

that they were probably present in other hosts in the area and highlighting the importance of studying the diversity of plant viruses present in the vectors as a surveillance method of the diversity of plant viruses in a given area that may possibly forecast the emergence of new virus diseases. Financial Support: Embrapa, CNPq, FAP-DF, Rede Estrece, Rede Centro Oeste de Pesquisa em Biodiversidade Viral, INCTIPP.

#### PIV437 DETECTION OF TWO ENDORNAVIRUS IN COMMON BEAN GENOTYPES IN BRAZIL

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Common bean (*Phaseolus vulgaris*) is an important source of proteins and is a major grain legume consumed worldwide. Brazil is the third largest producer of beans, with different cultivars planted and consumed in the country. Viral pathogens play a significant role in reducing the productivity and quality of this crop. However, some viruses do not cause apparent damage and may play a beneficial role in the plants. Endornaviruses (*Endornaviridae*) are persistent viruses that infect important crops such as pepper, rice, broad bean, and beans. However, these viruses are poorly studied and have not yet been reported in Brazil. In this study, we investigated the occurrence of two endornaviruses, *Phaseolus vulgaris* endornavirus-1 (PvEV-1) and *Phaseolus vulgaris* endornavirus-2 (PvEV-2) in bean genotypes. Forty five bean genotypes from Embrapa's germplasm bank including Brazilian cultivars (25) and breeding lines (17) and three accessions of 'Black Turtle Soup' were selected. The seeds were germinated, and total nucleic acids were extracted from the first true leaves using the STE-phenol protocol. Duplex RT-PCR was conducted using a SuperScript® III One-Step RT-PCR Kit (Invitrogen) with primers to detect both endornaviruses simultaneously. The sizes of virus-specific PCR product were evaluated in 1.5% agarose gel electrophoresis. Based on electrophoresis results, PvEV-1 and PvEV-2 were present in high frequency, and 80% of the tested genotypes contained at least one of these viruses. PvEV-

1 was more frequent and was observed in 80% of tested genotypes. In contrast, PvEV-2 was detected in 40% of the tested beans and in double-infection with PvEV-1. Infection only with PvEV-2 was not observed. Only 20% of the bean genotypes were endornavirus-free. Further work will include molecular characterization and phylogenetic analysis of these viruses. Financial Support: EMBRAPA, CNPq, FAP-DF, Rede Estrece, Rede Centro Oeste de Pesquisa em Biodiversidade Viral, INCTIPP

#### PIV443 - GROUNDNUT RINGSPOT VIRUS (GRSV) INFECTING WATERMELON (*Citrullus lanatus*) IN CENTRAL BRAZIL

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Viral diseases are among the main cause of yield losses in cucurbit species around the world. In Brazil, at least ten virus species had already been reported infecting these crops. During 2013 and 2015, watermelon (*Citrullus lanatus*) plants exhibiting mosaic and leaf distortion symptoms, with incidence varying from 20% to 40%, were observed in fields of the state of Goiás, at the Central region of Brazil, one of the most important watermelon producers in the country. Samples were collected from symptomatic plants and submitted to serological and molecular tests. Simultaneously, 235 thrips specimens were collected for identification on leaves and in flowers of watermelon plants in many fields. Leaf extracts obtained from those plants were tested for *Papaya ringspot virus* - type watermelon (PRSV-W), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV), *Cucumber mosaic virus* (CMV) and, *Zucchini lethal chlorosis virus* (ZLCV) by NCM-ELISA, using polyclonal antibodies raised against the coat protein of each virus. Samples were also subjected to total RNA extraction and used as template for complementary DNA (cDNA) synthesis by reverse transcription (RT) with primer J13 that contains a conserved region present in all tospovirus RNA termini, followed by conventional PCR using the primer set BR60/BR65 that targets the non-translated region from the 3' end portion of the S RNA and the protein N-coding gene and, amplify fragments of at least three tospovirus species (453 bp). In addition, specific primers for ZLCV (ZLCV-P1/ZLCV-P2), *Tomato spotted*

wilt virus (TSWV - TSWV722/TSWV23) and *Groundnut ringspot virus* (GRSV - GRSVNv/GRSVNvc) detection were used in RT-PCR reactions. Serological tests results indicated that symptomatic plants were positive mostly for ZLCV and just a few plants showed to be infected with potyviruses (PRSV-W; ZYMV). More than 80% (32/40) of the symptomatic watermelon plants tested positive for tospovirus degenerate primers (BR60/BR65) and GRSV specific primers (GRSVNv/GRSVNvc) amplifying DNA fragments of 453 bp and 494 bp, respectively. No samples were positive for ZLCV or TSWV. *Frankliniella schultzei* (99%) was the most frequent thrips species found in watermelon fields, while no *F. zucchini* was identified among the thrips specimens collected. These results indicate the importance of monitoring viruses in watermelon crop in the field and the need of generating epidemiological studies of GRSV in this crop. Financial Support: EMBRAPA.

#### **PIV444 - MELON AND GHERKIN: TWO NEW NATURAL HOSTS OF ZUCCHINI LETHAL CHLOROSIS VIRUS (ZLCV)**

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*Zucchini lethal chlorosis virus* (ZLCV) belongs to the *Tospovirus* genus, in the family *Bunyaviridae* and is transmitted by thrips, in a circulative propagative manner. ZLCV is the solely tospovirus species that is reported on cucurbits in Brazil, causing yield losses, especially in watermelon and pumpkin fields. In 2012 and 2014, three gherkin (*Cucumis anguria* L.) and four melon (*Cucumis melo* L.) plants showing virus-like symptoms were observed in greenhouse cropping system, in the Federal District. Serological tests were performed with leaf extracts obtained from symptomatic melon and gherkin plants for Papaya ringspot virus - type watermelon (PRSV-W), *Watermelon mosaic virus* (WMV) and, *Zucchini yellow mosaic virus* (ZYMV), genus *Potyvirus* in the family *Potyviridae*, *Cucumber mosaic virus* (CMV), genus *Cucumovirus* in the family *Bromoviridae* and, *Zucchini lethal chlorosis virus* (ZLCV), genus *Tospovirus* in the family *Bunyaviridae*, using polyclonal antibodies, by NCM-ELISA. In addition, total RNA was extracted from leaves collected from gherkin and melon plants and tested for ZLCV, by two-steps RT-PCR, using ZLCV-P1/

ZLCV-P2 primer set, which amplifies a fragment of the S RNA, of 350 bp. Leaf extracts prepared in 0.01 M phosphate buffer at pH 7.0 were also rub inoculated onto leaves of indicator host plants *Datura stramonium*, *Nicotiana benthamiana* and, *Cucurbita pepo* cv. Caserta, previously dusted with carborundum. Serological analysis revealed that all three and four gherkin and melon plants, respectively, were infected with ZLCV. Samples also tested positive for ZLCV by RT-PCR with primers ZLCV-P1/ZLCV-P2, producing a 350 bp amplicon. Typical ZLCV systemic symptoms were observed in *D. stramonium*, *C. pepo* and, *N. benthamiana* plants, 10-12 days after inoculation. ZLCV infection in the inoculated plants was confirmed by serological tests. Cloning and sequencing of DNA fragments are underway to confirm ZLCV identification. These data indicate the importance of ZLCV to cucurbit crops. Considering that the virus is a threat to watermelon and pumpkin production in Brazil, further survey is needed to determine the frequency of ZLCV infecting melon and gherkin crops as well as its geographical distribution in the field. Financial Support: EMBRAPA.