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REMOVING OF LEAVES IN INDUCTION OF SYMPTOMS IN CITRON INOCULATED WITH CITRUS VIROIDS GROUPS CEV, CV II, CV III AND CV IV. Rodrigues, M. I. S.[1]; Barbosa, C. J.[2]; Santos Filho, H. P.[2]. [1]Plant Science Department/EAUFBA, C. P. 082, 44380-000, Cruz das Almas-BA. [2]Embrapa - Mandioca e Fruticultura . Virology Laboratory. C. P. 007, 44380-000 - Cruz das Almas, BA.

In July 1995 it was started an experiment on biological characterization of citrus viroids, groups CEV, CV II CV III and CV IV, in Citron 'Arizona 861 S1', under greenhouse and growth chamber conditions, at Embrapa - Cassava and Fruit Crops. The citrons were bud grafted on Rangpur Lime scions. Inoculations were performed three months after grafting, when the citron plants were about 68 cm tall. Three axillary buds from Sweet Orange infected plants were grafted to each citron plant. No symptoms were observed three months after inoculation. The leaves were then removed and symptoms were observed in the new developed leaves, only in the plants inoculated with the citrus viroid group CEV. Eight months after inoculation the leaves were removed again. Symptoms were then observed in new developed leaves of the plants inoculated with the citrus viroid groups CV II, CV III or CV IV. Infection was confirmed through RNA purification and RT - PCR analysis using primers specific for the above mentioned groups. Amplification was evaluated through agarose (2%) gel electrophoresis and polyacrilamide (5%).

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CHARACTERIZATION OF THE N-GENES OF TWO NEW TOSPOVIRUS SPECIES FROM ZUCCHINI AND ONION IN BRAZIL *Bezerra, I.C.[1]; Pozzer, L.[2]; Kormelink, R.[3]; Resende, R.O[2]; De Ávila, AC.[1] ([1]EMBRAPA Hortaliças, km 09 - BR 060 - BSB/Anápolis, Cx. Postal 218, CEP 70359-970 - Brasília - DF. E-mail: bezerra@cnph.embrapa.br; [2]Universidade de Brasília, Dep. Microscopia Eletrônica, Brasília - DF)

Tospovirus is a virus genus that infects more than 750 plant species. It is an important disease for ornamentals and vegetables crops causing million of dollars of losses when an epidemic occurs like in tomato, pepper, lettuce and other. The genus is worldwide distributed and composed of different species. In the world were described five different species: TSWV (tomato spotted wilt virus), TCSV (tomato chlorotic spot virus), GRSV (groundnut ringspot virus), INSV (impatiens necrotic spot virus) and WMSM (watermelon silver mottle virus). In Brazil were reported TSWV, TCSV and GRSV; INSV and WMSM were not found yet. Some species have been proposed based on the host range, serological relationship, vector specificity and the nucleocapsid homology. In the last years, in Brazil, were proposed new species CSNV (chrysanthemum stem necrosis virus) and ZLCV (zucchini lethal chlorotic virus), based on host range, serological relationship and partial nucleotide sequence of the N-gene. This year, the N-gene sequence of ZLCV and of a new isolate, from onion were obtained by RT-PCR of the S RNA, using specific primers for S RNA as well as non-specific. The PCR fragments were cloned and sequenced. Nucleotide and deduced protein sequence of both, zucchini and onion, showed to be different from the previously reported species. Nucleotide and protein sequence of these two new species is showed. The homology analysis with previously described Tospovirus showed that onion isolate is 90,51% identity to IYSV (iris yellow spot virus), a new specie found in Europe. ZLCV is a new Tospovirus specie as previously proposed and belongs to a new serological group. The onion isolate belongs to IYSV serological group.

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NATURAL OCCURRENCE OF SATELLITE RNA IN ISOLATES OF CUCUMBER MOSAIC VIRUS BELONGING TO SUBGROUP I. Boari, A.J.; Zerbini, F.M.; Maciel-Zambolim, E.; Carvalho, M.G. (Dep. de Fitopatologia/BIOAGRO, UFV, Viçosa, MG, 36571-000. E-mail: zerbini@mail.ufv.br

Besides three genomic RNAs and one subgenomic RNA, particles of cucumber mosaic cucumovirus (CMV) can be associated with a fifth RNA species. This satellite RNA (sRNA), which has 300-400 nucleotides, does not code for any protein, and depends on CMV (called the helper virus) for its replication and encapsidation. The presence of the sRNA can alter the replication rate and the pathogenicity of CMV, although the nature of its interaction with the virus and its hosts, and the mechanism(s) by which the sRNA affects replication and pathogenicity, remain unclear. The sRNA is distributed worldwide, although it is of rare natural occurrence. In Brazil, there are no reports of the occurrence of sRNA associated to CMV. The objective of this work was to verify the occurrence of sRNA among 30 CMV Subgroup I field isolates, collected in the states of Minas Gerais, Espírito Santo and Rio de Janeiro. CMV virions were concentrated from infected plants, and the RNA forms associated with the particles were purified and analyzed by agarose gel electrophoresis. RNAs approximately 350 nucleotides in length were found to be associated with one isolate from tobacco (*Nicotiana tabacum*), three isolates from bell pepper (*Capsicum annuum*) and one isolate from black pepper (*Piper nigrum*). The symptoms induced by these isolates on *N. tabacum* 'Havana 425' were milder than those induced by the other isolates analyzed, which did not have this fifth RNA species. After successive sap-inoculations under greenhouse conditions, symptoms became attenuated to the point of being difficult to observe. The presence of this fifth RNA species associated with CMV isolates and the attenuated symptoms induced by these isolates on tobacco strongly suggest the presence of satellite RNAs in naturally occurring field isolates of CMV in Brazil.

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WATERMELON MOSAIC VIRUS 2 - NATURAL INCIDENCE ON SQUASH, IN THE STATE OF RIO DE JANEIRO. *[1]Brioso, P. S. T.; [1]Bonis, M.; [2]Pozzer, L. & [2]Ávila, A. C. (*[1]Área Fitopatologia, Depto. Entomologia e Fitopatologia, IB, UFRRJ, Cx. Postal 74585, CEP 23890-000 - Seropédica, RJ; [2]EMBRAPA/CNPH, Cx. Postal 218, CEP

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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.

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

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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.

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**Fellow of CNPq

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

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TOSPOVIRUS DETECTION BY RT-PCR AND MOLECULAR PROBES.

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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.

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MOLECULAR CHARACTERIZATION OF GARLIC VIRAL COMPLEX.

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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