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ORIGINAL PAPER

W. Marczewski · D. Strzelczyk-Żyta
J. Hennig · K. Witek · C. Gebhardt

Potato chromosomes IX and XI carry genes for resistance to potato virus M

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Abstract Two new loci for resistance to potato virus M (PVM), *Gm* and *Rm*, have been mapped in potato. The gene *Gm* was derived from *Solanum gourlayi*, whereas, *Solanum megistacrolobum* is the source of the gene *Rm*. *Gm* confers resistance to PVM infection after mechanical inoculation. *Rm* induces a hypersensitive response in potato plants. Two diploid populations segregating for *Gm* and *Rm*, bulked segregant analysis (BSA) using random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR), and available potato molecular maps were instrumental for mapping the resistance loci. The novel locus *Gm* was mapped to a central region on potato chromosome IX. The locus *Rm* was placed on the short arm of chromosome XI, close to the marker loci *GP250* and *GP283*, where a hotspot for monogenic and polygenic resistance to diverse pathogens is located in the potato and tomato genome.

Introduction

Potato virus M (PVM; genus *Carlavirus*) is a devastating virus in the seed and ware potato production in Central and Eastern Europe. The virus may reduce tuber yields by 40–75% (Brunt 2001). Symptoms produced by PVM vary from mild to very severe and depend on the virus strain and the potato genetic background. Leaflet malformations, leaf vein clearing, petiole and stem necrosis, and mosaic symptoms can be observed in susceptible plants after PVM infection (Kowalska 1978; Ruiz de Galarreta et al. 1998). Genes for resistance to PVM found in wild and cultivated *Solanum* species have been summarized by Dziewońska and Ostrowska (1977, 1978) and Ruiz de Galarreta et al. (1998). The PVM resistance gene *Rm* originates from *S. megistacrolobum* and is responsible for a hypersensitive response of potato plants to PVM infection (Dziewońska and Ostrowska 1977). Potato plants possessing *Rm* do not get infected with PVM or develop only necrotic spots on leaves after mechanical inoculation when they are grown under greenhouse or field conditions. The necrotic reaction can be observed in graft inoculated plants (Mietkiewska 1994). The dominant gene *Gm* was derived from *S. gourlayi* and confers a different type of PVM resistance to potato than the *Rm* gene. Upon mechanical inoculation, PVM does not propagate in potato plants carrying *Gm*. The presence of PVM particles can be detected only incidentally in plants inoculated by grafting (Dziewońska and Ostrowska 1978; Waś et al. 1980; Swieżyński et al. 1981; Mietkiewska 1994).

Microsatellites, also called simple sequence repeats (SSRs), are an abundant and dispersed class of repetitive DNA throughout most eukaryotic genomes (Morgante and Olivieri 1993). However, the development of SSR markers for genetic mapping is still relatively expensive, laborious and requires sequence information to design specific primers. An inter-simple sequence repeat (ISSR) approach does not need knowledge of a sequence to be amplified. ISSR markers are generated by microsatellite-repeat primers with variations at 5′′ and 3′′ anchors, amplifying anonymous DNA regions between adjacent SSR loci (Zietkiewicz et al. 1994). Such regions, named SSR hot spots, serve as a source of ISSR markers (Rakoczy-Trojanowska and Bolibok 2004). Similar to random amplified polymorphic DNA (RAPD) markers (Williams et al. 1990), ISSR markers are a high

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W. Marczewski (✉) · D. Strzelczyk-Żyta
Plant Breeding and Acclimatization Institute,
Platanowa 19, 05831 Młochów, Poland
E-mail: w.marczewski@ihar.edu.pl
Tel.: +48-22-7299248
Fax: +48-22-7299247

J. Hennig · K. Witek
Institute of Biochemistry and Biophysics,
Polish Academy of Sciences, Pawińskiego 5A,
02106 Warsaw, Poland

C. Gebhardt
Max-Planck Institute for Plant Breeding Research,
Carl von Linné Weg 10, 50829 Cologne, Germany

throughput marker system for bulked segregant analysis, which is easy to handle, inexpensive and fast to detect. In the last decade, ISSR markers were successfully used for phylogenetic studies and gene tagging in several plant species (reviewed by Rakoczy-Trojanowska and Bolibok 2004). In potato, ISSR markers linked to genes for resistance to potato virus S (PVS), potato leafroll virus (PLRV) and potato virus Y (PVY) were used to identify the corresponding resistance loci on chromosomes VIII (Marczewski et al. 2002), XI (Marczewski et al. 2004) and XII (Flis et al. 2005). Here we report the use of ISSR and RAPD markers to identify on the potato genetic map the positions of the genes *Gm* and *Rm*, which are both new members of resistance gene families on chromosomes IX and XI.

Material and methods

Plant material

The diploid potato (*Solanum tuberosum*, $2n = 2x = 24$) F1 population "Ns" (Marczewski et al. 1998) was used for mapping the PVM resistance gene *Gm*. This population has been used previously to map the loci *Ns* and *PLRV.4* for resistance to PVS and PLRV (Marczewski et al. 2002, 2004). The family of 138 F1 individuals originated from a cross between clone DW 91-1187 used as the seed parent and clone DW 83-3121 used as the pollen parent. Accession INTA7356 of *Solanum gourlayi* provided by K.A. Okada (Instituto Nacional de Tecnología Agropecuaria collection, INTA, Argentina) was the source of *Gm* present in DW 83-3121 (Dziewońska and Ostrowska 1978). The population "Rm" was generated within the diploid potato line breeding program at the Laboratory of Genetics, Plant Breeding and Acclimatization Institute (IHAR), Młochów. One hundred twenty F1 individuals were obtained from a cross between the resistant seed parent I/28 and the susceptible pollen parent DG 95-1471. Parent I/28 was the source of the *Rm* allele and originated from the cross R.72.554 (*S. megistacrolobum* × *S. tuberosum* dihaploid) supplied by H. Ross (Max-Planck Institute for Plant Breeding Research, Cologne, Germany) (Dziewońska and Ostrowska 1977).

Tests for resistance to PVM

Leaves of five young plants of each clone were mechanically inoculated twice at 2-day intervals using an inoculum prepared from tomato leaves of cv. Najwcześniejszy infected with the M_{55a} isolate of PVM (Kowalska 1978). Inoculated plants of the "Ns" population were grown in the greenhouse during spring season, while the experiments for the "Rm" population were carried out in a growth chamber at 20°C and 16 h light per day. PVM infection was assayed by ELISA 4 weeks after inoculation as described by Chrzanowska and Zagórska (2001). Plants of the "Rm" population, in which PVM was below detection threshold after mechanical inoculation, were additionally tested by graft inoculation, according to Wasilewicz-Flis (2001). Non-inoculated plants were used as controls.

Plant DNA isolation, PCR, electrophoresis, DNA cloning and sequencing

Extraction of plant genomic DNA, PCR amplification of ISSR markers and electrophoresis were performed as previously described (Flis et al. 2005). PCR conditions for RAPD markers were according to Marczewski et al. (1998). The SCAR marker $SC878_{885}$ was amplified in 20 µl of 20 mM Tris–HCl pH 8.4, 50 mM KCl, 2.5 mM $MgCl_2$, 0.1 mM of each deoxynucleotide, 0.15 µM of each primer, containing 1 U *Taq* DNA Polymerase (Invitrogen, Carlsbad, CA, USA) and 30 ng genomic DNA as template. The PCR parameters for amplifying $SC878_{885}$ were: 94°C for 60 s followed by 40 cycles of 93°C for 15 s, 58°C for 20 s, 72°C for 60 s and a final extension time of 5 min at 72°C. The markers GP38 (XI), GP39 (IX), GP283 (XI), TG9 (IX) and TG18 (IX) were amplified using 1.5 mM $MgCl_2$ and 0.2 µM of each primer at the annealing temperature of 51°C. The annealing temperature of 53°C was applied for amplification of the markers $SC864_{816}$ and GP190. Forward and reverse primers for $SC878_{885}$ (NCBI GenBank accession AY960860), $SC864_{816}$ (AY974037), GP38 (AJ492255), GP39 (CG783251), GP283 (CG783215), GP190 (CG783121), GP250 (CG783183), TG9 (SGN database TG9FWD) and TG18 (SGN TG18FWD) are given in Table 1. The marker GP250 (XI) was amplified as described by Oberhagemann et al. (1999). Other primers were designed according to the Primer Select Program from Lasergene® softwere version for Windows, 3.04a (DNA STAR. Inc., Madison, WI, USA). The markers $UBC878_{962}$, $UBC822_{1079}$ and $UBC864_{816}$ were cloned using the pGEM®-T Easy Vector System (Promega, Madison, WI, USA). The sequences of the cloned amplified DNA's were determined using the Bigdye Kit on the ABI PRISM™ 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA).

Mapping of the Gm and Rm loci

DNA samples of parental clones P3 and P38 and eighty-seven F1 individuals of the diploid mapping population K31 (Schäfer-Pregl et al. 1998) were used to map the RAPD marker $OPF5_{1200}$ linked to the *Gm* gene. Segregation of $OPF5_{1200}$ was scored as presence or absence of the marker fragment. The map position was identified relative to the restriction fragment length polymorphism (RFLP) map existing for this population using the software package MAPRF (E. Ritter, NEIKER, 01080 Victoria, Spain). The map position of the *Rm* locus was initially found by detecting sequence homology of the

Table 1 Primers for PCR-based markers, lengths of amplicons or restriction fragments and enzymes used for CAPS markers

Marker	Primer sequence (5′ to 3′)	Amplicon or restriction fragment size (bp)	Marker assay
$OPF5^{a}_{1200}$	CCGAATTCCC	1,200	RAPD
$OPF15_{1800}$	CCAGTACTCC	1,800	RAPD
$UBC878_{962}$	GGATGGATGGATGGAT	962	ISSR
$SC878_{885}$	GGATGGATGGATGAGGAGGAAACT CCGACTAGCGATTTGGATGC	885	SCAR
GP39	TTTTTGCTTCTACCTTCTG TTGGCTAATACCCTTTCA	950	STS
GP190	ACGTGATGGGGGATAGATTG GGCAATCTGCAAAAAGGAAG	180	CAPS/*Rsa*I
TG9	TGCCTAGACTCTGGGATTTATTTT ACCTAGTTGTGTTTTCCAGTGTTG	400	CAPS/*Hin*fI
TG18	CCATTAAGGGTTGTTGATTCC AAAAGCACCAGTTCTCCAGTTG	120	CAPS/*Mse*I
$OPH18^{b}_{480}$	GAATCGGCCA	480	RAPD
$UBC822_{1079}$	TCTCTCTCTCTCTCTCA	1,079	ISSR
$SC864_{816}$	TGATGCATGATGACCATAACC GAACGAAATTTGGTCCACTGA	200	CAPS/*Hind*III
GP38	TGGAACTTACTTCACTGACAAACT TGCAGTAACTGAAAGCAACAGAT	380	CAPS/*Alu*I
GP283	CCCTCCCCATGAAAAAGGTA GCAACTTCCTGTCCGAATGT	320	CAPS/*Dde*I
GP250	AGTTCAACACCAGTAGGAC GACATCAAGTTACCTATGAC	510	CAPS/*Xap*I

[a]Markers linked to *Gm* on chromosome IX; [b]markers linked to *Rm* on chromosome XI

Rm-linked marker $UBC822_{1079}$ with tomato *I2C-1* resistance gene (AF004878.1). The position of *Gm* and *Rm* was subsequently confirmed by detecting linkage to anchor markers specific for potato chromosomes IX and XI in the population ''Ns'' and ''Rm'', respectively. The linkage groups of the resistant parents DW 83-3121 and I/28 were constructed by scoring RAPD, ISSR, SCAR (sequence characterized amplified region), STS (sequence tagged site) or CAPS (cleaved amplified polymorphic sequence) markers in the populations ''Ns'' and ''Rm''.

Results

PVM resistance tests

More than 1,300 plants of the two diploid potato populations ''Ns'' and ''Rm'' were tested altogether for resistance to PVM infection. A_{405} values obtained by ELISA tests for non-virus-inoculated control plants ranged from 0.01 to 0.04. Four weeks after mechanical inoculation with PVM, ELISA values similar as in the controls were recorded in 36 F1 hybrids and the resistant parent DW 83-3121 of the ''Ns'' population, and in 55 F1 hybrids and the resistant parent I/28 of the ''Rm'' population. No hypersensitive response was observed in the inoculated leaves. Two tuber progeny plants from each of the PVM-inoculated, resistant plants of the ''Ns'' population were additionally tested. They failed to become infected with PVM. The ''Rm''-plants expressed hypersensitivity after grafting with PVM-infected tomato scions. All these clones were considered as resistant to PVM infection. The remaining 102 and 65 F1 clones of the ''Ns'' and ''Rm'' population were infected by PVM and A_{405} values ranging from 0.6 to 1.2 were observed in ELISA tests. They were classified as susceptible. The segregation ratio of *Gm* resistant versus susceptible F1 offspring of the ''Ns'' population deviated significantly from the 1:1 ratio expected for the segregation of a single dominant gene and was distorted towards susceptibility ($\chi^2 = 31.6$, $P < 0.05$). Distorted segregation ratios are frequently observed in crosses of dihaploid potato clones and can be explained by selection against unfavourable allele combinations in F1 seedlings (Gebhardt et al. 1991). For the ''Rm'' population, the 1:1 segregation ratio of resistance versus susceptibility ($\chi^2 = 0.83$, $P > 0.05$) indicated the presence of a single, dominant gene present in the heterozygous state in the resistant parent I/28.

Mapping of the PVM resistance gene *Gm*

Polymorphism between DNA fragments amplified by 185 RAPD and 56 ISSR primers for the parents of the ''Ns'' population were described previously (Marczewski et al. 1998; Marczewski 2001). DNA bulks were formed from equal DNA amounts of eight resistant and eight susceptible F1 plants. Of 185 RAPD primers tested, two DNA fragments of 1,200 and 1,800 bp were amplified only in the resistant DNA bulk when using primers OPF5 and OPF15. Out of the 56 ISSR primers tested, UBC878 generated a specific 962 bp DNA fragment in the resistant parent DW 83-3121 and in the resistant bulk. When $OPF5_{1200}$, $OPF15_{1800}$ and $UBC878_{962}$ were

scored in the whole "Ns" population, all three markers were linked to *Gm*. $OPF5_{1200}$, $OPF15_{1800}$ and $UBC878_{962}$ were separated by 30, 27 and 3 recombinants, respectively, from the *Gm* locus. Only RAPD marker $OPF5_{1200}$ was suitable for mapping in the reference potato mapping population K31, as this was the only marker that was amplified in the parental clone P3. $OPF5_{1200}$ mapped to linkage group IX in the interval between RFLP loci CP20-a–GP167-b (Schäfer-Pregl et al. 1998; Gebhardt et al. 2001). To confirm the location of *Gm*, the additional, chromosome IX specific RFLP markers GP39, GP190, TG18 and TG9 were developed into PCR-based markers that segregated in the "Ns" population and were heterozygous in parent DW 83-3121 having *Gm*. Amplification of GP39 resulted in two DNA fragments of 900 and 950 bp, of which the 950 bp fragment was linked to *Gm*. The PCR-based assay for TG9 revealed a strong band of 400 bp also linked to *Gm* that was obtained after *Hin*fI digestion of the PCR product. This fragment co-segregated with a 120 bp fragment of the TG18 amplicon digested with *Mse*I. The GP190 amplicon was mapped relative to *Gm* based on a 180 bp *Rsa*I restriction fragment. The map position of *Gm* in relation to all DNA markers is shown in Fig. 1a. Based on the distance to the anchor markers GP39 and GP190 (Gebhardt et al. 2001), the *Gm* locus maps to a central region of chromosome IX. The most closely linked marker $UBC878_{962}$ was located 2 cM proximal to the *Gm* locus. This marker was cloned, sequenced (AY960860) and compared to the sequence database with the BLASTN program. $UBC878_{962}$ shared 94% sequence identity with a *S. tuberosum* genomic region on chromosome XII containing the *Rx1* gene for resistance to potato virus X (PVX) (van der Vossen et al. 2000), and 84% identity with the 5′ non-translated region of the *Gpa2* gene (AF195939.1) for resistance to the potato cyst nematode *Globodera pallida* which is physically tightly linked to *Rx1* (van der Vossen et al. 2000). Additionally, $UBC878_{962}$ shared 87% identity with the promoter region of the patatin gene (Liu et al. 1991) located on chromosome VIII (Gebhardt et al. 1991). The ISSR marker $UBC87_{962}$ was converted into the SCAR marker $SC878_{885}$ to obtain an easy scorable PCR marker linked to *Gm* (Fig. 2, lane 6).

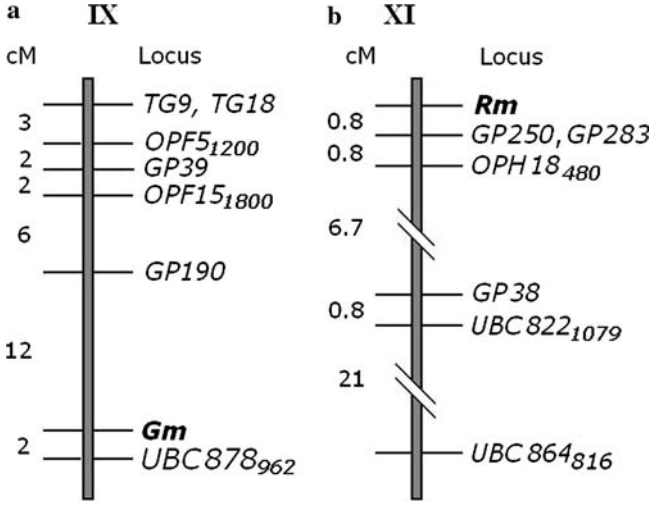

Fig. 1 **a**, **b** Genetic maps of linkage group IX of parent DW 83-3121of the "Ns" population (**a**) and linkage group XI of parent I/28 of the "Rm" population (**b**) with the positions of the loci *Gm* and *Rm*, respectively, conferring resistance to PVM. Map distances are in centimorgans (*cM*). The markers $UBC878_{962}$ and $UBC864_{816}$ co-segregated with the corresponding markers $SC878_{885}$ and $SC864_{816}$

Mapping of the PVM resistance gene *Rm*

The screening of the parental DNA of the "Rm" population with 56 ISSR and 185 RAPD primers resulted in detection of 121 (23%) and 485 (32%) polymorphic DNA fragments. However, only one 1,079 bp ISSR product, and one RAPD fragment of approximately 480 bp were observed as clear bright bands in the resistant parent I/28 and in the resistant DNA bulk. The markers $OPH18_{480}$ and $UBC822_{1079}$ were generated by primers OPH18 and UBC822. $UBC822_{1079}$ was cloned and sequenced (AY960859). $UBC822_{1079}$ shared 89% sequence identity with tomato *I2C-1* resistance gene (AF004878.1) to *Fusarium oxysporum* f. sp. *lycopersici* located on tomato chromosome 11 (Simons et al. 1998). To confirm the position of *Rm* on potato chromosome XI, markers GP250 and GP283 mapping to the corresponding end of the syntenic short arm of chromosome XI (Dong et al. 2000; Marczewski et al. 2004) were scored in the "Rm" population for linkage with the *Rm* locus. CAPS fragments of 510 bp (Fig. 2, lane 1) and 320 bp (Fig. 2, lane 3) obtained after *Xap*I and *Dde*I digestion of GP250 and GP283 amplicons, respectively, both revealed a distance of 0.8 cM from *Rm* on chromosome XI, thereby confirming the location of *Rm* (Fig. 1b). A CAPS product of 380 bp was generated after *Alu*I digestion of the GP38 amplicon, which mapped 8 cM proximal to *Rm*. The *Rm* locus was separated by 27 cM from the marker $UBC864_{800}$ on chromosome XI, which is linked to the locus *PLRV.4* for resistance to PLRV (Marczewski et al. 2004). $UBC864_{800}$ was scored based on a 200 bp *Hind*III restriction fragment of the $UBC864_{800}$ amplicon. The cloned and sequenced marker fragment $UBC864_{800}$ was 816 bp long (AY974037) with no apparent homology to any sequences available in GenBank. The order of markers *GP250, GP283, GP38,* $OPH18_{480}$, $UBC822_{1079}$ and $UBC864_{816}$ in relation to the *Rm* locus on chromosome XI is shown in Fig. 1b.

Discussion

A number of *R* genes and quantitative resistance loci have been mapped in the potato genome (reviewed in Gebhardt and Valkonen 2001). Integration of independent mapping experiments by means of common anchor markers, which tag the same genomic regions in potato

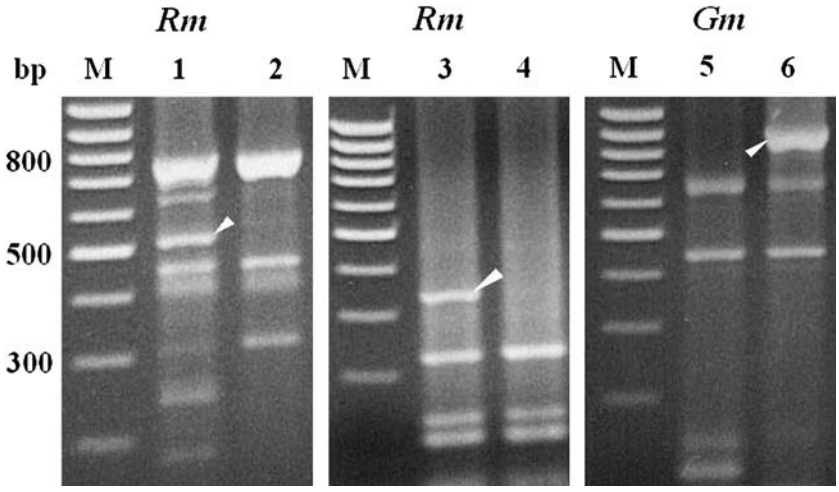

Fig. 2 Electrophoretic patterns of the CAPS and SCAR markers amplified for parental DNA of the "Rm" and "Ns" populations. *Lanes 1 and 2* CAPS marker GP250 digested with *Xap*I in the resistant (I/28) and susceptible (DG 95-1471) parents of the "Rm" population, respectively; *lanes 3 and 4* CAPS marker GP283 digested with *Dde*I in I/28 and DG95-1471, respectively; *lanes 5 and 6* amplicons obtained in the susceptible (DW 91-1187) and resistant (DW 83-3121) parents of the "Ns" population, respectively, amplified using the primer pair for $SC878_{885}$; M = 100-bp ladder. Marker fragments $GP250_{510}$ and $GP283_{320}$ (linked with *Rm*) and $SC878_{885}$ (linked with *Gm*) are indicated by *arrows*

and tomato revealed certain resistance hotspots where genes for resistance to different pathogens are clustered. Such a resistance hotspot is the distal end of the short arm of chromosome XI where genes for resistance to various pathogens have been located. The marker GP250 co-segregated with the *R3a* gene for resistance to *P. infestans* (Huang et al. 2004) and is tightly linked to four other *R* genes with distinct specificities to the late blight disease caused by *Phytophthora infestans* (El Kharbotly et al. 1996; Huang et al. 2005). QTL for resistance to late blight and *Erwinia carotovora* ssp *atroseptica* have also been mapped to the same region (Oberhagemann et al. 1999; Collins et al. 1999; Zimnoch-Guzowska et al. 2000). The co-linear region of tomato chromosome 11 harbors the *I2* locus conferring resistance to race 2 of *F. oxysporum* f. sp. *lycopersici* (Ori et al. 1997; Simons et al. 1998; Huang et al. 2005). The *Rm* locus reported here was at a close genetic distances of 0.8 cM from the marker GP250. The PVM resistance gene *Rm* is therefore a new member of this resistance gene cluster located on potato chromosome XI and the first one conferring resistance to a virus. Sequence analysis of the *I2* locus in tomato (Ori et al. 1997; Simons et al. 1998) and the *R3a* locus in potato (Huang et al. 2005) revealed a complex structure of clustered resistance gene homologs. Based on its position, the gene *Rm* could be another member of these gene families.

The PVM resistance gene *Gm* mapped to a central portion of potato chromosome IX. Due to the lack of an anchor marker tightly linked to *Gm*, the comparison of the *Gm* position to other known resistance loci on chromosome 9 of tomato and potato cannot be precisely done. A novel *R* gene controlling resistance to *P. infestans* has been recently identified in a similar region of potato chromosome IX (Sliwka et al. 2004), based on linkage of both resistance genes to the anchor marker GP39. In tomato, the genes *Tm-2a* for resistance to tobacco mosaic virus (TMV) and *Fr1* against *F. oxysporum* f. sp. *radicis-lycopersici* may be located in the syntenic central segment of tomato chromosome 9 (Vakalounakis et al. 1997; Young et al. 1988; Young and Tanksley 1989).

The sequence analysis of ISSR markers $UBC878_{962}$ and $UBC822_{1079}$ indicated evolutionary relationships of the genomic regions having the *Gm* and *Rm* resistance loci with other genomic locations of known potato and tomato resistance genes. The marker $UBC822_{1079}$ linked to *Rm* showed significant DNA sequence similarity to the *I2C-1* resistance gene against *F. oxysporum* f. sp. *lycopersici* located on tomato chromosome 11 (Simons et al. 1998). The marker $UBC878_{962}$ tightly linked to *Gm* shared high identity with sequences upstream of both the *Rx1* gene for resistance to PVX and the *Gpa2* gene for resistance to the potato cyst nematode *Globodera pallida* (van der Vossen et al. 2000). Both resistance genes are physically tightly linked (van der Vossen et al. 2000; Bakker et al. 2003) and reside in a resistance hotspot on potato chromosome XII (Gebhardt and Valkonen 2001). Additionally, $UBC878_{962}$ was homologous to the promoter region of the patatin gene (Liu et al. 1991) located on chromosome VIII (Gebhardt et al. 1991). In this case, the sequence similarity did not help in finding the correct chromosome IX. However, these sequence homologies may indicate duplications of genome segments of unknown size between potato chromosomes IX, XII and VIII. These findings are in agreement with other studies (Yu et al. 1996; Ratnaparkhe et al. 1998), suggesting that disease resistance loci in plants are often accompanied by microsatellite sequences.

There are only two cultivars Triada and Korona registered in Poland that express the hypersensitive response to PVM-infection under field conditions

(M. Chrzanowska, personal communication). Both the *Gm* and *Rm* genes are currently being used in diploid and tetraploid breeding programs at IHAR Młochów. The PCR markers described in this paper, $GP250_{510}$ and $GP283_{320}$ for *Rm*, and $SC878_{885}$ for *Gm* will be tested for usefulness to speed up selection of PVM-resistant potato clones.

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