and negeviruses for humans and their interference with arbovirus replication in competent vectors is largely unknown, however, recent studies suggest that the insectspecific viruses could alter the vector competence of the mosquitoes for some arboviruses resulting in superinfection exclusion or by alteration of the vector's immune response.

PIV219 - IN VIVO AND IN VITRO VIRULENCE ANALYSIS OF A BACULOVIRUS ISOLATED FROM CHRYSODEIXIS (=PSEUDOPLUSIA) INCLUDENS, A SOYBEAN PEST IN THE BRAZILIAN CERRADO

de Souza, M.L.; Sanches, M.M.; Sihler, W.; Sosa Gomes, D.R.

1. *EMBRAPA RECURSOS GENÉTICOS E BIOTECNOLOGIA*
2. *EMBRAPA SOJA*

Baculovirus are important biological control agents of Lepidoptera. In February 2014, larvae with symptoms of viral infection were observed in populations of Chrysodeixis (=Pseudoplusia) includens infesting soybean field at BuritisMG (S15o22.2′ W46o50.7′). Observations of larval tissue under optical microscope showed the presence of typical virus particles of Nucleopolyhedrovirus (NPV). The virus was identified as Pseudoplusia includens single nucleopolyhedrovirus (PsinSNPV) by transmission electron microscopy. The present work was carried out in order to investigate the potential of this viral isolate to control this insect pest. Two bioassays were performed. In the first, larvae infected with virus were macerated and incorporated to the artificial diet and offered to 432 C. inludens larvae (third instar). In the second, semipurified virus particles were incorporated to the artificial diet and offered to third instar C. includens larvae. The mortality was verified at 10 and 15 d.p.i. and the lethal concentration LC50 and LC99 were calculated using Probit analysis. To select a good candidate for in vitro multiplication of the virus, analysis was performed with six different lepidopteran cell lines: Bombyx mori (BM5), Lymantria dispar (IPLBLD625Y), two Trichoplusia ni (BTITn5B14 and TN368), and two Spodoptera frugiperda cells (IPLB-SF21AE and Sf9).The cells were seeded at a density of 1x106 per 60mm2 dish. The virus was obtained from infected larvae hemolymph at 4 d.p.i., treated and allowed to be adsorbed by cells during 1 hour. Then, the cells were kept in TNMFH complete medium and incubated at 270C. Morphological analysis was monitored by light microscopy during five days, using an Olympus CK2 optical microscope. The best results in the bioassays were obtained with the semipurified virus. The LC50 obtained was 10,918 polyhedra/ ml artificial diet (pol/ml) and the LC99 was 247,710 pol/ml, at10 d.p.i. Furthermore, at 15 d.p.i the LC50 was 6,709 pol/ml and LC99 was 146,880 pol/ml. In addition, preliminary analysis of the cell lines incubated with this isolate showed typical cytopathic effects as cell rounding, nuclear hypertrophy and the presence of polyhedra inTn5B14 cells at 4 d.p.i. The bioassays showed potential to use this virus isolate as a biopesticide. Moreover, the Tn5B14 cell line demonstrated to be prospective for further in vitro studies.

PIV220 - COMPARISON OF THE INFECTIVITY OF TWO SPODOPTERA FRUGIPERDA CELL LINES TO SFMNPV

de Souza, M.L.; Sihler, W.; Sanches, M.M.; Barros, A.M.R.; Valicente, F.H.

1. *EMBRAPA RECURSOS GENÉTICOS E BIOTECNOLOGIA*
2. *UNIVERSIDADE PAULISTA*
3. *EMBRAPA MILHO E SORGO*

The fall armyworm, Spodoptera frugiperda, is a severe pest in South America causing damage to different crops, especially in maize. The Spodoptera frugiperda Multiple Nucleopolyhedrovirus (SfMNPV), a baculovirus highly pathogenic to this pest, has been largely used as a biocontrol agent. So far the baculovirus production has been done by multiplication of the virus in its insect host in despite of difficulties as intense cuticle lyses and cannibalism behavior. Therefore, optimization of baculovirus in vitro production is essential as an alternative technology. In the present work, the SfMNPV production in IPLBSF21AE and Sf9 cell lines were compared. The polyhedra yield was determined as well as the kinetics of viral protein synthesis. In addition, larval mortality was determined by virulence assays with 3th to 4th instar larvae. Cells seeded at a density of 2X106 in a T25 flasks were incubated with the SfMNPV I19 isolate for 1h adsorption time and kept in TNMFH complete medium at 270C. At 5 dpi the cells were collected by centrifugation at 3000rpm for 5 min. The cell pellet was disrupted by treatment with 1% SDS, for 1h, at

270C. Polyhedra was placed in a Neubauer chamber and counted under an optical microscope. For kinetics of the protein synthesis both cell lines were seeded at a density of 1X106 per 60mm2 dish and incubated with the virus. After that, pulse labelling was carried out by addition of 50 uCi of [35S] methionine per dish at 0h, 24h, 48h and 72h pi. Analyses of the labelled proteins was done by SDS–PAGE followed by autoradiography. Comparison of the two cell lines infected with the virus showed that polyhedra production was similar in both cells ranging from 200400 polyhedra/cell. As expected, the kinects of radiolabed proteins showed that the cell protein synthesis was shut off while an intense band of aprox. 30 kD (polyhedrin) was synthesized in SF21 and Sf9 cells. Assays with Spodoptera frugiperda larvae showed that the virus produced in cell culture was pathogenic to its host. The present data indicates that cell culture is a viable system for baculovirus in vitro production and reinforces the need to optimize strategies for large production in bioreactors.

PIV221 - INFLUENCE OF THE AGE OF TOMATO TRANSPLANTS ON THE RATE OF INFECTION AND SYMPTOM SEVERITY CAUSED BY THE BEGOMOVIRUS TOMATO SEVERE RUGOSE VIRUS

Rodrigues, C.S.; Macedo, M.A.; Rêgo, C.M.; Michereff Filho, M.; Inoue Nagata, A.K.

1. *UNIVERSIDADE DE BRASÍLIA*
2. *EMBRAPA HORTALIÇAS*

High incidence of viral diseases is reported in tomato plants, particularly those caused by begomoviruses (Fam. Geminiviridae, gen. Begomovirus) in Brazil. These viruses are transmitted by the whitefly Bemisia tabaci. Tomato sereve rugose virus (ToSRV) is the most important begomovirus reported in the country. The control of these viruses is difficult, and usually rely on the use of resistant cultivars and the chemical control of insect vectors. There is a lack of information on the cultural practices that could be implemented to reduce the incidence of the begomovirus in the field. The objective of this study was to evaluate the influence of transplants age of tomatoes on the begomovirus infection rate and symptom expression. The susceptible cultivar H9553 was used with whitefly inoculation. Transplants of 20, 30, 40, 50 and 60 days after seeding (DAS) were distributed in three blocks (completely randomized) with 10 plants for each treatment. Inoculation was performed by B. tabaci biotype B viruliferous to ToSRV. Negative controls consisted of plants inoculated with aviruliferous whiteflies and plants without the presence of whiteflies. The evaluation was performed visually and by detection tests based on PCR and hybridization. There was a clear difference in the rate of infection and severity of the symptoms observed in plants inoculated at distinct ages. The treatment with plants of 20 DAS had the highest incidence of infection (50%), followed by plants with 30, 50, 60 and 40 DAS. Plants inoculated 40 DAS showed the lowest rate with 20% of infection. For symptom severity, plants inoculated at 40 DAS showed the least severe symptoms, followed by plants inoculated at 60 DAS. The results suggest that the age of the transplants influence the incidence rate and severity of a disease caused by a begomovirus. Further studies are in progress to confirm this result.

PIV223 - IMMUNOSTAINING OF ROOT TISSUE OF NICOTIANA BENTHAMIANA INFECTED WITH PEPPER RISNGPOT VIRUS

Tavares, M.L.; Blawid, R.; Nagata, T.; Inoue Nagata, A.K.

1. *UNIVERSIDADE DE BRASILIA*
2. *CENTRO NACIONAL DE PESQUISA EM HORTALIÇAS*

The genus Tobravirus is formed by three species: Tobacco rattle virus (TRV, type species), Pea early browning virus (PEBV) and Pepper ringspot virus (PepRSV). The genome of a tobravirus is formed by two segments of single stranded RNA molecules. Because of their ability 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, 5bromo4chloro3 indolyl phosphate (BCIP) and nitro blue tetrazolium