Detection and Genetic Fingerprint of Lethal Yellowing Disease Associated with Candidates Phytoplasma Palma in Egypt

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Abstract

The date palm *(Phoenix dactylifera* L.) is one of the oldest cultivated trees and is an important all-purpose tree. About 105 million date palms are planted in Africa and Central America, Spain and Italy, but in Egypt have 16 million. The date palm is one of the important crops that is considered a strategic crop, and the date palm suffers from diseases, the symptomatic tree streaking's and yellowing to phytoplasma infection. The most important of which is phytoplasma disease, which is difficult to identify, this study using Molecular genetics has recently entered detection and genetic fingerprint of lethal yellowing disease, Polymerase chain reaction (PCR) assays using primer pairs designed for identification of phytoplasma using universal (R16mF2/R16mR1) primer 16Sr DNA sequences and specific nested (R16F2n/R16R2) a sensitive means of detecting this phytoplasma pathogen and a wide array of. The universal primer pair specifically for initiated amplification of among phytoplasma strains resulted in a DNA size of approximately 1500 bp in using gel electrophoresis. Nested PCR assays using the specific primer of phytoplasma in infected tissues resulted on a DNA of approximately 1200 bp. and Detection of LY-phytoplasma by the pathogen-specific primer pair LY1 (5`-CAT ATT TTA TTT CCT TTG CAA TCTG-3`), LY2 (5`-TCG TTT TGA TGA TCT TTC ATT TGAC-3`) designed for genomic DNA isolated from lethal yellow of windmill palm and electronics microscopy by transmission electron microscope At 70 kV, and Applied SEM Technology at sections of the infected leaves. This is examination of a phytoplasma associated with streak yellows on date palm in Egypt.

Key words: Phytoplasma, lethal yellow, date palm, genetic fingerprint

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Introduction

The date palm is spread in most governorates of the Republic and in the western oases, north and south Sinai, and the climatic conditions from a desert to semi-desert climate to a Mediterranean climate and a dry climate in the south help determine the distribution of suitable varieties that can achieve good production, (Heselmans, 1997). Egyptian date products are exported to many different international markets. The number of palm trees in Egypt has reached nearly 16 million palm trees, producing more than 1.6 million tons of dates and dates annually. Palms are affected by important diseases that lead to a decrease in production and its deterioration, including the fatal yellow disease, which is caused by the fatal yellow phytoplasma. Lethal yellowing (LY) is a fast-moving, fatal phytoplasma disease

of coconut and numerous other palm species (McCoy *et al*., 1983; Harrison & Jones, 2004). Phytoplasmas from the obligate prokaryotic plant pathogens that do not possess cell walls, and they are pleiomorphic in profile, with a mean diameter of 200–800 nm. Inhabits the phloem sieve cells of their palm hosts. Phytoplasmas have genomes a relatively small genome compared with other prokaryotes – and biosynthetic functions (Marcone *et al*., 1999; Bai et al., 2006). The method using magnetic beads is generally performed in an automated DNA extractor (eg King Fisher from Thermo Scientific1).DNA extraction methods are good for a variety of plant palm trees. The choice of method depends on the host being tested and the availability of equipment and facilities. A number of universal PCR primers have been designed that allow amplification of the 16S rRNA gene for any known phytoplasma. The most common are the P1/P7 pairs (Deng and Hiruki, 1991;), which can be used in a nested PCR protocol, depending on the composition of the host and phytoplasma , It is possible to perform more specific PCR assays and the result is conclusive (eg, post-entry quarantine samples, new host record, new distribution), a conventional PCR product should be arranged. In addition to 16S rRNA gene amplification, PCR methods have also been used to amplify other regions of the genome for detection and classification of plant plasmas, including ribosomal protein genes (Lim and Sears, 1992; Quaglino *et al*., 2013).These primers may be useful when a second independent region of the phytoplasma genome is required. And even Egyptian dates to international markets. The different pathogens of palm trees must be combated and their causes and methods of detection must be identified. During this study, the identification of the phytoplasma that causes fatal yellowing was carried out in three specialized ways through genetic fingerprinting with an examination with the electron microscope with two methods, scanning and intracellular examination in order to examine the presence of phytoplasma inside phloem cells inside infected plants. And prove it. The aim of this study and identify the presumed causal agent of streaking and yellowing disease in date palms in Egypt.

Materials and methods

1. Samples collection

A total of 22 samples of date palm cultivars exhibiting BSE-like symptoms were collected from four types of palm trees at two sites of the Agricultural Research Center farm in Giza.Stem tissues, young leaves as well as leaves from mature and mature palm trees (Zagloul, Sammani, Amhate and Hayani cultivars) showing LY like symptoms, collected from two sites in Giza in June 2020. In addition, healthy samples on what should be a control were collected from the same sites. A portion of the combined microscopic studies were treated as new material. Stem tissue samples were obtained using the methods of Oropeza *et al*. (2011) with minor alterations as described by Córdova *et al*. (2014).

2. Detection and Identification (PCR)

2-1 DNA extraction

extracted total DNA was from samples collected from naturally infected palm trees (Zaghloul, Sammani, Amhate and Hyani cultivars) as well as healthy controls using the a protocol by Harrison *et al*., (2004) and modifications by Cordova *et al*. (2014).

2-2 Low nucleotide primers

Four low-nucleotide primers (R16mF2, R16mR1, R16F2n and R16R2) were designed based on the 16SrDNA sequence from the Michigan yellow ester strain (Lee *et al*., 1993). The small nucleotide sequence for the sites of these primers and bases is R16mF2,5`-CAT GCA AGT CGA ACG A-3`; R16mR1, 5`-CTT AAC CCC AAT CAT CGA C-3` (base 53 to 1487 on the 16 S rDNA genomic nucleotide sequence); R16F2n, 5`-GAA ACG ACT GCT AAG ACT GG-3` and R16R2, 5`-TGA CGG GCG GTG TGT ACA AAC CCC G-3` (base152 to1397 on 16 nucleotide of SrDNA) (Al-Awadi *et al*., 2002).

Polymerase chain reaction assay

Extracted DNA was analyzed initially using phyto-plasma universal primers R16mF2 and R16mR1. PCR assay was performed using 200 µM deoxynucleotide triphosphates (dNTPs) and 10 pmol of each universal primer pairs. Thirty five PCR cycles were conducted in a thermal cyler (Biometra Co.). The following parameters were used; 1 min denaturation step at 94°C, annealing for 2 min at 60°C and primer extension for 3 min at 72°C, with final cycle extension for 10 min at 72°C. For nested PCR, DNA products previously amplified by using the universal primer pairs were used as template. A reaction mixture of 50 µl which contained the same previously component but using oligonucleotide nested primer pairs R16F2n and R16R2. DNA samples were amplified for 35 cycles under the thermal cycling conditions of the initial denaturation step at 94°C for 1 min, annealing temperature at 55°C for 2 min and primer extension at 72°C for 3 min with final extension step at 72°C for 10 min. PCR products were analyzed by electrophoresis through a 1.2 % agarose gel followed by staining in ethidum bromide and visualization of DNA fragments using a UV translluminator. Step ladder, 50 bp DNA marker (50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, 2000 and 3000 bp) was used to determine the fragment sizes (Sigma).

2-3 Conventional nested PCR

The PCR primers used in this assay are P1 (Deng and Hiruki, 1991) and P7 (Schneider et al., 1995) for the first-stage PCR:

P1 (forward): 5′-AAG AGT TTG ATC CTG GCT CAG GAT T-3′
P7 (reverse): 5′-CGT CCT TCA TCG GCT CTT-3′

2-3-1 Conventional Real-time PCR

Real-time PCR is performed using the TaqMan assay designed for the 16S rRNA gene by Christensen et al. (2004):

Forward primer: 5′-CGT ACG CAA GTA TGA AAC TTA AAG GA-3′
Reverse primer: 5′-TCT TCG AAT TAA ACA ACA TGA TCC A-3′
TaqMan probe: 5′-FAM-TGA CGG GAC TCC GCA CAA GCG-BHQ-3′

Alternatively, the real-time PCR of Hodgetts *et al*. (2009) designed for the 23S rRNA gene can be used:

JH-F 1 (forward primer): 5′-GGT CTC CGA ATG GGA AAA CC-3′
JH-F all (forward primer): 5′-ATT TCC GAA TGG GGC AAC C-3′
JH-R (reverse primer): 5′-CTC GTC ACT ACT ACC RGA ATC GTT ATT AC-3′
JH-P uni (TaqMan probe): 5′-FAM-MGB-AAC TGA AAT ATC TAA GTA AC-BHQ-3′

The 25 µl reaction mixture consists of 1× TaqMan real-rime PCR master mix, 300 nM forward primer, 300 nM reverse primer, 100 nM FAM probe and 2 µl DNA template. All samples are tested in duplicate. The amplification conditions are an initial denaturation step of 95 °C for 3 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. These cycling conditions may vary depending on the type of master mix used (e.g. some mixes require a polymerase activation step at 95 °C for 10 min and mixes that contain uracil-DNA glycosylase (UDG) require an initial hold at 50 °C for 2 min). Realtime PCR results are analysed with the manufacturer's software provided with the instrument .The real-time PCR assay of Christensen *et al*. (2004) uses 900 nM of the reverse primer, and this was updated to 300 nM in a later report (Christensen *et al*., 2013). This assay will work equally well with either concentration of reverse primer. PCR products were analyzed by electrophoresis through a 1.2 % agarose gel followed by staining in ethidum bromide and visualization of DNA fragments using a UV translluminator. Step ladder, 50 bp DNA marker (50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, 2000 and 3000 bp) was used to determine the fragment sizes (Sigma).

2-3-2 Detection of PCR products

Amplifications were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 µg/ml) in 1X TBE buffer at 95 V. PCR products on photographed by using a Gel Documentation Systemwere visualized and UV light (BIO-RAD 2000).

2-4-1 Detection of LYphytoplasma by nested-PCR

The presence of LY-phytoplasma was confirmed in diseased plants by PCR according to methods described by Oropeza *et al*. (2011). The PCR was run for 35 cycles in a programmable PCR Express (Thermo Hybaid, Ashford, UK) using the universal phytoplasma rRNA primer pair P1/P7 (Deng and Hiruki, 1991) with the following parameters: denaturation for 60 s at 94 °C; annealing at 54 °C for 50 s; and extension at 72 °C for 1 min 20 s (10 min for final cycle). The products of the P1/P7-primed PCR were diluted 1:40 with sterile ultrapure water and re-amplified for 35 cycles using the universal phytoplasma 16S rRNA Gene primer pair R16F2n/R16R2 (Lee and Gundersen, 1998) or the LY-group 503f/LY16Sr specific primer pair as described by Harrison *et al*. (2004).

2-4-2 The Detection of LYphytoplasma PCR

Detection of LY-phytoplasma by the pathogen-specific primer pair LY1 (5`-CAT ATT TTA TTT CCT TTG CAA TCTG-3`), LY2 (5`-TCG TTT TGA TGA TCT TTC ATT TGAC-3`) designed for genomic DNA isolated from lethal yellow of windmill palm (Trachycarpus fortunei). Designed primers were used that allow specific detection of lethal yellow according to Harrison *et al*. (2004). The polymerase chain reaction was performed in a reaction mixture (50 µl) containing (50 ng) of each starting material. Forty cycles of polymerase chain reaction were required under denaturing step conditions at 70 °C for 80 s in a PCR machine as described previously. Reaction mixtures containing DNA extracted from healthy palm tissues, and sterilized distilled water as substituted for complete DNA, were unseeing the negative control that were used in each experiment for all PCR amplifications.

3- Transmission Electron Microscopy (TEM) and (SEM)

Specimens of each affected species; date palm trees (Zaghlol, Samany, Amhat and Hyani c.v.) and untreated samples (control) were prepared for TEM using the procedures described below (Amin, 2013 and 2016).

3-1. Examination in TEM

Electron microscopy: High-resolution and fine-thickness sections were made of samples with pathological symptoms, yellowing and normal to make comparisons between them in the case of infection and healthy plants and changes that occur at the anatomical level in the cell, the affected sections were examined by JEOL - JEM 1010 - transmission electron microscope At 70 kV, the Faculty of Science, Al-Azhar University Egypt.

3-2. Applied SEM Technology

Samples of palm leaves without disease symptoms and others with symptoms were prepared to compare between healthy and infected people in the shape and arrangement of the outer cells in the killing solutions and fixation after rinsing. Samples with a 2.5% solution of 0.2 M glutaraldehyde with a dilute solution of calcium oxide (pH 7.2-7.4) for 1 day at 4°C. After rinsing the samples Store, to dry, to increasing degrees of ethanol solution (30%, 50%, 70%, 95% and 100%) by absolute acetone, for 20 minutes in successive form of these solutions. Until the samples were dry, and after being dried in CO2 (critical point dryer, Samdry-PVT-3B), they were mounted on SEM stages with double-sided tape. Samples were coated with palladium in ion spray (Denton Vacuum, DESK II) and examined with an electron microscope (S-3000N, Hitachi, Japan) at different acceleration voltages (5-20 kV).

Results and discussion

Disease Symptoms

Trees infected with phytoplasma were identified during the inventory process by matching the symptoms recorded for them on palm trees and photographed and samples were taken for laboratory examination and identification by genetic fingerprint, at the stems and bases of leaves in infected palm trees. The disease was first observed in the Arab region by Nixon (1954) in the Al-Ahsa Oasis in eastern Saudi Arabia. The main symptoms of the disease are

stunted leaves with pallor, yellow streaks, a noticeable decrease in the size of the fruits, and the absence of it, and the stem is weak due to poor photosynthesis. The presence of streaks in palm fronds with intermittent or longitudinal pallor and yellowing facilitates detection of phytoplasma infection on palm trees, and this is clear from the picture (1) (b) the leaves become completely yellow and their age decreases. Stunting and yellowing increases with age and loses its properties from its ability to conduct photosynthesis, which leads to the death of leaves (C). Diseased plants are shorter than healthy ones and end in complete death (D, E), as mentioned by Al-Hudayb *et al*. (2007).

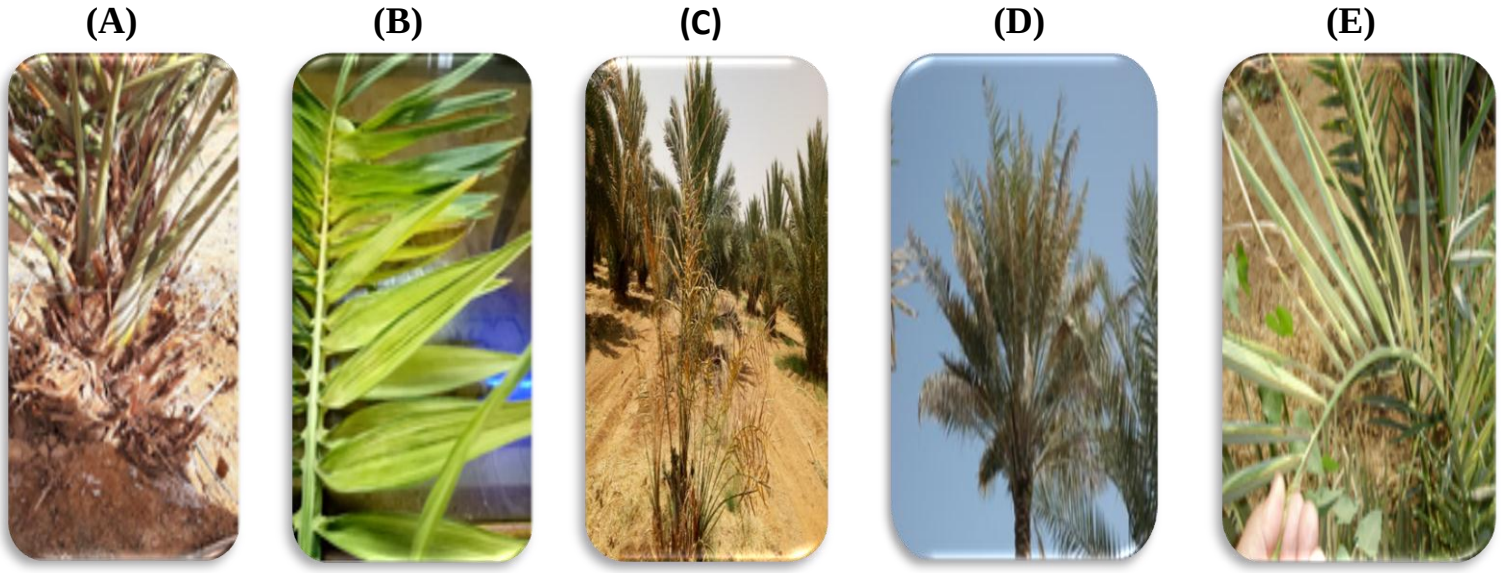


Fig (1): Symptoms of having phytoplasma (A) Plant infections on palm tree leaves Plant stem wrap. (B) Planning on plant leaves (C) Yellowing on plant leaves (D) The size of the plant is dwarf (E) Deterioration of plant color

2-1 Detection of PCR products 16SrDNA sequences

The identification and molecular characterization of phytoplasma associated with wejam. A fingerprint was made for the genetic structure of the samples that showed symptoms, and the Figure (2) image of the agarrose showed that the genetic structure of the samples' plasma matched with the items of the phytoplasma groups. The infected plant plasma was identified from the date palm, which clearly shows typical symptoms of wejam in the 16SrI "Ca.P.asteris" group.Which is supported by disease genotype data at (1500) molecular weight (Al-Hudaib *et al*., 2007).

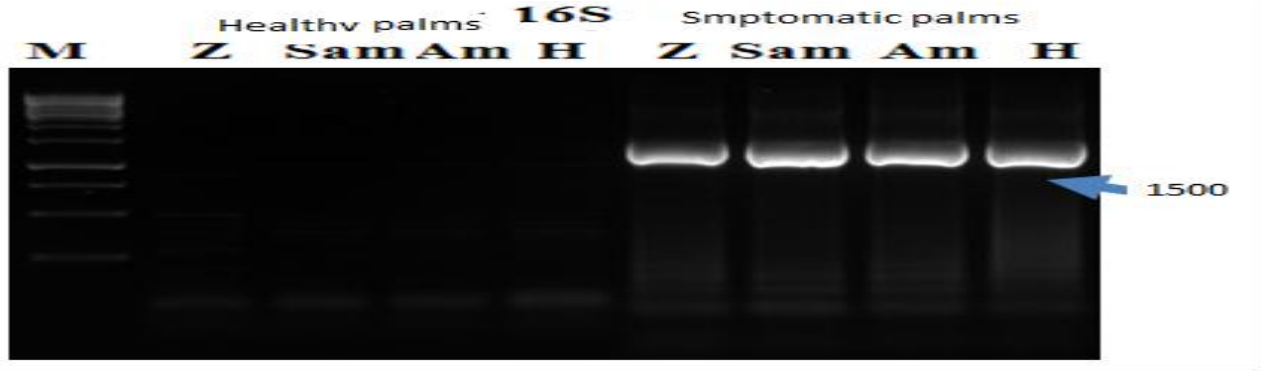


Fig (2): Polymerase chain reaction (PCR) amplification of phytoplasmal 16SrDNA sequences from naturally infected date palm

Palm leaf samples collected in this survey showed a severe infestation group, showing yellow streaks and general stunting of new leaves and of plants in general (Fig. 2) and the lifespan of plants is reduced. The palm trees affected in the farm also showed more severity of symptoms than the healthy ones, as the most severe infections were related to the type of planted varieties. The analyzes of the DNA samples under study from different sites in this study showed positive results for nPCR, and the pathogen was identified in patients using 16S primers (Figure 1), which confirms the presence of phytoplasma infection. The data and sequence similarity ratios of nPCR products, in this study, reached the identity of 99-100% of the samples that were analyzed and tested.

2-2 Detection of PCR product nested DNA

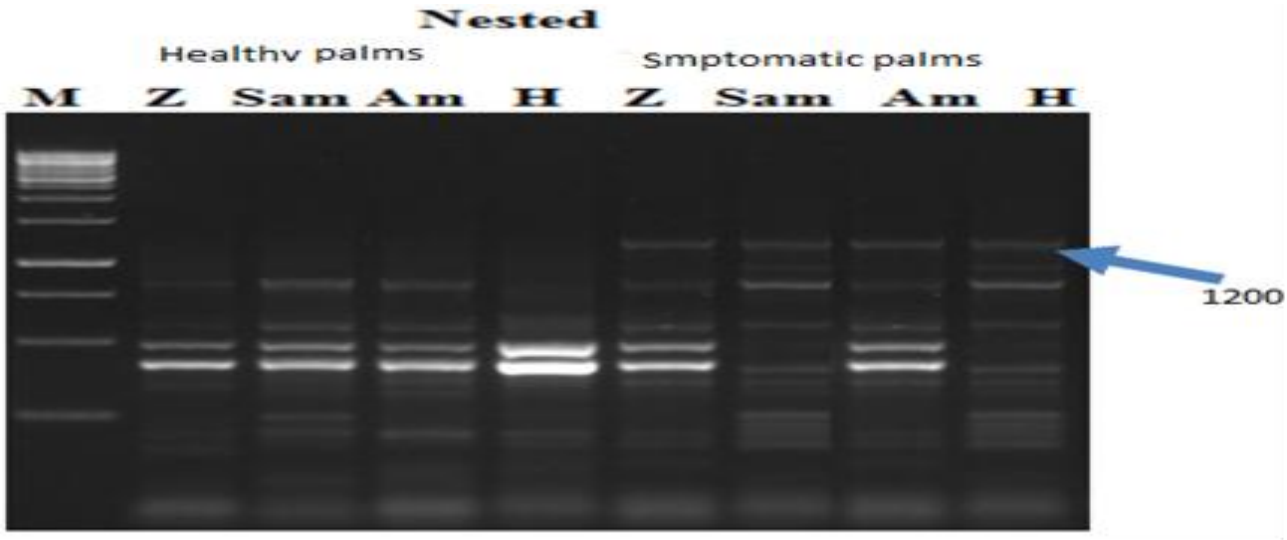


Fig (3): Using molecular genetics, genetic fingerprinting, and sequencing this interfering DNA with date palms infected with phytoplasmal Nested DNA.

The captured image of agarose showed the samples in Figure (3); Which was used interfering amplification of the sequences of phytoplasma 16SrDNA and also gene sequencing using the overlapping primer pairs (R16F2n / R2) for naturally infected palms that clearly showed symptoms of pathology that questioned their infection, which was analyzed with 16SrDNA first and then tested the primer-overlapping reaction to confirm (from Giza samples on respectively). Lines 1, 2, 3 and 4 are sequential belonging to the phytoplasma group, where the former includes all bacterial groups, while the latter is specific to phytoplasma. (Al-Awadi *et al*., 2002). As there is little information available about the yellowing diseases of date palms in Egypt (Ammar *et al*., 2005) and it needs verification and confirmation so that sound and effective measures are taken.

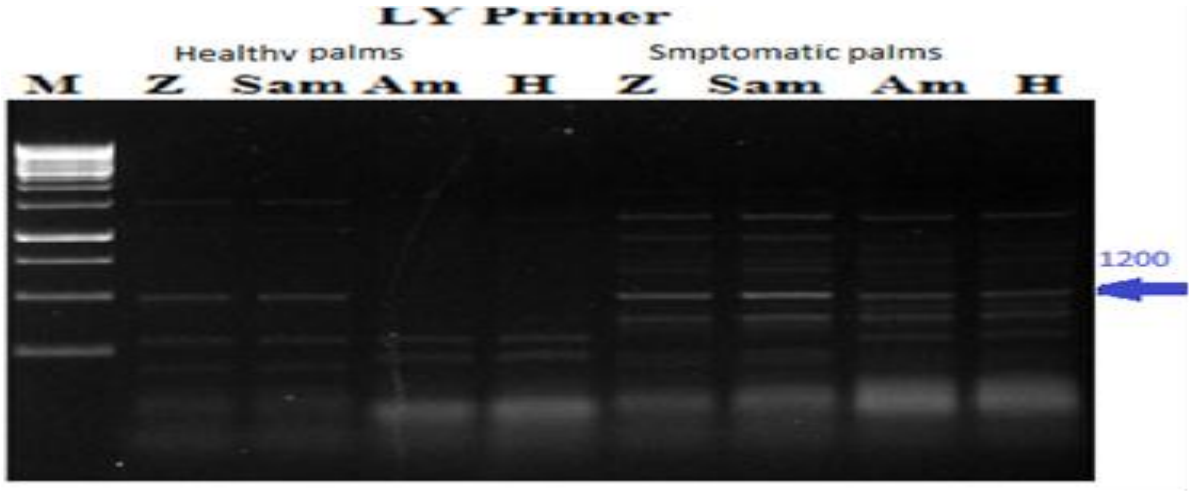
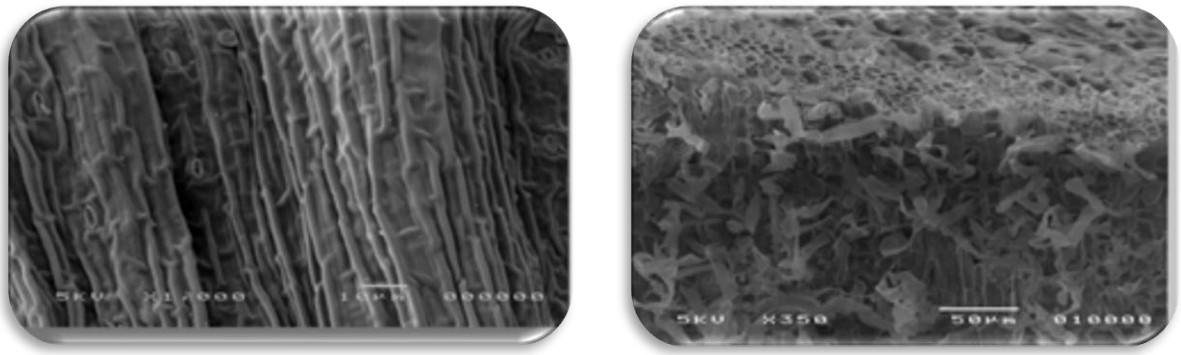


Fig (4): Using of (PCR) to identify the phytoplasma that causes yellowing dead in palm by sequencing LY Phytoplasmal DNA from naturally infected date palms

Samples analysis images with LY primers. The Figure (4) using of (PCR) to identify the phytoplasma that causes yellowing dead in palm by sequencing LY Phytoplasmal DNA from naturally infected date palms, This is to confirm that infection with phytoplasma causing fatal yellowing at (1200) molecular weight, where positive samples showed 16S primers and Nested DNA with a LY primers .Various methods are available to establish the presence of phytoplasma in diseased plants (Hibben *et al*., 1986). Symptoms are one of the main criteria for the initial diagnosis of presumed phytoplasmic diseases. Symptoms on a random sample of infected palm trees of various cultivars showed similar patterns of infection, which led to the conclusion that the plants were affected by lethal yellow phytoplasma disease and this agrees with what was mentioned (Nixon, 1954 and Al-Awadi *et al*., 2002). Here, we conclude that the symptoms alone are not sufficient to diagnose this disease, as they are insufficient evidence to discover and identify Phytoplasma as a supposed cause and is linked to the fatal yellowing disease in palms, which is consistent with the results of the research and the use of more than one initiator to confirm the disease, which is consistent with (Al-Awadhi *et al*., 2002).

3- Detection of phytoplasmas in plants by scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

(A) **(B)**



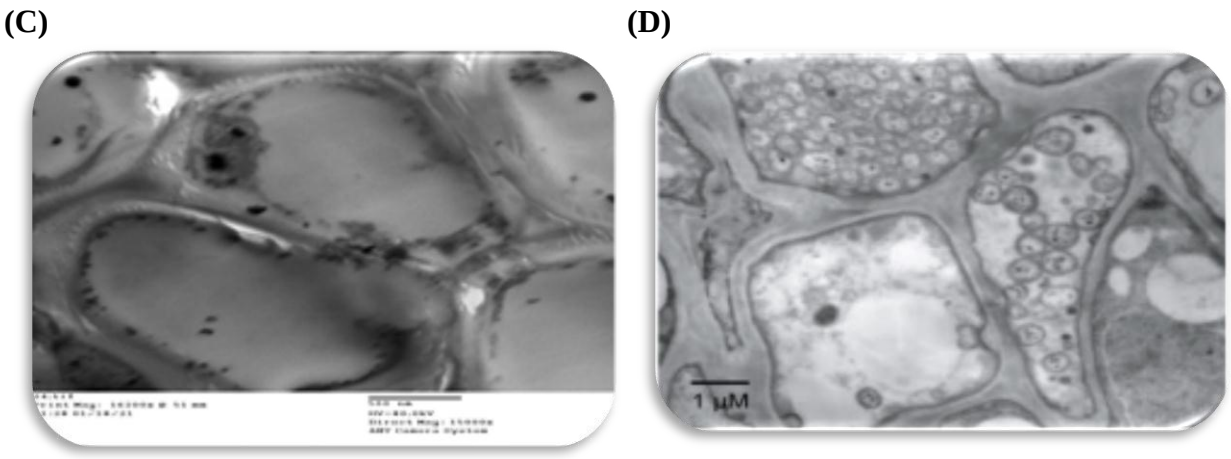


Fig (5): Electron micro photo graph of (Candidatus Phytoplasma palmae) infecting the plant phloem. (A) scanning control cells, (B) section of phloem tissue from an infected plant shown. Several of the plant cells contain, (C) wood cells and (D) phytoplasma cells in phloem

3-1 Scanning electron microscopy (SEM)

Electron microscopic scanning was performed with an electron microscope on the surfaces of infected and healthy leaves to identify the shape of the infected cells compared to the healthy cells and to ensure the presence of plant cells in the phloem cells. Plant phtoblsamasts were morphologically characterized by SEM on infected plant cells and were found to have short, branched filamentous forms in the phloem cells of infected plants in agreement with (Marcone *et al*., 2004).

3-2 Detection of phytoplasmas in plants by Transmission electron microscopy (TEM)

Detection of phytoplasm by examining plants using a transmission electron microscope (TEM). The electron microscope has high characteristics and capabilities. With this feature and capabilities, we can detect healthy and unhealthy cells, as phytoplasma appeared inside the phloem tissues, as in Picture No. 5, where there are cells with phytoplasma cells and healthy cells next to them for arbitration.

Recommendations

The overlapping PCR can be used to detect secondary phytoplasma in mixed infections, so it is important in identifying the pathogen in patients, and it turned out to be more specific than the general PCR and this is consistent with what was mentioned (Al-Awadhi *et al*., 2002). Also, the polymerase chain reaction (PCR) allowed the detection of plant plasma in some suspicious trees where the observed symptoms are weak. The present study can conclude that the yellow streak may be caused by a single phytoplasma and this is consistent with the results obtained by (Lee *et al*., 1993) and (Al-Awadhi *et al*., 2002). Phytoplasma cells are small and have multiple shapes, and we need to magnify the electron microscope and its resolution for examination and direct observation, and to study the morphological characteristics of Phytoplasma and its shapes inside the sieve tubes of the infected host plant as mentioned (Lee *et al*., 2000). For more than 30 years, TEM has remained one of the most reliable and effective methods for detecting plant plasmas (Poghosyan *et al*., 2004). There are

other methods using the electron microscope, which are also good in diagnosing and tracing inside cells and its effect on cell shapes,TEM was first used with success to establish its presence and to describe the phytoplasma associated with wejam (Doi *et al*., 1967).

Conclusion

This technique is good for identifying the diseased pathogen in the bark of the host plants. And we need to continue studies in this direction to increase and enrich the nature of this type of pathogens inside plants during the occurrence of infection. And using of host palm cultivar resistance represents the most practical in long-term solution for LY control.

References

Al-Awadhi, H., A. Asma Hanif and P. Suleman, 2002. Molecular and microscopical detection of phytoplasma associated with yellowing disease of date palms Phoenix dactylifera L. in Kuwait. Kuwait Journal of Science and Engineering. 29 (2): 87-109.

Alhudaib, k., Y. Arocha, M. Wilson and P. Jones, 2007. Al-wijam", a new phaytoplasma disease of date palm in Saudi Arabia . bulletin of insectology. 60(2): 285-286.

Ammar, M.I., M.A. Amer, and M.F. Rashed, 2005. Detection of Phytoplasma Associated with Yellow Streak Disease of Date Palms (*Phoenix dactylifera* L.) in Egypt, Egyptian J. Virol. 2, 74-86.

Bai, X., J. Zhang, A. Ewing, S.A. Miller, A. Jancso Radek, D.V. Shevchenko, K. Tsukerman, T. Walunas, A. Lapidus, J.W. Campbell, and S.A. Hogenhout, 2006. Living with genome in stability: The adaptation of phytoplasmas to diverse environments of their insect and plant hosts. Journal of Bacteriology, 188: 3682–3696.

Basma, H. Amin 2013. Studies on antifungal agents extracted from some desert and cultivated plants, Ph.D. Thesis, Botany and Microbiology Department, Al – Azhar University

Basma, H. Amin 2016. Isolation and characterization of antiprotozoal and antimicrobial metabolite from Penicillium roqueforti. Afr. J. Mycol. & Biotech. (2016) 21(3), 13-26.

Christensen, N.M., H. Nyskjold, and M. Nicolaisen, 2013. Real-time PCR for universal phytoplasma detection and quantification. In: M. Dickinson & J. Hodgetts, eds. Phytoplasma: Methods and protocols. Methods in molecular biology series, Vol. 938, pp. 245–252. New York, NY, Humana Press. 421 pp.

Christensen, N.M., M. Nicolaisen, M. Hansen and A. Schulz, 2004. Distribution of phytoplasmas in infected plants as revealed by real-time PCR and bioimaging. Molecular Plant Microbe Interactions, 17: 1175–1184.

Cordova, A., L. Iban, S. Jesus, V. Gerardo, R.F. Javier, 2014. Physiological Responses during Cycling With Oval Chainrings (Q-Ring) and circular chainrings. Journal of Sports Science and Medicine 13, 410-416

Deng, S. and C. Hiruki, 1991. Amplification of 16S rRNA genes from culturable and non-culturable Mollicutes. Journal of Microbiological Methods, 14: 53–61.

Doi , Y.M., M. Teranaka, K. Yora, and H. Asuyama, 1967. Mycoplasma or PLT-group like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches'broom, aster yellows and paulownia witches' broom. Annals of the Phytopathological Society of Japan, 33: 259–266.

Harrison, N.A. and P. Jones 2004. Disease caused by a phytoplasma: lethal yellowing. In Compendium of Ornamental Palm Diseases and Disorders, pp. 39–41. Eds M.E. Elliott, T.K. Broschat, J.Y. Uchida and G.W. Simone. St Paul, MN, USA: American Phytopathological Society Press.

Hibben, C.R., C.A.Lewise, and J.D. Castello, 1986. Mycoplasma- like organisms, cause of Lilac Witches-Broom. Plant Dis. 70: 312-345.

Hodgetts, J., N. Boonham, R. Mumford, and M. Dickenson, 2009. Panel of 23S rRNA gene-based real-time PCR assays for improved universal and group-specific detection of phytoplasmas. Applied and Environmental Microbiology, 75: 2945–2950.

Lee I.M., R.E. Davis, and D.E. Gundersen-Rindal, 2000. Phytoplasma: phytopathogenic mollicutes. Annu Rev Microbiol 54, 221-255.

Lee, I. M., D.E.Gundersen-Rindal, R.E. Davis, and I.M. Bartoszyk, 1998. Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. International Journal of Systematic Bacteriology, 48: 1153–1169.

Lee, I. M., K.D. Bottner-Parker, Y. Zhao, R.E. Davis, and N.A. Harrison, 2010. Phylogenetic analysis and delineation of phytoplasmas based on secY gene sequences. International Journal of Systematic and Evolutionary Microbiology, 60: 2887–2897.

Lee, I.M., R.W. Hammond, R.E. Davis, and D.E. Gundersen, 1993. Universal amplification andanalysis of pathogen 16S rDNA for classification and identification of mycoplasma like organisms. Phytopathology, 83: 834–842.

Lim, P.O. and B.B. Sears, 1992. Evolutionary relationships of a plant-pathogenic mycoplasmalike organism and Acholeplasma laidlawii deduced from two ribosomal protein gene sequences. Journal of Bacteriology, 174: 2606–2611.

Marcone, C., B. Schneider, E. Seemuller, 2004. 'Candidatus Phytoplasma cynodontis', the phytoplasma associated with Bermuda grass white leaf disease. International Journal of Systematic and Evolutionary Microbiology, 54(4):1077-1082.

Marcone, C., H. Neimark, A. Ragozzino, U. Lauer, and E. Seemüller, 1999. Chromosome sizes of phytoplasmas comparing major phylogenetic groups and subgroups. Phytopathology, 89: 805– 810.

McCoy R.E., F.W. Howard, J.H. Tsai, H.M. Donselman, D.L., Thomas, H.G. Basham, R.A. Atilano, F.M. Eskafi, L. Britt and M.E. Collins, 1983. Lethal Yellowing of Palms Agricultural Experiment Station Bulletin No. 834. Gainesville, FL, USA: University of Florida, IFAS.

Nixon, R.W. 1954. Date culture in Saudi Arabia. Ann. Date Growers' Instit. 31: 15-20.

Oropeza C, I. Cordova, A. Chumba, M. Narváez, L. Sáenz, R. Ashburner, N. Harrison, 2011. Phytoplasma distribution in coconut palms affected by lethal yellowing disease. Ann Appl Biol 159:109–117.

Poghosyan T.D., H. Koga, M. Taga, 2004. Scanning electron microscopy of mitotic nuclei and chromosomes in filamentous fungi. Mycologia 96:208–210

Quaglino, F., Y. Zhao, P. Casati, D. Bulgari, P.A. Bianco, W. Wei, and R.E. Davis, 2013. 'Candidatus Phytoplasma solani', a novel taxon associated with stolbur- and bois noir-related diseases of plants. International Journal of Systematic and Evolutionary Microbiology, 63: 2879–2894.

Schneider, R., M. Massow, T. Lisowsky, H. Weiss, 1995. Saccharomyces cerevisiae after inactivation of the gene encoding the mitochondrial acyl carrier protein. Curr Genet 29(1):10-7

Zouine, J., M.j. El Bella, A. Meddich, J. Luc Verdeil, and I. ElHadrami, 2005. Proliferation and germination of somatic embryo from embryogenic suspension cultures in Phoenix dactylifera. Plant Cell, Tissue and Organ Culture, 82: 83-89.

الكشف عن مرض الاصفرار المميت بالبصمة الجينية المصاحب لفيتوبلازما النخيل في مصر

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الملخص العربى

نخيل التمر (*Phoenix dactylifera* L.) من أقدم الأشجار المعروفة وهي شجرة عديدة الاغراض والنفع منزرع حوالي ١٠٥ مليون نخلة تمر في مناطق إفريقيا وأمريكا الوسطى وإسبانيا وإيطاليا ، ويوجد في مصر حوالى (١٦) مليون نخلة تمر.و نخيل التمر من المحاصيل المهمة التي تعتبر محصولاً استراتيجياً ، ويعاني نخيل التمر من بعض مسببات الأمراض ، أهمها واخطرها مرض الفيتوبلازما المسبب للاصفرار المميت وأعراضه عديدة وهى وجود تخطيط على اوراق الأشجار مع الأصفراروتقزم العام فى النبات مع التفاف الاوراق . ، و يصعب تحديدالمسبب المرضى لتداخل تلك الاعراض مع كثير من غير المختصين ، وفى هذه الدراسة التييتم الاستفادة من علم الوراثة الجزيئية حيث ادخلت مؤخرًا في الكشف بالبصمة الجينية لمرض الاصفرار القاتل ،حيث يتم تفاعل البوليميراز المتسلسل (PCR) باستخدام أزواج أولية مصممة لتحديد البلازما النباتية باستخدام (عام R16mF2 / R16mR1) .(التمهيدي ١٦ Srتسلسل DNA و بريمرات متداخلة محددة (R16F2n / R16R2) حيث هذه وسيلة حساسة للكشف عن مسببات الأمراض النباتية فى مجموعة واسعة من المجموعة النباتية . ونتج عن الكشف بالبادىء العام المتخصص للتضخيم المبدئي فى سلالات الفيتوبلازما الكشف عن الحمض النووي عند موقع تقريبًا ١٥٠٠ pb مع استخدام الهجرة الكهربائية على الاجاروز. أدت فحوصات PCR المتداخلة باستخدام البريمر المحدد للفيتوبلازما في الأنسجة المصابة إلى الحصول على بندات DNAعند موقع ١٢٠٠ pb نقطة أساس ومع البرايمر LY-phytoplasma بواسطة زوج التمهيدي الخاص بالعوامل الممرضة (`5-CAT ATT TTA TTT CCT TTG CAA TCTG-3`) ، LY1 (`5-TCG TTT TGA TGA TCT TTC ATT TGAC-3`) LY2 وصممت البريمرات للحمض النووي الجيني المعزول من العينات التى بها اعراض الأصفر القاتل للنخيل وتم الفحص المجهري للقطاعات عن طريق المجهر الإلكتروني النافذ عند ٧٠ كيلو فولت ، وتكنولوجيا SEM التطبيقية في مسح الخلايا المصابة لدراسة شكل الخلايا المصابة بالفيتوبلازما . وبهذا تمت عملية فحص واثبات الفيتوبلازما النباتية المرتبطة بالأصفرار المتقطع والمميت على نخيل التمر في مصر.

الكلمات المفتاحية: الميكرسكوب الاكتروني، فيتوبلازما، الإصفرار القاتل، نخيل التمر، البصمة الوراثية