

# Molecular identification, characterization and transmission of phytoplasmas associated with sesame phyllody in Turkey

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**Abstract** Phyllody is a destructive disease of sesame in Turkey. The disease has been causing significant economic losses by stunting the plants and altering their floral parts into leafy structures with no capsule and hence no seeds in sesame fields of the country. This research was undertaken to examine symptomatology, etiology, taxonomy and transmission of two recently discovered phyllody phytoplasmas infecting sesame in Turkey. Direct and nested PCR amplifications of 16S rRNA gene with the phytoplasma-specific universal primers P1/P7 and R16F2n/R2, respectively were employed for identification of the phytoplasmas associated with sesame phyllody. Phytoplasma-specific PCR amplicons of 1.8 kb and 1.2 kb were amplified only from symptomatic sesame plants and insect vector samples. Sequencing of the PCR amplicons and computer simulated restriction fragment length polymorphism analysis allowed classification of the phytoplasmas with pigeon pea witches'-broom (16SrIX-C) and peanut witches'-broom (16SrII-D) groups. The sequence homology and phylogenetic analyses further confirmed this classification. Among the insects collected from the sesame fields, the leafhopper

*Orosius orientalis* Matsumara (Syn: *O. albicinctus* Distant) was the only vector proven to transmit the sesame phyllody phytoplasmas from diseased to healthy sesame plants in transmission assays. The results demonstrated that the 16SrIX-C and 16SrII-D group phytoplasmas were the agent of sesame phyllody and *O. orientalis* was the vector insect of the disease in Turkey.

**Keywords** *Sesamum indicum* L · Disease · Phylogeny · Transmission · 16SrRNA gene · Sequencing · Vector insect

## Introduction

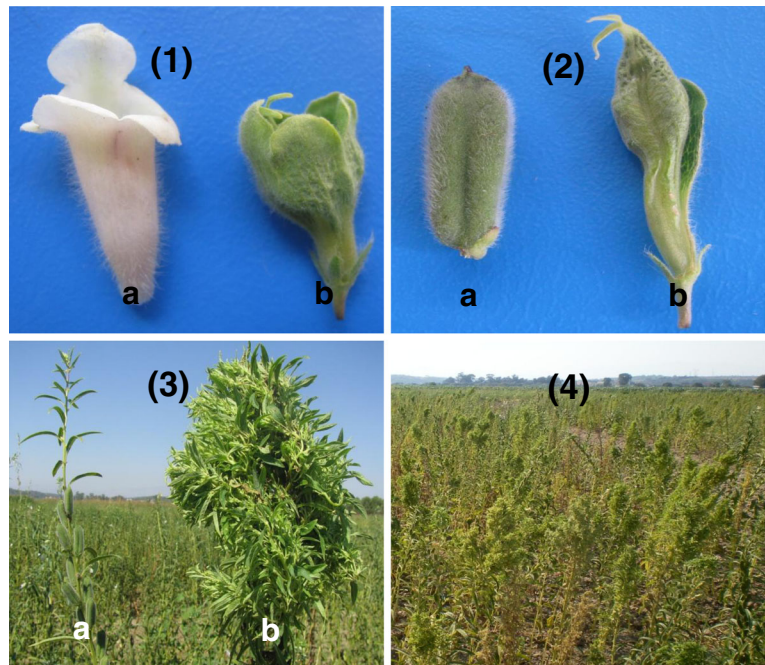
Phytoplasmas are intracellular wall-less obligate pathogens of plants in the class Mollicutes of prokaryotes (Seemuller et al. 1998; Bertaccini 2007). These pathogens inhabit sieve cells in plant phloem tissue and spread from infected plants to healthy ones by the sap-sucking insect vectors (Lee et al. 2000; Hogenhout et al. 2008). Phytoplasmas cause a wide variety of diseases in hundreds of plants species. The symptoms of diseases associated with phytoplasmas include yellowing, decline, witches'-broom, leaf curl, abnormal elongation of internode, floral virescence and distortion, shoot proliferation, plant sterility, and phyllody. Phyllody is the most common and prominent symptom of phytoplasma diseases (Bertaccini et al. 2005; Al-Zadjali et al. 2007; Win et al. 2011; Kaminska et al. 2011; Salehi et al. 2011). Phytoplasma diseases cause significant yield losses in many economically important crops in the world (McCoy et al. 1989; Kirkpatrick 1992; Lee et al. 2000; Bertaccini 2007).

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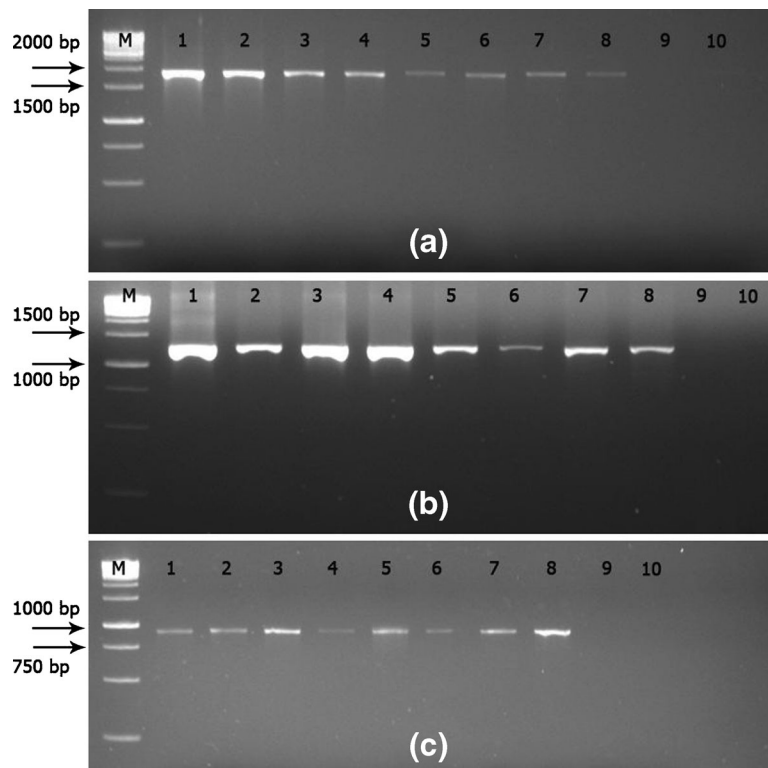
**Fig. 1** Symptoms of sesame phyllody disease. (1a) healthy flower, (1b) infected flower. (2a) healthy capsule, (2b) infected capsule. (3a) healthy sesame plant, (3b) infected sesame plant. (4) the phyllody affected sesame plants in the field



Phytoplasmas are, in general, poorly understood plant pathogens due to their inability to survive *in vitro* (Seruga et al. 2003). Traditional methods which were largely used

in the past are not appropriate for detection and differentiation of phytoplasmas (Lee et al. 1998; Hodgetts et al. 2007; Cervantes et al. 2008). DNA-based molecular

**Fig. 2** Polymerase chain reaction (PCR) amplification of 16S rDNA gene from sesame phytoplasma infected sesame plants and the insect vector. Direct PCR amplification with the universal primer pair P1/P7 of total DNA (a), nested PCR amplification with the universal primer pair R16F2n/R2 (b) nested PCR amplification with the universal primer pair Fu5/Ru3 (c) from infected sesame plants (1–4); *Orosius orientalis* (5–8); healthy sesame plant (9); water control (10); 1-kb DNA ladder (Promega) (M)



methods have been widely used for detection, characterization and phylogeny of phytoplasmas in recent years (Lee et al. 1993; Bhat et al. 2006). The 16S rRNA gene is highly conserved among phytoplasma groups, present in two copies and easy to amplify; hence, universal phytoplasma-specific and group-specific primers have been employed extensively for molecular detection, identification and phylogenetic analysis of phytoplasmas (Seemuller et al. 1998; Lee et al. 2000; Marcone et al. 2000; Hodgetts et al. 2007; Al-Zadjali et al. 2007; Manimekalai et al. 2011). Up to date, 31 16S rRNA (16Sr) groups and more than 100 subgroups have been identified (Martini and Lee 2013).

Sesame (*Sesamum indicum* L.), is the economically most important species of genus *Sesamum* due particularly to its high oil content (50–60 %) with a high ratio of unsaturated fatty acids (Uzun et al. 2008). Sesame oil also contains antioxidant lignans such as sesamin and sesamol that are highly resistant to oxidative deterioration (Yoshida and Takagi 1997; Moazzami and Kamal-Eldin 2006; Erbas et al. 2009). Despite its long history and nutritional value, sesame production falls behind other major oilseed crops because of low seed yield, seed shattering, indeterminate growth habit, and susceptibility to diseases (Ashri 1998).

Phyllody is one of the most important diseases of sesame plants and causes significant economic losses around the world (Sertkaya et al. 2007). The disease, characterized by stunting of the plants and alteration of the floral parts into leafy structures bearing no capsule and seeds, is now a serious threat for the production of sesame in many countries. Sesame phyllody has now been reported from India, Iran, Iraq, Israel, Burma, Sudan, Nigeria, Tanzania, Pakistan, Ethiopia, Thailand, Uganda, Upper Volta, and Mexico (Akhtar et al. 2009). The disease has also been found in several sesame producing regions of Turkey (Kersting 1995; Sertkaya et al. 2007; Ikten et al. 2011).

Several molecular studies based on the 16S rRNA gene have been conducted to identify and characterize phytoplasmas infecting sesame plants. PCR-RFLP analyses have shown that sesame grown in various parts of the world were infected with distinct strains of phytoplasmas in different 16 Sr groups such as 16SrI (India) (Khan et al. 2007a), 16SrI-B (Myanmar) (Win et al. 2010), 16SrII (Iran) (Esmailzadeh-Hosseini et al. 2007), 16SrII-D (Pakistan) (Akhtar et al. 2009), 16SrII-D (Oman) (Khan et al. 2007b), and 16SrVI-A (Turkey) (Sertkaya et al. 2007). Several leafhoppers have also been reported as insect vectors of sesame phyllody phytoplasma including *Orosius orientalis* [= *albicinctus* (Distant)] (Esmailzadeh-Hosseini et al. 2007; Sertkaya et al. 2007; Akhtar et al. 2009), *Circulifer haematoceps* (Salehi and Izadpanah 1992; Kersting 1993), and *Neoliturus haematoceps* (Salehi and Izadpanah 1992). These previous findings indicated that sesame phyllody was caused by different strains from a diverse group of phyllody phytoplasmas and transmitted by different vectors, although the disease symptoms observed in various regions of the world were similar.

The objective of the present study was to identify and characterize the phytoplasma strains and vectors associated with sesame phyllody in Antalya province of Turkey.

## Materials and methods

### Sesame plants and insects

The samples were collected from sesame fields in Aksu, Bogazkent, Denizyaka, Beskonak and Dosemealti districts of Antalya province from June to September in 2011 and 2012 for this study. The map of the districts was given in a previous publication by Ikten et al. (2011). Sesame plants displaying phyllody symptoms

**Fig. 3** Adults of *Orosius orientalis*, the vector of sesame phyllody in Turkey



**Table 1** Insect vector *Orosius orientalis* samples tested by PCR from each field visited

Vector	Surveyed districts of Antalya province														
	Aksu			Bogazkent			Denizyaka			Beskonak			Dosemealti		
	Number of insect sampled	Number of PCR positive samples	Number of insect sampled	Number of PCR positive samples	Number of insect sampled	Number of PCR positive samples	Number of insect sampled	Number of PCR positive samples	Number of insect sampled	Number of PCR positive samples	Number of insect sampled	Number of PCR positive samples			
<i>Orosius orientalis</i>	64	21	40	14	40	9	35	7	48	15					

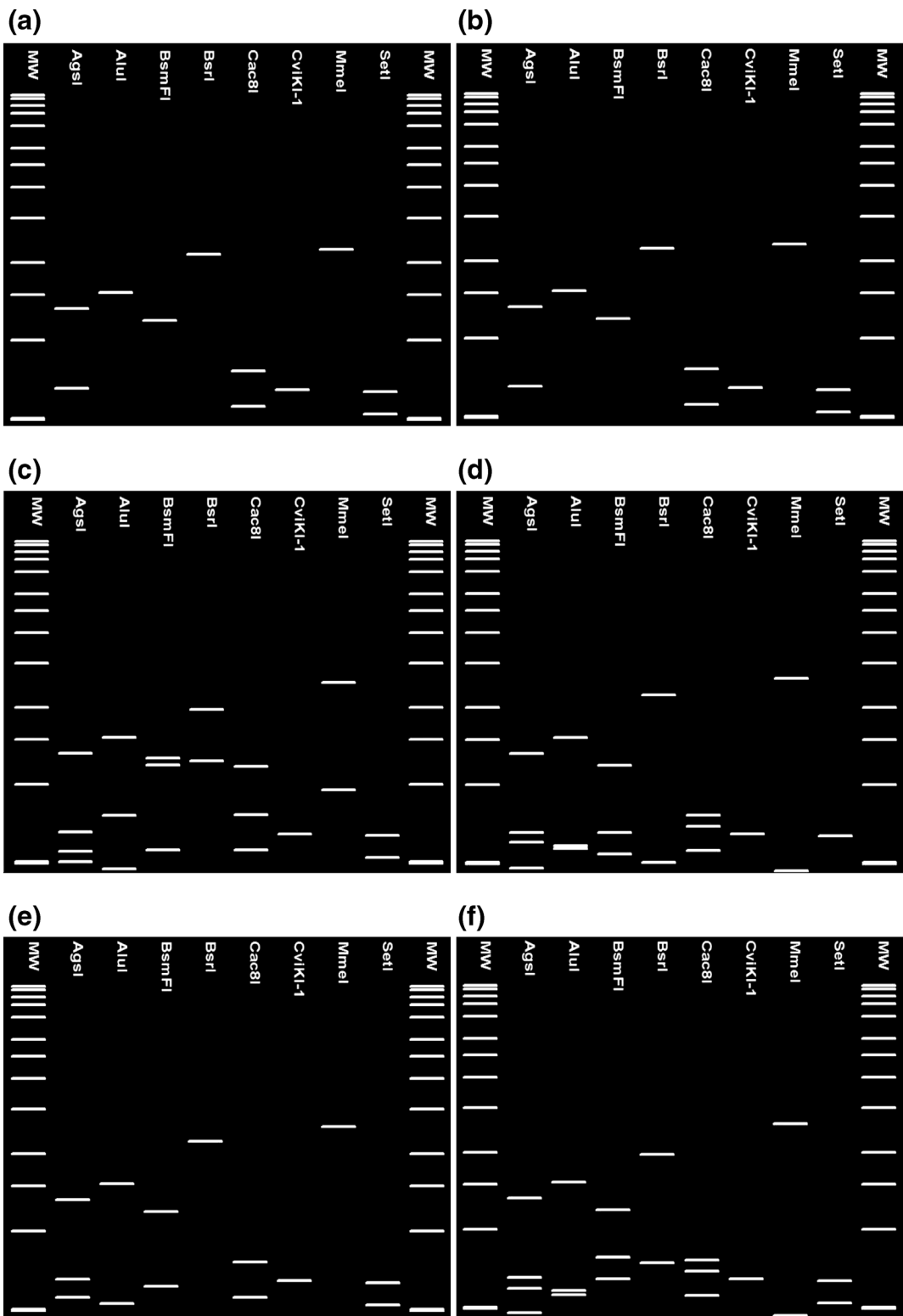
**Fig. 4** Virtual restriction fragment length polymorphism (RFLP) analysis with restriction enzymes *AgsI*, *AluI*, *BsmFI*, *BsrI*, *Cac8I*, *Cvkl-1*, *MmeI*, and *SetI*. **a** Sesame phyllody phytoplasma (ANT2) **b** Echinacea witches' broom phytoplasma (JF340080) (16SrII-D) **c** Faba bean phyllody (X83432) (16SrII-C), **d** Sesame phyllody (EF193357) (16SrII-A), **e** *Picris echioides* phyllody (Y16393) (16SrII-E), **f** Cotton phyllody (EF186827) (16SrII-F), MW: 1 kb—DNA Ladder

were visually identified by foliar, floral and capsular symptoms, collected and transferred to -80 °C for molecular analyses. Insect samples were collected using a hand-held vacuum apparatus in the morning hours between 08.00 and 10.00. A portion of each insect samples collected was stored at -20 °C for DNA extraction and for phytoplasma testing by PCR and the remaining was cage-reared along with sesame plants under greenhouse conditions. Insect samples were identified to species by morphological characteristics using a stereomicroscope. At least ten insect samples from each species were tested by PCR for sesame phyllody phytoplasma. The rearing of the insect species that were negative for the phytoplasma in PCR assays was discontinued. On the other hand, *Orosius orientalis* species was continuously reared in cages as the phyllody was identified by molecular methods in the samples of this leafhopper. Representative leafhopper samples were sent to Prof. Dr. Saban Guclu, an expert on Hemiptera identification and taxonomy at the Department of Plant Protection in Bozok University in Yozgat of Turkey, for further confirmation.

#### Phyllody transmission assays

For transmission assays with *Orosius orientalis*, seeds of sesame test plants were sown in pots placed in the cages free of insects. A total of 36 sesame plants were grown and 12 healthy plants were placed in each of three cages. A total of 50 *O. orientalis* adults continuously fed on phyllody-infected sesame plants was released into each cage except the control one. One cage with 12 healthy sesame plants was used for control and no insect was released on them. The plants in each cage were continuously monitored for symptom development and leaf samples were collected several times for molecular analyses. The insect samples were also collected for the analysis following the appearance of first phyllody symptoms on the plants.

Graft transmission assays were also carried out to transmit the phyllody phytoplasma from infected to





healthy sesame plants. A total of 10 healthy sesame plants were grafted with infected ones. A 10-cm long piece of sesame branch exhibiting typical phyllody symptoms was cut from an infected plant and grafted onto the stem of a healthy sesame plant (Akhtar et al. 2009). The graft unions were wrapped with a piece of parafilm and grafted plants were monitored for symptom development under greenhouse conditions. The transmission of the phytoplasma to grafted plants was further confirmed using molecular analyses.

### Molecular analyses

Total DNA was extracted from sesame plant and insect samples using a CTAB method (Doyle and Doyle 1990). The quality and quantity of the DNA extracts were checked by agarose gel electrophoresis with a DNA standard. The DNA extracts were suspended in milli-Q PCR water and stored at  $-20^{\circ}\text{C}$ .

Total DNA extracts from all plant and insect samples were amplified in a direct PCR assay using primer pairs P1/P7 in the first round of amplification. The amplicons of the direct PCR assay were re-amplified with internal primers R16F2n/R16R2 and/or Fu5/Ru3 in nested PCR assays as described by Gundersen and Lee (1996) and Smart et al. (1996). Healthy sesame plant samples and pure PCR water were used as negative controls. The amplifications were carried out in a programmable thermocycler (BIONEER, MyGenie™).

The direct PCR amplicons of sesame phytoplasmas were purified and ligated onto a pTZ57R/T vector and cloned in the *Escherichia coli* strain DH5 $\alpha$  cells using the INsT/Aclone PCR Product Cloning kit according to the manufacturer's instructions (MBI, Fermentas). Cloned direct PCR and purified nested PCR amplicons were sequenced by Beckman Coulter Sequencer 8000 CEQ Genetic Analysis System using the dye cycle sequencing kit. Representative 16S rRNA gene sequences were deposited at GenBank. The sequences of other closely related phytoplasmas were retrieved from GenBank database and compared with the sesame phytoplasma sequences.

A phylogenetic tree was constructed by the neighbour-joining method with 1,000 replications for each bootstrap values using MEGA software version 5 (Tamura et al. 2011). *Acheloplasma laidlawii* was used as outgroup to root the tree. The virtual RFLP profiles of the 16S rRNA gene sequences were analyzed using pDRAW32 program developed by AcaClone Software (<http://www.acaclone.com>).

**Fig. 5** Virtual restriction fragment length polymorphism (RFLP) analysis with restriction enzymes *AgsI*, *AluI*, *BsmFI*, *BsrI*, *MmeI*, and *SetI*. **a** Sesame phyllody phytoplasma (ANT1) **b** Khafr (Iran) almond witches'-broom phytoplasma (DQ195209) (16SrIX-C), **c** Periwinkle phyllody (JN792516) (16SrIX-A), **d** Blueberry stunt disease (16SrIX-E) (JN791268), **e** 'Candidatus Phytoplasma phoenicium' (AF515636) (16SrIX-D), **f** Lethal disease of almond trees (AF515637) (16SrIX-B), MW: 1 kb—DNA Ladder

[www.q-bank.eu/phytoplasmas/](http://www.q-bank.eu/phytoplasmas/)). Sub-groups of 16Sr were identified by using interactive tools, *iPhyClassifier* (Zhao et al. 2009) and Q-BANK ([www.q-bank.eu/phytoplasmas/](http://www.q-bank.eu/phytoplasmas/)).

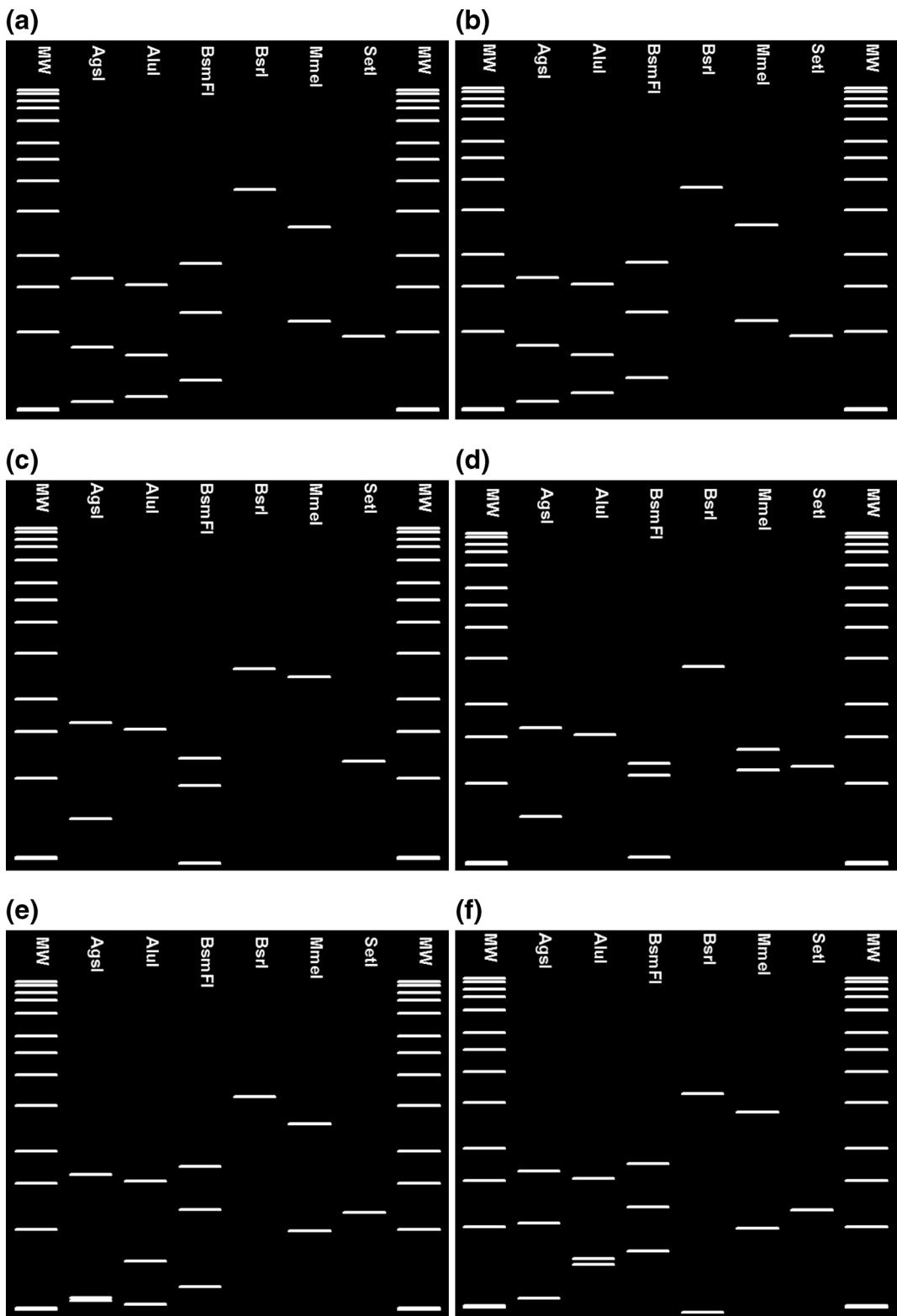
### Results

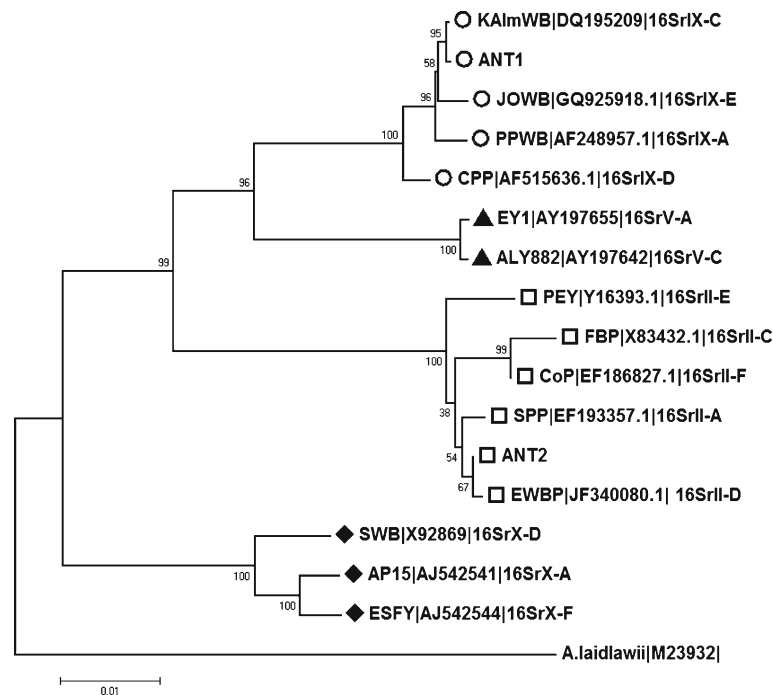
#### Phyllody phytoplasma identification by symptoms and PCR assays

Infected sesame plants exhibited typical phytoplasma symptoms such as phyllody, virescence, asymptomatic shoot proliferation, infertile flowers, reduced leaf size, and weak capsules without seeds (Fig. 1). Symptoms of the disease initially developed on the upper part of the canopy. The incidence of the disease and symptoms increased significantly during flowering and extended rapidly to the older leaves. A large number of sesame plants with typical phyllody symptoms were collected from the fields and tested in direct PCR assays using universal primer pair P1/P7. Although PCR amplicons of expected size (1.8 kb) were obtained in direct PCR amplifications with most of the symptomatic plant samples, single-step direct PCR occasionally failed to amplify or resulted in weak amplifications of the target phytoplasma DNA. However, nested-PCR amplifications always yielded phytoplasma-specific PCR amplicons (1.2 kb) from all sesame plants with phyllody symptoms (Fig. 2). Symptomless sesame plants and water controls yielded no amplicon in nested PCR assays.

#### Screening of field-collected insects by PCR assays

The surveys for possible insect vectors of sesame phyllody have shown that *Orosius orientalis*, *Empoasca decipiens* and *Bemisia tabaci* were the predominant insect species among a total of 11 different species found in the sesame fields of Antalya. A minimum of 10 individuals randomly chosen from each group of insect species were





**Fig. 6** Phylogenetic tree of 16S rDNA sequences from 16 phytoplasmas including the two sesame phyllody phytoplasmas (ANT1 and ANT2) and *Acholeplasma laidlawii* as an out-group. The tree was constructed using the software *MEGA 5*. Bootstrap values are displayed at tree nodes. GenBank accession numbers and 16Sr groups are given next to the phytoplasma name abbreviations. *KAlmWB* Khafr (Iran) almond witches'-broom phytoplasma; *JOWB* *Juniperus occidentalis* witches'-broom

phytoplasma; *PPWB* pigeon pea witches'-broom phytoplasma; *CPP* '*Candidatus* Phytoplasma phoenicium'; *EYI* elm yellows phytoplasma; *ALY882* Alder yellows phytoplasma; *PEY* *Pichris echiioides* phyllody; *FBP* Faba bean phyllody; *CoP* Cotton phyllody; *SPP* Sesame phyllody phytoplasma; *EWBP* echinacea witches'-broom phytoplasma; *SWB* spartium witches'-broom; *API5* '*Candidatus* phytoplasma mali'; *ESFY* '*Candidatus* Phytoplasma prunorum'

subjected to molecular analyses. Leafhopper *O. orientalis* were the only species that yielded the expected amplicon of phyllody phytoplasma (Fig. 3). Out of 227 *O. orientalis* samples collected from different sesame growing areas, 66 samples were positive for phytoplasma in direct or nested PCR assays (Table 1).

**Table 2** Sequencing results of phyllody phytoplasma 16S rDNA from 80 of symptomatic plant samples collected from the fields of Antalya province

District	Number of plant samples sequenced		
	16SrII-D	16SrIX-C	Total
Aksu	17	2	19
Bogazkent	15	0	15
Denizyaka	18	0	18
Beskonak	15	0	15
Dosemealti	9	4	13

#### Sequence analysis of 16S rRNA regions

A slight difference in amplicon mobility was observed for some rare samples such as sample 5 (Fig. 2) during initial screening with P1/P7 primer pairs. Therefore amplicons from such samples and from the samples that consistently yielded strong expected products were subjected to sequence analysis. The results from the samples revealed the presence of two distinct types of 16S rRNA gene sequences. The sequence types, hence the phyllody phytoplasma strains, were named as ANT1 and ANT2. Representative sequences from each sequence type were deposited in the GenBank database under the accession numbers KC139791 and KC756845, KC756846, KC756847, KC756848. The sequences were compared with those of phytoplasma species available in the GenBank using BLAST similarity search tool. ANT1 sequence was identical to those of 16SrIX group members including *Brassica rapa* phyllody phytoplasma (HM559246), pigeon pea witches'-broom phytoplasma



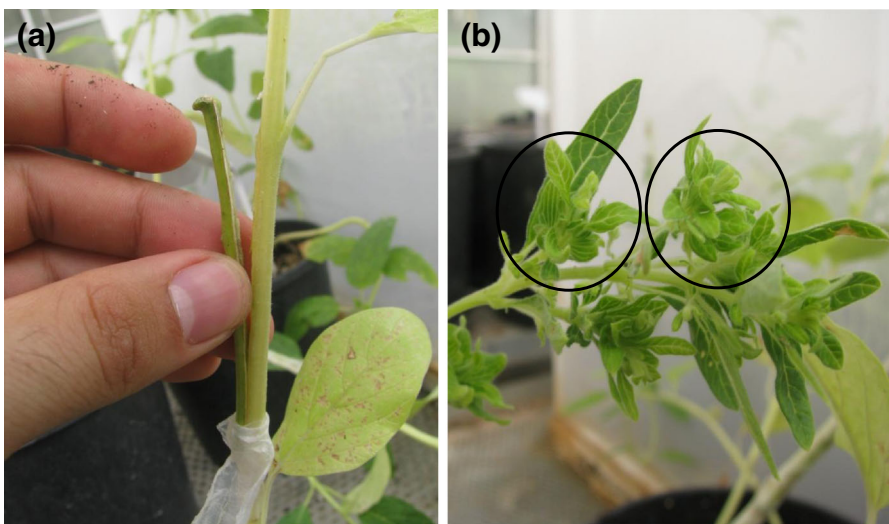


**Fig. 7** Symptoms of sesame phyllody on sesame plants exposed to *Orosius orientalis* under greenhouse conditions

(AF248957) and periwinkle virescence phytoplasma (HQ589191) and the ANT2 sequence to those of 16SrII group members including long bean phyllody phytoplasma (AB690306), crotalaria witches'-broom phytoplasma (GU113154), and sunn hemp witches'-broom phytoplasma (AB558143).

Virtual RFLP analysis

RFLP patterns of the nested PCR products showed that 16S rDNA sequence of ANT2 was identical to Echinacea witches'-broom phytoplasma that belongs to phytoplasma group 16SrII and sub-group "D" (JF340080) (Fig. 4). The



**Fig. 8** Graft transmission. **a** Grafting under greenhouse conditions, **b** graft-transmitted phyllody symptoms

*AgsI*, *AluI*, *BsmFI*, *BsrI*, *MmeI*, and *SetI* restriction enzyme digestions of the 1.8 kb amplicon of ANT1 yielded the same profile as Khafir (Iran) almond witches'-broom phytoplasma (DQ195209) which belongs to the group "16SrIX" and the sub-group "C" (Fig. 5).

Analysis of the phytoplasma ANT1 sequence with online tool *i*phyclassifier indicated that the virtual RFLP pattern derived from the query of F2nR2 fragment of 16S rDNA sequence was identical (similarity coefficient 1.00) to the reference pattern of 16Sr group IX and subgroup C. The sequences of the phytoplasma ANT2 analyzed using the Q-bank Phytoplasma database had 100 % similarity with those of 16SrII-D phytoplasmas, '*Candidatus* Phytoplasma aurantifolia' (Qbank code: QH37) and tomato big bud phytoplasma (NCBI id: JQ868448). These analyses further confirmed that phytoplasmas, ANT1 and ANT2 belonged to 16SrIX-C and 16SrII-D groups, respectively.

#### Phylogenetic analysis

The 16S rRNA gene sequences of ANT1 and ANT2 were compared with the sequences of different strains from 16Sr phytoplasma subgroups and *A. laidlawii* as an out-group (Fig. 6). While ANT1 grouped with pigeon pea witches'-broom members (16SrIX-C), ANT2 clustered with the peanut witches'-broom group (16SrII-D), in phylogenetic analyses.

#### Phytoplasma in the fields and transmission studies

Since the leafhopper, *O. orientalis* was found to be the only vector of the phyllody phytoplasma as indicated by the results of PCR and sequence analyses, transmission studies were focused on this insect species under greenhouse conditions. Sequence analysis of PCR amplicons from caged insects and plants indicated the presence of only 16SrII-D phytoplasma in transmission materials. Although phytoplasma samples belong to 16SrIX-C group were not apparent in transmission assays, they were rarely recorded in field collected samples (Table 2). Sesame and insect samples infected with 16SrIX-C group were mainly obtained from Kovanlık (a part of Dosemealti) province of Antalya which is away from the coastal regions. The other field survey regions where 16SrII-D was quite common were near to Mediterranean coast.

The leafhopper *O. orientalis* consistently transmitted the phytoplasma from infected sesame plants to healthy

plants as indicated by symptom development and nested PCR analysis (Fig. 7). In the transmission assays, all of 24 sesame plants in both cages infested with *O. orientalis* showed typical disease symptoms and were phyllody positive in PCR analyses. Twelve plants in the control cage with no insect infestations did not display any disease symptoms and were negative for the phytoplasmas in PCR assays.

The sesame phyllody phytoplasma was also successfully graft-transmitted from infected to healthy plants under greenhouse conditions (Fig. 8). All the grafted plants showed the phyllody symptoms while no symptoms were developed in the controls. The nested PCR assays further verified the results of graft transmission assay.

#### Discussion

The sesame phytoplasmas were detected by PCR with the universal primer pairs and characterized by virtual RFLP and phylogenetic analysis of 16S rDNA sequences. The analyses indicated that two different sesame phytoplasmas in two separate 16Sr groups were present in sesame plant samples collected from the fields of Antalya province in Turkey. One sesame phytoplasma, ANT2, was closely related to the members of peanut witches'-broom '16SrII group' and sub-group "D" and the other, ANT1, to group "16SrIX", in the pigeon pea witches'-broom and sub-group "C". These two sesame phyllody phytoplasmas from the different groups have not been previously reported from Turkey. Sesame phyllody disease was first studied by Turkmenoglu and Ari (1959) in the country. However, they reported the causal agent as viruses. The studies by Kersting (1993, 1995), Baspinar et al. (1993), and Kersting et al. (1997) were generally conducted on host-vector interactions, and symptomatology and etiology of the disease. Molecular identification of sesame phytoplasma from the country was first reported by Sertkaya et al. (2007). However, they designated the causative strain as a member of 16SrVI-A phytoplasma group. This contrasts with the current report of 16SrII-D and 16SrIX-C phytoplasma groups in our research. Phytoplasma classification by Sertkaya et al. (2007) was solely based on RFLP banding pattern with no sequence information and thus lack sequence homology and phylogenetic analyses. Therefore, direct comparison of our current findings with theirs was not possible.

Sesame phytoplasmas from 16SrII group were also identified in other countries. Al-Sakeiti et al. (2005), Esmailzadeh-Hosseini et al. (2007) and Akhtar et al. (2009) reported sesame phytoplasmas from peanut witches'-broom '16SrII group' from Oman, Iran and Pakistan, respectively. Akhtar et al. (2009) even identified the subgroup of phytoplasma as 16SrII-D. The 16SrII group phytoplasmas were reported to affect not only sesame but also white clover (Hosseini et al. 2013) and pot marigold (Esmailzadeh-Hosseini et al. 2011) in Iran, alfalfa witches'-broom in Oman (Khan et al. 2002), tomato in India (Singh et al. 2012), solanaceous and cucurbit crops in Egypt (Omar and Foissac 2012), chickpea in Sudan (Alfaro-Fernández et al. 2012), and *Picris hieracioides* in Serbia (Mitrovic et al. 2012).

A sesame phytoplasma from the 16SrIX-C group was not known from the literature. However, there was a sequence available in GenBank (Jamshidi et al. 2011). As 16SrIX-C phytoplasma has not been reported as a causal agent in sesame grown throughout the world, the infection of this phytoplasma strain on sesame was announced as “a first report” by our group recently (Catal et al. 2013). Although 16SrIX-C phytoplasma has been detected in sesame in Turkey, it is highly destructive as “almond witches'-broom phytoplasma” in almond, peach and nectarine trees of Lebanon and Iran (Verdin et al. 2003).

The leafhopper species *Orosius orientalis* Matsumara (syn: *O. albicinctus* Distant) is the main vector of sesame phyllody phytoplasma in Asia and Africa (Ishihara 1982). The insect has also been reported to be the major vector for the alfalfa witches'-broom (Salehi et al. 1995), aster yellows (Tanne et al. 2001) and garden beet witches'-broom (Mirzaie et al. 2007) phytoplasmas. In our greenhouse studies, we have successfully demonstrated that sesame phyllody phytoplasma 16SrII-D was transmitted from diseased to healthy sesame plants by the leafhopper *Orosius orientalis* collected from the sesame fields (Figs. 3 and 7). PCR assays of the phytoplasma infected plants from fields and greenhouse transmission assays further confirmed that *Orosius orientalis* is a natural vector of sesame phytoplasma in the areas of this study. The leafhopper *O. orientalis* has also been reported as common vector species in Iran and Pakistan (Esmailzadeh-Hosseini et al. 2007; Akhtar et al. 2009). Similarly, Sertkaya et al. (2007) had identified the insect as the main vector of sesame phyllody in Turkey although they reported a different phytoplasma strain. *Circulifer haematoceps* has also been reported to be an

effective vector of the disease in Turkey (Kersting 1993; Baspinar et al. 1993). However, we have not observed this leafhopper as a vector of sesame phyllody in our locations.

In the past decade, phyllody disease has had a significant negative effect on the production of sesame in Turkey. Effective and reliable control methods are required to manage this serious disease. Selection and use of phyllody resistant sesame cultivars would be the most efficient means of controlling the disease in the field. Any cultivar resistant to the phyllody has been known yet even though the disease has been reported from Asia, Africa and North America (Akhtar et al. 2009). An extensive research on sesame breeding has currently been underway by our group for the development of sesame cultivars resistant to the phytoplasmas and/or its vectors in Turkey. Therefore, the outcomes of the study described here will provide basic and invaluable knowledge for future research on the disease resistance and host-vector interactions.

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