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W. Marczewski · J. Hennig · C. Gebhardt

The *Potato virus S* resistance gene *Ns* maps to potato chromosome VIII

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Abstract The dominant allele *Ns* confers in potato resistance to *Potato virus S* (PVS). To identify the chromosomal location of *Ns*, we mapped the *Ns*-linked marker $SCG17_{448}$ and the ISSR marker $UBC811_{600}$ to linkage group VIII of the RFLP map of a population that did not segregate for *Ns*. The map position of the *Ns* locus on chromosome VIII was confirmed with the detection of linkage between *Ns* and three RFLP markers, GP126, GP189 and CP16, known to be located in a corresponding region on potato chromosome VIII. PCR-based assays were developed for these RFLP markers. The PCR primers specific for GP126 generated polymorphic products (STS marker). In the case of markers GP189 and CP16, informative polymorphism was revealed in the Ns population after digestion with the restriction enzymes *Hae*III and *Hin*dIII, respectively. The genetic distance between *Ns* and the closest *CP16* locus was 4.2 cM.

Keywords *Potato virus S* · Resistance gene *Ns* · *Solanum tuberosum* · Genetic mapping · PCR markers

Introduction

The dominant *Ns* gene, originating from *Solanum tuberosum* ssp. *andigena* (Baerecke 1967), confers resistance to *Potato virus S* (PVS). Plants expressing *Ns* remain symptomless, and no PVS titers develop in enzyme-linked immunosorbent assays (ELISA). In graft inoculation tests, the resistant plants react with a fading of the foliage of shoots developed from the axillary meristems as a result of the hypersensitive reaction of the plants to PVS infection. Depression of tuber formation in such plants is also observed (Marczewski et al. 1998). A cross between diploid potato (*Solanum tuberosum* L.) clones DW 91-1187 and DW 83-3121 was used to identify random amplified polymorphic DNA (RAPD) (Marczewski et al. 1998), sequence-characterized amplified region (SCAR) (Marczewski et al. 2001b) and inter-simple sequence repeat (ISSR) (Marczewski 2001) markers linked to the *Ns* locus. The markers $SCG17_{321}$ (Marczewski et al. 2001b) and $UBC811_{660}$ (Marczewski 2001) are currently being used for indirect selection of the *Ns* resistance gene in diploid breeding programs at the Plant Breeding and Acclimatization Institute at Młochów.

Nineteen single dominant genes (*R* genes) for resistance to various pathogens have been positioned on the molecular maps of potato using DNA markers. Seven genes confer resistance to important potato viruses: *Potato virus Y* (PVY), *Potato virus X* (PVX) and *Potato virus A* (PVA) (reviewed in Gebhardt and Valkonen 2001). A single gene might also confer resistance to *Potato leafroll virus* (PLRV) in potato (Marczewski et al. 2001a)

Here, we report the chromosomal localization of the *Ns* locus based on mapping the *Ns*-linked markers on the restriction fragment length polymorphism (RFLP) map of population K31 (Schäfer-Pregl et al. 1998).

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W. Marczewski (✉)
Plant Breeding and Acclimatization Institute,
Platanowa 19, 05831 Młochów, Poland
e-mail: w.marczewski@ihar.edu.pl
Fax: 48-22-7299247

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

C. Gebhardt
Max-Planck Institut für Züchtungsforschung,
Carl von Linne Weg 10, Cologne, 50829 Germany

Materials and methods

Plant materials

The parental diploid potato (*Solanum* tuberosum L.) clones, DW 91-1187 (susceptible) and DW 83-3121 (resistant), and 119 F_1 hybrids (Ns population) were available to map the *Ns* locus. The resistance test to PVS infection has been described by Marczewski et al. (1998). Sixty and fifty-nine F_1 individuals were determined

as being susceptible and resistant to PVS infection, respectively. The 1:1 segregation ratio of resistant versus susceptible F_1 plants indicated that the resistant parent was heterozygous for the single dominant *Ns* gene.

Polymerase chain reaction (PCR) amplification

DNA extraction, PCR amplification with a primer UBC811 and electrophoresis were performed as previously described (Marczewski 2001). The PCR conditions used to amplify the DNA fragment $SCG17_{448}$ and patterns of the products generated after *Mun*I digestion were as described by Marczewski et al. (2001b).

PCR analyses of RFLP markers GP126, GP189 and CP16 were performed in 20 m*M* Tris–HCl pH 8.4, 50 m*M* KCl, 2.5 m*M* $MgCl_2$, 0.1 m*M* of each deoxynucleotide, 10 ng of each primer, 0.4 U *Tag* DNA polymerase (Gibco BRL) and 20 ng of genomic DNA in a final volume of 20 µl. The primer sequences, listed in Table 1, were designed according to the Primer Select Program (DNA STAR, Madison, Wis. version for Windows, 3.10) based on DNA sequences of the RFLP markers (C. Gebhardt, unpublished). The PCR parameters were: 94 °C for 60 s, followed by 35 cycles of 94 °C for 15 s, 55 °C for 15 s, 72 °C for 60 s, and a final extension time of 3 min at 72 °C.

Mapping of the *Ns* locus

DNA samples of parental lines P3 and P38 and 87 F_1 plants of the diploid mapping population K31 (Schäfer-Pregl et al. 1998) were used to map the two *Ns*-linked markers $SCG17_{448}$ (Marczewski et al. 2001b) and $UBC811_{660}$ (Marczewski 2001) on the RFLP map covering the 12 potato chromosomes that was constructed in the K31 population. Scoring and linkage analysis of PCR-derived DNA fragments in the K31 population was the same as for RFLP fragments and has been described by Ritter et al. (1990), and Schäfer-Pregl et al. (1998).

Linkage analysis between PCR markers and *Ns* was performed using LINKAGE-1 software (Suiter et al. 1983). Map distances in centiMorgans (cM) were calculated from recombination frequencies by using Kosambi's mapping function (Kosambi 1944).

Table 1 Primer sequences used for PCR amplification of the RFLP loci *GP126*, *GP189* and *CP16*

Marker name	Forward and reverse primer sequences (5′ to 3′)
GP126	TAGCCGTTGCCACCCTACA TTGTTGAAGTCTAAGAAATCTGTT
GP189	AGTTGAGGAGCTGTTTGTGA AGGCTTTAGTATTTCTGTGTATTT
CP16	CTTAAACGCGTCAAGTGAAACT TTAGGGACATACAAACAAACCTCA

Results and discussion

Polymorphism of a single *Ns*-linked PCR fragment of 448 bp, designated $SCG17_{448}$ (Marczewski et al. 2001b), was revealed after *Mun*I digestion in the parental lines of the K31 cross (Fig. 1, lanes 5 and 6). The *Ns*-linked ISSR marker $UBC811_{660}$ (Fig. 1, lane 2) was not amplified in the parental DNA samples. Instead, a 600-bp ISSR product was observed in the parent P3 (Fig. 1, lane 3), while a 800-bp ISSR product was amplified in the parent P38 (Fig. 1, lane 4). This indicated the presence of different insertion/deletion alleles of the $UBC811_{660}$ marker in the K31 population and/or the detection of different loci.

Eighty-seven F_1 clones of the K31 population were screened for segregation of both *Mun*I-digested $SCG17_{448}$ and the two ISSR fragments. The 800-bp ISSR fragment mapped on linkage group XII of the K31 map, linked by 1 cM to the RFLP locus *GP183*. The 600-bp ISSR fragment and the marker $SCG17_{448}$ both mapped to linkage group VIII. The two marker fragments were linked in repulsion by 2 cM. The most closely linked RFLP loci (1–5 cM) were *GP293(b)* and *GP171* (Schäfer-Pregl et al. 1998). To confirm its position on chromosome VIII, we mapped the *Ns* locus relative to three extra markers, GP126, GP189 and CP16, that map to the same region on potato chromosome VIII as GP171 (Gebhardt et al. 1991) in the Ns population. PCR performed with GP126-specific primers resulted in polymorphic PCR products (STS, sequence tagged site). Two DNA fragments, 750 bp and 700 bp in length, were amplified in the resistant parent DW 83-3121 (Fig. 1, lane 8). The 750-bp fragment was not amplified

Table 2 Summary of linked loci, phenotypic frequencies, χ^2 values for goodness-of-fit to expected segregation ratio (two-point: χ^2 AB) and recombination frequency between loci (*SE* standard error)

Locus		Phenotypes[a]				χ^2 AB[b]	Recombination frequency ± SE
A	B	AB	Ab	aB	ab		
GP126	*Ns*	55	9	4	51	73.2	10.9±2.9
GP189	*Ns*	56	8	3	52	79.7	9.2±2.7
CP16	*Ns*	58	4	1	56	100.1	4.2±1.9
$UBC811_{660}$	*Ns*	59	1	0	59	115.1	0.8±0.8
$SCG17_{448}$	*Ns*	58	1	1	59	111.1	1.7±1.2
GP126	GP189	62	2	2	53	103.5	3.4±1.7
GP126	CP16	58	6	4	51	82.4	8.4±2.5
GP126	$UBC811_{660}$	56	8	4	51	76.1	10.1±2.8
GP126	$SCG17_{448}$	55	9	4	51	73.2	10.9±2.9
GP189	CP16	59	5	3	52	89.2	6.7±2.3
GP189	$UBC811_{660}$	57	7	3	52	82.7	8.4±2.5
GP189	$SCG17_{448}$	56	8	3	52	79.7	9.2±2.7
CP16	$UBC811_{660}$	59	3	1	56	103.7	3.4±1.7
CP16	$SCG17_{448}$	58	4	1	56	101.1	4.2±1.8
$UBC811_{660}$	$SCG17_{448}$	59	1	0	59	115.1	0.8±0.8

[a] Phenotypes: A or B corresponds to the presence of a marker or to PVS-resistant clones; a or b corresponds to the absence of a marker or to PVS-susceptible clones

[b] Significant at the 0.01 probability level

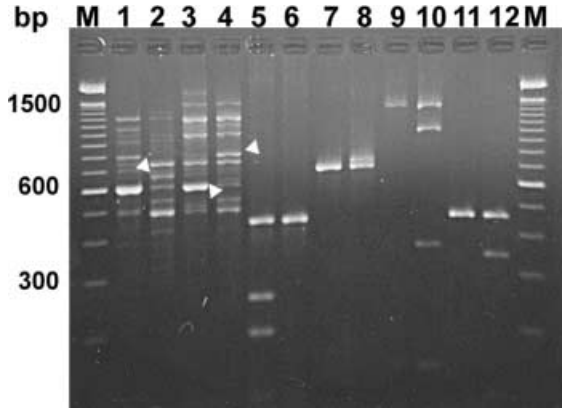

Fig. 1 Electrophoretic patterns of the ISSR, SCAR, STS and CAPS markers in the Ns and K31 populations. *Lanes*: *1*, and *2* PCR products amplified with ISSR primer UBC811 in the susceptible (DW 91-1187) and resistant (DW 83-3121) parents of the Ns population, respectively; *3*, *4* PCR products amplified with UBC811 from the parents P3 and P38 of the K31 population, respectively; *5*, *6* $SCG17_{448}$ digested with *Mun*I in P3 and P38, respectively; *7*, *8* STS marker GP126 in DW 91-1187 and DW 83-3121, respectively; *9*, *10* CAPS marker GP189 digested with *Hae* III in DW 91-1187 and DW 83-3121, respectively; *11*, *12* CAPS marker CP16 digested with *Hin*dIII in DW 91-1187 and DW 83-3121, respectively; *M*100-bp ladder. $UBC811_{660}$ (linked with *Ns*), $UBC811_{600}$ and $UBC811_{800}$ are indicated by arrowheads

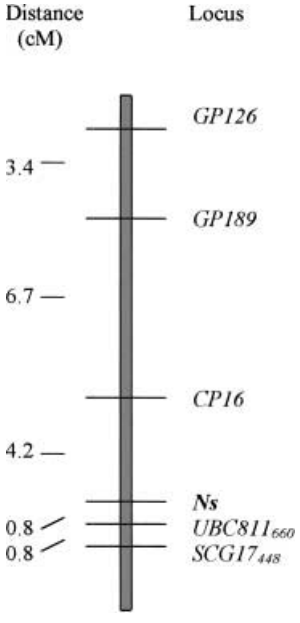

Fig. 2 Genetic map of the potato chromosome VIII region containing the *Ns* gene for PVS resistance

in the susceptible parent DW 91-1187 (Fig. 1, lane 7). PCR amplification with GP189-specific primers revealed a single band of 1,500 bp in both parents. Among the restriction enzymes tested for fragment length polymorphism of the PCR products, *Hae*III, *Eco*RI and *Alu*I generated informative, segregating DNA fragments (CAPS, cleaved amplified polymorphic sequence). The 1,500-bp PCR fragment amplified in the susceptible parent was not cleaved with *Hae*III (Fig. 1, lane 9), whereas three bands of 380 bp, 1,100 bp and 1,500 bp were observed after *Hae*III digestion of the PCR product obtained from the resistant parent (Fig. 1, lane 10). The PCR products obtained with primers for the cDNA marker CP16 were 460 bp long. Of the five restriction enzymes tested, an informative polymorphism was observed after *Hin*dIII digestion (Fig. 1, lanes 11 and 12).

The STS marker GP126 and the CAPS markers GP189 and CP16 were tested for linkage to the *Ns* locus in 119 progeny of the *Ns* population (Table 2). The map position of *Ns* was found to be 4.2 cM (5 recombinants) distal to *CP16* on chromosome VIII. The order of the *GP126*, *GP189* and *CP16* loci was the same as in the previously published map of a different mapping population (Gebhardt et al. 2001). The SCAR marker $SCG17_{448}$ and the ISSR marker $UBC811_{660}$ were located distal to *Ns* (Fig. 2).

Ns is located in a region of potato chromosome VIII where no other *R* gene has been identified so far (Gebhardt and Valkonen 2001). Among several putative QTL (quantitative trait loci) for leaf resistance to *Erwinia carotovora* ssp. *atroseptica* mapped in yet another population (Zimnoch-Guzowska et al. 2000), QTL *Eca8B* may be located in a similar segment of linkage group VIII as the *Ns* locus. The localization of the *Ns* locus extends the potato function map for pathogen resistance and is a useful starting point for the map-based cloning of the *Ns* gene, which will be the subject for our further studies.

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