

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, 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium chloride (NBT), were used for immunodetecting AP activity. BCIP is hydrolyzed 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 NBT-diformazan 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. benthamiana* 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 tobnaviruses, 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