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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