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MOLECULAR DETECTION OF HONEY BEE VIRUSES IN APIARIES OF SOUTHERN BRAZIL

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Resumo

Bees are very important insects for agriculture, fulfilling an important role in pollination and renewal of the ecosystem. However, in several countries significant losses of colonies and population decline of honeybees and native bees have been reported, which are influenced by biotic and abiotic factors, including the effects of multiple pathogen infection and/or pesticide exposure. The majority of the viruses that have already been isolated and characterized in bees are classified as positive-sense single-stranded RNA viruses within the order *Picornavirales,* comprising the families *Dicistroviridae* and *Iflaviridae.* Prominent viruses include acute bee paralysis virus (ABPV), deformed wing virus (DWV), black queen cell virus (BQCV), sacbrood bee virus (SBV) and israeli acute bee paralysis (IAPV). Thus, the objective of this study was to detect the main bee viruses in apiaries in southern Brazil. Samples of honeycomb (larva and pupa) and adult bees were collected in the apiaries and kept under refrigeration until transportation to the Laboratory. Immediately the larvae and pupae were homogenized to TRIzol Reagent and frozen at -70 °C. Adult bees collected and stored in closed bottles were directly frozen. Six pupae, larvae and adult bee abdomen were submitted to RNA extraction and cDNA synthesis, followed by two multiplex polymerase chain reaction (PCR) (1: ABPV, CBPV and SBV; 2: BQCV, DWV and IAPV). All cDNA samples were tested with the endogenous control (GAPDH) to verify the efficiency of the whole process. RNA extracted from the bees' *pool* was used as negative control and gBlock® Gene Fragments were used as positive control of honey bee viruses. To date, 75 samples were obtained, mainly from southern Rio Grande do Sul, two of which were positive for IAPV (3,5%), three for ABPV (5,4%) and twenty-five for BQCV (33,3%), totaling 40% (30/75) of positive samples. All detected viruses were obtained from adult bees, and the identity of these viruses was confirmed by nucleotide sequencing. No viruses were detected in samples of larvae and pupae. These results demonstrate that ABPV, BQCV and IAPV viruses are present in apiaries in the South region of Brazil, with a high percentage of positivity for BQCV, and may, together with other factors, contribute for the bee population decline. This study is underway in order to increase the number of samples collected and phylogenetic characterization of the viruses detected.

Financial Support: CAPES.

Palavras-chaves: ABPV, Apis mellifera, BQCV, IAPV, RT-PCR multiplex

BACULOVIRUS INFECTION TRIGGERS DIFFERENT CYTOSOLIC DNA SENSING PATHWAYS IN MAMMALIAN CELLS

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Resumo

The baculovirus AcMNPV is an enveloped virus with a dsDNA genome and is a pathogen of insects. The budded vir capable of transducing genes under the control of an adequate promoter in mammalian cells, although it cannot its genome in this host. Among the applications of baculoviruses as a biotechnological tool in mammals, it mentioning their use for vaccine development, gene delivery and as immunomodulators. BVs induce a strong innate response in mammals that is capable of generating an unspecific antiviral state, independent of TLR pathways. Our reports using a reporter BV showed that the cytoplasm is the main destination reached by their genome in diffe lines. Thus, this work aims to study the role of baculoviral cytoplasmic nucleic acids in the production of an antivira non-immune mammalian cells. We studied the involvement of different cytosolic DNA sensors in murine and hu infected with BV. We evaluated the production of cytokines by qPCR and antiviral activity by protection against s vesicular virus infection. In first place, we demonstrate that RNA Pol III does not participate in the establishme antiviral state. We then studied the cGAS-STING pathway by CRISPR-Cas9 gene editing in murine cells and complementation of cGAS or cGAS and STING in HEK293 and HEK293 T (human epithelial cells), respectively. Th showed that STING was required for the establishment of an antiviral state in mammalian cells. Moreover, at l different signaling pathways had an impact on STING and contributed to the baculovirus induced antiviral st detection of the viral genome by cGAS sensing induced the strongest cellular response and it was necessary production of IFN β. Additionally, the cGAS-independent STING activation produced an antiviral state in human cells where the production of IFN λ1 was involved. In conclusion, the results of this work show that the genom baculovirus AcMNPV has a relevant role in the establishment of an antiviral state and in the production of IFN through its impact on the nucleic acids sensing pathway cGAS-STING.

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Palavras-chaves: BACULOVIRUS, cGAS, INTERFERON, MAMMALS, STING

THE INHIBITORS OF APOPTOSIS GENES LIMITS THE IN VITRO HOST RANGE OF CHRYSODEIXES INCLUDENS NUCLEOPOLYHEDROVIRUS

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Resumo

The larvae of *Chrysodeixes includens* and *Anticarsia gemmatalis* are two important defoliators in soy fields of Brazil. The baculovirus biocontrol agents applied in the fields to control each have low efficacy on the other species and little is known regarding the exact cause of host restriction of each baculovirus. There are many potential barriers to infection *in vivo*, ranging from physical and chemical barriers within the midgut to innate cellular defenses that inhibits viral proliferation and systemic spread in the host. Apoptosis, or programmed cell death, is an efficient method to limit systemic infection by a baculovirus. The viral genes that function as inhibitors of apoptosis (*iap*) evolved to inactivate specific enzymes in the biochemical cascade that leads to apoptosis within the host cell. This makes the *iap* functions crucial to viral proliferation and fitness. In this work we isolated a new *Chin*NPV strain (*Chin*NPV-UNB1) and generated a recombinant virus containing the fluorescence reporter *gfp* gene controlled by the constitutive *hsp70* promoter (*Chin*NPV-GFP). We used these viruses to assess the infectivity in UFLAg-286 cell lines, derived from *A. gemmatalis*. Light and fluorescence microscopy revealed that the infection leads to apoptosis with subsequent increase of effector caspase activity as measured by chemiluminescent assays. To further our understanding of this phenomenon, we cloned *Chin*NPV-UNB antiapoptotic *iap2* and *iap3* genes in *p35* defective *Ac*MNPV bacmid vectors to assess their specific activities. While the *iap2* containing bacmid induced some apoptosis, both *iap2* and *iap3* were capable of infecting *Trichoplusia ni* derived Tn5B leading to the production of occlusion bodies, a hallmark of successful baculovirus infection. In contrast, both bacmids failed to inhibit apoptosis in UFLAg-286. These results demonstrate that *Chin*NPV inhibition of apoptosis genes limits its host range *in vitro* to Plusiinae derived cell lines. This suggests that *Chin*NPV *iaps* are strong determinants of host range *in vivo*.

Financial support: CAPES, CNPq, FINATEC and FAPDF.

Palavras-chaves: baculovirus, IAP, biocontrol, Anticarsia gemmatalis, Chrysodeixes includens

ULTRASTRUCTURAL STUDIES OF THE COTESIA FLAVIPES OVARIES AND ITS ENDOSYMBIOTIC POLYDNAVIRUS

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Resumo

The parasitoid wasp *Cotesia flavipes* has been introduced in Brazil to serve as a biocontrol agent of lepidopteran pests of corn and sugarcane. *C. flavipes* is an efficient hunter of *Diatreia saccharalis* larvae that lives within the sugarcane stems, effectively protected from chemical insecticides applied over the fields. Once a female *C. flavipes* finds the larval host, it injects its matured eggs and calyx fluid contained in the ovaries into the hemolymph of the host. The eggs then develop into larvae within the lepidopteran larvae, feeding on the nutrients of the hemolymph until they breach out of their hosts, to pupate outside and morph into an adult wasp. Lepidopteran larvae have innate immune defenses against parasitoids such as melanization that can encapsulate and asphyxiate the wasp's eggs. Throughout the evolutionary history of this parasite-host interaction, the Braconidae group of wasps has incorporated an ancestral Nudivirus into its genome and further evolved it into an endosymbiotic Polydnavirus. A specific group of calyx cells within the ovaries of *C. flavipes* produces abundant viral particles that contains circular segments of the Bracovirus genome (CfBV). These viral particles are secreted into the ovarian lumen and are also injected with the eggs into the larval host, acting as gene delivery agents into cells of the hemolymph, expressing viral genes that disrupts the metabolism and their defense functions for the organism. The study of the biology of CfBV and its symbiotic relationship to the wasp is crucial to understand the evolution of its parasitoid life. It should also yield new information on how to improve biological control methods. Here we present the first images of the *C. flavipes* CfBV particles within the wasp's ovaries by Transmission Electron Microscopy (TEM), demonstrating that the ovarian fluid possesses abundant viral particles composed of multiple nucleocapsids with a common envelope. We also purified viral particles by ultracentrifugation and visualized them by Negative Staining TEM. A single nucleocapsid has clear physical traits such as the end-cap, ring-like and helix-tail structures with variable lengths, possibly due to the different sizes of the DNA segments contained in each virion.

Palavras-chaves: Parasitoid, Biocontrol, Cotesia flavipes, Diatreia saccharalis, Poly DNA vírus

AN IN-SILICO APPROACH TO VALIDATE THE CAPSID ARCHITECTURE OF NEW PUTATIVE ICOSAHEDRAL VIRUSES: GEMINIVIRIDAE AS CASE STUDY.

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Resumo

With the advent of metagenomics approaches, a diversity of known and unknown viruses has been identified in various types of samples. The sequence-based only taxonomic classification of these viruses is a challenge, and more tools are needed to understand and predict biological aspects of this viral-dark matter. Many capsid proteins from icosahedral viruses have a positively charged domain (R-arm) that is important for genome packaging and particle stability but that have poor sequence conservation. Recently, we developed a computational approach based on the net charge calculation of discrete protein segments that can automatically identify R-arms and calculate its electrostatic net-charge. With this analysis, we identified various virus families with ssDNA, ssRNA, and dsDNA genomes, for each the genome packaging capacity is highly correlated with the total number of capsid subunits (capsid architecture). Therefore, we propose that by knowing the capsid protein sequence and the total genome size, we could apply this analysis to check if a putative new member of a given viral family complies with the particle morphology expected for that group. In this work, we tested this hypothesis with geminiviruses (*Geminiviridae*), that were among the viruses that use capsid R-arms to stabilize their capsids. These ssDNA plant viruses are divided into two main genera, *Begomovirus* and *Mastreovirus,* which contain the viruses with known tridimensional capsid structure: a geminated capsid, formed by two T=1 icosahedral capsids joined by a missing pentameric vertex, totalizing 110 repeated subunits. The family also comprises other 7 minor genera, 2 unsigned species and several new species. We applied our program to calculate the R-arm net charge for all *Geminiviridae* sequences included in the 10th ICTV report (n=466) and other putative isolates. We observed a linear correlation between the R-arm net charge and the genome size for most of the data-set. All minor genera had similar genome/capsid charge ratio, corroborating the assumption that they share the same geminated capsid architecture. Importantly, our plot was able to predict that a virus closely related *Genomoviridae* family (T=1, 60 subunits) did not comply with the geminated architecture. Moreover, our analysis indicated that mulberry mosaic dwarf associated virus that is yet unassigned to a genus in the family *Geminiviridae,* could have an alternative T=1 virus architecture based on the R-arm charge and genome size.

Palavras-chaves: Bioinformatics, Capsid, Geminiviridae

EMERGENCE AND ADAPTATION OF TOMATO BEGOMOVIRUSES IN BRAZIL: ASSESSING REPLICATIVE AND TRANSMISSION FITNESS

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Resumo

The prevalence of only a few begomoviruses infecting tomatoes in Brazil is an intriguing fact, on light of the great begomovirus diversity that has been reported in this crop. Most studies that have been performed in an attempt to understand the begomovirus emergence process and consequent epidemics in Brazil have focused on the genetic structure and dispersion patterns of viral populations. Studies addressing the underlying mechanisms leading to emergence and the current patterns of prevalence have not been conducted. Here, we quantified the replicative fitness of two begomoviruses infecting tomato in Brazil, *Tomato severe rugose virus* (ToSRV) and *Tomato yellow spot virus* (ToYSV), in tomato plants and in non-cultivated hosts to which each virus has often been associated, and quantified the transmission efficiency by *B. tabaci* Middle East-Asia Minor 1 (MEAM1) and *B. tabaci* Mediterranean (MED). Interestingly, ToSRV and ToYSV presented similar adaptation levels in tomato. No fitness trade-off across hosts was observed for ToYSV when viral accumulation was evaluated in tomato and in the wild host *L. sibiricus*. In contrast, ToSRV performed better in tomato than in *N. physalloides*. These results reinforce that ToSRV is well adapted to tomato and occasionally spills back to wild hosts, while ToYSV is well adapted to both tomato and *L. sibiricus*. We also compared the replicative fitness of ToSRV and ToYSV during single or mixed infection. Interestingly, while there were no differences in fitness between the two viruses at 14 days after inoculation (dpi), ToYSV had a gain in fitness from 21 to 28 dpi. Furthermore, a negative interference of ToSRV over ToYSV was observed, as ToYSV reached a higher accumulation in single infection than in mixed infection with ToSRV. Together, these results suggest that adaptation to the host does not explain the prevalence of ToSRV over ToYSV in the field. However, while ToSRV was transmitted by *B. tabaci* MEAM1 and MED, ToYSV was not, which could be the reason why this virus is not widespread in the field.

Financial Support: CAPES, CNPq, FAPEMIG

Palavras-chaves: begomovirus, Bemisia tabaci, geminivirus, mixed infection, transmission

ASPECTS OF THE ASSOCIATION BETWEEN LEONURUS YELLOW SPOT ALPHASATELLITE AND BIPARTITE BEGOMOVIRUSES: EFFECTS ON INFECTION AND TRANSMISSION BY BEMISIA TABACI MIDDLE EAST-ASIA MINOR 1

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Resumo

The genus *Begomovirus* (family *Geminiviridae*) includes plant viruses with circular, single-stranded DNA (ssDNA) genomes which are transmitted by the whitefly *Bemisia tabaci*. Begomoviruses in the New World can be found in association with alphasatellites, which are circular, ssDNA molecules capable of autonomous replication, but dependent on the helper begomovirus for encapsidation, systemic infection and insect transmission. The impact of the interaction between alphasatellites and begomoviruses is unknown. The objective of this work was to verify the effect of *Leonurus yellow spot alphasatellite* (LeYSA) in the infection of *Tomato yellow spot virus* (ToYSV), *Tomato severe rugose virus* (ToSRV) and *Euphorbia yellow mosaic virus* (EuYMV) in three hosts, *Leonurus sibiricus, Nicotiana benthamiana* and tomato. The plants were inoculated with each virus in the presence or absence of the alphasatellite. Infectivity and symptom development for each begomovirus alone or in the presence of LeYSA were evaluated, and viral DNA accumulation was quantified for each virus and virus-satellite combination. The association of LeYSA with ToYSV was less efficient in tomato than in *L. sibiricus* and *N. benthamiana*, as measured by a lower percentage of plants in which the presence of the alphasatellite was detected. The association between ToSRV and LeYSA was similar in both tomato and *N. benthamiana*. The association between EuYMV and LeYSA in tomato was the least efficient, and in *N. benthamiana* the presence of the alphasatellite was not detected in any of the plants infected with EuYMV. Together, these results indicate distinct levels of interaction between the alphasatellite and different begomoviruses. Quantification of ToYSV and ToSRV DNA-A accumulation indicated that LeYSA does not intefere in the accumulation of these begomoviruses. However, symptoms were more severe in the presence of LeYSA for both viruses and in all hosts. There was a variation in the accumulation of LeYSA relative to the host and the associated begomovirus. Together with previous studies, these results highlight the potential risk of the association between begomoviruses and alphasatellites in both cultivated and non-cultivated plants.

Financial Support: CAPES, CNPq, FAPEMIG

Palavras-chaves: alphasatellite, begomovirus, geminivirus, Leonurus, tomato

MALVAVISCUS YELLOW MOSAIC VIRUS, A BEGOMOVIRUS CARRYING A NANOVIRUS-LIKE NONANUCLEOTIDE AND A MODIFIED STEM-LOOP STRUCTURE

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Resumo

Begomoviruses (family *Geminiviridae*) are whitefly-transmitted viruses with a circular, ssDNA genome encapsidated in twinned icosahedral particles. In Brazil, a high number of begomoviruses infecting non-cultivated plants have been described. These plants may act as natural begomovirus reservoirs and as sources of genetic variability. Here we describe a novel bipartite begomovirus infecting *Malvaviscus arboreus* (Malvaceae) plants showing a bright yellow mosaic, collected at Campinas, São Paulo state in May 2005 and Rio de Janeiro, Rio de Janeiro state in August 2009 and February 2011. Total DNA was extracted and the viral genome was amplified by RCA, cloned and sequenced. Sequence analysis indicated that the virus corresponds to novel species, for which the name Malvaviscus yellow mosaic virus (MalYMV) is proposed. Successful infection by biolistic of *Nicotiana benthamiana* and *Malvaviscus arboreus* was confirmed by PCR, RCA and Southern blot hybridization. Symptoms observed in infected *Malvaviscus arboreus* plants consisted in bright yellow mosaic while in N. *benthamiana* showed slight mosaic and leaf deformation. The progeny virus population present in biolistic infected plants was isolated and identity to the original isolate was confirmed by sequencing. Therefore, Koch's postulates were fulfilled. Strikingly, MalYMV has a nanovirus- and alphasatellite-like nonanucleotide (5'-TAGTATTAC-3'). Moreover, a short sequence located 5' of the nonanucleotide potentially forms a minor hairpin structure embedded in the major hairpin. Intramolecular interactions involving the sequence of the atypical nonanucleotide were predicted. To biologically characterize the replication origin of this distinct begomovirus, three different mutants were obtained. The mutant that rescues the begomovirus nonanucleotide (TAATATTAC) was able to infect *N. benthamiana* plants, showing that the point mutation at the nonanucleotide does not disrupt MalYMV replication. On the other hand, the short sequence located 5' of the nonanucleotide seems to be essential for infection. Although MalYMV has been collected in Brazil, it is phylogenetically closer to viruses from Central and North America. The *M. arboreus* plant at Campinas has been displaying the observed yellow mosaic symptoms since at least the 1960's, which suggests that MalYMV may be poorly transmitted (or not transmitted at all) by local whitefly populations.

Financial Support: CAPES, CNPq, FAPEMIG

Palavras-chaves: Geminiviridae, Malvaceae, replication origin , hairpin structure

COMPOSITION OF BEGOMOVIRUS POPULATIONS IN CULTIVATED AND NON-CULTIVATED HOSTS DETERMINED BY HIGH-THROUGHPUT SEQUENCING

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Resumo

The genus *Begomovirus* (family *Geminiviridae*) includes single-stranded DNA plant viruses transmitted by whiteflies. Begomoviruses are among the most damaging plant pathogens, causing epidemics in economically important crops worldwide. Tomato-infecting begomoviruses emerged in Brazil in the early 1990's following the introduction of *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1, previously known as *B. tabaci* biotype B). Several lines of evidence suggest that these viruses evolved from indigenous viruses infecting non-cultivated hosts. However, tomato-infecting viruses are only rarely found in non-cultivated hosts, and vice-versa. It is possible that viral populations in a given host are composed primarily of viruses which are better adapted to this host, but also include a very small proportion of viruses which are poorly adapted. Then, after transfer to a different host by the whitefly vector, the composition of the viral population shifts rapidly, with the viruses which are better adapted to the new host becoming predominant. To test this hypothesis, we collected tomato and *Sida* sp. plants, growing next to each other, at two locations (Coimbra and Florestal, both in Minas Gerais state, Brazil), in 2014 and 2018. Viral infection was confirmed by polymerase chain reaction (PCR) using specific primers. Total DNA from one tomato and one *Sida* sp. sample from each location and year were subjected to high-throughput sequencing (HTS). Following a highly stringent set of criteria, reads were mapped to a data set including all New World begomoviruses. The reads were classified as (i) *Tomato severe rugose virus* (ToSRV), (ii) *Sida micrantha mosaic virus* (SiMMV) and (iii) *Sida common mosaic virus* (SiCmMV), when the first three hits were isolates of these species, or (iv) begomovirus, when the first three hits included isolates of different species. For the 2014 samples, >98% of the reads from *Sida* sp. mapped to SiMMV, but 0.01% of the reads mapped to ToSRV. Conversely, >99% of the reads from tomato mapped to ToSRV, with 0.001% mapping to SiMMV. For the 2018 samples, >99% of the *Sida* reads mapped to three *Sida*-infecting viruses, and 0.1% of the reads mapped to ToSRV. These results are consistent with the hypothesis that viral populations in a given host are composed primarily of the virus that is most adapted to this host but also includes a very small proportion of viruses that are less adapted.

Palavras-chaves: Geminivirus, host adaptation, viral population

THE OVEREXPRESSION OF SLDJ1 PROTEIN IN NICOTIANA BENTHAMIANA LEADS TO DECREASED INFECTION BY TURNIP MOSAIC VIRUS

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Resumo

Potyviridae is one of the largest and most important families of viruses that infect plants, being *Potyvirus* the largest genus in the family. Potyvirus genome consists of one single strand positive-sense RNA component, which encodes about eleven proteins that can interact with the host, manipulating the plant cell for the benefit of the virus. A previous analysis of genes differentially expressed in tomato plants infected by the potyvirus *Pepper yellow mosaic virus* (PepYMV) led to the identification of the gene *SlDj1* as induced by the infection. SlDj1 is a member of the DnaJ protein family, also known as Hsp40. These proteins may act as co-chaperones of Hsp70 proteins, regulating their activity, or they may also act as chaperones. The DnaJ-Hsp70 complex is involved in cellular processes such as protein folding, regulation of protein degradation and protein complexes assemble, among others. The downregulation of *SlDj1* homologs in *Nicotiana benthamiana* plants leads to decreased infection by both the potyviruses PepYMV and *Turnip mosaic virus* (TuMV). Silenced plants also present a phenotype similar to the symptoms of viral infection. Confocal microscopy analyses demonstrated the co-localization of SlDj1 with vesicles associated with TuMV replication in infected plants. In order to understand the role of SlDj1 in potyvirus infection, SlDj1 was fused to the GFP and overexpressed transiently in leaves of *N. benthamiana* by agroinfiltration. Twenty-four hours later, the same leaves were agroinoculated with TuMV and the virus infection was analyzed. The overexpression of SlDj1-GFP was confirmed by Western Blot, using antiserum anti-GFP, and the viral accumulation was quantified by qRT-PCR, using specific primers. The plants overexpressing SlDj1 accumulated fewer viruses than control plants. These results indicate that SlDj1 is involved in the process of infection by potyviruses. Further studies are necessary to unravel the role of SlDj1 in potyvirus infection. Financial support: CAPES, CNPq, FAPEMIG.

Palavras-chaves: Plant-virus interaction, Chaperones, Potyvirus

SPECIFIC NUCLEOTIDES IN THE COMMON REGION OF THE BEGOMOVIRUS TOMATO RUGOSE MOSAIC VIRUS (TORMV) ARE RESPONSIBLE FOR THE NEGATIVE INTERFERENCE OVER TOMATO SEVERE RUGOSE VIRUS (TOSRV) IN MIXED INFECTION

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Resumo

Natural mixed infections with two or more begomoviruses are common and may alter infectivity, symptom severity and viral accumulation in comparison to single infections. The begomoviruses Tomato rugose mosaic virus (ToRMV) and Tomato severe rugose virus (ToSRV) have genomes with a high degree of sequence identity, including the cis-elements (iterons) in the common region (CR) and their specific recognition sites within the Rep gene (iteron-related domain, IRD), which are essential for initiating viral replication. Previous work has shown that the interaction between these begomoviruses in mixed infection is complex, with ToRMV negatively interfering in infectivity and in the accumulation of ToSRV. In this work we investigated if divergent sites in the CR and IRD of these begomoviruses are involved in this interference. ToSRV DNA-A mutants containing the same nucleotides of ToRMV DNA-A at the divergent positions of the CR (ToSRV-A(CR)), of the Rep gene IRD (ToSRV-A(IRD)) and in both regions (ToSRV-A(CR+IRD)) were constructed. Infectivity and viral accumulation of ToSRV in single infection were not affected by mutations in either of the two regions. However, in mixed inoculation of ToRMV with ToSRV-A(CR), high infectivity of both viruses and high accumulation of ToSRV-A(CR) DNA in relation to wild-type ToSRV was observed. This was not observed when plants were inoculated with ToRMV and ToSRV-A(IRD). These results suggest that the mutated CR sites serve as specific recognition sites for Rep binding, increasing the viral replication rate and viral DNA accumulation. On the other hand, decreased viral accumulation in plants inoculated with ToSRV-A(CR+IRD) suggests that the divergent amino acids in the IRD do not offer an advantage for ToSRV replication efficiency.

Financial Support: CAPES, CNPq, FAPEMIG

Palavras-chaves: Geminiviridae, tomato, replication, interaction

RSIBR1, AN INOVIRUS THAT CAN MODULATE MOTILITY AND BIOFILM PRODUCTION OF THE PHYTOPATHOGEN RALSTONIA PSEUDOSOLANACEARUM

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Resumo

Filamentous bacteriophages contain a single-stranded DNA genome and have a peculiar lifestyle compared to other bacteriophages once they do not cause host cell lysis, but actually establish a persistent association with host, often causing behavioral changes, with unpredictable effects on bacterial ecology. In a previous work, we reported the RSIBR1, an Inovirus capable to remarkably attenuate the virulence of the phytopathogenic *R. pseudosolanacearum* GMI1000 besides provoking innumerable alterations in phenotypic and physiological parameters of the host bacterium. The presence of GMI1000 phage-infected (GMI1000 PI) in xylem vessels of plants without symptoms after 3 months confirms that the infected isolates are able to colonize the plant without causing disease, showing that the phage infection changed the behavior of these pathogens. However, it is not clear how the virus modulates the bacteria pathogenesis. Motility and biofilm production are important components for establishing bacterial wilt in plants caused by R. solanacearum. In this work, we evaluate the effects of RSIBR1 in R. solanacearum motility (swimming and Slipping) and the production as well as composition of biofilm. In order to better understand this phenotypic change, the isolate of *R. pseudosolanacearum* GMI1000 was infected with the inovirus RSIBR1 and its motility and biofilm production were evaluated. Motility was evaluated by motility assay in culture medium CP 0,3% agar, 0,7% agar and Minimal Medium. The biofilm production was evaluated on 96 well plates and biolfim composition was determined using (DNASE 1) to quantify eDNA, Proteinase K was used to quantify proteins while sodium periodate was used to quantify polysaccharide. The *R. pseudosolanacearum* GMI1000 infected with RSIBR1 showed an increase of motility (swimming and slipping) and a reduction in total biofilm production. We also observed a reduction of polysaccharides on biofilm produced by *R. pseudosolanacearum* GMI1000 infected with RSIBR1. These results can help us to explain the phenotypes of reduction or loss of virulence that have been reported for *Ralstonia* spp infected by inoviruses.

Palavras-chaves: Inovirus , Ralstonia pseudosolanacearum, Biocontrol, Biofilm, Phytopathogen

SYSTEMIC INFECTION OF PLANTS BY A GEMYCIRCULARVIRUS (FAMILY GENOMOVIRIDAE)

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Resumo

The *Genomoviridae* family is one of the most recently established ssDNA virus families. Genomovirids are non-enveloped, ssDNA viruses with circular genomes ranging from 2 to 2.4 kb, containing two ORFs separated by an intergenic non-coding region (IR). One of the ORFs, located in the virion-sense strand, encodes the putative coat protein (CP), and the other, in the complementary-sense strand, encodes a putative replication-associated protein (Rep) similar to the Rep found in members of the family *Geminiviridae*. Currently, the *Genomoviridae* family is comprised of 73 viral species classified into nine genera, with *Gemycircularvirus* as the largest genus. Members of the family have been identified in mammals, birds, invertebrates, in a variety of environmental samples, and also in plants. However, despite this large number of reports and their pervasive presence in the environment, the infectivity of genomovirids to specific hosts remains largely unknown. The gemycircularvirus SsHADV-1 remains the only genomovirid with a known (fungal) host. Interestingly, the genus *Gemycircularvirus* includes all plant-associated genomovirids found so far. It is possible that gemycircularviruses play relevant ecological roles in association with plants. Here, we report the first case of systemic infection of plants by a genomovirid, Euphorbia heterophylla associated gemycircularvirus (EuaGmV). A dimeric clone (˜4.4 kb) of EuaGmV was used for the biolistic inoculation of eight plants of *Euphorbia heterophylla* and 10 plants of *Nicotiana benthamiana*. Control plants (three of each species) were inoculated with the geminivirus Euphorbia yellow mosaic virus (EuYMV) or with water. At 21 and 28 days after inoculation (dai), non-inoculated upper leaves of the EuaGmV-inoculated plants (which did not show any symptoms) were collected for PCR-based analysis of the presence of the virus. Six plants of *E. heterophylla* and all 10 plants of *N. benthamiana* were PCR-positive for the presence of EuaGmV. Amplicons (612 bp) obtained from both plant species were sequenced, and a 99.6% identity with the sequence of the EuaGmV clone was obtained. These results demonstrate, for the first time, that a gemycircularvirus (family *Genomoviridae*) is capable of systemically infecting plants.

Financial support: CAPES, CNPq, Fapemig

Palavras-chaves: metagenomics, genomoviridae, gemycircularvirus, Euphorbia heterophylla, CRESS DNA vírus

INTERCEPTION OF BARLEY STRIPE MOSAIC VIRUS-BSMV: A QUARANTINE VIRUS ABSENT IN BRAZIL DETECTED IN IMPORTED BARLEY GERMPLASM

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Resumo

Barley stripe mosaic virus (BSMV), a quarantine virus absent in Brazil, was intercepted in 2019 in imported barley (*Hordeum vulgare*) seeds. Expressive and typical symptoms of virus infection were observed in young leaves in seedlings from the abovementioned barley material (confidential process). The presence of BSMV in these symptomatic leaflets was confirmed by Enzyme-Linked Immunosorbent Assay (ELISA), using antibodies against BSMV (Agdia SRA 19500/0096) in biological and technical independent duplicates, resulting in clear detection of BSMV, once the spectrophotometry value was 1,063 times higher in barley samples than the experimental control samples. Visual inspection of symptoms and Polymerase Chain Reaction (PCR) for phytoplasma detection were negative. As BSMV is a regulated quarantine pest absent in Brazil (according to the Normative Instruction n. 39, 01/10/2018- Brazilian Ministry of Agriculture, Livestock and Food Supply), destruction of the respective imported seeds was recommended (incineration) and a report with technical information justifying such recommendation was issued. Samples of BSMV infected barley leaves were stored and will be used as positive control for future detection procedures. The presently reported BSMV quarantine interception of barley to be imported into Brazil reinforces the need for research on quarantine intelligence, development and validation of more sensitive molecular diagnostic methods, and preventive genetic improvement to combat the vulnerability of introduction of quarantine pests absent in the country, which can seriously jeopardize the primary sector of the national economy. This work is aligned with the Brazilian policy, which promote prevention and surveillance of quarantine absent pests, according to the "Portaria nº 131, 27/jun/2019 - Programa Nacional de Prevenção e Vigilância de Pragas Quarentenárias Ausentes (PNPV-PQA)".

Financial support: Empresa Brasileira de Pesquisa Agropecuária - Embrapa

Palavras-chaves: Grass-plants, Phytovirus, Prevention, Serological-molecular diagnosis, Surveillance

AN ASYMPTOMATIC IFLAVIRUSES COVERTLY INFECTING BRAZILIAN STINK BUGS: MOLECULAR AND ULTRASTRUCTURAL CHARACTERIZATION

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Resumo

Iflaviruses belong to the picorna-like virus family *Iflaviridae* (order *Picornavirales*) and are classified within a single genus called *Iflavirus*. The members of this genus have a broad host spectrum and are characterized by a positive ssRNA genome organized into one single ORF that codes for one polyprotein organized into structural and non-structural peptides. *Iflavirus* infection is still unclear and seems to be different depending on the host type, but the majority of iflavirus infections do not result in visible signs or any apparent disease. An analysis of transcriptomes derived from the antennae of the South American stink bugs *Euschistus heros* (Fabricius, 1794), *Chinavia ubica* (Rolston, 1983), and *Dichelops melacanthus* (Dallas, 1851) revealed the presence of picorna-like virus genome-length RNAs with high sequence identity to the genome of Halyomorpha halys virus (HhV), originally discovered in the transcriptome of the brown marmorated stink bug, *Halyomorpha halys* (Stål). Features of the genome sequences, their phylogenetic relationships to other insect picorna-like viruses, and the appearances of virus-like particles isolated from host stink bugs all confirm that these viruses are iflaviruses. Comparison of the predicted capsid amino acid sequences of these viruses indicate that all four viruses are isolates of an undescribed species of genus *Iflavirus*. Iflavirus RNAs were present at high levels, with iflavirus reads significantly outnumbering actin mRNA reads in all stink bug transcriptomes that were examined. Approximately 40% to 90% of transcriptome reads in the stink bug antennal transcriptomes mapped to the virus genome sequences found in these transcriptomes. In whole-insect transcriptomes of *H. halys*, HhV reads were >500-fold more abundant in adults than in nymphs. No iflavirus sequences were detected in the genomic DNA datasets for *E. heros*, *C. ubica*, *D. melacanthus* or *H. halys*, suggesting that the iflavirus RNAs detected in the stink bug transcriptomes are not derived from copies of iflavirus sequences integrated into host genomes. The results of the analysis suggest that these iflaviruses are able to produce large quantities of their RNAs without causing any obvious pathology to their hosts.

Financial Support: CAPES

Palavras-chaves: covert infection, Halyomorpha halys, Iflaviridae, iflavirus, Pentatomidae

NOVEL VIRUSES IN SALIVARY GLANDS OF ANOPHELES MOSQUITOES FROM MATO GROSSO, BRAZIL

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Resumo

Anopheles species are the main vectors of Malaria. However these mosquitoes have been associated to the transmission of few arboviruses such as the alphaviruses Mayaro virus and O'nyong nyong virus to date. Metagenomic studies busted the discovery of novel insect viruses and their evolutionary relationships. Here we report the identification of three viruses in salivary glands of 353 female anopheles specimens allocated into 12 pools according to genus, capture location and climatic period (dry, transitional, rainy). In total, 6 pools (197 specimens of 13 species; 55.8%) from High Pantanal, 5 pools (126 specimens of 8 species; 35.7%) from Chapada dos Guimarães National Park and 1 pool (30 specimens of 3 species; 8.5%) from Cuiabá were subjected to RNA extraction, dscDNA synthesis and randomic PCR; the purified DNA was sequenced on an Illumina HiSeq 2500 plataform. Anopheles nimbus / thomasi (70; 19.8%) was the most frequent along with 18 sampled species. Three viruses were found: segments VP1 and VP3 of a putative novel reovirus named Purunga orbivirus, with 74% and 65% of identity with Changuinola virus and Urongo virus, was identified in an Anopheles pool (30 Anopheles benarrochi and 3 Anoplheles spp.) from Pantanal. This virus was isolated in Vero cells. A novel rhabdovirus named Coxipó dielmovirus was identified in an Anopheles spp. pool from Cuiabá and presents 54% identity with Merida virus. Also, Anopheles triannulatus orthophasmavirus (92% identity) was detected in this study in an Anopheles lutzi pool from Chapada dos Guimarães. Anopheles mosquitoes can be infected with insect-specific viruses and arboviruses. Evolutionary studies have shown that insect-specific viruses are more ancient than arboviruses. Although these agents comprise different phylogenetic groups, they share the same viral families and a common ancestral.

Financial Support: Capes, CNPq rede pró-centro oeste

Palavras-chaves: arbovirus, High throughput sequencing, ISV, novel viruses, phylogeny

COMPARISON OF PARAMETERS FOR CHRYSODEIXIS INCLUDENS NUCLEOPOLYHEDROVIRUS IN VIVO PRODUCTION

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Resumo

The *Chrysodeixis includens nucleopolyhedrovirus* (ChinNPV), genus *Alphabaculovirus*, family *Baculoviridae* is pathogenic to *C. includens* larvae, which is an important soybean pest. The ChinNPV-Buritis isolated from Brazilian savanna demonstrated potential for biocontrol of this pest. However, there are limiting factors for the large-scale *in vivo* production of ChinNPV, such as disruption of larvae integument and restraints on soybean looper mass rearing due to endogamy and colony depletion. In order to improve *in vivo* production of ChinNPV some parameters were tested. The first assay was conducted comparing larvae of 3rd instar and 4th instar, three different temperatures (23°C, 26°C and 29°C) for larvae incubation and two different viral concentrations (5x10^6 and 5x10^7 Occlusion bodies-OBs/ml). The experiment was performed with 30 larvae/treatment and 30 larvae for control without virus. The larvae were keep individually in 50ml plastic cups with artificial diet. OBs concentration in the larvae was determined using a Neubauer chamber under an optical microscope. The evaluations were performed at 3 to 7 d.p.i. The mass of infected and control larvae was measured in a precision balance. The second assay was performed comparing the incubation of 3rd instar larvae individually and groups of 30 larvae in 300ml plastic recipients and two temperatures for larvae incubation (23°C and 26°C). Each treatment was performed with 30 larvae inoculated with 5x10^6 OBs/ml and 30 larvae for control without virus. OBs and mass evaluation were performed at 6 d.p.i. Statistical analysis were performed in R program using ANOVA for mass and GLM-quasipoisson for OBs analysis. In the first experiment the best OBs production occurred at 6 d.p.i. No significant differences in OBs production (p*in vivo* production to avoid the disruption of larvae integument. The rearing of larvae in groups and lower concentration of virus as inoculum are indicated to reduce the costs of production.
Financial Support: FAP-DF

Palavras-chaves: ChinNPV, baculovirus, biological control, soybean looper

VIRAL METAGENOMICS OF HEMATOPHAGOUS INSECTS COLLECTED IN THE COMPLEXO MINERADOR DE CARAJÁS AREA, STATE OF PARÁ

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Resumo

The Amazon region presents favorable environmental factors for the maintenance of viruses and some anthropic actions contribute mainly to the dispersion of vectors. Metagenomic studies are essential to elucidate the arthropod virosphere and provide relevant data on viral diversity and evolution. A total of 40 hematophagous insects of 2 mosquito species (*Haemagogus janthinomys* and *Sabethes glaucodaemon*) and a genus of *ceratopogonidae* (Biting midges) were collected in 3 municipalities: Curionópolis, Marabá e Canaã dos Carajás, state of Pará (2014-2016). It was performed the extraction of viral RNA, double strand cDNA and sequencing illumina Miniseq. In the Bioinformatic step, the programs SortMeRNA, CD-HIT, assembly with IDBA-UD and SPADES, alignment with Diamond and curation with Geneious were used. The JModelTest and RaXML programs were used for phylogeny. Viral sequences belonging to more than 15 different RNA virus families were detected and annotated. The most abundant families were *Flaviviridae, Chuviridae, Rhabdoviridae, Phasmaviridae* and *Phenuiviridade*. The *Sabethes glaucodaemon* pool had the highest viral diversity and the highest number of contigs for the *Xincheng Mosquito Virus*, presenting a low identity (35-56%) in BLAST, which may indicate a new virus. Among the members of the *Flaviviridae* family were identified the *Mercadeo virus* and *Culiseta flavivirus* which are insect-specific flaviviruses also detected in North American countries. The viruses *Hubei tombus-like virus 28, Hubei diptera virus 17, Wuhan insect virus 8, Hubei dimarhabdovirus virus 2, Wuhan insect virus 8, Hubei virga-like virus 11, Hubei chuvirus-like virus 3 and Sanxia water strider virus 9 f*ound in this work were recently discovered in a chinese study made in 2016. A large number of non-classified contigs were observed in the database, and a detailed curation was necessary to identify the existing viruses. The majority of the viruses found in this work do not have a well defined biological importance because they are newly discovered viruses, thus other molecular biology studies are necessary to elucidate their role in the arthropod virome and possible pathogenic effects on vertebrates.

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Palavras-chaves: Amazon, Carajas, insects, metagenomic, viroses

NEW VIRUS IN MOSQUITOES FROM CANAA DOS CARAJAS, NORTH BRAZIL

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Resumo

New viruses and its variations keep on emerging and studies are needed to elucidate the virosphere profile and to locate new viruses in mosquito samples, as mosquitoes are vectors of many viruses that infect animals and humans. A total of 5 mosquitoes of *Sabethes glaucodaemon* specie, collected in Canaã dos Carajás (Pará) in 2016, was subjected to the extraction of viral RNA, double strand of cDNA and sequency with Illumina Miniseq technology. The softwares used in this work were: SortMeRNA and CD-HIT (preprocessing of the readings); Spades and IDBA-UD (assembly); Diamond (BLAST of the contigs); besides Geneious versão 9.1.4 program and BLAST in the NCBI site (curating of the contigs). The new virus found in this work was named C*anaa Virus* (VCAN). The assembling of the contigs revealed a low identity (35-56%) with the *Xincheng mosquito virus* when individually subjected to the BLASTx of the NCBI. The structure of this virus is similar to the structure of the *Xincheng mosquito virus*, which presents 3 non-structural genes (VP1, VP2 and VP3) and 2 structures, glycoprotein (G) and RNA polymerase (L). The molecular weight of which structure is given in kilodaltons (KDa): VP1 (46.1 KDa), VP2 (18,4 KDa), VP3(48,1 KDa), G (72 KDa) e L (231,1 KDa). To make the phylogeny of *Canaa Virus*, the gene corresponding to L, which is generally more conserved among the species, was used. The database was assembled with reference genomes of the viral families grouped in the order of Mononegavirales. The *Canaa Virus* showed a greater similarity with the *Xincheng Mosquito Virus*, an *Anphevirus* of the *Xinmoviridae* family, grouping in the same clade in the phylogenetic tree. The phylogenetic analysis has demonstrated that *Canaa Virus* is closely related to the *Xincheng Mosquito Virus*, being their genomic structure the same, presenting the regions VP1, VP2, VP3, G e L, which have similar sizes. This work emphasised the utility of a metagenomic sequencing approach to the discovery of a new virus, besides contributing, in the future, to the deposition of a cured sequence in Genbank.

Financial Support: Fundação Instituto para o desenvolvimento da amazônia- FIDESA, Capes-CNPQ, Instituto Evandro Chagas, Universidade do Estado do Pará.

Palavras-chaves: Amazon, metagenomic, vírus

NOVEL VIRUSES IN MOSQUITOES FROM BRAZILIAN PANTANAL

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Resumo

Viruses are ubiquitous and diverse microorganisms. Emerging viruses arise as a result of novel interactions between populations of hosts and pathogens, and can threaten the health and wellbeing of the entire spectrum of biodiversity. Here we herein report the presence of different viruses in salivary glands of 1,657 mosquitoes, major infectious disease vectors worldwide, classified over 28 culicinae species captured in the Brazilian Pantanal wetland. After identification in a dormant state, mosquitoes were allocated in 29 pools, which were subjected to RNA extraction, to randomic RT-PCR and to IlluminaHiSeq 2500 sequencing plataform. In total, 12 viruses were found after sequence analysis, eight putative novel viruses with relatively low similarity with the pre-existing species of viruses according to classification criteria within their respective families, named with traditional terms from Mato Grosso: Pirizal iflavirus, Furrundu phlebovirus and Pixé phlebovirus, Guampa vesiculovirus, Chacororé flavivirus, Rasqueado orbivirus, Uru chuvirus and Bororo circovirus. Viruses were additionally confirmed with iflavirus-like, rhabdovirus-like and circovirus-like and Phlebovirus-like RT-PCR protocols and through viral isolation in C6/36 cells. We also found the already described Lobeira dielmorhabdovirus, Sabethes flavivirus, Araticum partitivirus and Murici totivirus. Phylogeny revealed these viruses clustered with insect-specific viruses within their respective genus/family members and, surprisingly, ssRNA- viruses, which are evolutionarily more recent than dsRNA and ssRNA+, were more frequently detected in this study. Therefore, these findings underscore the vast diversity of culicinae and novel viruses yet to be explored in Pantanal, the largest wetland in the planet.

Financial support: *Capes, CNPq rede pró centro-oeste*

Palavras-chaves: arboviruses, brazillian pantanal, culicinae mosquitoes, insect-specific viruses, novel viroses

CHARACTERIZATION OF SPODOPTERA ERIDANIA NUCLEPOLYHEDROVIRUS ISOLATE VPN165 AND THE EVOLUTION OF A BACTERIAL CHONDROITIN LYASE HOMOLOG ACQUIRED BY BACULOVIRUSES

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Resumo

The Southern armyworm *Spodoptera eridania* (Cramer, 1782) (Lepidoptera: Noctuidae) is native to the American tropics and considered a pest with intensive polyphagous habit. In Brazil, *S. eridania* went from a secondary plague status to an expanding pest of great importance in crops of soybean, cotton, fruits, and weeds. In this work, we characterized a second baculovirus isolated from *S. eridania* at structural, biological, and molecular level. The virus was found in extracts of caterpillars died with symptoms of baculovirus infection and called Spodoptera eridania nucleopolyhedrivurus isolate VPN165 (SperNPV-VPN165). Analysis by Scanning electron microscopy and transmission electron microscopy showed that SperNPV-VPN165 OBs are polyhedral with a mean diameter of 2.7 µm ± 0.4 and contained several virions with multiple rod-shaped nucleocapsids per envelope. Bioassays confirmed that the virus was lethal to the caterpillars of *S. eridania* at third instar with an LC50 of $1.04x10^2$ OB/ml. SperNPV-VPN165 is member of a new tentative species inside *Alphabaculovirus*, with a genome of 137.373 bp in size with a G+C content of 42.8%. We annotated 152 ORFs with 16 ORFs unique in baculoviruses. The genome has no typical homologous region. SperNPV-VPN165 isolate is closely related to the *Spodoptera*-infecting viruses, which include SeMNPV, SlNPV-II, and SpeNPV-251 viruses. Surprisingly, SperNPV-VPN165 has only one copy of *odv-e66*, a bacterial acquired *chondroitin lyase* gene, whereas sister viruses present two copies. Therefore, we took advantage of this feature, and analyzed the evolution of *odv-e66* in *Baculoviridae*. We found 13 deletions, 16 acquisitions, and 1 duplication of *odv-e66* among baculoviruses. The analyses suggest that the SperNPV-VPN165 genome seems to have independently lost this gene. The study of baculovirus allows a better understanding of the virus evolution, providing important information for the development and improvement of tools for biological control.

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Palavras-chaves: Baculovirus genome, Alphabaculovirus, Spodoptera eridania nucleoplyhedrovirus, chondroitin lyase, odv-e66

A CPD-PHOTOLYASE-CONTAINING ALPHABACULOVIRUS INFECTIOUS TO THE PLUSIINAEAN SOYBEAN LOOPER RACHIPLUSIA NU PRODUCES TETRAHEDRAL OCCLUSION BODIES AND CLARIFIES THE EVOLUTION OF DNA REPAIR GENES IN BACULOVIRUS

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Resumo

We described a novel alphabaculovirus isolated from the polyphagous insect pest *Rachiplusia nu*. After analysis by scanning electron microscopy and transmission electron microscopy, we found a peculiar feature, never described in baculovirus before: the virus presented pyramidal-shaped occlusion bodies (OBs) with singly-embed nucleocapsids. The usual OB morphology of alphabaculovirus is polyhedral. The major protein responsible for OB formation is polyhedrin and RanuNPV polyhedrin presents one punctual mutation (A197N) in comparison to other closely related polyhedral OB-forming viruses, *i.e.* ChchNPV and ChinNPV. The tetrahedral OBs allowed for a dose mortality response of 6.9×10^3 OBs/ml to third-instar larvae of *R. nu*. Moreover, it is not clear the molecular basis of OB shape in baculoviruses. As remark for this viruses, we also found in its genome several auxiliary genes with homologs in other baculoviruses, such as a *CPD-photolyase* (*phr*), responsible for removing UV-caused pyrimidine dimers in the virus genome. The lack of *phr* by other plusiinaean-infecting baculovirus genomes is rather intriguing. Since the acquisition happened by HGT from lepidopteran to baculovirus and that solely some species retained the gene, this fact prompted us to investigate what could happen with the expression of a plusiinaean host *phr* in a context of infection by a plusiinaean-isolated baculovirus that lacks *phr.* Therefore, we used as infection model the AcMNPV infecting *T. ni* and checked the expression level of the host *phr* along the infection course in midgut cells. The majority of host transcripts is downregulated during baculovirus infection since early stages (6 h p.i.). Surprisingly, we found no difference in host *phr* transcription during baculovirus infection when compared to the mock-infection control. Indeed, the host *phr* transcription in the midgut is not changed by the virus infection and seems to be advantageous maintaining the transcripts level throughout the infection. Importantly, baculovirus infection triggers a positive phototactic response in caterpillars to induce 'tree-top' disease, a host behavior manipulation that enhances virus transmission and survival. Therefore, the UV exposure intensification might be counteracted by the virus to avoid DNA damage by pyrimidine dimer formation.

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Palavras-chaves: Tetrahedral occlusion bodies, Alphabaculovirus, Rachiplusia nu nucleopolyhedrovirus, Photolyase, Plusiinae

THE VIROME OF WILD ACCESSIONS OF CAPSICUM SPP.: LOW DIVERSITY OF VIRUS SPECIES MAY SUGGEST NEW SOURCES OF RESISTANCE TO PLANT VIRUSES.

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Resumo

The Andean region in South America is the primary center of origin and diversity of the *Capsicum* genus. To characterize the viral diversity in wild accessions of *Capsicum* spp., samples of *C. baccatum*, *C. chinense* and *C. frutescens* from several localities were cultivated under high inoculum pressure. Frozen leaves samples were used for total RNA extraction using the commercial kit RNeasy Plant Mini kit. The samples were pooled together according to the *Capsicum* species, and the libraries were prepared using TruSeq Library Prep Kit and sequenced by Illumina HiSeq 2500 platform at Macrogen Inc, South Korea. The resulting reads: 24.527.146 (MS3=*C. frutescens*), 31.495.892 (MS4=*C. chinense*) and 29.188.784 (MS5=*C. baccatum*) were quality trimmed, and *de novo* assembled using CLC Genomics Workbench version 6.3. All contigs were compared to a viral protein RefSeq database using Blastx implemented in Geneious R11. All sequences with hits matching the viral database were then subjected to a Blastx search against the nr database. To confirm the assembly results and further extend incomplete genomes, trimmed reads were mapped back to the viral contigs and reassembled, until genome completion or no further extension. The final sequences of the virus genomes were obtained from the majority consensus of the mapping assembly and annotated using Geneious R11. In MS3 pool, only one contig related to probably new *Solendovirus* was found with 170.651 reads mapped. In MS4 pool, only one contig related to Pepper mild mottle virus-PMMoV was found with 25.838.128 reads mapped. In MS5 pool, only one contig related to PMMoV was found with 19.393 reads mapped. Overall, we sequenced 36 accessions, and only three viruses were found. Even Capsicum frutescens endornavirus 1-CFEV 1, previously reported from wild accessions from South America was not found. Indeed, disease and infection risk increases with the level of human management and appears to be associated with low species diversity, low genetic diversity, and high host plant density. However, the reduced number of viruses found infecting the wild accessions may suggest they are resistant to common viruses described in *C. annuum* from Ecuador, since all accession, including *C. annuum*, were cultivated in the same area. Therefore, an initial virome characterization may assist the identification of accessions without viral infection and/or with a lower virus accumulation, helping in the search for sources of resistance to plant viruses.

Palavras-chaves: CAPSICUM, NGS, RESISTANCE, WILD SPECIES

HIGH-RESOLUTION METATRANSCRIPTOMIC REVEALS SEVERAL NEW VIRUSES IN CAPSICUM ANNUUM SAMPLES COLLECTED IN ECUADOR

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Resumo

The Andes is considered the primary center and diversity of *Capsicum* spp., therefore, it is likely that this region harbors great viral diversity. Aiming to reveal the viral diversity in *C. annuum* from Ecuador, several samples with typical viral symptoms were collected in Guayas, Manabí and Santa Elena provinces and stored at -80 °C. Frozen leaves samples were used for total RNA extraction using the commercial kit RNeasy Plant Mini kit. The samples were pooled according to the sampling location, and the libraries were prepared using TruSeq Library Prep Kit and sequenced by Illumina HiSeq 2500 platform. The raw reads (37.696.482=GP1, 29.129.762=MC1, 31.686.936=MC7 and 27.772.706=SE6) were quality trimmed, and de novo assembled using CLC Genomics Workbench version 6.3. The resulting contigs were compared to a viral protein RefSeq database using Blastx implemented in Geneious R11. All sequences with hits matching the viral database were then subjected to a Blastx search against the nt database. To confirm the assembly results and further extend incomplete genomes, trimmed reads were mapped back to the viral contigs and reassembled, until genome completion or no further extension. The final sequences of the virus genomes were obtained from the majority consensus of the mapping assembly and annotated using Geneious R11. Blastx comparisons revealed great plant viruses diversity. In SE6 pool, we were able to identify Bell pepper endornavirus-BPEV, Cucumber mosaic virus-CMV, Melon yellow spot virus-MYSV, Papaya ring spot virus-PRSV, Pepper mild mottle virus-PMMoV, Peru tomato mosaic virus-PTV, Tomato yellow vein streak-ToYVSV, two contigs were to news *Enamovirus* and *Solendovirus*. In MC7 pool, we found BPEV, Capsicum frutescens endornavirus 1-CFEV, Maize yellow dwarf virus-MYDV, PMMoV, and PTV. In MS1 pool, we found contigs related to BPEV, Pepper cryptic virus 1-PCV1, Pepper cryptic virus 2-PCV2, PMMoV and one contig related to a new *Solendovirus*. In GP1 pool, contigs related to BPEV, CMV, PMMoV, PTV and five contigs were related to probably novel viruses belonging to *Enamoravirus*, *Luteovirus*, *Potexvirus*, *Potyvirus*, and *Umbravirus*. Sequencing only 29 plants we identified 11 known viruses and six previously unknown viral species in *C. annuum*. Overall, our results suggest that many plant viruses remain to be discovered, and that sampling at the primary center of genetic diversity may increase the discovery rate of novel viruses of the cultivated plants.

Palavras-chaves: DISCOVERY, DIVERSITY, NGS, PEPPERS

CONSTRUCTION OF INFECTIOUS CLONE OF CUCURBIT APHID-BORNE YELLOWS VIRUS BRAZILIAN MELON ISOLATE

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Resumo

The Northeast region is the largest melon producer in Brazil, contributing about 90% of the national production. Brazil occupies the 11th place in the world ranking of melon production, being this fruit one of the most exported in recent years in the country. The main disease of melon crop in Brazil is caused by virus called “Amarelão do meloeiro”. This disease is associated with the carlavirus (*Betaflexiviridae*) *Melon yellow-associated virus* (MYaV) and the polerovirus (*Luteoviridae*) *Cucurbit aphid-borne yellows virus* (CABYV). CABYV was detected by the Next Generation Sequencing in Brazil in 2018. CABYV genomic RNA is about 5.7kb and encapsidated in icosahedral particle. The symptoms caused by solely CABYV in melon are not well-elucidated due to the mixed infection with MYaV. Therefore, the objective of this work was to construct an infectious CABYV clone. Total RNA was extracted from M3 isolate of CABYV using silica-based nucleic acid extraction protocol. The complete CABYV genome was amplified in two fragments by RT-PCR. For this, cDNA was synthesized with random or specific reverse primer using SuperScript IV reverse transcriptase. The 5' and 3' region fragments were amplified and, then, cloned in pCR4 Topo cloning kit using *Escherichia coli* (DH10B). The two fragments were reamplified and, then, joined by Gibson Assembly using pJL89 as background vector. The construct was cloned into *Agrobacterium tumefaciens* (GV3101). The sequence of clones obtained from *E. coli* and *A. tumefaciens* was confirmed by Sanger sequencing. To evaluate the infectious clone, CABYV clones were agroinoculated in cucumber (redneck and Japanese), melon and *Nicotiana benthamiana* plants and the infection was evaluated by RT-PCR 10 days post-agroinoculation (dpa). Strong chlorosis symptoms were observed in all *N. benthamiana* leaves. In cucumber and melon plants, symptoms of internerval chlorosis were observed 10 dpa. CABYV was detected by RT-PCR with specific primers in all plants evaluated in the experiment. In conclusion, the complete CABYV M3 isolate genome clone obtained from the Gibson Assembly methodology is infectious.

Finnatial Support: CNPq, UnB.

Palavras-chaves: CABYV, Infectious clone, Polerovirus

TWO NEW VIRUSES NATURALLY FOUND CO-INFECTING LEGUMINOUS FORAGE PLANTS IN BRAZIL, BELONG TO A NEW PUTATIVE GENUS OF THE POTYVIRIDAE FAMILY TRANSMITTED BY WHITEFLY

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Resumo

In Brazil, forage crops represent large areas of tropical pasture for cattle feeding. These pastures can be natural or planted and are composed of grasses and forage legumes. The use of forage legumes of the genus *Stylosanthes* has increased in Brazil due to its nutritional value and great potential of nitrogen fixation. Recently, virus-like mosaic symptoms were observed in *Stylosanthes guianensis* cv. Mineirão in the experimental fields of the Embrapa Gado de Corte. These samples were collected and submitted to high performance sequencing (HTS) in the Plataform Illumina HiSeq 2000. The sequences obtained were assembled using the CLC Genomics Workbench 7.0 program and after that, were analyzed in the BLASTX program against the database (GenBank). The results obtained in these symptomatic samples revealed the presence of two new viruses belonging to the *Potyviridae* family and have been tentatively named as Stylosanthes Mosaic-associated virus 1 (StyMaV-1) and Stylosanthes Mosaic-associated Virus 2 (StyMaV-2). Simultaneously, the contigs were assembled and then RT-PCR detection tools were developed with specific primers from the consensus. The fragments were amplified, sequenced by the Sanger method and comparative phylogenetic analyzes of nucleotides and amino acids of the viral proteins based on the polyprotein were performed. These analyzes demonstrated that these viruses are new genus within the family and have been tentatively named Stylomovirus. Based on the taxonomic criterion of demarcation of species of the *Potyviridae* family, which is related with the sequences identity of the nucleotide and amino acid of the protein coat (CP), the StyMaV-1 and StyMaV-2 viruses have 46% and 44% of the nucleotide sequence identity (respectively) with *Blackberry Virus Y* (*Brambyvirus*). In the biological assay these viruses were transmitted mechanically to *N. benthamiana* and different varieties of *Glycine max*. A whitefly transmission assay was performed and the results revealed transmission of the "Stylomovirus" by this vector. The present work aims to study the virus/host/vector interaction to understand the existing epidemiological relationship and propose effective control measures.

Financial Support: CAPES; CNPq; UnB.

Palavras-chaves: Beef Catle, forrage, legume, HTS, Stylosanthes

PEPPER MILD MOTTLE VIRUS (PMMOV) DETECTED IN IMPORTED QUARANTINE CHILI PEPPER GERMPLASM

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Resumo

Recently (2019) the presence of *Pepper mild mottle virus* (PMMoV) was detected in imported chili pepper (*Capsicum* sp.) seeds through visual analysis of symptoms in chili pepper plants (germinated seeds), mechanical inoculation of indicator plants with symptomatic leaf extract of chili pepper and Enzyme-Linked Immunosorbent Assay (ELISA). A symptomatic chili pepper plant was identified, whose extract mechanically inoculated in indicator plants generated typical symptoms of virus in sweet pepper (*Capsicum annuum*). ELISA confirmed the presence of PMMoV as well as absence of *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Tomato bushy stunt virus* (TBSV) and *Potyvirus*. Polymerase Chain Reaction (PCR) molecular tests confirmed absence of *Potyvirus, Tobamorivus and phytoplasma*. As PMMoV is a non-quarantine pest and is already present in Brazil, the corresponding chili pepper seed samples were released for importation, with the information of PMMoV presence. Consultation of scientific publications and discussion with virologist colleagues corroborated decision making. Therefore, the detection of PMMoV in this quarantine importation process of chili pepper seeds exemplifies the relevance of the plant quarantine service of import / export of materials for research. In addition, this service is effective preventive control of plant viruses and contributes to impacting scientific research on plant genetic improvement and phytosanity as well as for agriculture and national food security within Brazil. This work is aligned with the Brazilian policy, which promote prevention and surveillance of quarantine absent pests, according to the "Portaria nº 131, 27/jun/2019 - Programa Nacional de Prevenção e Vigilância de Pragas Quarentenárias Ausentes (PNPV-PQA)".

Financial support: Empresa Brasileira de Pesquisa Agropecuária - Embrapa

Palavras-chaves: Prevention, Quarantine, Serological-molecular diagnosis, Surveillance, Vegetables

P0 AND P4 FROM CLRDV SHOW SYNERGIST EFFECT WITH VIRUS FROM DISTINCT FAMILIES

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Resumo

Polerovirus are ssRNA+ virus largely distributed around the world imposing severe lost in agriculture. Virus of this genus impact potato, pepper and other solanaceas plants as well beet and even cotton crops. In South America, especially in Argentine and Brazil, cotton diseases associate to CLRDV are largely crops. The suppressor of gene silencing protein of CLRDV, the P0 protein, was already reported as a possible effector of disease and is associated to virulence. It was also already shown for PLRV and CABYV that the movement protein P4 may induce local silencing suppression (Fusaro et al., 2017). In order to understand the paper of CLRDV PO and P4 during virus infection, infectious clones of PVX and TRV viruses presenting a GPF insertion were agroinfiltrated in *Nicotiana benthamiana*plants in the presence of CLRDV P0 and/or P4. We observed the induction of necrotic areas in the agroinfiltrated leaves when PO was co-infiltrated with TRV. P0 also improved the systemic spread of TRV. CLRDV P4 however didn't affect TRV systemic movement but induced a faster local cell-cell movement. Necrosis wasn't induced by CLRDV P4. When TRV was co-infiltrated with both P0 and P4, a stronger local and systemic spread of the virus was observed, showing synergistic effects. Looking for PVX, we could observe the induction of strong necrosis in the infiltrated area and in systemic leaves in all proteins combinations. The induction of necrosis for PVX was associated with the presence of the SSP P19 from TSBG in all infections. However, it could be observe that the virus was able to leave necrotic tissues and spread to healthy parts of the leaves. The presence of P4 increased the number PVX spots both locally and systemically. P0 however seems to reduce PVX systemic spread. We can conclude that in P0 and P4 could act in a synergistic way with virus from these two other families, probably helping them to evade the anti-viral silencing defense.

Palavras-chaves: P0, P4, CLRDV, synergy