DISEASE NOTE

FIRST REPORT OF '*CANDIDATUS* PHYTOPLASMA ASTERIS' AFFECTING SESAME CULTIVATION IN INDIA

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Sesame (*Sesamum indicum* L.), family *Pedaliaceae* is one of the most ancient cultivated oilseed crops. India ranks first in area (46.5%) under sesame cultivation, producing about 27.9% of the total production of sesame in the world. A phyllody disease was noticed on sesame plants growing in various districts of Uttar Pradesh, India with a disease incidence of 12-20% in 2005. To detect the causal agent, the total nucleic acid isolated from infected and healthy leaf samples collected from Bahraich district were blotted on nylon membrane and hybridized with a probe prepared from a clone of Chilli little leaf phytoplasma 16S ribosomal RNA gene (GenBank Acc. DQ343288). All symptomatic plants showed strong hybridization but none was seen in symptomless samples, indicating an association of a phytoplasma with the phyllody disease. For molecular identification of this phytoplasma, PCR was performed using total DNA of infected samples and P1/P6 phytoplasma specific universal primers (Deng and Hiruki, 1991) which resulted in a ~1.5 kb amplicon. Further, nested-PCR was carried out with primers R16F2n/R16R2 (Gundersen and Lee, 1996) using diluted primary PCR product as template. The ~1.35 kb amplicon obtained was cloned and sequenced. Blast search analysis of sequence data (GenBank Acc. DQ431843) showed highest (99%) identity with the following phytoplasmas: Barley deformation (AY734453), Aster yellows (AY665676), Onion yellows (AP006628), Silene virescence (AY744070), Ash witches'-broom (AY566302) and Maize bushy stunt (AY265208), members of '*Candidatus* phytoplasma asteris' (16SrI) group. To our knowledge, this is the first report of molecular identification of '*Candidatus* phytoplasma asteris' (16SrI group) affecting sesame cultivation in India. Recently a witches'-broom disease of sesame in Oman has been assigned by RFLP analysis to a different group (16srII) (Al-Sakeiti *et al*., 2005).

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DISEASE NOTE

SPREAD OF *TOMATO YELLOW LEAF CURL VIRUS* AND *TOMATO CHLOROSIS VIRUS* TO A NEW AREA IN PORTUGAL FOLLOWING THE NORTHERN EXPANSION OF THE VECTOR *BEMISIA TABACI*

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The whitefly (*Bemisia tabaci*)-transmitted *Tomato yellow leaf curl virus* (TYLCV, genus *Begomovirus*) and *Tomato chlorosis virus* (ToCV, genus *Crinivirus*) have been detectable in the Algarve region of southern Portugal since 1995 and 1998 (Louro *et al*., 1996; 2000). In July 2005, an epidemic disease outbreak occurred in open field tomato crops near Campo Maior (Alentejo region). Affected plants showed leaf curling, rolling, brittleness, yellowing, and growth reduction, associated with unusually high populations of *B. tabaci* in what was an EU protected zone for this insect. Of 13 samples analysed, all reacted with a DNA probe specific for TYLCV-type strain (intergenic region, IR), and seven reacted with an RNA probe specific to ToCV (coat protein gene, CP). PCR with primers MA-250 (5'GGTGTCCCTCAAAGCTCTATGGCAATCG3')/MA-118 (5'GATCCCACATATTGCAAGAC3') (Monci *et al*., 2002) designed for amplification of the IR of TYLCV-type strain, produced a 310 bp DNA fragment with a sequence (GenBank DQ333307) 98% identical to that of a Spanish isolate (AJ489258) of TYLCV-type strain. RT-PCR with primers MA-380 (5'GTGAGACCCCGATGACAGAT3')/MA-381 (5'TACAGTTCCTTGCCCTCGTT3'), designed for amplification of the CP gene of ToCV, produced a 436 bp DNA fragment with a sequence (DQ335133) 100% and 99% identical to the CP genes of ToCV isolates from Algarve (DQ335134) and Spain (DQ136146) respectively. This is the first report of the type strain of TYLCV in Portugal, and of TYLCV and ToCV in Alentejo.

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DISEASE NOTE

FIRST REPORT OF *MELON NECROTIC SPOT VIRUS* AND *ZUCCHINI YELLOW FLECK VIRUS* IN CUCURBITS IN IRAN

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During the summer of 2006, 79 samples were collected from symptomatic leaves of cucumber, melon and squash plants growing in commercial fields in the Bahar area of Hamedan province, Iran. Leaf extracts were tested by DAS-ELISA (Clark and Adams, 1977) for the presence of *Cucumber mosaic virus* (CMV), *Melon necrotic spot virus* (MNSV), *Papaya ringspot virus* (PRSV), *Watermelon mosaic virus* (WMV), *Zucchini yellow fleck virus* (ZYFV) and *Zucchini yellow mosaic virus* (ZYMV), and used to mechanically inoculate herbaceous hosts. CMV, PRSV, WMV and ZYMV were detected serologically in 13, 5, 3 and 34 samples, respectively. Extracts from 5 melon and 4 squash plants, respectively, reacted strongly in ELISA with antibodies (Loewe, Biochemica Gmbh, Sauerlach, Germany) specific for MNSV or ZYFV. Mechanical inoculations with MNSV-infected samples resulted in chlorotic lesions and systemic chlorosis in *Cucumis sativus*, and necrotic lesions followed by systemic necrosis in *Cucumis melo*. ZYFV from squash induced necrotic lesions and mottling in *C. sativus*, and systemic necrosis preceded by chlorotic lesions in *Cucurbita pepo*. The results were similar to those reported previously for MNSV and ZYFV (Brunt *et al.*, 1995). This is the first report of the natural occurrence of MNSV and ZYFV in Iran.

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DISEASE NOTE

FUSARIUM OXYSPORUM CAUSING CROWN ROT ON *GAZANIA RIGENS* AND *PETUNIA* X *HYBRIDA* IN ARGENTINA

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Gazania rigens (treasure-flower) and *Petunia hybrida* (petunia) are common potted ornamentals grown in Argentina. During spring 2002, plants from commercial greenhouses in Moreno (Buenos Aires) showed sudden wilt and death due to crown and root rot. Leaf yellowing and decreased turgidity developed from the base. *Fusarium oxysporum* Schlechtend.Fr (Booth, 1971; Nelson *et al*., 1983) was consistently isolated from symptomatic plant pieces that were surface disinfected with 0.2% sodium hypochlorite for 2 min, washed with sterilised water and plated in potato dextrose agar at 22±2°C. For pathogenicity tests, six three-month-old potted plants of both treasure-flowers and petunias were removed from the substrate and re-planted after wounding their roots with a flamed scalpel. Pots were irrigated with a suspension of 2×10^6 fungal spores per ml of distilled sterilised water. Cross-inoculations (each host was inoculated with the fungal isolate obtained from the other) were made using the same method and number of replicates as above. Control plants (six for each combination host/isolate) were removed from the substrate, wounded, re-planted and irrigated with sterilised distilled water. All plants were individually enclosed in polyethylene bags, incubated at 22±2°C in a growth chamber and monitored for symptom appearance. Regardless of the fungal isolate, all inoculated plants showed wilting and root rot seven days post inoculation. The pathogen was successfully reisolated from all inoculated plants, whilst controls remained healthy. To the best of our knowledge, this is the first record of *F. oxysporum* causing crown rot of treasure-flower and petunia in Argentina.

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DISEASE NOTE

FIRST REPORT OF *APRICOT LATENT VIRUS* IN LEBANON

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Apricot latent virus (ApLV), a member of the genus *Foveavirus*, was first identified in Moldavia in a symptomless apricot of cv Silistra N4 (Zemchik and Verderevskaya, 1993). During a survey for the presence of this virus in the Mediterranean basin, 111 apricot samples were collected from twelve commercial orchards in the north Bekaa valley of Lebanon and were tested by RT-PCR using the ApLV-specific primers pairs H-ALV1 and C-ALV1 (Nemchinov *et al*., 2000). Two samples of cv Ajami yielded the expected amplification product of 200 bp. One of the samples came from a tree with necrotic spots and deformation of the fruits and yellowish rings on the kernels. ELISA tests for the presence of *Apple mosaic virus* (ApMV), *Plum pox virus* (PPV), *Prune dwarf virus* (PDV), *Apple chlorotic leaf spot virus* (ACLSV) and *Prunus necrotic ringspot virus* (PNRSV) detected only PNRSV. Multiplex RT-PCR tests for ApMV, PPV, PDV, ACLSV, PNRSV, ApLV, Plum bark necrosis stem pitting-associated virus (PBNSPaV) and *American plum line pattern virus* (APLPV) using tissues from another symptomatic tree of the same variety, coming from the same area, showed it to be mixedly infected by ApLV and PNRSV; none of the other viruses were detected. To our knowledge, this is the first report of ApLV in Lebanon.

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DISEASE NOTE

ERYSIPHE PLATANI, AN ANAMORPHIC POWDERY MILDEW ON *PLATANUS ORIENTALIS* IN TURKEY

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Oriental plane (*Platanus orientalis*) is a landscape and forest tree which, in Turkey, is planted in gardens of historical buildings and parks, and grows naturally along many streams and rivers. During surveys in the Şanlıurfa district (south eastern Anatolia), a severe case of powdery mildew was detected in early June of 2006 on *P. orientalis* trees in a park. Fungal mycelium extensively covered new leaves and shoots, desiccating them. Infections on mature leaves were localized, resulting in variously extended necrotic areas. The mycelium was epiphytic, white at the beginning, then greyish. Immature conidiophores were not septate, whereas mature ones were septate, mostly erect, and measured 90 to 220 µm in length. Longer conidiophores and those bearing more than one conidium became bent like an elbow. Immature conidia were hyaline and somewhat pointed, whereas mature conidia were cylindrical and slightly swollen in the middle, and were single, rarely double or in a short chain, and measured 27-43 × 12-17 µm. Although infected trees were surveyed fortnightly from early summer to late autumn and mycelium-bearing organs were carefully checked microscopically, no teleomorph structures were detected. Based on the anamorphic morphology, the agent of the powdery mildew was identified as *Erysiphe platani* Howe (Glawe, 2003). This appears to be the first report of *E. platani* on *P. orientalis* in Turkey.

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DISEASE NOTE

INTERNAL BACTERIAL ROT OF ONION BULBS CAUSED BY *BURKHOLDERIA CEPACIA* IN CHINA

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Onion (*Allium cepa*) bulbs of cv Fujia with symptoms of internal rot were observed in autumn 2005 in Hangzhou (Zhejiang Province, China). Rotting was initially confined to one to two of the inner fleshy scales, which turned pale-yellow to light brown, while the adjacent scales were not visibly affected. Eventually, tissues collapsed and the exuded juices infected adjacent bulbs, inducing substantial economic losses. Six bacterial strains isolated from infected bulbs showed characteristics similar to those of the standard reference strains of *Burkholderia cepacia* LMG 1222 and M 297 (Accession No. DQ989509) in phenotypic tests, including results of Biolog version 4.1, pathogenicity tests, and FAME, using the Microbial Identification System with aerobic bacterial library (TSBA 5.0). Bacterial isolates were aerobic, rod-shaped, gram-negative, had 1-4 polar flagella, and did not produce green-fluorescent diffusible pigment in King's Medium B. Colonies on nutrient agar were pale yellow, slightly raised with smooth margins. Hypersensitive reaction was observed in tobacco. All isolates were identified as *B. cepacia* with Biolog similarity of 0.751–0.902 and FAME similarity of 0.736-0.862. RFLP assays of the amplified *recA* gene with the enzyme HaeIII and species-specific PCR tests using primers BCRG11 and BCRG12 (Mahenthiralingam *et al*., 2000) confirmed identification as *B. cepacia* genomovar I. Inoculation of intact healthy onion bulbs of cv Fujia reproduced the symptoms observed in natural infections, which differ from the internal brown rot induced by *Pseudomonas aeruginosa* (Hao and Xie, 2006). The bacterium was re-isolated from symptomatic bulbs. Although *B. cepacia* genomovar I was recently isolated from the rhizosphere of rice and maize in China (Zhang and Xie, 2007), to the best of our knowledge this is the first report of internal onion rot caused by this bacterium in our country.

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DISEASE NOTE

FIRST REPORT OF *APPLE DIMPLE FRUIT VIROID* IN LEBANON

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In the North of Lebanon in 2006, apple fruits of cv Starking Delicious were observed that had depressed green spots 3-4 mm in diameter scattered on the whole fruit surface and around the calyx, where they sometimes merged to give large discoloured areas. These symptoms closely resembled those of apple dimple fruit and dapple apple diseases that are induced by *Apple dimple fruit viroid* (ADFVd) (Di Serio *et al*., 1996) and *Apple scar skin viroid* (ASSVd) (Hashimoto and Koganezawa, 1987), respectively. Total nucleic acids (TNA) were extracted from symptomatic fruits and from symptomless fruits of apparently healthy plants of the same variety and tested by dot-blot hybridization using digoxigenin-labelled riboprobes of cRNA to ADFVd or ASSVd. Hybridization signals were obtained only with TNA from symptomatic fruits exposed to the ADFVd-specific probe. Further evidence that ADFVd was associated with the observed symptoms was obtained by using a multiplex RT-PCR amplification assay specific for detecting this viroid in both single and mixed infections with ASSVd (Di Serio *et al*., 2002). An amplification product of 250 nt, the size expected for ADFVd, was obtained only when testing TNA preparations that had tested positive for ADFVd by dot-blot hybridization. Conclusive evidence that only ADFVd was present in diseased Lebanese apple trees was that the sequence of the 250 nt amplicon was that expected for part of ADFVd cDNA. To our knowledge, this is the first report of ADFVd in Lebanon and the first report of this viroid in a country other than Italy.

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DISEASE NOTE

FIRST REPORT OF *GROUNDNUT YELLOW SPOT VIRUS* INFECTING *CAPSICUM ANNUUM* IN CHINA

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During the summer of 2005, severe necrotic ring spot symptoms were observed in fruit of sweet pepper (*Capsicum annuum* L.) plants growing in fields in Yunnan province of China. Tospovirus-like spherical particles with diameters of 80 to 100 nm were observed in the ultra-thin sections of infected fruit tissues. Analysis of extracts of infected sweet pepper plants by double antibody sandwich enzyme-linked immunosorbent assay using specific antisera to *Tomato spotted wilt virus* (TSWV), *Watermelon silver mottle virus* (WSMoV), *Groundnut ringspot virus* (GRSV), *Impatiens necrotic spot virus* and Iris yellow spot virus (Agdia, Elkhart, IN) showed weak reactions with antibodies to TSWV and WSMoV. But RT-PCR tests of total RNA from infected plants using degenerate *Tospovirus* primers BR60 and BR65 to verify tospovirus infection (Eiras *et al*., 2001) did not yield detectable DNA. However, a DNA fragment of about 1.3 kb was amplified by RT-PCR tests with infected plant samples using the *Groundnut yellow spot virus* (GYSV)-specific primer GYSV-1669 (5'-GGCTATCAAGGACTGGTCTAAAC-3') and degenerate tospovirus primer J13 (Cortez *et al*., 2001). The PCR product was cloned and sequenced. All residues in the sequences were confirmed by comparing duplicate clones. Alignment of the sequences (GenBank Accession EF528556) showed that it was 90.4% identical in sequence to the nucleocapsid protein gene of GYSV (GenBank Accession AY013994). The results indicate that the virus associated with fruit necrotic ring spot disease of sweet pepper in Yunnan is an isolate of GYSV. To our knowledge, this is the first report of GYSV in China.

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DISEASE NOTE

FIRST REPORT OF *RHIZOCTONIA SOLANI* CAUSING CROWN ROT OF WORMWEED IN ITALY

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Wormweed (*Artemisia annua* L.), traditionally considered as a weed species, is now regarded as an emerging crop due to the increasing demand of the antimalarial drug artemisinin. Mostly cultivated in subtropical areas, wormweed has recently been proposed as an alternative crop in tobacco-growing areas of southern Italy. In the past couple of years, plants grown in several locations of Campania (Calvi, Montesarchio) and Apulia (Monteroni) were found to be affected by crown rot. Infected plants were located along the rows showing reduced size and necrotic tissues at the level of the soil line. Isolations on 2% water agar (WA) from infected tissues of samples collected in all locations, yielded 16 multinucleate *Rhizoctonia* isolates. Pairings on WA plates were made according to Carling *et al.* (1996) with tester isolates belonging to *Rhizoctonia solani* anastomosis groups (AG) 1 through 11. Observations for hyphal anastomosis carried out at 400x with a light microscope, showed high fusion frequency with tester strains Rh-74 and RG1 belonging to AG-4. Pathogenicity of three selected isolates was tested on plantlets at the 5-6 leaf stage, grown in sterilized soil in 60-well polystyrene containers. Each well was inoculated with millet kernels previously infested with mycelial plugs from PDA cultures. About 50% of the plants came down with damping-off in 7-10 days, while the rest showed a more or less extensive necrosis at the crown level. To our knowledge, this is the first report of a crown rot of *A. annua* incited by *R. solani* AG-4. This anastomosis group infects many plant species at the soil line, and was previously reported as the causal agent of damping-off and sore shin of tobacco in the above-mentioned areas (Nicoletti *et al.*, 1997).

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