Arch Virol (2006) 151: 1243–1247
DOI 10.1007/s00705-006-0760-9

Archives of Virology
Printed in Austria

Biological and sequence data suggest that potato rough dwarf virus (PRDV) and potato virus P (PVP) are strains of the same species

Annotated Sequence Report

G. A. Massa[1], **M. E. Segretin**[2], **M. Colavita**[3], **M. F. Riero**[3], **F. Bravo-Almonacid**[2], and **S. Feingold**[1]

[1]Laboratorio de Biotecnología Agrícola, PROPAPA, INTA Balcarce, Argentina
[2]INGEBI-CONICET, Vuelta de Obligado, Argentina
[3]Laboratorio de análisis de semillas de papa, PROPAPA, INTA Balcarce, Argentina

Received October 19, 2005; accepted March 2, 2006
Published online April 10, 2006

Virus provenance

Potato rough dwarf virus (PRDV, genus *Carlavirus*) was originally described in Argentina by Butzonitch et al. [1]. Potato virus P (PVP) had been reported previously in Brazil [3] and also catalogued as a member of the genus *Carlavirus* [5]. PRDV presents frequent asymptomatic infections, and when symptoms appear, causes dwarfism, leaf deformation, leaf roughness, and systemic interveinal chlorosis [5]. Until now, PVP has been reported to be asymptomatic [5]. *Nicotiana occidentalis* has been used as propagation host and inoculated mechanically at the 3–4 leaf stage with sap from infected leaves from original virus sources (cv. Sierra Volcán INTA for PRDV and from cv. Baronessa for PVP). Previous results using double antibody sandwich enzyme-linked immuno-sorbent assay (DAS-ELISA) [2] showed cross-reactions in PVP-infected plants with anti-PRDV and vice versa [1].

Biological and RNA sequence comparison

Artificial inoculations were performed in indicator plants and potato cultivars individually with each virus for the purpose of comparing them. Observed symptoms were associated with virus presence detected by DAS-ELISA. Symptomatology of

PVP and PRDV in indicator plants was almost identical. Minor differences were observed in two independent trials with *N. occidentalis*, where plants infected with PRDV showed systemic interveinal (sivc) and leaf deformation (ld), while the ones infected with PVP also presented leaf roughness (lr), these being the first PVP symptoms reported (Table 1). All potato cultivars were infected by PVP and PRDV as detected by ELISA, with the exception of Calén INTA, which was infected with PRDV, but not with PVP (Table 1). Despite this evidence, no symptoms were observed in primary infection in any of the ELISA-positive plants. However, plants obtained from infected tubers (secondary infection) of Primicia INTA and Sierra Volcán INTA showed symptoms with PRDV, but not with PVP (Fig. 1, Table 1). This is in agreement with previous observations on PRDV-infected plants that only showed symptoms two or three generations after primary infection (M. Colavita, pers. comm.). Cross-reactions were always detected using anti-PRDV in PVP-infected plants and vice versa (data not shown).

A partial genomic sequence of PRDV was determined from clones of a cDNA library made using an Universal RiboClone® cDNA Synthesis System (Promega, USA; [4, 6]). Comparison of PRDV clone sequences with complete genomes from related members of the genus *Carlavirus* [BlScV, NC_003499; LSV, NC_005138] revealed that they correspond to the genes encoding the coat (CP), 11 kDa, replicase, and triple block proteins (Fig. 2). Primers were designed to amplify homologous PVP regions by PCR amplification from purified viral single-stranded RNAs. The compared amino acid (aa) sequences comprised 47 aa from the 3′ end of CP with a stop codon at nucleotide 142 (nt); the second ORF, corresponding to the 11-kDa protein, spanned 106 aa from a methionine start codon (in position 137 nt) to a stop codon (318 nt downstream). Consequently, an overlap of 8 nt that defined a frame shift between CP and the 11-kDa protein genes was found. Six nucleotide changes were found in the 11-kDa protein gene, and three of them were silent. Consequently, the 11-kDa protein presented 98% aa identity between PRDV and PVP. The CP nucleotide sequences showed 100% similarity between both viruses. The 915-bp amplified fragment corresponding to the replicase gene showed two nt differences, one being silent, resulting in 99% aa similarity. The complete sequences of the TGB1 and TGB3 genes and a fragment of 276 bases corresponding to the TGB2 gene presented a 100% identity between PVP and PRDV. Overall, we have found that the amino acid sequence identity between PRDV and PVP was 99.5%.

Based on results on host range, similarity of symptoms and sequence comparison and following the criteria of Tsuneyoshi et al. [7] in classifying two *Allium* carlaviruses (SLV and GLV), we suggest that PRDV and PVP should be recognized as strains of the same virus species. Complete sequence comparison of PRDV and PVP will ultimately determine the real relationship between these viruses.

Sequences for the coat and 11-kDa proteins for PVP has been deposited in the GenBank database under accession number DQ023270. Replicase and TGB gene sequences were deposited in GenBank database (www.ncbi.nlm.nih.gov) under accessions DQ022557, DQ022558, DQ211578, DQ211579 for PRDV and DQ211580, DQ375244, DQ375245, DQ375246 for PVP.

Table 1. Results of the visual symptoms and DAS-ELISA for differential hosts and potato varieties. Lines in gray indicate differences observed between PRDV and PVP

DIFFERENTIAL HOST	SYMPTOMS		ELISA	
	PRDV	PVP	PRDV	PVP
Nicotiana xanthi	n/s	n/s	–	–
Nicotiana clevelandii	n/s	n/s	+	+
Nicotiana occidentalis	sivc/ld	sivc/ld/lr	+	+
Nicotiana benthamiana	n/s	n/s	–	–
Nicotiana tabacum cv samsun	n/s	n/s	–	–
Nicotiana bigelovii	n/s	n/s	+	+
Nicotiana tabacum cv white burley	n/s	n/s	–	–
Nicotiana glutinosa	n/s	n/s	–	–
Nicotiana debneyi	n/s	n/s	+	+
Nicotiana rústica	n/s	n/s	–	–
Nicotiana edwardsonii	n/s	n/s	+	+
Nicotiana megalosiphon	sivc	sivc	+	+
Chenopodium album	n/s	n/s	–	–
Physialis floridana	n/s	n/s	–	–
Physialis angulata	n/s	n/s	–	–
Datura metel	n/s	n/s	+	+
Datura stramonium	n/s	n/s	+	+
Petunia hybrida	n/s	n/s	+	+
S. lycopersicum cv ¨seyhene¨	n/s	n/s	–	–
S. lycopersicum cv ¨rutger¨	n/s	n/s	–	–
Nicandra physaloides	n/s	n/s	+	+

POTATO VARIETIES	SYMPTOMS				ELISA			
	PRDV		PVP		PRDV		PVP	
	G1	G2	G1	G2	G1	G2	G1	G2
Bintje	n/s		n/s	n/s	+		+	+
Calén INTA	n/s		n/s	n/s	+		–	–
Frital INTA	n/s		n/s	n/s	+		+	+
Kennebec	n/s	n/s	n/s	n/s	+	+	+	+
Primicia INTA	n/s	d/ld/lr	n/s	n/s	+	+	+	+
Sierra Volcán INTA	n/s	d/ld/lr	n/s	n/s	+	+	+	+
Spunta	n/s		n/s	n/s	+		+	+

Symbols:

d: dwarfism
ld: leaf deformation
lr: leaf roughness
sivc: systemic interveinal chlorosis
n/s: no symptoms
blank cells: not tested
+: ELISA positive
–: ELISA negative
G1: first generation
G2: second generation

Fig. 1. **A** Sierra Volcán INTA infected with PVP (left, symptomless) and PRDV (right, with symptoms) and **B** Primicia INTA infected with PVP (left, symptomless) and PRDV (right, with symptoms)

Fig. 2. Schematic representation of the genomic organization of the PRDV and PVP RNA encoding the viral RNA-dependent RNA polymerase (*RdRp*), triple block proteins (*TGBs*), coat protein (*CP*), and 11-kDa protein (*ORF6*)

Acknowledgements

We thank Dr. J. Daniels for supplying PVP virus and antiserum and Dr. I. Butzonitch for supplying PRDV virus used in this study. GM support was obtained from Consejo de Investigaciones Científicas de la Provincia de Buenos Aires (CIC) in the past and presently from a CONICET/INTA fellowship. MES holds a fellowship from CONICET and FB-A is a Research Scientist of CONICET (Argentina).

References

1. Butzonitch IP, Nieto A, Truol GAM, Colavita ML (1996) Características de un nuevo *Carlavirus* relacionado con el virus S de la papa hallado en Balcarce, Argentina. V Congreso Argentino de Virología y II Encuentro de Virólogos Latinoamericanos Tandil, Argentina
2. Clark MF, Adams AN (1977) Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J Gen Virol 34: 475

3. Daniels J, Carvalho TCP, Bisognin DA (1993) Um novo virus latente de batata encontrado no Rio Grande do Sul. Fitopat Bras 18: 287
4. Gubler U, Hoffman BJ (1983) A simple and very efficient method for generating cDNA libraries. Gene 25: 263–269
5. Jeffries CJ (1998) Technical Guidelines for the safe movement of germoplasm FAO/IPGRI No. 19, Potato
6. Okayama H, Berg P (1982) High-efficiency cloning of full-length cDNA. Mol Cell Biol 2: 161–170
7. Tsuneyoshi T, Matsumi T, Deng TC, Sako I, Sumi S (1998) Differentiation of *Allium* carlaviruses isolated from different parts of the world based on the viral coat protein sequence. Arch Virol 143: 1093–1107

Author's address: S. Feingold, Laboratorio de Biotecnología Agrícola, PROPAPA, INTA Balcarce CC276 (7620), Argentina; e-mail: sfeingold@balcarce.inta.gov.ar