Posters Plant and Invertebrates Virology - PIV
PIV734 - EFFECTS OF THE PYRA- CLOSTROBIN + METIRAM IN THE EXPRESSION OF ENZYMES RELATED TO THE RESISTANCE INDUCTION IN PLANTS INFECTED WITH TOSRV


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In the Americas, diseases caused by begomoviruses have been causing significant damage to tomato production, and the incidence can be severe enough to make impossible the commercial cultivation in certain regions. Therefore this study aims to verify the performance of the tomato treated with Cabrio Top® (pyraclostrobin + metiram) in terms of resistance induction. The experiment was carried out at the Department of Vegetal Production, UNESP, Botucatu Campus, during 30 days after viral inoculation (DAI) with ToSRV. 35 tomato seedlings of cv. Mariana were prepared, and at the time of sowing the plants were divided into two groups; one without product (T1, T2, T3), and the other receiving treatment (product Cabrio top® (metiram + pyraclostrobin) 3 g / L H2O + 0.3 g / L H2O of Cantus® (Boscalida)) (T4, T5, T6, T7). Seedlings derived from the group 1 received the following treatment: T1-No Viruses, and no application of Cabrio Top®, T2-Virus inoculated, no application of Cabrio Top®, and T3- Virus inoculated, and application of Cabrio Top® (3:18 DAI). Group-2: T4-Virus inoculated, and application of Cabrio Top® (3 DAI). T5-Virus inoculated, and application of Cabrio Top® (18 DAI). T6-Virus inoculated, and application of Cabrio Top® (3:18 DAI). T7-Virus inoculated, and no application of Cabrio Top®. The collection of material for peroxidase biochemical analysis (POD) and polyphenoloxidase (PPO), was carried out at 5 and 10 days after treatment application. The product used (Cabrio Top® at a dosage of 400 g/100L water) induced alterations in plants metabolism and growth, increased the chlorophyll content, and masked the symptoms of the disease, when compared with untreated controls. Higher levels of POD and PPO were observed in plants leaves treated at sowing, and subsequently sprayed two times (3 and 18 DAI), suggesting their involvement in the mechanisms of resistance induction. Therefore, the treatment 6 proved to have the best results, thus may be more appropriate.

PIV735 - EFFECTS OF THE APPLICATION OF CHEMICAL PRODUCTS IN TOMATO GROWN IN THE PERIOD OF INCIDENCE OF TOSPOVIRUS


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The tomato crop has great economic importance in Brazil and is subject of several diseases, and depending on the level of genetic resistance of the cultivar used, it can limit its production. The Tospovirus are considered causal agents of one of the most important diseases of tomato crops, resulting in enormous economic damage. The objective of this work was to evaluate the effect of resistance induction in the tomato crop, as well as the incidence of Tomato spotted wilt virus. The experiment was carried out in the Experimental Farm of UNESP Botucatu, in an area of historical occurrence of the disease. Were used 3200 tomato plants cv. Saladinha Plus. The trays were divided in two groups; those that received treatment (product Cabrio Top® (pyraclostrobin + metiram) 3 g / L H2O + 0.3 g / L of H2O Cantus® (Boscalida)) at sowing, and those that didn’t receive the application. The transplantation was followed by treatments with Cabrio Top® 200g/100 L H2O and 400g/100 L H2O, Serenade® (Bacillus subtilis) 400mL/100 L H2O and standard treatment (Tamaron®) control. The spraying was carried in all treatments at 20, 35, 50 and 65 days after transplanting. The crop was harvested at 80 DAT, and the analyses of total production were carried out, including average fruit weight and percentage of stain’s absence in fruits per treatment. By the end of the experiment it was observed that the disease incidence was 13.75% in the plants that have received the product in the sowing stage, and 16.75% in the plants that received the grower’s standard treatment. The Cabrio Top® 400g/100 L H2O treatment presented 8.00% incidence of the disease, higher percentage of stains absence in fruits, increased the total production and average fruit weight. Therefore, the treatment 2 showed the best results, thus this may be the more appropriate. Financial Support: FAPESP

PIV737 - GENETIC VARIABILITY OF BRAZILIAN PASSION-FRUIT COWPEA APHID-BORNE MOSAIC VIRUS ISOLATES REVEALED BY PHYLOGENETIC ANALYSIS OF THE CYLINDRICAL INCLUSION GENE


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The woodiness disease of passion-fruit (Passiflora spp.), induced by Cowpea aphid-borne mosaic virus (CABMV), is the main viral disease of this crop in Brazil. In order to evaluate the genetic variability of Brazilian passion-fruit CABMV isolates, leaves showing mosaic and blister symptoms, from different passion-flower producing areas in Sao Paulo (municipalities of Adamantina, Alvinlandia, Bauru, Campinas, Fernao, Garca, Jacupiranga and Monte Alegre do Sul), Parana (Ibaiti and Sao Jose da Boa Vista) and Goias (Planaltina and Urutai) were submitted to molecular analysis. After total RNA extraction and RT-PCR with degenerated primers, 700 bp DNA fragments correspondent to cylindrical inclusion (CI) gene were successfully amplified and sequenced. Comparisons with other CI sequences from Genbank (accession codes AF348210, HQ880242 and HQ880243) were done using Basic
Local Alignment Search Tool (BLASTn) program of the National Center for Biotechnology Information (NCBI), and multiple alignments for the nucleotide sequences were done manually. Trees were constructed by maximum likelihood using PAUP 4.0b10, with HKY+I nucleotide substitution model, base frequencies parameters f(A)=0.33, f(C)=0.21, f(G)=0.20, f(T)=0.26, and transition/transversion ratio of 6.1117. Passionfruit woodiness virus sequence (accession code HQ122652) was used as outgroup and bootstrap percentages values were computed after 1,000 resamplings. The phylogenetic analyses revealed that CABMV isolates from Sao Paulo, Parana and Goias clustered in three different clades of the other CABMV isolates stored in the Genbank. The isolates from Sao Paulo state were distributed in two monophyletic groups, supported by 100% bootstrap values. The isolates from Goias clustered together, while those isolates from Parana had a behavior similar to the Sao Paulo isolates. These results indicate a high genetic variability among Brazilian CABMV passion-fruit isolates. Financial Support: FAPESP (Proc. 2011/11796-5)

PIV743 - GENETIC ANALYSIS OF GARLIC VIRUS B AND THE DISTRIBUTION OF THE DIFFERENT SPECIES OF ALLEXIVIRUS IN GARLIC PRODUCING AREAS OF BRAZIL

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Garlic virus B (GarV-B) is one of several members of the genus Allexivirus (family Alphaflexiviridae), all of which have been detected only in Allium spp. and are thought to be transmitted by mites. The dissemination and accumulation in bulbs of garlic is favored by vegetative propagation. Thus, mixed infections between the different species of allexivirus were frequently found in garlic. In Brazil, Garlic virus A (GarV-A), Garlic virus B (GarV-B), Garlic virus C (GarV-C), Garlic virus D (GarV-D) and Garlic mite-borne filamentous virus (GarMbFV) are allexivirus found infecting garlic, but there is insufficient research about GarV-B. Therefore, a specific primer pair named CPBS2 and CPBA1 was designed to amplify the partial coat protein gene from the GarV-B and used for a preliminary detection. A specific primer pair called GarV-BS1 and GarV-BA2 was constructed to amplify the complete coat protein gene from GarV-B and utilized for genetic analysis. The CP nucleotide identity between the GarV-B isolates was 88% to 96% compared with GarV-B sequences deposited in GenBank. Two distinct phylogenetic groups were found for Brazilian isolates of GarV-B, and they were not correlated to the localities where samples were collected. One isolate collected in Sao Manuel (25.31) formed a phylogenetic branch with the isolate JN019813.1 of GarV-B from Australia. The others six isolates grouped in a separated branch. From a total of 340 symptomatic plants analyzed, 139 were positive for the presence of allexivirus and 61 samples had mixed infection. From the total, 76 were infected by GarV-A, 26 with GarV-B, 38 with GarV-C and 97 with GarV-D. GarV-A and GarV-D were frequently found in mixed infections.
PIV772 - ORCHIDS OF SÃO PAULO STATE: SURVEY OF THE VIRUSES OCCURRING AND THE CYMMV CP GENE BASED VARIABILITY

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Viruses are a major problem in orchid’s production systems, as they promote considerable damage to its commercial value, since they directly affect its aesthetic appeal as well as productivity. In world terms, the potexvirus Cymbidium mosaic virus (CymMV) and the tobamovirus Odontoglossum ring spot virus (ORSV) are of greater economic importance. A survey of the viruses present in 232 orchid samples belonging to different genres and localities from 12 São Paulo State cities was carried out. The diagnosis was made by direct ELISA test for the detection of CymMV, ORSV, TSWV (Tomato spotted wild virus), and CymRSV (Cymbidium ring spot virus), as well as indirect ELISA for Potyvirus detection, using universal anti serum against this genre. The results were confirmed by RT-PCR, with specific primers designed for the CP gene of the CymMV and ORSV, as well as for the detection of Orchid fleck virus (OFV). Of the total of samples tested, 153 were positive for CymMV presence (65,9%), and 40 were positive for ORSV (17,2%). CymRSV, TSWV, OFV and Potyvirus were not detected on the samples analyzed. In 90% of the samples in which ORSV was detected, CymMV was also detected in mixed infections. Nucleotide identity of greater than 97% with CymMV Genbank accession numbers AY571289.1 and AB197937.1 was observed for CymMV isolates. These results denote the CymMV importance in the orchid’s production systems in São Paulo state, as well as possible synergistic link between this virus and ORSV. Financial support: FAPESP

PIV798 - MICRORNAS AS REFERENCE GENES FOR QUANTITATIVE GENE EXPRESSION STUDIES USING RT-QPCR DURING VIRAL INFECTION IN COTTON

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Reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) is a technique largely used in the investigation of gene expression. The selection of reference genes is a crucial factor for correct analysis and interpretation of their results. Find good reference genes to the study of endogenous genes during plant virus infection is not easy. Here we propose the use of miRNAs as reference genes under virus infection in cotton. The miRNAs have important regulatory roles in eukaryotic acting in at post-transcription level at different biological functions. In the present study, we evaluated, for the first time, cotton microRNAs as candidates as reference genes. The stability of miRNAs and mRNAs in Gossypium
hirsutum in different tissues (root, stem, leaf and flower), different cultivars (FiberMax 966, Delta Opal and Cedro) and under biotic stress caused by infection of Cotton leaf roll dwarf virus (CLRDV) was analyzed. Cotton samples were organized in 12 sets where the expression stabilities of the six miRNAs and five mRNA reference genes were evaluated. Four algorithms, geNorm, NormFinder, BestKeeper and ΔCt were used to identify the stability of these genes. In 8 of the 12 sets tested, the miRNAs were the best reference genes. We validated them in expression assays performed in study cases. For that, we studied the expression of miRNAs and mRNA already know in leaves of “cv FM966” under stress biotic. We evaluated the miR2910, miR164, miR2118, miR3476, miR159, and the mRNAs GhDCL1, GhDCL2, GhDCL3 and GhDCL4. Statistical test p sustained the expression levels of the differentially expressed miRNA and mRNA even under biotic stress. These analysis also showed that to normalize mRNA we can use miRNA or mRNA as reference genes, although to normalize miRNA expression levels, miRNA reference genes seems to be better than mRNAs. The indication that for miRNA expressions analyzes we should use miRNA as reference genes instead of mRNAs was already well supported in human miRNAs studies, however this is the first study were this is shown in plants. Financial Support: FAPERJ, CAPES

PIV843 - BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF A CARLAVIRUS SPECIES INFECTING SOYBEAN IN BRAZIL

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Viral species of the Carlavirus genus (family Betaflexiviridae) have a positive ssRNA genome of 7.4-8.9 Kb, consisting of six ORFs, and are insect and mechanically transmissible. Symptomatic soybean samples (dwarfing and stem necrosis) were collected in Goiás state in the season 2000/01. The symptoms were associated with a disease caused by Cowpea mild mottle virus (CpMMV). To confirm this, the aims of this study were biological and molecular characterization of viral species infecting soybean in Goiás state, Brazil. For host range studies the sap from infected soybean leaves was mechanically inoculated into 21 species/cultivars belonging to 5 families (Amaranthacea, Chenopodiaceae, Cucurbitaceae, Solanaceae and Fabaceae). The symptoms were evaluated periodically and indirect enzyme linked immunosorbent assay was used to confirm infection. To amplify the complete genome, the total RNA was extracted from soybean plants cv. CD206, followed by RT-PCR. The nucleotide sequences obtained were assembled using DNA BASER sequence assembler v.3.5, and the ORFs were determined using ORF Finder. Additional pairwise nucleotide sequences comparisons were analyzed in MEGA v.5.0 and phylogenetic trees were constructed using Bayesian inference. The viral isolate was able
to infect Glicine max (cvs. CD206 and Pintado), Phaseolus vulgaris (cvs. Jalo and Manteigão), Vigna unguiculata, Nicotiana debney, N. benthamiana, Chenopodium quinoa and C. amaraticolor. Analysis of the complete genome showed typical characteristics of carlaviruses, with six ORFs and 8.198 nt in length. The pairwise nucleotide sequences comparisons for the different ORFs and phylogenetic analysis showed a higher closely related to Ghana isolate of CpMMV. Although for the capsidial protein the nucleotide sequence identity was 79% (according to ICTV when CP and replicase ORFs are ≥ 72% isolates can be considered the same species), while for the replicase identity was 61%, suggesting that this isolate is a new species. Financial support: Fapemig, CNPq

PIV901 - PRODUCTION OF POLYCLONAL ANTISERUM AGAINST THE MOVEMENT PROTEIN NSM OF BEAN NECROTIC MOSAIC VIRUS (BENMV): A TOOL FOR STUDYING VIRUS/HOST INTERACTIONS

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Given Tospoviruses’ great capacity to adapt to different host plant species, understanding both viral cell to cell movement and long distance movement in plants can shed light on the processes driving host-pathogen interactions. The elucidation of these processes can reveal the host proteins involved, as well as the dynamics of viral infection. Thus, the present study aimed the production of a polyclonal antibody against the NSm protein of Bean necrotic mosaic virus (BeNMV), as an essential tool for allowing the studies of host pathogen interaction. The NSm gene of BeNMV was amplified by RT-PCR with primers containing specific sites, then the segment was assigned to the input vector pENTR11 (InvitrogenTM) and finally introduced to an expression vector pDEST15 (InvitrogenTM). The NSm protein was expressed in E.coli cells and purified by gel electrophoresis. Purified fractions of 100ug were then used to immunize four mice, and 30 days after injection total proteins was collected from blood plasma. A western blot analyzed extracts from bacteria expressing the protein NSm BeNMV and the expected staining was clearly observed, demonstrating the efficiency of immunization and specificity of the antibody produced. The antibody produced will be used for further understanding the interaction of the NSm protein of BeNMV with host proteins employing techniques such as, co-immunoprecipitation and finally the identification of host proteins associated with NSm by amino acid sequencing. Financial support: Capes, CNPq, UnB, FAP-DF

PIV957 - ANALYSIS OF TRANSIENTE EXPRESSION OF THE MOVEMENT PROTEINS (NSM) OF DIFFERENT BRAZILIAN TOSPOVIRUS IN HOST PLANTS BY CONFOCAL MICROSCOPY

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The genome of plant viruses encodes one or more proteins involved in virus movement. In the genus Tospovirus, the movement protein (NSm) is encoded by the M segment of the viral genome. This protein seems to bind specifically to nucleic acids, however, has the capacity to modify plasmodesmata and is involved in tubule formation on the surface of protoplasts. The present study aimed to evaluate the cellular location and dynamic motion of the protein NSm of two species of tospovirus: Bean necrotic mosaic virus (BeNMV) and Tomato chlorotic spot virus (TCSV). These viruses show a significant difference compared to the spectrum of hosts, a biological characteristic that may be associated with the function of their movement protein. The NSm protein genes of BeNMV and TCSV were amplified by RT-PCR and cloned into vector target pENTR11 (InvitrogenTM) at specific sites using the Gateway recombination system, and subsequently, the insert was cloned into the viral vector PVX-GW-GFP. Host plant leaves were agroinoculated with the constructs and 10 days after, viral infection was monitor by fluorescence of the GFP protein fused to viral movement protein. The viral vector PVX-GW-GFP was used as mock control experiment. In TCSV, was observed an association of the movement protein with the chloroplast, type of location that hitherto has not been observed in Tospovirus and Potexvirus infection. In BeNMV, no association was observed with the chloroplast. Financial support: Capes, CNPq, UnB, FAP-DF Field of Virology: vegetable virus

PIV989 - CEREBRAL GANGLION OF BOMBYX MORI INFECTED BY ALPHABACULOVIRUS


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Bombyx mori (Lepidoptera; Bombycidae) is a holometabolous insect, which in the larval stage of its life cycle spins a cocoon of silk used in the production of yarns and fabrics. In the state of Paraná sericulture is an important part of family farming and the production of cocoons can be affected by a number of factors such as viral diseases. The Bombyx mori Nucleopolyhedrovirus (BmNPV), belonging to the Alphabaculovirus (AlphaBV) genus, has been isolated from larvae in Paraná and studies have demonstrated the susceptibility of various tissues of B. mori, including nervous tissues. However, the data did not show cytopathological details of infection in the cerebral ganglion of the insect and furthermore, previous studies have focused on multiple Bombyx mori Nucleopolyhedrovirus (BmMNPV). Thus, this study aims to analyze the infection caused by BmNPV in the cerebral ganglion of B. mori. To this end, we performed an experiment where larvae of 5th instar B. mori were fed with mulberry leaf discs containing...
the viral inoculum BmNPV and water (control). After ingestion of the disks, and on different post inoculation days (dpi), from the 3rd to the 9th dpi, the larvae were anesthetized, dissected, and the cerebral ganglia were collected and fixed in DuBosq Brazil. After fixation, the material followed routine histological processing for embedment in paraaffin. The slides were stained with Azan technique modified for viral occlusion bodies. Infected cells from the cerebral ganglion of B. mori were present from the 5th dpi and cytopathological characteristics were consistent with literature data for AlphaBV, i.e., nuclear hypertrophy containing the viroplasm and viral polyhedra in various quantities and sizes. Furthermore, the methodology used revealed that the cytopathology of BmNPV does not differ significantly different from that caused by BmMNPV.

PIV995 - MOLECULAR CHARACTERIZATION OF TOMATO-INFECTING BEGOMOVIRUSES IN THE AGRESTE REGION OF ALAGOAS STATE, BRAZIL


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Begomoviruses (family Geminiviridae) are transmitted by whitefly and have circular, single-stranded (ss) DNA genomes (DNA-A and DNA-B) encapsided in twinned quasisicosahedral particles. Begomoviruses constitute one of the most economically important groups of plant viruses because of their high incidence and severity of diseases they cause in vegetables and field crops throughout tropical and subtropical regions. In Brazil, begomoviruses are considered limiting factors for tomato production and since of their first report in tomatoes six species have been described. The emergence of novel begomoviruses in tomatoes has been attributed to the introduction and spread of the B biotype of Bemisia tabaci in mid-1990s. The present work aimed to accomplish the molecular characterization and analysis of genetic diversity of tomato-infecting begomoviruses from Agreste region in the state of Alagoas. Tomato samples were collected in county of Arapiraca in 2011. Total DNA was extracted from each sample and used for PCR reactions. Partial sequencing of 23 PCR amplicons revealed that at least two begomoviruses species was present. Five full-length viral genomes were amplified using the DNA polymerase from phage phi29, cloned into plasmid vectors and completely sequenced. DNA-A nucleotide sequences were compared to those of previously characterized begomoviruses species, and the ICTV-established 89% threshold identity level was used to identify the species. Sequence analysis indicates the presence of two species. Four full-length clones corresponded to an isolate of Tomato mottle leaf curl virus, a begomovirus species predominant. The analysis of 2400 nucleotides of clone BR:Ara1:11A, suggests that an isolate of Bean golden
mosaic virus may be present in tomato crops in Arapiraca. However, the actual taxonomic status of the virus isolate may be defined by complete sequencing of the genome.

PIV1008 - BOMBYX MORI MALPIGHIAN TUBULES ARE NOT INFECTED BY ALPHABACULOVIRUS


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Alphabaculovirus (AlphaBV) are entomopathogenic viruses that infect several insect orders, as the mulberry silkworm, Bombyx mori (Lepidoptera, Bombycidae). B. mori is an useful insect to man, since their cocoons are used for sericulture in the production of silk. However, this production can be affected by a number of factors such as viral disease. A virus belongs to Baculoviridae family and AlphaBV gender, was isolated from B. mori larvae, the BmNPV. Various tissues were established as targets of BmNPV, however, there is little information regarding the susceptibility of B. mori Malpighian tubules. This organ is responsible for body homeostasis, since it is involved in maintaining constant levels of salts, water, osmotic pressure of the circulating liquid as well as the disposal of toxic metabolic waste products. Thus, aiming to understand the behavior of the Malpighian tubules against viral infection, matrices of B. mori larvae M19-2, 5th age, obtained from the germplasm bank of the State University of Maringá, were inoculated with a viral suspension of BmNPV. On different days post inoculation (dpi), the larvae were anesthetized and prepared for light microscopy analysis. The material was stained by the Azan technique modified for viral occlusion bodies. The results revealed that, in any of the periods analyzed and the methodology employed, the cells of the Malpighian tubules not showed evidence of BmNPV infection. The presence of viral polyhedra into the extracellular medium, resulting from their multiplication in other cells, which are known targets of the virus undergo lysis at the end of their reproductive cycle, confirming the viability of the inoculum used.

PIV1011 - STUDIES OF THE SUBLOCATION AND PHYLOGENY OF A RECEPTOR-LIKE PROTEIN KINASE MODULATED BY PEPPER YELLOW MOSAIC VIRUS INFECTION IN TOMATO PLANTS


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Plants are often exposed to various biotic and abiotic stresses that interfere with endogenous gene expression patterns. Previous studies on differential gene expression in tomato plants infected with PepYMV (Pepper yellow mosaic virus) revealed a repressed expression of a gene encoding a receptor-like protein
kinase (RLPK). RLPKs are normally cell-wall associated proteins that allow the cell to recognize and respond to the extracellular environment, being an important component of the signal transduction pathway involved in resistance to pathogens and response to abiotic stress. The aims of the present study were: first, to investigate the subcellular localization of the mentioned tomato RLPK; and second, to determine its phylogenetic relationships with other known protein kinase receptors. For such, the entire RLPK open reading frame was fused to the green fluorescent protein (GFP) and transiently expressed in Nicotiana benthamina leaves using agroinfiltration. The localization of the fusion protein was examined 48-72 h after agroinfiltration using a laser scanning microscope. As a result, the RLPK:GFP fusion was found to be located at the plasma membrane of the cells. To establish the phylogenetic relationships of the tomato RLPK, the deduced amino acid (aa) sequence of its kinase domain was aligned with the corresponding domain of different receptor-like protein kinases, and a tree was constructed. The resulting tree showed that the investigated RLPK protein was closely related to a N. tabacum RLPK, named WRK, involved in N gene-dependent hypersensitive cell death in tobacco leaves. The functional role of this protein in viral infection is under investigation. Financial support: CNPq

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In the course of evolution, several plants have been developing sophisticated immunity mechanisms based on gene-for-gene interactions. In this way, some plant genes encode resistance (R) proteins that recognize specific avirulence proteins of pathogens. The Sw-5 gene isolated from tomato (Solanum lycopersicum L.) is a member of the CC-NB-LRR class of the R genes and confers resistance to various tospovirus species (Family Bunyaviridae). The objective of this work was the expression of the Leucine Rich Repeat (LRR) domain from Sw-5 protein in Escherichia coli and production of polyclonal antibodies for subsequent serological experiments. The C-terminal region of Sw-5 gene, which contains the LRR domain, was amplified from S. lycopersicum (variety Viradouro) genomic DNA by PCR using specific primers containing sites for NcoI and NotI. The amplicon (1135bp) was cloned into pGEM-T easy and subcloned into the entry vector pENTR-11. Then, the C-terminal region of Sw-5 gene was translocated into the expression plasmid pDEST-17, which fuses to the heterologous protein an N-terminal histidine tail (6xHys). The latter plasmid was introduced into E. coli (BL 21) by electroporation and the induction of the recombinant protein was performed using L-arabinose, under control of the T7 RNA polymerase promoter. SDS-PAGE and Western Blot were used to confirm the expression. The recombinant 409 amino acid protein with a predicted molecular mass of 47.3 kDa was removed from polyacrylamide gel and

PIV1037 - EXPRESSION OF THE LEUCINE RICH REPEAT DOMAIN FROM SW-5 TOSPOVIRUS RESISTANCE PROTEIN IN E. COLI

Vasconcellos, A.F., Oliveira, A.S., Resende, R.O., Leastro, M.O., Lima, R.L.
used to immunize mice. Furthermore, the produced polyclonal antibodies will be used to detect the Sw-5 protein in tomato plants through serological experiments. Such methodologies will allow assessment of Sw-5 protein expression and distribution, and characteristics of its signaling pathway involved in tospovirus resistance. Finantial support: Capes, CNPq, UnB, FAP-DF.

PIV1055 - EFFECT OF CONTIGUOUS PLANTING OF BUSHY-TYPE TOMATOES ON THE INCIDENCE OF VIRUSES TRANSMITTED BY WHITE-FLIES

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Viruses transmitted by whiteflies (Bemisia spp.) have a great economic impact in the tomato production worldwide. General control measures usually rely on the use of insecticides, but with low efficiency. The main objective of this work was to estimate the effect of contiguous planting of bushy-type tomatoes for the incidence of viral diseases in two regions, the Federal District and Goiás from March to May, 2012. Two central pivots with tomatoes (cultivar AP-533) were selected side-by-side with 15 days of planting date difference. Three plots of 15 x 15 plants were marked and weekly monitored by evaluating the presence or absence of viral symptoms. The incidence of symptomatic plants at DF in the first planting date was 95.4, and 83.8% in the second date. The incidence

in GO in the first pivot was 71.0, and 51.1% in the second. We confirmed the presence of begomovirus and/or crinivirus in symptomatic samples. In both areas, older plantings had higher incidence of viruses than the following plantings. It suggested that the presence of infected plants close to the area did not result always in a higher percentage of infected plants in subsequent plantings. A possible explanation is that viruliferous whiteflies from the first pivot may have migrated to the other pivot when the plants were older, and thus less sensitive to the viruses. The transplanting was done for the first pivot when the whitefly population was extremely high (from soybean fields), but which was followed by a drop in their population. When the new pivot was covered by young tomato seedlings, the plants from the older pivot were still attractive to the whiteflies and it did not promote the massive migration to other areas. It is preliminary concluded that contiguous tomato planting may be done if the migration of viruliferous whitefly can be controlled. It can be achieved by an efficient strategy to control the whitefly populations from tomatoes and from other cultures of the region.

PIV1064 - LOCALIZATION OF ENDOSYMBIONTS IN VIRULIFEROUS AND AVIRULIFEROUS BEMISIA TABACI POPULATIONS FROM BRAZIL

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Whiteflies Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae) harbor endosymbiotic bacteria that form intimate symbiotic associations with their host. They are responsible not only for providing supplemental diet products essential for the host survival but also to manipulate the reproduction rate of their host by male-killing, cytoplasmic incompatibility and feminization to enhance their own transmission. There are two types of endosymbiotic bacteria in whiteflies, the primary one “Candidatus Portiera aleyrodidarum” and six secondary symbionts (Arsenophonus, Cardinium, Fritschea, Hamiltonella, Rickettsia and Wolbachia), which are passed host to host by vertical transmission. The localization of the symbiotic bacteria in whiteflies depends on a series of factors as biotype population, sex, host plants and geographical locations. Here we studied the localization of three different endosymbionts (P. aleyrodidarum, Wolbachia and Hamiltonella) in viruliferous and aviruliferous whiteflies populations from Brazil using fluorescence in situ hybridization (FISH). Viruliferous whiteflies were obtained by feeding them on begomovirus-infected tomato plants and aviruliferous insects were fed in healthy cabbage plants. Adults, eggs, nymphs of various instars were collected directly into Carnoy’s fixative, decolorized and hybridized overnight in solution containing the fluorescent probes. All three endosymbionts were found to be located inside the bacteriocytes, however with a different distribution pattern. In addition, different bacterial concentration in adult males and females was found. Our results should shed light on the interactions occurring among bacterial species in whiteflies from Brazil under field conditions. Financial support: Conselho Nacional Científico e Tecnológico (CNPq), Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Embrapa

PIV1065 - A SIMPLE KIT OF PLATE-TRAPPED ANTGEN ENZYME- LINKED IMMUNOSORBENT ASSAY FOR IDENTIFICATION OF PLANT VIRUSES

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Tentative to control plant viruses without a previous diagnosis, usually results in one inadequate control. Many methods can be used for arriving in a correct virus diagnose, and the serological techniques are the most used methods for plant virus identification. The indirect enzyme-linked immunosorbent assay (indirect-ELISA) has been useful for detection of viruses in a wide range of situations, especially to test a large number of samples in a relatively short period of time. Immune-biological Companies have developed practical kits for direct ELISA or double antibody sandwich (DAS-ELISA), but neither Company has developed kits for indirect or plate-trapped antigen ELISA (PTA-ELISA). The PTA-ELISA requires a universal IgG enzyme conjugate composed of an IgG produced against the IgGs from the animal in which virus antibodies are raised. As a single universal antibody-
conjugate is used for detection of a wide range of plant viruses, the PTA-ELISA technique is more economical, practical and suitable for virus detection in disease diagnosis programs. Considering also the great problem of including infectious plant viruses in DAS-ELISA kits, a simple kit for PTA-ELISA was developed for plant virus identification. Extracts from infected plant tissues were added into the ELISA plate holes, which were sealed with plastics and maintained in the refrigerator and Laboratory conditions for different periods of time. After 10-days intervals the plates were tested by the regular PTA-ELISA method. After 60 days of incubation, the plate trapped antigen showed excellent results when used for detection of six virus species from the genera Comovirus (Squash mosaic virus, SqMV and Cowpea severe mosaic virus, CPSMV), Cucumovirus (Cucumber mosaic virus, CMV), Potyvirus (Cowpea aphidborne mosaic virus, CABMV and Zucchini yellow mosaic virus, ZYMV) and Sobemovirus (Papaya lethal yellowing virus, PL) in infected plant tissues. The ELISA absorption reading values for all the ELISA plate holes previously treated individually with SqMV, CPSMV, CMV, CABMV, ZYMV and PL were over three times the values obtained for the respective controls with extracts of healthy plants. The plate trapped virus together with its specific antiserum could constitute a simple PTA-ELISA kit, which will permit the ex-change of antisera between virologists without transferring infectious viruses from one laboratory to another to be used as control.

PIV1086 - MIXED INFECTION OF VIRUS AND FUNGI IN BOUGAINVIL-
PIF-2 GENE OF PSEUDOPLUSIA INCLUDES SNPV ISOLATES


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Pseudoplusia includens single nucleopolyhedrovirus (PsinSNPV) is a baculovirus pathogenic to Pseudoplusia includens caterpillars, a pest that cause considerable economic losses. Recently, seven isolates of this virus (PsinSNPV IA to IG) obtained from P. includens larvae in soybean and cotton in Brazil and Guatemala, respectively, were characterized based on sequences of genes considered as highly conserved among baculoviruses, as lef-8, lef-9, pif-2, and polh/gran. The gene pif-2 is a member of the (per os infectivity factor) PIF family, that encodes a protein essential for oral infectivity of ODVs, and belongs to the 31 core genes that are common to all of the sequenced baculoviruses. In the present study, pif-2 gene sequences of PsinSNPV isolates from different geographical regions were compared and analyzed by bioinformatics to better understand the origin and evolution of this gene. The pif-2 genes from PsinSNPV isolates were sequenced (454 sequencing) and the sequences were aligned by MUSCLE. It has previously been proposed in the literature that the pif-2 is the gene most conserved among the core genes of baculoviruses. Our results, in contrast showed a large number of polymorphisms in the pif-2 gene. The significant differences detected in the nucleotide sequence in pif-2 gene produced changes in the amino acid sequence that may cause differences in secondary structure of the corresponding protein. Maximum-likelihood models and HyPhy software were used to estimate the evolutionary rates among PsinSNPV isolates. Selection pressure analysis performed on the non-synonymous (dN) to synonymous (dS) substitution rate ratio (ω=dN/dS) revealed 3 positively selected sites (ω>1) and 56 negatively selected sites (ω<1). Therefore, the pif-2 gene may be involved in phenotypic variation that has been reported in these isolates and, these genes under positive selection pressure, may contribute to environmental stability and transmission efficiency of the virus. Financial support: CAPES/UnB; Embrapa/Cenargen/FAPDF

PIV1106 - GENETIC VARIABILITY BASED ON THE ANALYSIS OF DNA-A FRAGMENT OF BEAN GOLDEN MOSAIC VIRUS (BGMV) FROM COMMON BEAN (PHASEOLUS VULGARIS L.) IN THE STATE OF PARANÁ


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Common bean (Phaseolus vulgaris L.) is affected by several diseases,
among them the virus disease golden mosaic, caused by Bean golden mosaic virus (BGMV) belong to the Begomovirus genus, is the most important one. This virus induces several different symptoms, ranging from the characteristic mosaic to deformation, inducing curling, stunting and brooming. This multiplicity of symptoms indicates virus genetic variability or mixed infection with different strains or virus species. The objective of this work was to examine the variability of BGMV isolates that infect common bean by using PCR-RFLP and sequencing of a DNA-A fragment of the virus genome. Virus isolates that cause mosaic symptoms and brooming, obtained from different regions of Parana State, were maintained in common bean plants, under controlled greenhouse conditions. The DNA extracted from symptomatic plants was used as template for amplification of the viral DNA-A by PCR, using begomovirus universal primers PAL1v1978 and PAR1c496. The amplified fragments, of 11 kb, were digested with the restriction enzymes Bgl II, Rsa I, Sal I and Sst I. The digestions with the enzymes Sal I and Sst I revealed polymorphisms, allowing separation of the isolates in three groups. The PCR fragments of eight isolates, that induce different symptoms and representing the three groups, were sequenced. The partial sequencing of the fragment containing the common region and the ORF AC1, approximately 1 kb, revealed 96 % identity with the sequences of Brazilian isolates BGMV, deposited in the GenBank. The identity among the isolates ranged from 97 to 100 %. The sequence analysis was consistent with the results obtained by RFLP. DNA-A sequence analysis confirmed genome diversity among the isolates. Further studies may correlate the virus genetic variability with pathogenic patterns. Financial support: Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Paraná.

PIV1111 - INFECTION OF THE PYLORIC POSTERIOR INTERSTITIAL RING OF BOMBYX MORI BY ALPHA-BACULOVIRUS


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Bombyx mori Nucleopolyedrovirus (BmNPV) is an entomopathogenic virus that belongs to the Baculoviridae family and Alphabaculovirus (AlfaBV) genus, which infects Bombyx mori (Lepidoptera, Bombycidae). This virus is polyorganotrophic and several tissues are known as targets, however, little information is known regarding the piloric posterior interstitial ring, a region of digestive tube responsible for the passage of food to the hindgut. Bearing in mind the importance of the piloric posterior interstitial ring, a region of digestive tube responsible for the passage of food to the hindgut. Bearing in mind the importance of the piloric posterior interstitial ring, a region of digestive tube responsible for the passage of food to the hindgut. Bearing in mind the importance of the piloric posterior interstitial ring, a region of digestive tube responsible for the passage of food to the hindgut. Bearing in mind the importance of the piloric posterior interstitial ring, a region of digestive tube responsible for the passage of food to the hindgut. Bearing in mind the importance of the piloric posterior interstitial ring, a region of digestive tube responsible for the passage of food to the hindgut.
of 2.6 x 106 polyhedral occlusion bodies/mL BmNPV. On different post inoculation days (dpi), from the 2nd to the 9th dpi, segments of the digestive tract containing the end of the midgut region and the beginning of the hindgut were dissected, following the routine histological process for embedding in paraffin. The sections were stained using the Azan technique modified for viral occlusion bodies and analyzed by light microscopy. The symptoms of the larvae were observed daily, as an additional parameter in the confirmation of the infection. The cytopathological analyses showed the first signs of infection of the piloric posterior interstitial ring between the 5th and 6th dpi. However, only the anterior region of the interstitial ring showed itself susceptible to the virus and the posterior region did not reveal evidence of infection during the period of study. The infection characteristics demonstrated the hypertrophy of the nucleus of the epithelial cells, of the anterior of the interstitial ring, containing the viroplasm, the area in which the virions are produced, and polyhedra in various stages of development. After the cycle of infection, cellular lysis occurs, with the release of polyhedra into the hemocoel. These cytopathological characteristics are consistent with infection by BmNPV, in the same way as symptomatic analyses.

PIV1112 - POPULATION GENETIC ANALYSIS OF TOMATO LEAF DEFORMATION VIRUS IN ECUADOR


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The family Geminiviridae is characterized by a particle morphology of twinned incomplete icosahedra and a genome comprised of circular, single-stranded DNA. Whitefly-transmitted geminiviruses (begomoviruses) are responsible for serious agricultural threats in Latin America. We have recently reported the widespread occurrence of a monopartite begomovirus, Tomato leaf deformation virus (ToLDeV), in Ecuador. In this study we investigated the population structure of ToLDeV populations in Ecuador. A total of 28 full-length clones were obtained by rolling-circle amplification from field-collected samples. The isolates displayed 98.4-100% nucleotide sequence identity amongst them. Interestingly, subdivision analysis indicated the existence of two genetically cohesive populations. The Snn value was closer to 1, indicating that the two subpopulations are highly differentiated. Analysis of each gene yielded Fst values of 0.24-0.55, indicating strong genetic differentiation based on geographic location. Overall, subpopulation 1 (12 isolates) had lower genetic variability than subpopulation 2 (16 isolates). However, this was mostly due to a much more variable Rep/AC4 in subpopulation 2 compared to subpopulation 1, while the other three genes (Trap, Ren and CP) were slightly more variable in subpopulation 1 than in subpopulation 2. For example,
Watterson's estimates of the population mutation rate were 2.98 and 11.15 for the Rep genes of subpopulations 1 and 2, respectively, and 5.29 and 4.52 for the CP genes of subpopulations 1 and 2, respectively. Tajima’s D, Fu and Li’s D*, and Fu and Li’s F* statistics were not significant. The combination of a low degree of genetic variability with strong population subdivision based on geographical location indicates a recent introduction of the virus into Ecuador. Financial Support: INIAP, Fapemig, CNPq

PIV1119 - ILEUM RESISTENCE OF BOMBYX MORI TO ALPHABACULO-VIRUS

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Bombyx mori (Lepidoptera, Bombycidae) is a very important insect in Brazilian agribusiness because its cocoons are used in silk fabric production. Sericulture is an important part of family-run agriculture in the state of Paraná, and production is directly related to cocoon quality and B. mori health. The insect is susceptible to a virus of the Baculoviridae family, Alphabaculovirus (AlfaBV) genus known as BmNPV that has been isolated in Paraná and it attacks several tissues, but not all. However, there is no available information relating to the susceptibility of the hindgut, especially the ileum region. Thus, as a contribution to the establishment of the infectious cycle of this entomopathogen in the insect’s body, this study aimed to analyze the susceptibility of the ileum in relation to BmNPV. To that end, 4th instar B. mori larvae were inoculated per os with a viral suspension of 2.4 x 107 polyhedral occlusion bodies/mL. Over different days after inoculation (dpi), from the 2° to 9° dpi, segments of digestive tract containing the ileum region were dissected, fixed and colored using the Azan technique modified for viral occlusion bodies. Analysis by light microscopy showed that cells in the ileum region were resistant to BmNPV, geographically isolated in Paraná, in none of the analyzed dpi. However, viral occlusion bodies were observed in the fatty tissue adjacent to the extracellular environment, indicating the viability of the used inoculum. It should be highlighted that even though it was not targeted by BmNPV the metabolic function of the hindgut, in relation to the absorption of water; mineral salts, formation, compression and elimination of faeces, can be affected due to the susceptibility of others tissues that are known targets of BmNPV.

PIV1128-ASPECTS OF TCTP-PEPYMV INTERACTION IN SUSCEPTIBLE HOSTS

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Most plant viruses have small genomes
that code for only 4-10 proteins. For a successful infection, these viral proteins must interact with host factors, modulating metabolic pathways and coordinating networks of biochemical and molecular interactions in the pathogen’s favor. TCTP is a highly conserved protein in eukaryotes, and is involved in several cellular processes such as cell growth, cell cycle progression, programmed cell death and protection against different types of stresses. In tomato plants infected with PepYMV, TCTP is upregulated at 72 hours post-inoculation (hpi) and at 14 days post-inoculation (dpi). The functional role of TCTP during PepYMV infection was investigated using virus-induced gene silencing and TCTP-silenced transgenic plants. Silenced plants were inoculated with PepYMV and qRT-PCR showed that non-silenced plants were infected, while silenced plants had strongly reduced viral load in systemically infected leaves. These results suggest that TCTP contributes to viral establishment in susceptible plants. To determine which PepYMV protein(s) promotes TCTP upregulation, individual viral proteins were transiently expressed in N. benthamiana leaves. The results indicated that P3 and CP expression increased TCTP expression. The subcellular localization of TCTP was analyzed by confocal microscopy using a TCTP-GFP fusion. In healthy plants the subcellular localization of TCTP is both nuclear and cytoplasmatic. In infected plants (14 dpi), TCTP is localized only in the cytoplasm. Interaction between viral proteins and TCTP was analyzed by yeast two-hybrid assay. TCTP did not interact with any individual viral protein. Protein purification by affinity assays identified 30 plant proteins which possibly interact with TCTP. Together, our results suggests that TCTP is a susceptibility factor that contributes during both the initial and late steps of PepYMV infection. Financial support: CAPES, CNPq, Fapemig and International Foundation for Science (IFS)

PIV1160 - ENHANCED CYTOTOXICITY OF A BACULOVIRUS IN SEMIPERMISSIVE AND NONPERMISSIVE INSECT CELL LINES MEDIATED BY A PLANT VIRUS-ENCODED RNA SILENCING SUPPRESSOR PROTEIN (NSS)

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NSs is a Tomato spotted wilt virus (TSWV) gene that was first identified in plant cells as a silence suppressor gene. We have shown in a previous work that the NSs protein can suppress gene silencing activity associated to egfp transcripts in semi-permissive and permissive insect cell lines, infected with a recombinant baculovirus (vAcNSs) derived from Autographa californica multiple nucleopolyhedrovirus (AcMNPV). In this work, a cytotoxicity assay was carried out to evaluate cell viability of semi-permissive (UFL-AG-286) and nonpermissive (C6/36 and BM-5) insect cells infected with vAcNSs, wild type AcMNPV and vAcOCC-, a recombinant baculovirus derived from AcMNPV which does not contain the polh gene. vAcNSs induced higher levels of cytotoxicity in all cells at 48 h.p.i when compared to the other viruses tested. A decreased of 1.77 times in viability
was obtained in BM-5 cells compared to AcMNPV and 1.48 times compared to vAcOCC-; in C6/36 cell line, 1.73 times compared to AcMNPV and 1.9 to vAcOCC-; in UFL-Ag, vAcNSs resulted in a decreased of 2.65 times over AcMNPV and 3.71 to vAcOCC-. These results show that, even though C6/36 and BM-5 cells are nonpermissive to AcMNPV, this recombinant virus is still capable of inducing cell death, which is enhanced by the expression of the NSs protein.

PIV1161 - CONSTRUCTION OF A RECOMBINANT BACULOVIRUS ENCODING A BEGOMOVIRUS RNA SILENCING SUPPRESSOR PROTEIN

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RNA interference (RNAi) is a post-transcriptional gene silencing process. It acts in eukaryotic organisms for regulation of gene expression as well as in defense against exogenous genetic materials such as virus genomes. On the other hand, the viruses have been acquired exclusive proteins with ability to suppress the RNA silencing mechanism of host cells. Currently, there are several known viral suppressor proteins, which can interfere in different steps of the RNA silencing pathway. In this study, a recombinant baculovirus encoding a begomovirus RNA silencing suppressor gene [The Tomato severe rugose virus (TSRV)-AC2 gene] was constructed. For this, PCR amplification using the AC2F/AC2R primers designed to amplify AC2 gene of the DNA-A component were performed. PCR product was purified and then cloned into the cloning vector, pGEM®T-Easy (Promega) and the viral transfer vector, pFastBac™1 (Invitrogen). The heterologous gene is under the control of the viral polyhedrin (polh) gene promoter. The recombinant baculovirus in the form of a large plasmid (bacmid) was obtained by transposition of the transfer vector into E. coli DH10-Bac cells (Invitrogen). DNA from the bacmid was purified and transfected into Trichoplusia ni (BTI-4-Tn-5B) cells and the recombinant virus collected from the supernatant of transfected cells and viral DNA was purified. To confirm the insertion of the AC2 gene into the viral genome, 50 ng of the viral DNA were used in a PCR with the specific primers AC2F/AC2R. The recombinant baculovirus was named vAcAC2. The recombinant protein expression will be detected by immunoblot assay. In this way, the effect of the AC2 protein will be analyzed during baculovirus replication in permissive Trichoplusia ni (BTI-4-Tn5B1), semipermissive Anticarcia gemmatalis (UFL-AG-286) and nonpermissive Aedes albopictus (C636) insect cell lines.

PIV1162 - PHYLOGENETIC ANALYSIS OF HCPRO AND P1 PROTEINS OF A SUGARCANE MOSAIC VIRUS BRAZILIAN ISOLATE REVEALS COMMON ORIGIN WITH AN AUSTRALIAN ISOLATE

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Sugarcane mosaic virus (SCMV), Potyvirus, Potyviridae, primarily affects sugarcane, maize, sorghum and several other poaceous causing significant economic losses worldwide. Its genome comprises a positive sense ssRNA that encodes, among others, the proteins P1 and HCPro, known to act as posttranscriptional gene silencing (PTGS) suppressors in members of Potyviridae. In order to gain new insights towards understanding pathogenicity and evolution of a Brazilian highly virulent isolate of SCMV (Rib-1), we cloned and sequenced its P1 and HCPro. The sequences were aligned and submitted to phylogenetic analysis by neighbor joining (2000 bootstraps replicates), and verified for possible recombination events with other 15 SCMV isolates available in the GenBank using a variety of automatic and manual detection approaches from Recombination Detection Program RDP3 v3.44. Phylogenetic trees based on both P1 and HCPro grouped the Brazilian isolate in a separated clade with SMCV-Brisbane A isolate, from Australia, forming a separate lineage out of 16 SCMV isolates. The most variable region in RIB-1 HCPro compared to other isolates is located in the Central and the N-terminal portions of the protein, while in the Rib-1 P1 is located in its Central and C-terminal portions. On the other hand, analyses by RDP3 individual algorithms did not detect any potential intraspecific recombination event among these isolates. These results reinforce a common origin for Rib-1 and Brisbane A SCMV isolates. Financial support: CAPES and MICINN, Spain

PIV1175 - FLUORESCENT IN SITU HYBRIDIZATION METHOD FOR LOCALIZATION OF TOMATO CHLOROTIC MOTTLE VIRUS AND GROUNDNUT RINGSPOT VIRUS IN INFECTED PLANTS

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We optimized a method for localization of the begomovirus Tomato Chlorotic mottle virus (ToCMoV) transcripts in hand-cut leaf sections of tomato and Nicotiana benthamiana plants, and the localization of the tospovirus Groundnut ringspot virus (GRSV) in Datura stramonium and N. benthamiana plants using confocal microscopy. To this extent, short DNA oligonucleotides complementary to the viral RNA harboring a fluorescent molecule (Cy-5) at its 5’end were designed and used for the fluorescent in situ hybridization (FISH) analysis. The probe was designed to hybridize the viral RNA coding for the coat protein of ToCMoV and for the ribonucleoprotein of GRSV. In this method, hand-cut leaf sections were fixed in Carnoy’s fixative overnight, decolorized, washed in hybridization buffer three times for 3 minutes and hybridized overnight with the fluorescent probe at different conditions of temperature and probe concentration (room temperature vs.
48 C and 10pmol/µl vs. 0.1pmol/µl). Negative controls were healthy and RNase-digested plants. As result, an accumulation of ToCMoV transcripts were observed in the vascular tissue of the infected plants confirming that ToCMoV is limited to the phloem while GRSV transcripts were localized at the cytoplasm of epidermal cells of D. stramonium and N. benthamiana plants. The best condition of hybridization was obtained using the probe at 0.1pmol/µl at room temperature for localizing both viruses. This method allowed the localization of both DNA and RNA plant viruses in infected plants and might in future studies help to shed light on the knowledge of the field virus tissue-limitation/association and in pathways of viral pathogenicity in plants. Financial support: Capes, CNPq, FAP-DF, Embrapa

PIV1201 - STUDY OF LOCALIZATION OF P29 AND CP PROTEINS OF PEPRS V USING CONFOCAL LASER MICROSCOPY
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Pepper ringspot virus (PepRSV) is a tobravirus and was originally isolated from plants of Capsicum spp. showing ring spot symptoms in Brazil. This isolate had been considered as a strain of Tobacco rattle virus (TRV, Tobravirus). However analysis of their genomic sequence revealed that it is a distinct virus from TRV, which occurrence was reported only in Brazil. Virus particles are elongated and rigid and it genome is composed of two segments of single stranded RNA of positive sense. Unique feature that distinguishes it from other known viruses is the firm association of their virus particles with mitochondria of the host cell. Preliminary in silico studies of the viral protein p29 of PepRSV suggested that this protein is the candidate that has certain interaction with mitochondria. The coat protein (CP) is also thought to have interaction with mitochondria due to the observation of attached viral particles to this organelle. By these assumptions a strategy was developed to express the CP and p29 of PepRSV fused to different fluorescent proteins (YFP and GFP) to study the interaction of viral proteins p29 and CP with the mitochondria. For this purpose, both protein genes, cp and p29, were fused to YFP and GFP genes, respectively. Thus, agrobacteria containing the constructs were agroinoculated into Nicotiana benthamiana leaves and the location of both fused proteins was observed with confocal laser microscope. CP was located specifically in nuclei and p29 in periphery of cells, most likely in plasmodesmata. As the homolog of p29 of TRV had been shown its movement protein (MP) property, p29 of PepRSV also may act as MP due to the possible association of p29 in plasmodesmata. However, the (co-) localization of these proteins in mitochondria was not observed.

PIV1232 - SUBCELLULAR LOCALIZATION PROFILE OF THE CAPSID PROTEIN AND WILD AND NATURALLY MUTATED MOVEMENT PROTEINS OF CLRDV IN N. BENTHAMIANA L.
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Cotton leafroll dwarf virus (CLRDV) is a member of the genus Polerovirus, family Luteoviridae and is extensively found in cotton plants showing blue disease symptoms in Brazil. The disease is transmitted by Aphis gossypii and was first observed in Central African Republic in 1949. In Brazil the disease is found in almost all cotton fields and is an important economical problem for the national cotton production. In this work the viral movement protein and the coat protein were cloned in binary vectors under the control of the 35S promoter, through the Gateway system. The constructions were introduced in Agrobacteria GV3101 and then agroinfiltrated for transient expression in leaves of Nicotiana benthamiana. L. cv. CLRDV CP alignment analysis and Nod web server analysis identified a particular sequence involved in nucleolar targeting [the nucleolar localization signal (NoLS)]. Optical confocal fluorescence microscopy showed that CP containing NoLS was targeted to the nucleus. CP:GFP fused proteins showed localization into the nucleus probably in nucleolus. Stably transformed BY-2 cells expressing the CP:GFP construction showed a perinuclear localization. The localization of the CLRDV wild type movement protein (wtMP) and an natural variation of this protein found in some viral isolates associated with resistance breakdown (mtMP) were also analyzed through agroinfiltration. Both proteins were shown to be localized in the plasmodesmata membrane, but with different distribution profiles. Financial support: Facual, CNPq, CAPES

Tomato chlorosis virus (ToCV) is a crinivirus, family Closteroviridae, with long flexuous particles, and transmitted by whiteflies in a semi-persistant manner. ToCV has been considered an emergent virus in Brazil where it was reported in tomato crops from many states, which have different environment conditions. The efficiency of ToCV transmission by Bemisia tabaci B-biotype under distinct temperatures conditions is unknown. The knowledge on the influence of the temperature on the transmission of ToCV by the vector is important to delineate management strategies of the disease in tomato crops based in risk and prevision analyses. The objective of this study was to verify the influence of different temperatures on the transmission of ToCV by B. tabaci B-biotype under distinct temperatures conditions is unknown. The knowledge on the influence of the(212,880),(780,929)
different temperatures. Afterward, single whitefly was caged in individual tomato healthy plant for an inoculation access period of 2 days, under the same conditions described above. After that, plants were maintained at 25°C, during 60 days, when infection by ToCV was analysed by nested-PCR. The transmission efficiency of ToCV by whiteflies under 20/22°C was 60%, while under 25/27°C and 30/32°C were 42.5% and 35%, respectively. These results indicated that the temperature influence the transmission efficiency of ToCV by B. tabaci B-biotype, being lower when virus transmission by the vector occurred under higher temperature. Further studies are necessary for better understanding the influence of the temperature on the transmission of ToCV by B. tabaci biotype B in Brazil.

PIV1315 - TOSPOVIRAL TRADE ROUTES: THE PHYLOGEOGRAPHY OF TOMATO SPOTTED WILT VIRUS AND IRIS YELLOW SPOT VIRUS
Ferreira, F.A., de Oliveira, A.S., Resende, R.O., Melo, F.L.

Tospoviruses are an economically important genus of negative RNA viruses within the family Bunyaviridae whose members infect a wide range of hosts, including many major crop species. Tomato spotted wilt virus (TSWV), for example, accounts for over a billion dollars in crop losses, and has been ranked amongst the ten most damaging plant viruses. Despite the economic interest in Tospoviruses, there have been few studies devoted to mapping out the global trends in spatial dynamics of members of this genus. To address this dearth, we have carried out extensive Bayesian phylogeographic analyses of nucleoprotein (N) gene of two economically important tospoviruses: TSWV and Iris yellow spot virus (IYSV) sampled from different countries and continents and available in GenBank. The results were then compared to the level of export/import (calculated in tonnes) of an important susceptible crop (tomato and pepper for TSWV, onion for IYSV) for the countries present in the sample. Despite both TSWV and IYSV exhibiting more intense viral migration starting in the mid-1980s, correlating with trade liberalization policies of the time, the paths of trade between countries and the dynamics of viral evolution were distinct for the two species. In the United States, for instance, while TSWV appears to migrate primarily within the country and seems to stem from a single introduction, for IYSV the US has a higher exchange rate with other countries of the Americas, as well as Asia, Oceania and Europe. Within the European block, both virus species are heavily trafficked amongst specific countries (Italy, Serbia, France), while other countries (Spain) seem to present a contained, endemic infection dynamic, with low rates of viral exchange with other countries. Overall, our study reveals various hypothetical scenarios to explain the global tendencies of TSWV and IYSV infections, serving as a basis for both further molecular studies and government policies concerning viral control measures. Financial support: Capes, CNPq, UnB
PIV1323 - PRODUCTION OF POLYCLONAL ANTISERUM AGAINST COWPEA MILD MOTTLE VIRUS COAT PROTEIN AND ITS APPLICATION IN VIRUS DETECTION

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Cowpea mild mottle virus (CpMMV), the causal agent of stem necrosis disease, has drawn attention of soybean producers in recent years due to yield losses in the main producing regions of Brazil. Serological methods for viral detection require the use of an antiserum of good quality to achieve specificity and sensitivity. There is currently no alternative to serological detection of the virus in Brazil. The entire coat protein gene of a Brazilian CpMMV isolate was cloned into a bacterial expression vector and transformed into Escherichia coli BL21::DE3 for in vitro expression. The coat protein, fused to a His-tag, was purified under denaturing conditions by affinity chromatography using a Ni-NTA resin. After renaturation, the integrity and identity of the purified recombinant protein was confirmed by SDS-Page and MALDI-ToF/ToF mass spectrometer analyses. Two rabbits were immunized with increasing amounts of the recombinant protein. The specificity and sensitivity of the antisera were demonstrated by Western blot and indirect ELISA assays. The polyclonal antisera raised against recombinant coat protein proved to be a reliable tool for CpMMV detection. Financial support: Fapemig, CNPq

PIV1326 - A NEW BACULOVIRUS-MEDIATED STRATEGY TO EXPRESS A COAT PROTEIN FROM GARLIC VIRUS IN ORDER TO GENERATE A PLANT VIRUS-INDEXING DIAGNOSTIC KIT BY ELISA

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We developed a rapid and efficient method to express and purify full length virus coat proteins using a baculovirus expression system. Baculovirus presents a highly expressed protein that forms a crystal matrix surrounding an orally infective virion, the polyhedrin. Here, we fused the full coat protein (cp) gene from the Garlic Mite-borne Filamentous Virus (GarMbFV) to the polyhedrin gene 3-end from the baculovirus Autographa californica multiple nucleopolyhedrovirus. For this purpose we constructed a modified donor vector, derived from the commercial plasmid pFastBac1 (Invitrogen), containing a modified polyhedrin gene with one NcoI recognition site and a hexa-histidine-tag on the 3-end. The cp gene was amplified containing flanking NcoI restriction sites without the stop codon sequence and inserted into the modified donor vector. This plasmid was used to generate recombinant viruses through a bac-to-bac system (Invitrogen). The recombinant baculovirus was amplified in insect cell culture and the virus-containing supernatant was used to infect Spodoptera frugiperda larva by hemocoel-injection. The recombinant baculovirus was amplified in insect cell culture and the virus-containing supernatant was used to infect Spodoptera frugiperda larva by hemocoel-injection. The recombinant fused protein was purified from insect cadavers by sucrose gradient method, washed with non-ionic detergent and analyzed by immunoblotting assay.
using anti-his antibody and anti-polyhedrin antiserum. The chimeric protein was used to immunize rats and generate antibodies against the target protein. Infected-cell cultures and the recombinant crystals were analyzed by light and scanning electron microscopy, respectively. Amorphous crystals were produced in the cell cytoplasm infected with the chimeric-protein-producing virus, but not in the cells infected with the virus containing only the hexahistidine tagged polyhedrin gene. In the latter, crystals were present in the nucleus, and showed a regular form. Furthermore, the antiserum produced from chimeric-protein immunization was able to detect plants infected with GarMbFV, which had been initially confirmed by RT-PCR. Thus, the method to express full length coat gene fused to the baculovirus polyhedrin was proved efficient and may be an innovative strategy for the development of plant virus diagnostic kits.

PIV1330 - BEMISIA TABACI FROM THE NEW WORLD GROUP MAY HARBOR BRAZILIAN BEGOMOVIRUS SPECIES

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Bemisia tabaci is one of the most important global agricultural insect pests, being a vector of emerging plant viruses such as begomovirus and crinivirus that cause serious problems in many countries. Although knowledge of the diversity of populations of B. tabaci and viral species are important to control this pest and understand viral epidemics, the available information is limited in Brazil, mainly for whiteflies belonging to the New World group. B. tabaci populations were collected from different locations of São Paulo state, total DNA was extracted and the mtCOI gene was sequenced to select individuals of B. tabaci belonging to the New World group which comprises the New World 1 (NW-1) and New World 2 (NW-2) species. A rolling-circle amplification – RCA was used for amplification of the circular ssDNA of begomovirus possible present in the whiteflies, followed by PCR with the begomovirus detection primers PAL1v1978 / PAR1c496 and V324 / C889. The fragments were purified and directly sequenced. Among the NW1 species, one PCR product had higher identity with the species Sida micrantha mosaic virus and the other one with Sida mottle virus. Those whiteflies were collected from Ipomea sp. and Solanum gilo in the cities of Pariquera Açú and Jacupiranga. PCR fragments with higher identity with Sida micrantha mosaic virus were also found in two individuals of NW-2 collected from Euphorbia spp. in Bastos. These results indicate that whiteflies of the New World group may harbor Brazilian species of begomoviruses.

Financial support: FAPESP Área da Virologia: Vegetal

PIV1341 - A RT-PCR TEST BASED IN THE HC-PRO REGION FOR DETECTION OF SOYBEAN MOSAIC VIRUS AND FOR IDENTIFICATION OF IMPORTANT MUTATIONS FOR ITS VIRULENCE
Brazil is the second greatest producer of soybean in the world, following only the United States. The Soybean mosaic virus (SMV) is one of the major pathogens of the crop. The virus belongs to the genus Potyvirus and there are strains (G1 to G7) which are described according to the reaction of resistant cultivars. In Brazil only the G1 and G5 strains were found in the field. Recent outbreaks caused by the G5H and G7H strains were reported in Korea. The presence of recombination events and mutations in the genome of the SMV is considered responsible for the emergence of virulent strains. Due to seed transmission, a large number of soybean samples have been intercepted with SMV at the Embrapa Plant Quarantine Laboratory. The major challenges of quarantine are the sensitive detection and accurate identification of the pathogen. This requires efficient diagnostic tools to avoid the escape of severe strains. In order to check the specific primers for SMV detection, a search for the SMV isolates was made in the genbank. The sequences of 56 complete genomes and the sequences of specific primers described for SMV detection were aligned using the Clustal W program. The alignment showed the presence of gaps for some isolates. New primer pairs were designed from the HC-Pro sequences and used in RT-PCR tests. The fragments with 871 bp were efficiently amplified from three previously intercepted isolates and identified by the Elisa Test. The PCR product of each isolate was purified and sequenced. The three isolates had identities of 90-99% in the nucleotide sequences and 96-99% in the predicted amino acids sequences with other SMV isolates. They do not have the presence of important mutations for virulence described in literature. The phylogenetic analysis made with the MEGA 4.0.2 program showed that the three isolates are not close to the severe strains of SMV. The test proved to be a useful tool for diagnosis and genetic diversity studies of SMV. Financial support: Embrapa.
oleracea, Solanum lycopersicum, Lactuca sativa, Gossypium hirsutum, Cucurbita pepo, Ipomoea batatas, Phaseolus vulgaris, and invasive species Euphorbia heterophylla, Ipomoea sp. and Sonchus oleraceus in different sub-regions of the State of Alagoas in order to identify the species of B. tabaci present in the State. The analysis of individuals of B. tabaci was made based on the portion of the mitochondrial cytochrome oxidase gene (mtCOI). This gene was amplified by polymerase chain reaction, analyzed by RFLP using the restriction enzyme TaqI, sequenced and compared to consensus sequences for the demarcation of species. Middle East-Asia Minor 1 - MEAM 1 is the predominant species of B. tabaci found in Alagoas. This species comprises the biotype B responsible for begomovirus epidemics in different regions of Brazil. Two samples collected in Solanum lycopersicum were classified as New World species, which comprises the biotype A. Four species collected on Euphorbia heterophylla and Ipomoea sp. were identified as belonging to the New World 2, a species recently described in Argentina and also found in São Paulo State. The indigenous species NW-1 and NW-2 seems to be widely spread in different regions of Brazil and could have an importance as vectors of different species of begomoviruses.

PIV1378 - METHODOLOGIES FOR THE FAST AND SENSITIVE DIAGNOSIS OF HIGH PLAINS VIRUS THROUGH RNA EXTRACTION FROM SEEDS AND REAL-TIME PCR


1. Embrapa Recursos Genéticos

High Plains virus (HPV) causes a potentially serious economic disease of cereals and is of quarantine importance for Brazil. The virus is transmitted by the mite Aceria tosichella and by seeds of some cultures. Both the virus and the vector are present in Argentina, but only the vector was reported in Brazil. Because of the low rates of seed transmission reported in wheat and corn, the virus diagnosis is complex. The fast and sensitive diagnosis is essential to avoid the entry of HPV in Brazil through the frontiers or by imported seeds. There are commercial kits for Elisa tests available as well as primers for conventional RT-PCR were described. However, there are not primers for real-time RT-PCR (qRT-PCR) described for the diagnosis of HPV. In order to increase the speed and sensitive of analysis, new methodologies for RNA extraction and qRT-PCR for HPV diagnosis were developed. Protocols for RNA extraction direct from seeds were tested for different cultivars of wheat, triticale and corn. A protocol that combine the CTAB method and the silica column of the Rneasy mini kit (Qiagen) had better results compared with the protocols from commercial kits. The yield and quality of RNA extracted from triticale and corn seeds were close to the expected values. However, the wheat seeds had...
a low RNA yield. A primer pair for qRT-PCR was designed based on the sequences of HPV in the genbank. The leaves of infected and healthy wheat had the RNA extracted and the cDNA was prepared using MMLV-Reverse Transcriptase (Invitrogen). The SYBR Green Master Mix was used for qPCR in a Rotor gene 5plex HRM platform (Qiagen). Two Argentinean isolates of HPV were efficiently amplified by the method. The healthy wheat did not show amplification. The melting analysis showed only a peak for both HPV isolates. The association between the RNA extracted from seeds and qRT-PCR will be tested for the HPV diagnosis. Financial support: Embrapa.

PIV1388 - SCREENING FOR GENES DIFFERENTIALLY EXPRESSED IN THE INTERACTION SUGARCANE INFECTED WITH SUGARCANE MOSAIC VIRUS BY CDNA-AFLP TECHNIQUE

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The Sugarcane mosaic virus (SCMV) is one of the main sugarcane viruses causing yield losses up to 50 per cent in susceptible sugarcane varieties. The objective of this study was to identify possible candidate genes for SCMV resistance through cDNA-AFLP by comparative analysis between the transcript profile of a resistant (IACSP95-5000) and a susceptible variety (IAC91-1099). A preliminary evaluation was conducted to verify the potential of the technique to this pathosystem. The variety IACSP95500 (three replicates) was inoculated with SCMV RIB-1 isolate under greenhouse conditions and samples from the +1 leaf was collected at 24 hours after inoculation. After one month of inoculation the plants from the susceptible variety showed typical symptoms of the disease with expressive reduction in their height. Total RNA was extracted with Trizol (Invitrogen) and RT-PCR was performed using a specific primer pair for the SCMV capsid protein to confirm the infection by the virus. Total RNA from the resistant variety was extracted at 24 hours of inoculation and used for cDNA synthesis to screen for AFLP markers with two combinations of selective primers. The cDNA-AFLP technique showed a total of 41 fragments, of which 4 (9.76%) were differentially expressed between the inoculated and non-inoculated treatments based on two selective combinations. From the preliminary results here obtained a new experiment was carried out with the two varieties to screen for different expressed transcripts at four sample times (0, 24, 48 e 72 hours). Financial support: CAPES and FAPESP/BIOEN

PIV1411 - GENETIC DIVERSITY OF BEGOMOVIRUS IN COMMON BEAN IN BRAZIL

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Common bean (Phaseolus vulgaris L.) is a staple food and important protein source in developing countries in tropical and subtropical regions. Brazil is the world’s largest producer, and beans are grown by small and large producers being important for economic and social development. The golden mosaic disease, caused by Bean golden mosaic virus, BGMV, is the most important viral disease of the crop, causing yield losses of up to 100%. Aiming to study the genetic diversity of begomovirus infecting beans in Brazil, leaf samples of different bean cultivars, mostly with symptoms typical of golden mosaic, were collected in eleven Brazilian states. After DNA extraction and rolling circle amplification (RCA), begomovirus detection was done by PCR. Subsequently, virus fragments were cloned in pGEM-T Easy and sequenced. Of the 108 bean samples collected and tested, 82 samples (75.9%) were positive for the presence of begomovirus, indicating a high incidence of begomovirus in all sampled areas. The identity of most clones (85.9%) ranged from 84.9 to 99.3% with corresponding sequences from BGMV-GO (M88686) and BGMV-Soy (FJ665283). However, eleven clones had identities below 72.3% with BGMV isolates and from 86 to 99% with different isolates of Macroptilium yellow spot virus (MYSV). Our results indicate that although BGMV is prevalent in common bean in the field, MYSV is also present and represents a potential threat to bean production in Brazil. Financial support: Embrapa, Fap-DF and CNPq

PIV1417 - MICRORNA PROFILING OF RESISTANT TOMATO PLANTS INFECTED WITH BEGOMOVIRUS


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Tomato chlorotic mottle virus (ToCMoV), member of the genus Begomovirus, is efficiently transmitted by the whitefly Bemisia tabaci. Plants infected with ToCMoV generally develop symptoms of vein chlorosis, chlorotic spots, and yellow mottle. LAM 157 is a ‘Santa Clara’ near isogenic tomato line containing the monogenic recessive gene tcm-1. The resistance is manifested by either reduction or absence of symptoms and reduced viral
accumulation. MicroRNAs (miRNAs) are small endogenous RNAs that regulate the expression of several genes that are involved with many important biological processes. To verify a possible involvement of miRNAs in symptom expression in tomato inoculated with ToCMoV, the accumulation of miRNAs previously reported to be related to plant development and with stress responses was studied. Susceptible plants of the cultivar ‘Santa Clara’ (SC) and resistant plants of the line ‘LAM 157’ were inoculated by particle bombardment with infectious clones of ToCMoV. Total RNA was isolated from upper leaves 17 days post inoculation and loaded in polyacrylamide gel. Northern blots were performed using as probes gamma32P-ATP end-labeled oligonucleotides complementary to miRNAs. The amount of miRNAs 159, 162, 164 and 168 accumulated by control plants of SC and ‘LAM 157’ was compared among those plants infected with ToCMoV. The results showed differential accumulation of miRNAs between susceptible and resistant plants, infected and mock-inoculated controls. These results suggest that the expression of host miRNAs can be affected as a result of ToCMoV infection and may reflect differences in symptom expression intensity as well as in the levels of virus resistance. Financial Support: Convênio Embrapa-Monsanto, INCTPP, CNPq, FAP-DF.

PIV1428 - ESTIMATION VIA ABSOLUTE QPCR QUANTITATION OF THE LEVELS OF BEGOMOVIRUS REPLICATION IN RESISTANT TOMATO (TCM-1 GENE)

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Tomatochloroticmottlevirus (ToCMoV) is a bipartite begomovirus that infects tomato fields in Brazil. ToCMoV and other begomoviruses from New World are composed by two circular single stranded DNA components (known as DNA A and DNA B) that replicate in the nucleus of infected cells. The DNA A contains genes related to viral replication, transcription and encapsidation, while the DNA B harbors genes related with the viral movement. One of the best strategies to control the disease spread is the employment of resistant cultivars. Recently, a ‘Santa Clara’ near-isogenic line named ‘LAM 157’ carrying the resistance gene tcm-1 was obtained by Embrapa. The broad tcm-1 gene resistance to different begomovirus species is characterized by the reduction or absence of symptoms and restriction of virus accumulation in infected plants. This work aimed to study the resistance mechanism operating in ‘LAM 157’ in order to verify if it was related to viral replication. Agrobacterium tumefaciens containing a partial tandem repeat of ToCMoV DNA-A cloned in pCambia was infiltrated into the abaxial surface of leaves from...
the susceptible cultivar ‘Santa Clara’, resistant line ‘LAM 157’, and Nicotiana benthamiana. Samples were collected during seven days post infiltration. Viral replication was estimated by evaluating viral DNA accumulation in infiltrated areas by Southern blot and qPCR. The amount of viral DNA increased with time in all the infiltrated plants reflecting viral replication but it was much lower in ‘LAM 157’ implying that restriction of viral replication is one of the mechanisms associated with the resistant response. Financial Support: Convênio Embrapa-Monsanto, INCTPP, CNPq, FAP-DF.

PIV1434 - ANALYSIS OF TRANSIENT EXPRESSION OF THE MOVEMENT PROTEINS (NSM) OF DIFFERENT BRAZILIAN TOSPOVIRUS IN HOST PLANTS BY CONFOCAL MICROSCOPY

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The genome of plant viruses encodes one or more proteins involved in virus movement. In the genus Tospovirus, the movement protein (NSm) is encoded by the M segment of the viral genome. This protein seems to bind specifically to nucleic acids, however, has the capacity to modify plasmodesmata and is involved in tubule formation on the surface of protoplasts. The present study aimed to evaluate the cellular location and dynamic motion of the protein NSm of two species of tospovirus: Bean necrotic mosaic virus (BeNMV) and Tomato chlorotic spot virus (TCSV). These viruses show a significant difference compared to the spectrum of hosts, a biological characteristic that may be associated with the function of their movement protein. The NSm protein genes of BeNMV and TCSV were amplified by RT-PCR and cloned into vector target pENTR11 (InvitrogenTM) at specific sites using the Gateway recombination system, and subsequently, the insert was cloned into the viral vector PVX-GW-GFP. Host plant leaves were agroinoculated with the constructs and 10 days after, viral infection was monitored by fluorescence of the GFP protein fused to viral movement protein. The viral vector PVX-GW-GFP was used as mock control experiment. In TCSV, was observed an association of the movement protein with the chloroplast, type of location that hitherto has not been observed in Tospovirus and Potexvirus infection. In BeNMV, no association was observed with the chloroplast. Financial support: Capes, CNPq, UnB, FAP-DF Field of Virology: vegetable virus

PIV1441 - EXPRESSION PROFILE OF CMVIE1 PROMOTER DURING RECOMBINANT ANTICARSIA GEM-MATALIS MNPV INFECTION IN INSECT CELLS


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The baculovirus Anticarsia gemmatalis Nuclear Polyhedrovirus (AgMNPV) is a dsDNA virus that is used as a control agent of the larvae Anticarsia gemmatalis moth (Noctuidea), a soybean crop pest. It may also be used as a protein expression vector in insect cells and larvae. Protein expression using baculovirus vectors usually is based on the hyperexpression of the desired protein product, by cloning the gene downstream of the polyhedrin (POLH) gene promoter within the virus genome and infecting insect cells or larvae with the recombinant budded virus. However, the POLH promoter is not active in mammalian cells, and this restricts the use of the baculovirus as an expression vector in expression systems distinct from insect cells. A solution is to use the cytomegalovirus (CMV) major immediate early 1 (IE1) gene promoter, which allows strong and constitutive expression in mammalian cells of different types. In this work, we cloned the CMVIE1 promoter upstream of the firefly luciferase (FLUC) gene, a chemiluminescent reporter gene, and generated a recombinant AgMNPV baculovirus containing this construct. This allowed us to infect UFLAG286, TN5B, SF21, C636 and LD5 insect cells grown in vitro and quantify the activity of the CMVIE1 promoter during a AgMNPV baculovirus infection. Although there aren’t any complete baculovirus promoter-like sequence in the CMVIE1 promoter sequence, during infection of permissive cell lines UFLAG286 and TN5B and semipermissive cell line SF21, we detected an expression pattern that starts at 4-5 hours post infection (hpi) and stabilizes at 15 hpi. Total amount of protein produced was low. Non-permissive insect cell lines C636 and LD5 showed a 5 hours delay and lower expression. This recombinant AgMNPV baculovirus will also be used to test transient expression of proteins in mammalian cells and further explore the biotechnological potential of the AgMNPV baculovirus expression vector system.

PIV1484 - GLOBAL ALTERATION OF MICRORNAS AND TRANSPOSON-DERIVED SMALL RNAS IN COTTON (GOSSYPIUM HIRSUTUM) DURING COTTON LEAFROLL DWARF POLE- ROVIRUS (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. Financial support: CAPES, CNPq and FAPERJ

PIV1487 - THE NSS PROTEIN OF TOMATO SPOTTED WILT VIRUS (TSWV) IS ABLE TO INCREASE MORTALITY OF A BACULOVIRUS SEMI-PERMISSIVE HOST

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The baculoviruses are widely used as biological control. To obtain an efficient virus replication it is necessary to get over host defense mechanisms, as the RNA silencing. The NSs gene from Tomato spotted wilt virus (TSWV) works as a strong gene silencing by RNAi suppressor. In the present work, a recombinant baculovirus [vAcNSs(occ+)] derived from AcMNPV (Autographa californica nucleopolyhedrovirus) containing the NSs gene from TSWV and the polyhedrin gene (occ+) was produced and purified. Insertion of the polyhedrin gene allows oral infection. The vAcNSs(occ+) recombinant virus and AcMNPV were injected in permissive larvae to AcMNPV (Spodoptera frugiperda) in order to produce viral occlusion bodies (OB), also called polyhedra. The OBs from infected larvae were purified by centrifugation in continuous sucrose gradient. The polyhedra were used in oral bioassays with second instar semi-permissive larvae of AcMNPV (Anticarsia gemmatalis) by the “droplet feeding” method. The preliminary results showed that the recombinant baculovirus was able to kill a considerable amount of larvae in its highest concentration, with the concentrations of 0, 10, 100 and 1000 polyhedra/nL. The amount of dead larvae in each concentration was used to calculate the lethal dose (LD₅₀) by the program Polo plus. Interestingly, the AcMNPV was unable to kill enough larvae to calculate the LD₅₀, confirming that Anticarsia gemmatalis is resistant to AcMNPV. However, it is important to compare the mortality rate of vAcNSs(occ+) with AgMNPV (Anticarsia gemmatalis nucleopolyhedrovirus), which is the virus used in the biological control of Anticarsia gemmatalis.