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Identification, Molecular Characterization of a Phytoplasma Affecting *Dodonaea viscosa* Plants and Determination of Some Biochemical Constituents in Leaves

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ABSTRACT

During June-2019, *Dodonaea viscosa* (L.) Jacq. plants showing typical symptoms of phytoplasma infection such as leaf yellowing, severe malformation, and crinkling were observed in the Agricultural experimental fields of Giza, Egypt. The causal agent of the yellows disease was identified based on symptoms, light microscopic observations, graft transmission, and sequencing of the 16S rRNA gene. Light microscopy of hand-cut sections treated with Dienes' stain showed blue areas in the phloem region of infected plants and the xylem stained turquoise. Phytoplasma was successfully graft-transmitted to *D. viscosa* and *Catharanthus roseus* plants. Nested-PCR assay was used for detecting the phytoplasma in the naturally infected plants as well as in the grafted plants with the causal agent, which gave the expected products of 1202 bp with the primer pair R16F2n/R16R2. The 16S rRNA gene sequence of the amplified region was submitted to the GenBank database (NCBI), followed by the accession number "MT940834", under Egy-SAM name, and shown to be identical (100%) to the 16S rRNA gene of phytoplasmas that isolated from *Vitis vinifera* (FJ824597), *Muscari armeniacum* (MN080270), and *Lactuca sativa* (KF573456), in Canada, Hungary, and the USA. The aster yellows phytoplasma group (16SrI) was associated with the *D. viscosa* plants. On the other hand, changes in the chemical contents of infected leaves of *D. viscosa* plants showed an increase in total flavonoid content with decreasing the content of total phytosterol.

Keywords: *Dodonaea viscosa,* Aster yellows phytoplasma, Dienes' stain, 16S rRNA gene, PCR, Sequencing, Flavonoids, Phytosterols.

Introduction

Dodonaea or hop bush (*Dodonaea viscosa* (L.) Jacq.) is a species of flowering plant in the *Sapindaceae* family. These shrubs have a global distribution in tropical, subtropical, and warm temperate regions of Africa, the Americas, Southern Asia, and Australasia (Khan *et al*., 2013; Baruch *et al*. 2017). Leaves of *D. viscosa* is traditionally used as a promising herbal drug to treat rheumatism, joint pain, toothache, and snake bites (Venkatesh *et al*., 2008; Hamadi, 2017; Abdela, 2019). The phytochemical investigation of ethanol extract of *D. viscosa* leaves revealed the presence of flavonoids and sterols (Veerapur *et al*., 2004; Al-Snafi, 2017). Veerapur *et al*. (2004) suggested that the antiulcer properties of the extract might attribute to the presence of phytochemicals like flavonoids. Also, flavonoids have been considered as a secondary reactive oxygen species (ROS) scavenging system to maintain redox homeostasis state in plants suffering damage to the photosynthetic system, due to the excess excitation energy (Lucarini and Pedulli, 2010; Fini *et al*., 2011; Treml and Smejkal, 2016). They also have a role in scavenging singlet oxygen ($^1O_2$) and alleviate the damages caused to the outer envelope of the chloroplast membrane (Agati *et al*., 2012). Phytosterol as plant-derived compounds that can decrease cholesterol levels related to the risk of cardiovascular disease or hardening of the arteries (De Smet *et al*., 2012).

Phytoplasmas are bacteria-like organisms, have very small genomes (680-1,600 kb) lacking major metabolic pathways for the synthesis of compounds needed for their survival and multiplication, and they are obligate parasites restricted to the phloem tissue of the host plant (Bertaccini *et al*., 2014). Yellows disease, mainly aster yellows (AY), is a widespread disease and affecting over 300 plant species, including vegetable and flower crops, caused by the aster yellows

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phytoplasma (AYP) in the 16SrI group (Lee *et al*., 2000 and IRPCM, 2004). The AYP is transmitted persistently by aster leafhopper, *Macrosteles quadrilineatus* belonging to the genus *Macrosteles* (Hemiptera: Cicadellidae) (Zheng-Nan *et al*., 2013). Due to their uncultivable characteristic *in vitro*, phytoplasma can study only in association with their plant hosts. Thus, grafting is the fastest and most effective method to obtain infected plants (Aryan *et al*., 2016), and periwinkle (*Catharanthus roseus*) is known as a source plant that can harbor many phytoplasmas (Choi *et al*., 2004).

Since phytoplasmas-advent during the 1960s, the 16S rRNA gene sequencing has represented a fundamental step for accurate phytoplasmas identification and essential information for their classification (Seemuller *et al*., 1998). The light microscopy technique, however, has been used successfully as a preliminary method for diagnosis to verify the presence of phytoplasma in symptomatic plants, so they constitute the faster and less expensive step to detect phytoplasma in plant tissue than molecular assay (Bertaccini *et al*., 2014). In all sections of the infected-phytoplasma plant treated with Dienes' stain, the phloem will mark with blue, and the xylem will be stained turquoise when observed under the light microscope (Musetti, 2013).

Phytoplasma diseases have been reported in *D. viscosa* in several countries, such as the USA (Borth *et al*., 1995 and 1999), China (Yu *et al*. 2016), Saudi Arabia (Omar, 2016), and Iraq (Al-Kuwaiti *et al*., 2019). Therefore, the objectives of this research are to (1) investigate the infection of *D. viscosa* plants with phytoplasma disease using the light microscope and molecular tools; (2) evaluate the differences in the content of biochemical products such as flavonoids and phytosterols of the infected *D. viscosa* leaf comparing with the healthy ones.

Materials and Methods

1. Plant materials

During June 2019, branches carry leaves were collected from symptomatic and asymptomatic *D. viscosa* shrubs grown in the experimental fields of Giza, Egypt. Most shrubs exhibit phytoplasma disease symptoms, such as leaf malformation and yellowing of the leaves. The plant identification was confirmed by Dr. Emad Shafik, Herbarium Section, Orman Garden, Giza, Egypt, and the description in accordance with Sasidharan (2004).

2. Light microscopy and Dienes' stain

Phytoplasma was preliminary detected in petiole tissues of the symptomatic plants, but not in the asymptomatic ones, using the Dienes stain method (Deeley *et al*., 1979). Samples were taken from petiole tissues of infected and healthy plants. Freehand cross-sections were prepared by razor blade edge, then stained with 0.2 % of Dienes' stain for 10 min. The stained sections were later washed in sterile distilled water, mounted on a glass slide, and examined using light microscope. Dienes' stain was prepared by dissolving 2.5 g of methylene blue, 1.25 g of azure II, 10.0 g of maltose, and 0.25 g of sodium carbonate in 100 ml distilled water.

3. Transmission of phytoplasma by graft inoculation

The lower end portion of infected *D. viscosa* scions (10 scions) with phytoplasma was cut into a tapered shape as well as a vertical side cut was made on the stem of the healthy rootstock plants (*D. viscosa* and *Catharanthus roseus* plants) using a sterilized blade (70% ethanol). In the greenhouse, the tapered cut end of the scions was inserted into the cut side surface of the rootstocks, and then the union between the donor and recipient plants tightly was joined together with Parafilm-M (Sigma-Aldrich). Symptoms of phytoplasma were monitored daily for eight consecutive weeks by visual inspection. Three healthy rootstock plants were served as negative controls. Some of the symptomatic *D. viscosa* and *Catharanthus roseus* leaves were collected from successful grafts as well as healthy leaves and used for DNA extraction. The remaining leaves of *D. viscosa* were dried for three weeks at room temperature and stored in airtight containers to determine the contents of flavonoids and phytosterols.

4. Molecular detection and characterization of the phytoplasma

4.1. DNA extraction

Total DNA was extracted from leaf samples (0.5 g) of naturally infected *D. viscosa* plants with phytoplasma and plants infected by grafting as well as healthy control ones, according to Dellaporta *et al*. (1983). Nucleic acid was precipitated by adding 2.5 volumes absolute ethanol, then collected by centrifugation, washed in 70% ethanol, dried, and re-suspended in 50 µL TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). The DNA was stored at -20°C until PCR assays.

4.2. PCR assays

Direct PCR assay was performed using the universal primer pair P1/P7 (Schneider *et al*., 1995). Three µL of extracted DNA was used in 25 µL standard PCR reaction mixture contained 10 pmol of each the primers forward primer (P1) and reverse primer (P7); 12.5 µL Mangotaq DNA polymerase (Bioline GmbH, Luckenwalde). PCR product was diluted 1:10 with nuclease-free water (Promega, USA), and used as a template for the nested PCR with a forward primer (R16F2n) and a reverse primer (R16R2) (Deng and Hiruki 1991). The reaction mixture with template DNA extracted from healthy plants was used as a negative control. The DNA amplification was performed in a thermocycler as follows: 94°C/30 s [94°C/60 s, 53°C/60 s, 72°C/120 s] × 35 cycles, 72°C/7 min. PCR products were stained with EZ View nucleic acid stain (Biomatik, USA), analyzed by electrophoresis in 1.0% agarose gel, and visualized by UV light.

4.3. Nucleotide sequencing

Nested-PCR product was purified from agarose gels using Gel/PCR DNA Fragments Extraction Kit (Geneaid, Taiwan). DNA samples were sequenced in both directions in an ABI 3730XL automated sequencer using nested primer pairs. The sequence was then compared with other eighteen-phytoplasma sequences in the GenBank database using the DNAMAN program version 7 (Lynnon Corporation, Canada).

5. Chemical analysis

The chemical analysis of dried leaves was carried out on the Regional center for mycology and biotechnology (RCMB), Al Azhar University, using a Spectrophotometer (MILTON ROY Spectronic 1201, USA). All chemicals and solvents used in this study were of analytical grade (Merck and Sigma Aldrich).

5.1. Total flavonoids content (TFC) determination

The TFC was determined spectrophotometrically at 510 nm against the blank, following the aluminum chloride ($AlCl_3$) colorimetric method (Zhishen *et al*., 1999) and expressed as mg quercetin equivalents (QE). Briefly, the dried leaf samples (2 g) were pulverized into coarse powder and subjected to hydro-alcoholic extraction using the soxhlet apparatus for 6 hours. The crude extracts were condensed by using a rotary evaporator under reduced pressure. The contents were dissolved in 20 ml methanol (80%). An aliquot (5 ml) of extracts was added into 10 ml volumetric flasks containing 4 ml of distilled water. To the flasks, 0.30 ml of 5% sodium nitrite solution ($NaNO_2$) was added, and after 5 min, 0.3 ml of 10% $AlCl_3$ was added. After 5 min, 2 ml of 1M NaOH was added and the volume was made up to 10 ml with distilled water.

5.2. Total phytosterols content (TPC) determination

The TPC was determined using a Liebermann-Buchard (LB) based method (Araujo *et al*., 2013). Briefly, the phytosterols standard (50 mg) was dissolved with chloroform in a 100 ml volumetric flask. The hydroethanolic extract was prepared by maceration the dried plant (1 g) for seven days, using ethanol 70% (v/v) as a solvent. Hydroethanolic extract (25 ml) was dried under reduced pressure at 40°C, then the residue was re-suspended in 20 ml of chloroform, and the volume adjusted to 50 ml with the same solvent. A portion of 2 ml extract was taken and mixed with 2 ml LB reagent. The absorptions were measured in a spectrophotometer at 625 nm after 5 min of the addition of the reagent LB. The chloroform was used as a blank. The LB reagent was prepared by dissolving 5 ml sulfuric acid in 50 ml acetic anhydride (Kenny, 1952). The TPC was calculated as beta-sitosterol (g %) using the following standard photometric formula (Kim and Goldberg, 1969) :

$$TPC = C_s \times A_{sm} / A_{st}$$

Where: C_s= Standard Concentration; A_{sm} = Absorbance of the sample; A_{st} =Absorbance of the standard.

Statistical analysis

Each analysis was performed in triplicate. The chemical analysis data were subjected to analysis of variance using the WASP 2.0 software package (Agri. Stat., ICAR). The Least Significant Difference (LSD) method was used to test the differences between means at a 5% level of probability.

Results

1. Plant identification

The plant identification carried out in Herbarium Section, Orman Garden, Giza, Egypt, and referred to as a *Dodonaea viscosa* plant (*Dodonaea viscosa* (L.) Jacq.), as shown in Fig. 1.

Fig. 1: Healthy *D. viscosa* (*Dodonaea viscosa* (L.) Jacq.) shrubs, leaves, flowers, and seeds.

2. Symptomatology of phytoplasma

Characteristic phytoplasma-symptoms on the collected *D. viscosa* plants from the experimental fields of the Agricultural Experimental Station, Giza, Egypt included yellowing and malformed crinkled leaves with variations in the size and shape of leaves (Fig. 2) as compared to the healthy ones (Fig. 1).

Fig. 2: Symptoms of naturally infected *D. viscosa* plants with AYP, included leaf yellowing (A) and variations in the size and shape of leaves with a curved shape (cupping shape) (B).

3. Light microscopy of Dienes' stain

Light microscopy of Dienes' staining method was used to detect phytoplasma in the infected tissues of *D. viscosa* plants. Phytoplasma was detected in the phloem tissues of the infected *D. viscosa* plant, but not in the healthy ones. The section from diseased tissues of phloem cells of petioles was stained blue, whereas, the xylem appeared as a turquoise (Fig. 3).

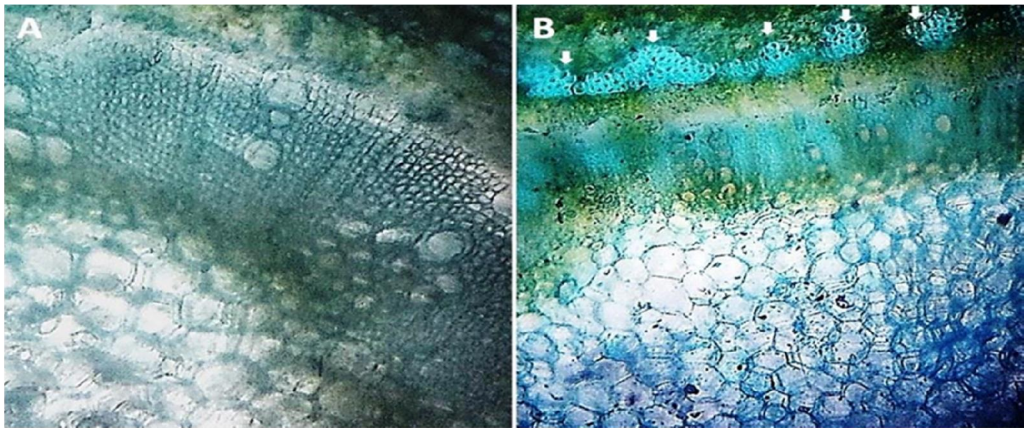

Fig. 3: Hand-cut sections of petioles of *D. viscosa* plant stained with Dienes' stain. A) Section of a healthy plant with unstained phloem and xylem tissues. B) Section of the infected phytoplasma-plant showing the phloem tissue is stained blue while the xylem tissue is turquoise. White arrows show the dark blue spots indicating phytoplasma presence.

4. Graft transmission

The grafting technique was used to transmit the phytoplasma from the naturally infected *D. viscosa* plants to healthy *D. viscosa* and *Catharanthus roseus* seedlings. Eight weeks after graft-inoculation, *D. viscosa* plants infected by phytoplasma showed leaf yellowing. Similarly, such symptoms have been observed in *Catharanthus roseus* (Fig. 4).

Fig. 4: Symptoms of yellows disease on leaves of *D. viscosa* (A) and *Catharanthus roseus* (B) plants following phytoplasma-transmission by grafting.

5. Molecular detection and characterization

Total DNA was successfully extracted from healthy and naturally infected *D. viscosa* plants as well as grafted plants with phytoplasma, then was used as a template for direct PCR using the universal primer pair P1/P7, and for nested PCR amplification using R16F2n/R16R2. Amplified products (1202 pb) were detected in all naturally infected samples of *D. viscosa* plants (Fig. 5) but not from the healthy ones using nested PCR (Fig. 5, Lane 4), which confirmed the phytoplasma infection. Amplified products (1202 pb) were also detected in both grafted *Catharanthus roseus* and *D. viscosa*

plants with phytoplasma (Fig. 6), which confirmed the readily of phytoplasma-transmission by grafting. No PCR product was obtained from the non-grafted sample of *D. viscosa* as a negative control (Fig. 6, Lane 6).

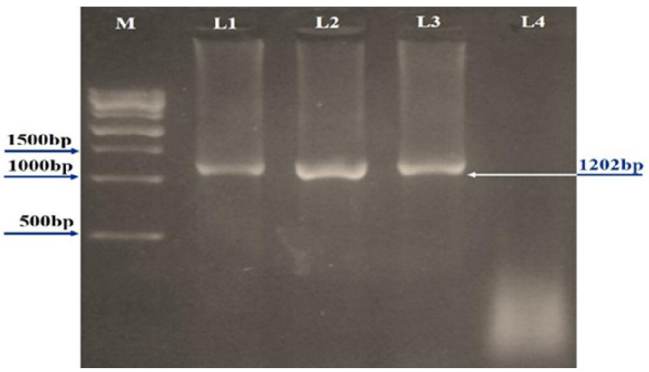

Fig. 5: Agarose gel electrophoresis (1%) of nested PCR products amplified from naturally infected *D. viscosa* plants with phytoplasma. L1-L3: Naturally infected *D. viscosa* plants that showed yellows disease symptoms, L4: Healthy *D. viscosa* plant. M: 1Kb DNA Ladder (Biomatic-USA).

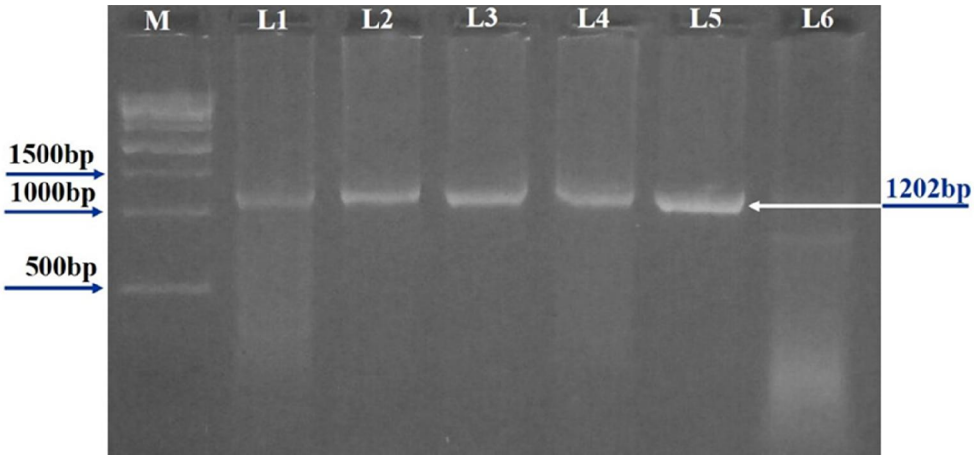

Fig. 6: Agarose gel electrophoresis (1%) of nested PCR products amplified from grafted plants with phytoplasma. Lanes 1 and 2: Samples of the grafted *Catharanthus roseus* plants with phytoplasma, Lanes 3 and 4: Samples of the grafted *D. viscosa* plants with phytoplasma. Lane 5: Positive control. Lane 6: Non-grafted sample of *D. viscosa* plant. M: 1Kb DNA Ladder (Biomatic-USA).

6. Sequence analysis

The nucleotide sequence for the phytoplasma detected from symptomatic *D. viscosa* plants was determined and compared with the corresponding sequences of the other 18 isolates of phytoplasmas available in the NCBI GenBank database (Fig. 7). The nucleotide sequence was submitted to the NCBI GenBank with accession number MT940834 as Egy-SAM isolate. Results of multiple sequence alignment and phylogenetic analyses showed that Egy-SAM-isolate has 100% similar to AY phytoplasmas and Lettuce yellows phytoplasma (BC-358, AJ14, and LetY-TX10) from Canada, Hungary, and the USA, respectively (ACC. Nos. FJ824597, MN080270, and KF573456). Also, the sequence results showed that the identity was about 99.5% with AYP (Cy-4) from Alexandria governorate, Egypt (ACC. No. MH985243), as well as 99% identity with *Candidatus* Sugarcane yellow leaf phytoplasma (Cane1) from Sharkia governorate, Egypt (ACC. No. JX157631). Additional comparisons showed that the nucleotide sequence from Egy-SAM-isolate has a similarity from 100 % to phytoplasmas belonging to group 16SrI (Table 1).

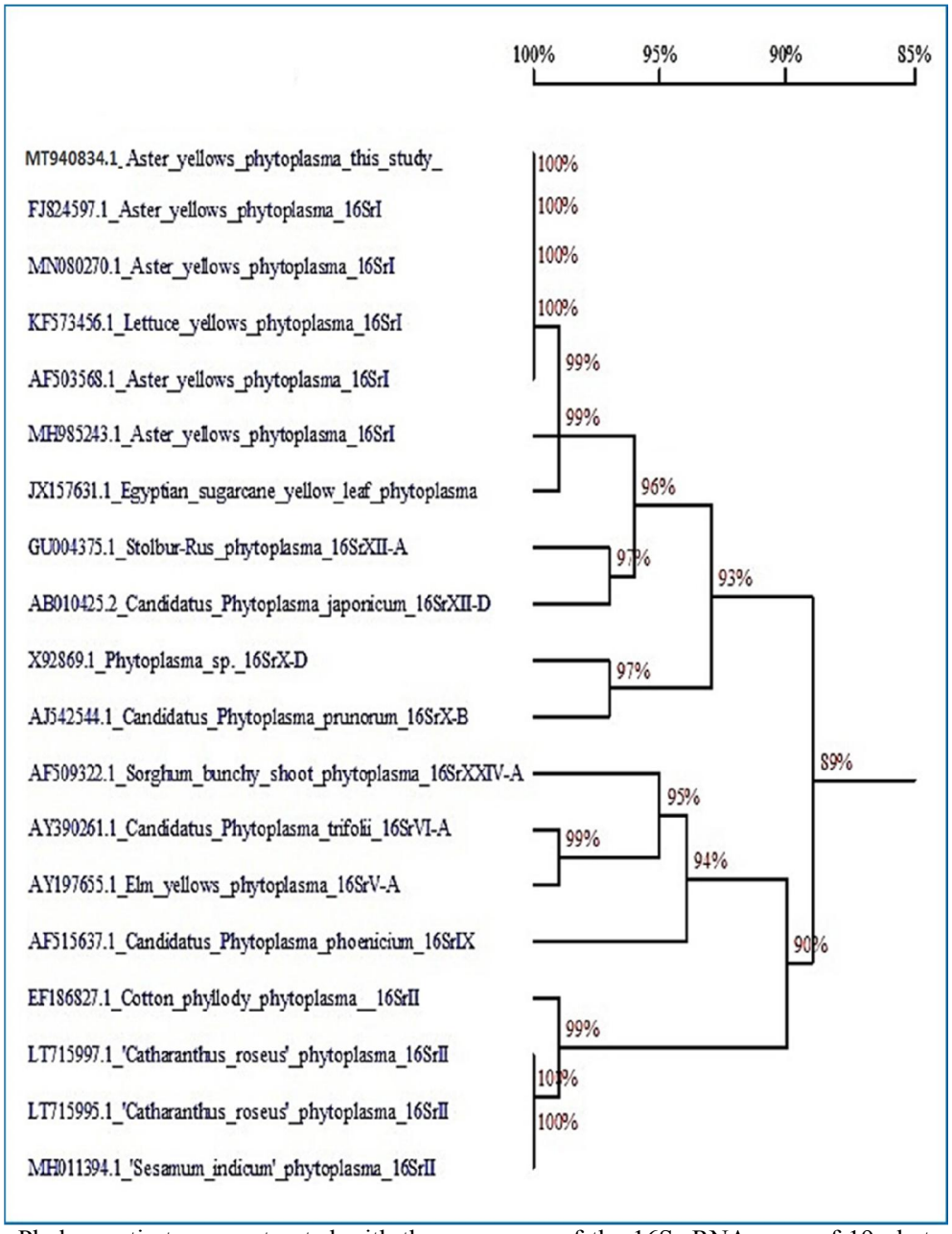

Fig. 7: Phylogenetic tree constructed with the sequences of the 16S rRNA gene of 19 phytoplasma isolates from different geographical regions belonging to different groups available in the GenBank and Egy-SAM-isolate in the present study (MT940834) using DNAMAN ver.7.

Table 1: Comparison of the nucleotide sequences of the 16S rRNA gene isolated in the present study (Acc. No. MT940834) with the corresponding sequences of phytoplasma isolates from Egypt and other countries.

Strain	Associated disease	Accession number	Geographical origin	Host plant	Group	% Id[2]
Egy-SAM	AY	MT940834	Egypt	*Dodonaea viscosa*	16SrI	100
BC-358	AY	FJ824597	Canada	*Vitis vinifera*	16SrI	100
AJ14	AY	MN080270	Hungary	*Muscari armeniacum*	16SrI	100
LetY-TX10	AY	KFS73456	USA	*Lactuca sativa*	16SrI	100
Cy-4	AY	MH985243	Egypt	*Cycas revoluta*	16SrI	99.5
1-P	AY	AFS03568	Croatia	*Populus nigra*	16SrI	99.5
Chanel3	AY	JXIS7631	Egypt	*Saccharum officinarum*	16SrI	99.0
COP	Phyllody	EF186827	Burkina Faso	*Gossypium Spp.*	16SrII	89.0
Per Kafr 1	WB5	LT715995	Egypt	*Catharanthus roseus*	16SrII	88.8
Sharkia-IID	Phyllody	MH011394	Egypt	*Sesamum indicum*	16SrII	88.8
Per-Kafr 3	Virescence	LT715997	Egypt	*Catharanthus roseus*	16SrII	88.6
EY1	Yellows	AY197655	USA	*Ulmus americana*	16SrV	90.0
CP	Proliferation	AY390261	Canada	*Trifolium hybridum*	16SrVI	89.5
21	Lethal yellows	AF515637	Iran	*Prunus dulcis*	16SrIX	88.3
ESFY-GI	ESFY[6]	AJ542544	Germany	*Prunus persica*	16SrX	93.5
SPAR	WB	X92869	Italy	*Spartium junceum*	16SrX	92.8
Ru93	Stolbur	GU004375	Russia	*Vitis vinifera*	16SrXII	96.5
JHP3	Phyllody	ABO 10425	Japan	*Hydrangea Spp*	16SrIX	95.8
NA4	Bunchy shoot	AFS09322	Australia	*Sorghum stipoideum*	16SrXXIV	89.3

Id[1]: Identity and the highest identity value indicated in bold. References[2] related to strain names (SPAR, JHP, Cane1, Per-Kafr 1, and 3) are Marcone *et al*. (1996), Sawayanagi *et al*. (1999), El Sayed and Boulila, (2014), and El-Sisi *et al*. (2017), respectively. Abbreviations: NA[3]: Not available; WB[4]: Witches' broom; ESFY[5]: European stone fruit yellows.

Effect of infection with phytoplasma on TFC and TPC

Illustrated results in Table 2 indicate a great effect of phytoplasma infection on some chemical constituents of infected *D. viscosa* leaves by grafting as compared with non-grafted plants. It was found that the TFC was significantly increased (111.2%). In contrast, the TPC was significantly decreased (37.9%).

Table 2: Total flavonoids content (mg /g dry wt.), and total phytosterols content (g %) in leaves of non-grafted (healthy controls) and grafted *D. viscosa* plants infected by phytoplasma.

Parameter	Healthy	Infected	Percentage of change (%)	LSD at 0.05
TFC	19.476	41.146	+ 21.67 (111.2)	1.576
TPC	4.203	2.610	- 1.593 (37.90)	0.253

Data are means of three independent measurements (n=3). **TFC:** Total flavonoids content; **TPC:** Total phytosterols content.

Discussion

This study aimed firstly to detect and characterize the causal agent of aster yellows (AY) disease on *Dodonaea viscosa* plants that showed leaf yellowing and malformation, or crinkling symptoms in Egypt. Aster yellows phytoplasma (AYP) was detected and identified in symptomatic samples using the Dienes' staining method along with light microscopy, phytoplasma-transmission by grafting, PCR-based assays using phytoplasma universal primers (P1/P7) followed by nested primers (R16F2n/R16R2), and 16S rRNA sequence identity. These symptoms are similar to those described for AY disease on *D. viscosa* or *Nasturtium officinale* (Borth *et al*., 1995; 1999; 2006). The diagnosis using the Dienes' staining method and light microscopy provided preliminary evidence indicating a possible infection by a phytoplasma in the infected *D. viscosa* tissues, which agreed with Musetti and Favali (2004) and Musetti (2013). Moreover, the possibility of phytoplasma transmission by grafting infected plant parts onto healthy plants provided evidence that phytoplasma is the causative agent of AY disease; the technique is similar to those described by Aryan *et al*. (2016) and Ahmed *et al.* (2016). Furthermore, PCR analysis of the nested PCR products (1202 bp) performed using the grafted *Catharanthus roseus* samples proved the accuracy of transmission by grafting. The sequence obtained

in this study (acc. no. MT940834) was compared with those of known phytoplasmas in the database (NCBI) and was found to be identical (100%) to the members of the 16SrI AY group, that contain phytoplasmas associated with grapevine yellows from Canada (Olivier *et al*., 2009), *M. armeniacum* yellows from Hungary, and lettuce yellows from the USA. The 16S rRNA gene sequence of the amplified region also shared 99.5% - 99% identity with the 16S rRNA gene of other isolates, such as AYP (Cy-4-isolate, MH985243) and Sugarcane yellow leaf phytoplasma (Cane1-isolate, JX157631) from Egypt (Alexandria and Sharkia governorates, respectively). Therefore, the results of sequence analysis confirmed that the AY disease, which occurred in *D. viscosa* plants in Egypt, is associated with AY-related phytoplasma in the 16SrI group. It is worth noting that, this disease affected *D. viscosa* plants and several host plants on all the major Hawaiian islands (Borth *et al*., 1995 and 1999), resulting in the emergence of new epidemics in other susceptible vegetable crops. Considering that, the aster leafhopper (*M. quadrilineatus*) as the main vector of AYP (Zheng-Nan *et al*., 2013); the potato leafhopper (*Empoasca fabae*) may be a potential vector for AYP (Stillson and Szendrei, 2020). Additional molecular survey studies are therefore required to investigate phytoplasma in the vegetable crops in Egypt since the *D. viscosa* plant became a reservoir of AYP in nature, from which AYP might spread in the future by different insect vectors.

On the other side, the active plant constituents are usually the secondary metabolites, derived from biosynthetic pathways present within the plant tissue. The pharmacological investigations carried out on *D. viscosa* plants proved the therapeutic potentials of this plant in the treatment of numerous diseases (Shafek *et al*., 2015 and Al-Snafi, 2017). The aerial parts of *D. viscosa* afforded many compounds such as flavonoids, tannins, sterols, and saponins (Rojas *et al*., 1996 and Getie *et al*., 2003), in particular, flavonoids showed promising antiulcer activity (Veerapur *et al*., 2004 and Venkatesh *et al*., 2008). Phytosterols derived from the plants remain the basis for a large proportion of the commercial medications used today for the treatment of a wide range of cardiovascular diseases and hardening of the arteries (De Smet *et al*., 2012). However, phytoplasma diseases associated with deformed leaves are a critical problem for *D. viscosa* plants. Hence as a second aim in this study to determine the biochemical changes that cause by AYP in *D. viscosa* plants; infected leaves using graft inoculation technique (8-week-old seedlings, PCR positive), as well as healthy leaves (PCR negative), were selected as materials for chemical analysis and comparison. Although effects of the AYP on the secondary metabolites of *D. viscosa* leaves are rare, flavonoids and phytosterols were determined. An examination of the content of both flavonoids and phytosterols revealed a difference between diseased and healthy plants. Grafting of the healthy plants with AYP caused decreasing the content of phytosterol by 37.9%, in contrast to the flavonoids content, which was significantly increased (111%) in infected plants compared with healthy ones. Since phytoplasma has a small-genome, they lost the genes for numerous biosynthetic pathways. Phytoplasmas lack the gene sets for the sterol biosynthesis, tricarboxylic acid cycle, phosphotransferase system, nucleotide de novo synthesis, and amino acid synthesis; thus, they must depend entirely on their host cells to supply them with the products of these pathways (Oshima *et al*., 2004; Bai *et al*., 2006). Also, several experimental data confirmed that there were profound taxonomically relevant properties of different phytoplasma strains connected with the nutritional requirement for sterol (Martini *et al*., 2014). The genomic features and the taxonomical properties imply that phytoplasmas may highly dependent on the sterol biosynthesis pathway, which may explain the reduced content of the plant phytosterols.

Besides, the best-described property of flavonoids is their capacity to act as antioxidants protecting the plants against insect predation and defense against pathogens, and action as sunscreens to absorb UV radiation and strong light (Mouradov and Spangenberg, 2014). Also, the reactive oxygen species (ROS) scavenging mechanism is adopted as one of such properties as demonstrated in the studies of Fini *et al*. (2011) and Agati *et al*. (2012). They reported that flavonoids could use as a direct secondary ROS-scavenging system, in which these compounds could easily donate a hydrogen atom and return to phenoxyl radicals, as the first step. Then, these radicals could react with other free radicals and form a stable quinone structure (Lucarini and Pedulli, 2010; Treml and Smejkal, 2016), thus, the increase of these compounds may be enhanced the *D. viscosa* plant defense system against AYP. Since levels of phytosterols and flavonoids were only measured, further investigation is needed to determine whether other secondary metabolites are affected by phytoplasma infection in *D. viscosa*.

Conclusion

Aster yellows phytoplasma (AYP)-isolate (Egy-SAM, MT940834) in 16Sr group I, was reported in *Dodonaea viscosa* plants grown in the experimental fields of the Agricultural Experimental Station, Giza, Egypt exhibiting yellowing symptoms and leaf malformation. There is no evidence to infer that AYP could have been introduced through imported plants or through planting infected seedlings since there are no reports of infecting *D. viscosa* with AYP elsewhere in Egypt. However, the 16S rRNA gene sequence of the amplified region in this study shared 99.5% and 99% identity with the 16S rRNA gene of AYP and Sugarcane yellow leaf phytoplasmas (such as Cy-4-isolate and Cane1-isolate) infecting both *Cycas revoluta* and *Saccharum officinarum* plants from Alexandria and Sharkia governorates in Egypt. This finding has a significant phytosanitary impact and serious threat to other economic plant species growing nearby. Grafting of the healthy *D. viscosa* plants with AYP caused decreasing in the content of some natural (medicinal) compounds such as phytosterol, whereas increased the total content of flavonoids in the leaves.

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