

GRSV and TCSV isolates were completely sequenced by Illumina HiSeq 2000. The sequence reads obtained were assembled by CLC Genomic Workbench program and the final contigs were analysed by the GenBank® database. Extensive phylogenetic analysis made by the software PhyML showed that the genetic variability between GRSV and TCSV M RNA is less than in Tomato spotted wilt virus (TSWV) species, the virus type in the genus. The M RNA phylogeny does not separate GRSV and TCSV segments in different groups, signaling that probably they share the same M RNA. As result, the qPCR analysis showed that TCSV was more efficient in replication, even when mixed with a higher amount of GRSV.

PIV208 - A NEW CLOSTEROVIRUS FOUND IN ARRACACIA XANTHORRHIZA BY NEXT GENERATION SEQUENCING

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Arracacia xanthorrhiza, known as mandioquinhasalsa (MS) in Brazil, is a root vegetable originally from the Andes and belonging to family Apiaceae. It is a vegetatively propagated plant and viral infection symptoms are frequently observed. Next generation sequencing (NGS) has proven to be an efficient tool for viral metagenomic analysis, without the need of previous viral genome knowledge. Here we describe the identification and genome analysis of a novel closterovirus found in *A. xanthorrhiza* by NGS. RNA from viral enriched preparation after differential centrifugation of plant extracts were sequenced by Illumina HiSeq 2000 platform. Reads were analyzed, assembled, and submitted to blastx analysis against the RefSeq Viral database. The contig of 15.756 bp with coverage of 4464 reads share a high identity to closteroviruses, and similar genomic organization. The genus Closterovirus (family Closteroviridae) comprises species with monopartite positive singlestrand RNA genome whose size varies from 14.5 to 19.3kb. Based on the sequence information obtained by metagenomic analysis, specific primers that amplify overlapping regions of all genome were designed. Initially, the presence of this new closterovirus was confirmed in 21 MS plants from 47 total plants based on RTPCR. Then, one sample (MS#6)

was selected and used for complete genome sequence through Sanger sequencing. To determine the 5' and 3' terminals the RACE approach was successfully used. The complete genome of this closterovirus encodes 9 potential open reading frames and shows the typical organization of closteroviruses. The putative heat shock protein 70 homolog (HSP70h), RNA dependent RNA polymerase, and coat protein genes showed 3744, 2633, and 1835% amino acid sequence identities with other closteroviruses genome, respectively. A phylogenetic tree based on HSP70h gene showed that Beet yellows virus and Grapevine leafroll associated virus 2 are their closest relative to this virus. In conclusion, this study shows evidence of the presence of a putative new species in genus Closterovirus in *Arracacia xanthorrhiza* of Brazil. Considering that the sequence similarities of all taxonomically relevant proteins between this new *Arracacia* virus and recognized closteroviruses are far below the species demarcation threshold proposed by the Closteroviridae Study Group, we propose this virus to be representative of a new species in the genus, for which we propose the name "Arracacha virus 1".

PIV210 - IDENTIFICATION BRUGMANSIA SUAVEOLENS MOTTLE VIRUS IN BRUGMANSIA SP

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Plants of the genus *Brugmansia* (Solanaceae) are bushy-like trees that can reach up to 4.6 metres high. In Brazil, plants of this genus are popularly known as 'trombeteira' (trumpet) or 'saia branca' (white skirt). They are used as ornamental plants, because of their beautiful, large and tubular shape flowers. A leaf sample of *Brugmansia* sp. with mosaic and vein clearing symptoms was collected in 2015, in Curitiba - PR (Parque do Papa, S 25°24'40" W 49°16'13"). There is a report of the infection of the potyvirus *Brugmansia suaveolens mottle virus* (BsMoV) in a *B. suaveolens* plant, observed in Campinas SP (isolate BsCampinas). As the symptoms observed in this 'trombeteira' plant was distinct from the one collected in Campinas, it was supposed that a different virus could be causing the viruslike symptom in this plant.

Initially, transmission electron microscopy analysis indicated the presence of long and flexuous particles, and cytoplasmic inclusions, typical of potyvirus. Then, biological characterization was performed by mechanical inoculations in twelve indicator plants and symptoms were recorded. The test plants inoculated with the BsCuritiba isolate showed symptoms similar to those induced by the isolate BsCampinas. The test plants *Nicotiana benthamiana*, *N. tabacum* cv. TNN and *N. rustica* reacted with symptoms of necrotic spots and necrosis in the veins. The virus produced strong necrosis on *Physalis pubescens*, *Datura metel* and *Nicandra physaloides*, resulting in plant death. The molecular identification of the BsCuritiba was initiated by a search of a potyvirus using a potyvirusuniversal primer pair. Total RNA was extracted and subjected to RTPCR using primers that amplify a 1.7kb fragment in the 3' end of the genome. The amplicon was cloned and the insert sequence determined. The sequence share a nucleotide identity of 98% with the BsCampinas isolate (Accession AB551370). It was concluded that *Brugmansia suaveolens* mottle virus is also present in 'trombeteira' plants in Curitiba.

PIV217 - COMPARATIVE GENOMIC ANALYSIS OF TWO ANTICARSIA GEMMATALIS CLONES OBTAINED FROM A NATURAL ISOLATE FOUND IN THE FIELD

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Baculoviruses are pathogenic to insects and have been effective in controlling agricultural and forest insect pests. In Brazil, the baculovirus *Anticarsia gemmatalis* multiple nucleopolyhedrovirus (AgMNPV) has been used as a biological insecticide since the early 80's to control the soybean caterpillar, *Anticarsia gemmatalis*, a major pest of this crop. In this study, we sequenced the entire genome of two clones (Ag01 and Ag16) derived from a natural population of AgMNPV occurring in the field (Ag79). The Ag01 and Ag16 clones were obtained by either using the plaque assay technique (Ag16) or by serial dilution (Ag01) of the Ag79. These isolates were selected for genomic analysis because they showed differences in the virulence pattern in the bioassay. DNA from both clones was extracted and sequenced using the pyrosequencing technique, and the data was analyzed using the Geneious software R6. The entire genome

sequence of the Ag2D (genebank) was used as a reference. The results showed that the major discrepancy between the Ag01 and Ag16 and the genome of reference Ag2D occurred in the pe38 gene. This gene is involved in viral DNA replication, and transactivation of viral early genes transcription. In the Ag2D isolate, the p38 gene presents itself divided in two ORFs while in the Ag01 and Ag16 clones only one ORF was found. Similarly, the he65 gene (unknown function) also have a single ORF arrangement compared to the Ag2D genome, in which the gene is split into two ORFs. Interestingly, in the broa (ORF6) and bro-b (ORF7) genes of the Ag16 clone, the major difference was a deletion of about 700 bps, resulting in a fusion of both genes into a single ORF. This was not observed neither in the Ag2D nor in the Ag01 clone. These clones have polymorphism in genomic regions which have not been described in the literature yet. The comparative genomic analysis is an approach that can bring relevant information on virus diversity and the mechanisms used by the virus to adapt to a new environment.

PIV218 - DETECTION OF CULEX QUINQUEFASCIATUS NATURALLY INFECTED BY INSECTSPECIFIC VIRUSES IN CUIABÁ, MATO GROSSO, BRAZIL

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Insectspecific viruses comprise a new group of viruses capable of replicating only in invertebrate cells. *Culex flavivirus* is an insectspecific virus of the *Flavivirus* genus isolated from *Culex* spp in several countries. This study aimed to investigate the frequency of natural infection of adult mosquitoes by viruses in Cuiabá city, Mato Grosso, Brazil. To achieve that, *Culicidae* females (n=4,556) belonging to 14 species sampled in 200 urban census tracts pooled according to collection site, species and gender were subjected to RTPCR for a NS5 region, nucleotide sequencing, viral isolation in C6/36 cells and IonTorrent platform. *Culex Flavivirus* (CxFV) was detected in 16/403 (MIR=4.6) pools of *Cx. quinquefasciatus*, the most abundant species. The nucleotide sequences presented a high similarity with CxFV sequences from México, Uganda and Brazil.