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Molecular Characterization of Different Phytoplasma Isolates Affecting New Hosts from Ornamental Plants in Egypt

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ABSTRACT

During 2021-2022, Phytoplasma-like infection symptoms were seen on various ornamental plants such as *Euphorbia milii*, *Catharanthus roseus*, *Duranta erecta*, and *Carpobrotus edulis* in four Egyptian governorates, Mansoura, Giza, Ismailia, and Matrouh. Natural symptoms associated with phytoplasma infections include leaf yellowing, leaf deformation, and plant stunting. Dienes' stain-treated hand-cut sections examined under a light microscope revealed blue patches around the affected plant regions. The primer pairs P1/P7 and R16F2n/R16R2 were used in PCR and nested-PCR assays to detect and identify phytoplasma in diseased ornamental plant samples. The amplified products of nested PCR were purified and then sequenced. The sequences were submitted to the GenBank database under the accession numbers OP723297, OP730893, OP723296, and OP723295, which were named EGY-SEAM1, EGY-SEAM2, EGY-SEAM3, and EGY-SEAM4, respectively. According to a comparative analysis using the iPhyClassifier database at the level of groups/subgroups with 41 references, sequences showed that one phytoplasma isolate was classified as related to (AF510323) Aconitum proliferation phytoplasma (16SrI-A) in Lithuania (99.7%), another isolate of phytoplasma belongs to the 16SrI-B type, the closest relative (98.8%) to Potato purple top phytoplasma (EU333396) in Russia, and other two isolates were similar or identical to the 16SrII-D type (98.6% or 100%) to Papaya yellow crinkle phytoplasma (Y10097) in Australia. In conclusion, the findings may suggest that *Catharanthus roseus* or *Carpobrotus edulis* plants in Egypt could serve as new hosts for the 16SrI group, subgroup A or B, as well *Euphorbia milii* and *Duranta erecta* plants for the 16SrII group, subgroup D. The presence of phytoplasma-related diseases in different Egyptian governorates shows the existence of numerous new hosts that may affect the production of several vegetables crops.

Keywords: Ornamental plants, Phytoplasma, Light microscope, Dienes' stain, Molecular techniques, iPhyClassifier.

1. Introduction

Phytoplasmas are prokaryotic organisms or pleomorphic bacteria and lack a cell wall. Phytoplasma possesses a minimal genome of about 680-1600 kb. Phytoplasma species comprise 37 groups and more than 150 subgroups (Bertaccini *et al*., 2022; Wei and Zhao, 2022). Numerous phytoplasma symptoms, including little leaves, phyllody, virescence, floral malformation, stunting, and the proliferation of axillary buds resulting in a witches broom-like structure are associated with phytoplasma diseases in ornamental plants (Mokbel *et al*., 2013; Alp *et al*., 2016; Kumari *et al*., 2019; Mokbel, 2020). Additionally, different crops may be associated with symptoms not particular to phytoplasma diseases, which may cause losses to reach 100% (El-Mahrouk *et al*., 2021). Recently, lily plants were infected with phytoplasma of the 16SrI group (Abdel-Salam *et al*., 2022). The

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presence of ornamental plants as reservoirs of phytoplasma in Egypt could explain why disorders caused by phytoplasma diseases were uncontrolled to date. Consider that this disease has been confirmed to have been present in ornamental plants in Egypt for seven years (Mokbel *et al*., 2020). Therefore, the authors believe that ornamental trees or shrubs are the primary cause of phytoplasma diseases spread in diverse crops, assisted by winds and phloem-feeding insects (Wei *et al*., 2021). To date, ornamental plants from all over the world have been found to contain more than 40 phytoplasmas from nine groups (Chaturvedi *et al*., 2010). Restriction fragment length polymorphism (RFLP) is the most appropriate molecular biology tool revels the differences between homologous DNA from differing the restriction enzyme locations sites and act as a molecular marker attributed to it is highly locus-specific (Chaudhary and Kumar 2020). The virtual RFLP analysis requires actual enzymatic gel electrophoresis as well as visual comparisons of different banded patterns, therefore, a new approach to phytoplasma taxonomy (IPhyClassifier) was created based on RFLP analysis, enabling or facilitating database-guided phytoplasma classification and identification, which also, provides high levels of resolution and chances to form new groups/subgroups. In the present study, 120 symptomatic samples with yellowing leaves or plant stunting and non-symptomatic samples were gathered of ornamental plants in four Egyptian governorates to identify and classify a phytoplasma isolate (s) at the level of groups/subgroups using the IPhyClassifier database. The results will, most importantly, serve as an indicator for the new hosts of phytoplasmas, which aids in the prevent-implantation of diseased plants and preserve significant crops from infections in Egypt.

2. Materials and Methods

2.1. Plant materials and appearance of phytoplasma diseases

Thirty samples from each of four species of ornamental plants i.e. (*Euphorbia milii* (Christ thorn), *Catharanthus roseus* (Periwinkle), *Duranta erecta* (Gold Edge), or *Carpobrotus edulis* (Hottentot-fig)) with symptomatic and some asymptomatic leaves belonging to different plant families (Euphorbiaceae, Apocynaceae, Verbenaceae, and Aizoaceae, respectively) were collected in 2021-2022 from four Egyptian governorates (Mansoura, Giza, Ismailia, and Matrouh). Most affected plants display symptoms such as leaf yellowing, leaves deformation, and plant stunting. The Orman Garden's herbarium section confirmed the previous plants' identities in Giza, Egypt.

2.2. Light microscopy detection with Dienes' stain

Using Dienes stain method, phytoplasmas were initially found in the leaf midribs of just the symptomatic plants (Deeley *et al*., 1979). Freehand cross-sections were created by razor blade edge, and then stained with Dienes' stain (0.2 %) for 10 minutes). At the Seed Pathology Lab in the Virus and Phytoplasma Research Department, the stained sections were washed in sterile distilled water, placed on four glass slides, and examined under a light microscope (Musetti, 2013).

2.3. Molecular methods for phytoplasma detection and identification

2.3.1. DNA extraction and PCR assays

Molecular tests were conducted at the plant virus identification and diagnosis lab (PVIDL) in the Virus and Phytoplasma Research Department. Total DNA was extracted from leaf midribs of samples (30) of the four studied ornamental plant species using symptomatic leaves and non-symptomatic ones, according to Dellaporta *et al.,* (1983). PCR was performed on the isolated DNA using the universal primer pair P1/P7, Table 1 (Deng and Hiruki 1991). The PCR products were utilized as templates in a nested PCR using the forward primer R16F2n and the reverse primer R16R2, Table 1 (Lee *et al*., 1993) after being diluted in 1:20 in nuclease-free water (Promega, USA). The DNA amplification was carried out in a thermocycler in three steps as follows: (i) denaturation 94°C/30s, (ii) annealing [94°C/60s, 53°C/60s, 72°C/120s] × 35 cycles, and (iii) extension 72°C/7 min. The resulting PCR products were analyzed by electrophoresis through 1.5% agarose gel, stained with EZ View nucleic acid stain (Biomatik, USA), and PCR product bands were then visualized using a UV transilluminator. Amplified fragments size standard was determined by comparison with a 1kb ladder.

2.3.2. Sequences analysis

The nested PCR products were sequenced in both directions on an ABI 3730XL automated sequencer with R16F2n/R16R2 primers, and then sequences were aligned through the MEGA-X program.

2.3.3. Evolutionary analysis by Maximum Likelihood method

For classification at the subgroup level, the R16F2n/R2 region sequences were used in the evolutionary analysis (Kumar *et al.,* 2018) with the MEGA-X program and the iPhyClassifier tool (http://www.ba.ars.usda.gov/data/mppl/iPhyClassifier.html). The four phytoplasma sequences were compared with 41 reference phytoplasma strains (Table 3). The Tamura-Nei model and the Maximum Likelihood approach were used to infer the evolutionary history (Tamura and Nei, 1993). A comparison between virtual RFLP patterns and the subsequent calculation of similarity coefficients was done and calculated, the pDRAW32 software was utilized to compare the virtual RFLP patterns, which were obtained through in silico digestions with 17 enzymes, using its multiple layer function. The similarity coefficient (F) was computed for each pair of phytoplasma strains based on the previously described formula by (Nei and Li, 1979; Lee *et al*., 1998).

Table 1: Nucleotide sequences of primers used for first and amplification rounds

Primer	Nucleotide Sequences (5'….3')	Expected size	Reference
P1	AAG AGT TTG ATC CTG GCT CAG GAT T	1800 bp	Deng and Hiruki (1991)
P7	CGT CCT TCA TCG GCT CTT		
R16F2n	CAT GCA AGT CGA ACG GA	1200 bp	Lee *et al*. (1993)
R16R2	TGA CGG GCG GTG TGT ACAAAC CCC G		

3. Results

3.1. Symptoms of phytoplasma on ornamental plants

Thirty samples of ornamental plants, such as *Euphorbia milii*, *Catharanthus roseus*, *Duranta erecta*, or *Carpobrotus edulis*, showed symptoms of leaf yellowing, leaves deformation, and plant stunting from four Egyptian governorates, Mansoura, Giza, Ismailia, and Matrouh (Figure 1 and Table 2). Leaves with these symptoms raised suspicion of phytoplasma disease infection thus; samples from affected plants were removed and tested using light microscopy and molecular assays.

Fig. 1: Collected symptomatic ornamental plants with phytoplasma from four Egyptian governorates: Mansoura, Giza, Ismailia, and Marsa Matrouh. (A) *Euphorbia milii*, (B) *Catharanthus roseus*, (C) *Duranta erecta*, and (D) *Carpobrotus edulis*, respectively.

3.2. Rates of Phytoplasma infection

The PCR assay results demonstrated that the plant species that harbor phytoplasma (symptomatic), with the highest rates, were *Catharanthus roseus* plants in Giza (66%), *Euphorbia milii* in Mansoura (56%), and *Duranta erecta* in Ismailia (40%) whereas, *Carpobrotus edulis* in Matrouh (26%) had the lowest rates (Table 2).

Table 2: Symptoms from naturally infected ornamental plants and phytoplasma detection in various Egyptian governorates.

Plant species /governorate	Symptoms	No. of Positive samples	No. of Negative samples	Total samples	%
***Euphorbia milii* /Mansoura**	Yellow leaves	17	13	30	56
***Catharanthus roseus* /Giza**	Yellow leaves	20	10	30	66
***Duranta erecta* /Ismailia**	Yellow leaves Reduced leaf size	12	18	30	40
***Carpobrotus edulis*/ Matrouh**	Stunting Malformation	8	22	30	26

Based on PCR assay using P1/P7-phytoplasma universal primer pair

3.3. Phytoplasma detection

3.3.1. Dienes' stain under light microscopy

Phytoplasma-infected ornamental plants were examined using light microscopy and Dienes' staining technique. The infected *Euphorbia milii* leaves (Fig. 2), as well as the other three symptomatic plants (data not shown), all had phytoplasma in their midribs, whereas the asymptomatic plants did not. The section from diseased midrib tissues was densely stained blue.

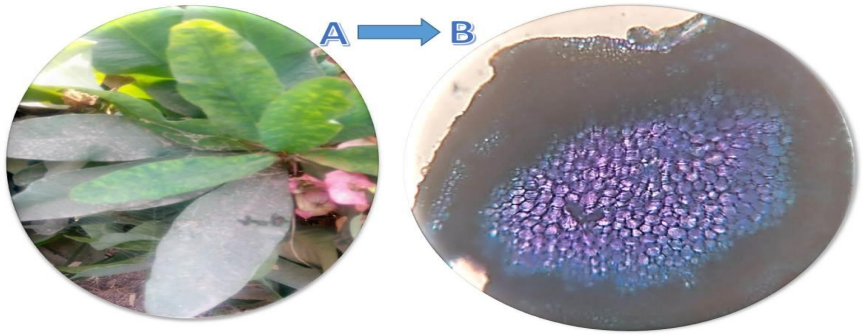

Fig. 2: Affected Euphorbia plant by phytoplasma. (A): Leaf yellowing symptom, compared to tissues stained with Dienes stain that showed a dark blue color, indicating the presence of phytoplasma (B).

3.3.2. Phytoplasma molecularly detection, identification, and characterization

The presence of phytoplasma in 57 samples with symptoms collected from naturally infected ornamental plants was investigated by nested PCR amplification. The phytoplasma-specific amplicons were consistently obtained with R16F2n/R16R2 primer (~1.2 kb), while no DNA fragments were amplified when asymptomatic sample DNAs were used as templates (Fig.3).

3.3.3. Sequence analysis

The nested-PCR products were purified and then sequenced in both directions using R16F2n/R16R2 primer pair. The sequences were deposited in the GenBank database with the accession numbers OP723297, OP730893, OP723296, and OP723295. Each phytoplasma isolate was given a name. For example, the phytoplasma isolate identified from *Euphorbia milii* sample was named EGY-SEAM-1.

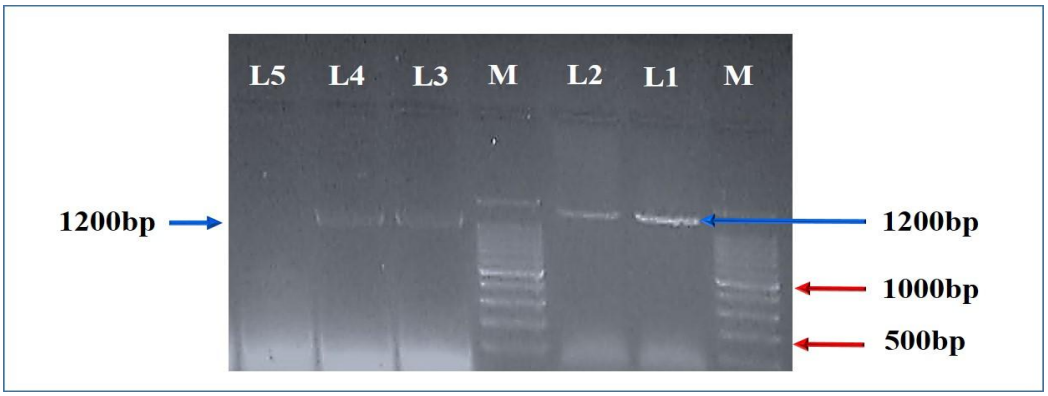

Fig. 3: Agarose gel electrophoresis (1.5%) of nested PCR products amplified from naturally infected ornamental plants with phytoplasmas. L1: Naturally infected *Euphorbia milii* plants with phytoplasma; L2: *Catharanthus roseus*; L3: *Duranta erecta*; L4: *Carpobrotus edulis*. L5: Asymptomatic plant sample. M: 1Kb DNA Ladder (Biomatic USA).

3.4. Phylogenetic analysis

The analysis of the 16S rRNA genes on the studied Egyptian phytoplasmas showed that the two Egyptian isolates, EGY-SEAM1 and EGY-SEAM3, belonged to the group (II), the same subgroup (D) and shared 100% and 98.6% identity with Papaya yellow crinkle phytoplasma from Australia. Whereas, the other two Egyptian isolates, EGY-SEAM2 and EGY-SEAM4, belonged to the group (I), subgroups (A and B) and shared 99.7% and 98.8% identity with Aconitum proliferation phytoplasma and Potato purple top phytoplasma from Lithuania and Russia, respectively (Table 3). The classification of groups was determined by the convention that had been previously established, wherein the coefficients of 16S rRNA gene RFLP pattern similarity between two distinct groups were should be equal to or less than 90%. The RFLP patterns of 45 16S rRNA gene sequence accessions from 41 phytoplasma strains, representing 28 groups, are displayed in Figure 5. Out of the 45 phytoplasma strains, 41 had already classified for group and subgroup using the means of the conventional phytoplasma 16S rRNA gene RFLP analysis. The virtual RFLP patterns of the previously classified strains matched the RFLP patterns observed on real gels with absolute accuracy (data not shown). These findings suggest that virtual RFLP analysis can serve as a convenient and reliable alternative to the conventional RFLP analysis.

Analysis performed with iPhyClassifier revealed that the phytoplasmas isolated from *Catharanthus roseus* and *Carpobrotus edulis* plants were clustered together within a group (I) and belonged to Aster yellow phytoplasma, while the phytoplasmas isolated from *Euphorbia milii* and *Duranta erecta* plants were clustered together within a group (II) and belonged to *Candidatus* Phytoplasma australasia. The EGY-SEAM2 and EGY-SEAM4 isolates are genetically related to each other and unlinked to the SEAM1 and EGY-SEAM3 isolates, as shown in Table 3 and Figure 4.

Table 3: Details of the phytoplasma isolates in the present study and the 41 reference phytoplasma strains used in building the phylogenetic tree.

Phytoplasma/disease common name	16S rDNA group-subgroup	GenBank no.	Country	Identity (%)			
Catharanthus roseus	This_study	OP730893	Egypt	100%			
Ca. Phytoplasma	This_study	OP723295	Egypt	99.5%	100%		
Ca. Phytoplasma	This_study	OP723296	Egypt	89.1%	89.0%	100%	
Ca. Phytoplasma	This study	OP723297	Egypt	90.5%	90.4%	98.6%	100%
Potato Purple Top	16SrI-B	EU333396	Russian	99.5%	99.8%	88.8%	90.2%
Aconitum proliferation	16SrI-A	AF510323	Lithuania	99.7%	99.6%	89.0%	90.4%
Rhododendronxhybridum' phytoplasma	16SrI-C	KC009838	Czech	99.6%	99.3%	89.3%	90.7%
Aster yellows (AY)	16SrI	M30790		99.5%	99.7%	88.8%	90.2%
WB disease of lime	16SrII-B	U15442	France	90.8%	90.7%	97.6%	99.0%
Papaya yellow crinkle	16SrII-D	Y10097	Australia	90.5%	90.4%	98.6%	100.0%
Western X-disease	16SrIII-A	L04682	USA	91.1%	90.8%	91.1%	92.4%
Palm lethal yellowing	16SrIV-A	U18747	USA	91.2%	90.9%	90.6%	91.9%
Elm yellows	16SrV-A	AY197655	USA	91.2%	91.0%	89.2%	90.6%
Jujube WB	16SrV-B	AB052876	Japan	91.2%	91.2%	89.2%	90.6%
Flavescence doree	16SrV-C	AF176319	USA	91.2%	90.9%	89.1%	90.5%
Clover proliferation	16SrVI-A	AY390261	Canada	91.0%	90.7%	89.7%	91.1%
Ash yellows	16SrVII-A	AF092209	USA	90.7%	90.8%	90.0%	91.2%
Loofah WB	16SrVIII-A	AF086621	Taiwan	91.0%	90.9%	89.9%	91.2%
Almond lethal disease	16SrIX-D	AF515636	Lebanon	89.5%	89.2%	90.0%	91.2%
Apple proliferation	16SrX-A	AJ542541	Germany	93.0%	93.1%	88.9%	90.3%
Pear decline	16SrX-C	AJ542543	Germany	93.2%	93.3%	89.0%	90.4%
Spartium WB	16SrX-D	X92869	Germany	93.2%	93.1%	89.0%	90.4%
European stone fruit Y	16SrX-F	AJ542544	Germany	93.7%	93.6%	89.2%	90.6%
Rice yellow dwarf	16SrXI-A	AB052873	Thailand	91.1%	90.8%	90.7%	92.0%
Stolbur phytoplasma	16SrXII-A	AF248959	USA	95.7%	95.8%	88.1%	89.3%
Australian GY	16SrXII-B	L76865	USA	96.2%	96.3%	88.5%	89.9%
Hydrangea phyllody	16SrXII-D	AB010425	Japan	95.7%	95.8%	88.3%	89.5%
Strawberry yellows	16SrXII-E	DQ086423	Lithuania	97.1%	97.1%	88.3%	89.5%
Mexican periwinkle Vir	16SrXIII-A	AF248960	USA	96.3%	96.4%	88.1%	89.5%
Bermuda grass WL	16SrXIV	AJ550984	Italy	90.9%	90.6%	90.2%	91.5%
Hibiscus WB	16SrXV	AF147708	USA	90.0%	89.9%	96.0%	97.3%
Sugarcane yellow leaf	16SrXVI	AY725228	Cuba	95.0%	95.2%	87.3%	88.5%
Papaya bunchy top	16SrXVII	AY725234	Cuba	93.8%	93.9%	86.5%	87.7%
Potato purple top wilt	16SrXVIII	DQ174122	USA	95.8%	95.9%	88.6%	90.0%
Chestnut WB	16SrXIX	AB054986	Japan	89.9%	89.8%	89.8%	91.0%
Buckthorn WB	16SrXX	X76431	Germany	92.7%	92.6%	89.1%	90.4%
Pine shoot proliferation	16Sr XXI	AJ632155	Spain	90.8%	90.7%	90.1%	91.4%
Nigerian Awka disease	16Sr XXII-A	Y14175	UK	90.7%	90.6%	90.8%	92.1%
Buckland Valley GY	16SrXXIII-A	AY083605	Australia	97.0%	96.9%	88.8%	90.2%
Sorghum bunchy shoot	16SrXXIV-A	AF509322	Australia	90.9%	90.6%	90.0%	91.3%
Weeping tea WB	16SrXXV-A	AF521672	Australia	89.7%	89.4%	91.2%	92.5%
Sugarcane yellows	16SrXXVI-A	AJ539179	Mauritius	94.5%	94.2%	89.8%	91.2%
Sugarcane yellows	16SrXXVII-A	AJ539180	Mauritius	93.4%	93.1%	90.1%	91.4%
Cassia italica WB	16SrXXIX	EF666051	Oman	89.4%	89.3%	90.2%	91.5%
Salt cedar WB	16SrXXX	FJ432664	China	93.7%	93.8%	88.9%	90.3%

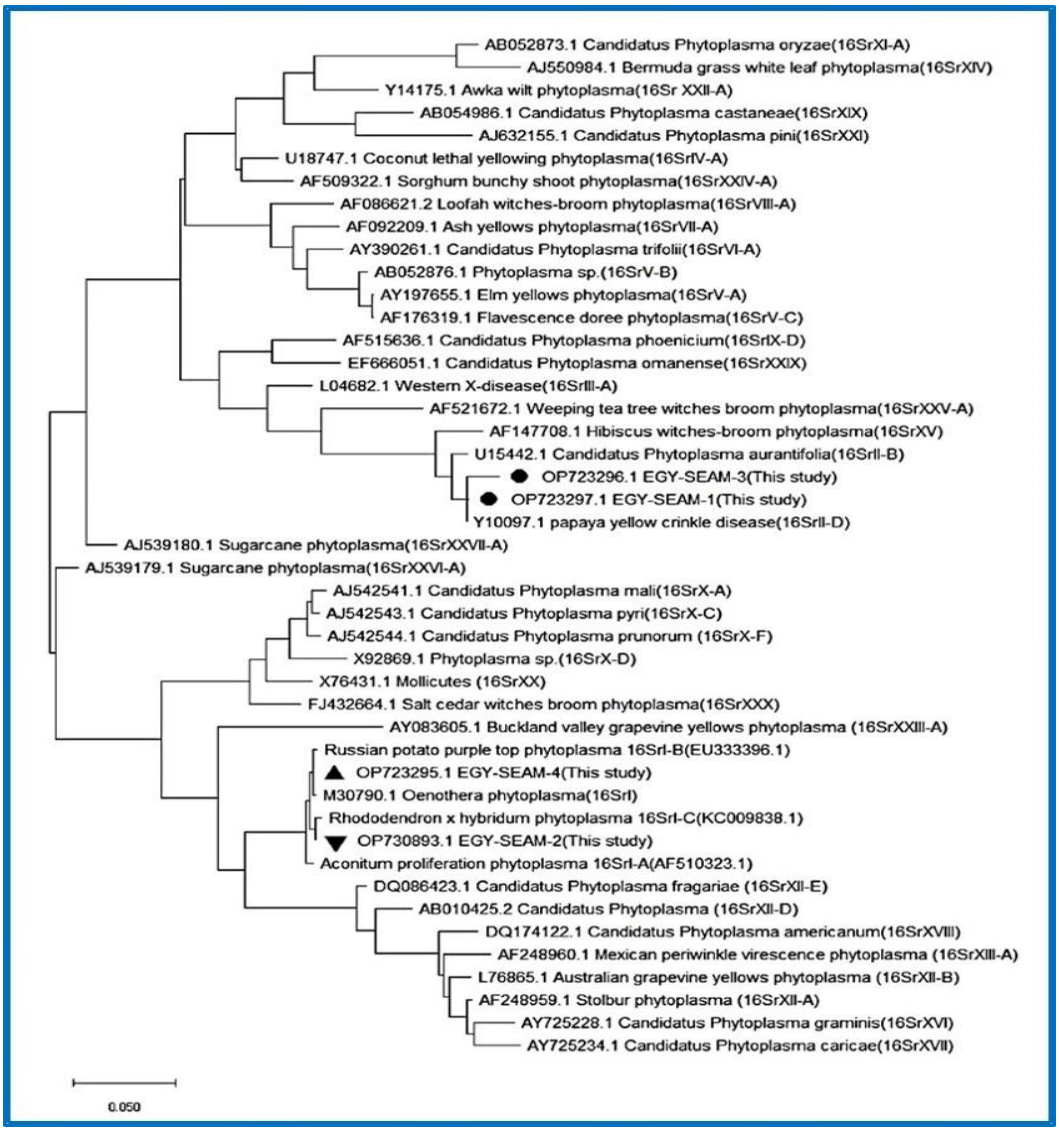

Fig. 4: The phylogenetic tree was constructed using forty-one previously identified "*Candidatus* Phytoplasma species" reference strains. The current study's discovered phytoplasma isolates in Egypt are displayed in hexagonal and triangular geometries. The Tamura-Nei model and the maximum likelihood approach (1000 bootstrap replicates) were used to infer the evolutionary history (Tamura and Nei, 1993). MEGA-X software was used to conduct evolutionary assessments (Kumar *et al*., 2018). The scale bar displays the evolutionary distance in nucleotide substitutions for each base position.

4. Discussion

Phytoplasma is a destructive infection for ornamental plants all over the world. Infected ornamental plants (*Euphorbia milii*, *Catharanthus roseus*, *Duranta erecta*, and *Carpobrotus edulis*) by phytoplasmas in the current study exhibited unspecific symptoms in their hosts to the Phytoplasma taxon. The ornamental tissues that were infected with phytoplasma were corroborated by preliminary results from Dienes' staining approach and light microscopy (Deeley *et al*., 1979; Musetti, 2013). Generally, the presence of phytoplasma in Euphorbia species was reported (Lee *et al*., 1997) and *C. roseus* (Favali *et al*., 2008; Su *et al*., 2011). The phytoplasma recognized within the infected *C. roseus* plays an essential role in the secondary metabolism of diseased plants (Favali *et al*., 2004). Moreover, Singh *et al.,* (2011) have also detected the presence of phytoplasma in *Duranta* plants in Indian gardens (Uttar Pradesh and Uttarakhand). Also, at Gorakhpur Gardens in Eastern Uttar Pradesh in India, the phytoplasma associated with the South African *C. edulis* plant was discovered to be an isolation of the pigeon pea witches' broom phytoplasma (Shukla *et al.,* 2014).

In recent years, an online software (iPhyClassifier) has been developed for the classification of phytoplasmas in groups and subgroups, which doesn't rely on symptoms only that appear on plants but depend on using molecular techniques (Zhao *et al*., 2009; Bertaccini *et al*., 2022; Wei and Zhao, 2022), that enabled the phytoplasmas classification by simulating restriction enzyme digestions and electrophoresis to generate "virtual" RFLP patterns for submitted query sequences (Liu *et al*., 2017). Because some authors assign the 16Sr group number incorrectly due to the lack of a standard framework for group nomenclature (Zhao and Davis, 2016).

The IPhyClassifier program provided distinct tools for the identification and characterization of the phytoplasmas under research. Ornamental plants in some governorates of Egypt showed that phytoplasma is a high probability of belonging to aster yellow phytoplasma or belonging to witches broom phytoplasma in other governorates. Where, all of the samples obtained from Giza and Matrouh (EGY-SEAM2, EGY-SEAM4) were more appropriate for the aster yellows phytoplasma disease. Witches broom phytoplasma was identified as the right phytoplasma disease from all samples collected in Mansoura and Ismailia (EGY-SEAM1, EGY-SEAM3) utilizing analysis of phytoplasma sequences based on the 16S rRNA gene using the program (IPhyClassifier).

Hundreds of Phytoplasma isolates have been classified into 28 phytoplasma groups based on its diverse of 16SrRNA gene restriction fragment length polymorphism (RFLP). Moreover, iPhyClassifier was also specific for the subgroups of different phytoplasma isolates under study based on RFLP analysis previously classified. In addition to The F2nR2 regions of 524 phytoplasma 16S rRNA gene sequence accessions underwent in silico digestion using 17 restriction enzymes. Virtual RFLP analysis were sorted into 28 groups and around 100 subgroups. Therefore, three subgroups were identified in the present study. These subgroups include subgroups 16SrI-B (represented by Acc. no. OP730893), subgroup 16SrI-A (represented by Acc. no. OP723295), and subgroup 16SrII-D (represented by Acc. nos. OP723296 and OP723297).

Several phytoplasma isolates would be expected to share common epidemiological characteristics related-diseases (Bertaccini, 2007). Results from the current study revealed that the three sequences of Potato purple top phytoplasma, Rhododendron x hybridum' phytoplasma, and papaya yellow crinkle disease that originated from Russia, Czech Republic, and Australia (GenBank accession numbers EU333396.1, KC009838.1, and Y10097.1) were 99.8%, 99.6%, and 100%, respectively with the identified phytoplasma isolates (OP730893, OP723295, and OP723296 or OP723297) in this study. The Russian Potato purple top phytoplasma strain may therefore represent an epidemiological isolate (Leyva-López *et al*., 2002; Khadhair *et al*., 2003) and is also known as the purple top phytoplasma of the Columbia Basin potato (Crosslin *et al*., 2005; Munyaneza *et al*., 2006).

Furthermore, the findings demonstrated that the phytoplasma strain (OP723297) isolated from *Euphorbia milii* plants in Mansoura was 100% identical with Papaya yellow crinkle phytoplasma (Y10097.1), which is a member of the 16SrII-D subgroup and was formerly known as Ca. P. australasiae, as reported by White *et al.,* (1998). Recently, the Australian isolate (Y10097.1) was reported to affect papaya, tomato crop, and Echinacea pallida over the world (White *et al*., 1998; Pearce *et al*., 2011) as well as tomato, eggplant, and squash plants in the North of Egypt, Elsharkia, and Ismailia, or central Egypt, Minya, and Baniswef (Omar and Foissac, 2012). Unfortunately, phytoplasma, which belongs to the 16Sr II group, is widespread in Egypt up to Aswan and specifically infected the tomato crop (Behiry, 2018).

The presence of ornamental plants as reservoirs of phytoplasma in Egypt (Mokbel, 2020; Mokbel *et al*., 2020) could explain why disorders caused by phytoplasma diseases were uncontrolled to date. Moreover, the vegetative propagation of the infected cuttings by a phytoplasma (Caglayan *et al*., 2019) or their spread through infected seeds (Botti and Bertaccini, 2006), specifically the phytoplasma belonging to a group (I) or (II), which is one explanation for the spread of numerous phytoplasma diseases in Egypt. Specifically, the phytoplasma isolate (Y10097) has already been present in Egypt since 2012 (Omar and Foissac, 2012).

In conclusion, information about some ornamental plants in Egypt harboring phytoplasma was given. Egyptian phytoplasma isolates were verified to be identified as belonging to aster phytoplasma in Giza and Matrouh and witches broom phytoplasma in Mansoura and Ismailia. The four phytoplasmas were clustered into two groups (I) and (II), as well as three subgroups 16SrI-B (OP730893), 16SrI-A (OP723295), and 16SrII-D (OP723297 and OP723296). Subgroups are crucial for distinguishing closely related strains that have critical biological traits. Therefore, there is an urgent need for additional research on managing any possible spread of these phytoplasma isolates into other plants and verifying their identity. Needless to say that it is impossible to rely on identifying phytoplasma according to symptoms only that appear on plants only and the management of phytoplasma-related diseases is dependent upon the precise diagnosis of plant infections infected plants.

Competing Interests

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.

Author (s) contribution

All four authors discussed the results and the final version of the manuscript. All four authors collected plant samples from different Egyptian governorates. While the second and fourth authors contributed to the manufacturing samples and the last one performed the Phytoplasmas characterization. The corresponding author and third author performed the writing of the manuscript.

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