2,882,857 and 95,736 contigs respectively. Blastx search of these contigs with viral reference genomes resulted in 4,981 contigs detected as viral sequences. The category of these sequences varied from bacteriophages to plant viruses. In plant viruses, some viral families of DNA (Caulimoviridae) and RNA viruses (Tombusviridae, Luteoviridae, Rhabdoviridae, Potyviridae and Umbravirus) were more evident. Viral genomes were assembled in silico using the Geneious (R9) software, resulting in six possible new viral species, including three from Rhabdoviridae, one from Potyviridae, one from Tombusviridae and one from Umbravirus. With these results, we can assume that important entities of plant virus are present in ornamental plants produced in the DF, which can be a risk to ornamental production as well to other crops distributed around the area.

PIV72 - SEQUENCING OF THE COTTON ANTHOCYANOSIS VIRUS BY SMALL RNA DEEP SEQUENCING AND ITS SIVRNAS PROFILE IN COTTON

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Small RNAs or siRNAs (interfering RNAs) are small RNA molecules originated when plants and animals are infected by viruses. After virus entry into the cell, its genome is released and recognized by cellular proteins called Dicerlike. These proteins fragment viral genome producing small interfering viral RNA (sivRNA), sequences that exhibit at approximately 2124 nucleotides (nts). The sequences of the siRNA are complementary to the viral genome. Total siRNA from Cotton anthocyanosis virus (CAV) infected plants were sequenced by deep sequencing in order to obtain the complete sequence of the CAV genome. The disease caused by CAV is restrict to Brazil, where is called "Vermelhão do algodoeiro". Symptoms are the intense reddening of leaves and stems. Until now, its agent causal was not known at molecular level. CAV was describing in Brazil in 1961 at Brazil by Santos and collaborators as belonging to the Luteoviridae family, Polerovirus genus. Polerovirus have ssRNA + genomes with seven ORFs. In a previous work we sequenced part of CAV genome corresponding to viral capsid (ORF3) and part of its replicase (ORF2) and observed a a high homology between these ORFs and ORFs 2 and 3 from Cotton leafroll dwarf virus (CLRDV) responsible for Cotton blue disease, reaching more than 90% identity. Using siRNA libraries obtain through deep-sequencing performed in Illumina platform at Fasteris Co., Geneva, Switzerland, almost complete genome of CAV was mapped using SearchSmallRNA software. The analyzes showed that siRNA generated during the process of infection range from 1826 nts, with siRNA of 22 nts as the most abundant, followed by 24 nts. Some small genomic portions were not covered by mapping (gaps) corresponding to less than 5% of the genome. For gaps sequencing, sets of primers were design for reverse transcription followed Reaction Polymerase Chain (RTPCR) and subsequent sequencing by Sanger. CAV genome has about 6000 nucleotides. Mapping results were validate by Sanger nucleotide sequencing. Alignment of the CAV ORFs nucleotide and amino acids sequences with other members of Luteoviridae family confirmed that it is a Polerovirus.

PIV99 - DSRNA DEEP SEQUENCING REVEALS FIVE VIRAL SPECIES IN COMMON BEANS

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Common bean (Phaseolus vulgaris L.) is an economically important leguminous crop cultivated worldwide. Viral pathogens play a significant role in reducing the productivity and quality of this crop. Transgenic bean golden mosaic virusresistant common bean plants were recently developed in Brazil. However, field experiments with transgenic lines presented diverse types of symptoms, probably due to infection by RNA virus. To investigate which viruses were present in these plants, we performed highthroughput sequencing from preparations enriched for viral dsRNA. Leaves from transgenic BGMVresistant common bean breeding line CNFCT16207 showing severe crinkling were collected in Goiás, Brazil. dsRNA extraction was conducted using

STEPhenol and cellulose column protocol. Pooled dsRNA samples were pairedend sequenced using MiSeq Ilumina® high performance platform. Sequencing results were analyzed in CLC Genomics Workbench and Geneious® program software for contig construction and comparison with viral sequences in public database and gene annotation. A total of 27,897 contigs were assembled from 13,780,310 reads obtained in the Illumina sequencing. Six viral RNA genomes were recovered and identified as Cowpea mild mottle virus (CpMMV; Carlavirus, Betaflexiviridae), Bean rugose mosaic virus (RNA 1 and RNA 2 BRMV; Comovirus, Secoviridae), two species of the genus Endornavirus, Phaseolus vulgaris endornavirus 1 and 2 (PvEV1 and PvEv2; Endornavirus, Endornaviridae), and a new Cytorhabdovirus (Rhabdoviridae). The size of the viral contigs ranged from 3.7 to 14.8 kb. Based on the consensus sequences obtained through nextgeneration sequencing, specific primers were designed for each virus species identified. Primers were used in PCR reaction to recover virusderived fragments, confirming the presence of all viruses in the plants. Largescale sequencing technology and advanced bioinformatics platforms have allowed the discovery of new viral species and four other RNA viruses in common bean plants from the state of Goiás, being an attractive tool for studying viral diversity in plants. Additionally, dsRNA enriched samples permitted recover the RNA genomes in the replicative form, selecting specifically RNA viruses. This is the first report of a Cytorhabdovirus infecting common bean plants.

HV2 - DETECTION OF THE EMERGING ROTAVIRUS G12P[8] GENOTYPE AT HIGH FREQUENCY IN BRAZIL IN 2014: SUCCESSIVE REPLACEMENT OF PREDOMINANT STRAINS

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INSTITUTO ADOLFO

The continuum characterization of circulating RVA genotypes is essential to understand how vaccine introduction could impact virus epidemiology. In the present study, an unexpected rapid changing pattern of RVA genotypes distribution in Brazilian population during three followed seasons is described. From January/2012 to December/2014, a total of 3441 fecal specimens were collected from collaborating centers across Southern, Southeastern and Midwest Brazil, and likely to be representative of Brazilian population. All specimens were screened for RVA using ELISA, and genotyped by RTPCR. Differences in proportions were tested using Chi Squares. A pvalue of less than 0.05 was considered statistically significant. RVA was detected in 19.7% (677/3441). G3P[8] remained prevalent in 2012 (37.6%, 69/185) and 2013 (40.1%, 74/186) (?2=0.107, p=0.743), but declined markedly in 2014 (3.5%, 10/281) (?2=71.770, p=0.000). G12P[8] was second highest strain in 2012 (22.7%, 42/185), decrease rapidly in 2013 (2.7%, 5/186) (?2=26.224, p=0.000) and re-emerged as the predominant genotype in 2014 (86.6%, 243/281) (?2=118.299, p=0.000). From July/2014, G12P[8] was the single genotype detected in all regions studied. The present study raised the hypothesis of a possible G12 outbreak being in progress. Nationally, the Hospitalbased Information System surveillance data confirmed the long term decline in gastroenteritis hospitalization observed in Brazil after RVA vaccine introduction. Nevertheless, the sharp increase in diarrhea hospitalization prevalence from 2013 to 2014 observed in Southern and Southeastern regions is consistent with what appears to be an outbreak of G12P[8]. Furthermore, in 2014, the FIFA World Cup was held in Brazil, and the introduction a novel RVA strain was a real threat, given large numbers of visitors from areas with ongoing G12P[8] genotype transmission. Moreover, this event occurred right before the beginning of the RVA seasonality in the country. Worldwide, the emergence of genotype G12P[8] as an epidemiologically important strain could raise new concerns for RVA vaccine development. However, despite the possible emergence of new strains, vaccination has been shown to reduce the disease incidence of RVA infection and remain below prevaccination levels. Continued surveillance is needed to verify the effectiveness of the RotarixTM vaccine in Brazil together with potential emergence of unusual genotypes.