ISSN 1519-2563

VIRUS

Reviews & Research

SEPTEMBER 2002
VOL. 07, Nº 1

SBV

Não Falei.

JOURNAL OF THE BRAZILIAN SOCIETY FOR VIROLOGY

XIII NATIONAL MEETING OF VIROLOGY
September 30 – October 3, 2002
Águas de Lindóia, SP, Brazil

SEPTEMBER – 2002
VOL. 07, Nº 1
SUPPLEMENT 1

determined by transferring five whiteflies per plant using tomato cv. Santa Clara as a host. For the AAP and IAP, nine different time periods were tested: 15 min, 30 min, 1, 2, 4, 8, 16, 20 and 24 h. The vector was able to transmit the virus after 15 min AAP, resulting in 6% of infected plants. The rate of transmission increased as the length of AAP, reaching 65% within 24 h. Regarding the IAP, it was observed 18% of infected plants after 30 min. The infection rate increased to 67% after an IAP of 24 h. The latent period was considered to be 16 h, when 3% of the inoculated plants were infected. To detect the GO-ANPL isolate in the vector, more than 2.500 specimens were tested by PCR. The presence of the virus was detected in the vector from the 1st to the 4th instar grown on infected plants, in adults under different AAPs and in adults from immature stages that were reared on infected tomato plants. No virus was found in eggs from aviruliferous females that had been laid on infected plants. The GO-ANPL isolate was transmitted to the progeny of viruliferous females, since the virus was detected in all stages of insect development from eggs to adults. However, no virus transmission was observed from these adults. High frequency of viral detection was observed in newly emerged adults from immature forms reared on virus-infected plants. These adults infected 33% of tomato plants in virus transmission assays performed subsequently. The results of the virus retention, the AAP, IAP, latent period and vector virus detection, indicates that the interaction between virus and vector starts at early stages of insect development. The higher levels of GO-ANPL isolate transmission efficiency with the longer AAP or IAP fit the persistent circulative mode of virus transmission.

PL 11 - FUNCTIONAL DISSECTION OF THE TMV (*TOBACCO MOSAIC VIRUS*) REPLICASE USING GREEN FLUORESCENT PROTEIN.

Figueira, A.R.[1]*; Golem, S.[2]; Goregaoker, S.P.[2], Culver, J.N.[2] ([1]Depto de Fitopatologia - UFLA, Lavras- MG, Brasil; [2]Center for Agricultural Biotechnology, University of Maryland Biotechnology Institute, College Park, USA). *E-mail: Antonia@ufla.br

Transgenic plants containing segments of the TMV replicase displayed resistance against several different tobamoviruses in a non-homology dependent fashion. There are two different forms of protection derived from segments of the TMV replicase genes. A low level derived from replicase RNA sequences via a sequence-specific host defense mechanism, termed post-transcriptional gene silencing (PTGS), and a high level derived from translatable segments of the 54-Kda polymerase domain. It seems that multiple mechanisms may contribute to the resistance conferred by TMV replicase domains. The goal of this work is to investigate the functions of specific replicase domains and how these functions can be utilized to confer protection in transgenic plants. Initially a conserved nuclear localization signal (NLS), encoded by the amino terminus of the 126 kDa protein, was investigated for its functionality using fusion constructs containing the green fluorescent protein (GFP). Fusion of the amino terminal 63 amino acids of the 126 kDa protein, containing the NLS, to a ß-glucuronidase-GFP open reading frame (ORF) directed the accumulation of fluorescence to the nucleus. It did not happen with the constructs lacking the NLS or containing a mutated NLS. Deletion constructs with the NLS motif were created and fused to the N-terminus of the GFP ORF. Two GFP fusion constructs (126^{1-178}-GFP, mw = 71.9-kDa) containing the first 178 and 388 N-terminal amino acids of the 126 kDa protein, respectively, were found to primarily localize to the nucleus but could also be observed associated as strands within the cytoplasm. In contrast, fusion constructs carrying the first 781 amino acids or the full-length 126 kDa ORF (126^{1-781}-GFP, mw = 115,7 kDa and FL126-GFP, mW152,9 kDa) did not localized to the nucleus but instead associated with the endoplasmic reticulum (ER), forming spot-like inclusions. It indicates that regions of the 126-kDa protein beyond amino acid 388 act in a dominate fashion over the N-terminal NLS to prevent the nuclear localization of the 126-kDa protein. Another 126-kDa-GFP fusion construct containing a non-functional NLS mutation also localized to ER but did not form inclusions. In addition, TMV mutant containing the same non-functional 126-kDa NLS failed to replicate in protoplasts. These findings suggest that the NLS contributes to the cellular localization of the 126-kDa protein and may play an important role in virus replication.

Financial support: CAPES *bolsista da Capes

37463

PL 12 - IDENTIFICATION OF *BANANA STREAK VIRUS* STRAINS THROUGH ANALYSIS OF PCR AMPLICONS.

Figueiredo, D.V.[1], Souza, M.G.[2], Gasparotto, L.[2], Teixeira, E.A.[3] e Brioso, P.S.T.[1]* ([1]Laboratório de Virologia Vegetal e Viróides/DEnF/UFRRJ, Seropédica, RJ; [2]EMBRAPA Amazônia Ocidental, Manaus, AM; [3]AGDER, Goiânia, GO). *E-mail: brioso@whouse.com.br

BSV belongs to the family *Caulimoviridae* (genus *Badnavirus*) and banana is its natural host. The banana crop has considerable economic importance in Brazil, but no BSV diagnoses molecular method has been utilized to index banana germplasm in this country. A polymerase chain reaction (PCR) assay using degenerate primers, designed to amplify Badnavirus genus, has been standardized to detect and characterize BSV strains in banana cultivars from Brazil. The cycling parameters consisted of an initial denaturation cycle of 94 °C for 5 min., 42 °C for 2 min., 72 °C for 3 min.; followed by 25 cycles of 94 °C for 1 min., 42 °C for 2 min., 72 °C for 3 min., and finally, 1 extension cycle of 94 °C for 1 min., 42 °C for 2 min., 72 °C for 10 min. A screening of DNA samples isolated from banana leaf crude sap of plants collected in Brazil was carried out. The virus was detected in diploid (AA - 'Khai nai on'), triploids (AAA - 'Caipira', 'Grand Naine', 'Nanicão'; AAB - 'Maçã', 'Mysore', 'Prata', 'Prata anã', 'Terrinha' e 'Thap maeo'; ABB -

'Marmelo' e 'Prata Zulu') and tetraploids (AAAB - 'FHIA-18', 'FHIA-21', 'Pacovan Apodi', 'Pioneira' e 'PV-03-44') banana cultivars. Four BSV strains were found, and distinguished by their electrophoretical patterns of PCR amplified products. One strain, BSVBR-1, was found to be distributed in the states of Amazonas, Bahia, Ceará, Goiás, Minas Gerais, Piauí, Rio de Janeiro, Rondônia and São Paulo, what suggests the same origin of infected banana crops in these States. The three others strains, BSVBR-2, BSVBR-3 and BSVBR-4, were detected only in the state of Ceará, while BSV-BR2 was found in Amazonas state. These results could be employed to the production of BSV-free banana germplasm, that will contribute to the increase of productivity of this crop in Brazil.

Financial support: CNPq

PL 13 - MOLECULAR CHARACTERIZATION OF A NEW BEGOMOVIRUS ISOLATED FROM TOMATO IN THE STATE OF GOIAS, BRAZIL.

Santos, C.D.G.[1]; Nagata-Inoue, A.[2]; Ávila, A.C.[2]; Resende, R.O.[3] ([1]Deptº de Fitopatologia - UNB, Brasília-DF; [2]EMBRAPA-Hortaliças, Brasília-DF; [3]Deptº de Biologia Celular, UNB, Brasília-DF). E-mail: carmelo@ufc.br.

The whitefly-transmitted viruses from the family *Geminiviridae*, genus *Begomovirus*, have been reported as an economically important pathogen group that affect important crops in tropical and subtropical countries. Since the last decade, the occurrence of the whitefly associated to *Begomovirus* infection has drastically increased worldwide. In Brazil, these pathogens have been responsible for severe economical losses in tomato fields and the production has hampered since 1994. In this work, infected tomato plants showing symptoms, such as mosaic, intervein clearing, leaf curling and growth reduction were collected in tomato fields in Anápolis, State of Goiás. The virus named GO-ANPL, was identified as a member of the genus *Begomovirus* by PCR reaction, using specific primers to amplify fragments of A and B components of the virus DNA genome. The host range was determined by mechanical inoculation and vector transmission of the virus isolate onto 46 plant species from nine different botanical families. The GO-ANPL isolate preferentially infected plants of the family Solanaceae as *Nicotiana benthamiana*, *Datura stramonium* and *Nicandra physalodes*. The number of infected plants was higher when it was inoculated by the virus vector, and the results were distinct from those obtained for other begomoviruses reported in Brazil. Virus infection was confirmed by dot blot hybridization using specific molecular probes to the virus. The virus was cloned and sequenced. Part of the sequenced genome (2.130 nucleotides long) corresponded to the coat protein and Rep genes and comprised the entire intergenic region. Sequence comparison revealed that the GO-ANPL isolate is distantly related to the begomoviruses found in Asia, Europe and Africa, and it is related to other begomoviruses reported in Brazil. The virus isolate showed to be more closely related to viruses found in the State of Minas Gerais (TRMV isolate) and in the Federal District (isolate DF-Br2). The highest homology (98,2%) was observed with the isolate DF-Br2 and it may represent a new specie of the genus *Begomovirus*.

Financial support: CAPES

PL 14 - MOLECULAR CLONING AND CHARACTERIZATION OF *TOMATO CHLOROTIC MOTTLE VIRUS* (TCMV), A NEW TOMATO-INFECTING BEGOMOVIRUS.

Andrade, E.C.[1], Ambrozevicius, L.P.[1], Calegario, R.F.[1], Fontes, E.P.B.[2] and Zerbini, F.M.[1]* ([1]Dep. de Fitopatologia, [2]Dep. de Bioquímica e Biologia Molecular, BIOAGRO/UFV, Viçosa, MG). *E-mail: zerbini@ufv.br

Geminiviruses comprise a large group of plant viruses, which infect a variety of economically important crops. Their genome is packaged in twinned, icosahedral virions as circular, single-stranded DNA. Members of the *Geminiviridae* family are divided into four genera based on insect vector, host range and genome structure. Species of the genus *Begomovirus* usually have a bipartite genome composed of two 2.6 kb components designated DNA-A and DNA-B. The DNA-A encodes for proteins involved in replication (Rep and Ren), gene expression (TrAP) and encapsidation (CP), whereas the DNA-B encodes two proteins (NS and MP) required for cell-to-cell and systemic movement of the virus. The incidence of geminivirus diseases in Brazil has increased dramatically in the last decade. A PCR-based assay was used to detect geminiviruses in tomato plants collected in Minas Gerais state. Preliminary sequence analyses of the PCR-amplified fragments suggested that a new begomovirus, named *Tomato chlorotic mottle virus* (TCMV), was present in samples collected at Igarapé, MG. Full-length cloning of the DNA-A and -B was carried out, and the complete nucleotide sequence of the TCMV-Ig1 isolate was determined. Sequence analysis indicated that the TCMV-Ig1 DNA-A shares greater than 80% nucleotide sequence homology with other begomoviruses, including viruses that infect only leguminous crops, such as BGMV and BDMV. The results indicate that TCMV is most closely related to *Tomato rugose mosaic virus* (TRMV), another tomato-infecting begomovirus from Minas Gerais. Sequences of their capsid and Rep proteins are 97 and 80% homologous, respectively, which suggests a recombinant origin. Infectious clones of TCMV-Ig1 were obtained, and will be used in pseudorecombination assays to determine the relationship of this isolate with other begomovirus species, including TRMV, and other isolates of TCMV from Bahia and Minas Gerais.

Financial support: CAPES, CNPq, PADCT, FAPEMIG