

**ORIGINAL PAPER**

## Prevalence and genetic diversity of phytoplasmas infecting different crops in Egypt

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### ABSTRACT

Phytoplasma-related symptoms such as big bud, witches' broom, phyllody, virescence, flower proliferation, little leaves, yellowing, and stem fasciation were observed in 9 plant species during a field survey in Fayoum Governorate, Egypt, in 2021 and 2022. Disease incidence on various crops ranged from 1 to 21.3% in 2021, with the percentage rising in 2022. In 2022, the highest incidence percentage (23 %) was recorded on sesame while on cowpea the lowest percentage of incidence was recorded being (1.2%). A nested PCR assay employing the universal primer pair P1/P7 followed by R16F2n/R16R2 was used to detect the presence of phytoplasmas by amplifying a segment of the phytoplasma 16S rRNA gene. The resulting amplicons were purified and sequenced. The sequences were deposited in GenBank. Sequence comparison, phylogenetic study, and virtual RFLP analysis of 16S rRNA indicated the presence of diverse phytoplasma ribosomal groups and subgroups infecting different crops (16SrVI-A, 16SrIII-A, 16SrII-D, 16SrII-A, 16SrI-A, and 16SrI-B). The 16SrVI-A phytoplasmas were detected in symptomatic tomato plants, the 16SrII-D phytoplasmas subgroup was identified in sesame, faba bean, and cowpea exhibiting phyllody symptoms, the 16SrII-A subgroup was detected in cauliflower. The 16SrIII-A and 16SrI-A phytoplasmas subgroups were identified in alfalfa witches' broom and chamomile, respectively for the first time in Egypt. Moreover, subgroup 16SrI-B was detected for the first time in Egypt in chrysanthemum and cabbage. This study showed the genetic diversity of phytoplasma strains infecting a variety of crops in Fayoum Governorate.

**Keywords:** Phytoplasma; 16Sr RNA; PCR; Sequence; phylogenetic analysis; virtual RFLP

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### INTRODUCTION

Vegetables and field crops are high in minerals, phytochemicals, vitamins, and fiber content. One of the key factors limiting crop output is its vulnerability to

biotic and abiotic stressors. Phytoplasmas are plant-pathogenic mollicute that infect many major crops, causing substantial economic losses in the productivity and quality of vegetable and cereal crops, decorative and medicinal crops, and fruit trees (Bertaccini et al., 2014). Due to large economic losses in several crops, the importance of phytoplasmas as plant pathogen has expanded quickly over the last three decades (Bertaccini et al., 2014; Bertaccini and Lee, 2018). Plant-pathogenic phytoplasmas lack cell walls and have a pleomorphic form, a diameter of 200-800 nm, and a very tiny genome of approximately 680 – 1600 kb. They are Gram-positive Mollicutes that live in plant phloem tissues and insect hemolymph, particularly in leafhoppers and plant hoppers (Weintraub and Beanland, 2006; Bertaccini et al., 2014; Yang et al., 2020; Wang et al., 2022 Wang et al., 2024). Phytoplasmas can cause a variety of symptoms, including shoot proliferation, stunting, phyllody, virescence, witches' broom, big bud, little leaf, giant calyx, floral malformation, and vascular

discoloration (Bertaccini 2007; Omar et al., 2014; Kumari et al., 2019; Wang et al., 2022; Bertaccini, 2022; Kirdat et al., 2023). The symptoms of infected plants might differ depending on the phytoplasma strain, the host plant and its age, the stage of the disease, the period of infection, and the climatic conditions (Seemüller et al., 1998; Lee et al., 2000).

Phytoplasmas have been difficult to be identified and characterized for several decades because phytoplasmas cannot be cultured. Phytoplasmas detection has greatly improved with molecular DNA-based techniques such as PCR/RFLP and nested PCR on 16S rRNA (Lee et al., 1998; Zhao et al., 2009a). The success of PCR application is mostly dependent on the presence of enhanced phytoplasma DNA. Because of phytoplasma's low titer and uneven distribution in host plants, nested PCR is most employed to overcome phytoplasma's low concentration in infected plants (Bertaccini et al., 2014). Molecular investigations of conserved genes, particularly 16S rRNA genes, are currently utilized to classify phytoplasmas into 37 groups and more than 150 subgroups (Bertaccini et al., 2022). Based on a unique 16S rRNA gene sequence, the novel '*Candidatus phytoplasma*' species can be determined if it has <98.65% similarity to any of the previously described species (Wei and Zhao 2022). Many factors influence the geographical distribution of phytoplasma strains, including human activity, insect vector feeding behavior (monophagous, oligophagous, or polyphagous), and phytoplasma host range (Hogenhout et al., 2008; EPPO, 2017). Phytoplasma diseases have been reported in various countries worldwide (Bertaccini and Duduk, 2010; Marcone et al., 2016; Kumari et al., 2019; Pierro et al., 2019; Hemmati et al., 2021; Wang et al., 2022; Xiaoyan et al., 2023; Wang et al., 2024). Phytoplasma diseases have previously been documented in Egypt on tomatoes (El-Banna et al., 2007; Omar and Foissac 2012; Ahmed et al., 2014), faba beans (Hamed et al., 2014; Ahmed et al., 2022b), and sesame (El-Banna et al., 2013; Youssef et al., 2018;

and Ahmed et al., 2022a). The purpose of this study was to look at the prevalence of phytoplasmas in some winter and summer crops grown in Fayoum governorate. This research includes the identification of detected phytoplasmas using molecular approaches.

## MATERIALS AND METHODS

### The incidence of phytoplasma diseases:

In 2021 and 2022, a field survey was conducted at 18 locations of six districts of Fayoum Governorate (Fayoum, Senours, Itsa, Ibshway, Youssef El-Seddik, and Tamyia) in Egypt to determine the occurrence and distribution of phytoplasma diseases in vegetables, ornamentals, and field crops and to identify the phytoplasma strains associated with the observed symptoms. Phytoplasma symptoms were observed on 9 plant species including winter crops such as tomato (*Lycopersicon esculentum*), cauliflower (*Brassica oleracea*), cabbage (*Brassica pteracea*), , chamomile (*Matricaria chamomilla*), chrysanthemum (*Chrysanthemum morifolium*), faba bean (*Vicia faba*), and summer crops such as cowpea (*Vigna unguiculata*), alfalfa (*Medicago sativa*), and sesame (*Sesamum indicum* L.) where the incidence of symptom expression was estimated at flowering stage by visual inspection of 500 plants in each field, following "W" pattern (Taloh et al., 2020) using the formula below:

$$\% \text{ Disease Incidence(DI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \times 100$$

### Extraction of nucleic acids and detection through nested PCR:

The collected symptomatic samples for each plant species mentioned in Table 1 were divided into groups according to the observed symptoms then total DNA was extracted from all groups of symptomatic leaf samples using the procedures reported by Dellaporta et al. (1983). Also, DNA was extracted from an asymptomatic sample for each plant species and used as negative controls in PCR assays. P1/P7 primers were used for the first PCR to amplify a 1.8 kb product of 16S rRNA gene (Deng and

Hiruki 1991; Smart et al., 1996), followed by R16F2n/R2 primers for the second PCR to amplify 1.2 kb (Gundersen and Lee 1996). A PCR reaction was conducted in 25  $\mu$ L containing 3  $\mu$ L of extracted DNA, 1.5  $\mu$ L of 10 pmol of each primer, 12.5  $\mu$ L of amaR OnePCR™ (Genedirex) and 6.5  $\mu$ L of sterile distilled water. PCR reaction was conducted in (Proflex PCR system; Applied Biosystems, Waltham, MA, USA). The reaction started with one cycle of 3 min at 94, followed by 40 cycles consisting of denaturation annealing, and extension (94°C for 30s, 53°C for 30s, at 72°C for 1min, and a final extension of 10 min at 72°C). PCR products were electrophoresed (45 minutes at 100 volts) in 1% agarose gel, stained with EZView Stain (Biomatik Canada), and visualized using a UV transilluminator.

#### **Cleaning and sequencing of PCR product**

Following the manufacturer's instructions, the amplified PCR products with R16F2n/R2 primers were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). Macrogen Company (South Korea) performed direct sequencing on purified DNA. After analyzing all phytoplasma sequences infecting each plant species in all locations, the nucleotide sequences were submitted to GenBank. To determine the phytoplasma groups, the sequences were examined and compared with the other Sequences available in the phytoplasma classification database in GenBank using MEGA X Sequence Analysis Software version 10 (Kumar et al., 2018).

#### **Virtual RFLP analysis:**

Virtual RFLP analysis of partial 16S rDNA sequences were performed, iPhyClassifier was utilized to conduct in silico RFLP analysis (<https://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cg>) as described by (Zhao et al. 2009b).

### **RESULTS**

#### **Symptoms and disease incidence of phytoplasma**

In 2021, phytoplasma symptoms were observed in 13.3%, 6.3%, 11.1%, 2.9%, 9.4%, 8.1%, 27.6%, 7.4%, and 33.3% of

total surveyed fields of tomato, cauliflower, cabbage, cowpea, chamomile, chrysanthemum, faba bean, alfalfa, and sesame, respectively. As shown in Table 1, these percentages increased in most locations during 2022. Tomato plants showed witches' broom, phyllody, and big bud symptoms (Figures 1A and B), compared to healthy plants (Figures 1C). Cauliflower plants displayed virescence, proliferation, and phyllody (Figures 1D, E, and G) when compared with healthy ones (Figures 1F and H).

Flat stem fasciation and phyllody symptoms were observed in cowpea (Figures 2A and B) in comparison with healthy plants (Figures 2C), and various abnormalities on cabbage leaves (Figures 2D and E). Phyllody, witches'-broom, little leaves, and flower virescence were observed in infected faba bean, alfalfa, and sesame plants (Figure 3A - I). In chrysanthemum and chamomile, floral proliferation, virescence, phyllody symptoms, and various malformations in flowers were also present (Figure 4A-G), compared to healthy plants (Figure 4H).

In terms of incidence in vegetable crops, tomato plants had the highest infection rate (Table 1), with a percentage of incidences reaching 11.8% in 2022, while cowpea had the lowest (1.2%). The prevalence in ornamental crops (Chrysanthemum and chamomile) is similar. Interm of field crops, the percentage of incidence varied by field and location, with sesame ranging from 12% to 23% in all examined areas in 2022, followed by faba bean (3.4% to 17.3%) and alfalfa (1.5% to 2.9%) in (Table 1).

#### **Nested -Polymerase Chain reaction (nested-PCR) Detection:**

During the years 2021 and 2022 a total of 413 samples were tested for phytoplasma presence using nested -PCR. Results confirmed the presence of phytoplasma in 9 plant species (Table1) growing in Fayoum Governorate; approximately 1200bp was amplified from 269 out of 413 tested samples. No PCR products were obtained from asymptomatic plants when used as negative control (Figure S1).

**Table (1): The incidence of phytoplasma on different crops during 2021 and 2022, in Fayoum Governorate.**

Host plant	Districts	Symptomatic fields/total		Visual inspection Symptomatic/total		Incidence %		Symptomatic Fields %		Symptom	PCR Positive/total
		2021	2022	2021	2022	2021	2022	2021	2022		
Tomato	Fayoum	0/10	0/10	0/5000	0/5000	0%	0.0%	13.3%	14%	BB,WB, Ph	45/60
	Itsa	1/10	2/10	100/5000	150/5000	2%	3%				
	Tamyia	4/15	5/15	490/7500	890/7500	6.5%	11.8%				
	Ibshway	1/10	0/15	50/5000	0/7500	1%	0.0%				
Cauliflower	Senours	2/10	3/15	150/5000	335/7500	3%	4.5%	6.3%	16.2%	V, Ph, SP	30/45
	Itsa	0/10	3/12	0/5000	90/6000	0%	1.5%				
	Ibshway	0/12	0/10	0/6000	0/5000	0%	0.0%				
Cabbage	Fayoum	2/15	2/15	150/7500	195/7500	2%	2.6%	11.1%	6.6%	LM	28/40
	Senours	1/12	0/15	75/6000	0/7500	1.25	0.0%				
Cowpea	Fayoum	1/10	2/10	190/5000	235/5000	3.8%	4.7%	2.9%	8.5%	BP, Ph, FS	20/35
	Itsa	0/10	0/10	0/5000	0/5000	0%	0%				
	Senours	0/15	1/15	0/7500	90/7500	0%	1.2%				
Chamomile	Fayoum	0/10	1/10	0/5000	100/5000	0%	2%	9.4%	17.1%	V, Ph,	25/30
	Youssef el- Seddik	3/15	5/15	175/7500	210/7500	2.3%	2.8%				
Chrysanthemum	Itsa	0/8	2/10	0/4000	80/5000	0%	1.6%	8.1%	15%	Ph,V,FP	28/38
	Tamyia	0/12	0/15	0/6000	0/7500	0%	0%				
	Ibshway	2/10	3/10	135/5000	155/5000	2.7%	3.1%				
	Youssef el- Seddik	1/15	3/15	50/7500	110/7500	0.7%	1.5%				
Faba bean	Fayoum	5/12	7/15	900/6000	1300/7500	15%	17.3	27.6%	29.1%	Ph,WB	25/50
	Senours	2/15	3/15	620/7500	695/7500	8.3%	9.3%				
	Ibshway	2/10	1/10	190/5000	235/5000	3.8%	4.7%				
	Itsa	4/10	5/15	160/5000	260/7500	3.2%	3.4%				
Alfalfa	Itsa	2/12	3/15	165/6000	220/7500	2.75%	2.9%	7.4%	12%	WB, LL, Y	35/70
	Tamyia	0/15	2/10	0/7500	75/5000	0%	1.5%				
Sesame	Itsa	6/15	7/15	1600/7500	1730/7500	21.3%	23%	33.3%	36%	Ph, V, FP	33/45
	Senours	3/12	2/10	457/6000	600/5000	7.6%	12%				

(BB)=Big bud, (WB)=Witches'-broom, (Ph)=Phyllody, (V)=virescence, (SP)= sprout proliferation (LM)= leaves malformation, (BP) = bud proliferation, (FS)= flat steam, (FP)= Floral proliferation, (LL)=little leaves, (Y) =Yellowing

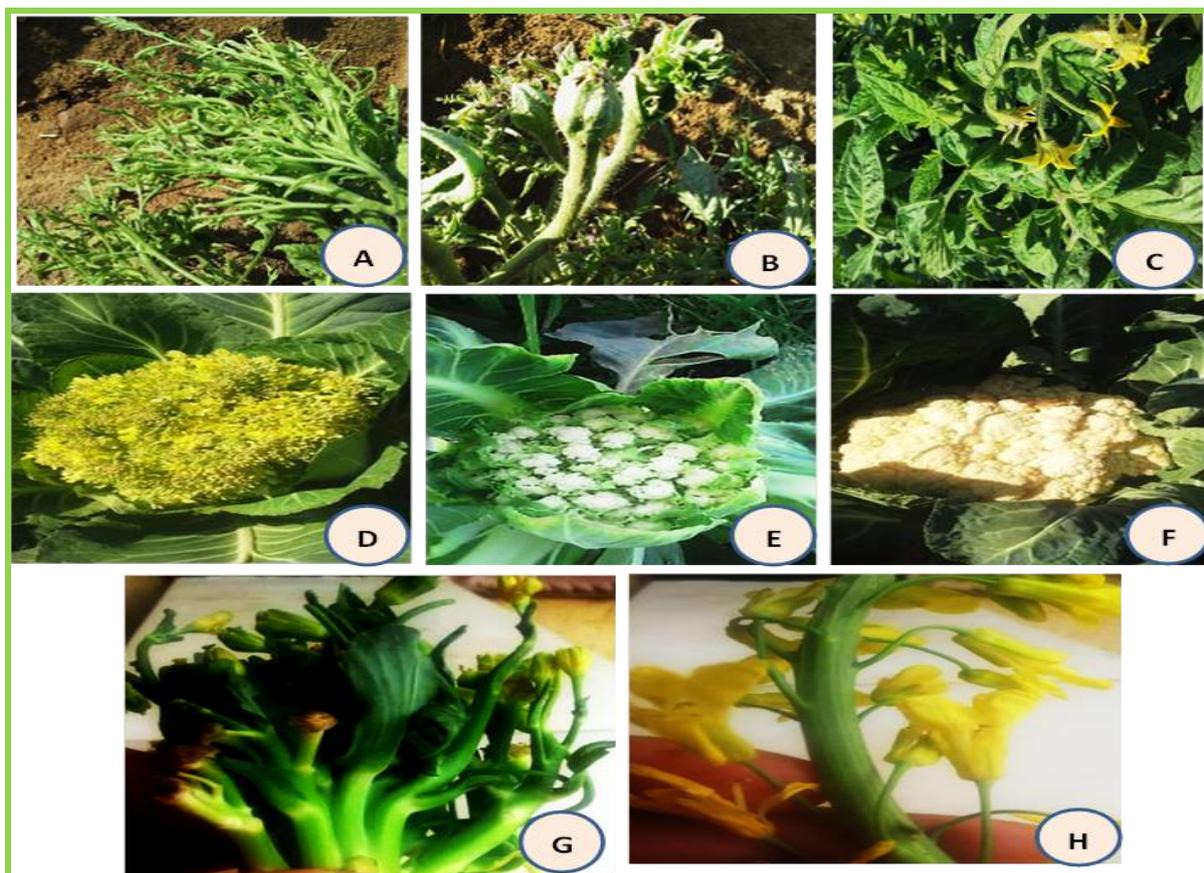
### Sequence analysis:

Purified and sequenced 16S rRNA amplified products were deposited in GenBank under nine accession numbers (ON921682.1, ON924755.1, MW945416.1, MW510021.1, OP659008.1, MW514054.1, OP687045.1, OP687044.1, and ON.922997.1) (Table S1).

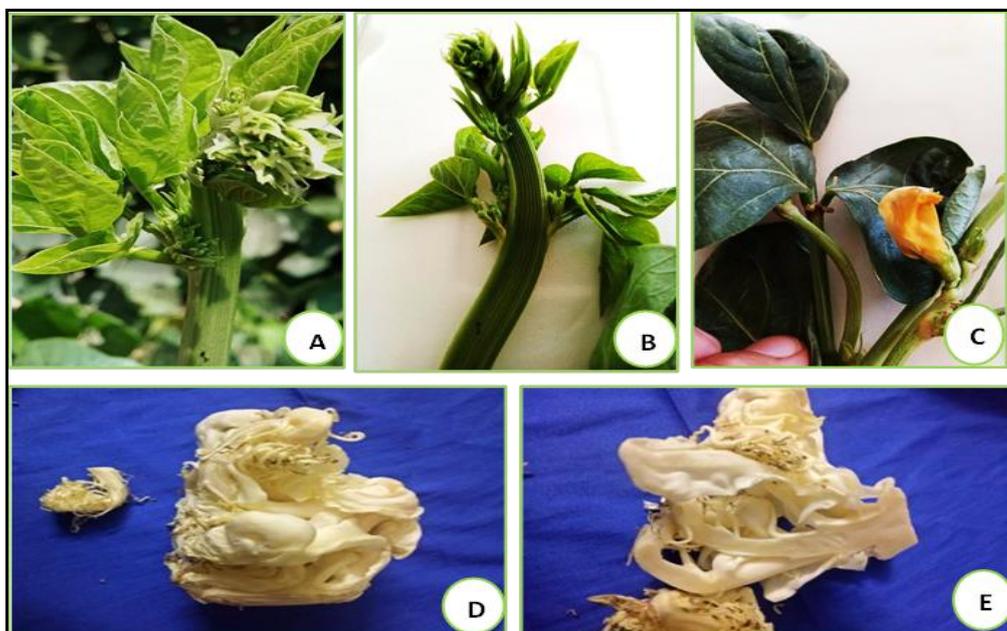
Sequence analysis and phylogenetic tree (Figure 5A) revealed the Egyptian tomato phytoplasma isolate (Accession no. ON921682.1) clustered with potato purple top phytoplasma which classified as 16SrVI group and shared 99.8% identity with it (Figure 5A and (Table S1). The sequences from the Egyptian Alfalfa phytoplasma isolate (Accession no. ON924755.1) shared 98% nucleotide identity with Canadian peach X mycoplasma of the 16SrIII group (Table S1). The phylogenetic analysis also showed the Egyptian phytoplasma isolates detected in sesame (Accession no. MW945416.1), faba bean (Accession no. MW510021.1), cowpea (Accession no.

OP659008.1), and cauliflower (Accession no. MW514054.1) shared nucleotide identity 98.8% between them and most closely related to *Corchorus olitorius* phytoplasma (99.9%) which belongs to 16SrII. Sequences Analysis of phytoplasma infecting Chamomile (Accession no. OP687045.1), Chrysanthemum (Accession no. OP687044.1), and cabbage (Accession no. ON.922997.1) showed 16S rDNA sequence similarity ranging from 99.6 to 100% with 16SrI group (Table S1).

The sequence comparison and phylogenetic analysis of phytoplasmas'16S rRNA in the present study with other Egyptian phytoplasmas available in the GenBank (Figure 5B) revealed that phytoplasmas of group 16SrVI infecting tomato was first documented in Egypt, while cowpea and cauliflower are new hosts of the 16SrII. The 16SrIII Phytoplasma group was identified in alfalfa witches' broom for the first time in Egypt.



**Figure 1:** Phytoplasma symptoms on naturally infected vegetable plant species; witches' broom symptoms on tomato (A); phyllody and big bud (B) and asymptomatic tomato plants (C). virescence and floral deformation symptoms on cauliflower (D); sprout proliferation (E); phyllody and petal greening symptoms (G) and asymptomatic cauliflower plants (F and H) confirmed by PCR detection.



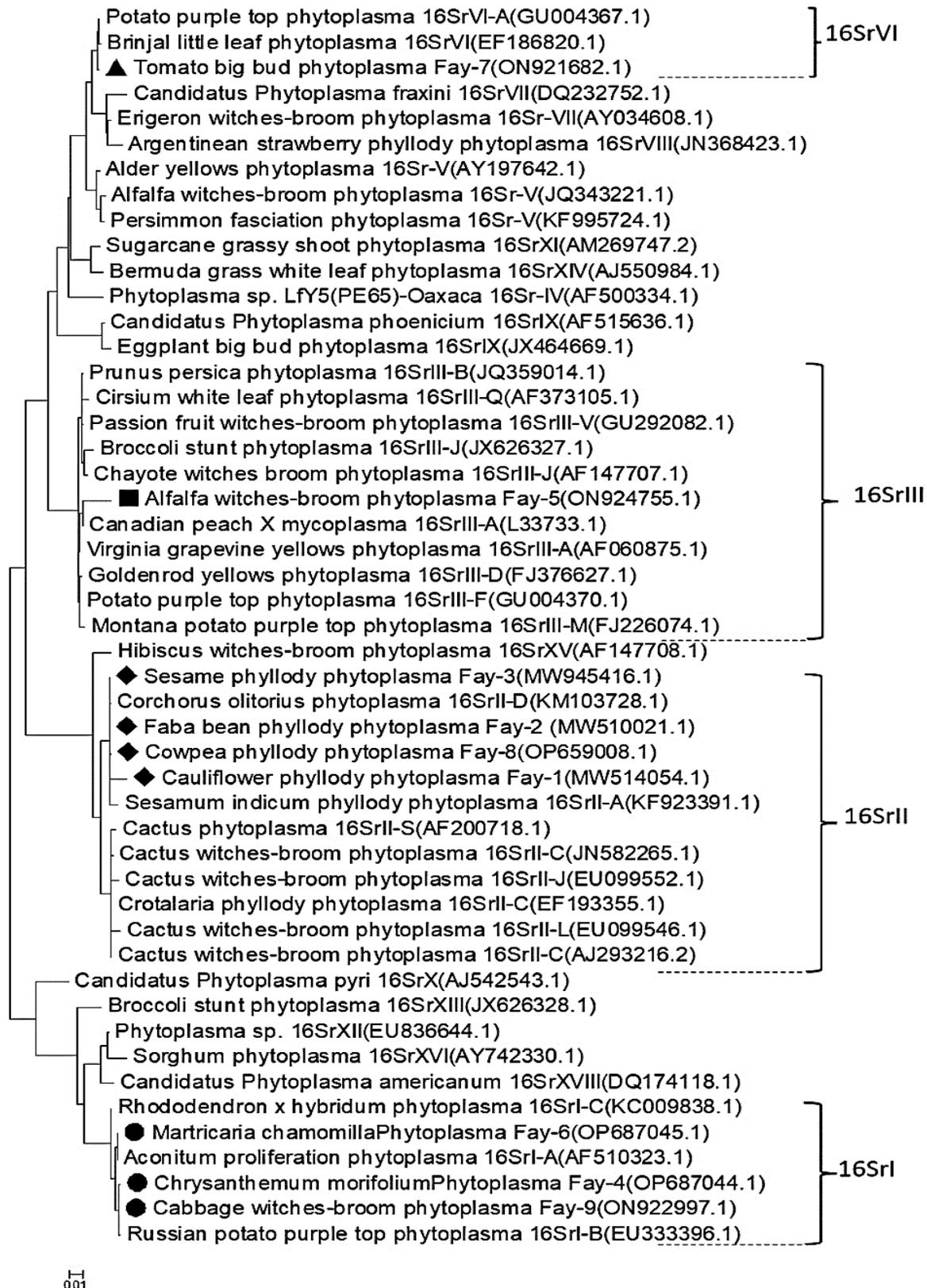
**Figure 2:** Phytoplasma symptoms on infected vegetable plant species; phyllody and flat stem faciation on cowpea (A and B); asymptomatic cowpea (C). Malformations on internal leaves of cabbage (D and E).



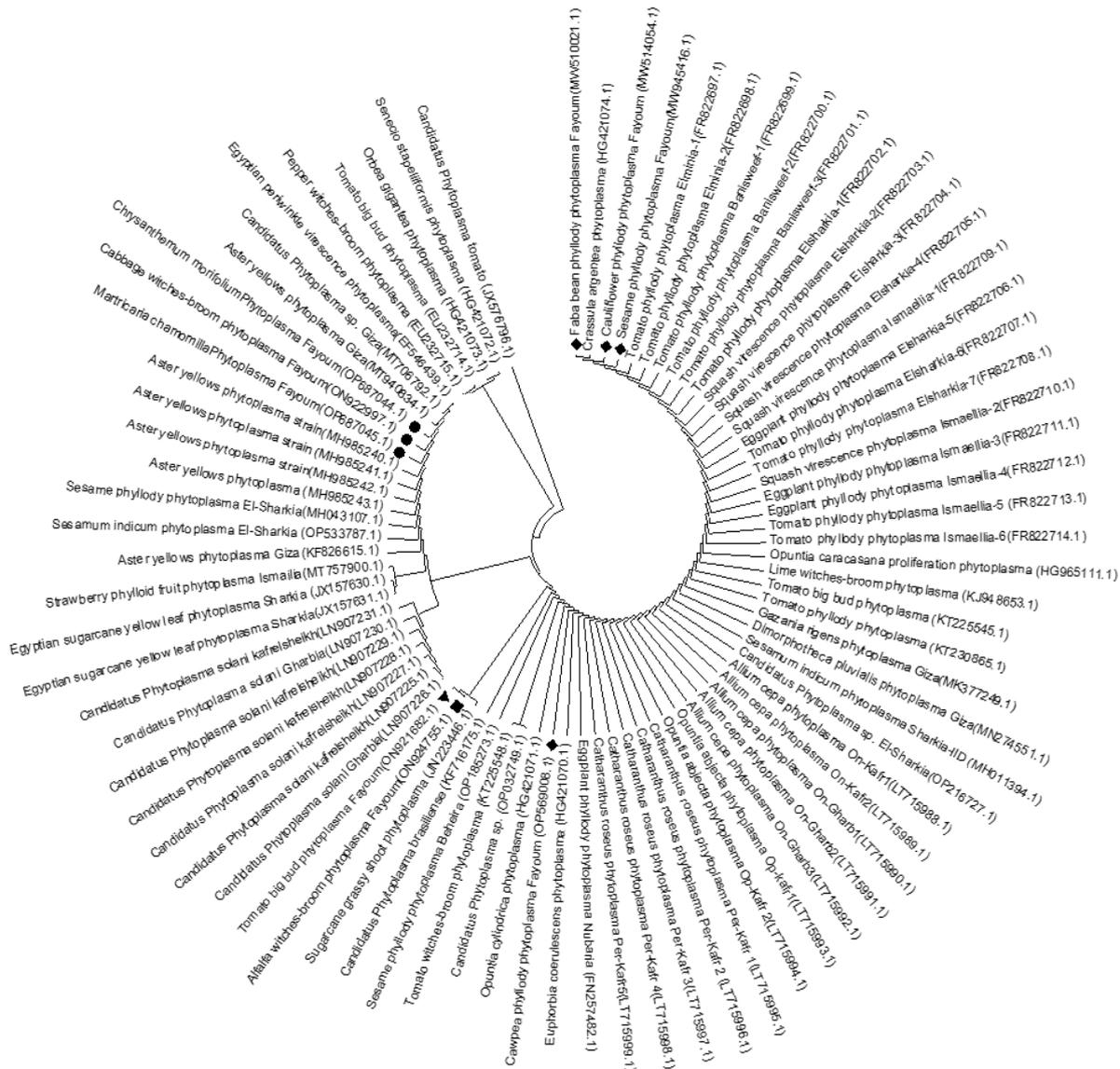
**Figure 3:** Phytoplasma symptoms on naturally infected field crops; phyllody symptoms in faba bean (A); little leaves and shoots proliferation (B) and asymptomatic faba bean plants (C). Phyllody symptoms on alfalfa (D); Witches'-broom and little leaves (E); and healthy alfalfa plants (F); Phyllody symptoms on sesame plants (G); virescence (H) and asymptomatic plants (I).



**Figure 4:** Symptoms of phytoplasma on infected ornamental plants: phyllody symptoms on Chamomile plants (A); virescence (B) and asymptomatic chamomile (C). virescence on chrysanthemum (D); flower proliferation (E); phyllody symptoms (F); different malformation in floral parts (G) and asymptomatic chrysanthemum flower (H).



**Figure 5A:** Phylogenetic tree performed based on sequences of the 16S rRNA gene using MEGA X Sequence Analysis, showing the relation between the nine phytoplasma Egyptian isolates (highlighted with black color) and other reference phytoplasma isolates belonging to different groups available in the GenBank database.



**Figure 5B:** Phylogenetic tree showing the relation between the nine phytoplasma isolates under the present study and other isolates recorded in Egypt.

Phytoplasmas from the 16SrI group were detected for the first time in Egypt in Chamomile, Chrysanthemum, and Cabbage. Virtual RFLP analysis (Fig. S2) succeeded in differentiating the phytoplasma strains in this study into six subgroups: 16SrVI-A (Tomato), 16SrII-D (Faba bean & cowpea, & sesame), 16SrII-A (cauliflower), 16SrI-A (Chamomile) and 16SrI-B (Chrysanthemum & Cabbage), and 16SrIII-A (Alfalfa).

**DISCUSSION**

Plant diseases are one of the most common problems limiting crop

productivity worldwide. Phytoplasma symptoms were found in nine plant species growing in six districts in Fayoum governorate (Egypt) during a field survey in 2021 and 2022. Phytoplasma infections were frequent in a variety of plant species and locations across Fayoum governorate, according to the presented findings. The emergence of active insect vectors in all affected areas could be related to the spread of phytoplasma infections in various crops and localities. Many factors influence phytoplasma dispersal, including host range, use of infected plant material for grafting or planting, and insect vector

feeding behavior (EPPO, 2017; Hogenhout *et al.*, 2008). Furthermore, climate change has an impact on plant disease epidemiology by influencing the insect life cycle, where vectors might become more active and phytoplasma infections could spread into new locations (Jones and Barbetti, 2012).

In this study Phytoplasmas induced different symptoms in different plant species probably due to many factors such as phytoplasma strain, and the climatic conditions (Seemüller *et al.*, 1998; Lee *et al.*, 2000; Kumari *et al.*, 2019).

Interestingly, some fields may remain completely free from phytoplasma disease symptoms, this could be due to factors such as low titer of phytoplasma in plants caused they are symptomless even though the pathogens are present or plant's resistance, moreover time of infection and plant age (early and/or late infection), vector control, or the use of disease-free planting materials. However, understanding the molecular mechanisms of phytoplasma-host interactions remains crucial for effective disease management. Our results agree with (Nair, and Manimekalai, 2021)

Phytoplasma was confirmed to infect 9 plant species in this study utilizing nested-PCR with universal primers P1/P7 followed by nested primers R16F2n/R16R2n. phytoplasma groups and subgroups were determined by comparing 16S rDNA sequences, phylogenetic analysis and virtual RFLP, results revealed that our phytoplasma isolates belonged to 16SrVI-A, 16SrIII-A, 16SrII-D, 16SrII-A, 16SrI-A, and 16SrI-B. The classifying of phytoplasmas into subgroups in this study based on virtual RFLP. Computer-simulated virtual RFLP analyses have been used and succeeded in classification of phytoplasmas into groups and subgroups (Zhao *et al.*, 2009b) (pDRAW32 ver. 1.1.144 software).

Tomato big bud (TBB) has been linked to a variety of phytoplasma groups and subgroups in Iran, including 16SrIX,

16SrXII, 16SrI, 16SrII, and 16SrVI (Salehi and Hosseini, 2016). TBB was also linked to 16SrVI-A and 16SrVII-A in Turkey (Sertkaya *et al.*, 2007). Phytoplasmas from the 16SrII-D subgroup have also been found infecting tomato crops in Egypt (Omar and Foissac, 2012), Saudi Arabia (Alhudaib and Razq, 2011), Iraq (Alkuwaiti *et al.*, 2017), Iran (Esmaeilzadeh-Hosseini *et al.*, 2022). The detected phytoplasmas of the 16SrVI group in tomatoes are consistent with those from Lebanon (Choueiri *et al.*, 2007), China (Du *et al.*, 2013), Syria (Khalil *et al.*, 2019), and Turkey (Usta *et al.*, 2022). Phytoplasmas of group 16SrVI infecting tomatoes, on the other hand, were first described in Egypt.

Phytoplasma detected in sesame, faba bean, cauliflower, and cowpea in this study were found to be associated with subgroup 16SrII-D, this subgroup has already been identified in sesame from Egypt (Ahmed *et al.*, 2022a), Pakistan (Akhtar *et al.*, 2009), Oman (AlSakeiti *et al.*, 2005), Turkey (Cengiz *et al.*, 2014) and India (Madhupriya *et al.*, 2015). Multiple phytoplasma groups and subgroups have been identified associated with sesame phyllody, including 16SrI, 16SrII, 16SrVI, and 16SrIX (Rao *et al.*, 2015).

The presence of 16SrII-D subgroup phytoplasmas in faba bean has been previously reported in Sudan (Alfaro-Fernandez *et al.*, 2012), Saudi Arabia (Al-Saleh and Amer, 2014; Omar, 2017), and Iran (Salehi *et al.*, 2016), but phytoplasma infecting faba bean was found belong to the 16SrIII group in Spain (Castro and Romero, 2004) and Peru (Torres Suarez *et al.*, 2021).

Results of 16S rRNA sequence analysis (Fig. 5 B) show that cowpea is a new host of 16SrII-D subgroup phytoplasmas in Egypt. Several phytoplasma groups and subgroups, including 16SrV and 16SrXII-B, were identified in cowpea from Australia (De La Rue *et al.*, 2001; Saqib *et al.*, 2006) and 16SrI-B, 16SrII-D, and 16SrIX from India (Kumar *et al.*, 2012; Thorat *et al.*, 2016; Rao *et al.*, 2018). In

addition, sequencing analysis results of the present study indicated that cauliflower is a new host of the 16SrII-A subgroup in Egypt. This subgroup was previously reported in cauliflower in China (Cai et al., 2016). However earlier phytoplasma groups and subgroups were found infecting cauliflower, 16SrVI-A subgroup in Iran (Salehi et al., 2007), 16Sr III-J and 16SrXV-A subgroup in Brazil (Rappussi et al., 2012; Canale and Bedendo, 2013), 16Sr VI-D and 16SrXIV-A subgroups in India (Gopala and Rao 2018; Sajeena et al., 2021).

The 16SrIII-A phytoplasma subgroup was detected in alfalfa witches' broom for the first time in Egypt in this study, although the presence of the 16SrV group was previously reported in alfalfa witches' broom from China (Li et al., 2012). Different phytoplasma groups and subgroup have been associated with alfalfa witches' broom from different countries such as 16SrI in Argentina and Bolivia (Conci et al., 2005; Jones et al., 2005), 16SrII-D in Oman, Iran and Saudi Arabia (Khan et al., 2002; Esmailzadeh-Hosseini et al., 2016; Al-Saleh and Amer, 2014), 16SrXII-A in Iran (Esmailzadeh-Hosseini et al., 2016).

In this work, Phytoplasmas belonging to the 16SrI-A subgroup were identified in chamomile for the first time in Egypt. On the other hand, chrysanthemum and cabbage were found to be hosts of phytoplasmas belonging to the 16SrI-B subgroup. In Egypt, this group was previously reported on periwinkle (Omar et al., 2008), date palm (Alkhazindar 2014), and *Dodonaea viscosa* plants (Mokbel 2020). The association of the 16SrI-S subgroup has been detected in chamomile from Argentina (Torres et al., 2011), although the 16SrXIV, 16SrVI-D, 16SrII-D and 16SrI phytoplasma groups and subgroups have been reported in chrysanthemum (Taloh et al., 2020).

The presence of 16SrI in cabbage witches' broom in Egypt is consistent with recent findings in China (Mou et al., 2012). Various phytoplasma groups and

subgroups have been found in cabbage, including 16SrVI (Salehi et al., 2007), 16SrI (Arocha et al., 2008; Gkavaleka et al., 2012), 16SrII-D, and 16SrII-X (Omar et al., 2020).

The presented data revealed the genetic diversity of phytoplasmas that infect different crops in different regions of Egypt's Fayoum governorate. The differences between strains could be attributable to the presence of different sources of phytoplasmas or the presence of different vectors in different areas. Furthermore, these genetic differences may be due to the emergence of point mutations in the 16S rRNA gene area, which signals the creation of new strains (Usta et al., 2022).

Finally, the field survey in this study revealed the large presence of phytoplasmas infecting several crops in different parts of Fayoum Governorate (Egypt). Sequence analysis of phytoplasmas 16S rRNA and computer-simulated virtual RFLP analysis succeeded in differentiating groups and subgroups of phytoplasma, for all recorded isolates used in this study. Results confirmed that phytoplasma isolates detected in the present study, belong to four groups and six subgroups. the phytoplasma infecting tomato is a member of the 16SrVI-A, 16SrIII-A phytoplasmas subgroup was detected in alfalfa witches' broom, phytoplasma strains associated with sesame, faba bean, and cowpea phyllody were members of 16SrII-D, and phytoplasmas of 16SrII-A have been found in cauliflower. The 16SrI-A was identified in chamomile, 16SrI-B in chrysanthemum, and cabbage. The findings of this study will be useful for phytoplasma epidemiology, allowing for more research into the diversity and epidemiology of phytoplasmas infecting crops in Egypt. Further research is needed to discover the wild plant species that serve as reservoirs for phytoplasmas and/or phytoplasma insect vectors and the vectors responsible for phytoplasmas transmission in various areas of the surveyed governorate.

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**Conflict-of-interest statement**

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

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