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ANNOTATED SEQUENCE RECORD

Comparison of complete sequences of potato rough dwarf virus and potato virus P and their relationships to other carlaviruses

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Potato rough dwarf virus (PRDV) was originally described in Argentina [2] while potato virus P (PVP) was first reported from Brazil [4]. These viruses were essentially indistinguishable in test plant reactions when inoculated onto 21 different solanaceous species [7]. Of the 10 solanaceous species susceptible to both PRDV and PVP, the majority did not display symptoms. Only *Nicotiana megalosiphon* and *N. occidentalis* showed systemic symptoms consisting of leaf deformations, leaf rugosity and/or interveinal chlorosis. Six of the 7 potato varieties tested were susceptible to both PRDV and PVP, but visible symptoms consisting of plant stunting and the aforementioned leaf symptoms were only evident in second-generation plants of the potato cvs. Primicia and Sierra Volcán infected by PRDV [7].

When amplicons were obtained from PRDV- and PVP-infected tissue using carlavirus-specific primers for polymerase chain reaction (PCR) these viruses were initially classified as members of tentative species of the genus *Carlavirus* [6]. Recent biological, serological and partial sequence comparisons have suggested that PRDV and PVP should be considered strains of the same carlavirus species [7, 8]. This report describes the complete genomes of PRDV and PVP, and their relationships with other carlaviruses.

The PVP and PRDV isolates studied were those from the potato cvs. Baronessa and Sierra Volcán [7]. They were inoculated onto plants of *Nicotiana occidentalis,* and one month after inoculation, leaves of these plants were used for virion purification following the International Potato Center protocol (CIP Training Manual, Techniques in Plant Virology). RNA extracted from purified PRDV and PVP particles was used for cDNA synthesis using the Universal RiboClone® cDNA Synthesis System (Promega, USA). After cloning of cDNA in pZErO®-2 (Invitrogen, USA), the recombinant clones were sequenced using SP6 and T7 vector-specific primers in a MegaBACE 750 Sequencer (GE, Sweden) with DYEnamic ET Dye Terminator Cycle Sequencing kits (GE, Sweden). The 5′ rapid amplification of cDNA ends (RACE) method was employed to obtain the 5′-terminal ends of both viral genomes [9]. Amplicons were cloned into a pGEM-T vector (Promega, USA). At least four independent clones were sequenced for each virus as described above. To obtain the complete sequence of the 3′ non-translated region (NTR), we used a tailed oligo-dT ["oligo-dTail"; 5′-GCTGAAGACGGCCTATGTGGCC $(T)_{16}$-3′] as a reverse primer for PCR amplification, followed by cycle sequencing of the amplicon with the primer "Tail" (5′-GCTGAAGACGGCCTATGTGGCC-3′).

Using the ContigXpress module from Vector NTI 9.0 suite (Invitrogen, USA), sequences from multiple inserts were assembled into two contigs to generate consensus sequences of the PRDV and PVP genomes, in which each nucleotide (nt) position was based on sequences derived from at least two independent clones (with a minimum quality index of 93 according to MegaBace Sequencing ScoreCard software, GE, Sweden). Sequence similarity searches were performed using the BLAST program. A dendrogram was constructed using the neighbor-joining method with 1,000 bootstrap replicates. Sequences of other carlaviruses used for the comparison were obtained from GenBank. For comparison of the PRDV and PVP genomes, we used a genome alignment package called Mauve [5].

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The genomes of PRDV (acc. no. EU020009) and PVP (acc. no. EU338239) had 8,404 and 8,392 nts, respectively. Each of them contained six open reading frames (ORFs) and had a genomic organization typical of viruses of the genus *Carlavirus* [1] (Fig. 1). PRDV and PVP had an overall nt sequence identity of 97.7% to each other. Figure 1 shows the alignment between PRDV and PVP, where the height of the similarity profile corresponds to the average level of conservation of the genome sequence. The white regions show sequences that were dissimilar and specific to each virus. While the 5′ and 3′ NTRs of PRDV were 18 and 3 nt longer than those of PVP, the two viruses shared 5′ and 3′ NTR sequence identities of 41.1 and 71.2%, respectively. The PRDV and PVP replicases showed 96.1 % amino acid identity, and major discrepancies were found in the N-terminal region of the RdRp domain (Fig. 1). The aa sequences of triple gene block (TGB) proteins were completely conserved between PRDV and PVP, and their coat proteins (CP) showed 99.7% aa identity. ORF6 potentially encodes a nucleic-acid-binding protein (NABP) of 105 aa (Mr of 11.9 kDa). PRDV and PVP shared 96.2% aa sequence identity in this protein, differing only in four C-terminal residues. Chiba et al. [3] showed that silencing suppressors in diverse filamentous viruses possess four highly conserved cysteine residues in a zinc ribbon motif plus three conserved aas (SXXXXXXRA). The ORF6 proteins of PRDV and PVP have these conserved motifs and may thus potentially act as a silencing suppressor. Therefore, the observed variation in ORF6 sequences between PVP and PRDV might be associated with the virulence differences observed previously [7, 8].

The genome sequences of PRDV and PVP differed strikingly (identities of 44–55%) from those of all other carlaviruses. Blueberry scorch virus (BlScV) showed the highest overall nt sequence identity (54.7%) with PRDV, while passiflora latent virus (PLV) was 55.3% identical to PVP. Consequently, PRDV and PVP shared highest aa identities with BlScV in all proteins, with the exception of TGB 3, which was most similar (only 34% aa identity) to that of potato virus S (PVS). The replicase protein of PVP presented an additional exception, showing the highest identity with PVS (50%). However, the distance tree grouped PRDV and PVP in a clade different from that of BlScV and PLV, which were grouped with PVS and lily symptomless virus (LSV) (Fig. 2) due to the high similitude among them. This indicates that BlScV, PVS, LSV and PLV are equally distant to PRDV and PVP (Fig. 2). The topology of the distance tree was essentially identical when using translated sequences.

The mean overall nt sequence identity between the PRDV-PVP clade and the BlScV, PVS, LVS, PLV clade was 54%. The aa identity between the replication protein of the members of this group and PRDV-PVP was around 50%, whereas the CP aa identities ranged from 63 to 68%. Thus, according to Adams et al. [1], the PRDV-PVP clade should be regarded as a different species within the genus *Carlavirus*. PRDV and PVP had a 97.7% nt identity and an overall aa identity of 97%. Virus strain delineation based solely on nt or aa sequences is cumbersome, and different authors have proposed different boundaries. The seventh ICTV report, cited by Adams et al. [1] states that strains of individual viruses share 75–90% identity in the CP core region. Based on previous biological and serological differences [7, 8] and despite the high sequence similarity, we propose that PRDV and PVP be considered strains of a new species of the genus *Carlavirus.* In the course of this research, these viruses were assigned as strains of Carlavirus species under the name PVP by ICTV. Accordingly, the former PRDV strain from Argentina can be referred to as PVP-Ar and the Brazilian strain as PVP-Br to denote their origin.

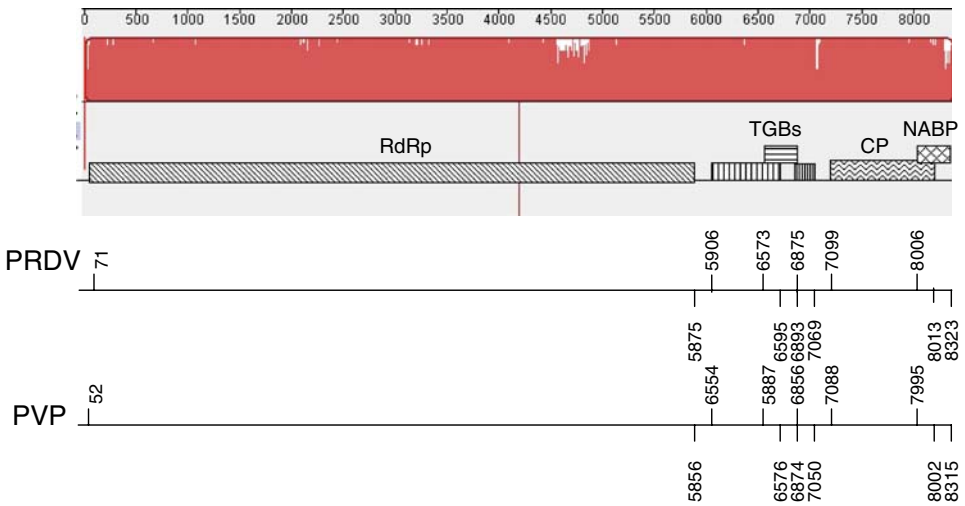

Fig. 1 Schematic representation of the PRDV and PVP genome organization and the alignment of the complete genome of PVP (*above*) and PRDV (*below*) obtained with the MAUVE software. The height of the similarity profile corresponds to the average level of conservation in that region of the genome sequence. *White areas* denote sequence variation between genomes

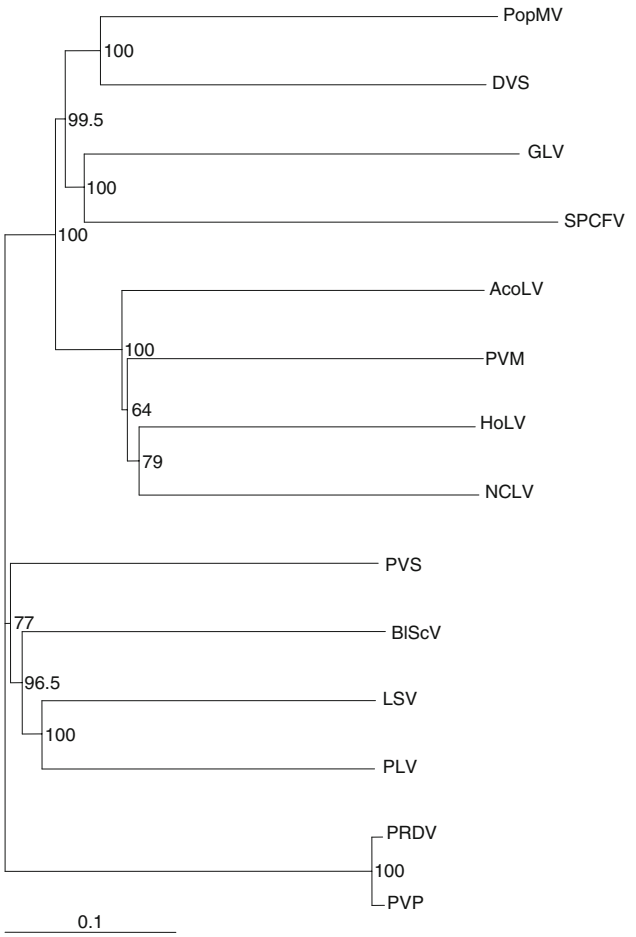

Fig. 2 Distance tree illustrating the relationships between the complete nt sequences of potato rough dwarf virus (PRDV), potato virus P (PVP) and other completely sequenced carlaviruses [aconitum latent virus (AcoLV), NC_002795; blueberry scorch virus (BlScV), AY941198; daphne virus S (DVS), AJ620300; garlic latent virus (GLV), NC_003557; hop latent virus (HpLV), NC_002552; lily symptomless virus (LSV), AJ516059; narcissus common latent virus (NCLV), NC_008266; passiflora latent carlavirus (PLV), NC_008292; poplar mosaic virus (ATCC PV257) (PopMV), X65102; potato virus M (PVM), NC_001361; potato virus S (PVS), AJ863509; and sweet potato chlorotic fleck virus (SPCFV), NC_006550]. For bootstrapping, 1,000 datasets were generated by the program PHYLIP. Bootstrap values are given at the nodes. The scale bar corresponds to 0.1 substitutions per site

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