control strategies are under way.
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EXPRESSION OF THE COAT PROTEIN OF TOMATO INFECTING GEMINIVIRUS IN *Escherichia coli* FOR SEROLOGICAL STUDIES. *Inoue-Nagata, A. K.[1]; Nagata, T.[2]; Miranda, J. D. R.[1]; Bezerra, I. C.[2]; Ribeiro, S. da G.[1] & de Ávila, A. C.[2] (1EMBRAPA Recursos Genéticos e Biotecnologia, Cx. Postal 2372, CEP 70770-900, Brasília, DF, 2EMBRAPA Hortaliças, Cx. Postal 218, CEP 70359-970, Brasília, DF. e-mail: alicenag@cenargen.embrapa.br, Fone: 61-3484711).

The recent outbreaks of geminiviruses in tomato crops throughout Brazil urged an efficient and simple diagnostic system, such as serology. However, their diagnosis relies on hybridization or PCR, both costly and time-consuming procedures. Production of geminivirus specific antibodies is hampered by the difficulties encountered in virus purification, usually resulting in poor purity and low yield. Therefore, serological methods are rarely used due to the lack of specific antisera. A geminivirus was isolated from a tomato field in the Federal District and a preliminary test using the available antibodies of several geminiviruses proved not to be suitable for ELISA-based detection of this virus. This study aimed to produce polyclonal antibodies using bacterially expressed geminivirus coat protein for serological studies. Primers flanking the coat protein in regions of high homology among several geminiviruses were designed and used to amplify a DNA fragment of ca. 1Kbp by PCR. This DNA fragment was cloned in an expression vector pQE (Qiagen) and transformed in *Escherichia coli* M15 cells. The expression of the protein was induced and total protein extract was loaded in a polyacrylamide gel. The gel revealed the production of high amounts of a ca. 30kDa protein, which was absent in the controls. Western blot analysis using polyclonal antibodies raised against bean golden mosaic virus showed a strong positive reaction with the 30kDa protein confirming the successful expression of the geminivirus coat protein. Large amounts of this protein was expressed and purified using a Nickel affinity column under denaturing conditions. Rabbits were immunized with fractions of the purified protein for polyclonal antisera production. The antibody raised in this study reacted only with the denatured protein but not with intact virus particles in tests of DAS-ELISA and Dot-ELISA.

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MAIZE VIRUS IN BRAZIL RELATED TO WHEAT STREAK MOSAIC VIRUS. Oliveira, E.; I.R.P., Souza & Paiva, E. (Embrapa Milho e Sorgo, C.P. 151, CEP 35701-970, Sete Lagoas, MG, Brazil. E-mail: beth@cnpms.embrapa.br, Fone: (31) 779-1192, Fax: (31) 779-1088).

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The presence of filamentous and flexuous particles, potyvirus-like, was detected in maize leaves showing epinasty, short internodes, reduced ears size and discontinuous discoloration over the leaf veins. These discoloration symptoms evolves to necrotic spots. The particles in "leaf dip" preparations stained with 2% PTA, were observed using a transmission electron microscope. Leaf samples from these plants were analyzed by Western blotting using a policlonal antiserum against wheat streak mosaic virus. Also, a polyclonal IgG against the potyvirusis that induces mosaic in maize (MDMV, SCMV, SrMV e JGMV) was used. All samples reacted positively only with the antiserum against wheat streak mosaic virus. The virus was purified from these analyzed plants and a specific antiserum was obtained. Several infected plants were detected in different cultivars using this specific antiserum. In 1997, leaf samples from corn and sweet corn, showing symptoms from a virus-like disease were analyzed by Western blotting using an IgG or antiserum against different virusis, including the potyvirus that induces mosaic. However, a positive reaction was just detected with the antiserum against wheat streak mosaic virus. The analysed plants exhibited reduced growth and ears size, and interveins discoloration symptoms. In sweet corn the discoloration symptoms evolved to necrotic spots. Unfortunately, these plants were not analyzed to verify the presence of virus particles. Improvement in the virosis characterization and complementary studies to determine its occurrence in the maize crop are in current development.

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TRANSGENIC TOBACCO PLANTS HARBORING ANDEAN POTATO MOTTLE COMOVIRUS REPLICASE IN ANTISENSE ORIENTATION SHOW VIRUS RESISTANCE F_1 GENERATION Vidall, M.S.[1]; Monteiro, A.L.V.W.[1]; Corrêa, L. R.[1]; Margis, R. [1-2] & Vaslin, M.F.S.[1] (1- Depto.Genética, Inst.Biologia; 2- Depto Bioquímica, Inst.Química, UFRJ, Rio de Janeiro, Brasil e-mail: margis@uol.com.br and mvaslin@chagas.biof.ufrj.br)

Chimeric constructs containing the Andean potato mottle virus (APMoV) replicase gene, in sense and antisense orientation, were introduced in tobacco plants via *Agrobacterium* transformation. The transgenic plants were screened in greenhouse for virus resistance and several independent transgenic lines have shown viral protection, as reported in a previous work. Here we show that the resistance was maintained in F_1 generation for the plants harboring the replicase in antisense orientation (repAS plants). Four of these lines presented resistance in inoculated leaves characterizing an immunity to the APMoV. Before viral inoculation total RNAs were extracted from repAS transgenic plant lines and northern-blot and RT-PCR experiments were performed. We were not able to detect the transgene expression in the virus resistant plants suggesting that the resistance observed is mediated by gene silencing. In line 3. 1, we observed a segregation of virus resistance phenotype that was inversely correlated with the presence or absence of the transgenic transcript. Otherwise, all analyzed transgenic lines harboring the replicase in sense orientation lost the resistance phenotype in F_1 generation. Analysis of homozigous F_2 transgenic plants will allow us to better characterize the resistance mechanism and the process of gene silencing.
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ANDEAN POTATO MOTTLE COMOVIRUS MAJOR COAT PROTEIN PRESENTS A TRANSCRIPTION ACTIVATION DOMAIN. Vidal, M.S.[1]; Monteiro, A.L.V.W.[1]; Vaslin, M.[1] and Margis, R.,[1-2] (1-Depto. Genética, Inst. Biologia; 2-Depto Bioquímica, Inst.Química, UFRJ, Rio de Janeiro. e-mail margisr@uol.com.br)

The first step in the two-hybrid system method is verify i the proteins that we are interested to study are able to activate th transcription of the reporter genes used by this system. In previous study with Andean Potato Mottle Virus (APMoV) coa protein we have observed that HF7C yeast cells, transformed wit the pGBT9CP42 construct, are able to grow on yeast drop-ou selection media lacking tryptophan and histidine (SD-Trp-His supplemented with 50 mM 3-amino-1,2,4-triazole. These clone also exhibited ß-galactosidase activity in both, qualitative an