RESUMO - VEGETAL E INVERTEBRADOS

CLERODENDRUM CHLOROTIC SPOT VIRUS AND BREVIPALPUS YOTHERSI MITE: A MODEL FOR THE STUDY OF THE DICHORHAVIRUS-VECTOR INTERACTION

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Clerodendrum chlorotic spot virus (ClCSV), genus Dichorhavirus, family Rhabdoviridae, is a bi-segmented (-)single-stranded RNA virus commonly occurring in some ornamental plants in Brazil. ClCSV causes localized chlorotic lesions restricted to the feeding sites of its vector, false spider mites of the species Brevipalpus yothersi. However, as in the case of other dichorhaviruses, features of the interaction between ClCSV and its vector are still poorly understood. To address this, we undertook in-depth biological and molecular tests to assess the viral transmission parameters, to identify ultrastructural modifications in the infected cells, and to reveal the kinetics of accumulation of antiviral genomes in viruliferous mites. Biological transmission parameters were studied using common bean (Phaseolus vulgaris) as indicator plants of the ClCSV infection. The viral acquisition occurred by all active life stages of the mite with a minimum access period of four hours, while the inoculation by all mite developmental stages, except for larva, occurred after an access period of one hour. Upon acquisition, mites were able to transmit the virus only after ˜

eight days (latent period), and they remained viruliferous during the next 26 active lifespan days. Presumed rod-shaped viral particles of ClCSV were detected inside vesicles in the cytoplasm of mite cells that also showed large electron-lucent nuclear inclusions. Essentially, ultrastructural modifications of mite cells resembled the cytopathic effects observed in the leaf tissues of clerodendrum plants infected by ClCSV. Since the accumulation of the complementary sense molecule of any viral gene could be considered as a sign of viral propagation, i.e. replication and gene expression, a strand-specific RT-PCR (ss-RT-PCR) assay for the detection of the complementary RNA strand (positive sense) of the gene N (ORF1: RNA1) was carried out in viruliferous mites. To avoid false positives, convenient tags were added to the 5'-end of primers, and the cDNA extracts were treated with exonuclease I before the PCR amplifications. After the acquisition, time course assays including all active phases of the mite indicated the presence of antisense genomic molecules in individuals reared by up to 18 days on Canavalia ensiformis, a non-viral host plant. Comprehensively, this work provides accurate data and compelling evidence about the propagation of ClCSV in B. yothersi, confirming a persistent-propagative virus-vector relationship previously suggested for this type of viruses. The detailed description and high reproducibility of the processes involving the biosystem ClCSV-B. yothersi suggests it as a model for further studies with other members of the genus Dichorhavirus.