

# A resistance gene against potato late blight originating from *Solanum* × *michoacanum* maps to potato chromosome VII

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**Abstract** *Solanum* × *michoacanum* (Bitter.) Rydb. is a diploid, 1 EBN (Endosperm Balance Number) nothospecies, a relative of potato originating from the area of Morelia in Michoacán State of Mexico that is believed to be a natural hybrid of *S. bulbocastanum* × *S. pinnatisectum*. Both parental species and *S. michoacanum* have been described as sources of resistance to *Phytophthora infestans* (Mont.) de Bary. The gene for resistance to potato late blight, *Rpi-mch1*, originating from *S. michoacanum* was mapped to the chromosome VII of the potato genome. It confers high level of resistance since the plants possessing it showed only small necrotic lesions or no symptoms of the *P. infestans* infection and we could ascribe over 80% of variance observed in the late blight resistance test of the mapping population to the effect of the closest marker. Its localization on chromosome VII may correspond to the localization of the *Rpi1* gene from *S. pinnatisectum*. When mapping *Rpi-mch1*, one of the first genetic maps made of 798 Diversity Array Technology (DArT) markers of a plant species from the *Solanum* genus and the first map of *S. michoacanum*, a 1EBN potato

species was constructed. Particular chromosomes were identified using 48 sequence-specific PCR markers, originating mostly from the Tomato-EXPEN 2000 linkage map (SGN), but also from other sources. Recently, the first DArT linkage map of 2 EBN species *Solanum phureja* has been published and it shares 197 DArT markers with map obtained in this study, 88% of which are in the concordant positions.

## Introduction

*Solanum* × *michoacanum* (Bitter.) Rydb. is a diploid, 1 EBN (Endosperm Balance Number) nothospecies, a relative of potato originating from the area of Morelia in Michoacán State of Mexico. Damp grassy fields amongst rocks, elevated 2000–2100 m above the sea level (Hawkes 1990) or areas of tropical deciduous forest among grasses, shrubs and cacti of old lava fields (website: *Solanaceae* Source—Natural History Museum) are its habitats. On the basis of morphology and experimental crosses, it is believed to be a natural hybrid of *S. bulbocastanum* × *S. pinnatisectum*, both of which naturally occur in the same region (Hawkes 1990). It was included into “diploid Mexican group” together with both parental species according to genetic diversity revealed by AFLP markers (Jacobs et al. 2008). However, in another study, alternative hypotheses that *S. michoacanum* is not a hybrid species at all or that it is a hybrid between *S. bulbocastanum* and *S. trifidum*, were also formed on the basis of genetic analyses with use of AFLP (Vriesendorp et al. 2007).

Both species generally assumed to be the parents of *S. michoacanum* and *S. michoacanum* have been described as sources of resistance to the potato pathogen, *Phytophthora infestans* (Mont.) de Bary and although neither of

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them can be directly crossed to potato, *S. bulbocastanum* has been widely exploited in potato breeding programs.

Clones highly resistant to *P. infestans* in detached leaflet test as well as the susceptible ones were identified within an accession of *S. michoacanum* VIR5763 and similar variation in tuber resistance was also noted (Jakuczun and Wasilewicz-Flis 2004a). The only documented effort to overcome the crossing barrier between *S. michoacanum* and *S. tuberosum* was through somatic hybridization, which resulted in obtaining several hybrids, three of them with enhanced late blight resistance (Szczerbakowa et al. 2010). Apart from the late blight resistance, clones that are suitable for the potato crisps production (Jakuczun and Wasilewicz-Flis 2004b) and resistant to the green peach aphid were also found within *S. michoacanum* (Radcliffe et al. 1974).

A major late blight resistance locus *Rpi1* was identified in the *S. pinnatisectum* and mapped to potato chromosome VII using an interspecific cross with *S. cardiophyllum* (Kuhl et al. 2001). Later, as a starting point to cloning of this gene, two BAC libraries were constructed from *S. pinnatisectum* and four markers linked to the *Rpi1* gene were hybridized to 14 BAC clones of these libraries (Chen et al. 2004). So far, the sequence of this gene has not been published. However, several attempts to exploit the resistance in *S. pinnatisectum* have been undertaken. A single aneuploid hybrid between *S. pinnatisectum* and *S. tuberosum* was obtained by double pollination and embryo rescue and it was shown to be resistant to late blight (Ramon and Hanneman 2002). Somatic hybridization has been used successfully several times in order to transfer late blight resistance from *S. pinnatisectum* to *S. tuberosum* (Thieme et al. 1997; Szczerbakowa et al. 2005; Greplová et al. 2008; Polzerová et al. 2010), although only one of the obtained resistant hybrids was further backcrossed to *S. tuberosum* (Thieme et al. 1997). *S. pinnatisectum* is also a source of resistance to Colorado potato beetle (Chen et al. 2003, 2004; Nandy et al. 2008) and tuber soft rot caused by *Pectobacterium carotovorum* (syn. *Erwinia carotovora* ssp. *carotovora*) (Hawkes 1994).

So far, four R genes for late blight resistance have been cloned from *S. bulbocastanum*. The fifth one, *Rpi-abpt* is likely, but not definitely originating from this species (Park et al. 2005b). The first *S. bulbocastanum* R gene for *P. infestans* resistance was identified on potato chromosome VIII, independently by two research groups. Under the name *RB*, it was mapped (Naess et al. 2001) and cloned (Song et al. 2003) using BC2 populations derived from somatic hybrids (Helgeson et al. 1998). The same gene, named *Rpi-blb1*, was cloned using an F1 population of the intraspecific *S. bulbocastanum* cross (van der Vossen et al. 2003). A highly similar gene originating from the same species, *Rpi-bt1*, was mapped also to the potato

chromosome VIII and then cloned (Oosumi et al. 2009). The *Rpi-blb2* gene was mapped on chromosome VI in several tetraploid backcrosses resulting from complex bridge crossing named ABPT (Hermsen and Ramanna 1973) that involved *S. acaule*, *S. bulbocastanum*, *S. phureja* and *S. tuberosum* and then cloned using the intraspecific cross (van der Vossen et al. 2005). Two other genes, *Rpi-blb3* and *Rpi-abpt* (Hermsen and Ramanna 1973) were mapped to potato chromosome IV (Park et al. 2005a, b) and also subsequently cloned (Lokossou et al. 2009). The materials from the bridge crossing mentioned above were involved in the potato breeding programs that yielded at least four cultivars: Biogold, Bionica, Kibama and Kisoro (website: Potato Pedigree Database, Wageningen University). The genes *Rpi-blb1* and *Rpi-blb2* are also being introduced into potato cultivars via cisgenesis (website: SeedQuest: Deliberate release into the E.U. environment of GMOs). Somatic hybridization was another method quite frequently applied for obtaining hybrids between *S. bulbocastanum* and *S. tuberosum* resistant to *P. infestans* (Thieme et al. 1997; Helgeson et al. 1998; Szczerbakowa et al. 2003; Bołtowiec et al. 2005; Greplová et al. 2008), but so far, this approach has not resulted in cultivar registration.

Since the resistance to *P. infestans* of both *S. pinnatisectum* and *S. bulbocastanum* is well known and a number of R genes have been identified in these species, *S. michoacanum* seemed to be a promising source of this trait. The goal of this study was to characterize the late blight resistance in an intraspecific *S. michoacanum* cross and to map the underlying R gene, named *Rpi-mch1*.

## Materials and methods

### Plant material

A resistant clone 99-12/8 and a susceptible clone 99-12/12 selected from the *S. michoacanum* accession VIR5763 were crossed to obtain an F1 mapping population of 164 individuals. Along with the mapping population and the parental clones, five standard cultivars: Eersteling, Tarpan (susceptible to late blight), Bzura (resistant, possessing *R1* and an unidentified other R gene), Escort [resistant, with *R1*, *R2*, *R3* and *R10* (Bormann et al. 2004)] and Robijn (moderately resistant to *P. infestans*) were included in tests of resistance to *P. infestans*.

### *Phytophthora infestans* isolates

A preliminary test on *S. michoacanum* parental clones and potato cultivars Tarpan and Bzura was performed in order to choose *P. infestans* isolates that can differentiate best the

**Table 1** Characteristics (origins, mating type, race defined on Black's differentials) of *P. infestans* isolates tested on the parental clones of the *S. michoacanum* mapping population and the standard cultivars

Isolate	Year, place and region of origin	Mating type	Race	99-12/8	99-12/12	Tarpan	Bzura
MP585	2004, Kurzętnik, warmińsko-mazurskie	A1	1.2.3.4.5.6.7.10.11	8.6 ± 0.5	5.1 ± 0.5	1.0 ± 0.0	1.0 ± 0.0
MP717	2006, Bystrz, warmińsko-mazurskie	A2	1.3.4.7.10.11	8.2 ± 0.4	7.0 ± 0.9	1.0 ± 0.0	5.3 ± 1.5
MP778	2006, Boguchwała, podkarpackie	A1	1.(2).3.4.(5.6).7.(8.9).10.11	7.0 ± 0.6	1.8 ± 0.4	1.0 ± 0.0	1.0 ± 0.0
<b>MP847</b>	<b>2007, Boguchwała, podkarpackie</b>	<b>A2</b>	1.3.4.7.10.11	<b>9.0 ± 0.0</b>	<b>1.1 ± 0.2</b>	<b>1.0 ± 0.0</b>	<b>9.0 ± 0.0</b>
MP919	2008, Babienica, śląskie	A2	1.3.4.(6).7.10.11	9.0 ± 0.0	7.7 ± 1.8	1.5 ± 0.8	4.3 ± 2.1
MP920	2008, Nadzów, małopolskie	A2	1.3.4.(6).7.10.11	8.5 ± 0.5	2.8 ± 1.7	1.0 ± 0.0	2.3 ± 0.6
<b>MP921</b>	<b>2008, Młochów, mazowieckie</b>	<b>A2</b>	1.3.4.(6).7.10.11	<b>8.5 ± 0.5</b>	<b>2.0 ± 0.9</b>	<b>1.0 ± 0.0</b>	<b>7.8 ± 1.1</b>
MP1161	2009, Boguchwała, podkarpackie	nt	1.3.4.7.10.11	6.0 ± 1.3	1.0 ± 0.0	1.0 ± 0.0	2.0 ± 0.8

The mean resistance (6–18 leaflets) of 99-12/8 (resistant parent), 99-12/12 (susceptible parent), cvs. Tarpan and Bzura to each of the isolates is shown in 1–9 scale, where 9 is the most resistant ± standard deviation. All isolates come from Poland; *nt* not tested. Virulence factors in brackets were not effective in each test. The isolates chosen for testing of the mapping population are bolded

resistant and the susceptible parent (Table 1). In 2009, the isolates MP585 and MP847 were tested, and in 2010 so were the isolates MP717, MP778, MP919, MP920, MP921 and MP1161. Some characteristics of these isolates are shown in Table 1. All the tested *P. infestans* isolates originate from Poland and belong to the IHAR-PIB Młochów Research Centre collection. Two chosen isolates were used in the late blight resistance tests of the mapping population: MP847 in 2009 and MP921 in 2010. The MP847 was isolated in 2007 in Boguchwała near Rzeszów, Poland, from the potato cv. Lawina, while the MP921 was collected in 2008 in Młochów near Warszawa, Poland, from the potato cv. Alicja. Both isolates were of A2 mating type and Ia mitochondrial haplotype, sensitive to meta-laxyl, race: 1.3.4.7.10.11 but only MP921 was able to infect cv. Bzura to some extent (Table 1). The *P. infestans* cultures were stored in vitro on rye A agar slopes (Griffith et al. 1995) covered with mineral oil, in darkness at 5°C. They were propagated and maintained for a short-term also on rye A medium, in Petri dishes kept in darkness at 16°C. In all resistance tests a set of Black's differentials obtained from Scottish Agricultural Science Agency, Edinburgh, UK was used to monitor the effective virulence of *P. infestans* isolates. Before each resistance test, the isolate was multiplied at least twice on susceptible potato tissue.

#### Late blight resistance assessment

The resistance of the parental clones, mapping population and standard cultivars to *P. infestans* was evaluated in two subsequent years 2009 and 2010 by the laboratory detached leaf test. The fully expanded leaves were detached from 6-week-old greenhouse-grown plants and placed on wet wood wool with the abaxial side up. They were inoculated by spraying with the sporangia and zoospore suspension (50 sporangia/μl) similarly as described by Kuhl et al.

(2001). Next day the leaves were turned over, the adaxial side up. After 4 days of incubation in conditions supportive for disease development (high relative humidity, 16°C and constant light of about 1,600 lx), six leaflets per leaf (three pairs adjacent to the terminal leaflet) were scored separately in a 1–9 scale, where 9 was the most resistant (Śliwka et al. 2007). In 2009, the test with the *P. infestans* isolate MP847 was performed on two dates and each time two replications × two leaves × six leaflets per genotype were scored giving in total 48 leaflets evaluated per genotype. In 2010, the test with the isolate MP921 was done on three different dates and each time two replications × one leaf × six leaflets per genotype (36 leaflets in total) were scored. A genotype was considered resistant, i.e. possessing the R gene, when its mean resistance score was ≥6.

#### DNA isolation, sequence-specific markers

Genomic DNA was extracted from 1 g of fresh, young leaves of greenhouse grown plants with the DNeasy Plant Maxi kit (Qiagen, Hilden, Germany). All sequence-specific markers listed in ESM 1 were amplified using the following conditions. The reaction mixture of 20 μl contained 2 μl of 10× PCR buffer, the four deoxynucleotides (0.1 mM; Sigma-Aldrich, St. Louis, MO, USA), MgCl<sub>2</sub> (1.5 mM; Invitrogen™ by Life Technologies™), primers (0.2 μM; Sigma-Aldrich, St. Louis, MO, USA), *Taq* polymerase (0.05U/μl; Invitrogen™ by Life Technologies™) and 10–30 ng of the template DNA. The PCR program was: 94°C–180 s; 40 cycles of: 94°C–30 s, 55°C–45 s, 72°C–90 s; 72°C–420 s, if the annealing temperature was modified, it has been marked in ESM 1. The reactions of PCR products with corresponding restriction endonucleases (Fermentas Life Sciences, Thermo Fischer Scientific Inc.), also listed in ESM 1, were performed according to the producer's recommendations.

## Diversity Array Technology

The analyses were performed by Diversity Array Pty Ltd. Canberra, Australia. The process of DArT marker discovery involved construction of 18 bacterial clone libraries using methods described by Wenzl et al. (2004), ranging in size from 384 to 4,992 clones and totalling 23,040 clones. These clones were tested for polymorphism using several hundred potato accessions and 7,680 most polymorphic markers were selected for re-arranging into a genotyping array. Each clone on the array was printed in duplication using equipment and methods described recently in detail by Sansaloni et al. (2010).

Genomic complexity reduction method is a critical component of DArT technology, selecting reproducibly the low copy fraction of a plant genome (Jaccoud et al. 2001). It usually involves using “methyl filtration” with *Pst*I Restriction Enzyme (RE) in combination with a frequently cutting, methylaton independent RE. After a number of tests performed in the preliminary experiment (data not presented) the *Pst*I/*Taq*I method was selected for potato marker discovery. The assay involving restriction digestion reaction combined with adaptor ligation followed by PCR amplification of the intact (and relatively small) *Pst*I fragments used methodology described by Akbari et al. (2006). Prior to performing the assay each DNA sample was tested for presence of DNAses through incubation with RE buffer for 2 h at 37°C. After passing this QC test each sample was processed in the same manner and resulting PCR products were run on 1.2% agarose gel to verify uniform digestion and amplification.

All successful amplification products were labelled with either Cy3 or Cy5 fluorescent dye and hybridised to the array developed by international potato-DArT consortium.

After overnight hybridisation at 63°C the slides were washed and scanned on Tecan LS300 scanner as reported by Akbari et al. (2006). Feature identification and marker classification was performed by DArTsoft version 7.4.4 developed by DArT Pty Ltd. (website: Diversity Array Technology Software). Binary (0/1) scores were used for map construction.

## Statistical and linkage analyses

The categorization of the individuals of the mapping population into classes of resistant and susceptible ones was done on the basis of weighted mean leaflet resistance (2009–2010) because of different numbers of leaflets tested in each year. Fitness to the normal distribution of the phenotypic data was checked by the Kolmogorov–Smirnov test. The reproducibility of the resistance tests between the years was evaluated by linear Pearson’s correlation coefficient. Marker-trait linkages and determination coefficients

( $R^2$ ) were estimated by the Student’s *t* test and analysis of variance, respectively. Fitness of segregation to the expected ratio was checked by the  $\chi^2$  test. All statistical analyses were performed using computer program STATISTICA for Windows (Stat Soft, Inc., Tulsa, OK, USA). Linkage analyses were performed using JoinMap<sup>®</sup> 4 (Van Ooijen 2006) with following settings: CP population type (creating of maternal and paternal linkage maps first and then creating a common population map), independence LOD as a grouping parameter (linkages with LOD > 3 were considered significant), regression mapping algorithm and Haldane’s mapping function.

## Results

### Late blight resistance assessment

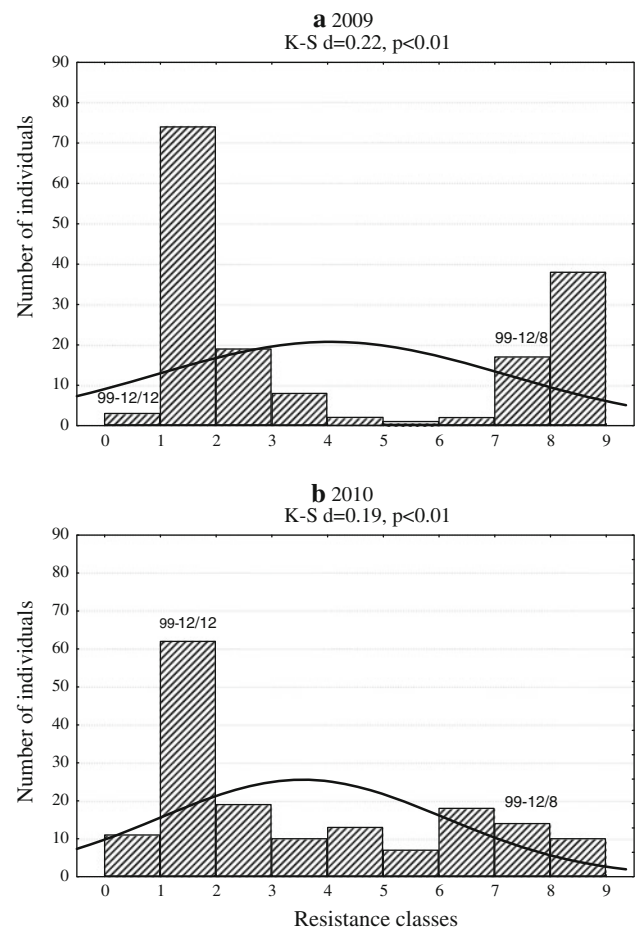
Parent 99-12/8 was resistant and parent 99-12/12 was susceptible to both *P. infestans* isolates used in 2 years of testing. In 2009 (*P. infestans* isolate MP847) mean scores of clones 99-12/8 and 99-12/12 were 7.6 and 1.0, respectively, while in 2010 (isolate MP921) the clones 99-12/8 and 99-12/12 were scored 7.3 and 1.4, respectively. Mean scores of the standard cultivars were according to the expectations and as follows: Eersteling (2009-3.2, 2010-6.2) and Tarpan (2.6, 5.8) were susceptible, Bzura (9.0, 7.8) and Escort (9.0 in both years) were resistant and Robijn (5.3, 6.0) was moderately resistant to *P. infestans*. Although the infection of the susceptible cultivars in 2010 was weak, the infection pressure estimated as mean score of all progeny, parents and standards was stronger in 2010 (score 3.5) than in 2009 (4.1). The mean results of the late blight resistance tests obtained for progeny in 2009 and 2010 were strongly correlated, with Pearson’s correlation coefficient  $r = 0.863$ ,  $P < 0.000$ . Analysis of variance showed significant effects of genotype, year, genotype  $\times$  year interaction and year  $\times$  date interaction on the resistance (Table 2). The genotype had the strongest influence on the resistance results explaining more than 76% of variance observed in the results (Table 2). Distributions of mean late blight resistance scores of the mapping population obtained in 2009 and 2010 deviated significantly from the normal distribution (Fig. 1) which was confirmed by the Kolmogorov–Smirnov test. The range of late blight grades observed in the mapping population was 1.0–9.0. The division of these results into two classes of resistant (mean score  $\geq 6$ ) and susceptible ones (mean score  $< 6$ ) was clearly noticeable in both 2009 and 2010, although it was sharper in 2009 (Fig. 1a). When weighted mean resistance result from both years was taken into account, 59 individuals from the mapping population were scored as resistant and 105 individuals were susceptible. That ratio is



significantly deviated ( $\chi^2 = 12.90$ ,  $df = 1$ ,  $P < 0.000$ ) from the 1:1 ratio expected under the assumption that a single resistant gene is segregating in this population. However, it is not significantly different from 1: 2 ratio ( $\chi^2 = 0.51$ ,  $df = 1$ ,  $P < 0.473$ ) indicating rather segregation distortion than the effect of other gene(s).

### Linkage map

The linkage map of *S. michoacanum* was constructed of 846 dominant markers, including 51 sequence-specific reference markers listed in ESM 1 and 792 DArT markers. Using both marker types and the JoinMap<sup>®</sup> 4, we obtained maternal and paternal genetic maps consisting of 12 linkage groups each (not shown). In a next stage, a common CP linkage map was made for 11 chromosomes, with an exception of chromosome VII, where due to a small number of markers (28), it was not possible (ESM 2). While only markers inherited from either 99-12/8 or 99-12/12 were assembled into, respectively, maternal and paternal map, the common CP map was enriched with large number of dominant markers which in both parents were in a heterozygous state. Within 51 sequence-specific reference markers, two marker bands derived from the PCR fragment C2\_At3g15430 (SGN) were mapped to a different chromosome than expected (chromosome II instead of VII) and one marker, GP78 (Śliwka et al. 2007) was scored but not mapped. The remaining 48 reference markers were paired in an expected manner, i.e. the each two of them derived from the same parent and from the same of the 12 chromosomes were linked together, which allowed us to identify the obtained linkage groups (ESM 2, Table 3). The order of the sequence-specific markers in most of the cases i.e. on chromosomes I, II, III, IV, VII, VIII, IX, X (SGN) and XII (GABI) corresponded well to the order on reference maps. There were the following exceptions: on chromosome V two markers inherited from different parents but derived from the same PCR fragment U227536 digested with two different endonucleases (ESM1) mapped



**Fig. 1** Distributions of mean leaflet resistance to *P. infestans* in whole leaf tests performed with the isolate MP847 in 2009 (a) and with the isolate MP921 in 2010 (b) in the mapping population of *S. michoacanum*. The resistance was assessed in 1–9 scale, where 9 means the most resistant. The fitness to the normal curve: K–S Kolmogorov–Smirnov test, d – coefficient calculated for this test, p – probability, the line indicates the normal curve. Resistance levels of parental clones are marked with their names: 99-12/8 and 99-12/12

to different positions 48 cM apart (ESM2); on chromosome VI markers C2\_At2g28690, C2\_At2g39690 and Mi closely linked on Tomato-EXPEN 2000 map (SGN: 3.7 cM, 5.3 cM

**Table 2** Analysis of variance in mean resistance scores in the mapping population of *S. michoacanum*, repeated in two dates in 2009 with *P. infestans* isolate MP847 and three dates in 2010 with *P. infestans* isolate MP921

Factor	$df^a$ effect	Mean sum of squares effect	$df$ error	Mean sum of squares error	$F$	$p$	$R^2$ (%) <sup>b</sup>
{1}date	2	0.54	1.38	77.40	0.01	0.993	n.s.
{2}year	1	127.75	1491	71.80	48.15	0.000	0.80
Interaction: 1 x 2	1	71.81	1655	9.61	7.47	0.006	0.45
{3} genotype	165	72.88	1491	6.37	27.47	0.000	76.65
Interaction: 2 x 3	165	6.05	1328	2.24	2.70	0.000	6.20

n.s. not significant

<sup>a</sup> Number of degrees of freedom

<sup>b</sup> Percent of variance explained

**Table 3** *S. michoacanum* linkage map and its comparison to the reference *Solanum phureja* diploid map 2010 (SGN)

Chromosome	Number of markers	Number of reference markers	Length (cM)	Number of markers common with <i>S. phureja</i> map:	
				located in concordant position	located in discordant position
I	133	4	116.3	23	3
II	75	4 (+2 <sup>a</sup> )	108.4	29	1
III	87	4	93.1	22	10
IV	73	4	99.7	17	–
V	32	3 (+1 <sup>b</sup> )	66.8	1	–
VI	74	5	128.2	2	–
VII	28	4	73.1; 64.1 <sup>c</sup>	2	4
VIII	99	4	68	41	–
IX	118	4	86	30	1
X	39	4	95.9	3	–
XI	48	4	54.5	2	1
XII	40	4	63	1	4
<b>Total</b>	<b>846</b>	<b>48 (+3<sup>ab</sup>)</b>	<b>1047</b>	<b>173</b>	<b>24</b>

<sup>a</sup> markers that on Tomato-EXPEN 2000 and Pepper-COSII maps were located on chromosome VII (SGN); <sup>b</sup> markers scored but unmapped; <sup>c</sup> values for the two linkage maps of the parents are given separately, because the amount of polymorphic and segregating markers did not allow us to construct the common map, the arithmetic mean of these two values was used to calculate total length of the map

and 5.5 cM, respectively) were further apart and in a different order on *S. michoacanum* map; markers C2\_At1g07960 and C2\_At2g28490 (Tomato-EXPEN 2000 map: 82.5 and 98 cM, respectively) from chromosome XI mapped to a different chromosome arm than expected (ESM2).

The total length of the *S. michoacanum* genetic map reached 1,047 cM. The length of particular chromosomes varied from 54.5 cM (chromosome XI) to 128.2 (chromosome VI) with the average value of 87.3 cM (Table 3). Chromosome I was one of longest but also the richest in markers, with 133 markers located on this chromosome, while the smallest number of markers (28) was mapped to the two parental linkage groups corresponding to the chromosome VII. The average number of markers per chromosome was 70.25 (Table 3). The theoretical mean interval calculated as the total map length (in cM) divided by the total number of markers was 1.2 cM and differed from the observed mean interval, calculated as a mean value of all intervals between the markers, that reached 2.2 cM, which indicated that the markers were not distributed evenly. The highest marker density was on chromosome VIII, where the observed mean interval between markers was 0.7 cM. The biggest observed mean interval between markers was 10.4 cM on chromosome VII of the clone 99-12/8.

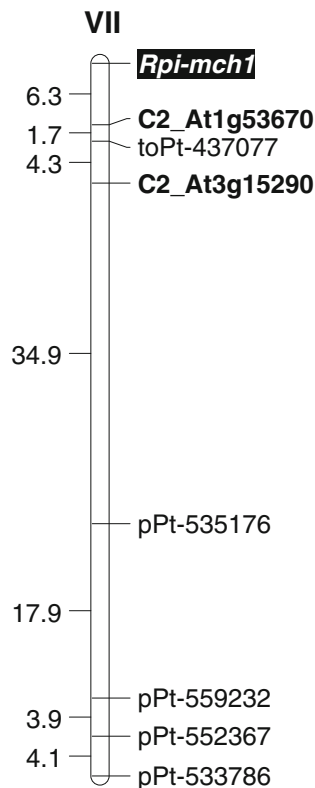
#### The *Rpi-mch1* gene

A resistance gene against potato late blight was mapped on chromosome VII of the resistant parent 99-12/8 (Fig. 2).

Only five DArT markers and two sequence-specific markers mapped to the same linkage group and all of them were located on the same side of the gene (ESM2). Three markers showed statistically significant linkage with the trait confirmed by the *T* test (Table 4). These marker-trait associations were highly significant irrespective of the year of testing and the isolate used for testing the resistance to *P. infestans* and were also clear when mean weighted resistance was taken into account (Table 4). The CAPS marker C2\_At1g53670 (Fig. 3) was the closest to the gene and located 5.7 cM from it. Its effect explained 82.7% of variance observed in phenotypic resistance assessment in 2009, when the *P. infestans* isolate MP847 was applied and 67.6% in 2010 when the isolate MP921 was used (Table 4).

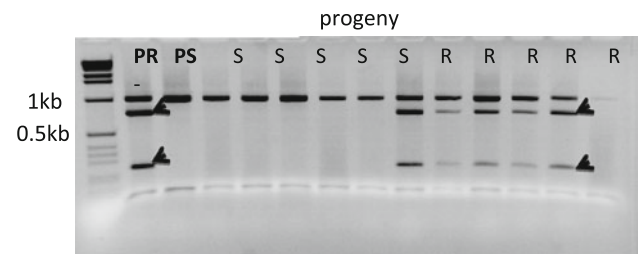
#### Discussion

A gene for resistance to potato late blight, *Rpi-mch1*, originating from *S. michoacanum* was mapped to the chromosome VII of the potato genome. It is a gene which confers high level of resistance since the plants possessing it showed only small necrotic lesions or no symptoms of the *P. infestans* infection. Its localization on chromosome VII possibly corresponds to the localization of the *Rpi1* gene from *S. pinnatisectum* (Kuhl et al. 2001) and it could be a second gene for resistance to *P. infestans* mapped in this region, although the lack of flanking markers prevents us from drawing conclusions. This position would be in a



**Fig. 2** Genetic linkage map of the *S. michoacanum* 99-12/8 chromosome VII showing the location of the late blight resistance gene marked by *black rectangle*. The reference PCR markers are bolded. On the left, interval genetic distances in cM are given

good agreement with the hypothesis that *S. michoacanum* is a natural hybrid of *S. pinnatisectum* and *S. bulbocastanum* (Hawkes 1990) and we still cannot exclude that both genes are homologous or even identical. Similarly to Kuhl et al. (2001) we noted in the mapping population resistance segregation ratio skewed towards the susceptibility, although in case of *S. michoacanum* population it was stronger. Even though in the Polish *P. infestans* population we find isolates that are virulent to the *Rpi-mch1* gene, it could be used in potato breeding, especially in the resistance gene pyramids with other *R* genes, if only the crossing barrier were overcome. Within the eight *P. infestans* isolates that were tested on the parents of the



**Fig. 3** The CAPS marker C2\_At1g53670 digested with *RsaI* mapped 5.7 cM from the *Rpi-mch1* gene. PR – resistant parent 99-12/8, PS – susceptible parent 99-12/12, R, S – progeny resistant and susceptible to *P. infestans*, respectively, - negative control. Arrows indicate the marker bands

*S. michoacanum* mapping population (Table 1), two were compatible (MP778 and MP1161), while the rest did not cause sporulating lesions on the leaves of the resistant parent, 99-12/8. On the basis of the virulence of these isolates we can conclude that the *Rpi-mch1* virulence spectrum might be similar or equal to the one conferred by *R8* and/or *R9* genes from *S. demissum* (Table 1) which also resembles the spectrum of the *Rpi1* gene (Kuhl et al. 2001). Several other resistance loci have been mapped in various *Solanum* species within the same chromosomal segment as *Rpi-mch1* and *Rpi1* genes. Among them there are *Gro1* gene for resistance to *Globodera rostochiensis* originating from *S. spegazzinii* (Barone et al. 1990), *I1* and *I3* genes from *S. pennellii* for resistance to *Fusarium oxysporum* (Sarfatti et al. 1991, Bournival et al. 1989) as well as potato QTL for resistance to *Erwinia carotovora* ssp. *atroseptica* (Zimnoch-Guzowska et al. 2000) and potato QTL for late blight resistance (Leonards-Schippers et al. 1994), indicating once again the tendency of resistance loci to cluster.

When mapping *Rpi-mch1*, we constructed one of the first genetic maps made of DArT markers of a plant from the *Solanum* genus and the first map of *S. michoacanum*, a 1EBN potato species. We identified particular chromosomes using sequence-specific PCR markers, originating mostly from the Tomato-EXPEN 2000 linkage map (SGN), but also from other sources (ESM 1). The first mapping of 1EBN *Solanum* species, *S. bulbocastanum*, with the use of DArT markers resulted in obtaining 12 linkage groups

**Table 4** *P* values (*T* student test) of the significant marker-trait linkages and the percentages of variance in *P. infestans* resistance ( $R^2$ ) explained by those linkages. All the markers are located on chromosome VII and are derived from the resistant parent 99-12/8

Marker	<i>P</i> , <i>T</i> student test			$R^2$ (%)		
	Resistance to MP847, 2009	Resistance to MP921, 2010	Weighted mean resistance 2009–2010	Resistance to MP847, 2009	Resistance to MP921, 2010	Weighted mean resistance 2009–2010
C2_At1g53670 <i>RsaI</i>	0.000	0.000	0.000	82.7	67.6	82.2
toPt-437077	0.000	0.000	0.000	75.4	61.4	75.2
C2_At3g15290 <i>TaqI</i>	0.000	0.000	0.000	68.5	54.6	67.4

consisting of 439 markers with a total map length of 403 cM. However, those linkage groups have not been assigned to the potato chromosomes, so far (Mann et al. 2011). Recently, the first DArT linkage map of 2 EBN *Solanum* species has been published: *Solanum phureja* diploid map 2010 (SGN), which enabled comparison between this map and ours (ESM 2, Table 3). The *S. phureja* diploid map 2010 consists of 2530 markers, including 1827 DArT markers, 316 Simple Sequence Repeat markers and 387 Single Nucleotide Polymorphisms. Out of 197 DArT markers that were common for both maps, 173 (88%) were in corresponding positions on both maps and 24 (12%) in discordant positions (ESM 2). Most of the common and concordant markers were located on chromosomes VIII, IX, I, II, III and IV, ranging from 17 to 41 per particular chromosome. On remaining chromosomes we found 1–3 DArT markers in positions concordant with the *Solanum phureja* diploid map 2010 (SGN). On chromosomes I, II, III, VII, IX, XI and XII we mapped 24 DArT markers that were located on different chromosomes on the *S. phureja* diploid map 2010 (SGN). Ten of them, located on chromosome III of *S. michoacanum*, were dispersed over chromosomes II, VII, X and XI of the *Solanum phureja* diploid map 2010 (SGN). The causes of this discrepancy could be either genuine differences between the genomes of these two distant *Solanum* species or mapping errors. Although the overall quality and resolution of the *S. michoacanum* linkage map obtained in this study was good (846 markers, 1,047 cM) in comparison to other genetic maps of diploid potato (Bonierbale et al. 1988; Kuhl et al. 2001; Costanzo et al. 2005; van Os et al. 2006), the chromosome VII that harboured the *Rpi-mch1* gene was poor in polymorphic and segregating markers. Moreover, both the *Rpi-mch1* gene and the linked markers showed strong distortion in segregation. That may be caused by the local suppression of recombination due to the hybrid nature of *S. michoacanum*. Reduction of recombination and distorted segregation was observed before in other interspecific *Solanum* hybrid obtained from the cross *S. tuberosum* × *S. spegazzinii* (Kreike and Stiekema 1997). Another explanation could be that while the DArT platform used in this study was developed using clones derived from cultivated potato genomes and *S. michoacanum* chromosome VII differed possibly more than the other chromosomes from the *S. tuberosum* ones, we were able to map only a few markers in this region of the genome. Nevertheless, the CAPS marker C2\_At1g53670 that was mapped 5.7 cM from the *Rpi-mch1* gene can be a useful tool for tracking the gene, for example in somatic hybrids with potato, as it was in case of the marker GP94 applied for selection of potatoes with another late blight resistance gene *Rpi-phu1* that was located in 6.4 cM distance (Śliwka et al. 2010).

The results obtained in this study will be exploited further in several ways. We plan to enhance map resolution of *S. michoacanum* chromosome VII using DArT markers derived from a panel of wild *Solanum* species. The mapping data can support the transfer of the *Rpi-mch1* gene into cultivated potato gene pool through marker-assisted selection of the resistant somatic hybrids *S. michoacanum* + *S. tuberosum*, and such work is in progress in our group. They can also be helpful in cloning of the *Rpi-mch1* gene and its subsequent transfer via cisgenesis into potato cultivars which can widen the pool of the late blight resistance genes available for potato breeding. That can enable building *R* gene pyramids which seem to be a promising strategy for obtaining potato cultivars highly and durably resistant to *P. infestans*.

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- SOL Genomics Network (SGN) Database <http://solgenomics.net/>
- Solanaceae* Source - Natural History Museum <http://www.nhm.ac.uk/research-curation/research/projects/solanaceaesource/>