

# Next generation sequence analysis of the forage peanut (*Arachis pintoï*) virome

Secuenciación de nueva generación del viroma del maní forrajero  
(*Arachis pintoï*)

doi: 10.15446/rfna.v69n2.59133

Pablo Andrés Gutiérrez Sánchez<sup>1</sup>, Helena Jaramillo Mesa<sup>1</sup> and Mauricio Marín Montoya<sup>1\*</sup>

## ABSTRACT

### Key words:

*Alphaflexiviridae*  
*Polerovirus*  
*Potyvirus*  
Viral genomes

Forage peanut (*Arachis pintoï*) is one of the forage crops best adapted to tropical agroecosystems where it is used as ground cover in urban areas and slopes, in the preservation of soils cultivated with coffee, African oil palm and citrus and as animal feed in combination with gramineous plants. *A. pintoï* is considered to be highly resistant to plagues and diseases; however, in recent years there has been a marked increase of plants showing symptoms typical of viral infection. In this work, Next Generation Sequencing (NGS) was used to confirm the presence of virus in symptomatic *A. pintoï* plants collected in urban areas in Medellín (Colombia). Transcriptome analysis revealed the presence of 3,291,089 reads related to viruses in the families *Potyviridae*, *Luteoviridae* and *Alphaflexiviridae* and resulted in the complete genome assembly of *Peanut mottle virus* (9707 nt), *Turnip yellows virus* (5578 nt) and two variants of a virus with phylogenetic affinity to the genus *Allexivirus*. These two variants lack ORF6 present in *Allexivirus* and probably belong to an uncharacterized genus within the family *Alphaflexiviridae*. The presence of at least three viruses infecting *A. pintoï* in Colombia highlights the importance of starting a germplasm clean-up program of the plant material used as seed in this crop.

## RESUMEN

### Palabras claves:

*Alphaflexiviridae*  
*Polerovirus*  
*Potyvirus*  
Genomas virales

El maní forrajero (*Arachis pintoï*) es uno de los cultivos forrajeros mejor adaptado a los agroecosistemas tropicales, donde se utiliza en mezclas con gramíneas para alimentación animal, como alternativa para la cobertura del suelo en áreas urbanas y talúdes y para la conservación de suelos en plantaciones de palma africana, café y cítricos. Aunque el maní forrajero se considera una planta tolerante a plagas y enfermedades, en los últimos años se ha observado en Colombia el aumento de síntomas asociados a enfermedades virales. Con el objeto de evaluar la ocurrencia de virus en dichos materiales sintomáticos, en el presente estudio se utilizó la metodología de Secuenciación de Nueva Generación (NGS) del transcriptoma de un grupo de muestras de *A. pintoï* procedentes de zonas urbanas en Medellín (Colombia). Los resultados indicaron la presencia de 3.291.089 reads asociados a genomas virales de miembros de las familias *Potyviridae*, *Luteoviridae* y *Alphaflexiviridae*; siendo posible obtener los genomas completos del *Peanut mottle virus* (9707 nt), *Turnip yellows virus* (5578 nt) y de dos variantes de un virus filogenéticamente relacionado con el género *Allexivirus*. Estas dos variantes carecen del ORF6 presente en *Allexivirus*, por lo que posiblemente se trata de miembros de un género hasta ahora no caracterizado en la familia *Alphaflexiviridae*. La ocurrencia de al menos tres virus que infectan plantas de *A. pintoï* en Colombia, llama la atención sobre la necesidad de emprender un trabajo de limpieza de germoplasma en el material de siembra utilizado para el establecimiento de este forraje tropical.

<sup>1</sup> Facultad de Ciencias. Universidad Nacional de Colombia. A.A. 3840, Medellín, Colombia.

\* Corresponding author <mamarinm@unal.edu.co>

Forage peanut (*Arachis pintoii* Krapov. & Gregory), locally known as 'Mani Forrajero Perenne' is a Leguminosae (section *Caulorrhizae*) plant native of the states of Goiás, Bahia and Minas Gerais in Central Brazil (Krapovickas and Gregory, 1994; Palmieri *et al.*, 2009). *A. pintoii* is a diploid perennial herb ( $2n=20$ ) of tetrafoliolate leaves that can reach heights between 20 to 40 cm and is well adapted for clonal propagation by means of stolons (Rincón *et al.*, 1992; Lavia *et al.*, 2011). This plant is widely used as forage to improve the nutritional quality of pastures and also as ground cover in substitution of common grasses in tropical and subtropical regions of Australia, Bolivia, Brazil, Colombia, Costa Rica, Honduras and Venezuela (Valls, 1996; Palmieri *et al.*, 2010). *A. pintoii* was introduced in Colombia as part of an investigation aimed at finding tropical legumes adapted to Oxisol savanna conditions. Currently, the most important cultivars is CIAT 17434, which was selected from a set of more than 40 accessions of wild species of the genus *Arachis* from collections in the United States (University of Florida and USDA) and Australia (CSIRO) (Rincón *et al.*, 1992).

Forage peanut has a high nutritive value (13-25% crude protein, 60-70% dry matter digestibility), low levels of condensed tannins, and is well adapted to acid and low fertility soils (Lascano and Thomas, 1988). Moreover, *A. pintoii* has good legume dry matter yields, can withstand heavy grazing conditions and is compatible with mat-forming species such as *Brachiaria humidicola* and *B. dictyoneura* (Rincón *et al.*, 2001). *A. pintoii* is resistant to a wide range of pests and diseases; however, there are reports of fungal infections with *Cercospora* sp., *Phomopsis* sp., *Periconia* sp., *Cylindrocladium* sp., *Colletotrichum gloeosporioides*, *Sphaceloma arachidis* and *Rhizoctonia solani*, none of which causes serious damage (Rincón *et al.*, 1992). Even though some studies have reported the presence of yellowing, ringspots and mottle symptoms, there are few investigations regarding viruses infecting *A. pintoii*. The only work available dates from 1991 and described the natural infection of *A. pintoii* affected by a potyvirus related to *Peanut mottle virus* (PeMoV) in various localities in the province of Valle del Cauca in Colombia (Morales *et al.*, 1991). Electron microscopy analysis of leaves exhibiting ringspot symptoms demonstrated the presence of filamentous flexuous particles of about 750 nm in length and 15 nm in diameter that induced the

formation of cytoplasmic cylindrical inclusions (Morales *et al.*, 1991). PeMoV, is transmitted non-persistently by aphids and seeds in peanut (*Araquis hypogaea*) and can also infect soybean and pea. In 2006, this virus was also found in rhizoma peanuts (*Arachis glabrata*), a forage crop with increasing acreage (>10,500 ha) in the coastal plain region of the United States, causing chlorotic ringspots (Nischwitz *et al.*, 2007).

With the commercial availability of Next-Generation sequencing platforms, the strategies for detecting viruses changed dramatically as it is now possible to obtain detailed genome information without previous knowledge of specific sequences and at very low cost (Rossinck *et al.*, 2015). Currently, the systems most widely used are 454 GS FLX+ (Roche), HiSeq2000, 2500 and MiSeq (Illumina) and SOLiD (ABI), which differ in their sequencing principles and methods of sample preparation. The illumina HiSeq and MiSeq platforms are characterized by the generation of very high throughput of short mate-paired-end reads generated by a bridge PCR that can result in millions of sequence clusters in a flow-cell. In this platform, reads of approximately 100 bp are obtained by synthesis using fluorescently-labeled nucleotides, resulting in about 1 Tb of sequence data per run (Adams *et al.*, 2009; Wu *et al.*, 2015). Using NGS in the last five years at least 50 plant viruses have been discovered, 36 of which were classified into new families and nine were chosen as type species for an equal number of genera (Wu *et al.*, 2015). In Colombia, the NGS study of plant viromes allowed the identification and characterization of new virus species, most of them infecting solanaceous crops of economic importance such as *Tamarillo leaf malformation virus* (TaLMV), *Potato virus Y* (PVY), *Potato virus V* (PVV), *Potato yellow vein virus* (PYVV), *Potato virus X* (PVX), *Potato virus S* (PVS) and *Andean potato latent virus* (APLV) (Kreuze *et al.*, 2013; Villamil-Garzón *et al.*, 2014; Gutiérrez *et al.*, 2014a, 2014b, 2016).

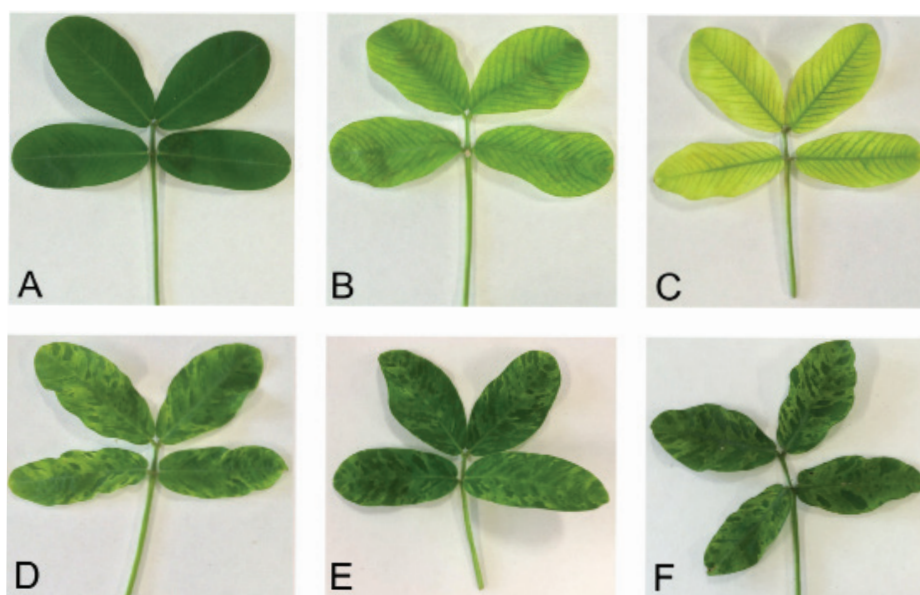
Close to 31 viruses representing 14 genera have been reported to naturally infect groundnut in different countries, including *Tomato spotted wilt virus* (TSWV), *Groundnut bud necrosis virus* (GBNV), *Tobacco streak virus* (TSV), *Groundnut rosette assister virus* (GRAV), *Groundnut rosette virus* (GRV), satellite RNA associated with GRV and/or GRAV, *Peanut clump virus* (PCV), *Bean common mosaic virus* (BCMV), *Peanut mottle*

virus (PeMoV) and *Cucumber mosaic virus* (CMV) (Sreenivasulu *et al.*, 2008). As a detailed description of viruses infecting *A. pintoi* is lacking at present, we present a genome sequence analysis of the forage peanut virome in Colombia using Next-Generation Sequencing (NGS).

## MATERIALS AND METHODS

This work was performed on a bulk sample of the *A. pintoi* leaves showing symptoms of leaf yellowing and green vein banding, as well as irregular dark islands and mottling on young leaves. Samples were collected in Medellín (Antioquia, Colombia) (Figure 1). Fresh

leaf tissue was ground using liquid nitrogen and RNA extracted with the GeneJET Plant RNA Purification kit (Thermo, EEUU). The integrity of total RNA was determined using a 2100 Bioanalyzer (Agilent Technologies, EEUU). rRNA was depleted with the TruSeq Stranded Total RNA with Ribo-Zero (Illumina, EEUU) while the TruSeq RNA Sample Preparation kit was used for cDNA library construction (Illumina). Sequencing was performed with the Illumina HiSeq 2000 system provided by Macrogen (South Korea). Low-quality bases were trimmed from both ends using the Phred algorithm implemented in the program SeqTk v.r82 (<https://github.com/lh3/seqtk>).



**Figure 1.** Symptoms of viral infection in *Arachis pintoi*. **A.** Asymptomatic leaf. **B, C.** Yellowing and green vein banding. **D, E, F.** Irregular dark islands and mottling on young leaves

Preliminary identification of viral sequences was performed with a BLASTN search against a local database containing all plant virus species currently accepted by the ICTV. *Potyvirus* and *Polerovirus* were assembled by mapping reads against the complete genomes of PeMoV (gb: NC\_002600) and *Turnip yellows virus* (TuYV) (gb: NC\_003743), respectively. The alphaflexivirus genomes were assembled using an iterative Perl routine that used BLASTN to identify reads with 30 nt overlapping segments at both ends of a seed sequence. Genomes were confirmed by *de novo*

reconstruction using Trinity (Grabherr *et al.*, 2011) and mapping with Bowtie2 (Langmead *et al.*, 2009). Open reading frames (ORFs) were identified using BLASTX and ORF finder (Gish and States, 1993). Sequences have been deposited in GenBank under accession codes KU708532 (PeMoV), KU726090-1 (TuYV), KX058345 (*Arachis pintoi* virus A) and KX058346 (*Arachis pintoi* virus B).

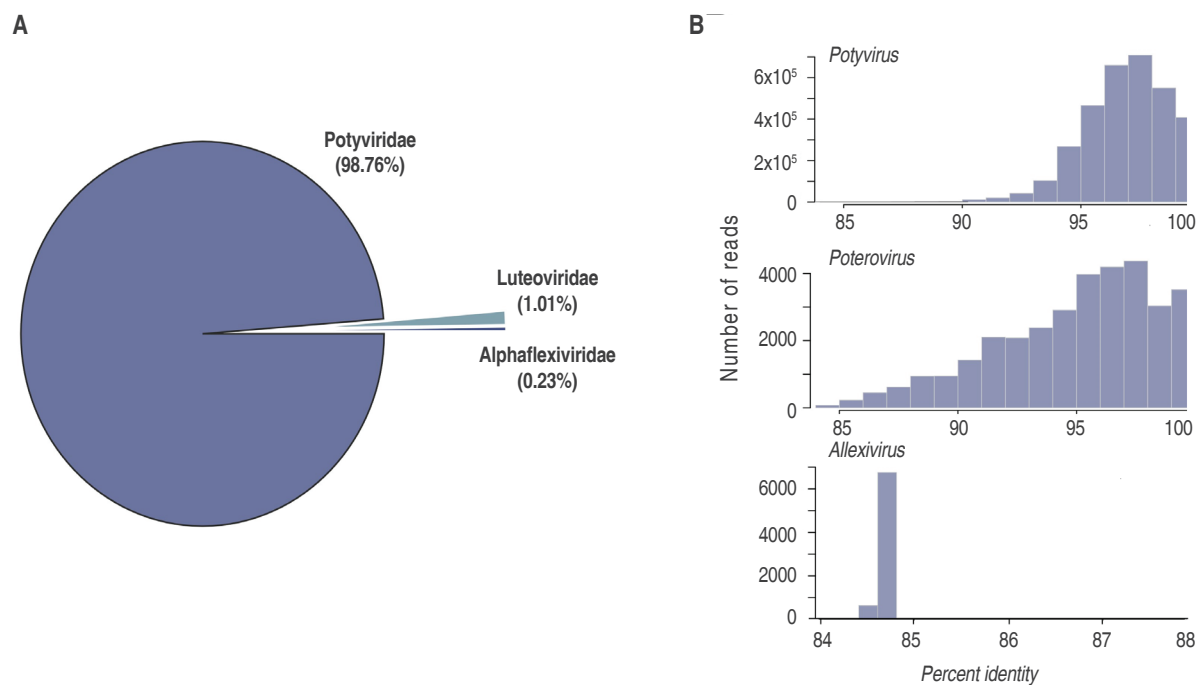
Phylogenetic reconstruction of amino acid sequences were inferred by the Maximum Likelihood (ML) method

using the LG+G+I substitution model (Le and Gascuel, 2008). ML analyses using nucleotide sequences were calculated with the HKY+G+I model (Hasegawa *et al.*, 1985). Sequence alignments were performed with MUSCLE (Edgar, 2004) and models selected with Modeltest (Posada and Crandall, 1998). Evolutionary analyses were conducted in MEGA6 using 1000 bootstrap replicates (Tamura *et al.*, 2013).

## RESULTS AND DISCUSSION

NGS analysis of the *A. pintoii* transcriptome resulted in a paired-end library of 40,712,018 reads for a total of 8,142,403,600 nt. BLASTN analysis indicated that 7.2 percent of the reads (3,291,089) were significantly similar to plant viruses within the *Potyviridae*, *Luteoviridae* and *Alphaflexiviridae* families (Figure 2).

Sequences related to *Potyviridae* members comprised 98.76 % percent of all viral sequences, which were further classified as belonging to the genus *Potyvirus*. Reads shared an average 96.67% (84 - 98.02%) percent identity with species of this genus; the most abundant hit corresponded to PeMoV (3,250,148). Reads in the family *Luteoviridae* shared identities ranging from 84 to 100% (average 94.82%) with members of the genus *Polerovirus*, the most abundant hit corresponding to TuYV (21,575). A group of 7,593 reads were classified as related to members of the family *Alphaflexiviridae* with the highest similarity to *Shallot virus X* (ShVX) (7,521), a species in the genus *Allexivirus*. However, the low average percent identity observed (84.7%), suggests that these sequences are probably significantly different to currently known members of this family.



**Figure 2.** Analysis of viral sequences present in the *A. pintoii* transcriptome. **A.** The pie chart shows the percentage of reads classified as belonging to the *Potyviridae*, *Luteoviridae* and *Alphaflexiviridae* families. **B.** Distribution of reads as a function of percent identity and classified at the genus level. *Potyvirus*, *Polerovirus* and *Allexivirus* were the only genus with significant similarity to the viral sequences present in the forage peanut transcriptome.

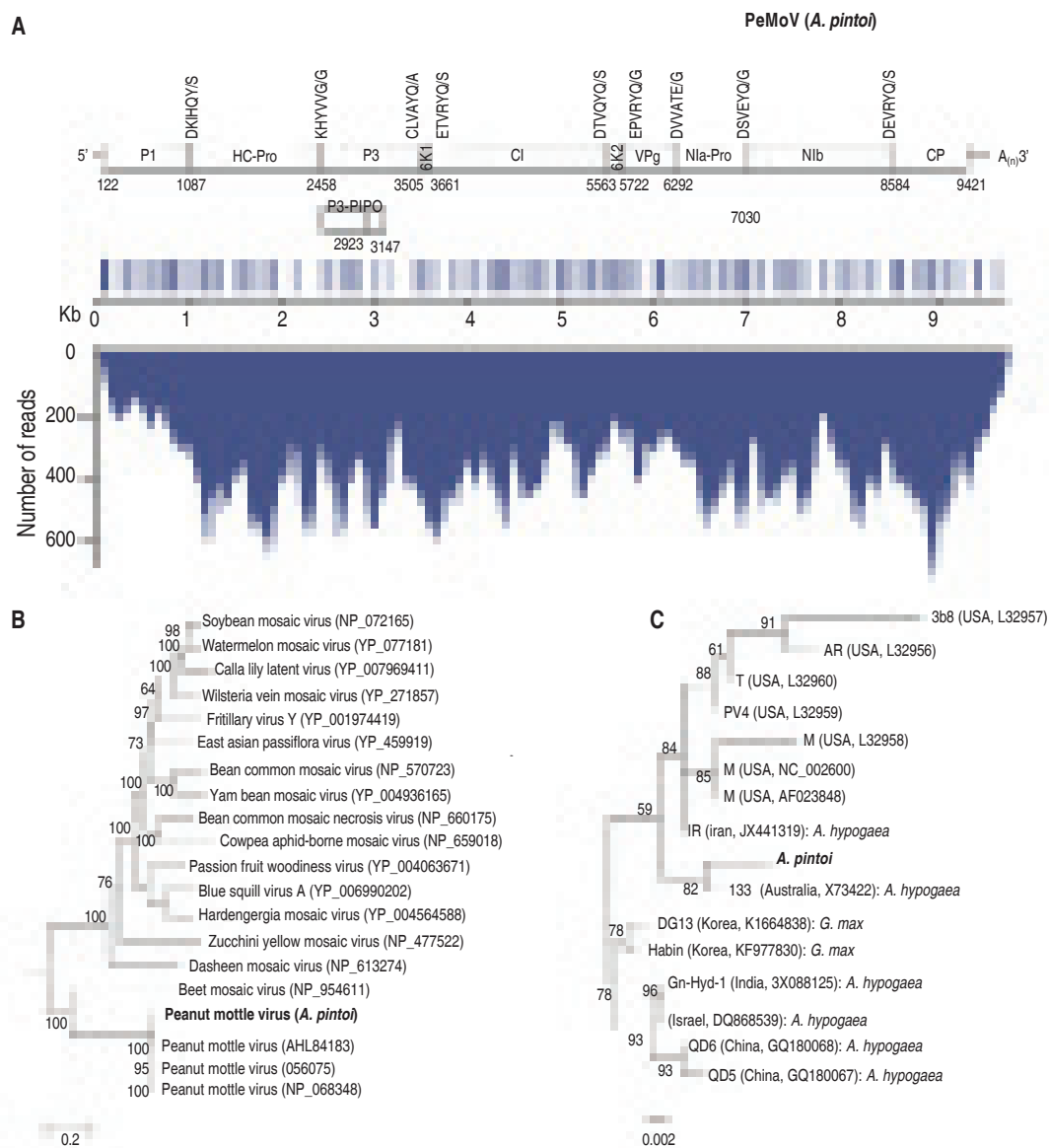
### *Potyvirus*

Mapping of the *A. pintoii* transcriptome to a PeMoV reference sequence resulted in a consensus assembly of 9707 nt (excluding the polyA tail) with an average coverage of 367x encoding a putative polyprotein of

3099 amino acids between positions 122 to 9421 (Figure 3). A BLASTN search using the complete assembly as query against the NCBI database confirmed that the assembled contig was closely related to *Peanut mottle virus* strain M (gb:AF023848) and *Peanut mottle virus*

isolate Habin (gb:KF977830) with a global percent nucleotide identity of 96% against both sequences. The consensus assembly was named *Peanut mottle virus* isolate pinto (PeMoV-pinto) to highlight the isolation host. In the PeMoV-pinto assembly, 242 polymorphic positions were identified, with a transition/transversion

ratio of 7.96. Within the coding region, most nucleotide changes (159), occurred in the third codon position, in contrast to 41 in the first position and 29 in the second. The following are the 61 amino acid substitutions observed: P1 (10); HC-Pro (10); P3 (10); CI (10); 6K2 (2); Nla-VPg (3); Nla-Pro (3); Nlb (10) and CP (3).



**Figure 3.** Characteristics of the *Peanut mottle virus* (PeMoV) genome found in *A. pintoi*. **A.** Genome structure of PeMoV illustrating the cleavage sites of each mature protein. The location of polymorphic sites (middle) and sequence coverage are indicated below. **B.** Molecular phylogenetic analysis using the complete polyprotein against selected virus species within the genus *Potyvirus*. **C.** Phylogenetic analysis using partial PeMoV CP sequences; when available, isolation host is shown after colons. Trees are drawn to scale, with branch lengths measured in number of substitutions per site.



Protease cleavage sites within the polyprotein were identified at amino acid positions 322, 779, 1128, 1180, 1814, 1867, 2057, 2303 and 2821 giving rise to putative mature proteins P1 (36.99 kDa), HC-Pro (51.21 kDa), P3 (40.27 kDa), 6K1 (5.72 kDa), CI (70.89 kDa), 6K2 (6.15 kDa), NIa-VPg (21.35 kDa), NIa-Pro (27.72 kDa), Nib (59.75 kDa) and CP (31.53 kDa). P1 and HC-Pro are predicted to be processed autocatalytically at recognition sequences DKIHQY/S and KHVVVG/G in agreement with previous reports (Adams *et al.*, 2005). The fusion product P3N-PIPO (26.3 kDa) is predicted to result from transcriptional slippage in the GA<sub>7</sub>T motif at nucleotide position 2918 within the P3 coding region and ending with a TAA stop codon at position 3147 (Olsper *et al.*, 2015). NIa-Pro, the cysteine protease responsible for cleavage at the P3/6K1, 6K1/CI, CI/6K2, 6K2/VPg, VPg/NIa-Pro, NIa-Pro/Nib, Nib/CP junctions is predicted to recognize the consensus [DEC]-x-V-x-[YT]-[QE]/ [AGS].

Phylogenetic analysis of the putative polyprotein confirmed that PeMoV-pintoi is a member of the PeMoV clade while phylogenies constructed using the coherently evolving CP region showed PeMoV-pintoi to be most closely related to Australian isolate PeMoV-AU found to infect *A. hypogaea* (Teycheney and Dietzgen, 1994). The PeMoV-pintoi/PeMoV-AU group is part of a larger clade comprising sequences isolated in the United States and Iran, all infecting peanut and clearly separated from strains infecting soybean. PeMoV was first described in 1965 in the United States and since then it has been reported to infect peanut in Africa, Asia, Australia and South America (Kuhn 1965; Sreenivasulua and Demski, 1988; Soumya *et al.*, 2014). PeMoV generally induces greenish mottle symptoms in peanuts and is also known to infect common bean and *Cassia* sp. (Kuhn, 1965; Lim *et al.*, 2014). PeMoV is of quarantine significance, can be transmitted by seeds and in a non-persistent manner by aphids such as *Aphis craccivora* and *Myzus persicae* (Adams and Kuhn, 1977; Sreenivasulua and Demski, 1988).

The closest PepMoV strain comes from Australia, where it is widely cultivated as forage in New South Wales and South-Eastern Queensland (Jones 1993; Bowman *et al.*, 1998). Australia was a source of *Arachis* accessions used in the selection of the best cultivars suited for use in Colombia (Rincón *et al.*, 1992); therefore, in spite of the large geographical distance between these two countries,

the similarity between the Colombian and Australian PepMoV isolates comes as no surprise. In 1991, Morales and collaborators characterized the causal agent of foliar ring spot symptoms in *A. pintoi* to be serologically related to PeMoV and which could also cause systemic infections in other legumes such as common bean, cowpea, peanut and soybean (Morales *et al.*, 1991). Unfortunately, monoclonal antibodies failed to confirm this suggestion. Our results confirm that PeMoV can naturally infect *A. pintoi* and, to our knowledge, this is first complete PeMoV genome sequence reported in a host different to *A. hypogaea*.

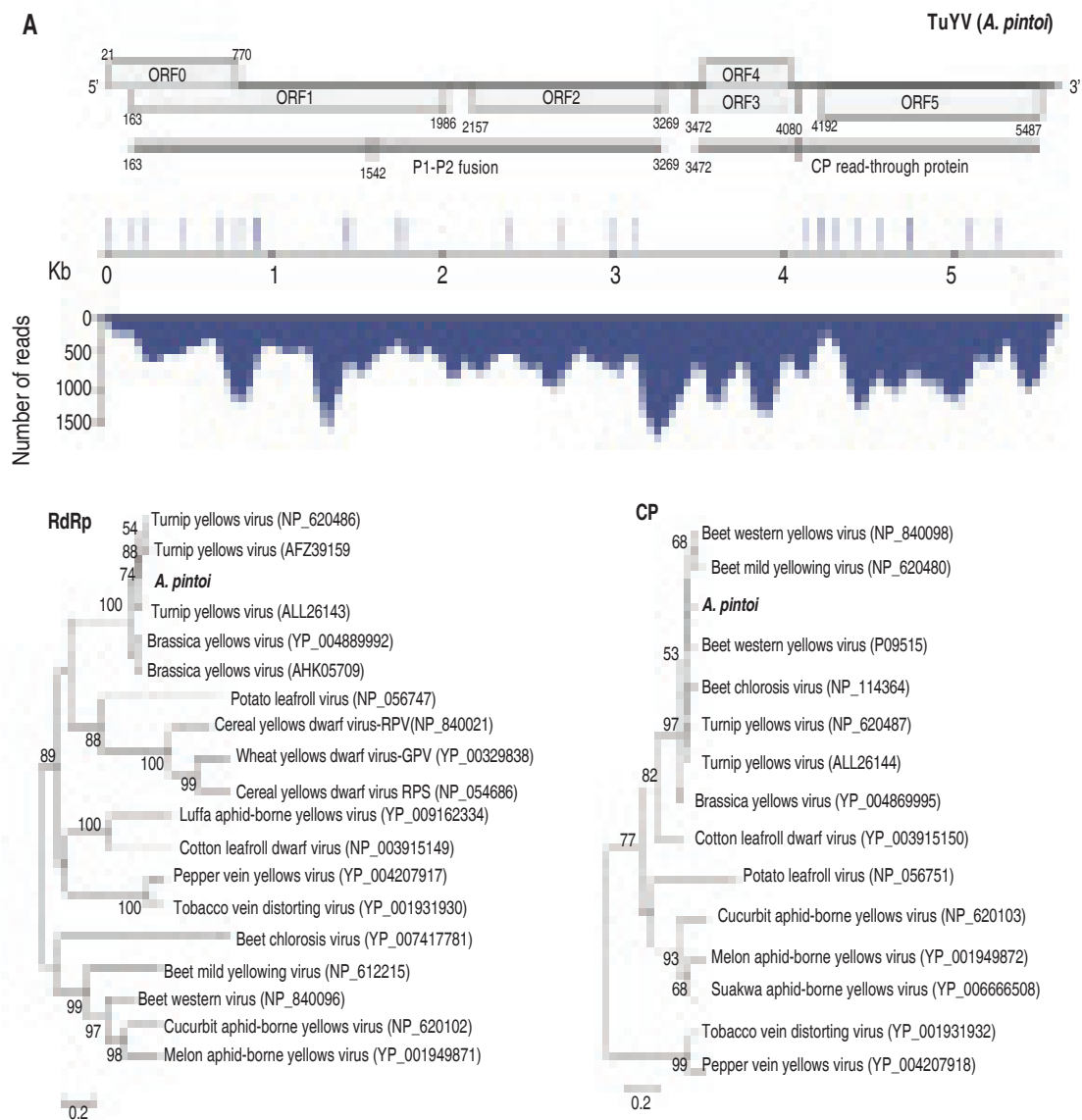
### **Polerovirus**

Poleroviruses are members of the *Luteoviridae* family, which are characterized by isometric virions of about 25 to 30 nm in diameter that encapsidate a positive single stranded genomic RNA of 5.6 kb to 6.0 kb with a Vpg protein linked to the 5' end and lack of a poly-A tail (King *et al.*, 2012). The assembly of the polerovirus present in the sample resulted in a contig of 5578 nt with an average sequence coverage of 724x, which was named TuYV-pintoi. Twenty seven polymorphic sites were found at positions 14, 161, 234, 434, 662, 778, 882, 888, 900, 1410, 1428, 1702, 1745, 2355, 2660, 2960, 3095, 4115, 4181, 4199, 4292, 4425, 4561, 4702, 4725, 5078 and 5226; the observed transition/transversion ratio was 3.5 (Figure 4). The best BLASTN hits were *Turnip yellows virus* isolates BWYV-FL1 (gb:X13063, 91%) and WA-1 (gb: JQ862472, 91%) identified in France (Veidt *et al.*, 1988) and Australia (Wylie *et al.*, 2013), respectively.

TuYV-pintoi contains six open reading frames ORF0-ORF5 at positions 21-770, 163-1986, 2157-3269, 3472-4080, 3503-4030 and 4192-5487, respectively, in agreement with the genomic structure of the genus *Polerovirus* (King *et al.*, 2012). ORF0 is predicted to encode protein P0 (28.9 kDa) that functions as a suppressor of RNA silencing and is involved in symptom and host range determination (Pfeffer *et al.*, 2002). ORF1 has a 607 nt overlap with ORF0 and codes for a 66.21 kDa protein (P1) containing an endopeptidase domain (pfam02122) at residues 206-408. This protein is believed to play a major role in the replication cycle by promoting the maturation of the genome-linked virion protein VPg (Nickel *et al.*, 2008). ORF2 is the RNA-dependent RNA polymerase (RdRp, 41.40kDa); however, as observed in other poleroviruses, a P1-P2 fusion protein of 115.56 kDa resulting from a

-1 frameshift at position 1542 within ORF1 is predicted (Xiang *et al.*, 2011) (Figure 4). ORF3 encodes a CP protein of 22.44 kDa protein expected to be produced by leaky scanning (King *et al.*, 2012). ORF4 codes for a 19.71 kDa protein with luteovirus VPg genome linked protein motif (pfam01659) at positions 45-148 (Rathjen *et al.*, 1994); this ORF is completely embedded within ORF3. ORF5 is predicted to encode a 47.89 kDa protein product; however,

a P3-P5 read-through protein has been observed as a result of suppression at the amber stop codon of ORF3 (Stevens *et al.*, 2005). With respect to the polymorphic sites in the consensus sequence, two resulted in amino acid substitutions in P0 (N47K and Y72H), six in the P1-P2 fusion (A91V, T167M, V206I, N462K, N414S, I732V) and six in the CP read-through (P215L, A237V, Q243L, S274F, N364Y, Y421H, I418M and L536P).



**Figure 4.** Characteristics of the *Turnip yellows virus* (TuYV) genome found in *A. pintoi*. **A.** Genome structure of TuYV illustrating the location of each encoded ORF. The location of polymorphic sites (middle) and sequence coverage are indicated below. Molecular phylogenetic analysis using RdRp and CP sequences are shown in panels **B** and **C**, respectively. Trees are drawn to scale, with branch lengths measured in number of substitutions per site.

Phylogenetic analysis using the RdRp protein confirmed TuYV-pintoi to be closely related to *Turnip yellows virus* and, more distantly to *Brassica yellows virus*. With respect to the CP analysis, TuYV-pintoi clustered within a group comprising *Beet western yellows virus*, *Beet mild yellowing virus*, *Beet western yellows virus* and *Beet chlorosis virus*. This clade also includes TuYV and *Brassica yellows virus* with a bootstrap of 97%. In the RdRp phylogenetic tree these viruses were separated into a different group. Interspecific and intraspecific phylogenetic studies suggested that beet-infecting polerovirus arose by recombination between a CABY-like ancestor contributing to ORFs 0, 1, 2 and a beet polerovirus parent that provided the 3'ORFs (Hauser *et al.*, 2002; Beuve *et al.*, 2008; Zhou *et al.*, 2011) thus explaining the conflicting results between CP and RdRp trees (Figure 4).

TuYV was originally identified in the United States in the late 1950s and has been reported to infect a wide range of hosts such as *Brassica napus* (oilseed rape), peanut, *Gomphrena globosa* (globe amaranth), *Crambe abyssinica*, *Trifolium subterraneum* (subterranean clover), *Montia perfoliata* (Indian lettuce), *Lactuca sativa* (lettuce), *Capsella bursa-pastoris* (Shepherd's purse), *Pisum sativum* (common pea), *Glycine max* (soybean), *Lens culinaris* (lentil), *Senecio vulgaris* (common groundsel) and *Spinacia oleracea* (spinach) (King *et al.*, 2012). Symptoms may include systemic leaf reddening, leaf yellowing and stunting (King *et al.*, 2012; Lim *et al.*, 2014). TuYV can be carried and transmitted in a persistent manner by aphids (Homoptera, Aphididae) such as *Macrosiphum euphorbiae*, *Aphis fabae*, *A. gossypii*, *Myzus ascalonicus* and *M. persicae*, which seems to be the most efficient (Stevens *et al.*, 2005). The transmission of TuYV-pintoi by aphids should be addressed in future studies. The genome presented here is the first complete genome of a putative polerovirus naturally infecting *A. pintoi*.

### **Alphaflexiviridae**

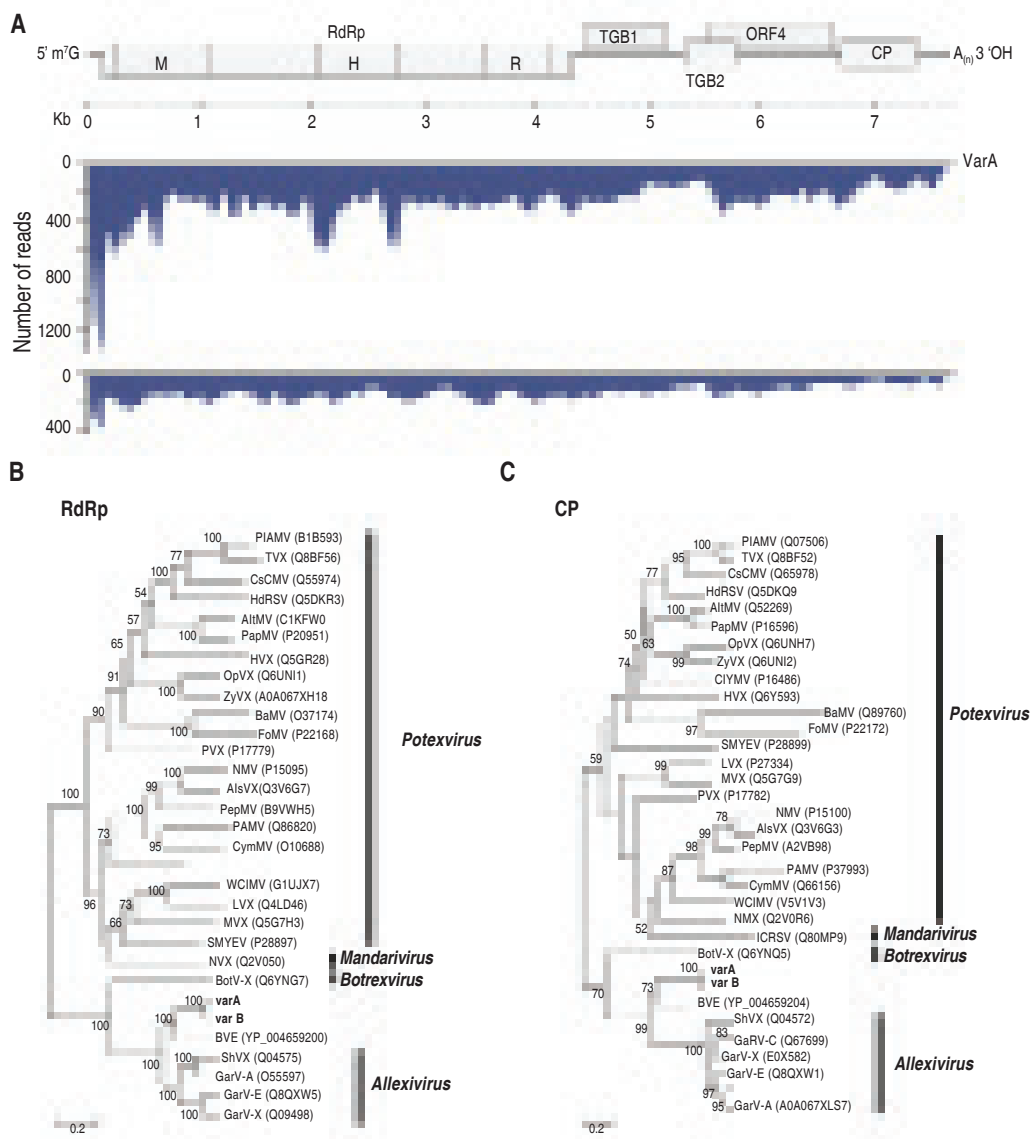
The analysis of reads evidenced the existence of viral sequences related to the family *Alphaflexiviridae* with an overall nucleotide percent identity of 85% to currently accepted genera by the ICTV (Figure 5). This family comprises viruses with genomes consisting of a single positive linear ssRNA of about 5.9-9.0 kb, capped with m<sup>7</sup>G at the 5' end and containing a 3' poly(A) tract (Martelli

*et al.*, 2007). *Alphaflexiviridae* typically encode 5 to 6 ORFs which include a RNA polymerase, triple gene block (TGB) proteins involved in cell-to-cell movement, a coat protein and, in some genera, a sixth ORF containing a zinc finger motif with nucleic acid binding activity. Currently, the *Alphaflexiviridae* family is divided into six Genera: *Allexivirus*, *Botrexvirus*, *Lolavirus*, *Mandarivirus*, *Potexvirus* and *Sclerodarnavirus* (King *et al.*, 2012).

*De novo* assembly resulted in two distinct contigs of 7561(135x) and 7538 (260x) nt, that share 85% of nucleotide identity and representing two variants (A and B). The most similar viruses in the NCBI database are *Blackberry virus E* (BVE) and *Garlic virus A* with 54 and 50 percent nucleotide sequence identity, respectively. BVE belongs to an unassigned genus within the family *Alphaflexiviridae* related to allexiviruses but lacks a 3' end-proximal ORF (Sabanadzovic *et al.*, 2011). *Garlic virus A* (GarV-A), on the other hand, is a member of the genus *Allexivirus* which also includes ShVX, *Garlic mite-borne filamentous virus* (GarMbFV), *Garlic virus B* (GarV-B), *Garlic virus C* (GarV-C), *Garlic virus D* (GarV-D), *Garlic virus E* (GarV-E) and *Garlic virus X* (GarV-X). The assembled genomes contain five ORFs identified at positions 129-4307 (ORF1), 4396-5109 (ORF2), 5087-5407 (ORF3), 5509-6597 (ORF4) and 6650-7345 (ORF5). ORF1 encodes a putative RNA-dependent RNA polymerase of 1392 residues (157.61kDa) with characteristic methyltransferase (pf01660, 40-343), helicase (PF01443, 636-869) and replicase (PF00978, 991-1338) domains. ORF2 codes for a 26.46 kDa protein and contains an ATPase (pfam13191) and viral RNA helicase (pfam01443) domains at positions 12-138 and 26-227, respectively. ORF2 and ORF3 are homologous to the triple gene block proteins TGBp1 and TGBp2. ORF4 encodes a 40.84 kDa protein homologous to the 40kDa protein of *Alphaflexiviridae*. ORF5 encodes a 25.52 kDa protein homologous to the viral coat protein of *Alphaflexiviridae*. No sequences homologous to ORF6 of allexiviruses were identified in the assembled genomes. The same number of ORFs was found in Variant B.

Phylogenetic analysis using the RdRp and CP proteins revealed that variants A and B are a sister group of BVE and are closely related to the genus *Allexivirus* (Figure 5); we propose the names *Arachis pintoi virus A* and *Arachis pintoi virus B* for these isolates. Our data indicates that





**Figure 5.** Characteristics of the *Alphaflexiviridae* genomes present in *A. pintoi*. **A.** Predicted genome structure of the assembled *Alphaflexiviridae* genome variants A and B and their corresponding sequence coverage. Molecular phylogenetic analysis using RdRp and CP sequences are shown in panels **B** and **C**, respectively. Trees are drawn to scale, with branch lengths measured in number of substitutions per site.

variants A and B probably belong to a new genus within the family *Alphaflexiviridae*; however future work should confirm the existence of this proposed new genus using specific primers and by satisfying Koch’s Postulates.

### CONCLUSIONS

Using Next-Generation sequencing of the *A. pintoi* transcriptome from a bulk sample of leaves, three different virus species from the *Potyviridae*, *Luteoviridae*

and *Alphaflexiviridae* families were identified. PeMoV and TuYV have been previously reported infecting peanut; in contrast, the *Alphaflexiviridae* member probably corresponds to a new virus distantly related to the genus *Allexivirus* and which would be a first report of this family in *A. pintoi*.

The presence of at least three viruses infecting *A. pintoi* in Colombia suggests that it is important to

initiate a germplasm clean-up program in the seed and propagation material used for this plant. Further studies should also address the transmission mechanisms of these viruses, their incidence across the country, as well as their host range.

## ACKNOWLEDGMENTS

This work was supported by the Faculty of Sciences, Universidad Nacional de Colombia Sede Medellín (Código Hermes 32098 “Desarrollo de una plataforma bioinformática para la detección y el estudio del procesamiento postraducciona de Potyvirus”. Convocatoria Apoyo a Grupos de Investigación - Facultad de Ciencias).

## REFERENCES

- Adams DB and Kuhn CW. 1977. Seed transmission of *Peanut mottle virus* in Peanuts. *Phytopathology* 67(9): 1126-1129.
- Adams MJ, Antoniw JF and Beaudoin F. 2005. Overview and analysis of the polyprotein cleavage sites in the family Potyviridae. *Molecular Plant Pathology* 6(4): 471-87. doi: 10.1111/j.1364-3703.2005.00296.x
- Adams IP, Glover RH, Monger WA, Mumford R, Jackeviciene E, Navalinskiene M, Samuitiene M and Boonham N. 2009. Next-generation sequencing and metagenomic analysis: a universal diagnostic tool in plant virology. *Molecular Plant Pathology* 10(4): 537-545. doi: 10.1111/j.1364-3703.2009.00545.x
- Beuve M, Stevens M, Liu HY, Wintermantel WM, Hauser S and Lemaire O. 2008. Biological and molecular characterization of an American sugar beet-infecting *Beet western yellows virus* isolate. *Plant Disease* 92(1): 51-60. doi:10.1094/PDIS-92-1-0051
- Bowman AM, Wilson GPM and Gogel BJ. 1988. Evaluation of perennial peanuts (*Arachis* spp.) as forage on the New South Wales coast. *Tropical Grasslands* 32: 252-258.
- Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32(5): 1792-1797. doi: 10.1093/nar/gkh340
- Gish W and States DJ. 1993. Identification of protein coding regions by database similarity search. *Nature Genetics* 3(3): 266-272. doi:10.1038/ng0393-266
- Gabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N and Regev A. 2011. Full-length transcriptome assembly from RNA-seq data without a reference genome. *Nature Biotechnology* 29(7): 644-652. doi: 10.1038/nbt.1883
- Gutiérrez PA, Alzate JF and Marín M. 2014a. Genome sequence of a virus isolate from tamarillo (*Solanum betaceum*) in Colombia: evidence for a new potyvirus. *Archives of Virology* 160(2): 557-560. doi: 10.1007/s00705-014-2296-8
- Gutiérrez PA, Alzate JF and Marín M. 2014b. Caracterización del viroma de ARN de tejido radical de *Solanum phureja* mediante pirosecuenciación 454 GS-FLX. *Bioagro* 26(2): 89-98.
- Gutiérrez PA, Alzate JF and Marín M. 2015. Complete genome sequence of an isolate of *Potato virus X* (PVX) infecting Cape gooseberry (*Physalis peruviana*) in Colombia. *Virus Genes* 50(3): 518-522. doi: 10.1007/s11262-015-1181-1
- Gutiérrez P, Mesa H and Marín M. 2016. Genome sequence of a divergent Colombian isolate of *Potato virus V* (PVV) infecting *Solanum phureja*. *Acta Virologica* 60(1):49-54. doi:10.4149/av\_2016\_01\_49
- Hasegawa M, Kishino H and Yano T. 1985. Dating the human-ape split by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* 22(2): 160-174. doi:10.1007/BF02101694
- Hauser S, Stevens M, Beuve M and Lemaire O. 2002. Biological properties and molecular characterization of *Beet chlorosis virus* (BChV). *Archives of Virology* 147(4): 745-762. doi: 10.1007/s007050200023
- Jones RM. 1993. Persistence of *Arachis pinto* cv. Amarillo on three soil types at Samford, South-Eastern Queensland. *Tropical Grasslands* 27:11-15.
- King AMQ, Adams MJ, Carstens EB and Lefkowitz EJ. 2012. Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, San Diego, EEUU. 1338 p.
- Krapovickas A and Gregory WC. 1994. Taxonomía del género *Arachis* (Leguminosae). *Bonplandia* 8(1): 1-186.
- Kreuze J, Koenig R, De Souza J, Vetten HJ, Muller G, Flores B, Ziebell H and Cuellar W. 2013. The complete genome sequences of a Peruvian and a Colombian isolate of *Andean potato latent virus* and partial sequences of further isolates suggest the existence of two distinct potato-infecting tymovirus species. *Virus Research* 173(2): 431-435. doi:10.1016/j.virusres.2013.01.014
- Kuhn CW. 1965. Symptomatology, host range and effect on yield of seed transmitted peanut virus. *Phytopathology* 55(8): 880-884.
- Langmead B, Trapnell C, Pop M and Salzberg SL. 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biology* 10(3): R25. doi:10.1186/gb-2009-10-3-r25
- Lascano CE and Thomas D. 1988. Forage quality and animal selection of *Arachis pinto* in association with tropical grasses in the eastern plains of Colombia. *Grass and Forage Science* 43(4): 433-439. doi: 10.1111/j.1365-2494.1988.tb01900.x
- Lavia GI, Ortiz AM, Robledo G, Fernández A and Seijo G. 2011. Origin of triploid *Arachis pinto* (Leguminosae) by autopolyploidy evidenced by FISH and meiotic behavior *Annals of Botany* 108(1): 103-111. doi:10.1093/aob/mcr108
- Le SQ and Gascuel O. 2008. An improved general amino acid replacement matrix. *Molecular Biology and Evolution* 25(7): 1307-1320. doi: 10.1093/molbev/msn067
- Lim S, Lee YH, Igori D, Zhao F, Yoo RH, Lee SH, Baek IY and Moon JS. 2014. First Report of *Peanut mottle virus* infecting soybean in South Korea. *Plant Disease* 98(9): 1285. doi: 10.1094/PDIS-04-14-0356-PDN
- Martelli GP, Adams MJ, Kreuze JF and Dolja VV. 2007. Family Flexiviridae: a case study in virion and genome plasticity. *Annual Review of Phytopathology* 45(1): 73-100. doi: 10.1146/annurev.phyto.45.062806.094401
- Morales FJ, Castano M, Velasco AC and Arroyave J. 1991. Natural infection of tropical forage legume species of *Arachis* and *Stylosanthes* by potyviruses related to *Peanut mottle virus*. *Plant Disease* 75(11): 1090-1093. doi: 10.1094/PD-75-1090
- Nickel H, Kawchuk L, Twyman RM, Zimmermann S, Junghans H, Winter S, Fischer R and Prüfer D. 2008. Plantibody-mediated

- inhibition of the *Potato leafroll virus* P1 protein reduces virus accumulation. *Virus Research* 136(1-2): 140-145. doi: 10.1016/j.virusres.2008.05.001
- Nischwitz C, Maas AL, Mullis RM, Culbreath AK and Gitaitis RD. 2007. First report of *Peanut mottle virus* in forage peanut (*Arachis glabrata*) in North America. *Plant Disease* 91(5): 632. doi:10.1094/PDIS-91-5-0632A
- Olspert A, Chung BY, Atkins JF, Carr JP and Firth AE. 2015. Transcriptional slippage in the positive-sense RNA virus family Potyviridae. *EMBO Reports* 16(8): 995-1004. doi: 10.15252/embr.201540509
- Palmieri DA, Bechara MD, Curi RA, Monteiro JP, Valente SES, Gimenes MA and Lopes CR. 2010. Genetic diversity analysis in the section Caulorrhizae (genus *Arachis*) using microsatellite markers. *Genetics and Molecular Biology* 33(1): 109-118. doi:10.1590/S1415-47572010005000001
- Pfeffer S, Dunoyer P, Heim F, Richards KE, Jonard G and Ziegler-Graff V. 2002. PO of *Beet western yellows virus* is a suppressor of posttranscriptional gene silencing. *Journal of Virology* 76(13): 6815-6824. doi:10.1128/JVI.76.13.6815-6824.2002
- Posada D and Crandall KA. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14(9): 817-818. doi: 10.1093/bioinformatics/14.9.817
- Rathjen JP, Karageorgos LE, Habili N, Waterhouse PM and Symons RH. 1994. Soybean dwarf luteovirus contains the third variant genome type in the luteovirus group. *Virology* 198(2): 671-679. doi:10.1006/viro.1994.1079
- Rincón CA, Cuesta PA, Pérez R, Lascano CE and Ferguson J. 1992. Maní forrajero perenne (*Arachis pintoi* Krapovickas y Gregory). Una alternativa para ganaderos y agricultores. *Boletín Técnico* No. 219. ICA/CIAT, Palmira. 23 p.
- Rincón A. 2001. Potencial productivo de ecotipos de *Arachis pintoi* en el Piedemonte de los Llanos Orientales de Colombia. *Pasturas Tropicales* 23: 19-24.
- Roossinck MJ, Martin DP and Roumagnac P. 2015. Plant virus metagenomics: Advances in virus discovery. *Phytopathology* 105(6): 716-27. Doi: 10.1094/PHYTO-12-14-0356-RVW
- Sabanadzovic S, Abou N and Tzanetakis IE. 2011. *Blackberry virus E*: an unusual flexivirus. *Archives of Virology* 156(9): 1665-1669. doi:10.1007/s00705-011-1015-y
- Soumya K, Yogita M, Prasanthi Y, Anitha K, Kavi-Kishor PB, Jain RK and Mandal B. 2014. Molecular characterization of Indian isolate of *Peanut mottle virus* and immunodiagnosis using bacterial expressed core capsid protein. *Virus disease* 25(3): 331-337. doi: 10.1007/s13337-014-0210-3.
- Sreenivasulu P and Demski JW. 1988. Transmission of Peanut mottle and Peanut stripe viruses by *Aphis craccivora* and *Myzus persicae*. *Plant Disease* 72:722-723. doi: 10.1094/PD-72-0722
- Sreenivasulu P, Reddy CVS, Ramesh B and Kumar PL. 2008. Groundnut viruses. pp. 47-97. En: Rao GP, Khurana SMP and Lenardon SL. Characterization, diagnosis & management of plant viruses. Volume 1: industrial crops. Studium Press, India.
- Stevens M, Freeman B, Liu HY, Herrbach E and Lemaire O. 2005. Beet poleroviruses: close friends or distant relatives? *Molecular Plant Pathology*. 6: 1-9. doi:10.1111/j.1364-3703.2004.00258.x
- Tamura K, Stecher G, Peterson D, Filipski A and Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30(12): 2725-2729. doi: 10.1093/molbev/mst197.
- Teycheney PY and Dietzgen RG. 1994. Cloning and sequence analysis of the coat protein genes of an Australian strain of Peanut mottle and an Indonesian 'blotch' strain of peanut stripe potyviruses. *Virus Research* 31(2): 235-244. doi:10.1016/0168-1702(94)90006-X
- Valls JFM. 1996. Variability in the genus *Arachis* and potential forage uses. pp 15-27. En: Springer TL and Pittman RN (eds). Identifying germplasm for succesful forage legume-grass interations. Proceedings of a Symposium of the Crop Science Society of America. USDA, Washington.
- Veidt I, Lot H, Leiser M, Scheidecker D, Guilley H, Richards K and Jonard G. 1988. Nucleotide sequence of *Beet western yellows virus* RNA. *Nucleic Acids Research* 16(21): 9917-9932. doi: 10.1093/nar/16.21.9917
- Villamil-Garzón A, Cuellar WJ and Guzmán-Barney M. 2014. Natural co-infection of *Solanum tuberosum* crops by the *Potato yellow vein virus* and potyvirus in Colombia. *Agronomía Colombiana* 32(2): 213-223. doi: 10.15446/agron.colomb.v32n2.43968
- Wu Q, Ding S, Zhang Y and Zhu S. 2015. Identification of viruses and viroids by Next-Generation Sequencing and homology dependent and homology independent algorithms. *Annual Review of Phytopathology* 53: 1-20. doi:10.1146/annurev-phyto-080614-120030
- Wylie SJ, Li H, Dixon KW, Richards H and Jones MG. 2013. Exotic and indigenous viruses infect wild populations and captive collections of temperate terrestrial orchids (*Diuris* species) in Australia. *Virus Research* 2013 171(1): 22-32. doi: 10.1016/j.virusres.2012.10.003
- Xiang HY, Dong SW, Shang QX, Zhou CJ, Li DW, Yu JL and Han CG. 2011. Molecular characterization of two genotypes of a new polerovirus infecting brassicas in China. *Archives of Virology* 2011 156(12): 2251-2255. doi: 10.1007/s00705-011-1091-z
- Zhou CJ, Xiang HY, Zhuo T, Li DW, Yu JL and Han CG. 2011. A novel strain of *Beet western yellows virus* infecting sugar beet with two distinct genotypes differing in the 5'-terminal half of genome. *Virus Genes* 42(1):141-149. doi: 10.1007/s11262-010-0553-9

