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Detection and molecular characterization of phytoplasmas infecting sesame and solanaceous crops in Turkey

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Abstract

A survey was carried out during 2002-2004 in Turkey to screen for phytoplasmas in sesame and periwinkle plants with phyllody symptoms, in pepper with stolbur symptoms, in eggplant with little and yellow leaves and in tomato with big bud symptoms. *Empoasca* sp. and *Orosius orientalis* (Matsumura) [= *albicinctus* (Distant)] leafhoppers were collected from infected sesame fields in 2003. Phytoplasmas detected in sesame, periwinkle, pepper, eggplant and *O. orientalis* showed 16S rDNA RFLP patterns that were most closely related to strains belonging to clover proliferation group (16SrVI-A). Phytoplasma associated with tomato big bud in Turkey belonged to the stolbur phytoplasma group (16SrXII-A). This is the first time that a phytoplasma associated with sesame phyllody has been detected and characterized by molecular techniques and the first report of phytoplasmas in solanaceous plants in Turkey.

Key words: sesame phyllody, stolbur of pepper, *Orosius orientalis*, clover proliferation.

Introduction

Phytoplasma symptoms have been observed in Turkey in numerous cultivated crops such as sesame, pepper, eggplant and tomato. Turkey is one of the most important sesame producing countries in the world and sesame phyllody has been known since 1959 and was considered to be caused by a virus (Turkmenoglu and Ari, 1959). Sesame phyllody is also present in neighboring countries like Iran (Salehi and Izadpanah, 1992; Esmailzadeh-Hosseini *et al*., 2007) and Israel (Klein *et al*., 1977). In Turkey and Iran, sesame phyllody is transmitted by *Circulifer haematoceps* (Mulsant and Rey) (Salehi and Izadpanah, 1992; Kersting 1993). In India, Thailand and Upper Volta sesame phyllody is transmitted by *Orosius orientalis* (Matsumura) [= *albicinctus* (Distant)] and the associated phytoplasma in Thailand belongs to peanut witches' broom group (16SrII) (Schneider *et al*., 1995). Recently the transmission of the phytoplasma associated with sesame phyllody (16SrII group) by *O. orientalis* was reported in Iran (Esmailzadeh-Hosseini *et al*., 2007). Diseases of tomato and pepper associated with phytoplasmas have been reported from several areas in the Mediterranean basin (Choueiri *et al*., 2007).

The aim of the present work was to investigate the phytoplasma etiology of sesame phyllody, and solanaceous crops showing mainly stolbur symptoms in Turkey using molecular tools.

Materials and methods

Symptomatic samples were collected from the Adana and Hatay provinces of the Eastern Mediterranean region of Turkey during the growing seasons of 2002 to 2004. Samples were: sesame with phyllody symptoms (3 samples in 2002 and 15 in 2003); periwinkle with phyllody symptoms exposed to disease in infected sesame fields (3 samples, 1 for each year); pepper with stolbur symptoms (3 samples in 2002 and 10 in 2003); eggplant with little and yellow leaf symptoms (1 sample in 2004) and tomato with typical big bud symptoms (1 sample in 2003). Total DNA was extracted from approximately 1g of fresh plant material by a phytoplasma enrichment CTAB protocol. Phytoplasmas maintained in periwinkle used as reference strains were: stolbur of tomato (P-TV) belonging to the 16SrXII-A subgroup; clover proliferation (CP, '*Candidatus* Phytoplasma trifolii', Canada) and vinca virescence (VR or BLTVA, California, USA) both belonging to the clover proliferation group (16SrVI-A). *Empoasca* sp. and *O. orientalis* leafhoppers were collected from diseased sesame fields in 2003 and maintained in alcohol. Total DNA was extracted from batches of 5-10 leafhoppers using a CTAB procedure. The presence of phytoplasmas in symptomatic samples and insects was determined by a PCR procedure using the universal phytoplasma primer pair P1/P7 in direct PCR followed by primer pair R16F2n/R16R2 in nested PCR (Lee *et al*., 2004). RFLP analyses of R16F2n/R16R2 amplified PCR products were carried out using the restriction enzymes *Tru1*I and *Hha*I. RFLP patterns were compared with reference strains profiles. To further characterize the phytoplasmas detected, another nested PCR using the P1/16S-SR primer pair (Lee *et al*., 2004) followed by RFLP analyses with *Tsp509*I endonuclease, was adopted.

TEM observations were performed on ultra-thin sections of symptomatic sesame and pepper samples.

Results

All symptomatic sesame samples tested in 2002 and 2003 (3 and 15 samples respectively), all 3 periwinkle samples, all 3 symptomatic pepper samples collected in 2002 and one symptomatic eggplant collected in 2004 gave positive results in nested-PCRs with both primer

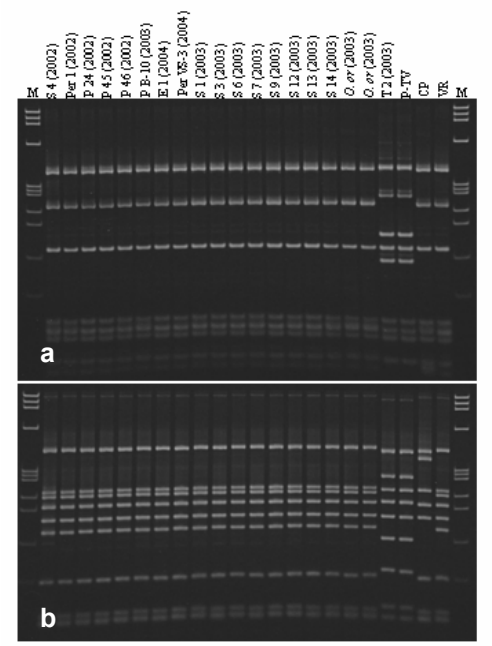

Figure 1. RFLP analyses with *Tru1*I (a) of R16F2n/R16R2 PCR products and with *Tsp509*I (b) of P1/16S-SR PCR products obtained from sesame (S), periwinkle (Per), pepper (P), eggplant (E), tomato (T) and *O. orientalis* (*O. or*). Reference strains P-TV, CP and VR. M: ΦX174 DNA *Hae*III digest.

pairs R16F2n/R16R2 and P1/16S-SR. Pepper samples analyzed in 2003 gave only one positive result from 10 tested samples, probably because the plant material was poorly preserved. All R16F2n/R16R2 PCR products amplified from sesame, periwinkle, pepper and eggplant plants gave identical RFLP profiles with *Tru1*I (figure 1a) and *Hha*I (data not shown), clearly indistinguishable from reference strains CP and VR (16SrVI-A). RFLP analyses of P1/16S-SR products (that cover the entire 16S rDNA gene) with *Tsp509*I (figure 1b) showed that all infected samples gave identical RFLP patterns particularly to the VR reference strain. All *Empoasca* leafhoppers were negative (14 batches), while *O. orientalis* were positive (2 out of 2 batches) for the same phytoplasma described above. One tomato plant with big bud symptoms collected in 2003 was positive in nested-PCR and was shown to be the stolbur phytoplasma (16SrXII-A).

TEM observations confirmed the presence of phytoplasma bodies in pepper samples. On the other hand, in sesame samples it was not possible to observe phytoplasmas probably because the samples were poorly preserved.

Discussion

Sesame phyllody is a widespread disease in Turkey and this is the first reported association with a phytoplasma related to '*Ca.* P. trifolii'. Molecular analyses revealed that the phytoplasma detected in sesame is more closely related to VR or the BLTVA phytoplasma strain from California than to CP strain. Both phytoplasmas are vectored by *Circulifer* spp., *C. haematoceps* and *Circulifer tenellus* (Baker) respectively, confirming their close molecular relationship (Salehi and Izadpanah, 1992; Kersting 1993; Golino *et al*., 1987). Our survey results suggest that *O. orientalis* is a potential vector of the sesame phyllody phytoplasma (16SrVI group) in Turkey. The same phytoplasma detected in sesame has also been found in pepper and eggplant plants, confirming that a phytoplasma closely related to '*Ca.* P. trifolii' can be associated with stolbur-like disease of pepper and other solanaceous plants (Choueiri *et al*., 2007). Recently in Iran, a phytoplasma belonging to the clover proliferation group and transmitted by *C. haematoceps* has been associated with cabbage yellows (Salehi *et al*., 2007). This work represents the first report of phytoplasmas detected in pepper, eggplant and tomato in Turkey using molecular techniques.

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