PIV401 - TOSPOVIRUSES GLOBAL SPREAD: THE PHYLODINAMIC AND PHYLOGEOGRAPHY OF TOMATO SPOTTED WILT VIRUS, TOMATO CHLOROTIC SPOT VIRUS AND IRIS YELLOW SPOT VIRUS

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The diversity of the genus tospovirus comprises 11 recognized species with a variable host range and geographic distribution, besides the brief reports of new species. Tospoviruses are responsible for considerable crop loses. Decipher the global trends in tospoviruses spread is an economic necessity, since it may be permit the formulation of better quarantine methods. Therefore, we performed phylogeography reconstructions of three selected species. Tomato spotted wilt virus (TSWV) is the type member of the genus, Iris yellow spot virus (IYSV) occurs occasionally in most locations that is reported, but has few endemic occurrences all over the world, and Tomato chlorotic spot virus (TCSV) was first describe in the early 1990's and nowadays can be found only in the americas. To investigate the global spread trends of TSWV, TCSV and IYSV, publicly available (Genbank) genomes with distinct dates and location of sampling were used. Viral phylogenies based on the nucleoprotein sequences of isolates were estimated using maximum likelihood implemented in Phyml and using Beast. Through Beast we carried out extensive Bayesian phylogeography analyses. Five hundred TSWV sequences, around eighty sequences for IYSV and thirty-three for TCSV were used. The resulting trees were summarized in the maximum clade credibility (mcc) tree using Treeannotator and the ancestral reconstruction were visualized with Figtree. The supported routes were then compared to the level of export/import of an important susceptible crop of involved countries. Analyses of the phylogenetic trees allow noting that viral migration for all three species intensified beginning in the mid-1980s, correlating with trade liberation policies of the time. Despite this similarity, the dynamics of viral trade and the countries involved were distinct to each species. IYSV seems to be much more mobile than the others are. TCSV disappeared for some years and now reemerged in the Caribbean and USA. TSWV has two different behaviors; one is circulating in USA and the other between Europe and Asia. Financial Support: CNPq.

PIV404 - URTHER EVIDENCE OF LOCAL EVOLUTION OF WEED-INFECTING BEGOMOVIRUSES IN BRAZIL: MOLECULAR CHARACTERIZATION AND PRODUCTION OF INFECTIOUS CLONES OF A NEW SPECIES INFECTING SIDA SP. FROM THE STATE OF PIAUÍ

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Begomovirus diseases are caused by numerous species, leading to significant losses in many important crops in the world, and new species have been frequently and continuously reported in both cultivated and non-cultivated plants. Begomoviruses are commonly found in mixed infection, which may lead to the occurrence of recombination and pseudo-recombination events between their genome components. Plants of genus Sida are common weeds found in agricultural areas in Brazil, and it is not rare to find sida plants infected with begomoviruses. The objective of this work was to molecularly characterize a new begomovirus species found infecting *Sida* sp. collected in the state of Piauí. Leaf tissue from a symptomatic *Sida* sp. plant was applied into an FTA card, the total DNA was extracted, PCR analysis was performed with degenerate primers for begomovirus A and B components and the amplified PCR fragment was sequenced. The total genomic DNA was also used to amplify the full DNA genome of both components by rolling circle amplification (RCA). RCA products were digested with restriction enzymes to identify restriction enzymes that linearize these components and full-length clones obtained. Sequence comparisons and recombination analysis were performed using SDT and RDP programs, respectively. Dimeric clones of DNA-A and DNA-B components were generated with a partially digested RCA products and transformed into *Agrobacterium tumefaciens. Nicotiana benthamiana plants*, agroinoculated with the DNA-A and DNA-B clones, developed symptoms including stunting and yellow spots and leaf curl. PCR analyses with DNA-A and DNA-B specific primers confirmed the

presence of both components. The complete sequence of DNA-A was determined to be 2694 nucleotides, with a genome organization typical of New World bipartite begomoviruses. The full-length sequence of DNA-A had the highest identities of 85% to *Sida mosaic Alagoas virus*, a begomovirus previously reported in *Sida* sp. plants in Brazil. The DNA-B component was 2622 nucleotides and encoded two open reading frames (BV1 and BC1). It had 79% sequence identity with the DNA-B component of *Okra mottle virus*. In a recombination analysis, no significant recombination event was detected from the DNA-A component of this isolate. Thus, a novel bipartite begomovirus species was found infecting a *Sida* sp. plant in Brazil, and the implications of this finding in begomovirus evolution will be discussed. Financial Support: UNIVERSITY OF BRASÍLIA, UNIVERSITY OF DAVIS, EMBRAPA VEGETABLES

PIV408 - DETECTION AND WHOLE GENOME SEQUENCING OF CPMMV IN COMMON BEAN RESISTANT TO BGMV FROM PARANÁ STATE

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Cowpea mild mottle virus (CPMMV) is a *Carlavirus* from the family *Betaflexiviridae* which has a linear single stranded positive sense rna genome of approximately 8,200 nt and infects a wide range of cultivated plants from the *Fabaceae* family. It is transmitted by the whitefly *Bemisia tabaci*. During the field tests required for the release of a new common bean (*Phaseolus vulgaris*) cultivar resistant to bean golden mosaic virus by rna interference, some plants with mild mosaic and distortion in the leaves were tested for the presence of CPMMV by ELISA. These plants were collected in the cities of Cambará and Londrina, Paraná state. In order to confirm the etiology of the disease and compare the genome of these isolates with the CPMMV isolates from soybean which we had obtained a couple of years previously, the positive samples from ELISA were used for rna extraction and RT-PCR with primers previously used for sequencing of CPMMV in soybean. One of the samples was used for the sequencing of the whole genome of the virus, using also the race protocol for the amplification of the 5' end of the genome. Another four samples were confirmed as infected by CPMMV with ELISA and RT-PCR of the 3' end portion of the genome. The complete genome showed 99% identity with some of the CPMMV isolates from soybean in Brazil and also with the complete genome of CPMMV obtained from whiteflies in Florida. The sequences also clustered with each other in the filogenetic analyses performed in mega v6.06. This work helps to elucidate the etiology of the virus causing disease in the newly developed common bean cultivar with resistance to BGMV by rnai and goes further in the investigation of CPMMV distribution and potential impact in Brazil. Financial Support: CNPq.

PIV416 - HOMOLOGY MODELING OF TOSPOVIRUS NUCLEOPROTEIN: STRUCTURE AND FUNCTION

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The rapid progress in the understanding of protein folding mechanisms and the advances in the bioinformatics field have provided reliable tools to modeling and predict three dimensional structures of plant virus proteins. recently, the nucleoprotein (np) crystal structures of related rna virus families (arena/orthomyxo/bunyaviridae) were elucidated and despite having different sizes and distinct np-folding structures, these proteins share common features and architectural principles when forming np-np multimers and np–rna complexes. therefore, due to their genetic relationship, the la crosse virus (lacv-orthobunyavirus) crystal structure in complex with ssrna (pdb id 4bhh) was selected as template for a homology modeling approach to predict a three dimensional model for the np of the tospovirus groundnut ringspot virus (grsv). the grsv np monomer was predicted to possess thirteen helical segments and two small beta-sheets organized in a globular core domain (33-223 aa) containing a deep positively charged groove with the two terminal chains forming a n-terminus arm (1-32 aa) and a c-terminus arm (224-258 aa). both n- and c-arms extend outwards from the globular core domain and they interact with the globular core domain of neighboring monomers to mediate the multimerization, supporting the "head-to-tail" model. the rna is primarily bound at the central rna-binding groove and the key residues for this interaction are mainly located in this groove. rna is strongly bent at each np–np interface and is largely solvent-inaccessible in the tetramer structure. the