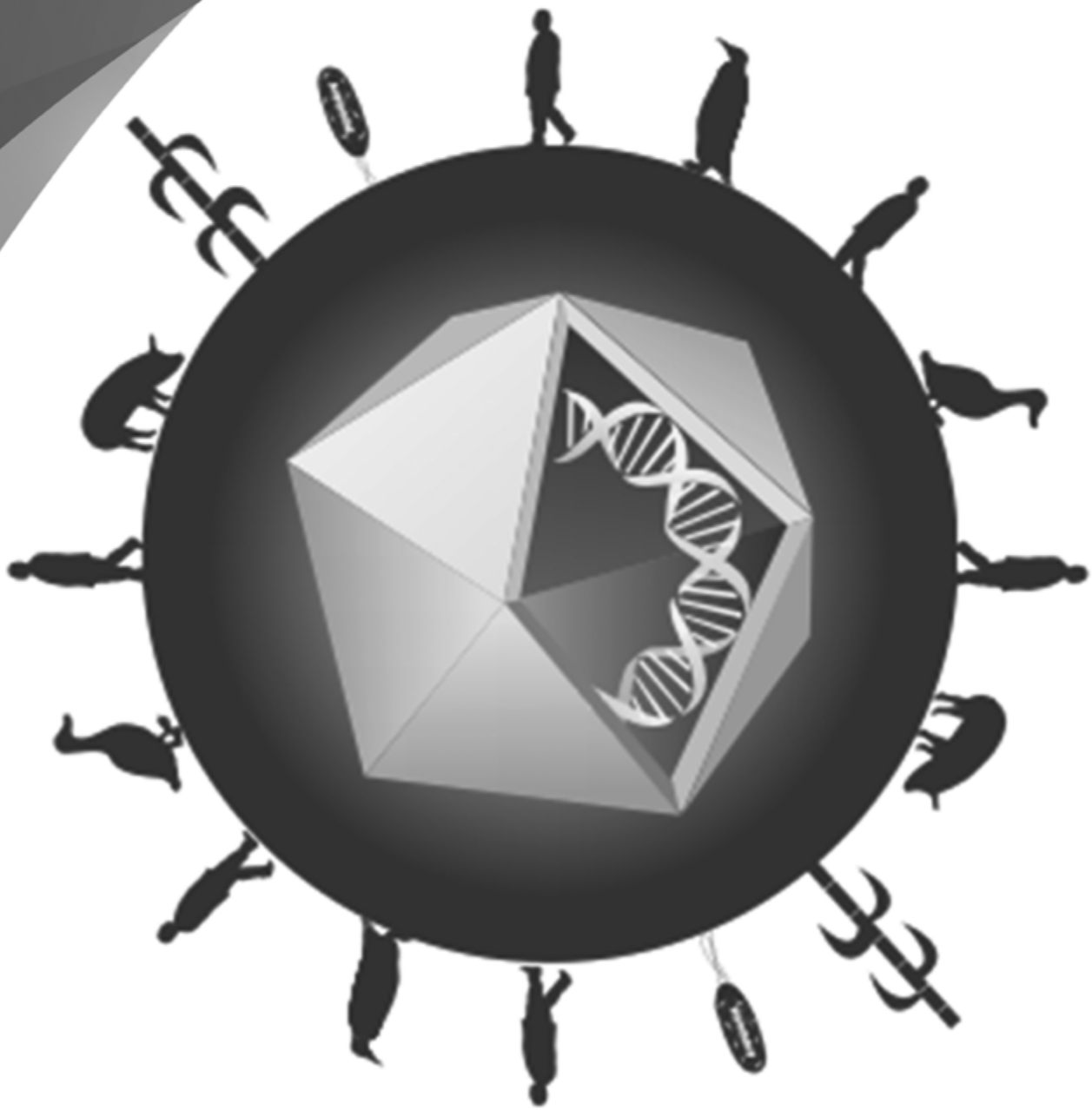
PLANT AND INVERTEBRATE VIROLOGY - PIV



PIV38 - EXPRESSION OF PARASPORIN PROTEINS FROM BACILLUS THURINGIENSIS IN INSECT CELLS

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Bacillus thuringiensis (Bt) is a pore-forming bacteria able to produce a crystalline protein inclusions during sporulation. Some strains exhibit entomopathogenic activity which makes Bt a largely-used bioinsecticide to control both agricultural insect pests and human disease vectors. Interestingly, somo Bts produce non-insecticidal crystalline inclusion-forming proteins called parasporins which are preferentially toxic to tumor cells. Bt proteins are efficiently expressed in insect cells using the baculovirus/insect cell-based expression system. Therefore, in this report, we have successfully expressed two Bt-derived parasporin genes using recombinant baculoviruses and insect cells. For this, both parasporin-2 gene and a newly discovered parasporin-3-related gene (41%) derived from a Brazilian Bt strain were inserted into a commercial vector pFastBac1™ (Invitrogen) and recombinant baculoviruses were constructed using the commercially available Bac-to-Bac system (Invitrogen). Recombinant viruses were used to infect cells and the recombinant proteins were detected by SDS-PAGE and immunobloting. Heterologous proteins will be analyzed by transmission and scanning electron microscopies to verify possible crystalline formations in the cytoplasm of insect cells. Moreover, the recombinant proteins are being purified by affinity chromatography based on a hexahistidine-tag for further toxicity tests against tumor cells.

PIV82 - HIGH HYDROSTATIC PRESSURE EFFECT ON THE EPITOPE MAPPING OF THE TOBACCO MOSAIC VIRUS COAT PROTEIN

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This study was aimed to investigate the effect of high hydrostatic pressure on Tobacco Mosaic Virus (TMV), a virus model for immunology and one of the most studied virus to date. When subjected to treatment with pressures it was observed a significant change in the recognized epitopes in comparison to sera from immunized mice with the native form of the virus. These alterations were further studied by combining the high pressure treatment with urea or low temperatures and inoculation of these altered virions in Balb-C mice, the collected sera titers were determined by ELISA and cross referenced between the groups tested. The titter obtained showed that the antigenicity of viral particles was maintained after treatments using monoclonal antibodies against the native form. The epitope prediction algorithms could not infer the some observed changes in the epitope profile suggesting conformational changes in the protein structure. The antigenicity of canonical epitopes was maintained, although binding intensities were different among the treatments used. Patterns of recognition from the epitope mapping were then cross checked with the prediction algorithms for the TMVcp amino acid sequence to infer which alterations might have occurred. Our findings suggest that different cleavages sites were exposed after the treatments; this was verified via epitope mapping using sera from mice immunized with the virus after the high pressure condition was imposed.

PIV151 - A BEGOMOVIRUS EXISTING AS A COMPLEX OF WELL DEFINED SUBPOPULATIONS IN A NON-CULTIVATED HOST

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Begomoviruses (family Geminiviridae) have a circular, ssDNA genome encapsidated in twinned icosahedral particles. In Brazil, a number of begomoviruses have been described infecting weeds. Here, we describe two

novel begomovirus species infecting Sida acuta plants collected from a small area (about 10,000 m2) at Viçosa, state of Minas Gerais in December 2011. Total DNA was extracted from S. acuta samples and the viral genome was amplified by RCA, cloned and sequenced. A total of 12 full-length DNA-A component were obtained from four samples, and the ICTV-established 89% DNA-A identity threshold was used for taxonomic placement. This analysis indicated that the cloned components correspond to two novel species, for which the names Sida golden yellow mosaic virus and Sida yellow spot virus (SiGYMV and SiYSV, respectively) are proposed. The DNA-A components exhibited a highly divergent 5´ half , including part of the intergenic region, the putative CP gene and an AV2-like ORF (present only in Old Word begomoviruses). The deduced amino acid sequence of the CP had very low identity with other begomoviruses, but the presence of conserved motifs in the CP and Rep coding regions, characteristic of OW begomoviruses, was detected. Although New World-like begomoviruses have been found in the OW, this is the first time that OW-like begomoviruses are found naturally in the NW. FINANCIAL SUPPORT: FAPEMIG, CAPES AND CNPQ

PIV156 - EVALUATION OF BRAZILIAN SUGARCANE GENOTYPES RESISTANCE TO SUGARCANE MOSAIC VIRUS UNDER GREENHOUSE AND FIELD CONDITIONS

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Sugarcane mosaic virus (SCMV), the causal agent of mosaic, is one of the main viruses infecting sugarcane in Brazil, which is mainly controlled by the use of resistant cultivars. Favorable epidemiological conditions to mosaic dissemination and recent descriptions of new isolates reinforce the current importance of the disease. In addition, there is little information about genetic parameters and heritability associated to mosaic resistance in sugarcane. In this regard, the present study aimed to evaluate the resistance of 79 sugarcane genotypes (varieties and elite clones) to a severe SCMV strain and also to estimate genetic parameters associated with mosaic resistance. Buds were collected from sugarcane stools without mosaic symptoms, maintained at the IAC Sugarcane Breeding Station, Ribeirão Preto, from which leaf samples of each stool were tested by PTA-ELISA (Plate Trapped Antibody-ELISA) in order to verify the virus sanity. Three weeks old seedlings, planted in stiff plastic tubes (volume of 50 cm3) in a two complete block design, were artificially inoculated with SCMV in aphid proof greenhouse conditions. Symptoms were evaluated by a grade scale and the confirmation of infection by the serological test PTA-ELISA were performed, being six months later validated under field conditions, adopting the same experimental design. Symptom data were submitted to variance analysis, adopting the Split plot temporal arrangement. The mean incidence of mosaic was low under greenhouse conditions, with higher values in the field assay. Based on field results, variance analysis revealed significant genotype and genotype x environmental effects. The interaction of sugarcane genotypes with days of evaluation revealed a differential behavior in mosaic symptom expression, including the recovery in some of them. The broad-sense heritability at individual level and means based were 19.37% and 62.18%, respectively, indicating that the resistance to mosaic tends to be a quantitative trait. The combination of symptom evaluation by grade scale with serological test ELISA for SCMV detection proved to be efficient for selection of sources of resistance to mosaic, detecting the virus in symptomless genotypes, and pointing out twenty two genotypes as resistant to SCMV strain in study. Financial support: FAPESP (BIOEN 2008/56146-5), IAC (Instituto Agronômico de Campinas) and CAPES.

PIV159 - SEQUENCING AND PHYLOGENETIC ANALYSIS OF PROTEINS P1 AND HC-PRO OF ISOLATES OF SUGARCANE MOSAIC VIRUS

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In Brazil, sugarcane, maize and sorghum are quite affected by Sugarcane mosaic virus (SCMV), responsible for the mosaic disease in these crops. The introduction of new maize hybrids and its cultivation in off-season near sugarcane fields has resulted in further spread of virus

strains in the country. The P1 and HCPro SCMV proteins play an important role in the virus infection, replication and transmission. The objective of this work was to better understand the genomic organization and variability of sugarcane and corn isolates of SCMV by means of sequencing and sequence analysis of these proteins. The infected plant material of sugarcane and maize were maintained in dehydrated and frozen at -20oC plant tissue. Isolates SCMV "PIR-2", "RIB-1" and "M-Campinas" were transmitted by mechanical inoculation to sorghum "Rio" seedlings using sodium phosphate buffer 0.01 M, pH 7.2, 0.1 % of sodium sulphite; symptoms of infection became evident about six weeks after inoculation, and total RNA was extracted using Trizol reagent. Amplification of the viral genome, was firstly targeted by synthesizing a cDNA template with a oligo dT and reverse transcriptase (RT), followed by a regular PCR. Fragments of 1210, 1274 nt corresponding to the target proteins were amplified with primer pairs 5UTR+HCP1R and HCP1R+HCP3R respectively, designed in this work. The products were properly identified, aliquot and analyzed by electrophoresis on 1.2% agarose gel. After identification of the target fragments under UV light, respective bands were excised from the gel, purified and sequenced. Nucleotide sequences were analyzed and the consensuses were submitted to BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) algorithm for comparison to homologous sequences. The generated sequences and the obtained from GenBank were aligned using the BioEdit program. Analyses of the sequences confirmed that the designed primers allowed the amplification of the viral genome corresponding to proteins P1 and HC-Pro. Sequences were deposited in "GenBank" providing information generated in the project to the scientific community. Multiple alignments and phylogenetic analysis by Neighbor Joining, based on P1 and HCPro sequences of different SCMV isolates, showed that Brazilian isolates studied in this work showed higher sequence identity and common origin with Australian and Argentinean isolates. Financial support: FAPESP (BIOEN 2008/56146-5). FCB was recipient of a CNPq PIBIC fellowship.

PIV190 - MOLECULAR CHARACTERIZATION OF A NOVEL SIDA-INFECTING BEGOMOVIRUS FROM SOUTHERN BRAZIL

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Begomoviruses (whitefly-transmitted, single-stranded DNA plant viruses) are among the most damaging pathogens causing epidemics in economically important crops worldwide. The incidence of begomovirus infections in crops increased in Brazil during the 1990s following the introduction of Bemisia tabaci Middle East-Asia Minor 1 (MEAM1, previously Bemisia tabaci biotype B). It is believed that MEAM1 transmitted begomoviruses from non-cultivated plants to crops with greater efficiency than the indigenous B. tabaci species. Non-cultivated plants harbor many begomoviruses, and it is believed that these hosts may act as mixing vessels where recombination may occur resulting in novel species adapted to new hosts. In this study we molecularly characterized a novel begomovirus infecting Sida sp. plants showing typical symptoms of begomovirus infection, collected in the state of Rio Grande do Sul in March 2010. The viral genome was amplified by rolling-circle amplification (RCA), cloned and sequenced. The DNA-A is 2601 nucleotides (nt) long and has a genomic organization similar to those of other begomoviruses, except for the presence of an additional open reading frame (ORF) in the complementary strand (AC5). Pairwise sequence comparisons indicated a maximum nucleotide sequence identity of 81.5% with Tomato dwarf leaf virus (ToDLV, GenBank access number JN564749). Phylogenetic analysis placed this isolate in a monophyletic cluster with other begomoviruses from the Americas. No reliable recombination events were detected by the RDP4 program. Therefore, based on the criteria established by the International Committee on Taxonomy of Viruses, this isolate represents a novel begomovirus species for which the name Sida chlorotic mottle virus (SiCMoV) is proposed. The continuing detection of new begomovirus species highlights the remarkable genetic diversity of this group of viruses, as

well as the power of the RCA technique in assessing this diversity. FINANCIAL SUPPORT: CAPES, CNPQ, FAPEMIG

PIV215 - EXPRESSION OF REPLICATION COMPLEX DOMAINS OF TOMATO BLISTERING MOSAIC VIRUS (TOBMV) IN ESCHERICHIA COLI

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Tomato blistering mosaic virus is a tymovirus member and possesses a single-stranded RNA genome in positive polarity. Tymovirus infection causes small vesicles along the chloroplast periphery. These vesicles in chloroplast were shown to be the replication site, forming viral replication complex (VRC). Despite knowing the replication site, little is known about the tymovirus replication mechanism. In order to elucidate the replication process, the overall aim of this project is antibody production for the major replication complex proteins as metyltransferase, helicase and RNA dependent RNA polymerase (RdRp). For this purpose, the expression of domains of these three proteins was performed using Escherichia coli system via Gateway technology (Invitrogen). The three domains were cloned in the entry plasmid pENTR2B and recombined to destination plasmid pDEST17. E. coli BL21AI was transformed with selected clones confirmed by sequencing. The protein expression of RdRp, Helicase and Metyltransferase at 2 and 4 hours post-induction was confirmed by Western blotting using antibody against HisTag. These results confirmed that the system chosen for protein expression is effective. The improved solubility of these proteins is ongoing interest at moment. Financial support: CNPq.

PIV232 - MOLECULAR CHARACTERIZATION OF BACULOVIRUS IDENTIFIED FROM CULEX QUINQUEFASCIATUS LARVAE IN SÃO PAULO STATE, BRAZIL

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Some mosquito species are humans and animals pathogens vectors, such malaria, encephalitis, dengue, yellow fever, and heartworm disease. A broad spectrum larvicides and adulticides have been used to combat these insects. However, the environmental harmful effect and the drug-resistance rate increasing in mosquitoes population forces new vector-control approach development. The baculovirus use could be an alternative to chemical methods. Culex quinquefasciatus baculoviruses were identified in São Paulo, Brazil. The objective was to perform parcial molecular characterization of helicase, polymerase, odv-e27, vlf-1 and lef-8 baculovirus genes from São Paulo (Brazil). The Samples DNA was amplified, the sequences were aligned, using ClustalX 2.0, with partial sequences of helicase, polymerase, odv-e27, vlf-1, and lef-8 from reference baculoviruses genes of other insect orders (Lepidoptera and Hymenoptera) deposited in GenBank database. Phylogenetic analysis was performed. Previous genes analysis results showed that the viruses found in the six larvae in this study showed 98% similarity with Culex nigripalpus NPV, the only species from Deltabaculovirus genus previously described. This specie shows dipteran infection and high mortality, showing remarkable potential as biological control. FINANCIAL SUPPORT: 2012/23947-0

PIV299 - BOMBYX MORI COLON RESISTANCE TO ALPHABACULOVIRUS

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Caterpillars of Bombyx mori (Lepidoptera, Bombycidae) build a cocoon of silk, from which is extracted yarn, used in the production of various tissues. This productive activity, known as sericulture, presents a major economic, social and environmental impact in the state of Paraná. However, the same can be affected by nuclear polyhedrosis disease, caused by Bombyx mori nucleopolyhedrovirus (BmNPV), an entomopathogenic virus (Baculoviridae, Alphabaculovirus), which infects B. mori, compromising the production of cocoons and causing damage to the productive chain. Studies have demonstrated that BmNPV is poliorganotrophic and various are the target-organs; however, some have shown to be resilient to pathogens and the present study aims to analyze the behavior of the colon region, in the hindgut, of B. mori to BmNPV, providing support to the establishment of the viral infectious cycle. The colon is an important region of the hindgut that acts in the absorption of water and salts and in the formation and elimination of fecal pellets in the insect. Caterpillars of B. mori 5th instar were inoculated with a viral suspension of BmNPV, and 2 to 9 day post inoculation (dpi) colon segments were processed for light microscopy. The sections obtained were stained by the cytochemical technique Azan, for viral identification. The analysis revealed that colon cells were not susceptible to BmNPV, at all times examined. However, viral occlusion bodies were observed in adipose, tracheal tissue and into the extracellular medium, indicating the viability of the inoculum used. Thus, despite the resistance to BmNPV, infection of other targets potentially compromises the metabolic function of the hindgut, on absorption of water and minerals and in the formation and elimination of feces.

PIV300 - CYTOPATHOLOGY OF THE TRACHEAL SYSTEM OF BOMBYX MORI (LEPIDOPTERA:BOMBYCIDAE) CAUSED BY ALPHABACULOVIRUS

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Bombyx mori is an insect of the order Lepidoptera that is only found in germplasm banks; it is used in scientific research and for commercial purposes. In this case, the silk cocoon, which is produced at the end of the 5th larval instar, is used in the production of various yarns and fabrics, constituting the sericicola industry. One factor that affects the national sericulture, compromising the commercial production of cocoon, is a nuclear polyhedrosis disease caused by the virus from the Baculoviridae family, Bombyx mori multiple nucleopolyhedrovirus (BmMNPV), genus Alphabaculovirus (AlphaBV. Studies have proved that BmMNPV is polyorganotropic and there are several target organs, such as the tracheal system; however, details of its cytopathology aren´t known. The tracheal system is responsible for the aeration of the tissues of the insect. The study described the cytopathology of the tracheas of hybrid larvae of B. mori, infected experimentally with BmMNPV, isolated geographically in the state of Paraná. Fifth instar hybrid larvae were divided into two groups; one control, and the other inoculated. After ingestion, and on different days post-inoculation (dpi), from the 2nd to the 9th dpi, the larvae were anesthetized and dissected. Segments of organs containing branches of the trachea, were collected and fixed in Karnovsky modified for transmission electron microscopy. On the 2 st dpi, fresh hemolymph analysis was conducted to determine the susceptibility of the hemocytes. The results revealed that the hemocytes were infected from the 2 nd dpi and the epithelial cells of the trachea were infected from the 4th dpi. The cytopathology of the tracheal cells showed hypertrophic nucleus, containing the viroplasm, the site of the synthesis of the nucleocapsids. Subsequently, the formation and development of the polyhedra occured, accentuating the nuclear hypertrophy and culminating in cell lysis. Virions were also observed, immersed in the basal lamina of the trachea, which appeared to be disorganized. Thus, the cytopathology of the trachea was consistent with the infection caused by AlphaBV, and the data that was obtained provides a better understanding of the infectious cycle of BmMNPV in the body of the insect. The time of infection, later for the hemocytes, and the presence of virions in the basal lamina of the trachea, indicated that this system is a secondary target for

infection, and also that the hemolymph is an important dispersant of viral infection.

PIV338 - GENOME ANALYSIS OF AN ATYPICAL ISOLATE OF LETTUCE MOSAIC VIRUS (LMV)

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Virus diseases are considered a key challenge for the production of lettuce (Lactuca sativa L.) in Brazil, where the losses in infected plants can reach 100%, depending on weather conditions and cultivars. The Lettuce mosaic virus (LMV) is currently considered the most important virus infecting lettuce in Brazil, and the symptoms observed in infected plants are mosaic, leaf distortion and even death of the more susceptible cultivars. In this study, an isolate of LMV, named LMV-Cf, was partially sequenced and analyzed in order to investigate the genomic variations which could potentially be linked to the induction of atypical symptoms in infected lettuce plants cv. Regina 579, characterized by closure of the head. For genome amplification by RT-PCR, the primers were designed based on the nucleotide sequences of LMV isolates, available in the GenBank, and the amplified genome fragments were purified and sent for sequencing. The 5'UTR, P1, HC-Pro, P3, 6K1, CI, 6K2, NIa VPg, CP and 3'UTR regions were completely sequenced and analyzed. Among those sequences, two regions showed significant changes: the first one was found in the LMV-Cf P3 protein, which showed a deletion of three nucleotides, resulting in the exclusion of a glutamic acid in the C-terminal of the protein. The comparison of the LMV-Cf P3 nucleotide sequence with the similar genomic region of other LMV isolates, available in the GenBank, ranged from 93% to 97%, which was very similar to those observed among the LMV isolates used for comparison. However, there was a deletion of one amino acid in the LMV-CF sequence, positioning it in a separate branch of the phylogenetic tree. Another difference was found in the nucleotide sequence of 6K2 protein coding-gene, which usually is highly conserved among the LMV isolates from the database, showing 100% identity. The 6K2 nucleotide sequence of LMV-Cf showed only 92% identity when compared with those LMV isolates. Since there are several evidences of the involvement of P3 and 6K2 proteins in the symptoms expression by host plants, they are considered candidates to be investigated regarding their probable correlation with the atypical symptoms of closed head showed by the infected plant. Site-directed mutagenesis and protein expression in host plants are required to get this information. FINANCIAL SUPPORT: CAPES, CNPQ, FAPEMIG

PIV342 - CIRCULAR SSDNA SATELLITES ASSOCIATED WITH BEGOMOVIRUSES IN THE AMERICAS

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Begomoviruses (genus Begomovirus, family Geminiviridae) are plant ssDNA viruses that are transmitted by the whitefly Bemisia tabaci. Begomoviruses cause serious diseases in economically important crops in tropical and subtropical regions. Two types of circular ssDNA satellites half the size of the helper virus components have been described: betasatellites and alphasatellites. Betasatellites are associated with monopartite begomoviruses from the Old World and are dependent on them for replication, movement in plants and transmission. Betasatellites consist of an A-rich region, a non-coding satellite conserved region and a single ORF coding for a multifunctional protein. Alphasatellites, also typically associated with Old World begomoviruses, contain a single ORF coding for a replication-associated protein with similarity to those of nanoviruses and an A-rich region. Unlike typical satellites, alphasatellites are capable of self-replication in host plants but require a begomovirus for movement within the plant and for insect transmission. In screening symptomatic non-cultivated plants collected in Brazil and Cuba for the

presence of begomoviruses by rolling circle amplification (RCA), we identified two alphasatellites and a novel class of ssDNA satellites half the size of betasatellites and alphasatellites. The alphasatellites were amplified from Euphorbia heterophylla and Sida sp. plants sampled in Chapada (state of Rio Grande do Sul, Brazil) infected by the bipartite begomovirus Euphorbia yellow mosaic virus and were found to be phylogenetically related to alphasatellites recently reported from the state of Mato Grosso do Sul (Brazil), Cuba and Venezuela. The small ssDNA satellites were found associated with bipartite begomoviruses infecting malvaceous species in Cuba. They do not possess any ORFs, contain an A-rich region, and share a short conserved region with betasatellites. Our results extend the diversity and geographical range of ssDNA satellites associated to bipartite begomoviruses in the Americas, suggesting that they may be widespread in the continent. Financial support: CAPES, CNPq (fellowship Pesquisador Visitante Especial to JNC) and FAPEMIG (fellowship Pesquisador Visitante to EFO).

PIV344 - FAUNA STUDY AND VIRUS ISOLATION ATTEMPTS IN CULICIDAE CAUGHT IN THE MOCAMBO AREA, EMBRAPA, BELEM, PARÁ, BRAZIL

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The Culicidae family insects are flies belonging to the Insecta class, Diptera order, suborder Nematocera and Culicomorpha infraorder, also known as mosquitoes. These insects transmit disease agents in humans, some of which can cause high mortality and morbidity of endemic or epidemic form.Material and Methods: Four excursions were conducted in the Mocambo forest, EMBRAPA, Belém city, Pará state, Brazil (August and October 2013, January and March 2014), lasting five days each. The arthropods collection was conducted by protected and enlightened human attraction in soil and canopy modalities. Arthropods were identified and separated in inoculation groups and stored at -70 ° C freezer. The groups were inoculated in newborns albino Swiss mice, and alternatively in VERO and C6/36 cells. Results: In the excursions it was collected 2.772 culicidae, divided into 185 groups for virus isolation attempted. In total 12 genera with 36 species of Mosquitoes were identified. Among the identified species were considered,as follows. Constants during the excursion time: Aedes hortator and Aedes serratus, among others; some of the Accessory: Johnbelkinia longipes, Limatus durhamii; Accidental: Aedes fulvus, Aedes taeniorrhynchus, Aedes oligopistus, among others. The species caught, Coquillettidea venezuelensis were classified as Eudominant; as Dominant: Coquillettidea arribalzagae, as Subdominant had Aedes serratus; Eventually: Mansonia titillans species; Finally had some Rare species like Sabethes belisarioi, Coquillettidea albicosta and Aedes fulvus. Groups of inoculated insects into newborn mice tested shows negative. However there was viral isolation of a new arbovirus of the Bunyaviridae family, Orthobunyavirus genus, a group of Aedes fulvus (strain AR800584) inoculated into C6/36 cells. Conclusion: Important arboviruses vectors were collected as Sabethes belisarioi, Coquillettidea venezuelensis, Haemagogus janthinomys, Sabethes glaucodaemon, Sabethes cloropterus, among others. A new arbovírus was isolated belonging to the Bunyaviridae family, Orthobunyavirus genus. The study demonstrates the need to conduct further research into the Mocambo forest, emphasizing the arboviruses main vector species, entomological monitoring and performing viral isolation attempts. Financial support: PIBIC/CNPQ/IEC

PIV360 - NEXT-GENERATION SEQUENCING APPLIED FOR IDENTIFICATION OF VIRUSES IN WATERMELON (CITRULLUS LANATUS) PLANTS

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The culture of watermelon (Citrullus lanatus Thunb) belongs to the Cucurbitaceae family and it is susceptible to several viral pathogens. These viral infections do not cause obvious symptoms in its host plants and, frequently,

the viral particles occurs in low concentrations. However, the capacity to analyze asymptomatic viral infections or coinfections increased with the appearance of high throughput sequencing. Thus, this study was designed to identify viruses present in symptomatic watermelon plants. The watermelon leaves with viral symptoms were collected from September to December 2013 in three producing regions of Tocantins state. The plant material was subjected to semi-purification of the viruses using centrifugation through a sucrose cushion. Total RNA was extracted from this viral enriched fraction using "RNeasy Plant Mini Kit" (Qiagen). Next, the RNAseq library was prepared and sequenced by Illumina HiSeq 2000 platform. Forty million reads were generated, and after quality trimming and de novo assembly using CLC Genomics Workbench software, nearly fifty thousand contigs were obtained. All contigs were submitted to blastx against the viral RefSeq database and 4,912 contigs produced hits with viral sequences. To further confirm these results, we submitted these selected contigs to blastx against the GenBank non-reduntant database (nr database), and only the plant viruses were selected. We were able to identify viral genomes belonging to the families Potyviridae, Partitiviridae and Bunyaviridae. The largest contig (10,371 bp) showed 91% identity with Papaya ringspot virus. One contig of 1,704 bp contig presented 98% identity with Zucchini yellow mosaic virus. We also found two contigs of 1,575 and 1,606 bp related to the segments 1 and 2 of Partitiviridae. Interestingly, these contigs presented an overall identity of 60%, suggesting these contigs represent a new viral species belonging to the Partitiviridae family. Moreover, we found highly divergent contigs (6,596 and 2,674 bp) related to Bunyaviridae L and S segment, respectively. Overall, we were able to identify viruses already described infecting watermelon as well as novel viral species that are currently been characterized in our laboratory.

PIV366 - DETECTION OF POTATO VIRUS Y (PVY) STRAINS IN PLANTS WITH SINGLE AND MIXED INFECTION

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Potato virus Y (PVY) is currently one of the most important viruses in potato worldwide, mainly due to its high field dissemination capacity and the constant appearance of new genetic variants. The detection and field management of PVY has been increasingly complex. Among the mechanisms responsible for the genomic variability, the recombination is the main one, probably due to the occurrence of mixed infections in the field. In this study, the DAS and TAS-ELISA and RT-PCR multiplex technique were compared, aiming at determining its efficiency for diagnosing and discriminating the main strains present in Brazil (PVYO, PVYN-Wi and PVYNTN), in single and mixed infections. In order to get plants with single and mixed infections, Nicotiana tabacum cv. Turkish plants were inoculated with these strains, individually and in combination. In addition, probes were developed for detection and quantification of these strains by real time PCR (qPCR). Willing to detect mixed infections in the field, ninety potato tubers PVY infected were collected and analyzed by DAS and TAS-Elisa and RT-PCR multiplex. The N. tabacum plants inoculated with the different strains reacted with the expected symptoms, ranging from light mosaic to necrosis, according to the strain. However, in all combinations in which the PVYO was inoculated with the necrotic strains, the plants did not present the expected vein necrosis, indicating a predominance of the symptoms induced by the common strain. The serological tests revealed a tendency of higher concentration of strains with O serology (PVYO and PVYN-Wi) when N. tabacum presented mixed infections. The RT-PCR was capable of discriminating the tree strains, presenting the patterns of bands specific to each of them. Among the 90 tubers tested 5 were negative, 11 were positive for the PVYNTN, and 74 were positive for the common and/or Wilga strain, with 8 being positive for mixed infections. The occurrence of mixed infections and highly favorable climate for the multiplication and dissemination of the PVY could explain the high genomic variability of this virus derived from recombination among virus isolates. The probes and primers designed were effective in discriminating each strain by qPCR, either in single or mixed infections. As qPCR presents a higher sensitivity, the designed probes revealed a good potential to be employed for the detection and quantification of these

strains in infected potato plants.FINANCIAL SUPPORT: Capes, CNPq, Fapemig

PIV377 - DEVELOPMENT OF MOLECULAR TOOLS TO STUDY TOSPOVIRUS REVERSE GENETICS

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The use of methods based on recombinant DNA technology such as reverse genetics (RG) to study the role of viral gene products in inducing disease has increased substantially our knowledge of the complex steps and interactions involved in the infectious cycle of plant viruses. Tospovirus [(-) ssRNA], are worldwide spread pathogens mainly of vegetable, causing severe economical losses, to which a RG system has not been developed yet. Currently, our research line focuses on the development of both a mini-genome approach and a classical full-length infectious clone strategy towards tospovirus RG. This work presents the development of highly specific and sensitive ribo-probes generated to support our RG research. First, primers for RT-PCR amplification were synthesized for specific RNA regions of the L, M (NSm gene) and S (NSs and N genes) segments of tomato spotted wilt virus (TSWV). Subsequently, total RNA was obtained from Datura stramonium plants infected with TSWV (BR-01 isolate) using TRIzol reagent (Invitrogen) followed by RT reaction with SuperScriptIII (Invitrogen) and standard PCR. The amplicons were cloned in the vector pGEM-T easy (Promega) and positive colonies were selected by standard restriction enzyme digestion of extracted plasmid DNA and automated Sanger sequencing. Ribo-probes were labeled with digoxigenin (Dig RNA labeling mix, Roche) by in vitro transcription with T7 and SP6 RNA polymerases according to manufacturer's specifications. The detection sensitivity of the probes was measured by dot-blot analysis of serial dilutions of total RNA extracted from leaf tissue of Nicotiana benthamiana and D. stramonium infected with TSWV and GRSV (Groundnut ringspot virus) and healthy plants. Probes showed high specificity to TSWV and in general limit of detection was 20 ng of total RNA. Therefore, molecular ribo-probes were produced for each segment and strand orientation of TSWV which will support to distinguish between introduced RNA transcription and that derived from viral replication complex of RG system. This work is essential in our RG research strategies and can provide important data regarding strand and segment specific accumulation and replication of the viral genome. This information is necessary to place in time the many events that are part of the infectious process, such as cell-to-cell infection, systemic infection, accumulation and activity of defective-interfering RNAs and their effects over the other viral genomic segments.

PIV385 - MOLECULAR ANALYSIS OF SQUASH MOSAIC VIRUS (SQMV) ISOLATES FROM TOCANTINS - BRAZIL

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Several viruses can affect cucurbits causing considerable losses in quality and quantity of production. High incidences of the mosaic caused by Squash mosaic virus (SqMV) have been detected in the field of some Brazilian regions, and those incidences depend on numerous factors such as temperature, field location and vectors. In this study the coat protein gene of fourteen Brazilian isolates of Squash mosaic virus (SqMV) collected in Tocantins state, named PTY1, PTY2, PTY4, PTY5, PTY10, PTY12, PTY14, PTY15, FA, GR2, LC2, LC3, PN1 and PN2, were amplified by RT-PCR, analyzed and compared with other isolates available in the GenBank. The identity between the nucleotides of Brazilian SqMV isolates ranged from 86% to 100%, while the identity of these isolates with SqMV isolates from the GenBank varied between 86% and 88%. The lowest identity (87%) was observed among the isolates AB054689 from Japan and PTY1 PTY10, PTY12, PTY14, PTY15, FA1, GR2 and LC2, and also among the PN2 and the SqMV isolates from the database. The amino acid identities of the Brazilian isolates ranged from 95%, among the isolate PN2 and all the other isolates, to 100% among PTY1 and PTY10, PTY12, PTY14, PTY15, FA1, GR2 and LC2 isolates; PTY2 and PTY5; LC3 and PN1. When compared with isolates from the GenBank, identities ranged from 91% (between isolated PN2 and DQ868881.1 and EU421060.1, both

Chinese isolates, and the American isolate AF059533.1) to 97% (among several isolates). The phylogenetic tree based on the nucleotide sequence showed two major clusters, one containing the Brazilian isolates PTY2, LC3, PTY5, PN1, PN2 and PTY4 and the SqMV isolates from the database, and the second group composed by the remaining Brazilian isolates. However it changed when the phylogenetic tree was constructed based on the sequence of amino acids. Several Brazilian isolates showing nucleotide substitutions resulted in amino acid changes, characterizing the nom synonymous substitutions type and confirming the large variability existing among Brazilian SqMV isolates. To confirm this variability, genomic studies of SqMV isolates should also include their RNA2 fragment, in order to provide the necessary support for breeding programs which aim to develop SqMV resistant cucurbits plants. FINANCIAL SUPPORT: CAPES, CNPQ, FAPEMIG

PIV398 - NOVEL VIRAL GENUS AND VIRAL SPECIES INFECTING FORAGE PLANTS IN BRASIL

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Panicum sp., Brachiaria sp and Stylosanthes sp. showing mosaic symptoms on leaves and growth reduction were collected in the State of Mato Grosso do Sul in the experimental field of Embrapa Beef Cattle. To obtain a viral enriched fraction, the leaves were ground in PBS buffer, filtred and centrifuged through a sucrose cushion. Viral RNA was extracted using RNeasy Mini Kit following the manufacturer's instructions. The RNA samples were pooled and sequenced at Macrogen INc. (Korea) using Illumina HiSeq 2000 technology. We obtained approximately 20,299,626 of reads, which were trimmed and assembled de novo using CLC Genomics Workbench 7.0. The assembled contigs (3,254) were submitted to blastx against the RefSeq Viral database and the contigs related to plant viruses were selected. We were able to identify complete genomes of viruses from some plant virus families: Potyviridae, Secoviridae and Tymoviridae. Among Potyviridae, we identified contigs related to Johnsongrass mosaic virus and two highly divergent genomes related to Rose yellow mosaic and Blackberry virus Y, which probably constitute two novel genera within Potiviridae family. Moreover, we found genomes related to Maize chlorotic dwarf virus (Seconviridae) and to a novel species/genus in the family Tymoviridade. We design specific primers to detect each virus in the surveyed hosts Panicum sp., Brachiaria sp and Stylosanthes sp. Johnsongrass mosaic virus, Rose yellow mosaic virus, Blackberry virus Y and Maize chlorotic dwarf virus were already amplified back by RT-PCR Host range and phylogenetic analysis are being conducted for further characterization of these viruses. FINANCIAL SUPPORT: CNPQ/REDE PRO-CENTRO-OESTE, FAP-DF, CAPES

PIV406 - PARTIAL CHARACTERIZATION OF BEGOMOVIRUS STRAIN FROM CLITORIA FAIRCHILDIANA ON RIO DE JANEIRO STATE, BRAZIL

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The Sombreiro (Clitoria fairchildiana) is a plant used in urban landscaping and for medicinal purposes (by extracting rotenoids from seeds that has anti-inflammatory activity). The objective of this study was to identify the viral genus associated with mosaic in leaves of C. fairchildiana, existing in Rio de Janeiro. Mechanical inoculation for Chenopodium amaranticolor were performed in sodium phosphate buffer pH 7.5 containing 0.1% sodium sulfite at dilutions 1:5, 1:10 and 1:20; transmission by grafting cuttings of diseased to healthy plant; transmission by seed originating from diseased plant; PCR test with primers for Begomovirus. Negative results were obtained in the mechanical inoculation and transmission by seed, however, positive results have occurred in transmission by grafting and in the PCR test. This is the first occurrence in the world of Begomovirus on this plant species.

PIV435 - IDENTIFICATION OF THE P26 GENE AND ITS EVOLUTION IN THE BACULOVIRIDAE FAMILY

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The family Baculoviridae comprises a group of arthropods-specific viruses with circular double-stranded DNA. Baculoviruses exhibit rod-shaped nucleocapsids embedded in a crystalline protein matrix composed of polyhedrin in nucleopolyhedroviruses (NPVs) and granulin in granuloviruses (GVs). The Baculoviridae family is composed of genera Alphabaculovirus (lepidopteran-specific NPVs), Betabaculovirus (lepidopteran-specific GVs), Gammabaculovirus (hymenopteran-specific NPVs) and Deltabaculovirus (dipteran-specific NPVs). Alphabaculoviruses are also divided into Groups I and II based on their envelope fusion proteins GP64 and F, respectively. The p26 gene is present in all Group I and II NPVs. However, the majority of the virus with more than one p26 copy belongs to Group II NPVs. The function of the p26 gene is not well-defined, but p26 is thought to be required for optimal virion occlusion in the polyhedron. The p26 gene was identified within NPVs genomes so far sequenced and was used bioinformatics tools in order to characterize the P26 protein and elucidate the evolution this gene in Baculoviridae family. The p26 copies found in baculoviruses are conserved in position, adjacent to the p10 gene (P1) in all the NPVs containing a single copy, adjacent to iap-2 gene (P2) in all Group II NPVs containing the second p26 copy and adjacent to the ptp1 and ptp2 genes (P3) in Group I NPVs with the second p26 copy. The isoelectric point and molecular weight of the deduced P26 protein was calculated and is presented in the context of their genomic positioning. The Bayesian phylogenetic tree obtained from the p26 copies found in NPVs genomes showed four clearly defined clades (IA, IB, IIA and IIB) supporting the hypothesis for the occurrence of three independent capture events of the p26 gene by baculoviruses. The presence and location of signal peptide cleavage sites in P26 amino acid sequences was analysed and only in the clade IB was found signal peptide. Although the function of P26 is not well understood, the signal peptide may lead to differences in activity of the clade IB proteins indicating a possible distinct function from other classes of P26. However, further investigations are needed for a better understanding of this protein in baculoviruses. FINANCIAL SUPPORT: Capes/Embrapa

PIV446 - GENOME SEQUENCING OF TWO POTEXVIRUS INFECTING PRICKLY PEAR (OPUNTIA COCHENILLIFERA)

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Viruses from the genus Potexvirus are widely spread in different genera of the family Cactaceae. Prickly Pear (Opuntia cochenillifera) plants collected in Pernambuco State were propagated and cultivated in the green house. RNA from symptomless plants was extracted from 45 plants and used for whole transcriptome shotgun sequencing, using an Illumina Hi-seq 2000 platform, aiming to study water stress related genes. De novo assembly of the reads was made with VELVET program and several contigs were found to be potexvirus-derived. To further characterize these virus sequences, we used Geneious software to perform Blastn searches. Comparisons of the nucleotide (nt) and the predicted amino acids (aa) sequences with other potexvirus members were made with SDT and CLUSTALW programs. Two distinct potexviruses genomes were identified. One genome comprises 6.636 bp and was identified as Schlumbergera virus X (SchVX), with a nt identity of 94% for the entire genome sequence. The identities for the nt sequences of the polymerase and CP genes are 93% and 94%, respectively. The predicted aa sequences for the polymerase and CP genes share 97% identity with SchVX. The other assembled genome (OcPotex) is 6.664 bp. The nt sequence of the entire genome has highest identities of 73% to 74% with different strains of Cactus virus X (CVX) and Zygocactus virus X (ZyVX). The nt and aa sequences of OcPotex polymerase and CP genes also share similar identities with those of CVX

and ZyVX, requiring additional studies for a conclusive taxonomic assignment of OcPotex. FINANCIAL SUPPORT: EMBRAPA, CNPq, FAP-DF

PIV465 - INVOLVEMENT OF TMV RESPONSE RELATED-PROTEIN IN THE DEFENSE OF TOMATO TO TOMATO CHLOROTIC MOTTLE VIRUS

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Begomoviruses currently represent one of the most serious problems for the tomato cultivation worldwide. In Brazil, breeding for resistance to bipartite tomato begomoviruses allowed to the development of the resistant line 'LAM-157' (carrying tcm-1 locus) which is a near-isogenic line to the susceptible Santa Clara cultivar. To study the interaction and the differential genotype response to Tomato chlorotic mottle virus (ToCMoV), a transcriptomic analysis using RNA sequencing (RNAseq) was carried out. Several genes showed differential expression in resistant 'LAM-157' plants inoculated with ToCMoV. One of these genes, the TMV response-related protein (TMV-RRP) gene, putatively involved in plant response to Tobacco mosaic virus infection, showed significant up-regulation (log2 fold change > 2.5). RNAseq results were confirmed by qPCR over a time course of virus infection in 'LAM-157', highlighting the possible involvement of TMV-RRP in the process of tomato defense/resistance to ToCMoV. To validate this hypothesis in planta, LAM-157- derived TMV-RRP gene was cloned and transferred to Nicotiana benthamiana plants by Agrobacterium transformation. Transgenic plants will be inoculated with ToCMoV to check for the influence of TMV-RRP super-expression in the disease phenotype and virus accumulation. FINANCIAL SUPPORT: EMBRAPA, CNPQ, INCTIPP, FAPDF.

PIV466 - IN SILICO ANALYSIS OF BACULOVIRUS CORE GENE PROMOTERS

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The baculoviruses are large dsDNA insect viruses with a high number of described species infecting several hosts, classified in four genus, the Alphabaculovirus, Betabaculovirus, Gammabaculovirus and Deltabaculovirus. To date, 61 species have been completely sequenced and are publicly available in GenBank. The analysis of these genomes revealed 37 core genes shared by all of them. These core genes participate in basic biological functions, such as RNA transcription, DNA replication and the formation of the virion structure. Moreover, most baculovirus genes can be classified in two groups: those transcribed by cellular RNApol II (early genes) and those transcribed by viral RNApol (late genes). Recently, the AcMNPV transcriptome was published and the transcription start sites (TSS) for its ORFs were mapped and putative motifs were described. However, it remains to be determined if the observed pattern is conserved through baculovirus evolutionary lineages. Therefore, we performed an in silico analysis of baculovirus core gene putative promoters. Firstly, the core genes (+ 1000 bp upstream of ATG) from all Alphabaculovirus and Betabaculovirus (57) was extracted and classified in early, early/late and late (6,4 and 27, respectively) according to AcMNPV data. The predicted TSS were manually annotated for all late genes (TAAG motif). Subsequently, we calculated the distance of TSS to ATG and the variation was higher for Alphabaculovirus than for Betabaculovirus. Moreover, the distance variation was higher for those genes with TSS that does not overlap with another ORF. We screened the early promoter for known insect transcription bactor binding sites (TFBS). We found two recurrent TFBS: HSF and Dfd, both TFBS described in insects. Finally, we analyzed the early data set in MEME and CentriMo, and we found different motif for each early core gene, thus, we suggest that the early promoters are more complex and variable than late promoters and other news motifs play an important role in transcription regulation in addition to TATAA box motif. Overall, the late promoter was more conserved than early promoters, suggesting that the promoters of genes transcribed by host RNA polymerase are adapted to the host gene regulatory network. Financial suport: CNPq

PIV471 - SEQUENCING OF NEW POLEROVIRUS COTTON VIRUS FROM BRAZIL USING SMALL RNA DATASET OBTAINED BY DEEP-SEQUENCING

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In a previous work, our group identified three recombinants virus isolates infecting cotton plants in Brazil which symptoms and transmission mechanism are closely related with the Cotton blue disease (CBD). CBD is an important cotton crop pathology present in America, Africa and Asia that causes significant economic losses. In Brazil it obligate the cotton farmers to plant only CBD resistant cultivars now days. The disease is transmitted by Aphis gossypii and is caused by a Luteoviridae virus, genus Polerovirus, the Cotton leaf roll dwarf virus (CLRDV). Typical symptoms include stunting due to internodal shortening, leaf rolling, intense green foliage. The isolates identified in this previous works, using CLRDV molecular diagnosis, were recovered from plants showing CBD atypical symptoms. Analyses of a partial polymerase sequence of these virus isolates showed that they presented low identity with CLRDV and can be considered as representatives of a new species of the genus Polerovirus. In orther to better characterize this new species, named Cotton red leaf virus (CoRLV), we submitted the isolate CoRLV-P01 to a deep sequencing by "Illumina". All small RNAs of an infected plant were sequenced and "contigs" were constructed. However, the contigs weren't able to reconstruct the viral genome. So, a new analysis of the small RNA data set libraries was performed using the software SearchSmallRNA (Andrade & Vaslin, 2014). Using CLRDV Brazilian and Argentine in independent analysis as reference genome, we were able to obtain 69% of the complete virus genome. With this new analysis, the 3' block of this putative polerovirus genome was almost completely recovered. Only a small portion of the 3'UTR region is absent. The complete CP, MP and RT proteins sequences are almost identical of those of the CLRDV isolates. However, the intergenic region and the almost complete polymerase (P2) show identities ranging from 73-82% with CLRDV. P2 amino acid sequence share similar ID levels with Brassica yellow virus, Pepper yellow leaf curl virus and Beet western yellows virus. Primers were design based in the genome reconstruct and were used to RT-PCRs using three virus independent isolates. The nucleotide sequence comparison of the amplicons showed the maximum identity of 73% with CLRDV and 71% with Pepper yellow leaf curl virus. In order to obtain the complete genome sequence of this virus, more primers combinations are been tested. FINANCIAL SUPPORT: FAPERJ AND CAPES

PIV474 - THE PHYLODINAMIC AND PHYLOGEOGRAPHIC OF TOMATO CHLOROTIC SPOT VIRUS: VIRAL SPREAD IN THE AMERICAS

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Viruses in the genus Tospovirus (family Bunyaviridae) are plant pathogens transmitted by insects known as thrips, with a trisegmented single-stranded ambisense RNA (S, M and L) genome and the ability to replicate in vector to. The diversity of the genus comprises 8 recognized species with a variable host range and geographic distribution. The genus Tospovirus are widespread in the Americas, where Tomato chlorotic spot virus (TCSV), Tomato spotted wilt virus (TSWV), Groundnut ringspot virus (GRSV), Chrysanthemum stem necrosis virus (CSNV), Zucchini lethal chlorosis virus (ZLCV), Impatiens necrotic spot virus (INSV), Iris yellow spot virus (IYSV), Alstroemeria necrotic streak virus (ANSV), Melon severe mosaic virus (MeSMV) and Bean necrotic mosaic virus (BeNMV) have been reported. TCSV was first noticed in Brazil in the early 1990's, with GRSV. Nowadays, GRSV is present in both new world and the old world, whereas TCSV is find only in the Americas. A phylodinamic study was conducted to infer the TCSV spread. Thirty-three sequences corresponding to RNA S with coding (N protein) and no-coding regions were downloaded from GenBank. The alignment was carried out using MUSCLE software. The phylogenetic and phylodinamic relationships were inferred by BEAST package using Bayesian Evolutionary Analysis. According to the sampling year, the spatial phylogenetic reconstruction and the dynamic evolution

were determined by SPREAD program. Probably, TCSV has two different origins in South America, one in Brazil (S45325) and the other in Argentina (U49709, U49707), in 1996. Brazilian lineage spread to the country to North America and Caribe. Once in Caribe, it was introduced back in North America. The first introduced in North America most likely in 1994, led to the first reassortant intra-species isolates. This new isolate has the S and L RNAs from GRSV and the M RNA from TCSV. The isolates originated from the second introduction in the North America around the year 2006 are so close related to Caribbean isolates, at least 97% identical. Comparing the first report of TCSV in Brazil with the last (JQ034525) sampled in 2009, the nucleotide sequence accumulated modifications resulting in 93% identity each other. That isolate is more related to Argentine lineage sharing 99% identity, with a clearly well supported phylogenetic reconstruction. There is not an updated information about the strain that are present nowadays in Brazil.

PIV479 - CHARACTERIZATION OF SOYBEAN-INFECTING SIDA MICRANTHA MOSAIC VIRUS

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The occurrence of begomoviruses in soybean (Glycine max) in Brazil has been sporadically reported and has not been associated with yield losses. However, the list of soybean-infecting begomoviruses it is growing, and there is a concern that yield losses may increase, since soybean infecting begomoviruses causing moderate to severe losses has been recently reported in Argentina. This potential threat has intensified the surveys for begomovirus infection in soybean-producing areas. Samples obtained from soybean plants collected at Federal District, showing typical symptoms of begomovirus infection were analyzed. Viral DNA from infected plants was subjected to rolling-circle amplification (RCA), cloning and sequencing. Phylogenetic analyses and sequencing comparisons of the DNA-A and DNA-B clones, confirmed infection by Sida micrantha mosaic virus (SimMV). Cloned DNA-A and DNA-B components were introduced into seedlings of Phaseolus vulgaris 'Pérola', Glycine max 'Conquista' and 'Williams 82', Solanum lycopersicum 'Santa Clara', Nicotiana benthamiana and Sida rhombifolia by a biolistic method to confirm their infective capacity. Four weeks after inoculation, symptoms of begomivrus infection appeared in almost all plants species inoculated, only S. lycopersicum and Phaseolus vulgaris 'Pérola' showed no symptoms and no infection. Leaves from soybean and Sida rhombifolia exhibited yellow and golden mosaic, yellow vein, chlorotic mottling, necrosis, blistering, leaf distortion and dwarfing symptoms; symptoms such as dwarfing, leaf distortion and blistering were observed in N. benthamiana plants. The infection was confirmed by PCR amplification using begomovirus universal primers and sequencing of the obtained amplicon. This is the first report of infectious clones of soybean-infecting SimMV and these clones will be a valuable tool to study soybean-begomovirus interaction.FINANCIAL SUPPORT: EMBRAPA, CNPQ, INCTIPP, FAPDF.

PIV485 - HIGH GEMINIVIRUS-INFECTION ON A POTATO FIELD GROWN FOR SEED PRODUCTION IN BRAZIL CENTRAL

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Virus diseases are one of the main causes of yield losses in potato (Solanum tuberosum L.) production worldwide. The main viruses associated with this crop belong to the genera Potyvirus, Polerovirus, Carlavirus and Potexvirus; however, emerging diseases caused by geminivirus (Family Geminiviridae) transmitted by whitefly (Bemisia tabaci) had been detected affecting this crop over the last years. In July, 2014, a 50-day-old potato field, cvs. Agata, Cupido, Manitou, Faluka, Mustang and Ambition of ca. 50ha showing geminivirus-like symptoms such as strong yellow mosaic, leaf reduction size with the edges turned upwards and stunting of plants, was observed in the State of Goiás, Brazil, one of the most important potato growers in the country. A variable range (2%-40%) of

symptoms incidence was observed depending on the cultivar planted and its position in the field. Forty-eight leaf samples were collected from symptomatic plants and subjected to total DNA extraction for polymerase chain reaction (PCR) using universal primers and rolling circle amplification (RCA) for geminivirus detection, besides restriction fragment length polymorphism (RFLP) to access diversity of the isolates. In addition, sample were also tested by using serological methods (DAS-ELISA) for detection of Potato virus Y (PVY), Potato virus X (PVX), Potato virus S (PVS) and Potato leafroll virus (PLRV) using polyclonal antibodies. All samples did not react with neither of the polyclonal antibodies tested, indicating that none of these viruses was involved in the cause of the symptoms observed in potato plants. However, a band of ca. 1,2 kb was visualized on a 1,2% agarose gel stained with ethidium bromide from the majority of the potato samples collected confirming they were geminivirus-infected. These data are of great concern considering that that potato field was grown for seed production. Also, PCR-positive samples produced an RCA product, indicating the high sensitiveness of the technique. Restriction enzyme profiles suggest the existence of low variability among these geminivirus isolates. Cloning and sequencing of these isolates are in progress. These data indicate that despite the existence of some reports of occurrence of geminivirus in potato fields, however, is it necessary to keep the monitoring of these viruses in the crop, considering the presence of virus sources as well as high whitefly populations in the field and its ability to colonize many plant species.

PIV520 - COTTON MICRORNAS GHR-MIR2910 AND GHR-MIRNA162 ARE UPREGULATED DURING COTTON LEAFROLL DWARF VIRUS (CLRDV) INFECTION

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Small RNAs (sRNAs) are a class of non-coding RNAs ranging from 20- to 40-nucleotides (nts) that are present in most eukaryotic organisms. In plants, sRNAs are involved in the regulation of development, the maintenance of genome stability and the antiviral response. Viruses, however, can interfere with and exploit the silencing-based regulatory networks, causing the deregulation of sRNAs, including small interfering RNAs (siRNAs) and microRNAs (miRNAs). To understand the impact of viral infection on the plant sRNA pathway, we deep sequenced the sRNAs in cotton leaves infected with Cotton leafroll dwarf virus (CLRDV), which is a member of the economically important virus family Luteoviridae. A total of 60 putative conserved cotton miRNAs were identified, including 19 new conserved miRNAs. Some of these miRNAs were clearly misregulated during viral infection, and their possible role in symptom development and disease progression is discussed. Furthermore, we found that the 24-nt heterochromatin-associated siRNAs were quantitatively and qualitatively altered in the infected plant, leading to the reactivation of at least one cotton transposable element. This is the first study to explore the global alterations of sRNAs in virus-infected cotton plants. Our results indicate that some CLRDV-induced symptoms may be correlated with the deregulation of miRNA and/or epigenetic networks. Ghr miR2910 was 7 to 7.5 times more expressed during infection. His role is still unknown, and its possible target would be the 18S ribosomal RNA. Besides him, the GHr miR-171, 157, 172 and 162 expression were also increased in the infection. On the other hand, the miRNA miR319, 393, 3476, 2111, 355, 159 have been drastically reduced in the infection. RT-PCR reactions in real time were carried out and confirmed the differential expression of these different miRNAs. MiRNAs exclusive cotton, as the 2110 and the 3476 are now being studied to try to understand its function. Dysregulation of metabolic pathways regulated by these miRNAs might explain the symptoms observed in plants showing the disease in cotton blue. FINANCIAL SUPPORT: CAPES, CNPQ AND FAPERJ

PIV521 - DETECTION OF TOSPOVIRUSES IN PEANUT ON MAIN PRODUCER AREAS OF THE STATE OF SÃO PAULO, BRAZIL

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The peanut (Arachis hypogaea) is a leguminous plant with a high socioeconomic importance. It constitute a food source rich on protein, vitamin, and minerals, used as cooked or toasted grains and in industrialized products. Furthermore, it has been used for producing cosmetics and biofuels. Worldwide, peanut is produced on tropical and subtropical regions and is considered the fifth leguminous in yield. In Brazil, it is very important, generating income and contributing for sustainability of small, medium and big farms. Despite the high economic relevance, several factors as pests and diseases affect this crop. The diseases caused by viruses, especially those belonging to the genus Tospovirus, may induce drastic yield losses, nearly 100 per cent, and low quality of the product when highly susceptible cultivars are used in presence of high population of thrips vectors. The tospoviruses in peanut induce a range of symptoms from small reduction on plant height to severe stunting, top necrosis, mild to strong mottling, ring spot, as well as plant death in severe cases. The virus infection may also affect the peanut pods and kernels that become stunted, deformed, and discolored. The first report of a tospovirus infecting peanut in the world was done in 1915, in Australia. In Brazil, it was initially found in 1941. The objective of this work was to analyze the occurrence of tospoviruses in the peanut producing areas of the State of São Paulo. Symptomatic and asymptomatic samples in 10 counties were collected and submitted to biological, serological and molecular tests. Virus transmission was obtained by mechanical inoculation and by grafting, being observed typical symptoms on peanut and other indicator hosts. The enzyme-linked immunosorbent assay (ELISA) and ImmunoStrip tests showed positive reaction to three tospovirus species and the RT-PCR determined the presence of the Tomato spotted wilt virus (TSWV), Groundnut ring spot virus (GRSV) e Tomato chlorotic spot virus (TCSV).