Molecular characterization of Potato virus Y (PVY) and Potato virus V (PVV) isolates naturally infecting cape gooseberry (*Physalis peruviana*) in Antioquia, Colombia

Caracterización molecular de aislamientos del virus Y (PVY) y virus V (PVV) de la papa infectando naturalmente uchuva (*Physalis peruviana*) en Antioquia, Colombia

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ABSTRACT

Due to the increase of the international demand for functional fruits, cape gooseberry (*Physalis peruviana*) has become one of the crops of highest expansion in Colombia and the Andean region of South America. Unfortunately, the emergence of fungal and unidentified viral diseases has slowed down the cultivation of cape gooseberry in Colombia and, particularly, in the department of Antioquia. In this work, a next-generation sequencing virome analysis of cape gooseberry plants from eastern Antioquia was performed, using leaves exhibiting symptoms such as mosaics, leaf deformation and greening of veins. The complete genomes of Potato virus Y (PVY) and Potato virus V (PVV) were obtained in the assembled data. The presence of both viruses was confirmed in the samples obtained at two commercial cape gooseberry fields by real time RT-PCR (RT-qPCR) and partial Sanger sequencing of the coat protein (CP). Sequence analysis revealed significant sequence similarity between PVY and PVV isolates infecting *P. peruviana* to previously identified strains infecting potato (*Solanum tuberosum* and *S. phureja*) and tomato (*Solanum lycopersicum*) in the same geographical region. This study suggests that cape gooseberry could be an alternate host to viruses of other economically important solanaceous crops in the Andean region of South America.

Key words: genomics, potyviruses, *Solanaceae*, virus diseases.

RESUMEN

Dado el incremento en la demanda internacional por frutas con características funcionales, la uchuva (*Physalis peruviana*) ha sido uno de los cultivos con mayor expansión en los últimos años en Colombia y otros países suramericanos. Desafortunadamente, la emergencia de enfermedades micóticas y virales ha reducido dichos planes de siembra, especialmente en el departamento de Antioquia. En este trabajo, se realizó un análisis de secuenciación de nueva generación del viroma asociado a tejidos foliares de plantas de uchuva del oriente antioqueño, con síntomas de mosaicos, deformación de brotes y verdeamiento de venas. Los genomas completos de los virus Y (PVY) y V (PVV) de la papa fueron obtenidos a partir de las secuencias ensambladas. La presencia de ambos virus fue confirmada en dos cultivos comerciales de uchuva, utilizando RT-PCR en tiempo real y secuenciación Sanger de una porción del gen de la cápside. Los análisis filogenéticos de dichas secuencias revelaron la existencia de altos niveles de similitud entre los aislamientos de PVY y PVV obtenidos en uchuva y cepas previamente identificadas para esta misma región geográfica en cultivos de papa (*Solanum tuberosum* y *S. phureja*) y tomate (*Solanum lycopersicum*). Este estudio sugiere que las plantas de uchuva pueden servir como hospedantes alternos de virus de importancia económica en cultivos de solanáceas de la region Andina de Suramérica.

Palabras clave: enfermedades virales, genómica, potyvirus, *Solanaceae*.

Introduction

Cape gooseberry (*Physalis peruviana* L.) is a solanaceous fruit crop native to the South American Andes that has recently become one of the most promising agricultural export trades in Latin American countries such as Colombia, Ecuador and Perú (Fisher *et al.,* 2014). The cape gooseberry plant produces a fruit with excellent nutritional properties, which is also a good source of phosphorous, dietary fiber and vitamins A, B and C (Ramadan, 2011). Due to its high content of phenolic acids, flavonoids and other bioactive compounds with antibacterial, anti-inflamatory, anti-tumorigenic and antioxidant properties, *P. peruviana* is also considered to be a functional fruit (Wu *et al.,* 2005; Ramadan, 2011). In Colombia, the cape gooseberry crop comprises a total cultivated area of 952 ha with an estimated

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yield of about 13,260 t per year; the departments of Boyacá, Antioquia and Cundinamarca are the main producers, accounting for 58.4, 17.4 and 17.5 percent of the national production (Agronet, 2016). Recently, the production of cape gooseberry has declined from 13.76 t ha^{-1} yr^{-1} in 2010 down to 9.81 t ha^{-1}yr^{-1} in 2014 (Agronet, 2016). This drop has been attributed to several factors, which include climate change, the increase in the incidence and severity of fungal diseases caused by *Fusarium oxysporum* and *Phoma* sp. (Fisher *et al.,* 2014) and the infection by several viruses inducing chlorosis, mosaics, leaf deformation, dwarfism and greening of veins (Zapata *et al.,* 2005; Aguirre *et al.,* 2014; Gutiérrez *et al.,* 2015; Rodríguez *et al.,* 2016).

P. peruviana can be host to a wide range of viruses such as tobamovirus (Tobacco mosaic virus, TMV) (Capoor and Sharma, 1965; Gómez *et al.,* 1997), polerovirus (Potato leafroll virus, PLRV) (Natti *et al.,* 1953), cucumovirus (Cucumber mosaic virus, CMV) (Chamberlain, 1939; Gupta and Singh, 1996; Daza and Rodríguez, 2006), potexvirus (Potato virus X, PVX) (Horvath, 1970; Zapata *et al.,* 2005; Gutiérrez *et al.,* 2015), crinivirus (Tomato chlorosis virus, ToCV) (Trenado *et al.,* 2007), tospovirus (Tomato chlorotic spot virus, TCSV and Tomato spotted wilt virus, TSWV) (Da-Graça *et al.,* 1985; Eiras *et al.,* 2012), several potyviruses (Peru tomato mosaic virus, PTV; Colombian datura virus, CDV; Potato virus Y, PVY and Bean yellow mosaic virus, BYMV) (Horvath, 1970; Salamon and Palkovics, 2005; Kaur *et al.,* 2014; Kisten *et al.,* 2016; Cutler *et al.,* 2018) and the viroid Potato spindle tuber viroid (PSTVd) (Hadidi *et al.,* 1976; Verhoeven *et al.,* 2010). The capacity of *Physalis* species to serve as virus hosts was investigated by Horváth (1996), who demonstrated the susceptibility of *P. alkekengi* to 10 viruses, *P. ixocarpa* to 14 viruses and *P. pubescens* to two viruses. This work also showed that *P. peruviana* is systemically susceptible to Alfalfa mosaic virus (AMV), Potato aucuba mosaic virus (PAMV) and PVY, and locally susceptible to Tobacco rattle virus (TRV). The role of *P. peruviana* as an alternate host of viruses affecting economically important crops has been demonstrated for PVY, PSTVd, ToCV in tomato (*Solanum lycopersicum*) (Trenado *et al.,* 2007; Verhoeven *et al.,* 2009; Kisten *et al.,* 2016) and CDV in tobacco (*Nicotiana tabacum*) (Salamon and Palkovics, 2005).

In the Andean region of Colombia, cape gooseberry is frequently inter-cultivated with other solanaceous crops, such as tamarillo (*S. betaceum*), tomato, bell pepper (*Capsicum annuum*) and potato (*S. tuberosum* and *S. phureja*) and can also grow as a weed within these crops (Fischer *et al.,* 2014). A recent next generation sequencing (NGS) study suggested that *P. peruviana* could be a natural reservoir host of PVX (Gutiérrez *et al.,* 2015); in this work, the role of cape gooseberry as an alternative virus host to other solanaceous crops in the municipality of La Unión (Antioquia) was furtherly investigated using NGS and RT-qPCR tests on cape gooseberry plants exhibiting mosaics, leaf deformation and greening of veins.

Materials and methods

Sample collection

Ten samples were collected at two commercial cape gooseberry plots in the municipality of La Unión (Antioquia) (5°58′22′′ N, 75°21′40′′ W and 2500 m a.s.l.), where some plants exhibited typical symptoms of viral infection. In the first plot, rugose mosaic and leaf deformation symptoms were detected in leaves; in the second plot, rugose mosaics and greening of veins were observed (Fig. 1). Six

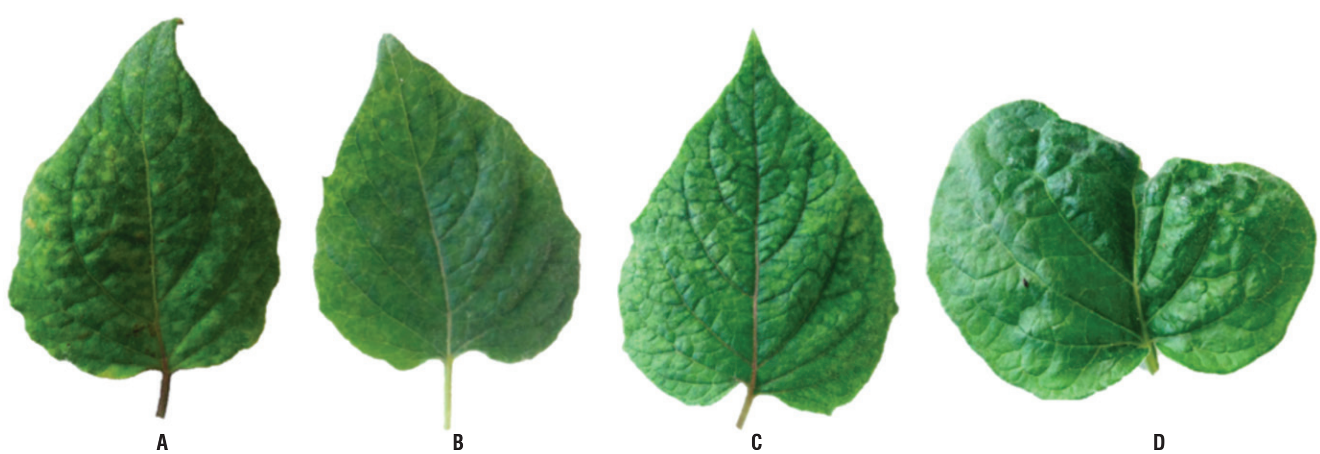

FIGURE 1. Symptoms of viral infection in the cape gooseberry leaves observed in this work. Rugose mosaics (**A** and **B**), greening of veins (**C**) and leaf deformation (**D**).

additional asymptomatic leaf samples were included for the RT-qPCR tests.

Next generation sequencing

High-throughput sequencing of the *P. peruviana* transcriptome was performed on a bulked sample of symptomatic leaves. The total RNA was extracted by the Trizol method (Chomczynski, 1993) following the manufacturer's instructions (ThermoFisher Scientific, Waltham, MA, USA) and the integrity was determined with a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The ribosomal RNA was removed with the TruSeq Stranded Total RNA with a Ribo-Zero Plant kit (Illumina, San Diego, CA, USA). The TruSeq Stranded Total RNA LT Sample Prep Kit (Illumina, San Diego, CA, USA) was used for the preparation of cDNA libraries and the ligation of adapters. Sequencing was performed with the Illumina HiSeq 2000 system service provided by Macrogen (South Korea).

Once the sequencing data was obtained, adapter sequences and low quality bases (Phred score < 30) were removed from the dataset with SeqTK v.r82 (GitHub, 2015). Sequences were assembled with Trinity (Grabherr *et al.,* 2011) and viral contigs were identified with a local BLASTN (Gish and States, 1993) search against a database containing all plant virus species currently accepted by the International Committee on Taxonomy of Viruses (ICTV). Genome assemblies were verified by mapping them against the reads with Bowtie2 (Langmead and Salzberg, 2012) and checked for inconsistencies and assembly artifacts with Tablet (Milne *et al.,* 2010). Protein coding regions were annotated using BLASTX (Gish and States, 1993) against reference PVV (NC_004010) and PVY (NC_001616) genomes. Putative protease cleavage sites were confirmed by comparison to published P1, HC-Pro and NIa-Pro consensus cleavage sites (Adams *et al.,* 2005). The complete genome sequences were deposited in GenBank under accession codes KY711363 and KY711364 with PVY_physalis and PVV_physalis as isolate names, respectively.

RT-qPCR tests

Primer specifity was first evaluated in bulk samples comprising either symptomatic (SL1 or SL2) or asymtomatic (AL1 and AL2) leaves collected at each plot. For these bulk samples, the total RNA was extracted from 100 mg of ground tissue with the GeneJET Plant RNA Purification kit following the manufacturer´s protocol (ThermoFisher Scientific, Waltham, MA, USA) and eluted in 40 µL of DEPC treated water. Purity and concentration were determined by absorbance readings at 260 and 280 nm using a Nanodrop 2000C (ThermoFisher Scientific, Waltham, MA, USA).

Synthesis of cDNA and RT-qPCR were performed using the method reported by Muñoz-Escudero *et al.* (2016a). PVY was detected with the primers PVY-1 FP (5´-CCAATCGTTGAGAATGCAAAAC-3´) and PVY-1 RP (5´-ATATACGCTTCTGCAACATCTGAGA-3´) (Singh *et al.,* 2013) after amplifying a 74 bp segment of the CP region. The primers PVV_phu_F (5'-ATGCTGGAAAAGATCCAGC-3') and qPVV_phu_R (5'-CATCCCGCTCCTCAAC-3') were used to target an 89 bp region of CP for PVV (Álvarez *et al.,* 2016).

After primer validation, each leaf sample was tested individually by Immunocapture Real-Time RT-PCR (IC-RT-qPCR) using an antigen-coated ELISA plate (ACP-ELISA, SRA 27200/0096) containing the PTY 1 monoclonal antibody for a generic detection of the potyvirus. Positive (LPC 27200) and negative (LNC 27200) controls were purchased from Agdia (Elkhart, IN, USA). Absorbance was measured at 405 nm in a Multiskan plate reader (ThermoFisher Scientific, Waltham, MA, USA). Samples were considered positive for ELISA when the absorbance value has higher than the cut-off value defined by the formula: Cut-off = (average OD405 + 3 s.d.) x 1.1 as recommended by Bioreba (Reinach, Switzerland).

For the RT-qPCR step, virus particles were released from the ELISA plate with 70 µl of a 10 mM Tris-HCl buffer (pH 8.0) containing 1% Triton X 100 and incubated at 70°C for 10 min (Wetzel *et al.,* 1992). RT-qPCR reactions included a negative control lacking template cDNA and a positive control containing cDNA from infected potato leaf tissue. Samples were considered positive after exhibiting fluorescence values higher than the threshold before the 35th cycle. Amplicon specificity was verified by High Resolution Melting (HRM) in the 50 and 99°C range and confirmed by Sanger sequencing of three samples plus the positive control.

To confirm the phylogenetic affinity of PVY and PVV isolates from *P. peruviana*, the RT-PCR amplification was performed on three positive RT-qPCR samples using primers to target the CP region. These amplicons were sequenced afterwards. The RT-PCR reaction was performed following the procedure reported by Henao-Díaz *et al.* (2013) with the primers PVYCPF (5´-ACCATCAAGSAAATGACACA-3´) and PVYCPR (5´-CGGAGAGACACTACATCACA-3´) (Glais *et al.,* 2002) for PVY. For PVV, the primers PVV_phu_F and PVV_phu_R

(5'-TGAAAGTGGGCTTTGCG-3') were used instead (Álvarez *et al.,* 2016). In each case, amplicons of the expected size were obtained (PVY: 801 pb; PVV: 459 pb). Samples were gel purified using the QIAquick Gel Extraction kit (Qiagen, Hilden, Germany) and sequenced at Macrogen using an ABI Prism 3730xl sequencer (PE Applied Biosystems, Foster City, CA, EEUU). PVY and PVV CP partial sequences were deposited in GenBank under accesion codes KY711356-62.

Phylogenetic analyses

Phylogenetic trees using the polyprotein coding segments of PVY and PVV were inferred by the Maximum Likelihood method using the General Time Reversible model (Nei and Kumar, 2000) and were modelled with a discrete Gamma distribution with 5 categories plus invariable sites and a gamma parameter of 1.02. The phylogenetic analysis using the polyprotein coding segments of PVY and PVV was inferred by the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993) with a discrete Gamma distribution of 5 categories plus invariable sites and a gamma parameter of 1.42. Positions with less than 95% site coverage were eliminated in each case. Nucleotide substitution models were selected with MODELTEST (Posada and Crandall, 1998) and sequences were aligned with MUSCLE (Edgar, 2004). Evolutionary analyses were conducted in MEGA6 (Tamura *et al.,* 2013).

Results and discussion

Next generation sequencing

Sequencing of the *P. peruviana* transcriptome resulted in a paired-end library of 13,420,698 reads (101nt/read) for a total of 1,355,490,498 sequenced nucleotides. Two potyviruses, Potato virus Y (PVY) and Potato virus V (PVV) were identified in the assembled data. The PVY contig (PVY_physalis) comprised 9,675 nt and had an average sequence depth of 1,683x (Fig. 2A). A total of 219,155 reads were mapped after the PVY assembly for an abundance of 1,679 reads per kilobase per million reads (RPKM). Forty one polymorphic sites (39 transitions and 2 transversions) were identified in the assembled genome. The sequence identified as PVV (PVV_physalis) corresponded to a contig of 9,832 nucleotides assembled from 108,629 reads (823 RPKM) and an average sequence coverage of 1,091x (Fig. 3A). In contrast to PVY_physalis, the PVV_physalis assembly did not contain any polymorphic sites.

Characterization of the PVY_physalis isolate

The ORF encoding the potyviral polyprotein in PVY_physalis was identified at the nucleotide positions 169-9,354, and corresponds to a protein of 3,061 a.a. (Fig. 2A). The PVY_physalis polyprotein is cleaved into a ten mature proteins product by the action of potyviral proteases. The P1 and HC-Pro cleavage sites were identified at positions 284 (RRMVQF/S) and 740 (KHYRVG/G), respectively. P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb and CP are cleaved by NIa-pro at the positions 1,105, 1,157, 1,791, 1,843, 2,031, 2,275 and 2,794, respectively, These are the sites with the consensus sequence V-x-[HE]-[QE]/[AGSR] (Revers and García, 2015). The GA_7T sequence inducing frameshift protein product P3N-PIPO (247 a.a.) was identified at the nucleotide position 2,899 within the P3 segment (Chung *et al.,* 2008) (Fig. 2A). Six nucleotide polymorphisms in the PVY_physalis assembly were translated into amino acid changes within the HC-Pro (T326A, I335M), 6K1 (V1118I), VPg (G1944S, H1964N), and CP (A2809E) segments. A BLASTN search against the complete nucleotide collection at GenBank revealed that PVY_physalis is closely related to the isolates LaUnionT (99.3%, KX531041), mar7 (99.1%, KR270797), VarA (98.7%, KT290511) and VarB (96.7%, KT290512) that are infecting *S. tuberosum* and *S. lycopersicum* in the department of Antioquia (Muñoz-Baena *et al.,* 2016; Muñoz-Escudero *et al.,* 2016a, b).

A phylogenetic analysis of complete PVY genomes clustered the sequences into well-defined clades corresponding to the strains PVY^N, PVY^C, PVY^O and PVY^{NP} plus the recombinant strains PVY^{NTN} and $PVY^{N:O/N\text{-}Wi}$ (Fig. 2B). PVY_physalis was part of a clade that includes some of the Colombian isolates identified using BLAST. This clade is sister to a group comprising isolates RRA-1, SASA-61, NTNHO90, NTND6 and NTNO92, which are non-recombinant PVY strains, but some of them have shown to induce the tuber necrotic ringspot disease in potato (Lorenzen *et al.,* 2006; Ogawa *et al.,* 2008). PVY_physalis is clearly different from recombinant PVY isolates identified in potato crops in northern (Yarumal_varB) and eastern Antioquia (La_Union) (Muñoz-Escudero *et al.,* 2016a, b). A phylogenetic analysis using partial CP sequences revealed a similar topology for the complete genome tree with some clades collapsing as a result of recombination as shown in a previous research (Karasev and Gray, 2013). The PVY_physalis is different to isolate PVY-KZNU from South Africa, and it was identified in *P. peruviana* plants exhibiting mottling, mosaic, and chlorosis symptoms on a tomato farm moderately infested with cape gooseberry weeds (Kisten *et al.,* 2016). The PVY-KZNU was identified as a recombinant PVY^C strain with spliced PVY^O-type RNA fragments in the coat protein region (Kisten *et al.,* 2016), a result that agreed with the phylogenetic analysis performed in this research (Fig. 2C). The identification of

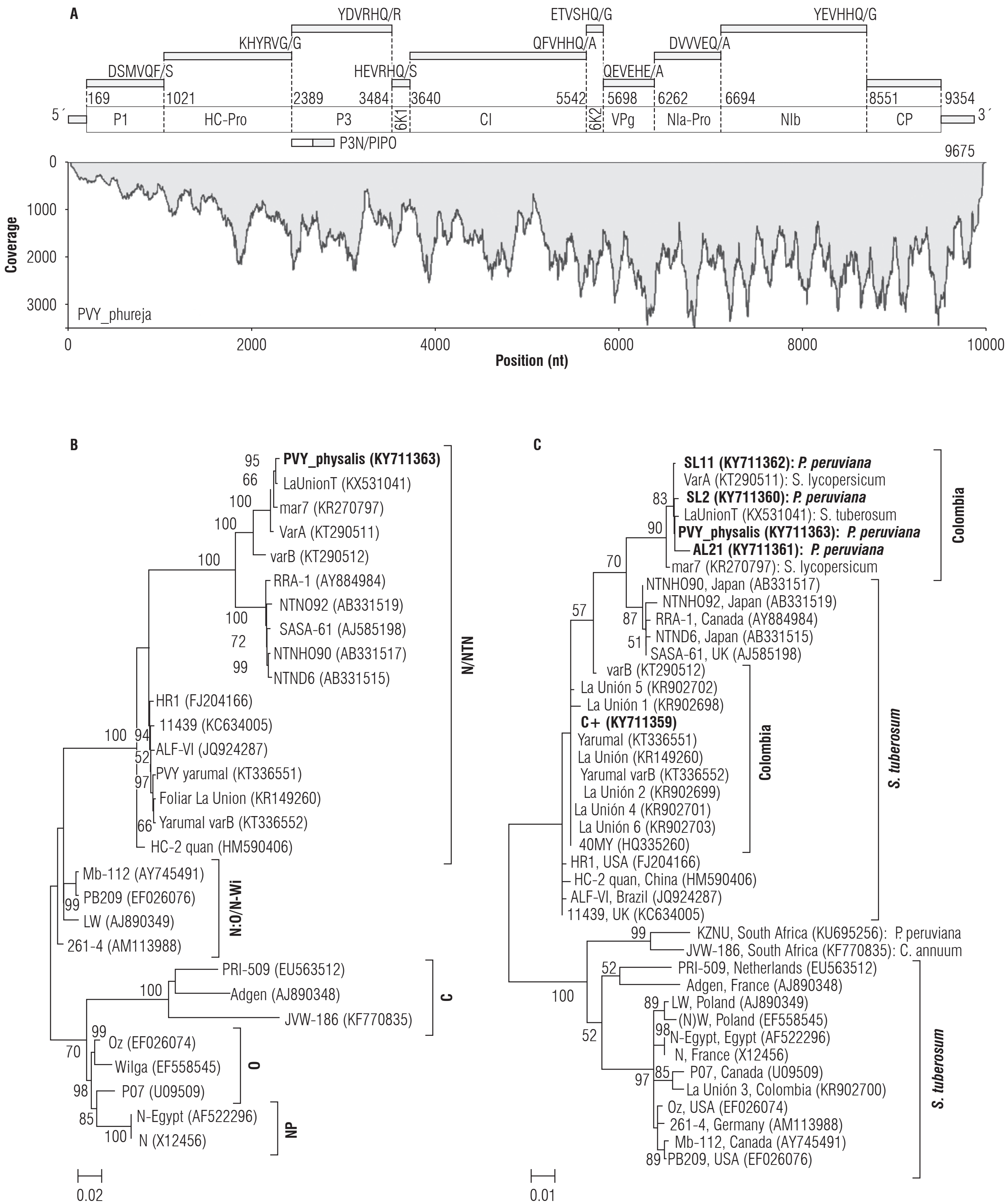

FIGURE 2. Genome annotation of the PVY_phureja genome and the phylogenetic relationship to other PVY isolates. **A)** Sequence coverage of PVY_phureja assembly and location of some important molecular features. The relative position of each mature protein with their corresponding protease cleavage sites is shown. Phylogenetic analysis of PVY isolates using complete (**B**) and partial CP (**C**) sequences confirms the close relationship between PVY_physalis and non-recombinat PVY isolates infecting *S. lycopersicum* and *S. tuberosum* in eastern Antioquia. Trees are drawn to scale with branch lengths in units of number of base substitutions per site as indicated in the bar at the bottom. Bootstrap values are shown above the branches.

these different PVY variants clearly demonstrates that *P. peruviana* can serve as a reservoir to PVY strains infecting other solanaceous crops of economic importance such as tomato and potato. So far, and according to different sources, this genome represents the first complete PVY sequence naturally infecting *P. peruviana*.

Characterization of PVV_physalis

The PVV_physalis polyprotein (3,065 a.a.) was encoded at nucleotide positions 186-9,383. P1 and HC-Pro cleavage sites were identified at positions 289 (RRMVQF/S) and 745 (IKHRVG/G), respectively. NIa-Pro cleavage sites contained the same V-x-[HE]-[QE]/[AGSD] motif observed in PVY_physalis and were located at the amino acid positions: 1102 (P3), 1,154 (6K1), 1,788 (CI), 1,840 (6K2), 2,028 (VPg), 2,274 (NIa-Pro) and 2,793 (NIb). P3N-PIPO (231 a.a.) is predicted to result from the frameshifting at the GA_7C sequence at the nucleotide position 2,907 with the P3 coding region (Chung *et al.,* 2008) (Fig. 3A). The closest homologs to PVV_physalis in GenBank are the PVV isolates La Union_varA (99.9%, KT985458), phureja (99.8%, KP849483) and La Union_varB (99.6%, KT985459) infecting *S. phureja* in Antioquia (Álvarez *et al.,* 2016; Gutiérrez *et al.,* 2016), isolate KER.LAL.P (83.6%, KC433411) from Iran and isolate DV 42 (83.0%, AJ243766) from Scotland (Oruetxebarria *et al.,* 2000; Shamsadden-Saeed *et al.,* 2014). A comparison of PVV polyproteins reveals two aminoacid changes unique to PVV_ physalis. In the first change, at the position 1,393 within the CI, a serine residue replaced a threonine/alanine observed in the PVV isolates infecting potato; this was followed by a second change at the position 2,903 within the CP, where a glutamic acid observed in the PVV strains infecting potato was replaced by lysine in the PVV_physalis.

A phylogenetic analysis of PVV genomes confirms that the PVV_physalis isolate has higher affinity to the PVV^{phu} lineage infecting *S. phureja* in eastern Antioquia and, more distantly, to the PVV isolates DV42 and KER.LAL.P infecting *S. tuberosum* in Eurasia (Scotland and Iran). Both clades are cleary differentiated and supported by a 100% bootstrap (Fig. 3B). The phylogenetic analysis of the partial CP sequences was in agreement with the complete genome analysis. Again, the CP sequences isolated from *P. peruviana* (PVV_physalis, SL1 and SL2) formed a distinct clade, along with the sequences infecting *S. phureja* (PVV_phureja) (Fig. 3C). All these sequences have been isolated in the municipality of la Unión in eastern Antioquia. The *S. phureja* / *P. peruviana* group is sister to a divergent PVV isolate identified in *S. tuberosum* cv. Papa Amarillo, which has distinct serological properties from other isolates infecting *S. tuberosum* (Shiel *et al.,* 2004).

The PVV^{phu} genetic lineage was first identified in a 454 GS-FLX pyrosequencing study of *S. phureja* root tissue in Colombia (Gutiérrez *et al.,* 2014) and its existence was confirmed with follow up genome sequencing studies (Álvarez *et al.,* 2016; Gutiérrez *et al.,* 2016). The PVV^{phu} lineage was originally believed to be exclusive to *S. phureja,* but its detection here in *P. peruviana* suggests that this lineage might be present in a wider range of hosts. Finally, a recently discovered PVV strain, TamarilloEc, was found to infect tamarillo (*S. betaceum*) in Ecuador (Insuasti *et al.,* 2016) and proposed to be the first PVV isolate infecting a host different from potato; our analysis contradicts this claim as PVV_TamarilloEc seems to be more closely related to the Ecuatorian rocoto virus than to any member of the PVV group. We believe that this strain has been misclasified and should be renamed.

Detection of PVV and PVY by RT-qPCR

Infection of *P. peruviana* by PVY and/or PVV was confirmed by RT-qPCR using specific primers for each species. In a preliminary experiment, the amplification reaction was performed on total RNA extracted from bulks containing either symptomatic (SL1 and SL2) or asymptomatic (AL1 and AL2) samples from each plot (L1 and L2) (Tab. 1). PVY was detected in all four samples with Ct values between 9.97 and 27.12 and very similar melting temperatures (77.5±0.5°C). A Ct of 13.39 was observed in the potato sample used as positive control with slightly lower Tm (76.48), (Fig. 4A). Individual amplification reactions using IC-RT-PCR confirmed the PVY results using the bulked samples. In this case, all samples tested positive, with higher Ct values (26.55-32.47) but with the same distribution of Tm (77.5±0.5°C). Regarding symptoms, leaves exhibiting mosaics (S1-L1 and S1-L2) had the lowest Ct values (26.55-27.52), followed by the samples with mottling (S2-L1, Ct=28.60) and greening of veins (S2-L2, Ct= 28.75). As expected, the highest Ct values were observed in the majority of asymptomatic samples (28.30-32.47) (Tab. 1). Previous work on PVY infecting potato (Medina *et al.,* 2016) and tomato (Muñoz-Baena *et al.,* 2016) in Colombia by RT-qPCR using the same primers reported in this research also resulted in similar Tm values (77.5°C±0.5°C). The identity of RT-qPCR amplification product was confirmed by the Sanger sequencing of four samples which were identical to the CP region of PVY isolates from Colombia and Cuba isolated from potato, tamarillo, tomato and pepper (KT290511, JF939837, HQ335262, HQ335245, KY050811).

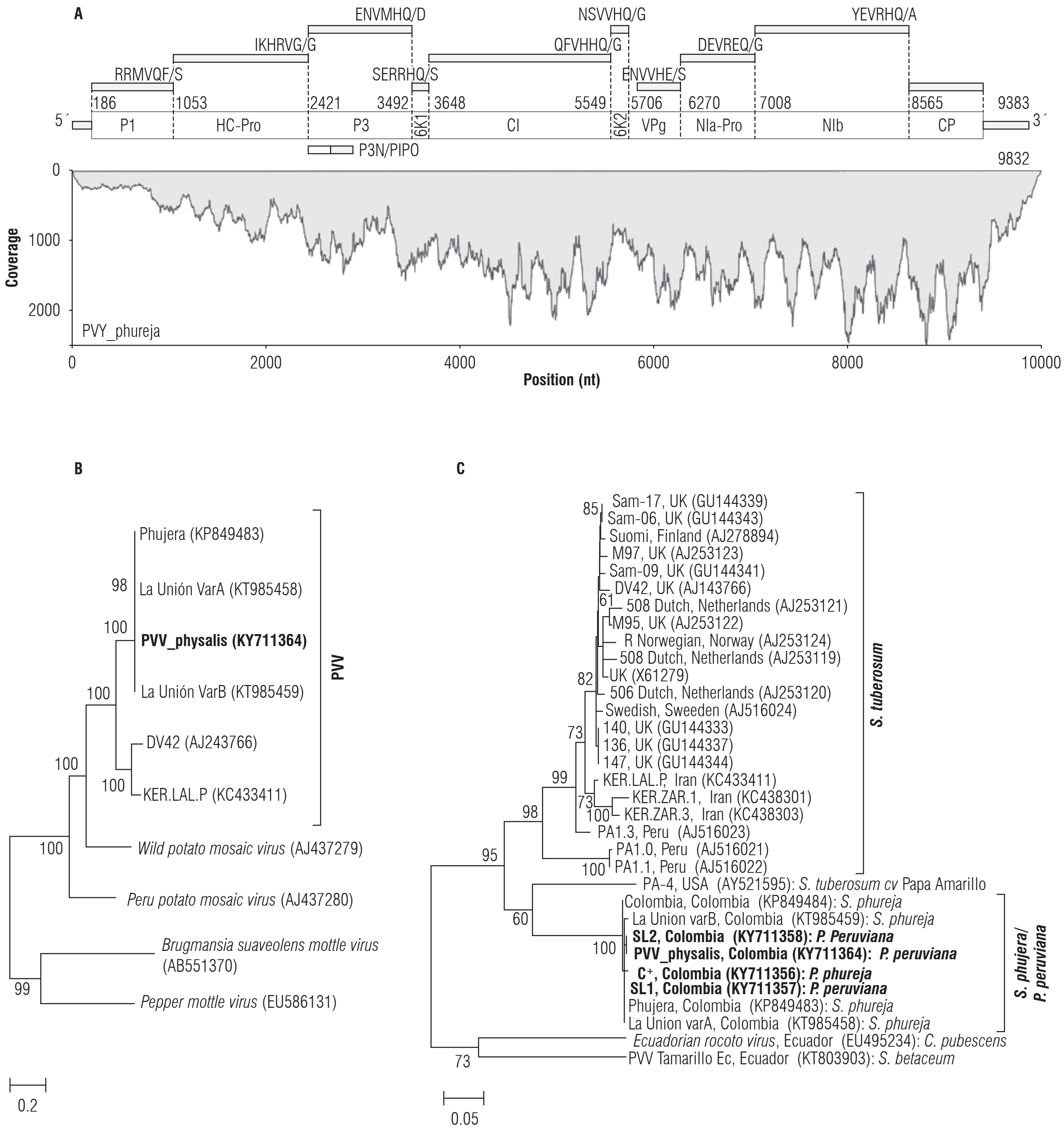

FIGURE 3. Genome annotation of the PVV_phureja genome and phylogenetic relationship to other PVV isolates. **A)** Sequence coverage of PVV_phureja assembly and location of some important molecular features. The relative position of each mature protein with their corresponding protease cleavage sites is shown. Phylogenetic analysis of PVV isolates using complete (**B**) and partial CP (**C**) sequences confirms the close relationship between PVV_physalis and PVV isolates infecting *S. phureja*. Trees are drawn to scale with branch lengths in units of number of base substitutions per site as indicated in the bar at the bottom. Bootstrap values are shown above the branches.

In contrast to PVY, the incidence of PVV was lower in both plots. PVV was detected in the asymptomatic and symptomatic bulks from the first plot but tested negative in both samples from the second plot. Surprisingly, a lower Ct value was observed in the asymptomatic sample (A-L1, Ct=22.38) than in the symptomatic one (S-L1, Ct=28.69) (Fig. 4B). Tm values were in good agreement with the positive control suggesting a sequence similarity. IC-RT-PCR

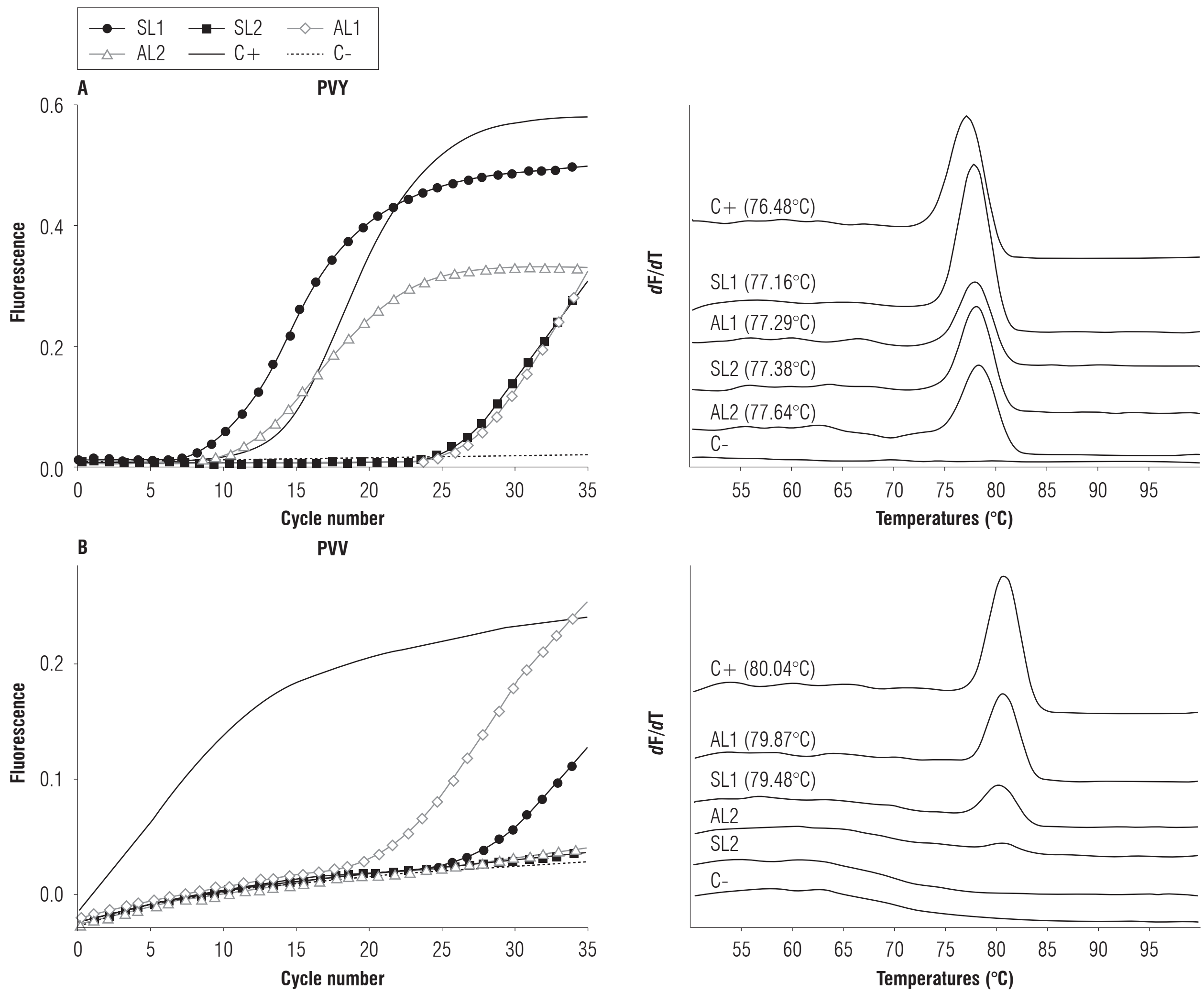

FIGURE 4. Molecular detection of Potato virus Y (PVY) and Potato virus V (PVV) in *P. peruviana* samples from La Unión (Antioquia). RT-qPCR amplification curves of PVY (**A**) and PVV (**B**) of symptomatic and asymptomatic leaf samples SL1-2 and AL1-2. Corresponding melting curve profiles are shown to the right with Tm values indicated in parentheses.

results differed from the RT-qPCR as only one sample in the first plot (S2-L1, Ct=26.83) tested positive for PVV, contrasting three positive samples in the second plot (A2-L2, A3-L2 and S1-L2) (Fig. 4B). There was no significant correlation between the symptoms and Ct values for PVV. The Tm values of 80±0.5°C are in good agreement with a previous study on PVV isolates infecting *S. phureja* isolates in Colombia (Álvarez *et al.*, 2016). Sequencing of RT-qPCR amplicons reveals a nucleotide sequence identity between 95 to 99% to PVV GenBank accesions KT985459, KT985458 and KC438304.

The natural ocurrence of potyviruses in cape gooseberry was first reported in Hawaii in 1953 (Sakimura, 1953) and later confirmed by serological and electron microscopy studies in India (Prakash *et al.*, 1988). *P. peruviana* has been thoroughly shown to be an alternate host to several viruses of tomato (Trenado *et al.*, 2007; Verhoeven *et al.*, 2010; Kisten *et al.*, 2016), tobacco (Schubert *et al.*, 2006) and potato (Prakash *et al.*, 1988; Gutiérrez *et al.*, 2015) and there is an increasing number of reports of viruses infecting *P. peruviana* in comercial plots all over the world such as the tospovirus TCSV in Rio Grande do Sul State of Brazil (Eiras *et al.*, 2012) and the potyvirus BYMV in Barabanki (India) (Kaur *et al.*, 2014). In Colombia, PVY was first identified in the department of Cundinamarca in a study of *P. peruviana* plants with leaf mosaics and mottling in 2006 (Daza and Rodríguez, 2006). In the mentioned research, PVY was detected using a combinantion of serological assays and electron microscopy and it was demonstrated to be transmitted through the aphid *Myzus persicae* acting as vectors from the cape gooseberry plants and by mechanical

TABLE 1. RT-q PCR detection of Potato virus Y (PVY) and Potato virus V (PVV) in leaf samples from *P. peruviana* plots in Antioquia (Colombia).

RT-qPCR (Total RNA)		PVY		PVV	
Sample name	**Type of sample**	**Ct***	**Tm****	**Ct**	**Tm**
C-		**>35**			
C+	Infected Potato leaf	13.39	76.48	80.04	4.66
SL1	Symptomatic bulk	9.97	77.16	28.69	79.48
SL2	Symptomatic bulk	26.37	77.38	>35	
AL1	Asymptomatic bulk	27.12	77.29	22.38	79.87
AL2	Asymptomatic bulk	12.49	77.64	>35	
RT-qPCR (Immunocapture)		**PVY**		**PVV**	
Sample name	**Type of sample**	**Ct***	**Tm****	**Ct**	**Tm**
C-		**>35**			
C+	Infected Potato leaf	27.02	76.99	5.02	79.78
A1-L1	Asymptomatic	30.28	77.29	>35	
A2-L1	Asymptomatic	31.07	76.56	>35	
A3-L1	Asymptomatic	28.30	77.46	>35	
S1-L1	Mosaic	26.55	77.35	>35	
S2-L1	Mottling	28.60	77.39	26.83	79.27
A1-L2	Asymptomatic	30.48	77.56	>35	
A2-L2	Asymptomatic	31.21	77.34	29.97	78.62
A3-L2	Asymptomatic	32.47	77.29	26.82	79.79
S1-L2	Mosaic	27.52	77.51	28.59	79.44
S2-L2	Greening of veins	28.75	77.64	>35	

*Threshold cycle. **Melting temperature.

infection to indicator plants. A later investigation also detected potyvirus infecting cape gooseberry plants in the municipality of Mosquera (Cundinamarca) using generic antibodies for aphid transmitted potyvirus and confirmed their results by electron microscopy (Aguirre *et al.*, 2014). Based on ELISA tests, PVY was also recently reported in three *P. peruviana* samples from Cundinamarca and Boyaca (Cutler *et al*., 2018).

The great diversity of viruses shown to infect cape gooseberry highlights the importance to continue the virome research on this host, including different geographical regions and growth conditions such as mixed cropping, crop rotation systems and even considering this species as a weed for other crops. Our results support the notion that mixed cultivation of *P. peruviana* with other solanaceous plants should be avoided and its presence as weed should be controlled as vectors transmitting potyviruses, such as *M. persicae*, *Aphis gossypii* and *Macrosiphum euphorbiae* are insects frequently associated with *P. peruviana* (Afsah, 2015). Future work should address the cross pathogenicity of PVV and PVY in the South American Andes and other places where there is coexistence between *P. peruviana* and other solanaceous crops as well as their effect on yield, plant longevity and physicochemical properties of the cape goosberry fruit.

Conclusions

The analysis of next generation sequencing data from *P. peruviana* leaf samples and the symptoms of the viral disease revealed an infection caused by the potyviruses PVY and PVV in the municipality of La Unión (Antioquia). These results were confirmed by real time RT-PCR (RT-qPCR) and the Sanger sequencing of the capsid region. Phylogenetic analysis confirmed these potyvirus isolates to be closely related to PVY and PVV isolates identified previously in tomato and potato crops in Antioquia, respectively, which suggests that cape gooseberry could be an alternate host to viruses of other economically important solanaceous crops in the Andean region of South America.

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