J. Trop. Plant Pests Dis.
Vol. 25, No. 1, March 2025
Pages: 84–95

ISSN: 1411-7525
E-ISSN: 2461-0399
DOI : 10.23960/j.hptt.12584-95

RESEARCH PAPER

Analysis of sesame phyllody disease using transmission electron microscopy and intersimple sequence repeat-polymerase chain reaction

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Manuscript received: 2 July 2024. Revision accepted: 1 December 2024. Available online: 3 March 2025.

ABSTRACT

Sesame phyllody disease caused by phytoplasma has been identified in Egypt as a devastating phytopathogenic mollicute, However, the genetic diversity of phytoplasma infecting sesame plants in Egypt, as revealed by Intersimple Sequence Repeats-Polymerase Chain Reaction (ISSRs-PCR), has not been fully explored. During 2021–2022, sesame plants exhibiting virescence, phyllody, proliferation, and witches' broom symptoms were observed in various fields across the Faiyoum, Luxor, and Beheira governorates in Egypt. This study aims to identify phytoplasma using transmission electron microscopy (TEM) and ISSRs-PCR. The methodology includes sampling, pathogenicity testing, TEM analysis of ultrathin sections, nested polymerase chain reaction (nested-PCR) targeting the 16S rRNA gene, and clustering analysis using 15 ISSRs primers. Phytoplasma was successfully transmitted to healthy plants through grafting and dodder transmission, with success rates of 80% and 90%, respectively. TEM analysis revealed polymorphic phytoplasma bodies and extensive phloem necrosis. Nested-PCR produced 1250 bp amplicons for all isolates. Phylogenetic analysis showed that the Beheira isolate ("OP185273.1") is closely related to the peanut WB group. DNA polymorphic fragments ranged from 1 to 10 per profile, with fragment sizes between 150 and 1500 bp. Cluster analysis revealed that only two isolates (Faiyoum and Beheira) clustered together. This study demonstrates the effectiveness of ISSR-PCR in detecting and analyzing phytoplasma infecting sesame plants in Egypt.

Key words: Sesame, phytoplasma, TEM, 16S rRNA, ISSRs-PCR

INTRODUCTION

Sesame (*Sesamum indicum* L.) is rich in phytonutrients and is a natural source of bioactive substances such as fiber, minerals, proteins, phytosterols, vitamins, and carbohydrates (Shyu & Hwang, 2002). Additionally, sesame has a prolonged shelf life due to its high content of lipid-soluble lignans (Anilakumar et al., 2010). This is why sesame oils is increasingly consumed in its raw form and widely used in cooking (Bedigian & Harlan, 1986).

Sesame is native to India, where it was first domesticated, and is now cultivated in many parts of the world. Currently, approximately 13 million hectares are used for sesame cultivation, producing around 6741 million tons of sesame seed worldwide, with 45.4% of this production occuring in Africa (FAO, 2022). In Egypt, the total area dedicated to sesame cultivation reached 32 thousand hectares in 2022, yielding an estimated annual production of approximately 48 thousand tons (FAO, 2022).

As a vegetatively propagated plant, sesame is grown from seeds to maintain its traits. However, this exposes the plant to a wide range of bacterial, fungal, viral, and virus-like pathogens, some of which are major constraints in Egypt. One of the most significant diseases affecting sesame in Egypt is sesame phyllody disease, caused by a prokaryotic pathogen known as phytoplasma. The disease is characterized by symptoms such as phyllody, virescence, yellowing, stem proliferation, and floral sterility in the infected plants (Bertaccini & Duduk, 2009; El-Banna et al., 2013). The economic impact of sesame phyllody disease is severe, as it dramatically reduces yields, especially in warm climates (Salehi & Izadpanah, 1992). The

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disease poses a significant threat to sesame production, causing 80–100% crop loss in major sesame-producing regions, including India (Madhupriya et al., 2015), Thailand (Nakashima et al., 1995), Pakistan (Akhtar et al., 2008), Myanmar (Win et al., 2010), Taiwan (Tseng et al., 2014), Turkey (Ikten et al., 2014), and Iran (Salehi et al., 2017). Recently, sesame phyllody disease has emerged as a devastating threat to sesame cultivation in Egypt (El-Banna et al., 2013).

The phytoplasma responsible for sesame phyllody disease belongs to the class *Mollicutes* (Bertaccini, 2007). It is a wall-less, uncultivable microorganism enclosed by a membrane, appearing in sieve elements as rounded or ovoid units ranging in diameter from 400 to 900 nm (Khadhair et al., 2001). It is transmitted naturally and experimentally through grafting, dodder and insect vectors (El-Banna et al., 2013; Gad et al., 2019).

Because phytoplasma cannot be cultured in vitro, diagnostic methods are limited. Therefore, biological and histological analyses (El-Banna et al., 2020; Rocchetta et al., 2007), as well as molecular molecular techniques such as Nested-PCR, are used for detection and identification (Gad et al., 2019; El-Banna et al., 2024).

In recent years, the use of ISSRs (Inter-Simple Sequence Repeats) has significantly facilitated genetic diversity analysis in sesame for crop improvement (Kim et al., 2002). Among molecular markers, ISSRs have proven to be powerful PCR-based tools for plant diversity analysis due to their abundance, high variability, co-dominant nature, ease of scoring, and high reproducibility (Ellegren, 2004). However, there have been no reports of sesame phyllody disease identification using ISSR-PCR. Since the low titers of phytoplasma DNA may not be reliably detectable using primers targeting low-copy genes such as the 16S rRNA gene in infected sesame plants via Nested-PCR (Deng & Hiruki, 1991; Sinclair et al., 1996; El-Banna et al., 2024; Gad et al., 2019), we utilized ISSR primers to characterize the genetic diversity of infected sesame cultivars associated with phytoplasma in Egypt.

This study aims to: (1) Detect phytoplasma associated with disorders observed in sesame plants from three different locations in Egypt, (2) Investigate ultrastructural changes in infected sesame tissues using transmission electron microscopy (TEM), (3) Improve molecular detection methods for phytoplasma associated with sesame phyllody disease by developing ISSR-PCR using 15 ISSR primers.

MATERIALS AND METHODS

Research Site. Observation and collection of symptomatic sesame samples were conducted in three Egyptian governorates—Faiyoum, Luxor, and Beheira (Figure 1)—during the 2021–2022 growing season. These samples were subsequently planted

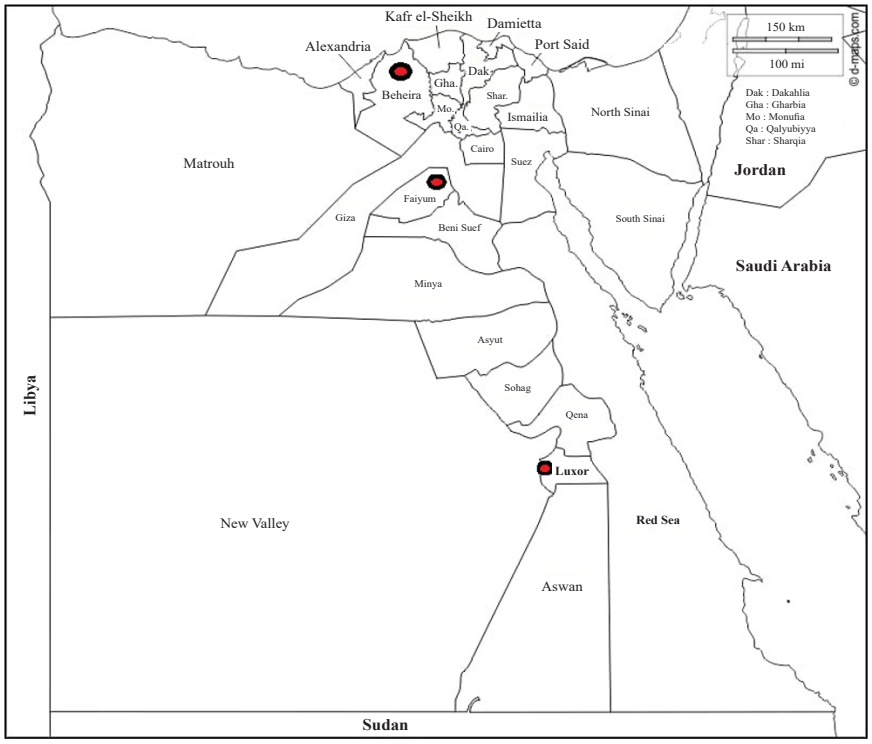

Figure 1. Egypt's map showing Governorates locations mentioned in this study in red circles.

in the Department of Virus and Phytoplasma, Plant Pathology Research Institute, Agricultural Research Center (ARC), Egypt, for further experiments.

Sampling. Four symptomatic sesame plants (70 days old) displaying typical phytoplasma infection symptoms were collected from the three governorates. These symptoms included virescence, phyllody, proliferation, and witches' broom. The presence of phytoplasma in all collected samples was confirmed using a nested-PCR assay.

All infected plants were maintained at the Department of Virus and Phytoplasma, Plant Pathology Research Institute, Agricultural Research Centre (ARC), Egypt, under controlled condition (35–38 ºC, 75–80% humidity, 13 h/11 h day/night) and used for subsequent experiments.

Detection and Characterization of Phytoplasma

Pathogenicity Test. The pathogenicity of the suspected phytoplasma was verified through grafting and dodder transmission, following the methods of El-Banna et al. (2013) and Gad et al. (2019).

Grafting transmission: Ten healthy sesame plants (4 weeks old) exhibiting no symptoms of phyllody were used as rootstocks. Naturally infected sesame plants displaying phyllody symptoms served as the source of scions. The graft unions were maintained in a greenhouse at 28±2 ºC, received standard care, and were monitored for symptom development.

Dodder transmission: Dodder (*Cuscuta reflexa*) seeds were germinated *in vitro* in Petri dishes (12 cm in diameter) lined with moistened filter paper for one week at room temperature (22–25 ºC). The germinated seeds were then transferred to naturally infected sesame plants (five germinated seeds per plant), where they parasitized 10 healthy plants under greenhouse conditions. Infection in the tested plants was confirmed based on symptom appearance.

Ultrastructural Changes. Transmission electron microscopy (TEM) was performed to detect phytoplasma units inside the leaf midribs of the infected sesame samples. Pieces of approximately 2 × 2 from infected sesame leaf midrib tissues were fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4) for one hour, following the method described by El-Banna et al. (2020, 2024). The samples were then refixed in 1% osmium tetroxide (OsO_4) for 15 hours at 4 ºC.

The samples were dehydrated using a graded ethanol series (15%, 30%, 50%, 70%, 80%, 95%, and 100%) and embedded in Spur's epoxy resin by adding one large drop into the sample tube every 15 min until reaching 75% resin overnight with continuous rotation. The samples were then placed in 100% resin for at least one day before being transferred into flat BEEM capsule molds and incubated in an oven at 60 ºC overnight.

The capsules were sectioned into ultrathin slices (90–100 nm thick) using an ultramicrotome (Leica model EM-UC6, Japan). The sections were mounted on copper grids (400 mesh) and double-stained with 2% uranyl acetate and 10% lead citrate (Haj Ali, 2020). Observations were carried out at the TEM laboratory, Research Park (FARP), Faculty of Agriculture, Cairo University, using a JEOL (JEM-1400 TE, Japan) at the appropriate magnification.

Molecular Characterization of Phytoplasma

Nested PCR for Phytoplasma Detection. DNA was extracted from 1 g of fresh sesame leaves exhibiting phytoplasma symptoms using a modified Dellaporta extraction method (Dehestani & Tabar, 2007). The types and structures of the two universal primer pairs used are shown in Table 1 (Sinclair et al., 1996).

Nested PCR was performed using two pairs of primers: P1/P7 and R16F2n/R16R2, in a two-step process: 1) Direct PCR: The first PCR Reaction was carried out in a 25 µL mixture containing 1 µL of template DNA, 25 pmol of each primer, 12.5 µL MangoTaq DNA polymerase (Bioline GmbH, Luckenwalde), and 8.5 µL of nuclease-free water (Promega, USA). The DNA amplification was performed with an initial denaturation at 94 ºC for 3 min, followed by 35 cycles of denaturation at 94 ºC for 30 s, annealing at 53 ºC for 1 min, and primer extension at 72 ºC for 2 min. A final elongation step was conducted at 72 ºC for 7 min; 2) Nested PCR: The PCR products obtained from the first reaction were diluted (1:10) with nuclease-free water

Table 1. Primers used for Nested-PCR and their coding, sequence and amplification size

Name	Coding	5' Sequence 3'	Amplification size (bp)
P1	Universal	5-'AAGAGTTTGATCCTGGCTCAGGATT-3'	1800
P7		5'-CGTCCTTCATCGGCTCTT-3'	
R16F2	Nested	5-'GAAACGACTGCTAAGACTGG-3'	1200
R16R2		5'-TGACGGGCGGTGTGTACAAACCCC-3	

(Promega, USA), and used as a DNA template for the second PCR step (nested PCR) with the R16F2n/R16R2 (Deng & Hiruki, 1991). The nested PCR was conditions included an initial denaturation at 94 ºC for 2 min, followed by 35 cycles of denaturation at 94 ºC for 30 s, annealing at 50 ºC for 2 min, and primer extension at 72 ºC for 3 min. A final elongation step was performed at 72 ºC for 10 min.

All amplification reactions were carried out using a Labnet MULTIGENE MINI thermal cycler. The nested PCR products were analyzed on a 1% agarose gel, compared with a 100 bp DNA ladder (BIOMATIK), and visualized using a gel documentation system (TR201 UV transilluminator, Acculab, USA).

PCR Purification, Nucleotide Sequencing, and Phylogenetic Analysis. The PCR product of the 16S ribosomal RNA gene from the Beheira isolate (ES Be) was purified using a QIAquick® PCR Purification Kit (QIAGEN, Germany) following the manufacturer's instructions. The purified gene was sequenced by Sigma Company.

The sequence of the Beheira isolate was submitted to the NCBI GenBank. Nucleotide sequencing was assembled and analyzed using DNAMAN Sequence Analysis software (version 7, Lynnon BioSoft, Vaudreuil, QC, Canada). Then sequence was then compared with 20 different phytoplasma isolates from various geographical regions deposited in the NCBI GenBank.

Inter-Simple Sequence Repeat Polymerase Chain Reaction (ISSRs-PCR) Analysis. Total DNA was isolated from infected fresh sesame leaf tissue using the cetyltrimethylammonium bromide (CTAB) method, following Doyle & Doyle (1990). ISSRs-PCR reactions were conducted using 15 primers (Table 2). PCR was performed in a total reaction volume of 25 µL, containing: 1 µL of template DNA, 2 µL of each primer, 12.5 µL of DreamTaq Green PCR Master Mix (2X) (Thermo Scientific), and 9.5 µL of nuclease-free water (Thermo Scientific). The PCR was carried out using a Veriti® 96-Well Thermal Cycler (Applied Biosystems, Catalog Number 4479071) following the protocol described by Kim et al. (2002) under the following conditions: Initial denaturation at 94 ºC for 4 min, followed by 35 cycles of denaturation at 94 ºC for 45 s, annealing at 45 ºC for 1 min, and primer extension at 72 ºC for 2 min, followed final extension at 72 °C for 5 min.

The PCR products were electrophoresed on 1.7% agarose gels alongside a 100 bp DNA ladder (Thermo Fisher). Gels were stained with ethidium bromide and visualized using a gel documentation system (G: BOX, SYNGENE model 680XHR, UK).

RESULTS AND DISCUSSION

Symptoms of Phytoplasma. The four phytoplasma-positive isolates caused characteristic symptoms of phytoplasma infection in each governorate: Behira isolate (ES Be): Virescence (Figure 2D) and phyllody

Table 2. Primers used for ISSRs-PCR and their nucleotide sequences

No.	Name	5' Sequences 3'
1	UBC811	GAG AGA GAG AGA GAG AC
2	UBC849	GAGAGAGAGAGAGAGAT
3	UBC-822	TCTCTCTCTCTCTCTCA
4	UBC-824	TCTCTCTCTCTCTCTCG
5	UBC-835	AGAGAGAGAGAGAGAGYC
6	UBC-845	CTCTCTCTCTCTCTCTRG
7	UBC810	GTGTGTGTGTGTGTGTCA
8	TA-3	AGGAGGAGGAGGAGGAGG
9	UBC-844	CTCTCTCTCTCTCTCTRC
10	UBC-827	TCTCTCTCTCTCTCTCRG
11	17898B	CACACACACACAGT
12	17899A	CACACACACACAAG
13	HB 15	GTGGTGGTGGC
14	HB 10	GAGAGAGAGAGACC
15	UBC834	GAGAGAGAGAGAGAGAGAGAT

symptoms (Figure 2C)]; Faiyoum isolates: (ES F1) Shortened internodes (Figure 2A) and (ES F2) Malformed growth with numerous stems (proliferation) (Figure 2B)]; and Luxor isolate (ES Lux): Leaves were abnormally small and crowded at the top of the stem, characteristic of witches' broom (Figure 2E).

In advanced stages, infected plants exhibited dark exudate structures on foliage and floral parts (Figure 2F). Virescence, phyllody, proliferation, and witches' broom were the early symptoms of phytoplasma-associated phyllody disease, followed by the appearance of dark exudates. These symptoms have been previously reported as typical of sesame phyllody disease in Egypt (El-Banna et al., 2013) and Iran (Salehi & Izadpanah, 1992).

Biological Characterization of Phytoplasma Pathogenicity Test. Sesame phyllody disease was successfully transmitted from infected to healthy sesame plants through grafting and dodder transmission. The pathogen was transmitted to eight healthy sesame plants, producing symptoms of yellowing, virescence, and phyllody within 60 days of grafting, with an 80% transmission rate. Phytoplasma transmission via dodder resulted in virescence and phyllody symptoms in nine new plant parts within 30 days, with a 90% transmission rate. This confirms that phytoplasma is responsible for sesame phyllody disease. These findings are consistent with previous reports by El-Banna et al. (2013, 2024), Gad et al. (2019), and Ranebennur et al. (2022).

Ultrastructural Changes. Transmission electron microscopy (TEM) of ultrathin sections of infected sesame leaf midribs revealed disorganization of phloem tissues (Figure 3A). Sieve elements contained numerous pleomorphic phytoplasma bodies next to the cell membrane, ready to pass through the sieve pores (Figures 3A, 3B, 3D, 3E, and 3F). These bodies utilize phospholipids in membrane structures and accumulate at high concentrations in mature phloem tissues. The number of phytoplasma units varied among different tissues, correlating with symptom severity, in agreement with Li et al. (2012).

Phytoplasma units were mostly rounded, elongated, or pleomorphic, bounded by a unit membrane (M), and lacked a cell wall (Figures 3A–3F), as previously reported by El-Banna et al. (2024). Budding of phytoplasma units was observed (Figures 3E, 3F), indicating a developmental stage of growth (El-Banna et al., 2015).

Furthermore, phloem cells walls were signicantly thickened necrosis (Figure 3A, 3C) and vacuolation (Figure 3A). This structural alterations, previously described by El-Banna et al. (2020), included uneven division of sieve elements, clumped necrotized cytoplasm, and increased plasmodesmata (Figures 3D, 3F). Similar observations were made by El-Banna et al. (2024) in phytoplasma-infected African daisy phloem tissues.

Infected sesame plants exhibited phloem necrosis (Figure 3C), indicating a reaction to phytoplasma infection (Uehara et al., 1999), with associated symptoms such as phyllody, virescence, proliferation, yellowing, and witches' broom (Figure 2) (Maejima et al., 2014b).

The mechanisms of phytoplasma-induced symptoms have been elucidated: 1) Glycolytic gene

Figure 2. Naturally infected sesame plants showing typical symptoms of phytoplasma infection. A. Short internodes with yellow, twisted, and reduced leaves; B. Sesame proliferation; C. Phyllody symptoms; D. Virescence of sesame flowers; E. Sesame witches' broom; F. Dark exudates structures on floral and foliage parts.

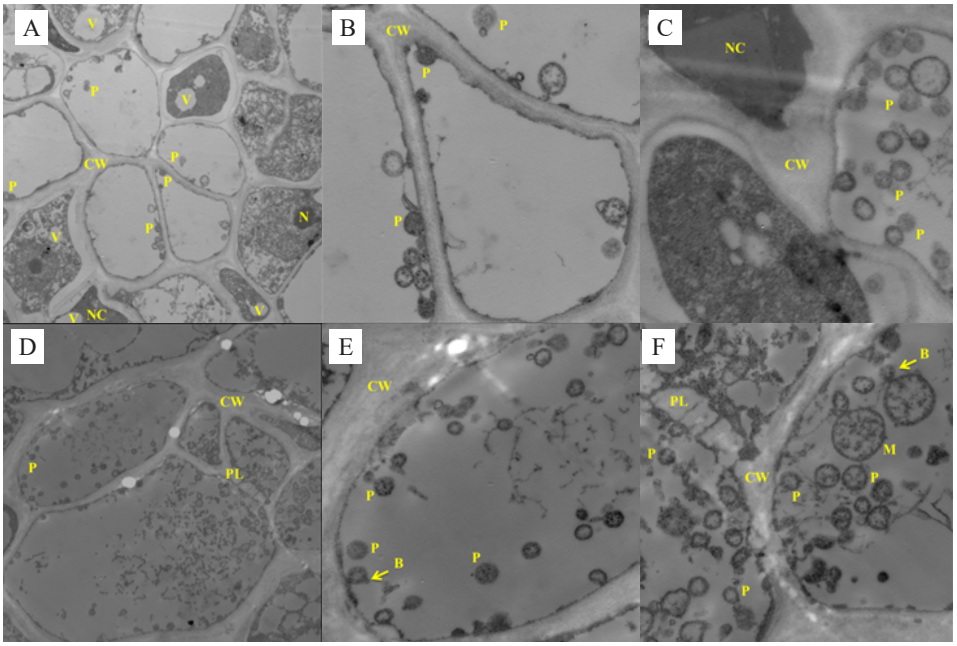

Figure 3. An electron micrograph of phloem sieve elements in an infected sesame leaf. A. Overview of phloem tissue in an infected sesame leaf, showing disorganization of the phloem tissue, necrotization (NC) and vacuolation (V) of the cytoplasm (5000×); B, D, E, F. The cytoplasm contains numerous phytoplasma unit (P) arranged next to the cell wall (CW), which begins to lose its integrity (20000×); A, B, C, D, E, F. These phytoplasma units (P) are mostly rounded, elongated or pleomorphic; C. Necrotic areas (NC) are observed between the cell wall of these areas (15000×); E, F. Some phytoplasma units exhibit budding (20000×); D, F. An uneven division of sieve elements in infected sesame tissues (6000×), with many plasmodesmata (PL) clearly visible.

cluster duplication increases carbon consumption, accelerating phytoplasma growth rates and leading to severe symptoms such as yellowing and plant decline (Oshima et al., 2007); 2) Phyllody, virescence, and proliferation result from the abnormal expression of floral homeotic proteins (ABCE-class MADS-domain transcription factors (MTFs)) in all floral organs except stamens (Himeno et al., 2011). The phytoplasma-secreted SAP54 and PHYL1 proteins induce phyllody by degrading A- and E-class MTFs via the ubiquitin–proteasome pathway (MacLean et al., 2011; Maejima et al., 2014a). These proteins also induce proliferation and virescence (Maejima et al., 2014a; Kitazawa et al., 2017). SAP54 and PHYL1 are conserved among different phytoplasma strains (Maejima et al., 2014a; Yang et al., 2015; Kitazawa et al., 2017); 3) Witches' broom formation is induced by the phytoplasma effector protein TENGU, a 38-amino acid peptide that inhibits the auxin pathway, disrupting apical meristem function (Hoshi et al., 2009; Sugawara et al., 2013).

Molecular Characterization of Phytoplasma Nested PCR. As shown in Figure 4, all four phytoplasma-infected sesame plants produced 1250 bp amplicons, while no visible bands were detected in healthy samples. These results align with findings by El-Banna et al. (2013, 2024) and Gad et al. (2019).

Nucleotide Sequencing Analysis. The 16S ribosomal RNA gene sequence of the Beheira phytoplasma isolate (ES Be) was submitted to NCBI GenBank (accession number OP185273.1). Phylogenetic analysis compared ES Be with 20 reference sequences (Figure 5).

Sequence identity: ES Be showed 96.7%–99.3% similarity with other phytoplasma isolates and 99.3% identity with Sesamum indicum phyllody phytoplasma (ON411187.1) from India (Table 3).

Comparative analysis: ES Be shared 92.2%–86.6% identity with various phytoplasma and mycoplasma isolates, including strains from USA, Iran, Japan, Taiwan, Italy, and Bolivia.

Phylogenetic grouping: ES Be was classified within the peanut witches' broom group (16SrII), sharing 99.3% homology with Scaevola witches' broom (AB257291.1) from Oman and papaya yellow crinkle disease (Y10097.1) (White et al., 1998). It also shared 99.1% similarity with alfalfa witches' broom (AB259169) from Oman (Al-Zadjali et al., 2007) and 99.0% similarity with sweet potato witches' broom

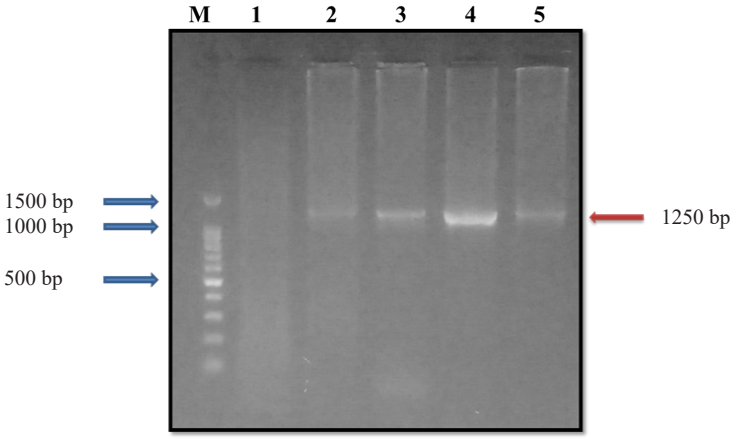

Figure 4. Nested PCR products of the 16S rRNA gene amplified using the R16F2n and R16R2 primer. Lane 1: Healthy sesame plant (negative control), Lane 2: Faiyoum sample (ES F1), Lane 3: Faiyoum sample (ES F2), Lane 4: Luxor sample (ES Lux), Lane 5: Beheira sample (ES Be), and M: DNA molecular weight marker (100 bp) (Biomatic-USA).

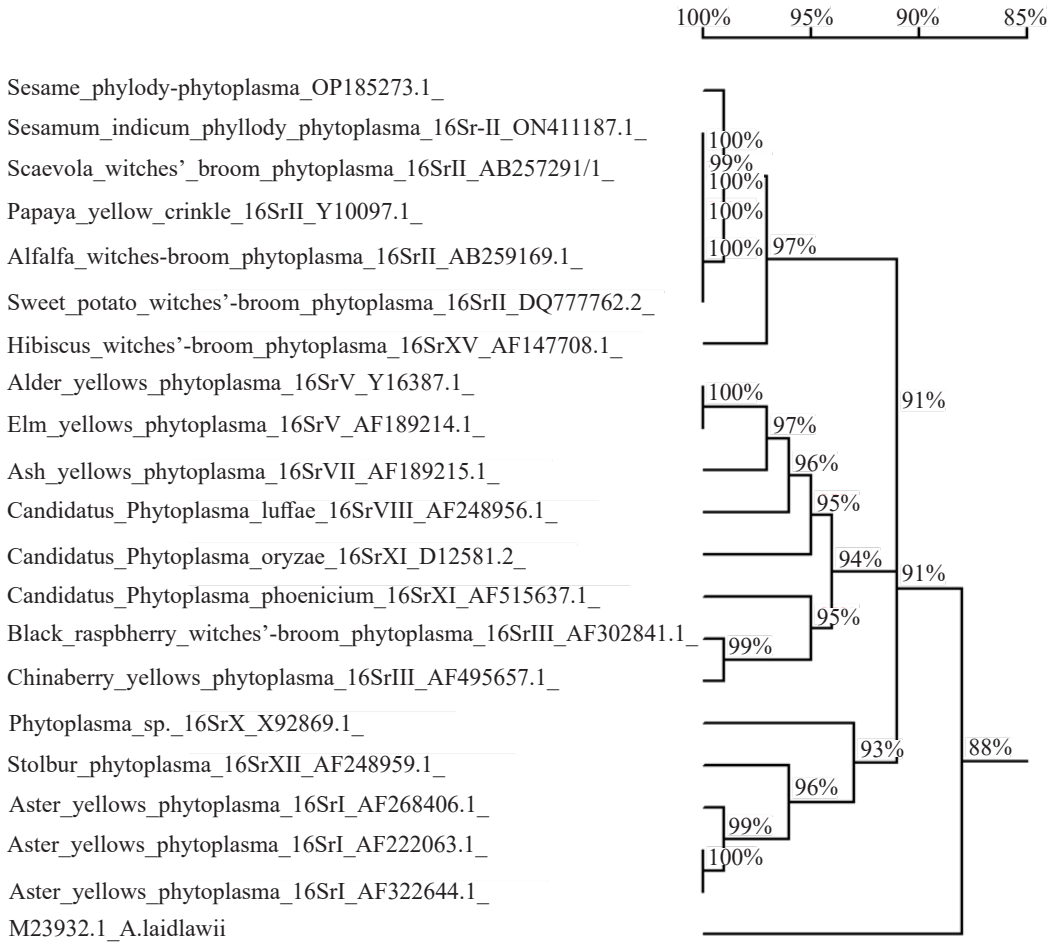

Figure 5. A phylogenetic tree of the sesame phyllody phytoplasma clone Beheira (ES Be) (Acc. No. OP185273.1) based on partial nucleotide sequence of the 16S ribosomal RNA gene, compared with 20 other isolates using DNAMAN Sequence Analysis software version 7 (Lynnon BioSoft, Vaudreuil, QC, Canada). The OP185273.1 sequence was obtained in this study, while the other reference sequences were retrieved from NCBI GenBank. *Acholeplasma laidlawii* (Mycoplasma) was used as an outgroup.

Table 3. Homology matrix of 16S rRNA gene sequences among phytoplasma isolates associated with phyllody disease. The Beheira isolate (ES Be) (Acc. No. OP185273.1) was compared with 20 other sequences available in the NCBI GenBank

Pathogen*	Group	Similarity																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
OP185273.1		100%																				
ON411187.1	16Sr-II	99.3%	100%																			
Y16387.1	16SrV	89.8%	90.2%	100%																		
AF189214.1	16SrV	89.7%	90.1%	99.7%	100%																	
AF189215.1	16SrVII	90.5%	90.8%	97.2%	97.1%	100%																
AF248956.1	16SrVIII	90.5%	90.8%	96.2%	96.1%	96.3%	100%															
D12581.2	16SrXI	91.0%	91.6%	94.5%	94.5%	94.9%	94.4%	100%														
AF515637.1	16SrIX	91.3%	91.7%	93.9%	93.8%	93.7%	94.0%	94.3%	100%													
AF302841.1	16SrIII	92.2%	92.6%	94.0%	94.0%	93.8%	94.0%	93.9%	94.8%	100%												
AF495657.1	16SrIII	92.0%	92.4%	94.0%	94.0%	93.8%	93.8%	93.8%	94.6%	99.3%	100%											
AF147708.1	16SrXV	96.7%	97.4%	90.2%	90.2%	91.0%	90.4%	91.0%	90.9%	92.3%	92.1%	100%										
DQ777762.2	16SrII	99.0%	99.7%	90.1%	90.0%	90.7%	90.7%	91.5%	91.6%	92.3%	92.1%	97.2%	100%									
AB257291.1	16SrII	99.3%	100%	90.2%	90.1%	90.8%	90.8%	91.6%	91.7%	92.6%	92.4%	97.4%	99.7%	100%								
Y10097.1	16SrII	99.3%	100%	90.2%	90.1%	90.8%	90.8%	91.6%	91.7%	92.6%	92.4%	97.4%	99.7%	100%	100%							
AB259169.1	16SrII	99.1%	99.8%	90.0%	89.9%	90.7%	90.7%	91.4%	91.5%	92.4%	92.2%	97.3%	99.5%	99.8%	99.8%	100%						
X92869.1	16SrX	90.4%	90.7%	91.7%	91.4%	91.6%	92.1%	92.1%	91.6%	91.4%	91.3%	90.4%	90.5%	90.7%	90.7%	90.6%	100%					
AF248959.1	16SrXII	90.3%	90.2%	89.4%	89.5%	89.4%	89.9%	89.9%	89.8%	90.7%	90.7%	90.3%	89.9%	90.2%	90.2%	90.0%	92.6%	100%				
AF268406.1	16SrI	90.9%	90.7%	91.1%	91.2%	90.9%	91.2%	91.0%	90.6%	91.8%	92.0%	90.6%	90.5%	90.7%	90.7%	90.6%	92.9%	95.8%	100%			
AF222063.1	16SrI	90.7%	90.6%	90.9%	91.0%	91.1%	91.2%	91.0%	90.6%	91.7%	91.8%	90.4%	90.3%	90.6%	90.6%	90.4%	92.9%	96.0%	99.5%	100%		
AF322644.1	16SrI	90.7%	90.5%	90.8%	90.9%	91.0%	91.1%	90.9%	90.5%	91.6%	91.8%	90.3%	90.2%	90.5%	90.5%	90.3%	92.9%	95.9%	99.4%	99.9%	100%	
M23932.1		86.6%	86.8%	87.3%	87.2%	87.0%	87.6%	87.8%	87.9%	87.7%	87.8%	86.2%	86.6%	86.8%	86.8%	86.6%	86.3%	89.4%	89.7%	90.1%	90.0%	100%

(DQ777762.2) from Australia.

ISSR-PCR Analysis. ISSR-PCR was used to assess the genetic deversity of phytoplasma infection in three Egyptian isolates (ES F1, ES Lux and ES Be). Using 15 ISSR primers, DNA polymorphic fragments were identified between repetitive sequences in the phytoplasma genome.

Fragment variation: The number of fragments ranged from 1 to 10, with sizes 150 to 1500 bp, depending on the primer-isolates combination, indicating variable repetitive elements in phytoplasma genomes. The ISSR analysis showed that phytoplasma isolates could be differentiated depending on their DNA polymorphisms (Table 4).

Cluster analysis: Only two isolates, Beheira (ES Be) and Faiyoum (ES F1), clustered together (Figure 6). This is the first study in Egypt to link ISSR polymorphisms with phytoplasma infection in sesame.

Comparison of Molecular Techniques: Nested PCR vs. ISSR-PCR. Nested PCR is widely used for molecular diagnostics but has limitations such as low specificity, low amplification efficiency, and time-consuming protocols. ISSR-PCR is a superior alternative due to its speed, accuracy, high reproducibility, and ability to detect high levels of DNA polymorphisms with minimal primers (Ellegren, 2004). It is useful not only for genetic diversity studies (Kim et al., 2002; Phui et al., 2014) but also for DNA fingerprinting of phytoplasma infections and phylogenetic studies.

CONCLUSION

Viral-like diseases have been relatively well studied globally, including Egypt. In this study, we used Inter Simple Sequence Repeat-Polymerase Chain Reaction (ISSR-PCR) to assess the genetic relationships among three phytoplasma isolates associated with phyllody disease from different governorates in Egypt using ISSR-PCR primers. Cluster analysis revealed that only two Egyptian isolates, Beheira and Faiyoum, were grouped together, while the Luxor isolate formed a separate group. Further research in other sesame-growing governorates of Egypt is recommended to map the detailed distribution of phytoplasma strains, which may aid in developing strategies for managing sesame phytoplasma.

ACKNOWLEDGMENTS

Acknowledgments are presented to the Agriculture Research Center (ARC) and International Centre for Agricultural Research in the Dry Areas (ICARDA) for supporting this research.

FUNDING

This study received no external funding.

Table 4. ISSR primers and DNA polymorphisms used to differentiate phytoplasma isolates

No.	Name	No. Amplified Fragments			Polymorphism
		Faiyoum	Luxor	Beheira	
1	UBC811	4	4	3	Unique
2	UBC849	2	2	2	Monomorphic
3	UBC-822	9	7	10	Polymorphic
4	UBC-824	2	2	2	Monomorphic
5	UBC-835	3	3	3	Monomorphic
6	UBC-845	1	6	2	Polymorphic
7	UBC810	1	2	8	Polymorphic
8	TA-3	7	4	7	Unique
9	UBC-844	6	9	8	Polymorphic
10	UBC-827	1	2	3	Polymorphic
11	17898B	1	1	1	Monomorphic
12	17899A	2	2	2	Monomorphic
13	HB 15	7	7	7	Monomorphic
14	HB 10	1	1	1	Monomorphic
15	UBC834	2	4	1	Polymorphic

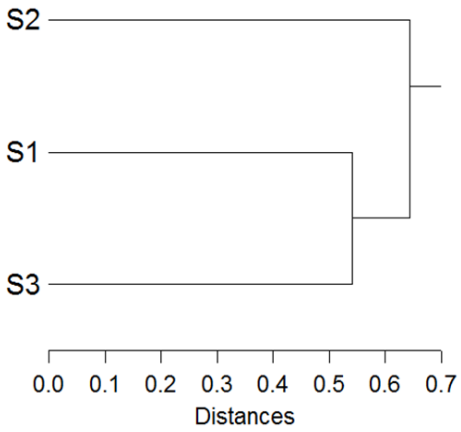

Figure 6. Dendrogram generated using cluster analysis, representing genetic relationships among three phytoplasma isolates associated with phyllody diseases using ISSR-PCR primers. S1: Faiyoum sample (ES F1), S2: Luxor sample (ES Lux), and S3: Beheira sample (ES Be).

AUTHORS' CONTRIBUTIONS

All the authors have contributed equally to the research and compiling the data as well as editing the manuscript.

COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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