J. Appl. Genet. 42(2), 2001, pp. 139-144

Inter-simple sequence repeat (ISSR) markers for the *Ns* resistance gene in potato (*Solanum tuberosum* L.)

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Abstract. Inter-simple sequence repeat (ISSR) polymorphism was used for finding markers linked to the *Ns* gene, responsible for a resistance of potato (*Solanum tuberosum* L.) to potato virus S (PVS). The ISSR markers $UBC811_{660}$ and $UBC811_{950}$ were found to be linked to *Ns*. Linkage distances were estimated to be 2.6 cM and 6.6 cM, respectively. $UBC811_{660}$ showed high accuracy for detection of PVS resistance in diploid potato clones. In tetraploids, among seventeen studied genotypes containing the resistance gene, this marker was revealed in eleven. $UBC811_{660}$ can be a powerful tool for detection of genotypes carrying the *Ns* gene in diploid potato breeding programmes.

Key words: *Ns* gene, potato, ISSR markers, potato virus S, resistance.

Introduction

Potato virus S (PVS) is considered to be one of the most important viruses for potato breeding (DE BOX 1970). *Solanum tuberosum* ssp. *andigena* cv. Huaca ñahui, PI 258907 was found to be a source of the dominant *Ns* gene, controlling a resistance of potato to PVS, associated with hypersensitivity (BAERECKE 1967). Plants carrying the *Ns* gene are not infected after mechanical inoculation. In agraft-inoculation test, fading of the foliage of shoots developed from the axillary meristems appears on a seedling as a result of the hypersensitive reaction of a potato plant to PVS infection.

Using the random amplified polymorphic DNA (RAPD) technique, four RAPD markers linked to the *Ns* gene have been identified in diploid potato (MARCZEWSKI et al. 1998). To overcome problems of poor sensitivity and

Received: October 9, 2000. Accepted: January 8, 2001.
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reproducibility of the RAPD method, the RAPD marker $OPG17_{450}$ was converted into sequence-characterized amplified region (SCAR) markers (MARCZEWSKI et al. 2001). In diploid potato, the genetic distance between *Ns* and the dominant SCAR marker $SCG17_{321}$ was estimated to be 3.2 cM, while the RAPD marker $OPG17_{450}$ was estimated to be 4.6 cM from *Ns* (MARCZEWSKI et al. 1998). $SCG17_{321}$ is currently being used in indirect selection of the *Ns* resistance gene in a diploid breeding programme at the Plant Breeding and Acclimatization Institute, Młochów.

Inter-simple sequence repeats (ISSRs) have been proposed as a new type of DNA markers (ZIĘTKIEWICZ et al. 1994) that has been found to be useful in gene tagging in plants (RATNAPARKHE et al. 1998b). ISSR markers are generated by microsatellite-repeat primers, which are short tandem repetitive DNA sequences with variations at 5' and 3' anchors, amplifying regions between adjacent SSR loci (ZIĘTKIEWICZ et al. 1994). These sequences are abundant, dispersed throughout the genome, and are highly polymorphic in plant genomes (SÁNCHEZ DE LA HOZ et al. 1996). ISSRs tend to be distributed in other regions of plant genomes than RAPDs (KOJIMA et al. 1998).

The present article demonstrates two ISSR markers $UBC811_{660}$ and $UBC811_{950}$ linked to the *Ns* locus in potato.

Material and methods

The diploid potato population, *Solanum tuberosum* L., consisted of 152 individuals derived from a cross between clones DW 91-1187 and DW 83-3121 used as the female (susceptible) and male (resistant) parents, respectively. This population was previously used by MARCZEWSKI et al. (1998) to identify RAPD markers linked to the *Ns* locus. The utility of the identified ISSR markers was verified in resistant and susceptible diploid clones (MARCZEWSKI et al. 2001) and tetraploid potatoes.

DNA was isolated from freeze-dried leaf tissue using a Genomic-tip 100/G kit (Qiagen GmbH). An equal quantity of DNA from 8 resistant and 8 susceptible F_1 individuals was pooled to form the resistant and susceptible bulks, respectively (MICHELMORE et al. 1991). The concentration of DNA in the two bulks and the two parental DNAs was adjusted to 20 ng/µl.

Most PCR analyses were performed in 20 mM Tris-HCl pH 8.4, 50 mM KCl, 0.1% Triton X-100, 1.5% formamide, 2.5 mM $MgCl_2$, 0.2 mM of each deoxynucleotide, 200 nM of primer, 0.8 unit of *Taq* DNA Polymerase (Gibco BRL), and 30 ng of genomic DNA in a total volume of 20 µl. The PCR parameters adapted from RATNAPARKHE et al. (1998a) were: 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 20 s and extension at 72°C for 2 min, with a 10 min of final extension at 72°C. Amplifications with some primers were carried out in 5 mM $MgCl_2$ and at 42°C. A Perkin Elmer Cetus 9600 thermal cycler

was used. PCR products were visualized by electrophoresis in 1.4% agarose gels in TBE buffer and ethidium bromide staining.

One hundred ISSR primers (UBC set ≠ 9) of 15-23 nucleotides in length were received from the Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, Canada.

Linkage analysis was performed using the LINKAGE-1 software (SUITER et al. 1983).

Results and discussion

Of the 100 UBC primers tested, 44 primers, mostly (AT) and (CT)-rich sequences, did not show amplification even at 42°C of annealing temperature. The remaining 56 primers gave 368 distinguishable bands after PCR amplification, ranging in size from 300 to 2000 bp, of which 145 (39%) were polymorphic between the parents. Most of these primers (40 out of 56) annealed to dinucleotide repeats, while the other sixteen annealed to tri-, tetra- and penta-nucleotide repeats. An average of 6.6 products per primer were amplified using ISSR sequences. It is a slightly higher value than that obtained in amplification of the two parental DNA with RAPD primers, where an average of 4.8 bands per primer were identified (MARCZEWSKI et al. 1998).

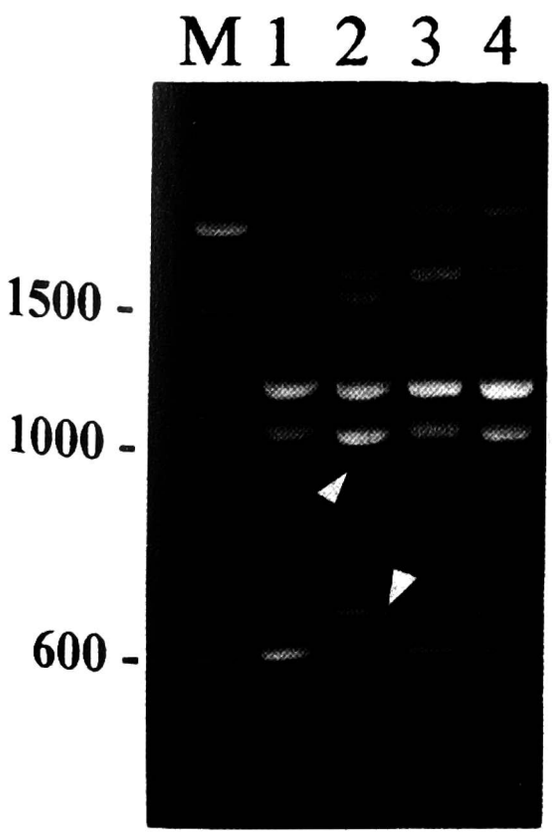

Figure 1. Patterns of amplified DNA of the parents DW 91-1187 (susceptible, lane 1) and DW 83-3121 (resistant, lane 2), and the DNA bulks presenting susceptible (lane 3) and resistant (lane 4) phenotypes, obtained with a primer UBC811. The ISSR markers $UBC811_{660}$ and $UBC811_{950}$ are indicated by arrows. M, a 100 bp DNA ladder

A primer UBC811 (5'GAGAGAGAGAGAGAGAC3') was found to be useful for amplifying two DNA fragments, 660 and 950 bp in size, both in the resistant parent, and in the resistant DNA bulk. They were designated as $UBC811_{660}$ and $UBC811_{950}$ (Figure 1). These DNA fragments were amplified at 42°C of the annealing temperature. $UBC811_{950}$ was visible at reduced intensity in comparison with the smaller DNA fragment, though the annealing temperature of 50°C yielded this band to be amplified with a higher intensity (data not shown).

The linkage relationships between the *Ns* locus and the identified ISSR markers, $UBC811_{660}$ and $UBC811_{950}$,

Table 1. Phenotypic frequencies and χ^2 values for goodness of fit to the expected ratio 1:1:1:1 (two-point: χ^2 AB) in pairwise combinations of the *Ns* and ISSR, RAPD and SCAR markers, and recombination frequency between loci in the F_1 population of diploid potato clones

Locus		Phenotype*				χ^2 AB**	Recombination frequency ± SE
A	B	AB	Ab	aB	ab		
Ns	$UBC811_{660}$	73	2	2	75	136.4	2.6 ± 1.3
Ns	$OPE15_{550}$	72	3	1	76	136.5	2.6 ± 1.3
Ns	$SCG17_{321}$	72	3	2	75	132.7	3.2 ± 1.5
Ns	$UBC811_{950}$	72	3	7	70	115.0	6.6 ± 2.0
$UBC811_{660}$	$OPE15_{550}$	73	0	2	77	144.2	1.3 ± 0.9
$UBC811_{660}$	$SCG17_{321}$	74	1	0	77	148.1	0.7 ± 0.7
$UBC811_{660}$	$UBC811_{950}$	74	1	5	72	129.3	4.0 ± 1.6
$OPE15_{550}$	$SCG17_{321}$	73	0	1	78	148.1	0.7 ± 0.7
$OPE15_{550}$	$UBC811_{950}$	72	1	7	72	122.5	5.2 ± 1.8
$SCG17_{321}$	$UBC811_{950}$	73	1	6	72	125.9	4.6 ± 1.7

* Number of seedlings; A and/or B = PVS resistant or presence of the markers; a and/or b = PVS susceptible or absence of the markers.

** Significant at the 0.01 probability level.

were estimated to be 2.6 and 6.6 cM from *Ns*, respectively (Table 1). The linkage of 2.6 cM for $UBC811_{660}$ and *Ns* is similar to the distance between the resistance locus and the RAPD marker $OPE15_{550}$ (MARCZEWSKI et al. 1998). $UBC811_{660}$ and $OPE15_{550}$ are located on the same side of the gene and linked to each other with a recombination frequency of 1.3 ± 0.9. Genetic linkage between $UBC811_{660}$ and the target locus is stronger than it has been recently detected for the SCAR marker $SCG17_{321}$ (MARCZEWSKI et al. 2001).

Similarly to the previous results obtained with $SCG17_{321}$ (MARCZEWSKI et al. 2001), the *Ns*-linked $UBC811_{660}$ marker was not amplified in five susceptible diploid clones, while it was present in eight resistant diploid genotypes tested (data not shown).

The amplification of $UBC811_{660}$ and $UBC811_{950}$ in tetraploid potatoes, in which the resistance to PVS originated from the clones G-LKS 678147/60 and MPI 65118/3 (MARCZEWSKI et al. 1998), is presented in Table 2. Of these clones or cultivars, eleven and seven out of the seventeen resistant genotypes could be identified with $UBC811_{660}$ and $UBC811_{950}$, respectively. In addition to seven resistant clones or cultivars revealed by the SCAR marker $SCG17_{321}$, four other resistant tetraploid genotypes could be detected with $UBC811_{660}$.

Cultivars Meduza and Barycz, resistant to PVS, are derived from the resistant clone PS 1501. In contrast to $SCG17_{321}$, which was not revealed in cv. Barycz,

Table 2. Presence (+) or absence (–) of the SCAR marker $SCG17_{321}$ and the ISSR markers $UBC811_{660}$ and $UBC811_{950}$, in tetraploid potato clones or cultivars

Tetraploid clones or cultivars	Reaction to PVS	SCG 17_{321}	UBC 811_{660}	UBC 811_{950}	A source of resistance
PS 662	R	–	–	+	MPI 65118/3
PS 646	R	–	–	+	
PS 150	R	–	–	+	
94-IX-4	R	–	–	–	
92-IX-63	R	–	+	–	
92-IX-79	R	–	+	–	
Barycz	R	–	+	–	
Klepa	R	+	+	–	
Meduza	R	+	+	–	
Omulew	R	+	+	–	
PW 286	R	–	–	+	G-LKS 678147/60
88-294	R	–	–	+	
88-444	R	–	+	–	
88-890	R	+	+	+	
PW 209	R	+	+	+	
PW 353	R	+	+	–	
PW 363	R	+	+	–	
Jagoda	S	–	–	+	
Lena	S	–	–	–	
Karlena	S	–	–	+	
Nicola	S	–	–	–	
Bila	S	–	–	+	
Bona	S	–	–	+	
Igor	S	–	–	–	
Bintje	S	–	–	+	

R = resistant, S = susceptible.

$UBC811_{660}$ proved to be amplified in both of them. Then, according to pedigree information, the *Ns* gene in clones 88-294 and 88-890 originated from clone 83-1366. However, $UBC811_{660}$ was observed only for 88-890 (Table 2). We cannot exclude the possibility that the tetraploid genetic background of potato resulted in the DNA amplification.

$UBC811_{660}$ was not amplified in eight susceptible cultivars representing a wide variety of germplasm (Table 2). The presence of $UBC811_{950}$ in some of these amplification patterns might be a result of too weak linkage (6.6 cM) between this marker and the *Ns* locus.

The amplification of $UBC811_{660}$ is highly repeatable, enabling clear visualization on agarose gels and unambiguous scoring of its presence or absence. It can be used, in addition to analysis of $SCG17_{321}$, in diploid potato breeding programmes, particularly in resolving some classification errors. Work is currently in progress

towards the mapping of the *Ns* gene on the genetic map of potato using AFLP technology (VOS et al. 1995). This technique, which is also highly efficient as the primary marker system, gives a chance to find other DNA markers linked tightly to *Ns* and extend this marker-assisted selection to all tetraploid potato crosses.

Acknowledgements. The study was in part supported by the State Committee for Scientific Research in Poland, project 5 PO6A 031 16.

REFERENCES

BAERECKE M. (1967). Überempfindlichkeit gegen das S-virus der Kartoffel in einem bolivianischen Andigena-Klon. Züchter 37: 281-286.

DE BOX J. A. (1970). Reactions of various plant species to inoculation with potato virus S. Neth. J. Plant Pathol. 76: 70-78.

KOJIMA T., NAGAOKA T., NODA K., OGIHARA Y. (1998). Genetic linkage map of ISSR and RAPD markers in Einkorn wheat in relation to that of RFLP markers. Theor. Appl. Genet. 96: 37-45.

MARCZEWSKI W., OSTROWSKA K., ZIMNOCH-GUZOWSKA E. (1998). Identification of RAPD markers linked to the *Ns* locus in potato. Plant Breeding 117: 88-90.

MARCZEWSKI W., TALARCZYK A., HENNIG J. (2001). Development of SCAR markers linked to the *Ns* locus in potato. Plant Breeding, in press.

MICHELMORE R.W., PARAN I., KESSELI R.V. (1991). Identification of markers linked to disease resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions using segregating populations. Proc. Natl. Acad. Sci. USA 88: 9828-9832.

RATNAPARKHE M.B., SANTRA D.K., TULLU A., MUEHLBAUER F.J. (1998a). Inheritance of inter-simple-sequence-repeat polymorphisms and linkage with a fusarium wilt resistance gene in chickpea. Theor. Appl. Genet. 96: 348-353.

RATNAPARKHE M.B., TEKEOGLU M., MUEHLBAUER F.J. (1998b). Inter-simple--sequence-repeat (ISSR) polymorphisms are useful for finding markers associated with disease resistance gene clusters. Theor. Appl. Genet. 97: 515-519.

SÁNCHEZ DE LA HOZ M.P., DÁVILA J.A., LOARCE Y., FERRER E. (1996). Simple sequence repeat primers used in polymerase chain reaction amplifications to study genetic diversity in barley. Genome 39: 112-117.

SUITER K. A., WENDEL J.F., CASE J.S. (1983). LINKAGE-1: A Pascal computer program for the detection and analysis of genetic linkage. J. Hered. 74: 203-204.

VOS P., HOGERS R., BLEEKER M., REIJANS M., VAN DE LEE T., HORNES M., FRIJTERS A., PELEMAN J., KUIPER M., ZABEAU M. (1995). AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407-4414.

ZIĘTKIEWICZ E., RAFALSKI A., LABUDA D. (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20: 176-183.