# Detection of RNA viruses in potato seed-tubers from northern Antioquia (Colombia)[1]

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## ABSTRACT

The most important viruses infecting potato in Colombia are PVY, PLRV, PVS, PYVV, PVV, PVX, PMTV, and PVB. Unfortunately, recent investigations on the health status of uncertified and certified seed-tubers of *Solanum tuberosum* cv. Diacol Capiro and *Solanum phureja* cv. Criolla Colombia used by farmers in eastern Antioquia (Colombia) have revealed alarming levels of infection by these viruses in all types of seeds distributed in the region. A similar study on northern Antioquia, testing all these eight viruses has not been performed. To close this gap, we present a complementary study on the prevalence of viruses in seed-tubers distributed and commercialized in this region using a combination of RT-qPCR and high-throughput sequencing. Our results further confirm the poor quality of seed-tubers used in Colombia and suggests infection by tobacco necrosis virus D (TNV-D), which is the first report for this virus in Colombia.

**Keywords:** plant viruses; RNAseq; RT-qPCR; seed certification; Solanaceae.

## INTRODUCTION

The province of Antioquia is the fourth most important potato producer in Colombia with fields comprising a total of 8619 hectares with annual yields estimated at 20.3 t/ha (Agronet, 2021) and dominated by the tetraploid cultivar *Solanum tuberosum* cv. Diacol Capiro, and the diploid *Solanum phureja* cv. Criolla Colombia (Porras & Herrera, 2015; MADR, 2019). Within Antioquia, potato production is concentrated in the eastern and northern regions, the latter of which comprises fields totaling 3585 ha across the municipalities of Santa Rosa de Osos, Entrerríos, San Pedro de los Milagros, and Belmira. Recent investigations on the viruses transmitted through seed-tubers in potato fields in eastern Antioquia have revealed a high prevalence of RNA viruses in formal- and informally- produced planting material, and it is believed that high viral loads are a major factor explaining the lower potato productivity of the region (Sierra *et al.*, 2020; 2021; Gallo *et al.*, 2019; 2021b). For example, in a study on seed-tubers from the Diacol Capiro cultivar it was found a prevalence for potato yellow vein virus (PYVV) of 73.3%, and up to 90% for potato virus Y (PVY) (Sierra *et al.*, 2021). With respect to the cultivar Criolla Colombia, the prevalence of viruses was more dramatic as some viruses such as potato virus S (PVS) and PYVV reached 100%, potato leaf roll virus (PLRV) 47%, PVY 47%, potato virus X (PVX) 40%, and potato virus V (PVV) 40% (Sierra *et al.*, 2020).

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It is a well-stablished fact that the widespread use of low-quality seed-tubers in Colombia is a major factor in the high prevalence of viral diseases in potato fields throughout the country (Gallo *et al.*, 2021b; Sierra *et al.*, 2021). It is estimated that only 3-5% of farmers in Colombia use certified planting material (Fedepapa, 2019; MADR, 2019) which, unfortunately, is not of sufficient quality with respect to viral infections either (Sierra *et al.*, 2021). To plant a new field, the average Colombian farmer uses seed-tubers produced by the same farmers from a preceding season or purchased in informal markets (MADR, 2019). Formal seed-tubers, on the other hand, are produced by specialized farmers in the highland plains of Cundinamarca, Boyacá and Nariño, where there is reduced insect pressure, from *in vitro* stock plants presumed to be free of viruses (ICA, 2015; Fedepapa, 2019). Unfortunately, the use of low sensitivity detection methods, and the circulation of local virus species and variants that are not detectable with available commercial serological kits has resulted in formal seeds with unacceptable levels of prevalence for some limiting viruses such as PLRV, PVY, PVX, PVS, and PYVV (Gallo *et al.*, 2021b; Sierra *et al.*, 2021).

PVY, PLRV, PVS, PYVV, PVV, PVX, potato mop-top virus (PMTV), and potato virus B (PVB) are the most prevalent viruses affecting potato in Colombia (Guzmán *et al.*, 2010; Vallejo *et al.*, 2016; Sierra *et al.*, 2020; 2021; Gallo *et al.*, 2019; 2021b; Giraldo *et al.*, 2022). However, only one study on the prevalence of these eight viruses in seed-tubers has been performed to this day (García, 2022). On that work, the author performed a pilot seed-indexing study using the tuber sprouting test through RT-qPCR with an updated set of primers on potato producing regions in eastern Antioquia. The author found that viruses such as PYVV and PMTV were highly prevalent in seed lots from both cultivars; PLRV, PVY, and PVB were highly prevalent in all cv. Diacol Capiro lots, but mostly absent in cv. Criolla Colombia, and PVV was almost exclusively found in cv. Criolla Colombia. With respect to PVS, and PVX these viruses were only found at low prevalence in the Diacol Capiro cultivar. In agreement, with previous work, multiple infections were also common in both cultivars, and no uninfected tubers were identified in any of the tested lots (García, 2022). Little is known about the health status of potato seeds commercialized in the northern producing regions of the province of Antioquia. To close this gap, we have performed this study on the prevalence of PLRV, PVY, PVX, PVS, PVV, PYVV, PMTV, and PVB in potato seeds stocks from the municipalities of San Pedro de los Milagros, Santa Rosa de Osos and Entrerríos by real-time RT-PCR (RT-qPCR) and high-throughput sequencing (HTS).

## MATERIALS AND METHODS

### *Plant material*

This work involved sampling and analysis of seed-tuber samples from the municipalities of San Pedro de los Milagros (6° 27′ 0″ N, 75° 33′ 0″ W), Entrerríos (6° 33’ 55.44” N, 75° 31’ 0.84” W), and Santa Rosa de Osos (6° 38’ 50.57” N, 75° 27’ 37.12” W) in northern Antioquia. Samples were collected from eight uncertified *S. tuberosum* cv. Diacol Capiro seed lots, eight uncertified *S. phureja* cv. Criolla Colombia seed lots, and eight certified *S. tuberosum* cv. Diacol Capiro seed lots. Due to the local nature and short dormancy period of the *S. phureja*, certified seeds for this cultivar were not available in the region. Sprouting in dormant seed-tubers of cv. Diacol Capiro was induced by spraying a diluted solution of gibberellic acid at 50 ppm (Sigma-Aldrich) in distilled water, air drying, and storage in darkness at room temperature for 15-20 days. Finally, from each seed-tuber lot, three separate tubers and three sprouts per tuber were used for virus indexing.

### *RT-qPCR*

Total RNA was extracted from 100 mg of the top two centimeters at the tip of each individual tuber sprout, following previous methods (García *et al.*, 2016; Sierra *et al.*, 2020). Prior to RNA extraction, plant tissue was ground in liquid nitrogen. RNA was purified using the GeneJET Plant RNA Purification Mini Kit (Thermo Fisher Scientific) and eluted in 40 µL of DEPC-treated water. RNA concentration and purity were determined using the A260/280 ratio measured in a Nanodrop 2000C spectrophotometer (Thermo Fisher Scientific). cDNA was synthesized using 80U of RevertAid Reverse Transcriptase (RT) (Thermo Fisher Scientific), RT buffer (1X), dNTPs at 1 mM, 20 U of RiboLock RNase Inhibitor, 1 µg of total RNA and 20 pmoles of the reverse primer in a reaction volume of 20 µL at 42 °C for 1 h. For PYVV, PLRV and PMTV, primers PYVV_R_CP, PLRV-R and 123-end were employed as reverse primers, respectively; for PVY, PVV, PVB, PVX

and PVS, an Oligo-(dT)18 was employed (Table 1). cDNA synthesis was performed in a T3 thermal cycler (Biometra) at 65 °C for 5 min, 42 °C for 60 min, and a final step at 70 °C for 10 min. The real-time PCR (qPCR) step was performed in a final volume of 14 µL with 6.25 µL of the Maxima SYBR Green/ROX kit (Thermo Fisher Scientific) using 100 ng of cDNA and primers at 0.3 µM concentration (Table 1). The temperature profile consisted of 10 min at 95 °C, followed by 35 cycles at 95 °C for 15 s, and 52 °C for 60 s. Amplifications were carried out in a Rotor-Gene Q-5plex cycler (Qiagen). In addition to threshold cycle (Ct), the specificity of the RT-qPCR results was confirmed by High Resolution Melting (HRM) in the 50-99 °C range. Positive and negative controls were included in each test and consisted of samples previously isolated from infected and virus-free potato tissues. The identity of amplicons was confirmed for selected samples by Sanger sequencing.

### *High-throughput sequencing*

cDNA libraries for HTS were prepared with the TruSeq Stranded Total RNA LT Sample Prep kit using bulked total RNA from certified (BST19) and uncertified (BST20) *S. tuberosum* cv. Diacol Capiro seeds, and uncertified (BSP18) *S. phureja* cv. Criolla Colombia. Ribosomal RNA (rRNA) was removed with the Ribo-Zero Plant kit (Illumina). The quality and quantity of the nucleic acid preparations was assessed with a 2100 Bioanalyzer (Agilent Technologies). Sequencing was performed with the Illumina NovaSeq system service provided by Macrogen (South Korea). The quality of each dataset was verified with fastqc (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and the low-quality bases (Phred < 30) were removed using Seqtk (https://github.com/lh3/seqtk). Preliminary detection of viruses was done with the PVDP platform (Gutiérrez *et al.*, 2021). Scaffolds from low coverage genomes were

**Table 1:** List of primers used in this study

| Virus | Primers | Sequence | Source |
|---|---|---|---|
| **PVY** | PVY_Col_RV2 | 5' GCA TAC TTG GAG ARA CAT CYT CG 3' | García et al. (2022) |
| | PVY_Col_qFV2 | 5' TCG GAT TGG AYG GYG GCA T 3' | |
| **PVV** | qPVV_phu_R | 5' CAT CCC GCT CCT CAA C 3' | Álvarez et al. (2016) |
| | PVV_phu_F | 5' ATG CTG GAA AAG ATC CAG C 3' | |
| **PVX** | PVX_101-2_RP | 5' GCT TCA GAC GGT GGC CG 3' | Agindotan et al. (2007) |
| | PVX_101-2_FP | 5' AAG CCT GAG CAC AAA TTC GC 3' | |
| **PVS** | PVS_gen_RV2 | 5' ATT GRA ANC CCA TRG CTT GC 3' | García et al. (2022) |
| | qPVS_gen_F | 5' CGG GRY TGA GRA ARG TGT G 3' | |
| **PLRV** | PLRV-Antisense | 5' TTG AAT GCC GGA CAG TCT GA 3' | Yang et al. (2014) |
| | PLRV-sense | 5' GCT CAA GCG AGA CAT TCG TG 3' | |
| | PLRV-R | 5' GCA ATG GGG GTC CAA CTC CAA CTC AT 3' | Singh et al. (1995) |
| **PYVV** | qPYVV_R_CP | 5' AGG TCT CAG GAT CTG GAT CAA CT 3' | Álvarez et al. (2017) |
| | PYVV_F_CP | 5' TCA GGT TAG AGC AGA CAG AGG 3' | |
| | PYVV_R_CP | 5' AGT TGC TGC ATT CTT GAA CAG G 3' | |
| **PMTV** | PMTV-1948F | 5' GTG ATC AGA TCC GCG TCC TT 3' | Mumford et al. (2000) |
| | PMTV-2017R | 5' CCA CTG CAA AAG AAC CGA TTT C 3' | |
| | 123-end | 5' GTG AAC CAC GGT TTA RCC CTG KAA GC 3' | Savenkov et al. (1999) |
| **PVB** | Neponew_pol_F | 5' TCG GCA CAA ACC CCT ATT CC 3' | Giraldo et al. (2022) |
| | Neponew_qpol_R | 5' TCA ATA GAC CAT CGA ACC CAC T 3' | |

assembled with Magic-BLAST (Boratyn *et al.*, 2019). Viruses with high coverage were assembled de novo with rnaSPAdes for each dataset (Bushmanova *et al.*, 2019). Contigs and scaffolds were verified for assembly errors with the Integrative Genomics Viewer (IGV) (Robinson *et al.*, 2011). Phylogenetic analyses were carried out in MEGA 11 (Tamura *et al.*, 2021) using the Neighbor-Joining method (Saitou & Nei, 1987) with 1000 bootstrap replicates. Sequences were aligned with MUSCLE (Edgar, 2004). Ambiguous positions were removed for each sequence pair. Evolutionary distances were computed using the Tamura-Nei method with a gamma distribution (Tamura, 1992).

## RESULTS

### *Detection of viruses by RT-qPCR*

With exception of PVV and PVB, RT-qPCR revealed high levels of prevalence for most viruses in *S. tuberosum* cv. Diacol Capiro tubers. For uncertified tubers of this cultivar PVX, PYVV, and PMTV were detected in all samples (100%); PVY and PVS were detected in seven samples (87.5%); and PLRV in five samples (62.5%). All uncertified *S. tuberosum* cv. Diacol Capiro tubers tested negative for PVV and PVB (Figure 1A). Viruses detected in uncertified Diacol Capiro tubers were also detected in certified tubers at 100% prevalence, with the addition of one sample from San Pedro that tested positive for PVB (Figure 1A). On the other hand, the eight viruses were detected in *S. phureja* cv. Criolla Colombia; PVY, PVV, PVS, PYVV, and PLRV were found in all samples (100%), PVX in seven samples (87.5%), PMTV in six samples (75%), and PVB in two samples (25%). The most remarkable difference was the high prevalence of PVV in *S. phureja* which was not detected in *S. tuberosum* (Figure 1B). Coinfections were common in all tested seed-tuber lots. In *S. tuberosum,* samples were coinfected with at least four viruses with the combination PVY/PVS/PVX/PYVV/PLRV/PMTV being the most common as it was observed in seven certified samples, and five uncertified samples. One certified sample was infected with all viruses, except for PVV (Figure 1C). With respect to *S. phureja*, all samples were infected with at least six viruses, however, the large majority were infected with the seven-virus combination PVY/PVV/PVS/PVX/PYVV/PLRV/PMTV.

### *High-throughput sequencing*

RNAseq analysis revealed a higher proportion of viral reads, 6.21% and 0.95% in the *S. tuberosum* uncertified and certified samples, respectively. For *S. phureja* the proportion of viral reads was 1.01% (Figure 2). In uncertified *S. tuberosum* cv. Diacol Capiro seed lots, the dominant viruses were PLRV at 2165 reads per million (RPM), the PMTV segments at 105-287.9 RPM and PVY at 59334 RPM (Figure 2). Reads attributed to all RNA segments of PYVV were also detected in this dataset at 2.9 (RNA1), 18.9 (RNA2), and 8.6 (RNA3) RPM. Viral loads were lower in the certified tubers, in this case, the dominant viruses were also PVY at 9398.6 RPM and PLRV at 51 RPM (Figure 2). The set of viruses present in *S. phureja* seed lots was different from that of *S. tuberosum*. In this case, the dominant viruses were PVX (8889 RPM), and PVV (1158.6 RPM); PVS was detected a lower load (15.4 RPM). Interestingly, a significant number of reads with about 96% nucleotide sequence identities to tobacco necrosis virus D (TNV-D) (*Betanecrovirus*, *Tombusviridae*) were also identified in this dataset at 10.3 RPM, which would be the first report for this virus in Colombia (Figure 2).

Phylogenetic analysis of scaffolds and/or contigs revealed the phylogenetic affinity of the viruses detected by RNAseq with previously known isolates in Colombia. For PLRV, sequences from both certified (BST19) and uncertified (BST20) *S. tuberosum* seed-tubers were part of the main clade found for this virus worldwide (Guyader & Ducray, 2002; Mesa *et al.*, 2016), and closely related to isolate M1 obtained from *S. quitoense* in Antioquia by Gallo *et al.* (2021a). Analysis of the PMTV RNA3 scaffold obtained from the uncertified *S. tuberosum* seed-lots (BST20) formed an independent clade between isolates of worldwide distribution (Gil *et al.*, 2011; 2016), and local isolates infecting *S. tuberosum* (CO2 and CO1) or *P. peruviana* (M7 and M2) recently reported by Gallo *et al.* (2020) (Figure 3).

PYVV sequence from the uncertified *S. tuberosum* seed lots (BST20) clustered within a clade containing isolates infecting different hosts such as *S. lycopersicum*, *S. phureja*, *S. tuberosum*, *S. quitoense,* and *P. peruviana* in Colombia (Guzmán-Barney *et al.*, 2012; Álvarez *et al.*, 2017; Gallo *et al.* 2020; 2021b) (Figure 3). Assembly of the PVY sequences resulted in three contigs clustering with the $PVY^{N}$, and the recombinant $PVY^{NTN}$ strains previously detected by Muñoz *et al.* (2016a) in *S. lycopersicum*, Álvarez *et al.* (2018) in *P. peruviana* and *S. tuberosum*, including the isolate Yarumal from northern Antioquia (Muñoz *et al.*, 2016b) (Figure 3). Surprisingly, uncertified seeds contained only the $PVY^{NTN}$ strain while, certified *S. tuberosum* seeds were also carriers of the $PVY^{N}$ strain.

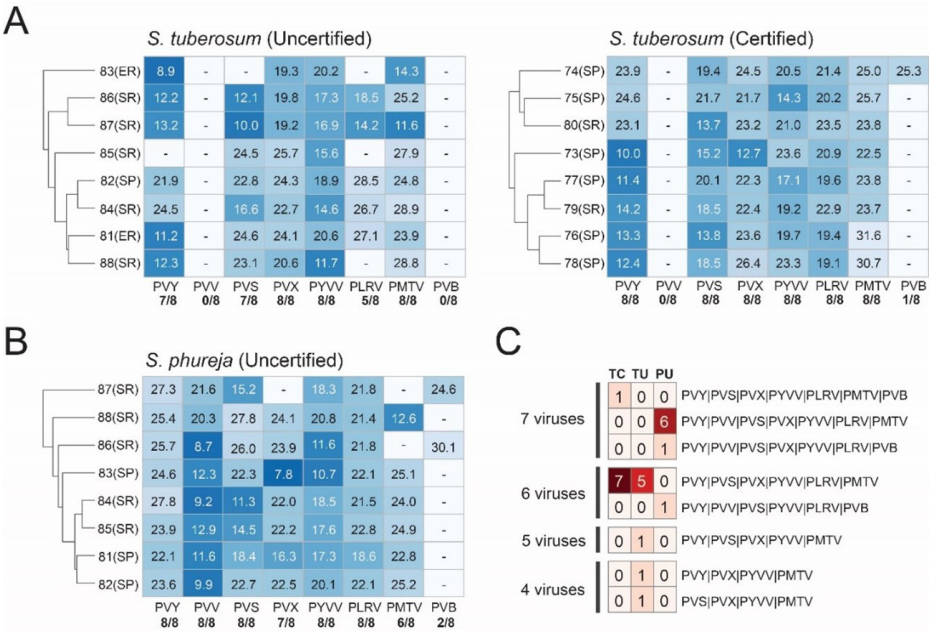


**Figure 1**: Virus prevalence in potato seed-tuber lots from northern Antioquia. A) Eight RNA viruses known to infect potato in Colombia were tested by RT-qPCR in certified, and uncertified seed-tubers from *S. tuberosum* cv. Diacol Capiro and B) *S. phureja* cv. Criolla Colombia. Numbers correspond to Ct values. C) Virus coinfections observed for each sample type. ER: Entrerríos; SP: San Pedro de los Milagros; SR: Santa Rosa de Osos. TC: *S. tuberosum* certified tubers; TU: *S. tuberosum* uncertified tubers; PU: *S. phureja* uncertified tubers.

Analysis of the *S. phureja* assemblies from the uncertified seed-tuber lots confirmed the presence of PVS, PVV, and PVX, in addition to TNV-D (Figure 4). The PVS scaffold (PVS-BSP18) clustered within the Andean PVS ($PVS^{A}$) group of strains infecting different solanaceous crops in Antioquia such as *S. tuberosum, S. quitoens*e, and *S. lycopersic*um but formed a distinct clade with isolate RL5 reported to infect *S. phureja* in Antioquia (Gutiérrez *et al.*, 2013; Vallejo *et al.*, 2016) (Figure 4). The PVV assembly (PVV-BSP18), on the other hand, clustered within a distinct clade comprising other *S. phureja* isolates (Álvarez *et al.*, 2016; Gutiérrez *et al.*, 2016) in addition to isolate from *P. peruviana* identified in Antioquia (Álvarez *et al.*, 2018). With respect to PVX, the assembled contig clustered within the Eurasian Clade at 100% bootstrap together with several worldwide isolates infecting *S. tuberosum* (García *et al.*, 2016) (Figure 4). Finally, the partial genome sequence of TNV-D from uncertified (BSP18) *S. phureja* seeds was part of a clade comprising several TNV-D isolates infecting a wide diversity of hosts such as *Valerianella locusta*, *Nicotiana tabacum* and *Glycine max;* this TNV-D isolate was also clearly separated from the other two approved members of genus *Betanecrovirus*: leek white stripe virus (LWSV) and beet black scorch virus (BBSV) (ICTV, 2021) (Figure 4).

## DISCUSSION

In this study, we have evaluated the prevalence of RNA viruses in seed-tubers from *S. tuberosum* cv. Diacol Capiro and *S. phureja* cv. Criolla Colombia, used by farmers in northern Antioquia. Our results suggest a high prevalence of viruses in seeds from all sources. With respect to cv. Criolla Colombia, our analyses confirmed infection with all the viruses tested and the high prevalence of PVV in contrast to *S. tuberosum* (Álvarez *et al.*, 2016). A comparison with similar studies performed in the eastern region of Antioquia (Sierra *et al.*, 2020; 2021; Gallo *et al.*, 2021b; García, 2022), confirms that seed-tuber degeneration resulting from the accumulation of viruses is a serious problem affecting the potato production in Antioquia that must be addressed urgently by the corresponding phytosanitary agencies. In perspective, the maximum productivity of cv. Diacol Capiro is estimated at 40 t/ha (Porras & Herrera, 2015) which is almost twice the productivity of northern Antioquia of 24 t/ha; with respect to *S. phureja* the maximum productivity is approximately 25 t/ha (Rodríguez *et al.*, 2009; Seminario *et al.*, 2018) but current productivity in fields from Antioquia rarely surpass 15 t/ha (Agronet, 2021). Of particular concern are the levels of infection of certified cv. Diacol Capiro tubers, which are expected to

have a lower prevalence of viruses but tested at 100% for PVY, PVS, PVX, PYVV, and PLRV as current legislation limits their prevalence at 5% for PLRV, PVY, PVS, and PVX; and 1% for PYVV (ICA, 2015); it is important to clarify that these values correspond to prevalence as measured by visual inspection and/or serological tests which are less sensitive than the methods used in this work. Another worrying fact is the high number of different viruses detected in certified seed lots, which were infected with at least six viruses. We strongly believe that the current potato seed certification program in Colombia should be reformulated to include more sensitive detection methods, particularly during the screening phase of the seed stock plants used to produce the material for further certification steps. The plantation of virus-free tubers can substantially improve yields in potato, particularly when combined with methods that reduce potential sources of inoculum such as early-season crop hygiene, roguing, and positive selection (Gildemacher *et al.*, 2011; Schulte-Geldermann *et al.*, 2012; Thomas-Sharma *et al.*, 2016). Some reports indicate that planting reliable certificated seed-tubers can result in about an initial 30% increment in yields (Halterman *et al.*, 2012; Frost *et al.*, 2013; Thomas-Sharma *et al.*, 2016; 2017), but can be increased further to more than 70% with the continued used of high-quality seed, as evidenced by data from western Europe, the United States, and Canada (Halterman *et al.*, 2012; Frost *et al.*, 2013; Thomas-Sharma *et al.*, 2016; 2017).

The production of healthy seed-tubers should be a concerted effort involving farmers, phytosanitary surveillance institutions, and basic research groups that results in the routine implementation of highly sensitive diagnostic techniques such as RT-qPCR or isothermal amplification tests such as RPA and LAMP for monitoring the prevalence

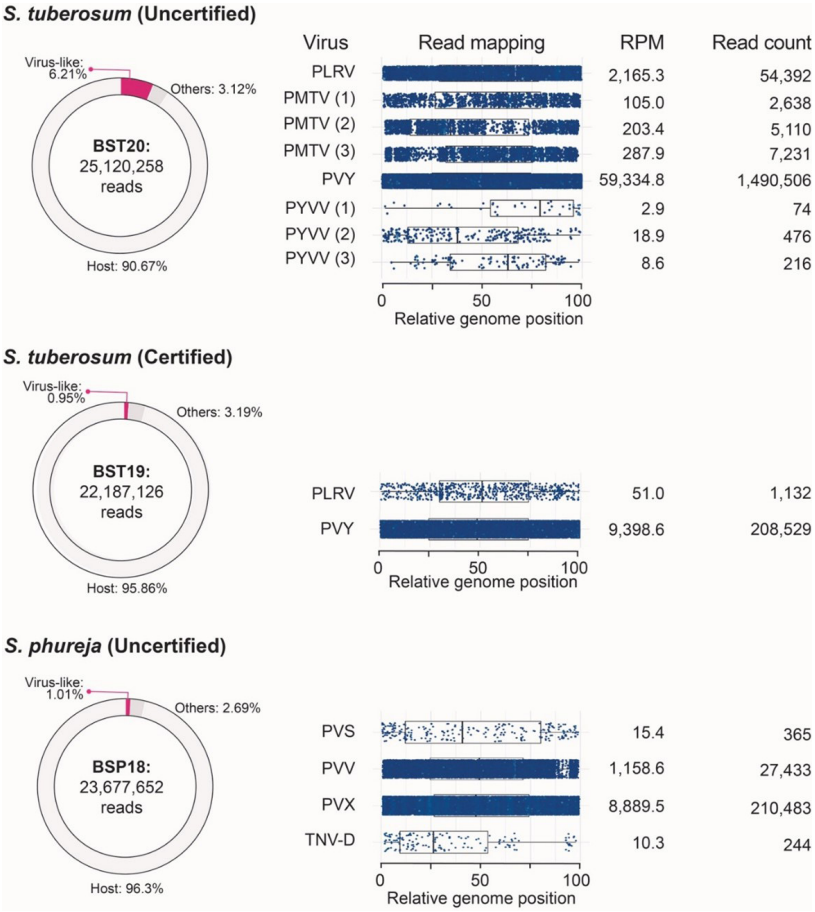


**Figure 2**: HTS detection of viruses in potato seeds-tubers in northern Antioquia. Detection of viruses was performed using the PVDP package. The proportion of virus-like sequences is shown on the left in magenta and corresponds to the proportion of sequences sharing high nucleotide sequence identities with currently known plant viruses at NCBI. Boxplots illustrate the relative genome position of mapped reads against each virus genome; darker colors indicate higher sequence identity. RPM and total number of reads for each identified virus are shown on the right.

of viruses in the field and the validation of virus clean-up protocols (Raigond *et al.*, 2020; Kumar *et al.*, 2021; Schumpp *et al.*, 2021). In addition, HTS methods should be used as a complementary technique for detecting the emergence of new virus strains and species and for monitoring the movement of viruses across regional or country borders. The usefulness of HTS as a complementary diagnostic method has been demonstrated in this work, allowing the detection of PLRV, PMTV, PYVV, and PVY in seed-tubers of *S. tuberosum* cv. Diacol Capiro, and of PVX, PVV, and PVS in *S. phureja* tubers. As a proof of concept, HTS also allowed the identification of TNV-D, a virus that was not initially included as part of this study and therefore should be investigated further to clarify its impact on *S. phureja.* TNV-D is a Betanecrovirus (*Tombusviridae*) first described in *N. tabacum* that can infect a wide spectrum of hosts since biological studies have shown that this virus can be transmitted to at least 88 dicotyledonous and monocotyledon species in 37 families (Price, 1938). TNV-D is naturally transmitted by zoospores of the root-infecting chytrid fungus *Olpidium brassicae* (Teakle & Gold, 1963) and despite its early discovery in 1935 (Smith & Bald, 1935), only a few isolates have been sequenced: TNV-D infecting french bean (*Phaseolus vulgaris*) from England, TNV-DH infecting *Nicotiana clevelandii* from Hungary, TNV-DP infecting olive trees from Portugal and the Manitoban isolate (TNV-D$^{MB}$) from soybean (Díaz-Cruz *et al.*, 2017). Genome analysis of TNV-D$^{MB}$ revealed a genome of 3,742 nt encoding six proteins: 22-kDa and 82-kDa proteins products required for RNA replication (p22 and p82), three 7-kDa proteins (71, 7a, and 7b) believed to be involved in cell-to-cell movement, and a coat protein (CP) gene (Díaz-Cruz *et al.*, 2017). Infection of potato with TNV-D is uncommon, however, it has been included as part of the EPPO Certification Schemes for potato seeds (PM4/028-1) and all nuclear stock microplants commercially produced in Europe should test negative for this virus (EPPO, 1999). Future work must investigate the prevalence

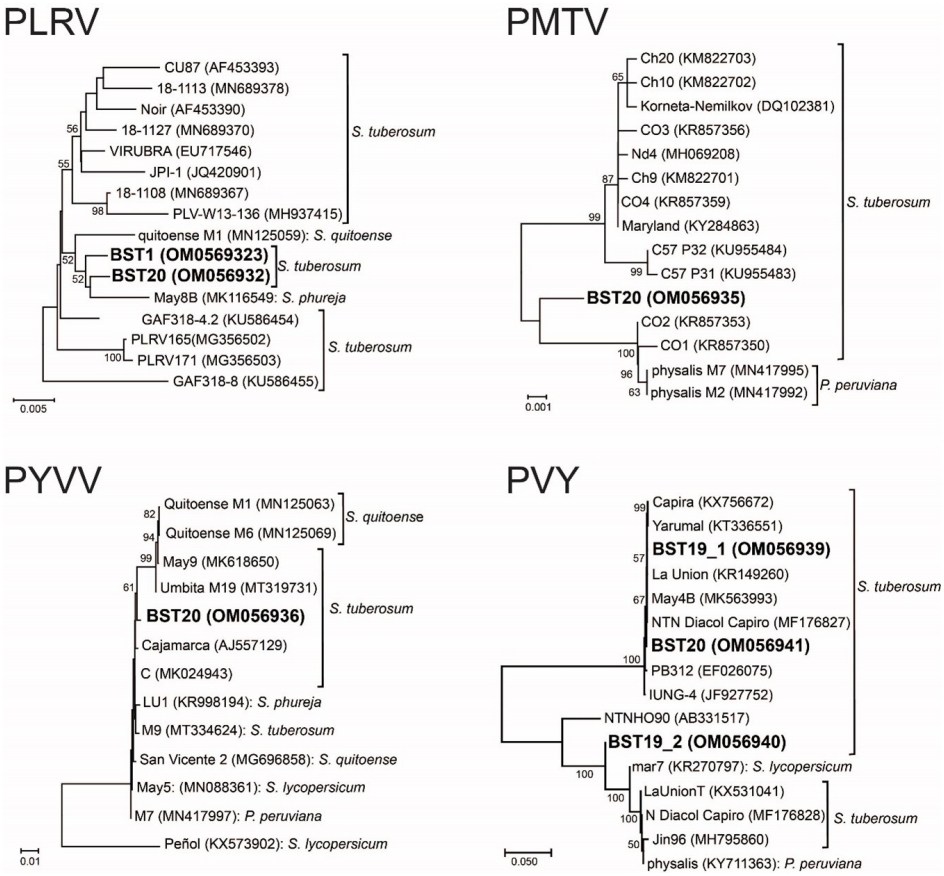


**Figure 3**: Neighbor-joining trees of viruses detected by RNAseq in *S. tuberosum* cv. Diacol Capiro in seed-tubers from northern Antioquia. Each panel shows the phylogenetic tree obtained for partial genome sequences (bold) derived from the RNAseq data from certified (BST19), and uncertified (BST20) potato seed-tubers. Bootstrap values are shown above the tree branches. NCBI accession

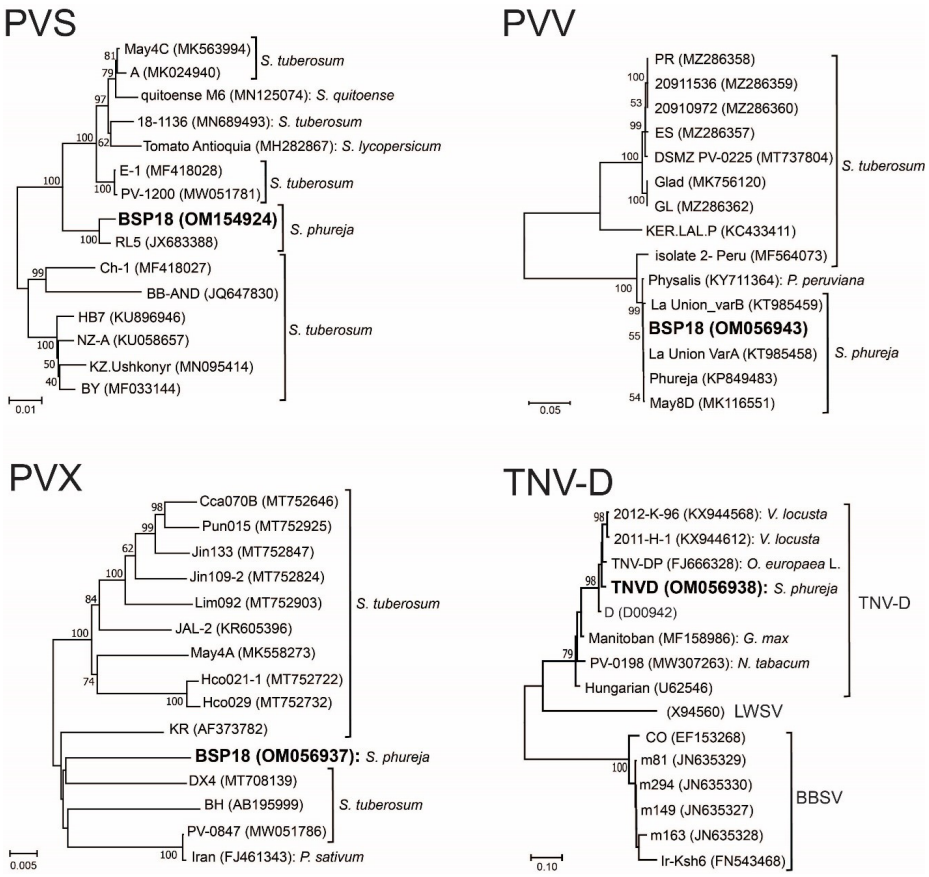


**Figure 4**: Neighbor-joining trees of viruses detected by RNAseq in *S. phureja* cv. Criolla Colombia in seed-tubers from northern Antioquia. Each panel shows the phylogenetic tree obtained for partial genome sequences (bold) derived from the RNAseq data from uncertified (BSP18) potato seed-tubers. Bootstrap values are shown above the tree branches. NCBI accession codes are indicated in

of TNV-D, and its associated vector *O. brassicae,* in potato fields in northern Antioquia.

In more general terms, we believe that similar studies like this one should be extended to other potato cultivating regions in Colombia and to include additional cultivars such as Parda Pastusa, Superior or Pastusa suprema, widely grown in Cundinamarca, Boyacá, and Nariño (MADR, 2019), to better understand the current phytosanitary status of this crop and have a more complete picture of the virome of potato in Colombia. Potato is affected by more than fifty viruses across the world (Kreuze *et al.*, 2020) and it is likely that more viruses remain to be discovered in Colombia and the rest of the South American Andes, the center of diversity of potato.

## CONCLUSIONS

A high level of prevalence of viruses: PVX, PYVV, PMTV, PVY, PVS and PLRV was found in potato seed-tubers from the northern region of Antioquia (Colombia) in both certified and uncertified material of cultivar Diacol Capiro. Additionally to these viruses, PVV and PVB were also found in uncertified tubers of *S. phureja* cv. Criolla Colombia. Using high-throughput sequencing, the partial genome sequence of tobacco necrosis virus D (TNV-D) was assembled from uncertified *S. phureja*. This is the first report for TNV-D in Colombia.

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