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NON-RADIOACTIVE MOLECULAR PROBES FOR TOSPOVIRUS DETECTION. M. EIRAS[1]; A.A. MISSIAGGIA[1]; R. de O. RESENDE[1]; I.C. BEZERRA[2] & A.C. de ÁVILA[2] . [1]UnB, Depto. De Fitopatologia, 70910-970, Brasília, DF; [2]CNP-Hortaliças/EMBRAPA. C.P. 0218, CEP: 70359-970, Brasília, DF. Utilização de sondas não radioativas para detecção de tospovirus.

In order to develop a fast and sensitive method for universal Tospovirus detection, non-radioactive molecular probes were developed. The probes were synthetized by RT-PCR with incorporation of the nucleotide labeled digoxigenine (dUTP-DIG), using total RNA extracted from *Nicotiana benthamiana* plants infected with tomato spotted wilt virus (TSWV) as template. Oligonucleotides were designed for 4 regions of the viral 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. Total RNA samples extracted from *N. benthamiana* plants infected with 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 were spotted on nylon membrane, and hybridized with non-radioactive molecular probes. 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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IDENTIFICATION OF TWO POTYVIRUSES IN MIXED INFECTION OF GARLIC PLANTS BY MOLECULAR TECHNIQUES. T.V.M. FAJARDO[1], A.C. de ÁVILA[2], J.A. BUSO[2] & R. de O. RESENDE[3]. ([1]Dept. de Fitopatologia, [2]CNPH/EMBRAPA, [3]Dept. de Biologia Celular, UnB, Brasília-DF). Identificação através de técnicas moleculares de dois potyvírus em alho com infecção viral múltipla.

Viral diseases of garlic are widespread throughout the world. Since garlic plants are propagated vegetatively the viruses are readily transmitted causing serious losses in crop yields due to virus infection. This disease known as the garlic viral complex is usually induced by simultaneous infection of several partially characterized viruses belonging to different genera, mainly potyvirus and carlavirus. This study aimed the molecular characterization of potyviruses in garlic viral complex. Degenerate primers previously designed from conserved regions of nucleotide sequence in the coat protein gene of potyviruses were used to amplify a 335 bp DNA fragments on potyvirus specific templates using RT-PCR. The amplified 335 bp DNA fragments were cloned into the pGEM-T vector. Eight clones of garlic viral complex were obtained and sequenced. An isolate of OYDV, from onion, a member of the potyvirus genus also occurring in garlic was used as control. Nucleotide sequences obtained were analysed by alignment to potyvirus coat protein sequences included in databases. Six clones showed high 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%). Amplified fragments were used in hybridization assays, using as samples total RNA from healthy and infected garlic plants, purified garlic viral complex and crude sap of plants. The radioactive probes displayed high specificity, being suitable for diagnostic purposes.

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MOLECULAR CHARACTERIZATION OF GARLIC COMMON LATENT VIRUS (GCLV), A CARLAVIRUS, IN GARLIC VIRAL COMPLEX. T.V.M. FAJARDO[1], A.C. de ÁVILA[2], J.A. BUSO[2] & R. de O. RESENDE[3]. ([1]Dept. de Fitopatologia, [2]CNPH/EMBRAPA, [3]Dept. de Biologia Celular, UnB, Brasília-DF). Caracterização molecular do garlic common latent virus (GCLV), um carlavírus, no complexo viral do alho.

Virus diseases of garlic are widespread all over the world. As garlic plants are propagated vegetatively, these diseases are responsible for significant yield losses. Most commercial garlic cultivars are infected with a complex of two or more viruses, mainly potyvirus and carlavirus. Identification of garlic viruses, however, is complicated because compontents of the garlic viral complex have been difficult to isolate whereas they infect a narrow host range. This study report the molecular characterization of a carlavirus infecting mosaic diseased garlic plants. Garlic viral complex was purified and RNA extracted from this viral preparation was used as template for cDNA production. Oligo dT-primed cDNA synthesis was conducted using a commercial kit. After the reaction, the viral cDNA was ligated to Eco RI adaptors and cloned into the pBluescript vector. Recombinant plasmids were introduced into *E. coli* cells and a cDNA library of about twenty clones was generated. Some clones were sequenced and aligned to other sequences included in databases. One clone, about 850 bp, enconding part of the coat protein gene, showed high identity (85.4%) with GCLV. Another clone, about 2.5 Kb, was partilly sequenced on both ends. Sequence comparison of 3' end showed high identity with the 850 bp cDNA clone (93.9%) and with GCLV (86.5%). Using these two clones as templates, radioactive probes were perfomed to verify specificity of hybridization. Total RNA of healthy and infected garlic plants, purified garlic viral complex and crude sap of plants were used as samples to spot on nylon membrane. The hybridization assays showed high specificity of the virus probes, being suitable to be used in diagnostic procedures.

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VARIABILIDADE EM ISOLADOS DE GEMINIVIRUS DE *Phaseolus*, NO BRASIL. J.C. FARIA[1] & D.P. MAXWELL[2]. ([1]*Embrapa Arroz e Feijão*, C.P. 179, 74001-970, Goiânia, GO; [2]Departamento de Fitopatologia da Universidade de Wisconsin, Madison, WI, 53706, USA). Variability of geminivirus isolates from *Phaseolus*, in Brazil.

Vírus do Mosaico Dourado do Feijoeiro (VMDF) é a principal virose da cultura no plantio "das secas". Foi realizado um levantamento, objetivando encontrar possíveis variantes do VMDF, tendo-se coletado amostras de *P. vulgaris* (Goiás, Minas Gerais, Mato Grosso, Bahia, São Paulo, Paraná), *P. lunatus* (Pernambuco) e *Leonurus sibiricus* (Mato Grosso do Sul). De todas as amostras foram amplificados fragmentos de DNA de geminivirus, e determinada a seqüência do DNA. As seqüências de amostras de *P. vulgaris*, todas continham o VMDF; a amostra de Taquarituba (SP) continha uma mistura de VMDF e vírus do mosaico do abutilon (AbMV). A amostra de *P. lunatus* e de *L. sibiricus*, contudo, continham geminivirus distintos daqueles cujas seqüências já se encontravam nas bases de dados GenBank, EMBL, DDBJ e PDB. Concluiu-se que o VMDF apresenta grande estabilidade, provavelmente devido a ser um vírus de replicação via DNA e a não existência de hospedeiro imune. A virose encontrada em *P. lunatus* potencialmente pode oferecer ameaça a *P. vulgaris*, mas não se tem informações de sua infectividade a esta espécie, no momento.

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PARTIAL HOST RANGE OF TGV-Ub, A NEW TOMATO-INFECTING GEMINIVIRUS FROM UBERLÂNDIA, MG. J.J. Fernandes[1,2], A.S.K. Braz[1], R. Krause[1], E.P.B. Fontes[3], I.C. Bezerra[4] and F.M. Zerbini[1]. ([1] Dep. de Fitopatologia e [3] Dep. de Bioquímica, UFV, Viçosa-MG, 36570-000; [2] Dep. de Agronomia, UFU, Uberlândia-MG; [4] EMBRAPA/CNPH, Brasília, DF. Gama de hospedeiros parcial do TGV-Ub, um geminivírus isolado de tomateiros em Uberlândia-MG.

A partial host range test was carried out with TGV-Ub (*tomato geminivirus, Uberlândia*), as part of the biological characterization of this new tomato-infecting geminivirus. Plants of zucchini squash (*Cucurbita pepo* 'Caserta'), cucumber (*Cucumis sativum* 'Caipira'), *Physallis floridana*, *Nicandra physaloides* and *Solanum nigrum* were inoculated with the insect vector (*Bemisia* sp.) and kept in a growth chamber at 28°C. All inoculated plants showed symptoms, including vein clearing and a mild mottling, two to three weeks after inoculation. Plants of zucchini squash showed typical silverleaf symptoms, indicating that the species of whitefly used was *Bemisia argentifolii*. However, PCR analysis of symptomatic plants, using degenerate primers for Subgroup III geminiviruses [*Plant Dis. 77*(4):340, 1993], failed to amplify A or B component DNA fragments, indicating that the plants were not infected. Therefore, it is concluded that the symptoms are due to the colonization of the whiteflies. Tests with aviruliferous insects are under way to confirm this hypothesis.

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PROGRAMA DE OBTENÇÃO DE MATRIAL BÁSICO DE CITROS LIVRES DE DOENÇAS PARA CERTIFICAÇÃO DE MATRIZES. H. P. Santos Filho (EMBRAPA/CNPMF, C.P.007, Cruz das Almas BA.) Basic material for developing a citrus diseases free plant certification program.

O método de microenxertia foi modificado e adaptado no CNPMF/EMBRAPA e se constitue na base científica para a obtenção de clones de citros livres dos virus da tristeza e da sorose, dos viroides da exocorte e da xiloporose e da disfunção vegetal, de causa ainda desconhecida, denominada sorose tBA. Para esta última, foi desenvolvido um método de diagnose rápida pela obtenção de um marcador bioquímico fluorescente, que sempre aparece em cromatografia de camada delgada de géis de sílica, com Rf de 0,55. Borbulhas de plantas microenxertadas e indexadas para as doenças, foram enxertadas em dois diferentes porta-enxertos, e se constituiram no material básico para a instalação de dois blocos de multiplicação e observação, os quais foram implantados em diversas instituições governamentais dos estados do Nordeste. Após quinze anos, as plantas matrizes foram comparadas àquelas que lhes deram origem e foram comprovados os valores de produtividade,