PIV196 - THE SW-5 GENE CLUSTER: ANALYSIS OF TOMATO RESISTANCE AGAINST TOSPOVIRUSES

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Tomato spotted wilt virus (TSWV) causes substantial losses on crop production around the world. So far only two natural resistance sources are available for commercial plant breeding against TSWV and other tospovirus species. One of them is the Sw-5b gene which encodes a CC-NB-ARC-LRR protein able to halt tospovirus infections in Solanum peruvianum L. and bred S. lycopersicum L., wild and commercial tomato species, respectively. Here we show that the cell-to-cell moment protein (NSM) of TSWV has been identified as the avirulence determinant (Avr) of the Sw-5b-mediated resistance. The transient expression of the NSM protein triggers a clear hypersensitive response (HR) in tomato and Nicotiana benthamiana L. harboring the Sw-5b gene. Moreover, it is shown that a high accumulation of the Sw-5b protein in N. benthamiana leaves achieved by its co-expression with RNA silencing suppressors (RSS) leads to auto-HR in the absence of NSM. In a similar approach Sw-5a, the highest conserved paralogous protein of Sw-5b from S. peruvianum, also triggered auto-HR while a Sw-5 orthologous protein from susceptible S. lycopersicum, named Sw-5aS, did not. None of those last two proteins, however, were able to trigger a NSM-dependent HR. Truncated and mutated versions of the Sw-5 proteins revealed that the NB-ARC domain is sufficient for HR-triggering and seems to be suppressed by the CC domain. Furthermore, a single mutation was sufficient to restore auto-HR activity within the NB-ARC domain of the Sw-5aS protein. When the latter was fused to the Sw-5b LRR domain, NSM-dependent HR triggering was regained, but not in the presence of its own Sw-5aS LRR domain. Finally, subcellular localization studies revealed that the Sw-5b protein has a nucleocytoplasmic distribution and its CC domain signalizes nuclear import. A model for the activation of the Sw-5b protein and the functionality of the other Sw-5 homologs will be discussed. Financial Support: CNPq, FAPDF, CAPES, UnB, WUR.

PIV246 - DISCOVERY OF POTENTIAL ENTOMOPATHOGENIC RNA VIRUSES IN THE WHITEFLY (BEMISIA TABACI) USING NEXT GENERATION SEQUENCING

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The whitefly (*Bemisia tabaci*) is one of the most devastating agricultural pests worldwide, due to its high reproduction rates, polyphagy, and its ability to vector dozens of plant viruses. Control methods largely rely on chemical pesticide use, which is environmentally detrimental and invariably leads to the development of insecticide resistant populations. The use of insect pathogens as biocontrol agents, such as RNA viruses belonging to *Dicistroviridae, Flaviviridae* and *Reoviridae* families, is a potential alternative to the current control methods. Here we used Illumina next generation sequencing to discover novel RNA viruses infecting *B. tabaci.* Nymphs were collected in commercial crops in Goiás and Distrito Federal, from February to November, 2014. Samples were extracted and treated with DNase I and RNase A in order to remove non-encapsidated nucleic acids. Total RNA was purified from the extract, amplified and sequenced in an Illumina sequencer. All the 286,182 reads generated were trimmed and assembled using CLC software, and the resulting 108 contigs were compared against a virus RefSeq database using Geneious software. Based on tBLASTx analysis, two contigs were similar to cripaviruses: the first sharing 33% amino acid identity with *Aphid lethal paralysis virus*, and the second presenting 61% identity with *Black queen cell virus*; and two other contigs were similar to aparaviruses, presenting 54 and 33% identity with *Israeli acute paralysis virus* and *Solenopsis invicta virus-1*, respectively. Both genera belong to the *Dicistroviridae* ssRNA virus family, carrying a poly-A tail. Therefore, a cDNA was generated by reverse transcription with an oligo-dT primer using the whitefly RNA sample. PCR targeting the four contig sequences confirmed the presence of the viruses in the sample. This work represents a first step towards discovering and characterizing these novel

viruses in order to assess their use as biological control agents. Financial Support: CNPq, EMBRAPA.

PIV273 - BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF TWO OLD-WORLD-LIKE BEGOMOVIRUSES INFECTING THE NON-CULTIVATED PLANT SIDA ACUTA IN BRAZIL

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The genus *Begomovirus* (family *Geminiviridae*) is comprised of viruses with one or two circular, single-stranded DNA (cssDNA) genomic components transmitted by the *Bemisia tabaci* sibling species group to dicotyledonous plants, and includes important plant pathogens responsible for severe losses in many economically important crops worldwide. Begomoviruses are divided into New World (NW) and Old World (OW) groups based on genomic organization and phylogenetic relationships. In this study, we performed the biological and molecular characterization of Sida yellow spot virus (SiYSV) and Sida golden yellow mosaic virus (SiGYMV), two OW-like begomoviruses isolated from samples of the non-cultivated plant *Sida acuta* collected in Viçosa, state of Minas Gerais, in December 2011. The viral genome was amplified by RCA, cloned and sequenced. Infectious clones (DNA-A and DNA-B) were generated to perform the biological characterization. The two genomic components of both viruses are phylogenetically related to NW begomoviruses. Nevertheless, their DNA-A components exhibited a highly divergent 5' half, including part of the intergenic region, the *CP* gene, and an *AV2*-like gene (which is present only in OW begomoviruses). The deduced amino acid sequences of the CP and AV2-like proteins had very low identities with either NW or OW begomoviruses, having greater similarity with a divergent monopartite geminivirus recently identified in apple trees in China. The presence of conserved motifs in the CP and Rep coding regions which are characteristic of OW begomoviruses was also detected. Both viruses infected plants in the *Malvaceae* and *Solanaceae* families (the latter with very low efficiency). Interestingly, SiYSV does not seem to be transmitted by *B. tabaci* MEAM1, a result that is not entirely unexpected considering the high level of divergence of its CP. Our results indicate that the origin of SiYSV and SiGYMV involves an ancient recombination event between a OW-like begomovirus and a divergent geminivirus. Further characterization of cssDNA viruses infecting non-cultivated hosts may shed additional light into their origin, and may lead us to reconsider the division of begomoviruses into NW and OW viruses. Financial Support: CAPES, CNPq, FAPEMIG.

VV99 - INFECTIONS AND COINFECTIONS BY RESPIRATORY VIRUSES IN SHELTER DOGS, RS, BRAZIL

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Canine infectious respiratory disease (CIRD) is associated with single or mixed virus infections, caused by pathogens that replicate sequentially or in synergism. The main viral agents involved in CIRD include *Canine distemper virus* (CDV), *Canine parainfluenza* (cPIV); *Canine adenovirus type 2* (CAdV-2) and Canid herpesvirus 1 (CaHV-1). These infectious are especially important in places with high animal density and constant movement. Although these viruses are distributed worldwide, little information is available about them in Brazil. The objective of this study was to investigate the occurrence of respiratory viruses in dog shelters in Rio Grande do Sul state (RS), Brazil, trying to correlate their occurrence with the environmental conditions. For this, nasal secretions were collected from asymptomatic and sick animals from three shelters of RS (Cachoeira do Sul #1 and 3; Passo Fundo #2) and tested by PCR for each virus, followed by nucleotide sequencing of the amplicons. Samples of shelters #1 and #3 were obtained during the cold season. Shelter #1 presented poor sanitary and nutrition conditions, high animal density and constant contact among dogs. In this shelter 78% (58/74) of the respiratory samples were positive, being 35% (26/74) in single infections and 43% (32/74) in coinfections. Shelters #2 and #3 presented satisfactory sanitary and nutrition conditions, with large outdoors exercise areas (#2) and animal separation by groups (#3). In shelter #2, 8% (5/35) of the samples were positive to cPIV and 6% to CaHV-1; in the shelter #3, 8% (7/77) samples were positive to CAdV-2 and 1% to CDV. The sequences obtained from the amplified products resulted in a 98 to 100% identity with sequences deposited in GenBank