Molecular characterization of phytoplasma associated with sesame plants in Egypt

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ABSTRACT

The significant loss of sesame harvests in Egypt in recent years can be attributed to phytoplasmas, a class of cell wall-less plant pathogenic bacteria (Mollicutes) that can infect sesame crops. Therefore, before developing and implementing control strategies, it is necessary to characterize the pathogen population. During the 2021 and 2022 growing seasons, samples of sesame leaves displaying phytoplasma symptoms were gathered from six governorate farms. By employing the universal primer pairs P1/P7 and R16F2n/R16R2, nested polymerase chain reaction (PCR) experiments effectively identified phytoplasma, and the resultant product of about 1800 bp was amplified. Its infection rate was the highest in Giza, at 29% and 25%, respectively, and the lowest in Ismailia, at 16.2% and 15.7%, respectively. Phylogenetic analysis, sequencing restriction profiling, and nested polymerase chain reaction (PCR) tests based molecular techniques demonstrated that our Egyptian isolate, accession number OR447565.1, is closely related to the 16SrII (Peanut WB group) and that (98.86%) is very closely identified with isolates of MN565885.1 and OP793488.1 from Iran and India, respectively, which belong to the same subgroup. In addition, the comparison showed that the Egyptian isolate (OR447565.1) shared 16SrVI (Clover proliferation group). A discernible degradation in the tissue and cell ultrastructure of phytoplasma-infected plants was found by Transmission Electron microscopy (TEM) analysis. Pleomorphic phytoplasma units were seen. Attached to the sieve elements of plasma membrane.

Keywords: Sesame; phytoplasma; 16SII; TEM.

INTRODUCTION

One of the first plants ever produced on the globe is sesame (Sesamum indicum L.). According to (Uzun *et al*. 2008; and Moazzami *et al*. 2006) sesame seeds are an abundant source of protein (20%), edible oil (50%), oleic acid (47%), and linolenic acid (39%). Cultivated sesame in Egypt is a diploid species with 2n= 2x= 26 chromosomes that belongs to the Pedaliaceae family and is the most cultivated edible oil crop species out of over 30 species of this *Sesamum* genus (Kobayashi *et al*. 1990), the primary places of production are Sudan, Paraguay, Nigeria, Tanzania, Uganda, Ethiopia, India, China, Myanmar, Pakistan, Niger, Turkey, and Thailand. Since most of the seeds are eaten raw, sesame is regarded as a food crop in Egypt as opposed to an oilseed crop. It is the most widely planted oil crop in the Ismailia Governorate and is grown in several governorates (El-Bramawy, 2006). 34,000 hectares were under cultivation in Egypt, yielding 44,000 tons of product (FAOSTAT, 2021).

Phytoplasma poses a major threat to the success of sesame production worldwide. The sesame crop is highly susceptible to phytoplasma infection (Singh *et al*. 2016). Phytoplasma disease is a significant disease that may cause up to 100% crop loss and appears to harm plants either partially or totally (Sahambi 1970; Salehi 1992). Phytoplasmas are gram-positive (low-G+C) bacteria of the Mollicutes family that do not have single-membrane cell walls (Weisburg *et al*., 1989; Woese, 1987; Hogenhout *et al*., 2008). According to (Doi *et al*. 1967) phytoplasmas are phloem-limited and cannot be cultivated outside of their hosts in a cell-free synthetic culture medium. Over a thousand plants species, including several agriculturally important crop species such as fruits, vegetables, cereals, trees, and legumes, have been reported to be infected with phytoplasma worldwide (Sridhar *et al*. 2013; Iftikhar 2011; Šeruga *et al*. 2003). Per Doi *et al*. (1967) the genomic size of phytoplasmas varies from 530 to 1350 kb. The typical phytoplasma has a diameter of less than 1 μm and a pleomorphic or filamentous form. Because phytoplasmas are prokaryotes, their DNA is dispersed throughout the cytoplasm as opposed to being localized in the nucleus. Many economically significant crops are susceptible to phytoplasma infection, which can be lethal to plants and cause enormous global losses in

agricultural output (Seemuller *et al*. 2002; Bertaccini 2007; Oshima *et al*. 2013). This work aims to study the genetic diversity in the groups of phytoplasma, which infected the cultivate sesame plants d in Egypt.

MATERIALS AND METHODS.

Source of samples and filed inspection.

Sesame plants used in the present work were collected from different locations in Egypt, known as the main production area of sesame in Egypt. Plant samples showing symptoms and symptomless were collected from six governorates (Giza, Beni Suef, Faiyum, Sharqiyya, Beheira, and Ismailia) during the spring and early summer of two successive seasons (2021/2022, and 2022/2023). 1127 samples were collected from different localities of cultivated sesame (Giza32, Chadwell3, Toshka1, and Sohage1) plants carrying different external symptoms like that caused by Phytoplasma and tested using nested-PCR.

Extraction of Total DNA.

Using a modified Dellaporta extraction technique, the total nucleic acids were extracted from fresh samples of identified sesame infected with phytoplasma (Dellaporta *et al*. 1983; Rojas *et al*. 1993).

Polymerase Chain Reaction (PCR).

The PCR template was created using DNA extracted from symptomatic and asymptomatic plants. The PCR reaction mixture of 25 µl contained (3 µl) extracted DNA, 0.5 µl of each primer and 15.8 µl ddH2O, 5 µl 5x MyTaq Reaction Buffer, and 0.2 µl Taq5000tmDNA polymerase, (STRATAGENE). The first PCR using P1/ P7 universal primer was initial denaturation for 3 min at 94°C, 35 cycles of the following steps: denaturation for 1 min at 94°C, annealing for 2 min at 53°C, extension for 2 min at 72°C and final extension for 5 min at 72°C. The second (nested and heminested) amplification consisted of 30 cycles of the following steps: denaturation for 1 min at 94°C; annealing for 1 min at 48°C (R16F2n/R16R2) According to (Youssef *et al*. 2017; Smart *et al*. 1996).

Electrophoresis analysis.

The PCR product was electrophoresed in 1% agarose gel in -0.5x TBE buffer (89 mM Tris-HCl, 89 mM boric acid, 2.5 mM EDTA, pH 8.5) at 120 volt. 100bp DNA molecular weight markers (ABgene, UK) for 1 hour and stained with ethidium bromide (0.5 µl/ml) Sambrook *et al*. (1989). The fragments were photographed using UV lamp in gel documentation (Bio Rad, Gel Doc XR system 170-8170).

Sequence and analysis.

The DNA fragments were sent to Macrogen Inc. (Seoul, Korea) for sequencing. In our investigation, we initiated a BLAST search employing the Blast algorithm, adhering to default parameter settings. This included utilizing the widely recognized Nucleotide collection database (nr/nt) and configuring the search parameters to optimize and identifying highly similar sequences, known as the "megablast" option. After retrieving relevant hits, the sequences were extracted and subsequently subjected to multiple sequence alignment (MSA). The MSA process was conducted using the MAFFT algorithm, a well-established tool for the alignment of multiple sequences, ensuring accurate alignment of our dataset.

Phytoplasma morphological.

Light and electron microscopy were carried out to study the anatomical and ultrastructural changes induced in tissue and cell components by phytoplasma infection.

Light Microscopy.

The stem and leaf midribs of infected and healthy sesame plants were killed and fixed for at least 48 h in formalin glacial acetic acid, before being dehydrated. They were then serially sectioned by a rotary microtome at 20 µ thickness, and finally double-stained with crystal violet and erythrosine, cleared in carbol xylene, and mounted in Canada balsam, (Nassar and El-Sahhar 1998).

Electron microscopy ultrastructure examination.

The control leaf samples, and all treatments were soaked in phosphate buffer than post-fixed in potassium permanganate solution for five minutes at room temperature, and then put in 3% glutaraldehyde for TEM analysis. The leaf samples were dehydrated for 15 minutes in a series of ethanol concentrations ranging from 10% to 90% and for 30 minutes in absolute ethanol. The samples were pierced using a graded series of acetone and epoxy resin, and then pure resin. Very tiny pieces were adhered to copper grids. After then, fragments were twice stained: first with uranyl acetate and again with lead citrate. At the RCMB, Al-Azhar University, stained pieces were inspected using a TEM (JEOL-JEM 1010) operating at 70 kV. (Yaseen and Amin 2021).

RESULTS AND DISCUSSION.

Source of samples and field inspection.

In various Egyptian governorates, a phytoplasma survey related to sesame plant disease was carried out in the growing seasons of 2021 and 2022. The samples obtained from the field showed symptoms of phytoplasma disease. Phytoplasma causes a wide range of symptoms on sesame plants from stunting, yellowing, witches' broom, phyllody, virescence, proliferation, shoots showing internodes with dense leaves and shoots showing green leaf-like floral organs with virescence. Many adventitious shoots with short internodes looking like aster-yellow symptoms were also observed Fig1.

Phytoplasma, which varied from location to region and from season to season, was found and identified in every studied governorate. Using the universal primer pairs P1/P7, collected samples were analyzed for 16S-23SrRNA. In samples of diseased sesame plants, phytoplasma was regularly found. In the first year, the governorate of Giza had the highest infection rate (25%), while the governorate of Ismailia had the lowest infection rate (15.7%) the second years infection rates were as follows: Giza Governorate had the greatest infection rate (29%), while Beheira Governorate had the lowest infection rate (16.4%). As shown in table 1.

Diseases caused by phytoplasmas are a serious production hindrance for commercially significant oilseeds, vegetables, fruit commodities, decorative plants, lumber, and shade trees. Their prevalence is rising daily due to illnesses with a wide geographic distribution, and novel symptomatology in recent years. According to the survey results, phytoplasma is widely distributed throughout Egypt's sesame-growing areas. But infection rates varied from one place to another site. The symptoms shown by sesame plants infected with phytoplasma are diverse, as per our research. These symptoms have been described by (El-Banna *et al*. 2013; and Youssef *et al*. 2018) in Egypt and other many countries.

The results obtained are consistent with the findings of (Venkataravanappa *et al*. 2017) who reported a significant incidence (35–50%) of sesame phytoplasma in the northeastern regions of Uttar Pradesh, India, which resulted in a crop loss in terms of revenue. PCR was used to confirm the presence of Phytoplasma infection in sesame plants. Universal primers for 16s rRNA (R16F2n/R16R2) and SecY gene (SecYF2 and SecYR1) were used for this purpose amplified SecY gene and 16s rRNA. The obtained results agree with (Singh *et al*. 2016) research on phyllody-affected sesame plants that were gathered from 21 different agroclimatic zones in India. Nine states saw a 40-100% incidence of infection and losses in sesame crop production. Sesame phyllody was seen in the experimental fields at a somewhat lower incidence of 5-10%. This contrasts with the findings of (Singh *et al*. 2018) investigation, which revealed that just 8% of India's sesame crops had no leaf or phyllody symptoms. On sesame fields, phytoplasma is thought to cause witches' broom symptoms; the incidence varies from 5 to 12% depending on the crop by using P1/P7 and R16F2n/R16R2.

Molecular detection of phytoplasma based on nucleic acid by PCR technique.

A modified Dellaporta extraction technique was used to extract total DNA from the leaves and flowers of infected sesame plants (Dellaporta *et al*. 1983). Accurate illness diagnosis depends on the detection and identification of phytoplasmas using the nucleic acid amplification approach. Compared to biological criteria that have been utilized for phytoplasma identification for a long time, molecular diagnostic approaches have shown to be more accurate and reliable in detecting phytoplasma introduced within the past two decades. The most effective method for finding phytoplasmas in their plant and insect hosts is polymerase chain reaction, or PCR. Nested PCR is one of the most widely used techniques for phytoplasma identification and characterization. The initial PCR detection was performed using universal phytoplasma primer P1/P7. All symptomatic samples gave positive results with a product size of approximately 1800 bp, while the healthy plants gave no results. Fig 2.

In this work, phytoplasma was effectively detected in all examined sesame plants using nested PCR with degenerate primer P1/P7, even in cases where the phytoplasma had low titers. Amplification of a 1.8 kb DNA fragment, visible on an agarose gel, proved the presence of phytoplasma. Utilizing universal primer pairs to amplify 16S-23SrRNA resulted in DNA bands characteristic of phytoplasmas. Our results were in agreement with those obtained (Deng *et al*. 1991; Lorenz *et al*. 1995; Samrat *et al*. 1996; Seemuller *et al*. 1998; Yossef *et al*. 2018; Ahmed *et al*. 2022). These used PCR primers P1/P7 and the result was 1.8 kb bands in gel electrophoresis. Screening of variations for the presence of phytoplasma is possible

since the PCR approach makes it easy to discriminate between plant and phytoplasma. Also, our results agreed with (Gundersen and Lee, 1996). When DNA from asymptomatic plants was used as a template for PCR, no amplification was seen.

Illustration of the typical symptoms of phytoplasma morphological structures using light and TEM microscopes.

Light microscope.

Light microscopy was used to determine whether phytoplasma infection had a significant impact on the morphological structure of sesame leaves, as shown in Fig 3. The mesophyll layer was compacted in infected plants when compared with healthy ones. It was observed also that the size of spongy cells was small characterized by large intracellular space in some parts of the section, and the cells became compacted in comparison with tissues of healthy plants. The regular palisade cells were observed in some parts of the healthy section while irregular palisade cells were observed in infected one. Moreover, they were separated and decreased in the height of the diseased than in the healthy one. The mesophyll layer was larger in infected leaves tissues compared with healthy ones, the spongy tissue was more compacted and characterized with large intracellular spaces in comparison with the healthy control.

Comparing the leaf tissues of sesame plants infected with phytoplasma to healthy plants reveals a variety of morphological alterations brought on by the infection. Among these alterations were irregularly spongy and palisade tissues. The results agree with (El-Banna and El-Deeb 2007; Randall *et al*. 2011; Mokbel, and El-Attar 2016; Ahmed e*t al*. 2022) who found that considerable differences between healthy and malformed samples (leaf blade, leaf petiole, and stem) and the most important changes are disorganization of phloem cells that accompanied by an increase in cell wall thickness, middle lamella, and size of spaces between cells, This could be caused by the quantity of starch and sugars building up as a result of phloem malformation, which has a notable impact on the translocation of molecules involved in photosynthesis. Further (Esau, 1977) reported that infection with phytoplasma led to an anatomical alteration in spinach phloem tissues such as phloem degeneration and necrosis of sieve tubes as well as abnormal cell proliferation. Similar results were obtained by (Salehi and Izadpanah 1992; Akhtar *et al*. 2009).

Transmission Electron Microscopy.

TEM was used to find phytoplasma in the sieve elements of infected phloem tissue in ultrathin sections of healthy and diseased sesame leaves. Phytoplasmas are spherical to filamentous pleomorphic bodies that are bordered by cell membranes without a cell wall. In contrast to the absence of phytoplasma bodies in healthy plants. Phytoplasma bodies were observed and present in dense amounts in various filamentous and circular shapes Fig 4. Pleomorphic phytoplasma units were detected by TEM investigation of the infected sesame plants. These units are connected to the sieve elements' plasma membrane and develop and divide by drawing energy from the sterols present in the cell membrane (Christensen *et al*. 2005; and Mou *et al*. 2013). Different numbers of phytoplasma units were discovered in phloem cells, and this has been connected to the severity of symptoms according to (Kesumawati *et al*. 2006; El-Banna *et al*. 2015). However (Kaminiska *et al*. 2001) could not discover a relationship between the number of phytoplasma units and the intensity of rose phyllody symptoms.

The results obtained agree with (Maust *et al*, 2003; El-Banna *et al*. 2015; Youssef *et al*. 2017; Ahmed *et al*. 2022) who mentioned that Phytoplasma infection caused various ultrastructural changes. These ultrastructural alterations brought on by phytoplasma infection might be linked to the buildup of sugars and starches, which interfere with the operation of sieve tubes and phloem movement. The functioning of the chloroplast is negatively impacted by these unfavorable alterations, which limit the photosynthetic process and lead to aberrant glucose buildup in the infected plants. Like the findings of our investigation, ultrastructural alterations brought on by phytoplasma infection have been documented by (Santi *et al*. 2013; Buxa *et al*. 2015).

Sequence Analysis.

Phylogenetic analysis was performed to compare our Egyptian isolate (deposited in the National Center for Biotechnology Information (NCBI) GenBank under an OR447565.1 accession number) with the sequences of other phytoplasma strains in the GenBank database (NCBI, Bethesda, MD, USA). The results indicate that our isolate was a member of the group 16SrII (Peanut WB group), and it very closely identified (98.86%) with isolates of MN565885.1 and OP793488.1 from Iran and India, respectively, which belong to the same

subgroup. In addition, the comparison showed that the Egyptian isolate (OR447565.1) shared 16SrVI (Clover proliferation group), Fig 5.

According to (Kakizawa and Yoneda, 2015) 16S rDNA is highly helpful in classifying phytoplasma and subgroups and identifying genetic diversity, both of which are essential steps in phytoplasma research. Nucleotide sequencing identified the phytoplasma groups in our investigation, and phylogenetic analysis verified that our phytoplasma isolates belonged to the 16SrII group. The results agreed with (Omar and Foissac, 2012) Who discovered Candidatus phytoplasma from group 16SrII on eggplant, tomato, and squash plants in Egypt, (El-Sisi *et al*. 2017) who discovered it on periwinkle, onion, and cactus plants; and (Gad *et al*. 2019) discovered it on gazania.

CONCLUSION.

The infection caused by phytoplasma causes significant losses in the production of sesame crops. Our Egyptian isolate (accession number OR447565.1) is categorized as a member of the 16SrII group, and phylogenetic research has validated the usefulness of phytoplasma identification by nested PCR experiments. The impacted sesame crops, which were gathered from many Egyptian governorates, included a variety of phytoplasma types. A range of morphological and ultrastructural changes were seen in the tissue and cellular components of sesame plants infected with phytoplasma. To safeguard sesame plants from potential phytoplasma infections in the future and boost their yield, we advise conducting more research to generate genotypes resistant to phytoplasma. Gaining more insight into their adaptable processes and phytoplasma infection response can help to achieve this.

REFERENCES

Ahmed, E.A., Farrag, A.A., Kheder, A.A., Shaaban, A., 2022. Effect of Phytoplasma Associated with Sesame Phyllody on Ultrastructural Modification, Physio-Biochemical Traits, Productivity and Oil Quality. *Plants, 11(4), 477.*

Akhtar, K.P., Sarwar, G., Dickinson, M., Ahmad, M., Haq, M.A., Hameed, S., Iqbal, M.J., 2009. Sesame phyllody disease: its symptomatology, etiology, and transmission in Pakistan. *Turk J Agric For, 33(5), 477-486*.

Buxa, S.V., Degola, F., Polizzotto, R., De Marco, F., Loschi, A., Kogel, K.H., Musetti, R., 2015. Phytoplasma infection in tomato is associated with re-organization of plasma membrane, ER stacks, and actin filaments in sieve elements. *Front. Plant Sci*, *6*, 650.

Cagirgan, M.I., Mbaye, N., Silme, R.S., Ouedraogo, N., Topuz, H., 2013. The impact of climate variability on occurrence of sesame phyllody and symptomatology of the disease in a Mediterranean environment. *Turk. J. Field Crops, 18(1),* 101-108.

Christensen, N.M., Axelsen, K.B., Nicolaisen, M., Schulz, A., 2005. Phytoplasmas and their interactions with hosts. *Trends Plant Sci, 10(11), 526-535.*

Dellaporta, S.L., Wood, J., Hicks, J.B., 1983. A plant DNA minipreparation: version II. Plant Mol. Biol. Rep., 1, 19-21.

Deng, S., Hiruki, C., 1991. Amplification of 16S rRNA genes from culturable and nonculturable mollicutes. *J. Microbiol. Methods*, *14*(1), 53-61.

Doi, Y.O.J.I., Teranaka, M., Yora, K., Asuyama, H., 1967. Mycoplasma-or PLT group-like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows, or paulownia witches' broom. *J.J.P, 33(4), 259-266.*

El-Banna, O.H.M., El-Deeb, S.H., 2007. Phytoplasma associated with mango malformation disease in Egypt. *J Phytopathol*, *35*(2), 141-153.

El-Banna, O.H.M., Toima, N.I., Youssef, S.A., Shalaby, A.A., 2015. Molecular and electron microscope evidence for an association of phytoplasma with Citrus Witches Broom disease. *Int. J. Sci. Eng. Res, 6, 127-133.*

El-Banna, O.H., Mikhail, M., El-Attar, A., Aljamali, A., 2013. Molecular and electron microscope evidence for an association of phytoplasma with sesame phyllody in Egypt. *E.J.P*, *41*(2), 1-14.

El-Bramawy, M.A., 2006. Inheritance of resistance to Fusarium wilt in some sesame crosses under field conditions. *Plant Prot. Sci, 42(3), 99.*

El-Sisi, Y., Omar, A.F., Sidaros, S.A., ElSharkawy, M.M., 2017. Characterization of 16SrII-D subgroup associated phytoplasmas in new host plants in Egypt. *Arch. Phytopathol. Pflanzenschutz*, *50*(9-10), 504-513.

Esau, K., 1977. Membranous modifications in sieve element plastids of spinach affected by the aster yellows disease. J. Ultrastruct. Res, *59*(1), 87-100.

F.A.O., 2021. Food and agricultural organization. Data base., WWW.fao.org.

Gad, S.M., Kheder, A.A., Awad, M.A., 2019. Detection and molecular identification of phytoplasma associated with Gazania in Egypt. *J. Virol. Sci*, *6*, 12-23.

Gundersen, D.E., Lee, I.M., 1996. Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. *Phytopathol. Mediterr*, 144-151.

Hogenhout, S.A., Loria, R., 2008. Virulence mechanisms of Gram-positive plant pathogenic bacteria. *Curr. Opin. Plant Biol.*, *11*(4), 449-456.

Iftikhar, S., Fahmeed, F.A.U.Q.I.A., 2011. Detection of phytoplasma from diseased potato sample. *Pak. J. Bot*, *43*(3), 1799-1800.

Kakizawa, S., Yoneda, Y., 2015. The role of genome sequencing in phytoplasma research. *Phytopathogenic Mollicutes*, *5*(1), 19-24.

Kamińska, M., Śliwa, H., Rudzińska-Langwald, A., 2001. The association of phytoplasma with stunting, leaf necrosis and witches' broom symptoms in magnolia plants. *J. Phytopathol*, *149*(11-12), 719-724.

Kesumawati, E., Kimata, T., Uemachi, T., Hosokawa, M., Yazawa, S., 2006. Correlation of phytoplasma concentration in Hydrangea macrophylla with green-flowering stability. *Sci. Hortic*, *108*(1), 74-78.

Kobayashi, T., Kinoshita, M., Hattori, S., Ogawa, T., Tsuboi, Y., Ishida, M., Saito, H., 1990. Development of the sesame metallic fuel performance code. *Nucl. Technol*, *89*(2), 183-193.

Lorenz, K.H., Schneider, B., Ahrens, U., Seemüller, E., 1995. Detection of the apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and nonribosomal DNA. Phytopathol, *85*(7), 771-776.

Maust, B.E., Espadas, F., Talavera, C., Aguilar, M., Santamaría, J. M., Oropeza, C., 2003. Changes in carbohydrate metabolism in coconut palms infected with the lethal yellowing phytoplasma. *Phytopathol*, *93*(8), 976-981.

Mokbel, S.A., El-Attar, A.K., 2016. Ultrastructural changes in tomato plants induced by phytoplasma infection and attempts for its elimination using tissue culture techniques. *Egyptian J. Virol*, *13*(1), 34-51.

Mou, H.Q., Lu, J., Zhu, S.F., Lin, C.L., Tian, G.Z., Xu, X., Zhao, W.J., 2013. Transcriptomic analysis of paulownia infected by paulownia witches'-broom phytoplasma. *PLoS One*, *8*(10), e77217.

Nassar, M.A., El-Sahhar, K.F., 1998. Botanical Preparations and Microscopy (Microtechnique). *Academic Bookshop, Dokki, Giza, Egypt. 219pp*.

Omar, A.F., Foissac, X., 2012. Occurrence and incidence of phytoplasmas of the 16SrII-D subgroup on solanaceous and cucurbit crops in Egypt. *Eur. J. Plant Pathol*, *133*, 353-360.

Oshima, K., Maejima, K., Namba, S., 2013. Genomic and evolutionary aspects of phytoplasmas. *Front. microbiol, 4, 230.*

Randall, J.J., Bosland, P.W., Hanson, S.F., 2011. Brote Grande, A New Phytoplasma Associated Disease of Chile Peppers in the Desert Southwest. *P.H.P*, *12*(1), 18.

Rojas, M.R., 1993. Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. *Plant Dis*, *77*(4), 340.

Sahambi, H.S., 1970. Studies on seasmum phyllody virus: virus-vector relationship and host range. In Plant Disease Problems, Proc. 1st Int. Symposium Plant Pathol., *New Delhi (340-351).*

Salehi, M., Izadpanah, K., 1992. Etiology and transmission of sesame phyllody in Iran. *J. Phytopathol, 135(1), 37-47.*

Sambrook, J., Frisch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual. 913 Cold Spring Harbour Laboratory Press. *New York, 914*.

Santi, S., De Marco, F., Polizzotto, R., Grisan, S., Musetti, R., 2013. Recovery from stolbur disease in grapevine involves changes in sugar transport and metabolism. *Front. Plant Sci*, *4*, 171.

Seemüller, E., Garnier, M., Schneider, B., 2002. Mycoplasmas of plants and insects. In *Molecular biology and pathogenicity of mycoplasmas* (pp. 91-115). Boston, MA: Springer US.

Seemuller, E., Marcone, C., Lauer, U., Ragozzino, A., Goschl, M., 1998. Current status of molecular classification of phytoplasmas. *Plant Pathol. J, 3-26.*

Šeruga, M., Škorić, D., Botti, S., Paltrinieri, S., Juretić, N., Bertaccini, A.F., 2003. Molecular characterization of a phytoplasma from the aster yellows (16SrI) group naturally infecting Populus nigra L.'Italica'trees in Croatia *For. Pathol, 33*(2), 113-125.

Singh, A.K., Gopala, Rao, A., Goel, S., Rao, G.P., 2018. Identification of 'Candidatus Phytoplasma asteris' causing sesame phyllody disease and its natural weed host in Jammu, India. *Indian Phytopathol*, *71*, 143-146.

Singh, V., Kumar, S., Reddy, N.B., Naik, M.K., Bhat, K.V., Lakhanpaul, S., 2016. Devastation of sesame (*Sesamum indicum* L.) crops in different agroclimatic zones of India by genetically diverse subgroups of phytoplasma. *Crop Prot, 86, 24-30.*

Sridhar, D., Patil, M.S., 2013. Survey for sesamum phyllody disease in Northern Karnataka. *Karnataka J. Agric. Sci*, *26*(2), 320-321.

Venkataravanappa, V., Reddy, C.N.L., Manjunath, M., Chauhan, N.S., Reddy, M.K., 2017. Detection, characterization and in-silico analysis of candidatus phytoplasma australasia associated with phyllody disease of sesame. *Advances in Plants and Agriculture Research.*7(3):00256.

Weisburg, W.G., Tully, J.G., Rose, D.L., Petzel, J.P., Oyaizu, H., Yang, D., Van Etten, J., 1989. A phylogenetic analysis of the mycoplasmas: basis for their classification. *J. Bacteriol*, *171*(12), 6455-6467.

Woese, C.R., 1987. Bacterial evolution. *Microbiol. Rev51*(2), 221-271.

Yaseen, R., Amin, B.H., 2021. The use of phosphorus nanoparticles synthesized by rhizospheric fungus Aspergillius fumigatus as a nanofertilizer for flax plant. *Malays. J. Microbiol*, *17*(3).

Youssef, A.S., Sayed, Y., Hassan, O., Safwat, G., Shalaby, A.A., 2017. Universal and Specific 16S-23Sr RNA PCR primers for identification of phytoplasma associated with sesame in Egypt. *Int. J. Adv. Res. Biol. Sci. 4(7): 191-200.*

Youssef, S.A., Safwat, G., Shalaby, A.B.A., El-Beltagi, H.S., 2018. Effect of phytoplasma infection on plant hormones, enzymes and their role in infected sesame. *Fresenius Environ. Bull.* (27):5727-5735.

Table 1: Survey of phytoplasma associated with disease in sesame plants collected from different governorates in Egypt, during 2021 and 2022 growing seasons.

Governorate	locality	2021			2022		
		Surveyed vineyards	Diseased vineyards		Surveyed vineyards	Diseased vineyards	
			No.	%		No.	%
Giza	Atfih	49	14	28.6	85	26	30.5
	El Saff	43	11	25.6	66	18	27.7
	El Ayyat	28	5	17.8	35	10	28.5
	Total	120	30	25	186	54	29
Beni Suef	Biba	44	9	20.5	55	10	18.1
	Ihanasya AL Madinah	27	4	14.8	35	6	17.1
	Sumusta	19	3	15.8	28	4	14.2
	Total	90	16	17.7	118	22	18.6
Faiyum	Itsa	51	12	23.5	63	10	27.8
	Ibsheway	22	5	22.7	33	5	15.1
	Tamiyya	27	4	14.8	40	7	17.5
	Total	100	21	21	136	22	16.2
Sharqiyya	Bilbeis	18	5	27.7	22	3	13.6
	Mashtol El souk	17	3	17.6	25	6	25
	Total	35	8	22.8	47	9	19.1
Beheira	Hosh Essa	30	7	23.3	40	8	20
	Abu Hummus	25	6	24	33	5	15.1
	Total	55	13	23.6	73	12	16.4
Ismailia	AL Qantara sharqiya	41	8	19.5	61	11	18
	Fayid	29	3	10.3	36	6	16.7
	Total	70	11	15.7	97	17	17.5
LSD at	0.05	1.44*		0.83*	1.80*		1.63*
	0.01	1.93**		1.11**	2.41**		2.17**

Figure 1: Phytoplasma different symptoms on sesame plants, naturally infected in various regions of the field: (A) Healthy sesame plant (arow red and yellow) and infected (arow blue), (B) Witches broom, (C) virescence, (D) Aster Yellow, (E) Germinated after cracked capsules devoid of seeds.

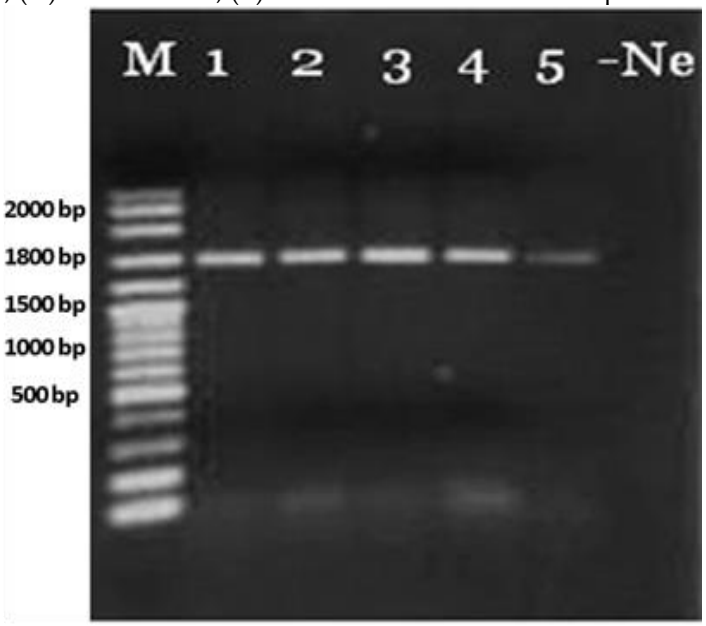

Figure 2: Agarose gel electrophoresis of PCR using the universal phytoplasma primer pair P1/P7, which is derived from highly conserved ribosomal sequences, amplifies a DNA fragment that is around 18,000bp. M= 100bp DNA marker, lanes (L1 - L2- L3- L4- L5) Symptomatic sesame leaf samples, -Ne= Healthy control samples.

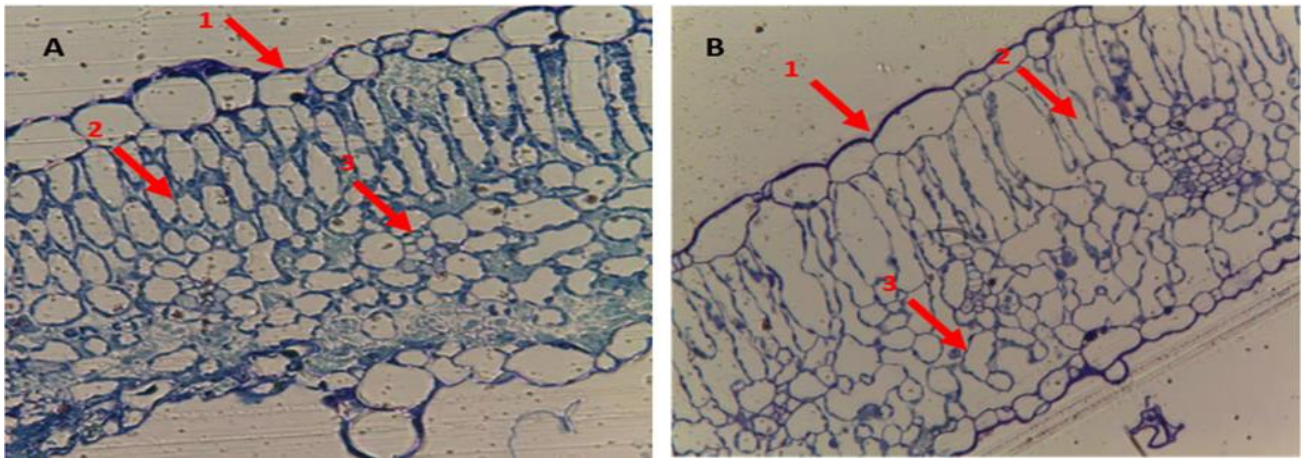

Figure 3: Light microscopy of phytoplasma-infected tissue, (A) healthy plant. mesophyll cells were compacted, spongy cells were small characterized and reduced intracellular space spongy tissues (B) infected plant. 1: epidermal cells, 2: Palisade tissue, 3: Spongy tissue.

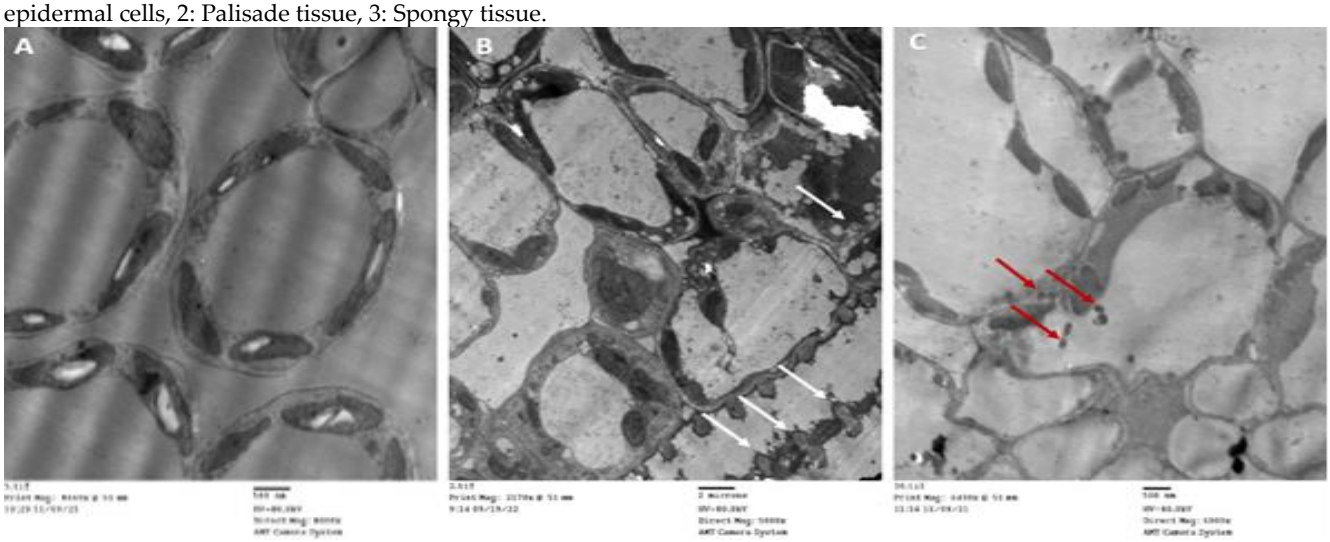

Figure 4: TEM of the phloem cells from the healthy and infected sesame leaves. (A) healthy plants, (B) Spherical or pleomorphic phytoplasma units in the sieve elements of infected phloem tissue (white arrows), (C) groups of phytoplasma particles attached to the plasma membrane of the sieve elements (red arrows).

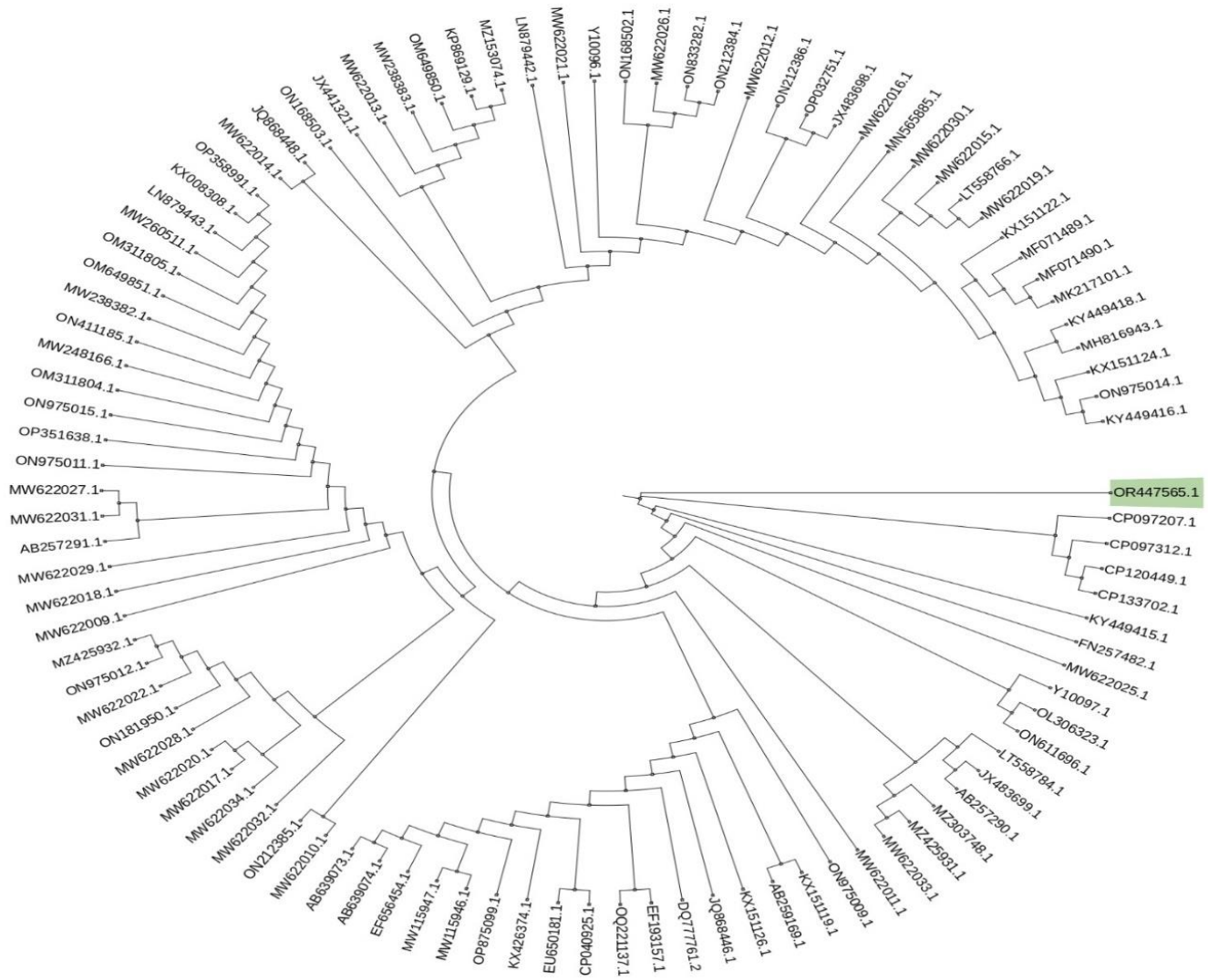

Figure 5: Phylogenetic tree constructed from partial 16S rDNA sequences for Egyptian isolate OR447565.1 compared to other isolates available in the GenBank database.

التوصيف الجزيئي للفيتوبلازما التى تصيب نباتات السمسم فى مصر.

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الملخص العربى

يعتبر السمسم من أقدم النباتات المنزرعة وبذوره مصدر غني للبروتين، ومضادات الأكسدة الطبيعية ويعتبر من أهم المحاصيل الزيتية من الناحية الاقتصادية ويزرع على نطاق واسع في مناطق مختلفة من العالم ولقد اتجهت مصر الى زيادة المساحة المنزرعة خلال الآونة الاخيرة ، للمساهمة في سد الفجوة من المحاصيل الزيتية وتوفير الاحتياجات اللازمة من الزيوت، ويصاب السمسم بالفيتوبلازما والتي تشكل واحدة من أهم وأخطر المسببات المرضية التي تؤثر على إنتاجيته. لذلك، قبل تطوير وتنفيذ استراتيجيات المكافحة السمسم ، تم جمع عينات أوراق التي تظهر عليها أعراض الفيتوبلازما من حقول 6 محافظات خلال موسمين متتاليين 2021 و2022. تم اكتشاف الفيتوبلازما بنجاح من خلال فحوصات تفاعل البلمرة المتسلسل المتداخلة (PCR) باستخدام أزواج التمهيدي العالمية P1/P7 وR16F2n/R16R2، وتم تضخيم المنتج بحوالي 1800 نقطة أساس. وسجلت أعلى معدل إصابة 25% و29% في الجيزة على التوالي، بينما سجلت أدنى معدل إصابة 15.7% و16.2% في الإسماعيلية والفيوم على التوالي. باستخدام الطرق الجزيئية المعتمدة على اختبارات تفاعل البلمرة المتسلسل المتداخلة (PCR) والتسلسل، وشجرة القرابة ، والتحليل الوراثي، كشف أن عزلتنا المصرية تحت رقم المدخل OR447565.1 ترتبط ارتباطًا وثيقًا بمجموعة SrII16 (WB) ، وهي وثيقة الصلة جدًا (98.86%) تم التعرف على عزلات MN565885.1 وOP793488.1 من إيران والهند، على التوالي، والتي تنتمي إلى نفس المجموعة الفرعية. بالإضافة إلى ذلك أظهرت المقارنة أن العزلة المصرية (OR447565.1) تتشابة مع مجموعة (Clover proliferation group) 16SrVI.كما تم ملاحظة تدهور في البنية التحتية للأنسجة والخلايا في النباتات المصابة بالفيتوبلازما من خلال تحليل الميكروسكوب الإلكترونى (TEM). واظهرت اشكال مختلفة من الفيتوبلازما داخل خلايا النبات.

الكلمات الاسترشادية: السمسم، الفيتوبلازما، الميكروسكوب الالكترونى.