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agrocolfabog@gmail.com
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Vallejo C., Daniela; Gutiérrez S., Pablo Andrés; Marín M., Mauricio
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Genome characterization of a *Potato virus S* (PVS) variant from tuber sprouts of *Solanum phureja* Juz. *et* Buk.

Caracterización del genoma de una variante de *Potato virus S* (PVS) obtenida en brotes de tubérculos de *Solanum phureja* Juz. *et* Buk.

Daniela Vallejo C.[1], Pablo Andrés Gutiérrez S.[1], and Mauricio Marín M.[2]

ABSTRACT

Potato virus S (PVS) is a prevalent virus in potato fields in Colombia and the rest of the world. PVS has been classified into two separate lineages, PVS^{O} (Ordinary) and PVS^{A} (Andean), which are genetically distinct. In this study, the complete genome sequence of a new PVS isolate (PVS_Antioquia) was obtained using High-throughput sequencing (Illumina HiSeq-2000) from tuber sprouts of *Solanum phureja* (var. Criolla Colombia). The PVS_Antioquia genome comprises 8,483 nt that code for six ORFs: RdRp (223 kDa), TGBp1-3 (25kDa, 12kDa, 7kDa) CP (32.3 kDa) and NABP (11 kDa) and share a high sequence identity with respect to the PVS_RVC (>95%) from Colombia, in contrast to 81 to 82% identity with respect to the PVS^{A} and PVS^{O} isolates from around the world. This genome information was used to design RT-qPCR primers that are specific for the Colombian PVS strains (RVC and Antioquia) which were validated in *S. phureja* leaf and tuber samples. These primers detected PVS in 80 and 60% of a set of fifteen leaf and tuber samples, respectively, suggesting a high incidence of this virus in the potato crops of Antioquia.

Key words: plant viruses, carlaviruses, diagnostic techniques, ELISA, PCR, potatoes.

RESUMEN

El *Potato virus S* (PVS) es un virus prevalente en los cultivos de papa de Colombia y otros lugares del mundo. Ha sido dividido en dos razas principales denominadas PVS^{O} (Ordinaria) y PVS^{A} (Andina), que representan a su vez dos linajes genéticos divergentes. En este trabajo se obtuvo la secuencia del genoma completo de un aislamiento de PVS denominado PVS_Antioquia, por secuenciación masiva de nueva generación (Illumina HiSeq-2000) realizada sobre extractos de transcriptoma de tubérculos de *Solanum phureja* (var. Criolla Colombia). A partir de este genoma se diseñaron primers específicos para la detección por RT-qPCR de variantes Colombianas de PVS (RVC y Antioquia), validándose su utilidad en pruebas de detección en tejido foliar y de tubérculos de *S. phureja*. El genoma de PVS_Antioquia tiene un tamaño de 8.483 nt que codifica para seis ORFs: RdRp (223 kDa), TGBp1-3 (25kDa, 12kDa, 7kDa) CP (32,3 kDa) y NABP (11 kDa), y comparte altos niveles de identidad con el aislamiento PVS_RVC (>95%) de Colombia y tan solo de 81 a 82% con representantes de PVS^{A} y PVS^{O} de diferentes países del mundo. Los primeros diseñados permitieron detectar el virus en 80 y 60% de 15 muestras foliares y 15 de tubérculos, respectivamente, lo que puede indicar la ocurrencia de altos niveles de incidencia de PVS y sus variantes en los cultivos de papa de Antioquia.

Palabras clave: virus de plantas; carlavirus, técnicas de diagnóstico, ELISA, PCR, papas.

Introduction

Potato virus S (PVS), a virus first described in the Netherlands in 1952 (de BruynOuboter, 1952), is a member of the *Carlavirus* genus (*Betaflexiviridae* family) with high incidence in potato fields around the world (Cox and Jones, 2010). PVS virions consist of flexuous rods that are 610-710 to 12-15 nm in size with a positive-sense RNA genome of approximately 8,500 nt in length and six open reading frames (ORFs) encoding an RNA-dependent RNA-polymerase (RdRp, 223 kDa), the triple gene-block proteins (TGBp1-3, 25kDa, 12kDa, 7kDa) involved in virus cell-to-cell movement, a viral coat protein (CP, 32.3 kDa) and a NABP (cysteine-rich nucleic-acid-binding protein) (11 kDa) (Martelli *et al*., 2007). PVS can be transmitted in a non-persistent manner by aphids, such as *Aphis fabae*, *A. nasturtii*, *Myzus persicae* and *Rhopalosiphum padi*; mechanical means and/or infected seed tubers (Wardrop *et al*., 1989; Lambert *et al*., 2012). Depending on the ability to cause systemic infection or not in the experimental host *Chenopodium* sp., PVS has been classified into two strains: PVS^{A} (Andean) and PVS^{O} (Ordinary), respectively

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[1] Laboratory of Industrial Microbiology, Faculty of Sciences, Universidad Nacional de Colombia. Medellin (Colombia)
[2] Laboratory of Cellular and Molecular Biology, Faculty of Sciences, Universidad Nacional de Colombia. Medellin (Colombia). mamarinm@unal.edu.co

(Hinostroza-Orihuela, 1973; Lin *et al*., 2009). More recently, Cox and Jones (2010) proposed the acronyms PVS^{O-CS} for isolates that invade *Chenopodium* sp. systemically, but are not within clade PVS^{A} and PVS^{A-CL} to divergent PVS^{A} isolates that cannot infect *Chenopodium* sp. systemically and suggested that the term PVS^{A} should be applied strictly to members of the genetically distinct clade. The natural host range of PVS seems to be limited because it has only been reported to infect the sweet cucumber (*Solanum muricatum* Ait.) (Dolby and Jones, 1988) and different potato species, such as *Solanum tuberosum* L. (Cox and Jones, 2010) and *S. phureja* Juz. & Buk (Guzmán *et al*., 2010; Gutiérrez *et al*., 2013).

Tipically, PVS strains do not induce visible symptoms in the majority of potato varieties, but, when they do, consist of mild leaf symptoms such as mosaics, leaf rugosity, vein deepening and leaf bronzing (Salari *et al*., 2011). Although the disease caused by PVS^{O} may be symptomless in the leaves and tubers of infected potatoes, the incidence may reach 100% with yield losses of 15% (Matoušek *et al*., 2005). Additionally, PVS co-infection enhances the severity of other viruses, such as *Potato virus A* (PVA), *Potato virus Y* (PVY) and *Potato virus X* (PVX) (Salari *et al*., 2011; Nyalugwe *et al*., 2012).

S. phureja, locally known as *papa criolla*, is a potato species widely grown in the South American Andes from western Venezuela to the center of Bolivia (Ghislain *et al*., 2006) and it has as a main center of diversity in the mountains in the province of Nariño (South of Colombia) (Rodríguez *et al*., 2009). This crop has received increasing attention in South America as a potential exotic export product due to its excellent organoleptic properties; tubers with yellow skin and medullary tissue; high levels of vitamins B and C, niacine and thiamine; short production cycles and reduced tuber dormancy (Rodríguez *et al*., 2013). Colombia is the country with the highest commercial explotation of potato varieties derived from *S. phureja*, which currently represents 8,500 ha and a yearly production of about 100 thousand t (Rodríguez *et al*., 2013).

Viral diseases are one of the most limiting factors in the production of *S. phureja* in Colombia, of which PVS has been shown to have a very high incidence (up to 40%) in the main potato producing provinces of Colombia: Antioquia, Boyaca, Cundinamarca and Nariño (Gil *et al*., 2013, Gutiérrez *et al*., 2012, 2013; Gutiérrez-Sánchez, 2014). The coat sequence analysis suggested the existence of at least two PVS variants in Colombia, related to PVS^{O} and PVS^{A} (Gil *et al*., 2013). A third strain was found by high-trough put sequencing of the foliar transcriptome of *S. phureja* var. Criolla Colombia (Gutiérrez *et al*., 2013). The high incidence of PVS in potato crops in Colombia, as well as the presence of new variants never detected elsewhere, highlights the need to better study this genetically distinct clade of PVS that infect *S. phureja*. In this study, we performed a high-throughput transcriptome sequencing analysis of *S. phureja* tuber sprouts infected by PVS in the province of Antioquia (Colombia), in order to characterize its sequence properties and to design a set of primers that would be useful in Real-time reverse transcription-PCR (RT-qPCR) for PVS detection.

Materials and methods

Plant samples and DAS-ELISA tests

For this study, fifteen *S. phureja* tuber samples from different storage cellars in the municipalities of La Union (six samples) and Yarumal (nine samples) and an equal number of leaf samples from *S. phureja* plots at the flowering stage in the municipalities of Entrerrios (fourth samples from one plot) and Marinilla (11 samples from three plots) were used (Tab. 1). Each tuber and leaf sample consisted of three tubers and six to eight leaflets, respectively. The samples were initially tested for PVS infection by DAS-ELISA using the PSA 40000 kit from Agdia (Elkhart, IN), which uses polyclonal antibodies and alkaline phosphatase enzyme conjugates as capture and detection reagents, respectively.

Solanum phureja transcriptome sequencing

High-throughput sequencing of the *S. phureja* transcriptome was performed on a bulk of the tuber-seed sprouts. The bulk sample was ground using liquid nitrogen followed by RNA extraction with the GeneJET Plant RNA Purification mini kit (Thermo Fisher Scientific, Waltham, MA). The library was constructed with the TruSeq RNA Sample Preparation kit (Illumina, San Diego, CA) and rRNA was depleted with the TruSeq Stranded Total RNA with Ribo-Zero Plant kit (Illumina, San Diego, CA). Sequencing was performed with the Illumina HiSeq 2000 System Provided by Macrogen (Seoul, South Korea), which resulted in a pair-end library of 24,817,068 reads for a total of 4,963,413,600 bp. Adapter sequences and low quality bases were removed with SeqTK (https://github.com/lh3/seqtk). The PVS genome was assembled with *de novo* reconstruction of the *S. phureja* transcriptome with Trinity (Grabherr *et al*., 2011) and confirmed by mapping with Bowtie2 (Langmead and Salzberg, 2012). The final contig was assembled from a total of 40,451 paired-end reads with an average depth of 949X. ORFs codifying for viral proteins were identified with BLASTX (Gish and States, 1993). Sequence alignments

TABLE 1. Samples of leaves and tuber-seeds of *Solanum phureja* used in this study to detect *Potato virus S* (PVS).

Samples	Location*	Tissue	Samples	Location*	Tissue
F1	Entrerrios, Tesorero	Leaflet	T1	La Unión, Town	Tuber-seed
F2	Entrerrios, Tesorero	Leaflet	T2	La Unión, Town	Tuber-seed
F3	Entrerrios, Tesorero	Leaflet	T3	La Unión, Town	Tuber-seed
F4	Entrerrios, Tesorero	Leaflet	T4	La Unión, Town	Tuber-seed
F5	Marinilla, Alto de Mercado	Leaflet	T5	La Unión, Town	Tuber-seed
F6	Marinilla, Alto de Mercado	Leaflet	T6	La Unión, Town	Tuber-seed
F7	Marinilla, Alto de Mercado	Leaflet	T7	Yarumal, Llanos de cuivá	Tuber-seed
F8	Marinilla, Alto de Mercado	Leaflet	T8	Yarumal, Llanos de cuivá	Tuber-seed
F9	Marinilla, Alto de Mercado	Leaflet	T9	Yarumal, Llanos de cuivá	Tuber-seed
F10	Marinilla, Alto de Mercado	Leaflet	T10	Yarumal, Llanos de cuivá	Tuber-seed
F11	Marinilla, Alto de Mercado	Leaflet	T11	Yarumal, Llanos de cuivá	Tuber-seed
F12	Marinilla, Alto de Mercado	Leaflet	T12	Yarumal, Llanos de cuivá	Tuber-seed
F13	Marinilla, Alto del Chocho	Leaflet	T13	Yarumal, Llanos de cuivá	Tuber-seed
F14	Marinilla, Alto del Chocho	Leaflet	T14	Yarumal, Llanos de cuivá	Tuber-seed
F15	Marinilla, Alto del Chocho	Leaflet	T15	Yarumal, Llanos de cuivá	Tuber-seed

* All samples were obtained in the department of Antioquia (Colombia).

were performed with MUSCLE (Edgar, 2004). Phylogenetic trees were calculated in MEGA6 (Tamura *et al*., 2013) using the Maximum Likelihood method based on the General Time Reversible model with a Gamma distribution (+G parameter = 0.4964). Nonsynonymous (Ka) and synonymous (Ks) substitution rates (denoted as Ka and Ks, respectively) were estimated using the software KaKsCalculator (Zhang *et al*., 2006) with the LPB method (Pamilo and Bianchi, 1993). Recombination analysis was done with the program RDP3 (Martin *et al*., 2010).

Primer design and RT-qPCR detection of PVS

Alignment of Colombian CP sequences, obtained in this and previous studies (Gil *et al*., 2013; Gutiérrez *et al*., 2012, 2013; Gutiérrez-Sánchez, 2014), allowed for the identification of sequences that are useful for designing RT-qPCR primers that are specific for the PVS variants found in Colombia with the aid of the program Primer3Plus (Untergasser *et al*., 2012). Validation of the RT-qPCR primers PVS_gen_F and qPVS_gen_R was performed on the fifteen tuber samples and fifteen leaf samples collected in the different municipalities of Antioquia. RNA was extracted from 100 mg of ground tissue using the GeneJET Plant RNA Purification kit (Thermo, Fisher Scientific, Waltham, MA) and eluted in 40 µL of DEPC treated water; the purity and concentration were determined by absorbance readings at 260 and 280 nm using a Nanodrop 2000C (Thermo Fisher Scientific, Waltham, MA). Retrotranscription was performed for 30 min at 50°C in 20 µL containing 200 U of Maxima Reverse Transcriptase (Thermo Fisher Scientific, Waltham, MA), 1X RT Buffer, 0.5 mM dNTP Mix, 100 pmol Oligo$(dT)_{18}$, 20 U de RiboLock RNase Inhibitor and 100-500 ng of total RNA. For the qPCR, the Maxima SYBR Green/ROX qPCR Master Mix (2X) kit (Thermo Fisher Scientific, Waltham, MA) was used in 25 µL of reaction containing 12.5 µLmix, 10 µL DEPC water, specific primers PVS_gen_F (5'ATG CCG CCY AAA CCA GAT CC 3') and qPVS_gen_R (5'AGC ATK GCT TCY TCA TTT TGC CCT G 3') at 0.3 µM and 50-100 ngc DNA. The amplification cycles consisted of 10 min at 95°C to activate the polymerase, followed by 35 cycles at 95°C for 15 s and 53°C for 45 s, using a Rotor-Gene Q-5plex Platform (Qiagen, Hilden, Germany); fluorescense was measured after each amplification cycle. The PVS positive control was obtained from a PVS infected potato leaf-tissue and a virus-free sample was used as the negative. The samples were considered positive if they exhibited fluorescence values higher than the threshold before cycle 35 (Schena *et al*., 2004). Primer specificity was verified by High Resolution Melting in the 50 and 99°C range; the identity of the amplicons was confirmed by Sanger sequencing of five amplification products (including the positive control), previously purified with the QIAquick Gel Extraction (Qiagen, Valencia, CA) kit. Sequences were edited manually with MEGA6 (Tamura *et al*., 2013) and compared against the NCBI database using BLASTN (www.ncbi.nlm.nih.gov/BLAST).

Results and discussion

Genome features of PVS_Antioquia

The analysis of High-throughput sequencing results confirmed the presence of a PVS strain, with a genome of 8,483 nt (excluding the 3' poly-A tail), closely related to strain PVS_RVC (95% nucleotide identity), that shares 81 to 82% nucleotide identity with respect to PVS^A and PVS^O isolates. The assembled sequence was deposited in GenBank under accession KR152654 with PVS_Antioquia as the strain name. No evidence of recombination was found with the program RDP3 (not shown). PVS_Antioquia has 5' and 3' untranslated regions (UTR) of 58 and 104 nt, respectively, and contains six ORFs, as expected. ORF1 (59-5986) codes for the RNA-dependent RNA polymerase (RdRp, 223 kDa) that contains the following functional domains: methyltransferase (Pfam:PF01660, residues 43-352), Carlavirus endopeptidase (Pfam:PF05379, 1000-1087), RNA helicase (Pfam:PF01443, 1175-1432) and RdRp (Pfam:PF00978, 1553-1967). ORF2 (5973-6704), ORF3 (6682-7008) and ORF4 (6972-7172) comprise the triple gene block (TGB): TGBp1 is predicted to be a 243 residue protein (26.9 kDa) with viral RNA helicase domain (Pfam:PF01443, 40-235), TGBp2 is composed of 108 residues (Pfam:PF01307, 11.7 kDa) and contains a plant viral movement motif (4-104) while TGBp3 comprises 66 residues (7.2 kDa) and contains a 7kDa viral protein motif (Pfam:PF02495, 6-65). The viral coat protein is predicted to be a 294 residues protein (32.3 kDa) encoded by ORF5 at positions 7214-8098 and contain Pfam domains PF08358 (48-99) and PF00286 (108-247), typical of Carlavirus CPs. ORF6 (8095-8379) codes for a 94 a.a. protein (10.6 kDa) and contains a cysteine-rich nucleic-acid-binding protein motif (Pfam:PF01623, 1-89). Ten polymorphic sites were detected in the PVS_Antioquia assembly: A1657G, A1933T, G3169A, T3817C, A3821G, A4828C, G4837A, A6050G, C6131T, C7600T; substitution A3821G results in amino acid change I1255V within RdRp (Fig. 1).

The sequence comparison between the PVS_Antioquia and PVS_RVC revealed a total of 310 transitions and 68 transversions for a global transition/transversion ratio of 4.56. With 60 amino acid substitutions, RdRp is the most divergent protein (96.96% aa identity); the most variable region corresponded to the one conecting the peptidase and methyl transferase domains (Fig. 2). The overall Ka/Ks ratio for the RdRp ORF was 0.12, suggesting a strong negative selection for the whole protein; however, a sliding window analysis revealed three regions undergoing neutral selection: 640-670, 739-778, 1196-1226 (Ka/Ks of 0.97, 1.00 and 0.84, respectively) and a segment within the helicase domain under positive selection (1263-1277, Ka/Ks of 3.22) (Fig. 2). The triple gene block proteins TGBp1-3 showed a high degree of conservation among the PVS lineages (Fig. 2), with measured Ka/Ks ratios of 0.08, 0.05 and 0.0 that suggested a strong negative selection. TGBp1 shares 98.3% identity at the amino acid level with respect to PVS_RVC and contains four aminoacid substitutions: Y123H, C138S, E140G and T228S. TGBp2 has only one aminoacid substitution E69G while TGBp3 is 100% identical to its homolog in PVS_RVC. The CP had a total of four amino acid substitutions (V31A, Q35E, P37S and E54G) with respect

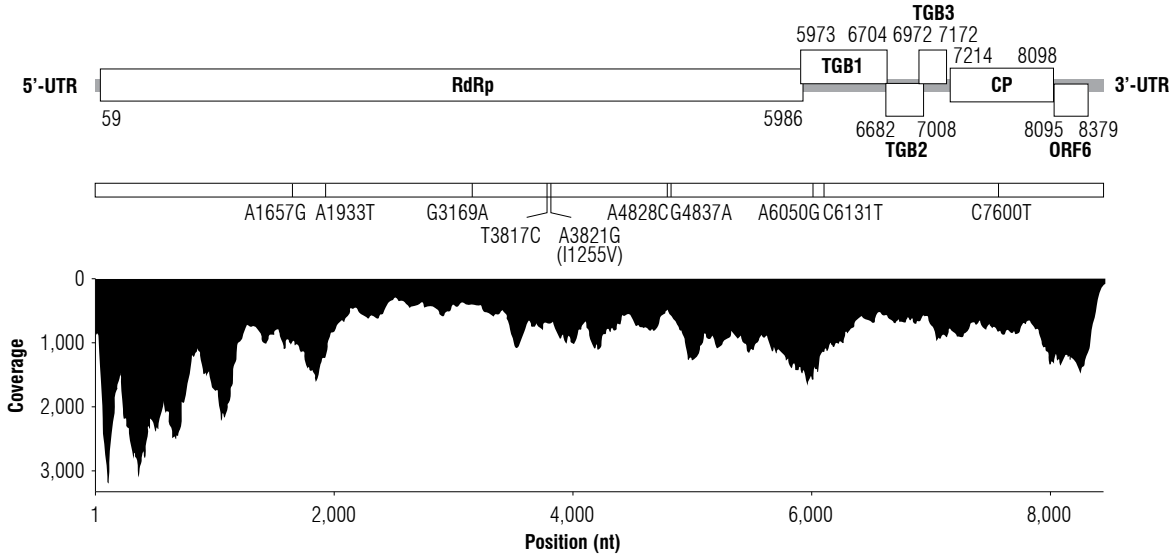

FIGURE 1. Sequence depth of the PVS_Antioquia genome assembly. Genetic features of the PVS_Antioquia genome are shown on the top diagram; nucleotide positions corresponding to each ORF are indicated. The diagram in the center shows ten nucleotide polymorphisms detected in the PVS_Antioquia assembly; only mutation A3821G translates into an amino acid change (I1255V).

to PVS_RVC and a sliding window analysis revealed a N-terminal region to be under neutral selection (Ka/Ks =1.08, 23-41). The 11K protein had the highest global Ka/Ks ratio with a value of 0.8, suggesting a mutation rate close to neutrality; however, residues 7-32 had a local Ka/Ks ratio of 1.23, indicating a slighty positive selection; the following substitutios were observed for this protein: G7S, Y14N, I28V, H40R and P81S. Recombination analyses performed on the PVC_Antioquia genome discarded recombination with other PVS strains as the mechanism of emergence for this new lineage. Failure to detect this PVS variant in previous studies was probably due to the lack of sequence information on Andean PVS strains; future studies would probably confirm the presence of this PVS lineage in other regions of Colombia and South America.

The phylogenetic analysis of the complete PVS genomes showed that isolate PVS_Antioquia clustered with PVS_RVC in an independent clade with a bootstrap of 100%. This clade is a sister group to Andean strains composed of BB-AND from Brazil (Geraldino *et al*., 2012) and RL5 from Colombia (Gutiérrez *et al*., 2012) (Fig. 3A). Using a partial segment of the CP sequence, the cluster analysis suggested the existence of four distinct groups (Fig. 3B). The first group comprised PVS^{O} strains and included three Colombian strains isolated in Colombia (Quincha-Cundinamarca, Valle de María-Antioquia and Chasques-Cundinamarca). The second group corresponded to isolates Peruvian and Vltava, of which the latter has been shown to be a recombinant strain between PVS^{O} and PVS^{A} (Geraldino *et al*., 2012). Group three comprised PVS^{A} isolates BB-AND from Brazil, Q5 from chile, Guizhou CP01 from China and four Colombian isolates (Suras-Nariño, and El Roble2, RL5, Aldana Bajo from Antioquia). The fourth clade seemed to be a lineage derived from the PVS^{A} group and comprised PVS strains infecting *S. phureja* such as

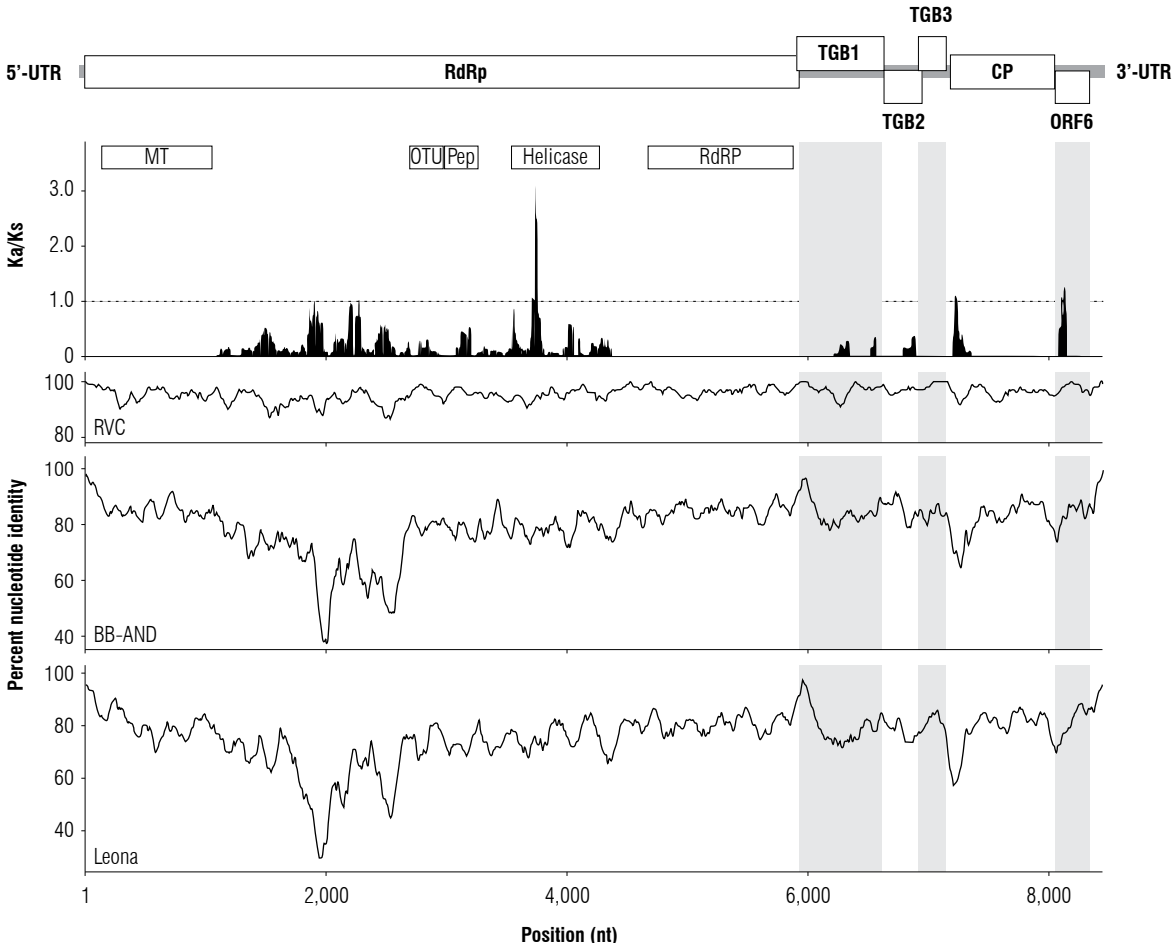

FIGURE 2. Sequence conservation profile of the PVS_Antioquia genome. The top panel illustrates the relative position of ORFs within the PVS_Antioquia genome. Sliding window Ka/Ks profile for each ORF in PVS_Antioquia with respect to PVS_RVC. Sites with Ka/Ks values above/below one indicate positive/negative selection, values close to one are indicative of neutral selection. The positions of the functional domains within the RdRp protein are indicated with squares. The lower panel shows the local conservation profile at the nucleotide level with respect to the PVS RVC, BB-AND and Leona genomes.

PVS_Antioquia as well as nine other Colombian isolates from Nariño, Antioquia and Cundinamarca, reported by Gil *et al*. (2103) and including PVS_RVC (Gutiérrez *et al*., 2013). CP sequences isolated from *S. tuberosum* (Gil *et al*., 2013) clustered in the same clade as PVS_Antioquia, suggesting that this PVS lineage does not have *S. phureja* as its unique host; however, we propose to name this derived lineage as PVS^P in reference to the first known host (*S. phureja*). Future studies on local *S. tuberosum* and *S. phureja* varieties from the South-American Andes will demonstrate whether PVS^P has a generalized distribution in countries that are part of the center of origin of the Potato or not. Additionally, it will be of great interest to determine the pathogenic characteristics of members of the PVS^P lineage (i.e local or systemic lesions) on *Chenopodium* sp., which will allow a biological comparison with respect to PVS^O and PVS^A.

Primer design and RT-qPCR detection of PVS

During the initial stages of this study, it was noticed that previously published primer sets designed to detect PVS failed to amplify some ELISA-positive samples even after exhaustive modifications of both RT-PCR and RT-qPCR protocols (data not shown). A similar situation was observed in a study by Gil *et al*. (2013) using RT-PCR where the authors where obliged to combine primers PVSCPF and PVSR reported in different papers (Nie and Singh, 2001; Ali *et al*., 2008) due to amplification problems using the original published sets. Sequence alignment of these primers with respect to the genomes of PVS_Antioquia, RVC and RL5 confirmed the presence of several mismatches at the annealing sites that could affect PCR efficiency (Fig. 4A, B). As these amplification problems could be detrimental to any PCR-based diagnostic tool aimed at detecting Colombian PVS isolates, a new primer set (PVS_gen_F and

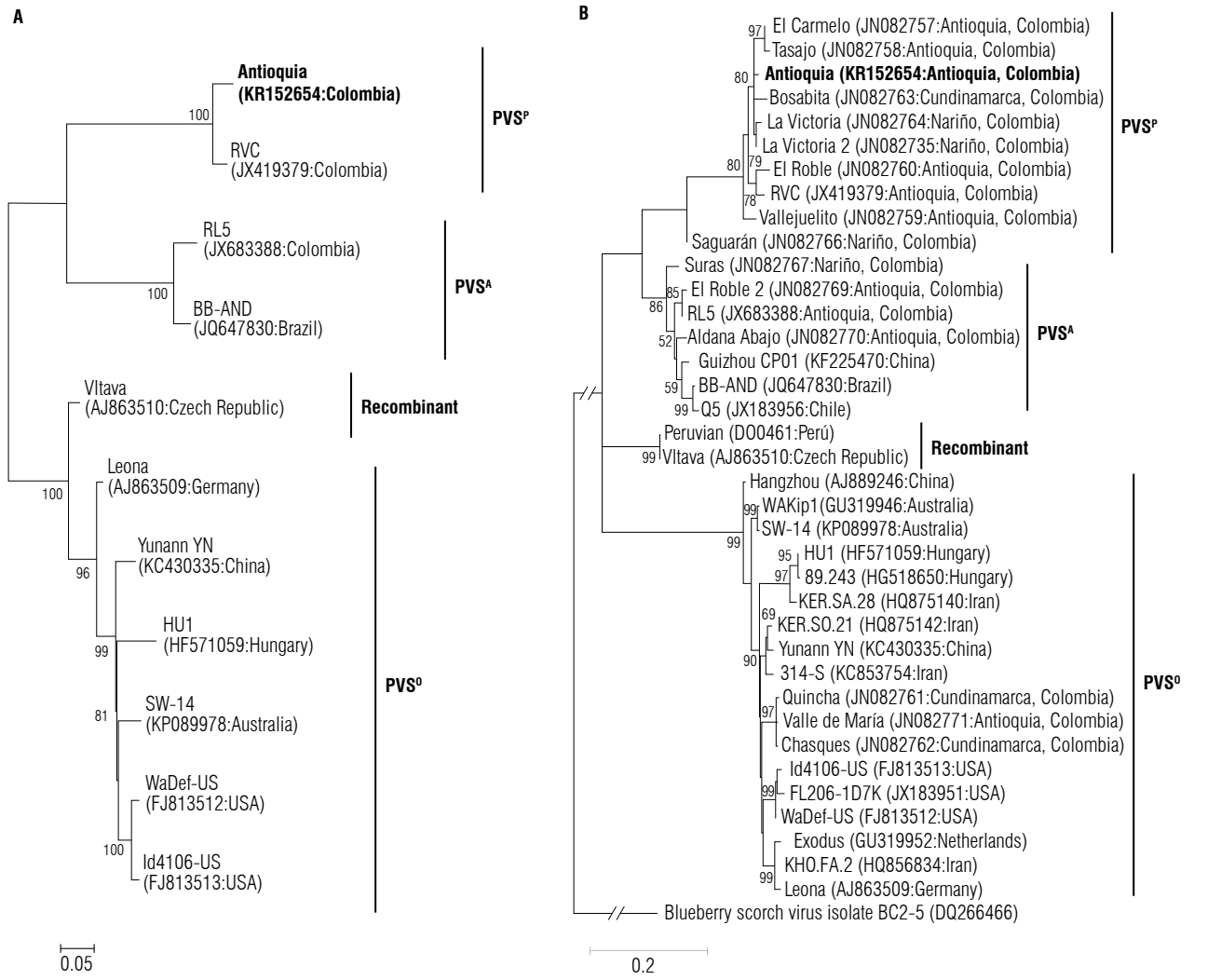

FIGURE 3. Molecular phylogenetic analysis with the Maximum Likelihood method tree showing the phylogenetic relationship of PVS_Antioquia to other PVS isolates from around the world using the complete genome sequence (A) and capsid (B) sequences. Bootstrap values > 50% are indicated at each node. GenBank (gb) accession numbers are shown in parenthesis.

qPVS_gen_R) was designed for specific detection by RT-qPCR of PVS variants from Colombia. Primers PVS_gen_F and and qPVS_gen_R were tested by RT-qPCR using fifteen tuber and leaf samples obtained at different locations in Antioquia. This trial resulted in positive amplification curves for 80 and 60% of the leaf and tuber samples analysed. The Ct values suggested a higher viral titer in the leaf tissues (Ct in the 7.12-30.08 range) than in the tubers (Ct 28.76-33.94). The specificity of the reaction was confirmed by the melting curve analysis, which resulted in Tm values in good agreement with the positive control (86.96±1°C) (Fig. 4C, D). Sanger sequencing of four randomly chosen samples and for the positive control confirmed amplification of the PVS coat region. Two sequences showed higher

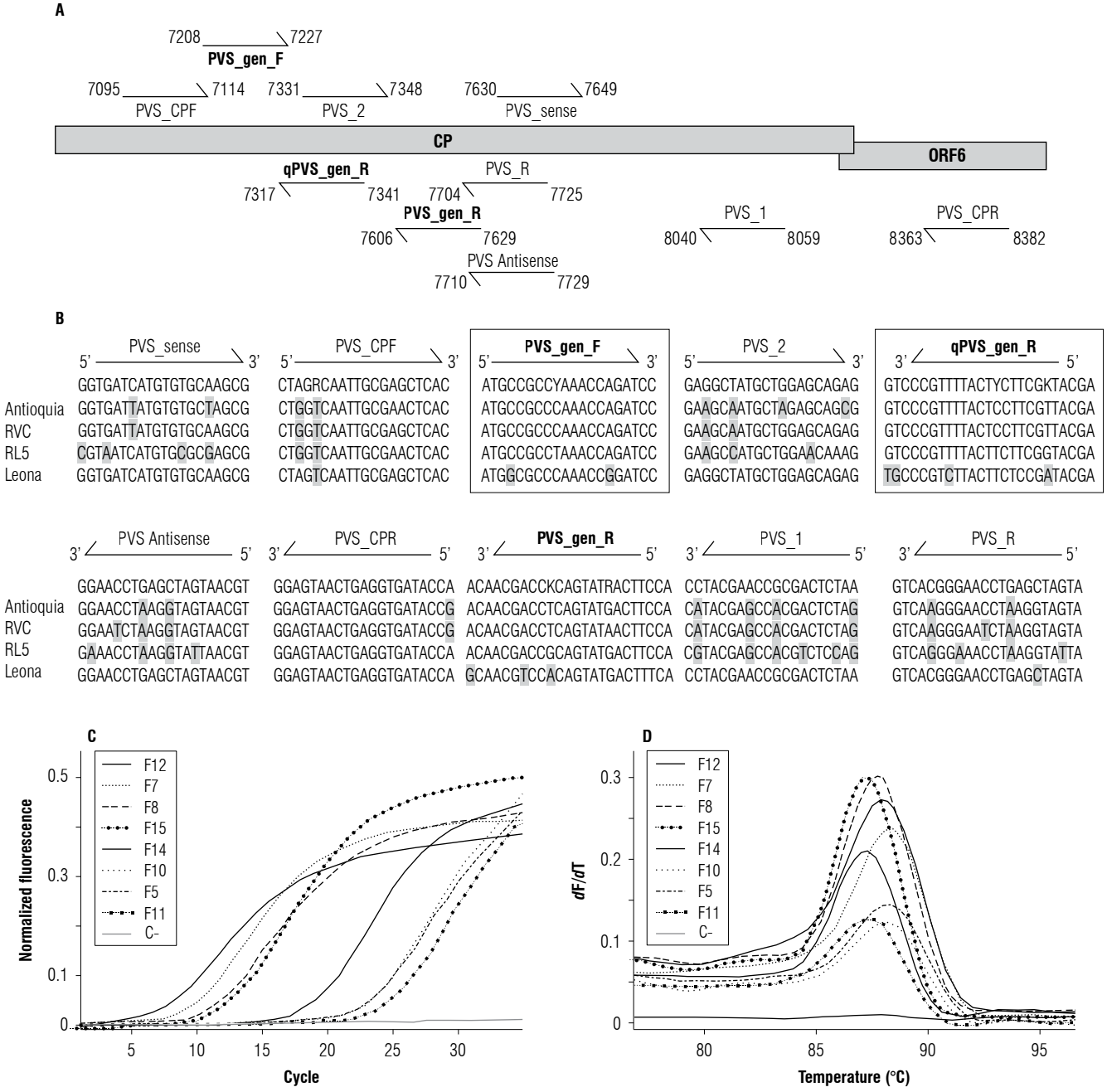

FIGURE 4. (A) Primer sets specificity with respect to the PVS strains (Tab. 1) present in the department of Antioquia (Colombia). The upper panel illustrates the location of primers for the detection of PVS previously reported in the literature: PVS_CPF and PVS_CPR (Ali *et al*., 2008); PVS_1 and PVS_2 (Crosslin and Hamlin, 2011); and PVS_Sense and PVS_Antisense (Yang *et al*., 2014). (B) Sequence alignment with respect to complete genomes from Colombia (includes isolate Leona for comparison); mismatches are highlighted with gray background. (C) amplification and (D) melting curves using primers PVS_gen_F and qPVS_gen_R designed in this study.

identity with regions at positions 7218-7301 of the coat of PVS-RVC (98% identity) while the remaining two samples did so with positions 7215-7300 of the PVS-RL5 genome (100% identity).

In a previous study, Gil *et al*. (2013) detected the presence of PVS in 40% of the 320 *S. tuberosum* and *S. phureja* leaf samples from the four main potato producing provinces of Colombia: Antioquia, Boyaca, Cundinamarca and Nariño. A separate study on the accessions from the Coleccion Central Colombiana de papa using immunoprinting and DAS-ELISA with polyclonal antibodies revealed infections levels of 61.3 and 85%, respectively (Franco-Lara *et al*., 2009; Guzmán *et al*., 2010). These reports underscore the need to use stricter parameters in the tuber seed certification program of Colombia, which tolerates PVS levels of 1, 2 and 5% in basic, registered and certified tuber seeds, respectively (ICA, 2015). Similar studies in the rest of the world have revealed high levels of PVS incidence when tuber-seed certifications programs do not reach all farmers in a region or due to the use of diagnostic tests with low sensitivity. For example, a three year study in seven provinces of Iran using two hundred and forty potato samples with one or more symptoms of leaf mosaic, distortion, mottling and yellowing, detected PVS in 18.2% of the samples with a predominance of the PVS^{O} lineage (Salari *et al*., 2011). In China, Wang *et al*. (2011) reported an average PVS infection level of 16.3%, reaching values of 22.6 and 26.7% in the provinces of Heilongjiang and Yunnan, respectively. In Costa Rica, Vásquez *et al*. (2006) evaluated the incidence of PVS and distribution at different altitudes, finding PVS to be present in 19% of the 600 tested samples. The highest PVS incidence was found at middle altitutes because the virus was detected in 60% of the plots of this region with an average infection of 75%.

Finally, as expected, the RT-qPCR proved to be of higher sensitivity with respect to ELISA; the latter only detected PVS in one tuber in contrast to RT-qPCR where PVS was found in nine samples (60%); with respect to the leaf samples, ELISA tested positive in eleven cases, while RT-qPCR did so for 12 samples (80%) (Fig. 5). These results suggest a higher PVS titer in leaves than tubers, which supports the notion that RT-qPCR is the method of choice in PVS seed certification programs. The absence of PVS symptoms in most of the potato cultivars and the use of non-certified seeds in different potato-growing regions likely contribute to the prevalence of PVS in many potato-growing areas (Lin *et al*., 2014a). The ordinary strain of PVS is very widespread in Europe and is considered symptomless for the majority of potato cultivars (Dolby and Jones, 1988). PVS^{A}, on the other hand, can produce much more severe reactions, such as premature senescence and defoliation, and higher losses might result from co-infection by other viruses, such as PVA, PVY and PVX (Salari *et al*., 2011; Nyalugwe *et al*., 2012). For this reason, PVS^{A} has been included in the European Union quarantine list for the potato (Jeffries, 1998). A recent study has also shown that PVS may break-down *Phytophthora infestans* resistance in the potato, making the late blight disease more severe, which may have an important impact in potato breeding programs (Lin *et al*., 2014b).

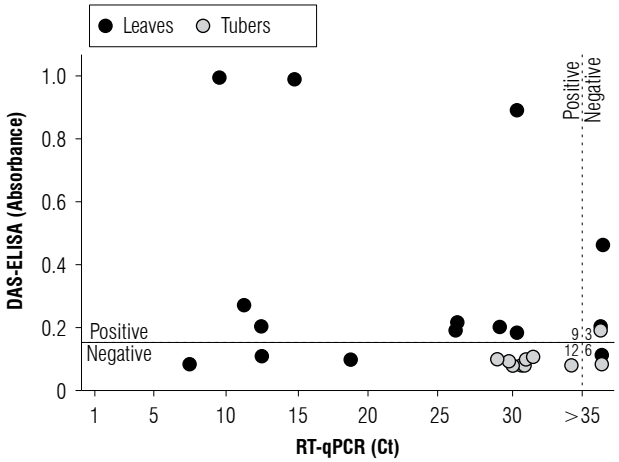

FIGURE 5. Correlation between the DAS-ELISA results and the RT-qPCR detection of PVS using primers PVS_gen_F and qPVS_gen_R. Open and closed circles correspond to leaf and tuber samples, respectively; lines indicate the threshold values at which each test was considered to be positive.

Due to the increasing importance of *S. phureja* for internal use as well as a potential export crop, it is important to establish appropriate certification guidelines using highly sensitive and specific techniques designed to detect local PVS variants. Especially in international trade, tubers and *in vitro* plants can carry PVS. It would be interesting to further investigate the biological features of isolates belonging to PVS^{P}, including the host range, transmission by aphids and overall agronomical effects as well as the ability of the members of new proposed lineage PVS^{P} to cause systemic and/or non-systemic infections in *Chenopodium* sp. and its synergistic interaction with other potato viruses.

Conclusions

Complete sequencing of a PVS strain obtained from *S. phureja* tuber sprouts in Antioquia (Colombia) confirmed the existence of a new lineage of this virus, for which we propose the acronym PVS^{P}. Based on this genome sequence, primers PVS_gen_F and qPVS_gen_R, targeting the CP

region, were designed for use in RT-qPCR detection of this virus. The validation of this molecular test on fifteen tuber samples and fifteen leaf samples revealed high levels of incidence of PVS in both tissues (60 and 80%, respectively), suggesting that urgent measures are required to strengthen current *S. phureja* seed certification programs in Colombia.

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