70359-970 - Brasília, DF. e-mail: paulobri@ufrrj.br, Fone: 021-682.1220 r. 574).

According to the cooperative program established between UFRRJ (Dr. Paulo S. T. Brioso) and ESALQ (Dr. J. A. M. Rezende) for the production of plants of zucchini (*Cucurbita pepo*) 'Caserta' pre-immunized against the PRSV-W, we have initially collected leaf samples of squash (*C. moschata*) and zucchini from different regions in Rio de Janeiro State, aiming to the detection of a virus which occurrence was not yet registered in the State. We have collected squash leaf samples showing mosaic and leaf malformation, that have not serologically reacted against PRSV-W. With the purpose of identifying the virus isolate, we have carried out several tests: mechanical transmission to zucchini plants ('Caserta'); observation of infected leaves by Leaf-Dip assay; indirect ELISA using antisera against CMV (*Bromoviridae - Cucumovirus*), CSNV and ZLCV (*Bunyaviridae - Tospovirus*), SqMV (*Comoviridae - Comovirus*), PRSV-W and WMV-2 and ZYMV (*Potyviridae - Potyvirus*); and dsRNA extraction. We have succeeded in mechanically transmitting the virus, which showed as flexuous filaments. The virus reacted, serologically, against WMV-2 antiserum and showed a dsRNA pattern compatible to WMV-2. The incidence of this virus was previously registered in the states of BA, MA, MG, PE, SP, and this is the first report in the state of RJ. The virus identification will be used as a strategy to be adopted in the control of cucurbits virus diseases in the state of Rio de Janeiro.

161

DETECTION OF A STRAIN OF TOBACCO STREAK VIRUS (TSV) IN COTTON PLANTS "IAC 22*"

Colariccio, A[1]; Frangioni, D[1]; Chagas, C. M[2]; & Vicente, M[1]** ([1] Seção de Virologia Fitopatológica e Fisiopatologia; [2] Microscopia Eletrônica, Instituto. Biológico, Cx. Postal 12898, CEP 04010-970- São Paulo, SP. Fone (011) 572-9822)

Cotton plants cv. IAC-22 from experimental field established in Instituto Biológico - SP. which showed symptoms similar to those caused by virus diseases, such as mosaic and necrotic rings, were observed on adult plants. The leaves with symptoms when submitted to mechanical transmission tests induced local reaction in *Chenopodium amaranticolor, C. quinoa, Gomphrena globosa* and *Nicotiana tabacum* cv. *White Burley*, TNN and . *Samsun*.In *Nicotiana* species the symptoms became systemic showing line patterns, vein necrosis and streak. The thermal inactivation point was ca 55°C, the dilution end point ca 10^{-3} and the *in vitro* longevity 24h at room temperature, in *N. tabacum* cv. Samsun plants. Electron microscopic observations revealed typical Ilarvirus particles in negativelly stained preparations, in thin sections of cotton plants no noticeable cytopatic effects were detected. Serologycal tests by DAS-ELISA and ISEM plus decoration tests gave positive reaction when AS-TSV (1) was used. After purification according to Fulton., 1985 (Descriptions of plant viruses, n° 307) isometric particles between 27 and 35 nm could be observed. Although TSV on cotton plants has been described as a disease without economical importance (COSTA, Divulg. Agron., 1966), the fact that many cultivars were introduced in the last few years could render them exposed to new viruses or to new strains of known viruses.

* Financial support: FAPESP and FINEP

**Fellow of CNPq

(1)antiserum produced by R. W.Fulton and furnished by E.W. Kitajima.

162

TOSPOVIRUS DETECTION BY RT-PCR AND MOLECULAR PROBES.

Eiras, M.[1]; Missiaggia, A.A.[1]; Resende, R. de O.[1]; Bezerra, I.C.[2] & de Ávila, A.C.[2] ([1]Depto. Fitopatologia UnB, 70910-970, Brasília, DF.; [2]CNP-Hortaliças/EMBRAPA. C.P. 0218, CEP: 70359-970, Brasília, DF).

In order to develop a fast and sensitive method for universal tospovirus detection, primers were designed to anneal at different conserved regions of the virus genome: **i)** S RNA (N gene), amplifying 430 bp fragment; **ii)** M RNA (G1/G2 gene), a 520 bp fragment; **iii)** M RNA (NSm gene), a 850 bp fragment; **iv)** L RNA (L gene), a 731 bp fragment. First strand cDNA was synthesized for 1h at 37°C, from purified virus RNA samples, purified nucleocapsid and from total RNA extracts of *Nicotiana benthamiana* plants infected with seven tospovirus species: tomato spotted wilt virus (TSWV), tomato chlorotic spot virus (TCSV), groundnut ringspot virus (GRSV), *Impatiens* necrotic spot virus (INSV), *Chrysanthemum* stem necrotic virus (CSNV), zucchini lethal chlorotic virus (ZLCV) and BR10 onion isolate. PCR was performed for 30 cycles (1,5 min denaturation at 94°C; 2 min annealing at 48°C; 30 sec extension at 72°C). The non-radioactives molecular probes were synthetized by PCR with incorporation of the nucleotide labeled digoxigenine (dUTP-DIG), using specific primers for the 4 regions of the viral genome mentioned above, using cDNA from TSWV as template. Total RNA samples extracted from *N. benthamiana* plants infected with TSWV, were spotted on nylon membrane, and hybridized with non-radioactive molecular probes. All virus species were detected by PCR from purified virus, using the primers for the S and M RNAs. The purified nucleocapsid samples were detected by PCR only with the S RNA primers. The primers designed for the L RNA were limited for the universal detection by PCR, being the larger number of tospovirus species detected by PCR with the primers for the S and M RNAs using total RNA as template. The 430 bp probe for the S RNA was virus specific, showing positive result only for TSWV. With the 850 bp probe for the NSm gene, positive results was achieved for the TSWV, TCSV and INSV species. Using the 520 bp probe for G1/G2 gene, hybridization was observed with the TSWV, TCSV, GRSV, INSV, CSNV and ZLCV species. With the 731 bp probe for L RNA, all tospovirus species were detected simultaneously.

163

MOLECULAR CHARACTERIZATION OF GARLIC VIRAL COMPLEX.

Fajardo, T.V.M.[1]; de Ávila, A.C.[2]; Buso, J.A.[2] & Resende, R. de O.[3] ([1]Depto. de Fitopatologia, UnB, [2]EMBRAPA-Hortaliças, [3]Depto. de Biologia Celular, UnB, Brasília-DF).

Garlic is a vegetatively propagated crop in which the greatest yield losses are attributed to viral complex infection. Typical symptoms include mosaics of different intensities, yellowing, and marked reductions in bulb weight and size. Molecular biology have provided new tools for identification

of viruses, being a convenient method for detecting mixed infections without the fastidious and many times unsuccessful work of separation of the components of viral complexes. This study evaluated garlic plants to identify individual garlic viruses in the complex. Garlic viral complex was purified and RNA extracted from this viral preparation was used as template for cloning of garlic virus. Two strategies were used either RT-PCR using degenerate primers to amplify a 335 bp DNA fragments on the coat protein of potyviruses or cDNA synthesis using oligo-dT and random primers. From PCR amplification, eight clones were obtained and sequenced. Six clones showed high nucleotide identity with leek yellow stripe virus, LYSV (84%). Other two clones showed high identity with onion yellow dwarf virus, OYDV-garlic strain (83%), with 335 bp OYDV-onion strain clone (85%) used as control, and with OYDV-onion strain (82%). Nucleotide sequences comparison between the clones of LYSV and OYDV-G showed low identity (69%). From the cDNA library, clones of about 2.5 kb were sequenced on both ends, showing a high nucleotide identity (86.5%) with a garlic common latent virus (GCLV). These DNA clones were used in hybridization assays, showing high specificity of the virus probes. The data obtained from molecular analysis of the clones showed mixed infections of garlic plants with at least three different virus, LYSV and OYDV belonging a potyvirus genus and GCLV, a carlavirus. Cloning of garlic viruses directly from diseased plants is an alternative approach to characterize them, whereas the amplified DNA fragments can be used for virus indexing by nucleic acid hybridization.

164

BEHAVIOR OF COWPEA GETOPYPES TO MECHANICAL INOCULATION OF COWPEA SEVERE MOSAIC VIRUS STRAINS. Lima, J. Albersio A.; Cavalcanti, F.R. and. Lima, R.C.A. (Federal University of Ceara, Plant Virus Laboratory. Cx. Postal 12168. Fortaleza, CE, 60.356-000. e-mail: albersio@ufc.br)

Cowpea, *Vigna unguiculata*, is one very important leguminous crop in North and Northeast of Brazil and the State of Ceara is the greatest producer, being responsible for more than 20% of the Brazilian production. In Ceara, the diseases caused by viruses constitute the most important plant pathological problems and the cowpea severe mosaic virus (CpSMV) family *Comoviridae* genus *comovirus* is one of the most severe pathogen. In the present paper, 44 cowpea genotypes were evaluated against artificial inoculations of two strains of CpSMV. The CpSMV-CE (prevalent in Ceara) and the CpSMV-MC isolated in the State of Piaui capable to infect 'Macaibo', a cowpea cultivar immune to all CpSMV isolates from different part of Brazil. Four potted plants of each genotype were inoculated with CpSMV-CE, four with CpSMV-MC and four were maintained uninoculated as control in greenhouse conditions. All inoculated plants were observed during 20 days for symptoms development and serologically tested against antiserum specific to each virus strain. According to the symptomatological reactions and the serological results the genotypes were classified as **Immune:** (CpSMV-CE= 4; CpSMV-MC= 5); **Tolerant:** (CpSMV-CE= 2; CpSMV-MC= 9); **Susceptible:** (CpSMV-CE= 2; CpSMV-MC= 9) and **Highly susceptible:** (CpSMV-CE= 36; CpSMV-MC= 19). The results confirmed the biological difference between these CpSMV strains and showed that the best way to control the virus is through the development of immune cultivars. Although biologically different, four genotypes showed to be immune to both strains

Financial support: CNPq

165

PURIFICATION OF CURLY TOP VIRUS TRANSMITED BY LEAFHOPPER, *Agallia albidula* Uhl AND CITOPATHOLOGICAL ASPECTS INDUCED IN DIFFERENT HOST PLANTS. *Nogueira, N.L.; Rossi, M.L. & Rodrigues, J.C.V. (CENA / USP, Cx, Postal 96, CEP 13400-970 - Piracicaba, SP. E-mail: nlnogue@pira.cena.usp.br, Fone: 019 429 4691)

Tomato Curly Top Virus (TCTV), is a tentative member of genera "Sub group II Geminivirus" and causes widespread losses to tomato plants. The agent is naturally transmitted by leafhopper, *Agallia albidula* Uhl. Symptoms induced by this virus consist of dwarfing, leaf rolling, or crinkling, leaf yellowing, vein swelling, and stimulation of growth of axillary buds. The leafhoppers were collected from the native hosts, near the tomato fields and transferred to the *Acantospermum hispidicum* DC., *Brassica oleracea* L.; *Datura stramonium* L. and *Lycopersicon esculentum* Mill in cages under greenhouse conditions. After symptoms development the viral purification procedures were carried out and specimens were prepared for electron microscopy (EM) analysis. The leaves of infected plants were ground in 0,01 M phosphate buffer pH 7,2 containing 0,01 Na_2SO_3 and 0,001 M EDTA at the rate of 2 ml/g of tissue. After squeezing homogenate through cheesecloth the filtrate was centrifuged at 10.000 g for 15 min. at 4°C. One volume of butanol was added to one volume of supernatant. The mixture was stirred for 90 min at 4°C, and centrifuged at 10.000 g for 15 min. The pellet was discarded and the virus was precipitated by adding polyethylene glycol (PEG, mw 6000) to a concentration 10% and NaCl at 1%. The mixture was stirred for 40 min and centrifuged at 12300 g for 15 min. The precipitated was resuspended in 0,001M phosphate containing 0,001 M EDTA at the rate 10ml/100g original host tissue. After sitting for 3 hr. at 4°C was centrifuged at 10.000 g for 15 min. The supernatant was saved and the pellet was washed three times by resuspension in 0,001 M phosphate -EDTA buffer and low-speed centrifugation. The three supernatants were combined with the original was centrifuged at 10.000 g for 15 min and concentrated by reprecipitation using the same conc. of PEG + NaCl used earlier. The final vol. was adjusted to 2ml/100g of original tissue. Virus preparations for E.M were stained with uranyl acetate. Isometric particles 16 to 20 nm and 17-21 in diam and paired particles 26 to 31 nm and 27 to 31 nm were observed on *D. stramonium* and *B. oleracea* preparations respectively. Ultrathin sections of infected leaves revealed marked modifications of phloem cells. These changes included cells prominently vacuolated with electron dense bodies in the nucleus; some cells with virus like particles showed chromatin confined to the vicinity of the nuclear envelope .Such ultrastructural changes were not observed in sections from healthy plants.

Financial support: FAPESP

166

DASHEEN MOSAIC VIRUS IN *ANTHURIUM* SPECIES. *Rivas, E. B.[1]; Duarte, L. M. L. [1], Alexandre, M. A. V.[1] & Galleti, S. R.[2] ([1]Seção de Virologia Fitopatológica e