**PL 26 - TEMPORAL AND SPATIAL ANALYSIS OF POST-TRANSCRIPTIONAL GENE SILENCING ON THREE TRANSGENIC TOBACCO PLANTS HARBORING DIFFERENT *ANDEAN POTATO MOTTLE VIRUS* SEQUENCES.**

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Post-transcriptional gene silencing (PTGS) is a mechanism of gene expression regulation based on specific RNA degradation in the cytoplasm. It was first found on plants and is now known to be a conserved phenomenon through several kingdoms. It's thought that PTGS in plants is a natural mechanism of protection against virus infection. There are numerous works concerning the state of PTGS during plant development. However, the existing data are very variable and sometimes contrasting. In this work, temporal and spatial analysis of PTGS input in transgenic tobacco plants lines containing different sequences of *Andean potato mottle virus* were done. These transgenic lines harbor one among the three following viral constructs: the replicase gene (REP lines), the minor coat protein (CP) gene (CP lines) and part of minor CP fused with 3'non-translatable region (DP3 lines). Plants exhibiting high PTGS or no PTGS of transgene from different generations were grown in vitro and collected at different times during the initial phases of development. Spatial analyses were done by collecting different leaf stands from the same adult plant. In order to detect transgene expression, northern blots were performed and hybridized with the transgene homologous fragments radioactively labeled. Autoradiography from different generations of the silenced DP3 line showed that PTGS is been triggered very early during plant development on that line. Northern blots from F3 generation plants showed that one-day after germination the DP3 transgene expression is no more detected. This result suggests a strong mechanism of RNA turn over and may be correlated with the high virus resistance phenotype observed in this line. Spatial analysis of DP3 line revealed that all leaves collected from adult plants were silenced. Autoradiography of replicase and CP22 lines showed that PTGS is triggered in a late phase of plant development. On these silenced lines, transgene expression was detected even on the 16th day post-germination. Although this later input of PTGS have no influence on viral resistance observed on these lines.

**PL 27 - *TOMATO MOSAIC VIRUS* (ToMV) IN *HEMEROCALLIS* IN BRAZIL.**

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The culture and commercialization of ornamental plants has considerably increased in the last years. To comply with commercial demands, several *Hemerocallis* varieties have been bred for appreciated qualities such as plant perenniality and flowers with a diversity of shapes and colors. Recently, imported *Hemerocallis* varieties kept under quarantine conditions were evaluated for viruses. Electron microscopic preparations from samples showing foliar chlorotic and necrotic spots contained tobamovirus-like particles, and inoculated host plants displayed symptoms indicative of TMV infection. One fragment corresponding to the coat protein gene with 480bp was obtained after RT-PCR using the primers PS1 and PSA2. This product was sequenced and aligned with 19 other tobamoviruses, showing 99% and 100% identity with *Tomato mosaic virus* (ToMV) isolates for nucleotides and amino acids, respectively. As the infected plants were kept under quarantine, they were destroyed. This occurrence shows the importance of the quarantine measures, avoiding the introduction and spread of the virus in the country.

**Financial support**: FAPESP (partially); CNPq



**PL 28 - TOMATO YELLOW LEAF CURL, A MONOPARTITE BEGOMOVIRUS, WAS NOT DETECTED IN A SURVEY OF TOMATO FIELDS IN SÃO PAULO STATE, BRAZIL.**

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*Tomato yellow leaf curl virus* (TYLCV) is a monopartite Begomovirus, Geminiviridae. This virus is widespread in several countries of Europe and North and Central America. The occurrence of TYLCV is still not reported in Brazil. A survey was carried out in tomato fields of São Paulo state, Brazil. Forty six leaf samples from plants exhibiting symptoms resembling those of TYLCV and bipartite begomovirus were collected throughout seven localities around Campinas in São Paulo State. The symptoms included leaf rugosity, mosaic, chlorosis and spoon-like shape of the leaves. The leaves were blotted and hybridized with probes to bipartite begomoviruses, TYLCV-Is from Israel and TYLCV-Sar from Sardinia. None of the samples reacted positively with TYLCV-Is and TYLCV-Sar. The respective positive controls as tissue print showed positive reaction confirming the specificity of the probes. Twenty seven plants out of 46 tested reacted positively with the probe to bipartite begomovirus. In order to confirm the results obtained by hybridization, seven leaf samples were selected and PCR was performed with total DNA extracts using primers specific to the intergenic region of TYLCV-Is and TYLCV-Sar separately, and to the conserved regions of the coat protein of geminiviruses. Only the positive controls TYLCV-Sar and TYLCV-Is resulted in amplification of specific PCR products using primers for the intergenic region of both isolates of TYLCV. When the coat protein was targeted for PCR, four hybridization positive

samples resulted in amplification. The remaining three leaf samples neither were hybridization positive nor PCR positive. The bipartite geminivirus and the two TYLCV isolates rendered positive by this method. The obtained results indicated that the monopartite TYLCV was not present in tomato fields around Campinas county. Although the surveyed area was restricted, the results suggests that a TYLCV-like symptom in tomato is not an indicative of its presence under our conditions.

**Financial support:** CNPq; 4CNPq fellows

### PL 29 - ULTRASTRUCTURE AND IN SITU IMMUNOLABELING STUDIES OF DIFFERENT SPECIES OF POTYVIRUSES OF APIACEAE.

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Comparison of NIb-CP region (650 kb) sequences of several isolates of potyviruses infecting Apiaceae, including celery throughout the world revealed 3 distinct but closely related species (Traicevski et al., 2001) respectively Celery mosaic virus (CeMV) with isolates from Europe, USA and Australia, Apium virus Y (APY) with isolates from Australia and Brazil and Carrot virus Y (CVY) with isolates from Australia. Leaf samples of plants infected by representatives of these 3 species (Conium maculatum/APY, carrot/CVY and celery/CeMV) from Australia were processed for ultrastructural studies. Leaf parenchyma cells infected by CVY and CeMV contained, as expected for potyviruses, cylindrical inclusions and aggregates of elongated particles in the cytoplasm, but those infected by APY also contained intranuclear fibrous masses, as previously described with the Brazilian isolate of CeMV (known as Celery yellow mosaic virus-CeYMV). *In situ* immunolabeling experiments using anti-CeYMV serum consistently labeled the elongated particles present in these cells. The results indicated that even though the NIb sequence analysis reveals enough differences to separate these 3 viruses as distinct species, they share common CP antigens. Phylogenetic analysis has placed CeYMV very close to APY and this was supported by the fact that both viruses produced similar intranuclear fibrous inclusion.

**Financial Support**: FAPESP; Pronex

### PL 30 - VIRUS RESISTANCE IN TRANSGENIC PLANTS THROUGH HOMOLOGY-DEPENDENT GENE SILENCING IS ABOLISHED BY PREVIOUS INFECTION WITH AN UNRELATED VIRUS.

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Post-transcriptional gene silencing (PTGS) is a ubiquitous mechanism for specific degradation of homologous mRNAs in cytoplasm of eukaryotic cells and has probably evolved as a natural system of defense against virus and transposons in plants, animals and fungi. Counteracting, several plant viruses encode proteins able to suppress PTGS at different stages. In this work, two transgenic tobacco lines harboring different sequences homologous to Andean potato mottle virus (APMoV) were used. These plants present concomitant transgene PTGS and APMoV resistance. Effect of a previous PVY infection in post-transcriptional transgene silencing suppression in both tobacco lines was analyzed by northern-blot and RT-PCR, while resistance to a further APMoV infection tested by ELISA. Different degrees of PTGS suppression were observed for individuals of both transgenic lines and lost of APMoV resistance confirmed in one of them.

**Financial support:** CAPES and CNPq

## Protein Expression and Vectors (PR)

### PR 1 - BICISTRONIC VECTORS FOR GLIOBLASTOMA GENE THERAPY STUDIES.

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Several studies show tumor suppressor gene transfer, like p16INK4a or p21WAF1, as a strategy for treatment of glioblastoma and other types of cancer. None however, has been totally successful, since no tumor suppressor gene is capable of acting alone as a general and complete tumor growth inhibitor. The action of a single tumor suppressor gene should not be expected to efficiently halt tumor cell growth since cancer results from multiple mutation events. Such mutations may destroy the endogenous targets of the therapeutic gene. Based on that, new proposals are being made, such as the simultaneous replacement of p16INK4a and p21WAF1 tumor suppressor genes that will be shown here. With the purpose of exploring advantages of both the retrovirus system and the approach of cytotoxic gene combinations, we have constructed a pCL bicistronic retroviral vector carrying p16INK4a and p21WAF1 cDNAs connected by an intercistronic IRES sequence: pCLp16IRESp21SEGFP. This pair of genes was chosen, as previous studies have shown stronger suppression of GBM cell proliferation when infected either with p16INK4a or p21WAF1, than through the action of p53, both in vitro and in vivo. The construction and production of a bicistronic virus encoding two potent cell cycle inhibitor genes is not simple and it is a principal challenge of this research. We will describe our results for cloning, production