to be transmitted by nematodes of genera Trichodorus and Paratrichodorus they are thought to be present in high titers in the root tissues. The objective of this study was to immunolocalize the PepRSV capsid protein (CP) in the root tissue of Nicotiana benthamiana. Plants of N. benthamiana were inoculated with the CAM isolate of PepRSV. After 14 days, the roots were collected, fixed (3% paraformaldehyde, 0.1% glutaraldehyde), treated with the polyclonal antibody against the CP of PepRSV, produced in the Laboratory of Virology of the ´Centro Nacional de Pesquisa em Hortaliças´, and later treated with antirabbit conjugated with alkaline phosphatase (AP). Chromogenic substrates, 5bromo4chloro3 indolyl phosphate (BCIP) and nitro blue tetrazolium chloride (NBT), were used for immunodetecting AP activity. BCIP is hydrolized by AP and intermediates undergo dimerization with the help of NBT. At the end of the reaction an insoluble darkblue precipitate is formed consisting of NBTdiformazan and 5,5`dibromo-4,4` dichloro indigo. The immunostained tissue was analyzed with the Leica TCS/SP5 confocal laserscanning microscope (Leica Microsystems, Wetzlar, Germany). Strong chromogenic signals were observed in the phloem cells of the root tissue in PepRSV infected N. benthamina plants suggesting that the virus efficiently translocate to root tissues. No positive signal was observed in noninoculated plants of N. benthamiana (negative control). We concluded that the CAM isolate of PepRSV translocates via vascular system (phloem) to the root tissue, similar to the other two tobraviruses, TRV and PEBV.

PIV226 - A NOVEL CYTHORHABDOVIRUS IN ARRACACHA (ARRACACIA XANTHORRHIZA)

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Arracacha (Arracacia xanthorrhiza) is one of the most important cultivated Andean roots, belongs to the family Apiaceae, which includes carrot, celery and parsley. It is vegetatively propagated, and therefore it accumulates high amounts of degenerative pathogens such as viruses. The viral metagenome sequencing brings many possibilities of identifying unknown viruses, overcoming previously technical barriers. Therefore, through next generation sequencing (NGS) and metagenomics analysis a novel plant virus related to Rhabdoviridae family was found infecting arracacha plants. Here we describe the molecular characterization of this putative new rhabdovirus genome. To this extent, based on NGS sequence information, primers were designed to amplify the full viral genome containing overlapping regions. Initially, the presence of this new putative plant rhabdovirus was confirmed by RTPCR in 36 arracacha plants out of 47 analyzed. One plant was selected and total RNA extracted aiming to amplify five overlapping regions of the genome. All amplified fragments were sequenced by Sanger sequencing. The RACE technology was used to determine both 5' and 3' terminals. The genomic organization resembles those of plant rhabdoviruses. Six open reading frames (ORFs) were identified in the antigenomic orientation of the negativesense, singlestranded viral RNA, in the following order 3\'NP4bM GL5\'. Amino acid sequence analysis of the putative nucleoprotein (N) showed 941% identity with N proteins encoded by other plant rhabdovirus genomes. Phylogenetic analysis of the N and polymerase (L) amino acid sequence indicated that this arracacha-infecting rhabdovirus is related to viruses belonging to Cytorhabdovirus genus, and are closely related to Alfalfa dwarf virus. According to Rhabdoviridae Taxonomy Group (ICTV), genus classification based on sequence diversity has thus far correlated 100% with classification by intracellular virus maturation. Giving these findings the novel virus found infecting arracacha should be considered as a new species of the Cytorhabdovirus genus.

PIV231 - DISCOVERY OF A NOVEL DICISTROVIRUS ISOLATE IN TOMATO LEAF SAMPLES

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The family Dicistroviridae is composed of viruses that infect invertebrates, including insects such as honeybees and hemipterans. Therefore, these viruses might provide practical applications for controlling agricultural arthropod pests. Our group is currently making efforts

to identify whiteflyinfecting viruses that can be used as biological control agents in integrated pest management programmes. The surveys are carried out directly in whiteflies and also in whiteflyinfested plants. Here, we report the identification of a dicistrovirus using a next generation sequencing (NGS) approach. Tomato leaves were collected in São Paulo state in 2013 and kept at 80 °C until processing. After semipurification of virus particles by differential centrifugation, total RNA was extracted and subjected to NGS sequencing at Macrogen, Inc. Reads were trimmed with an automatic Phred score on Trimommatic before contigs were assembled using the Velvet algorithm (91kmer) and analysed for their shared identities with other viruses in a RefSeq database using MegaBLAST on Geneious software. Forty contigs ranging from 190 to 1,228 nt shared high percentage identity (>80%) with the dicistrovirus Aphid lethal paralysis virus (ALPV). These contigs were used as reference for sequence extension using the Geneious mapper. By using this strategy, a 9,936 ntlong sequence was generated from 278,669 reads. The sequence presented 86% overall identity with ALPV (acc. JQ320375, 97% query cover, Evalue=0.0) and two major ORFs, the first ranging from nt 592 to 6699 and coding for a putative protein similar to ALPV nonstructural polyprotein (92% identity, Evalue=0.0) and the second ranging from nt 6896 to 9301, coding for an ALPVlike capsid protein (92% identity, Evalue=0.0). As this virus presumably infects insects, pathogenicity tests were initiated in insect cell lines. The extract containing semipurified particles used for NGS sequencing was filtered and inoculated into lepidopteran cell line UFLAg. Cytopathic effects such as vacuolization and cell rounding were evident following ten days postinoculation. Furthermore, reinoculation of the cell media into healthy cells consistently produced cytopathic effects. These results demonstrate the feasibility of using NGS methods for discovery of new virus isolates.

PIV233 - SEQUENCE VARIABILITY AND EVOLUTION OF TOMATO CHLOROSIS VIRUS IN BRAZIL

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Tomato chlorosis virus (ToCV, genus Crinivirus, family Closteroviridae) is a whiteflytransmitted crinivirus with a bipartite RNA genome (RNA1 and RNA2). The RNA1 encodes proteins involved in replication of viral RNA and suppression of gene silencing, while RNA2 encodes proteins likely involved in viral movement and encapsidation. In the last years, this virus is emerging as a serious threat to tomato crops in Brazil. During 2015, surveys were done on tomato (Solanum lycopersicum) virus diseases in three states of Brazil: Goiás (GO), Paraná (PR) and Rio de Janeiro (RJ). Samples exhibiting interveinal chlorosis on the basal leaves, typical symptoms of ToCV, were collected. Total RNA was extracted from 64 samples and the presence of ToCV infection confirmed by reverse transcriptionPCR (RTPCR) using the specific primers, which amplifies a fragment of approximately 463 pb. A total of 55 samples were PCR positive (14/14 from GO, 23/23 from RJ and 18/27 from PR). In order to study the variability and evolution of ToCV species infecting tomatoes in Brazil, 10 isolates from each Brazilian state were selected. Primers were designed to amplify three genomic regions coding three genes, p22 (RNA1), HSP70h and CP (RNA2), which amplify fragments of 720, 936 and 917 pb, respectively. PCR products were cloned and sequenced. Preliminary results based on p22 protein from a population of PR state indicate nucleotide diversity ranged between 0.1 and 0.3% and interpopulation between 0.1 and 3.2%. Virus evolution predictions based on synonymous/ nonsynonymous rates indicate that this population is under positive selection suggesting adaptation to a new ecological niche. Interesting, in phylogenetic analyses, these isolates grouped in a regular manner suggesting a geographicalbased evolution pattern. However, only one location has been completely analyzed, data from other regions (RJ e GO) will be useful to determine the true diversity of Brazilian isolates.