8* (Fig. 2). Two markers, CDP<sup>Sw5</sup>4 (277 bp) and CDP<sup>Sw5</sup>5 (165 bp), were identified using *Sw-5*-CDP. Both markers were located at 1 cM to the opposite side (distal) of the *R8* gene as CDP<sup>Tm2</sup>1-1, CDP<sup>Tm2</sup>1-2 and CDP<sup>Tm2</sup>2 (Fig. 2). Interestingly, one fully co-segregating marker, CDP<sup>Hero</sup>3 (500 bp; Fig. 3), was found using *Hero*-CDP out of 18 primer/enzyme combinations (Fig. 2). All CDP markers were excised from the gel and subjected to sequence analysis. CDP<sup>Tm2</sup>1-1 and CDP<sup>Tm2</sup>2 indeed showed similarity to *Tm-2*<sup>2</sup>. CDP<sup>Sw5</sup>4 and CDP<sup>Sw5</sup>5 were confirmed to be similar to *Sw-5*, a *S. lycopersicon* tospovirus resistance gene (Brommonschenkel and Tanksley 1997; Spassova et al. 2001). Unfortunately, the sequences of CDP<sup>Tm2</sup>1-2 and CDP3, remained unresolved due to technical reasons. The relative positions of the *Tm-2*<sup>2</sup> and *Sw-5* homologous markers in the *R8* map are in agreement with relative positions of *Rpi-moc1*, which is homologous to *Tm-2*<sup>2</sup> (Foster et al. 2009) and *Sw-5*, as inferred from publically available genetic maps of chromosome IX (Fig. 2). In addition, the draft sequence of the complete tomato chromosome IX shows that *Tm-2*<sup>2</sup> and *Sw-5* like sequences are located close to each other near the telomere (Fig. 1).

To further confirm the map position of *R8* and the newly identified profiling markers on chromosome IX, known markers (GP101, S2g3, TG591A, GP41, CT220, T0521, S1d11, S1d5-a, T1065, TG328, TG424, and St\_At3g23400) from the SGN and GABI databases on the long arm of chromosome IX were selected and tested for linked

**Fig. 2** Comparison of different genetic maps of chromosome IX. From left to right the potato SHxRH map (Van Os et al. 2006), the *R8* map produced in this study and the combined *Solanaceae* pathogen resistance map as extracted from the GABI website (May 20th, 2011). Only the long arms of chromosome IX are shown. The dotted arrows indicate relative positions of studied markers shared between the different maps



**Fig. 3** Morphology of marker  $CDP^{Hero3}$ . Part of a LI-COR gel containing co-segregating marker  $CDP^{Hero3}$  which was obtained with Hero4064F1/*Hae*III primer/enzyme combination. Pr, Ps, R, S, and M indicate the resistant parent, susceptible parent, resistant and

susceptible F1 genotypes, and molecular weight marker, respectively. The arrow on the left points at the  $CDP^{Hero3}$  band, the right arrow points at molecular weight marker

polymorphisms after digestion with 24 selected restriction enzymes. Only TG328 did display an informative SCAR type polymorphism. A segregation of 87 presence to 12 absence genotypes was found which fits a 5:1 ratio ( $\chi^2 = 0.23$ ,  $>0.05$ ), indicating that the TG328 marker allele is present in duplex in MaR8. TG328 located to SH9 BIN77 of the SHxRH map, was linked to *Rpi-moc1* in the GABI map, and located 2 cM proximal relative to *R8* (Fig. 2). Also three SSR markers (Stm1010, Stm1021, Stm0017) (Milbourne et al. 1998; Collins et al. 1999) were screened and one SSR marker, Stm1021, present in RH9 BIN65 of the SHxRH map located at 9 cM proximal to *R8*. Since no other useful polymorphisms could be found in known genetic markers in this region, we mined for potential polymorphic regions in the potato genome covering this region. Scaffold PGSC0003DM S000000184 which contained the flanking markers TG328,  $CDP^{Tm2-1-1}$  and  $CDP^{Tm2-2}$ , was aligned to the tomato genome and several polymorphic regions were identified. PCR screens within these regions eventually

identified an additional polymorphic marker (184-81), which located 1 cM proximal to *R8* (Fig. 2).

## Discussion

In this study, we report the genetic mapping of the *R8* late blight resistance gene from the differential clone MaR8 to the distal end of chromosome IX. Previously, it was suggested that *R8* was a *R3* family member located on chromosome XI (Huang et al. 2005). This suggestion was generally accepted in literature. However, from this study it is now clear that this is not the case. Rather, *R8* is located distal to Stm1021 and TG328, present in RH9 BIN65 and SH9 BIN77, respectively, of the SHxRH map (Fig. 2, Van Os et al. 2006), and proximal to *Sw-5*. Also *Rpi-edn2*, a gene from *S. edinense* conferring resistance to IPO-C (Verzaux 2010), was located distal ( $\sim 15$  cM) to Stm1021. Interestingly, Stm1021 was found to locate in between two known *R* gene clusters on chromosome IX, distal to the

cluster containing *Rpi-vnt1* (Foster et al. 2009; Pel et al. 2009) and proximal to the cluster containing *Rpi-moc1* (Smilde et al. 2005), which is probably the same as *Rpi-mcq1* (Foster et al. 2009). Both *Rpi-vnt1* and *Rpi-mcq1* were described to be homologous to *Tm-2<sup>2</sup>*, a tomato gene conferring resistance to Tomato Mosaic Virus (Lanfermeijer et al. 2003). This gene is located near the centromere on the long arm of chromosome IX. We conclude that *Tm-2<sup>2</sup>* like sequences are dispersed over the long arm of chromosome IX and are concentrated in at least three different clusters. Besides the *Rpi-vnt1*, *Rpi-mcq1*, *Rpi-edn2* and *R8*, the *Rpi-phu1* gene also was mapped to the long arm of chromosome IX (Sliwka et al. 2006), although more proximal than *R8*, as inferred from map comparisons. The multitude of late blight resistance genes in this genomic region raises the question whether they could represent different alleles of the same gene or whether they are indeed different genes. Mining for *R* gene homologs in this region has revealed several potential *R* gene clusters, suggesting that each cluster could potentially harbor different late blight resistance genes. Primer enzyme combination Tm19F and *Mse*I, used to produce CDP<sup>Tm22</sup>, was previously shown to link to *Rpi-edn2* at 6 cM distance (Verzaux 2010). CDP<sup>Tm22</sup>, however, produced a marker band of a different size. Further research is required to show whether *R8* and *Rpi-edn2* are allelic variants or different genes. Although we show that *Sw-5*, *Tm-2<sup>2</sup>* or *Hero* sequences are present in this part of chromosome IX (Fig. 2), we cannot yet clarify to which family the *R8* gene belongs.

In this study, we mapped eight profiling markers, of which six were *R* gene CDP markers. CDP is a refinement of the motif-directed profiling (MDP) marker technology (Van der Linden et al. 2004). However, it can easily be adapted to target other conserved gene families. For instance, it was adapted for Prx-Profiling (Peroxidase Profiling) in barley to map peroxidase genes and correlate them with QTL map positions for resistance (González et al. 2010). *R* genes from the same cluster usually have similarities in their sequences not shared with other *R* genes (McDowell and Simon 2006; Meyers et al. 2005). So it is possible to design specific primers for a particular *R* gene cluster. In this study, we have adapted the MDP technology to achieve marker saturation in an *R* gene cluster of interest, referred to as CDP. We show that comparative genomics tools can be used to predict chromosomal positions of the sequenced profiling markers. Besides the virtue of homology-based marker landing, an important pitfall is illustrated in this study. Highly similar sequences may sometimes be found in different clusters, as was shown for fragment NBS5a6H. For this fragment, high similarity was found with a sequence of tomato *Hero* gene

that had previously been mapped to tomato chromosome IV (Ernst et al. 2002). The putative map position on chromosome IV for this marker, inferred from the high sequence similarity to *Hero*, was incorrect.

The availability of the sequence assembly of the entire chromosomes from tomato has allowed us to identify multiple gene clusters on the long arm of chromosome IX. These clusters have high levels of homology (>90% identity) to *Sw-5* and *Tm-2<sup>2</sup>* respectively which are physically separated by millions of base pairs. This finding may provide indications as to how *R* gene clusters evolve. Several studies have indicated the role of unequal crossing over, resulting in local duplications leading to rapid evolution of *R* gene clusters (Leister 2004; Kuang et al. 2004; McDowell and Simon 2006). Duplication over long distances, as observed in this study, would suggest an excision and subsequent insertion mechanism. This could be associated with the excision and insertion of retrotransposons, which are present in many *R* gene clusters. An excision-insertion hypothesis for duplication is supported by the finding of a *Hero*-like gene on chromosome IX. This duplication cannot be a result from intra-chromosomal rearrangements such as unequal crossing over.

Finally, we would like to emphasize that differential clone MaR8 is durably resistant to many *P. infestans* isolates (Haynes et al. 2002), but it was not extensively used in breeding (Huang 2005). Localization of *R8* is a first important step for introgression breeding and for molecular cloning of this gene. The combination of several of the *R* genes from *S. demissum* and other wild potatoes using cisgenic modification or pyramiding breeding strategies offer good ways to protect the plant against late blight (Jacobsen and Schouten 2007; Zhu et al. 2011). In this perspective, marker CDP<sup>Hero3</sup> and its closely flanking markers are suitable for tagging *R8* introgressions in breeding material and to distinguish *R8* from other *R* genes in stacking approaches. Furthermore, the identified markers will be instrumental for the map based cloning of the *R8* gene.

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