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In vitro production of Phytoplasma (Al-Wijam) disease-free plants of Date palm (*Phoenix dactylifera* L.) in Egypt.

*Tarek E. Abdelbaset**, *Khaled I.A. Sakr, Ahmed E. Elshorbagy, and Amera M. Ismail*

Address:

Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt

*Corresponding author: ***Tarek E. Abdelbaset***, e-mail: drtarekelsayed71@gmail.com

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ABSTRACT

The Wijam (Phytoplasma) disease of date palms is an economic threat to date palm output in Egypt. Disease symptoms were identified in various date palm-grown orchards. The polymerase chain reaction (PCR) was used to analyze a total of ten samples from five different date palm cultivars. These samples came from both healthy and diseased trees. The findings of the nested PCR (nPCR) experiment revealed the presence of 16S rDNA phytoplasma in the trees that were infected. Furthermore, one date palm cultivar (Magdoul) showed no infection, while the other four (Khalas, Sukary, Barhi, and Zaghloul) exhibited varying degrees of infectivity. Chlorophyll a, b, and total chlorophyll content were dramatically reduced due to biochemical alterations. Meanwhile, proline and carotenoid levels in infected plants increased while ascorbic acid and soluble sugar levels decreased. The activities of antioxidative enzymes such as polyphenol oxidase, peroxidase, catalase, and oxidase were increased in phytoplasma-infected trees. A technique for in vitro organogenesis to propagate date palm cultivars was demonstrated. Isolated meristematic tip cultures were grown on MS media supplemented with 2,4-D 100 mg/L and 1.5 g/L charcoal for four to nine weeks, depending on the cultivar. For cvs. "Magdoul" and "Barhee," callus regeneration, shoot elongation, and multiplication were performed on MS media containing 1 mg/L NAA and 1.5 g/L activated charcoal.

Keywords: Date palm., Phytoplasma., antioxidative enzymes., Tissue culture.

INTRODUCTION

The date palm, scientifically known as *Phoenix dactylifera* L., is not only one of the oldest known fruit crops but also the most significant and vital fruit crop in the Middle East. The production of date palms reached 8.5 million metric tons in 2018, with Egypt, Saudi Arabia, and Iran being the top three producers, according to FAO statistics (FAO.FAOSTAT. 2020). According to FAO statistics (FAO.FAOSTAT. 2020), the production of date palm reached 8.5 million metric tons in 2018, with Egypt, Saudi Arabia, and Iran being the top three producers. Recently, Egypt has been considered one of the top ten producing countries for date palms, which explains the average productivity of date palms, which represents approximately 90 kg per tree, based on the base of the bearing palms, but this figure decreased to 75 kg when considering all the palm trees in the area. However, even after accounting for it in this manner, it is still quite high in comparison to the global average of 35 kg per tree. However, the national average yield of date palm is very low compared with world data. The most important reason for this low productivity is date palm disease. Lesser Date moth, Red Palm Weevil, Dubas Bug, and phytoplasma (Al-Wijam) disease are just a few of the illnesses that can affect the health, vigour, and productivity of date palm plantations in both commercial and dooryard plantings. Correctly identifying symptoms is crucial to management since improper therapeutic applications or actions can be expensive and occasionally harmful. Numerous Arab nations have reported date palm phytoplasma infection symptoms. It was observed and reported for the first time in Saudi Arabia, Sudan (Cronje *et al.,* 2000), Kuwait (Al-Awadhi *et al.,* 2002) and in Egypt (Abou-El-Einin, 2010., Alkhazindar, 2014).

Plant infections that are pleomorphic wall-less prokaryote organisms of the class Mollicutes are referred to as phytoplasmas. Phytoplasmas are distinguished by their small microscopic size and polymorphism shape as a result of the absence of cell walls (Moghaddam, *et al.,* 2017). It is impossible to develop phytoplasmas *in vitro* because they colonies the phloem of the plant. Phytoplasma has been linked to several hundreds of different plant diseases (Maejima *et al*., 2014; Kumari *et al*. 2019; Solomon *et al.,* 2019), and it is probably considered to be one of the most important plant pathogens, as it reduces the productivity of several economic crops all over the world, in countries ranging from tropical to temperate climates. (Weintraub and Beanland 2006., Linck and Reineke 2019., Quaglino *et al*. 2019; Jakovljevi *et al.,* 2020) It has been determined that leafhoppers, which belong to the order Hemiptera, are the most significant vectors responsible for the chronic transmission of the infection. 1967 was the year in which the phytoplasma pathogen was first described (Alhudaib, *et al*., 2018) causing many symptoms that may vary in an expression

such as., leaf yellowing, streaking, low yield, stunting and death in advanced severe infections symptoms in wide-range of susceptible host plants makes phytoplasma a serious economic threat to agriculture (Maharachchikumbura *et al.* 2016; Moghaddam, *et al.* 2017; Alhudaib, *et al*. 2018). Symptoms on date palm trees appeared on 5-8 years old palm trees which could die within 6-12 months of symptom appearance.

Date palms phytoplasma pathogen is placed in the genus Candidates, and its genome size can range anywhere from 0.530 to 1.350 Mbp (Namba *et al.,* 2005). The 16S rRNA gene is used to differentiate between species of the phytoplasma pathogen (Harrison *et al.,* 2011). The phytoplasma (Wijam) illness is responsible for more than a 30-40 percent drop in date production as well as the demise of a great number of palm trees (Sugio *et al.,* 2011). Date palms in Egypt are plagued by severe infestations of pests and diseases, which is the primary reason for the country's poor production levels. Additionally, the absence of effective disease control practices is another issue that contributes to the country's a low date palm productivity (Hill *et al*., 2000; Sugio *et al.,* 2014). Because of the phytoplasma infection, there was a significant rise in the levels of tocopherols, soluble sugars, and total phenols that were discovered in the leaves. Ahmad *et al,* (2019). When plants are attacked by phytoplasma, a few different antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT), contribute to the metabolism of reactive oxygen species. Phytoplasma (ROS). Somatic embryogenesis (Fki et al., 2011; Naik and Al-Khayri, 2016; Mazri *et al.,* 2017; Roshanfekrrad *et al.,* 2017; Hazubska-Przyby *et al.,* 2020; Hassan *et al.,* 2021) and organogenesis (Hazubska-Przyby T, *et al.,* 2017) are two methods that have been reported (Bekheet, 2013., Jazinizadeh *et al.,* 2015; Meziani *et al.,* 2015, 2016). In addition to offering a method for the speedy clonal propagation of date palms, this study presents: (Khierallah, 2007). Micropropagation of date palms in vitro has emerged as an indispensable and highly efficient method for ensuring the continual expansion and revitalization of palm estates (Smith and Aynsley, 1995). The aim of this study was initiated to (1) investigate the occurrence of date palm phytoplasma disease in Egypt using PCR. (2) illustrate the percentage of infection for date palm cultivars (3) production of pathogen-free and improved planting material. The adoption of such planting material will significantly improve the productivity and competitiveness of date palm production in Egypt.

MATERIALS AND METHODS:

Sample collection:

A total of 10 samples of 5 date palm cultivars (Magdoul, Khalas, Sokari, Barhi and Zaghlul) were collected in the winter seasons of 2017 and 2018 from both symptomatic and a symptomatic date palm tree, showing yellow streaks and stunting (Figure 1), from date palm plantation located in Giza governorate. The collected samples were kept in −20°C before to examine. The total nucleic acid was recovered from the leaf tissue and the wood from the interior basal trunks of five symptomatic date palms cvs, as stated in Doyle's 1987 research. The DNA that had been isolated was then redissolved in fifty microliters of sterile distilled water. The DNA samples were analyzed on agarose gels consisting of 1 percent agarose.

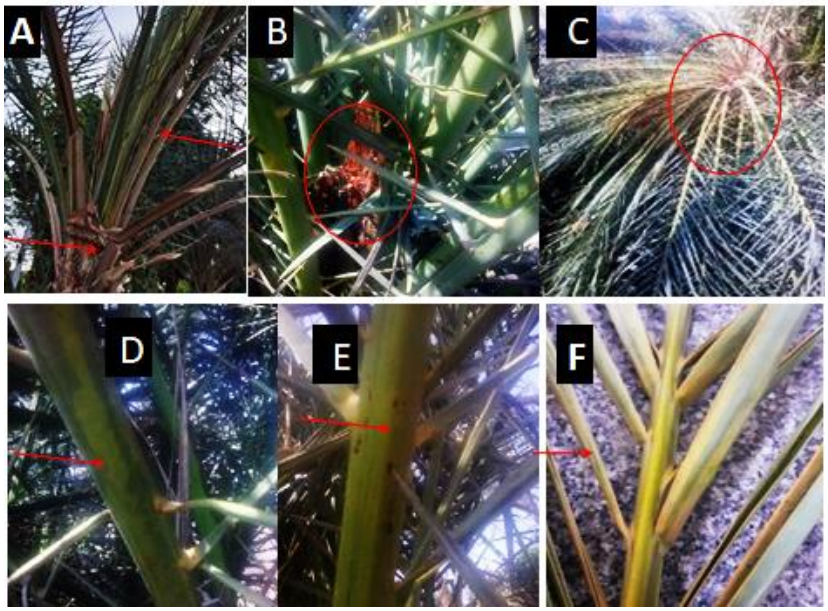

Fig. 1. Date palm phytoplasma pathogen infection symptoms. A date palm with severe indications of yellowing. B, leaves with light yellow striations. C, a date palm displaying fatal death signs. D, the leaf has yellow stripes visible. E, A date palm with significant yellowing and leaf stunting signs, and F, A date palm with yellow streaks on its leaves.

DNA extraction and PCR:

The modified Dellaporta method was used to extract total DNA from healthy and symptomatic leaves of date palm trees (Orlovskis *et al*., 2017). Using a mortar and pestle and liquid nitrogen in a 2 ml tube, about 100 mg of leaf sample were crushed or ground to a fine powder. Then, 33 l of 20 percent SDS was added, and the mixture was vortexed before being incubated at 65°C for 10 minutes. The extraction buffer was composed of 50 mM EDTA, 100 mM Tris-HCl, 500 mM NaCl, and 10 mM -mercaptoethanol. Following this, 700 ml of PCI (Phenol, Chloroform, and Isoamyl Alcohol, 24:24:1) and 160 ml of 5 M potassium acetate were added, vortexed, and kept on ice for 5 minutes before being centrifuged for 10 min. at 5000 rpm. To precipitate the nucleic acids, the purified top layer was transferred to a new tube, 1/10 volume of sodium acetate, 2.5 volumes of ice-cold ethanol, and 30 minutes at 80°C were all maintained. The precipitated DNA pellet was air-dried, twice-washed with 70 percent cold ethanol, and then re-dissolved in 50 l distilled water.

According to (Sugio *et al*., 2012), the first cycle of PCR was conducted in a 50 µl reaction using R16mF2/R16mR1 primers. The template was a 2 l DNA extract, to which were added 2 µl of each primer (10 pmol), 5 µl of 2.5 mM dNTP mix, 5 ul MgCl2 (25 mM), 5 µl of 10X Taq reaction buffer, 5 units of Taq DNA polymerase (Promega, TM, USA), and 50 µl of deionized water to bring the volume to 50 l. The thermal cycler's parameters were set to one cycle lasting two minutes at 94°C, 35 cycles lasting one minute each at 94°C, 58°C, and 72°, and a final extension lasting ten minutes at 72°C. The resultant PCR products were diluted 1:50 with deionized water and used as a template for a 50 l nPCR using the same PCR conditions and the fU5/rU3 primer pair (Du Toit, 2014).

The secondary and primary metabolism of date palms is influenced by phytoplasma infection:

The methods outlined by Arnon (1949), were used to measure the quantities of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in date palm plants. A leaf sample about 0.5 g was homogenized using a mortar and pestle in 80% acetone and filtered. A Pico drop spectrophotometer was used to test the filtrate's absorbance value at 645 and 663 nm for chlorophyll a and b, respectively (Hitachi-U-2001, Japan). According to Yoshida *et al.* (1976), description the chlorophyll a and b were measured. Chl. a (mg/g) = [12.7 (OD663)-2.69 (OD645)] × V/1000 × W Chl. b (mg/g) = [22.9 (OD645) - 4.68 (OD663)] × V/1000 × W. The plant samples were added with anthrone reagent to assess the amount of soluble sugars contents.

Additionally, the free proline was determined using the procedure of Bates *et al*. (1973). A sample of date palm leaves about 0.5 g was homogenised in 5 ml of 3 percent aqueous sulphosalicylic acid before being filtered through Whatman No. 2 filter paper. In a test tube, 1.25 g of ninhydrin was dissolved in 30 ml of glacial acetic acid, along with 1 ml of acid ninhydrin and around 1.0 ml of filtrate. The mixture was heated for an hour at 100°C in a water bath before being transferred to the ice bath. It was then briefly agitated in a vortex. Four millilitres of toluene were added, and the resulting liquid was vortexed for 15 to 20 seconds until it cooled. Proline-containing chromophore was taken out of the aqueous phase and allowed to incubate in a test tube at room temperature.

The absorbance at 520 nm was measured with a spectrophotometer. Using the same procedure, a blank was produced using 2 ml of toluene. Proline (moles) per gramme of fresh weight is calculated using the following equation: [(g proline/ml toluene)]/[115.5 g/u mole]/[(g sample/5] The phenolic content was determined using Julkenon Titto's formula. A 0.5 g sample of leaves was ground up in 5 ml of 80% acetone and centrifuged at 12,000 rpm for 15 minutes. Using 0.5 ml (500 ul) of the Folin-Ciocalteu' Phenol Reagent, the absorbance at 750 nm was measured using a picodrop spectrophotometer.

Using a spectrophotometer, the absorbance value was calculated at 520 nm. The same process was used with 2 ml of toluene to create a blank. The following formula was used to determine the free proline amount: Proline (µ moles)/g fresh weight = [(µg proline/ml × ml toluene)]/ [115.5 µg/u mole]/[(g sample/5] To determine the phenolic contents a 0.5 g of leaf samples were ground in 5 ml 80% acetone and centrifuged for 15 minutes at 12,000 rpm. The 0.5 ml (500 ul) of Folin-ciocalteu' phenol reagent was used to determine the absorbance value at 750 nm using a picodrop spectrophotometer. Eighty percent acetone was used to calibrate the instrument. The standard curve was created using the tannic acid standard solutions (100 ug/ml stock). Based on Bradford's (1976) methodology, the total soluble proteins of the collected samples were calculated. Leaf samples were treated with 2 percent dinitrophenyl hydrazine and one drop of 10 percent thiourea at 535 nm to determine the ascorbic acid concentration. Additionally, glycine betaine was calculated using Grieve and Grattan's techniques (1983)**.** A leaf sample 0.5 g was mixed with 20 ml of deionized water, homogenised, and shaken for 24 hours at 25°C. The extracts were then diluted with 2N H2 SO4 at a 1:1 ratio. Following that, 0.5 ml of the diluted extract was put into centrifuged tubes. After cooling down in an ice bath for one hour, the centrifuged tubes were added a cold IK-I2 reagent. The tubes' contents have undergone a

gentle vortex mixing. The tubes were then centrifuged at 1,000 rpm for 15 minutes at 0°C after being kept at 4°C for 16 hours. The fine-tipped glass tube was then used to extract the supernatant. The per iodide crystals were dissolved in 9 ml of 1,2-dichloroethane, and then the mixture was vigorously mixed in a vortex until the crystals were completely solubilized. After 2 to 2.5 hours of keeping the solution at room temperature, the absorbance value was measured at 365 nm. After that, the standard curve was used to compare the absorbance results.

Antioxidative enzyme activity is affected by phytoplasma infection:

Date palm leaf samples were spent centrifuged for 15 minutes at 4 °C at 15,000 rpm. Antioxidants have been isolated, including peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD). The efficacy of SOD to prevent the photoreduction of nitrobluetetrazolium (NBT) was then evaluated using the method of Giannopolitis and Ries technique (1977). While the protein weight of the extracts was quantified in accordance with Bradford's method, CAT, Poly-phenol oxidase (PPO), and POD were evaluated using Chance and Machly's method (1955), with minor changes, while the protein weight of the extracts was measured following the method of Bradford (1976).

Date palm Tissue culture:

The procedure of date palm tissue culture:

In our study the tissue culture of the date palm was achieved using organogenesis indirectly.

Firstly the offshoot (young seedlings) from the mother plant was separated carefully by cutting the bottom of the young palm seedling (3 years old) with a knife and discarding it. The offshoot length was 15 to 50 cm. Then, the obtained offshoot was cut until a soft whit meristematic part (with a lignified base) is appeared. The final size of the meristem explant was 3-4 cm in width and 6-8 cm in length.

Prior to the culture process, the explant with its lignified base was soaked in an antioxidant solution of 150 mg/l citric acid and 100 mg/L ascorbic acid to prevent tissue browning (Zaid *et al.,* 2011). The branch tip was then cleaned for 20 minutes with a 4 g/l Topsin M fungicidal solution. After that, rinse the tip three times with distilled water that has been sterilized. Then, place the tip under a weak vacuum for five minutes and under normal pressure for twenty minutes while dipping it in a 10% NaOCl (sodium hypochlorite) solution with a few drops of Tween 20.

1. **Explant Disinfection:**

Prior to the cultivation step, the meristem explant with its lignified base was soaked in an antioxidant solution of 150 mg/l citric acid and 100 mg/L ascorbic acid to stop the tissues from turning brown (Zaid *et al.,* 2011). The meristem explant was then cleaned for 20 minutes with a 4 g/l Topsin M fungicidal solution. After that, thoroughly clean the tip by rinsing it three times with distilled water. Next, immerse the meristem explant in a 10 percent sodium hypochlorite (NaOCl) solution containing Tween 20 for 20 minutes while maintaining a slight vacuum for the first five minutes.

2. **Explant Culture and starting stage:**

The explant meristem was dissected in the laminar flow hood without being rinsed under sterile circumstances, and it was then grown on the initiation (beginning) medium. Young leaf bases—the area between the leaf and the young tissue—are grown as explants. Starting media is made up of MS media that has been supplemented with 0.1–0.3 mg/L of growth regulators and small amounts of ammonium salts. The media's pH was then adjusted to 5.7 after being added to agar, but before autoclaving.

Then, all cultures have been incubated in computerized growth chamber in complete darkness., after 3-4 months, then the cultures transferred into a low light with 16 hours of photoperiod and 8 hours darkness.

3. **Multiplication Stage:**

Transferring the begun cultures to a multiplication medium. The initiation media's components also made up the multiplication media. The composition and kind of growth regulators are the only things that differ. IAA, NAA, BA, NOA, and Kinetin were added to the multiplication media in concentrations ranging from 0.5 to 5 mg/L. After 3 to 4 weeks, the produced plantlets were moved and sorted.

4. **Rooting and Elongation Stage:**

After three to four weeks, the generated plantlets are moved to a rooting medium, which is basic MS media that has been supplemented with auxins (hormones) for root formation, such as NAA (1 mg/L), BAP (0.5 mg/L), Kinetin (0.5 mg/L), and GA3 (0.5 mg/L), for 10-15 days. Following this, the plantlets were moved to a swelling medium, which was similar to the multiplication media stage with the exception of the increased sucrose concentrations (100-150 g/L).

5. Acclimatization of the produced Plantlets:

There are a few parameters that are used to decide whether the plantlets are ready to be sent to the greenhouse for the acclimatization process. These criteria consist of the following: 1) The plantlets ought to have between two and three healthy leaves that are either elongated or expanded and do not curl. 2) From the base up to the leaves, the stem height should be between 1 and 10 centimeters at the very least. 3) The crown of the shoot base should be in the shape of a pear. 4) A robust root system that is five centimeters in length.

If the produced plantlets have these characteristics, then they are ready for the acclimatization stage. For acclimatization process, the plantlets have been washed very well with distilled water to get rid of the agar residue from the roots. Then, the plantlets were soaking or sprayed with 0.5% Topsin M or Belnlet fungicide solution to protect the plantlets from fungal attacks, the plants were observed carefully daily. Healthy date palm plants are ready to the open field after a few weeks from the acclimatization stage.

RESULTS

Date palm phytoplasma identification:

The intensity of each symptom was variable in each sample of date palm leaves used in this investigation, including yellow streaks, stunted new leaves, and smaller fruits (figure 1). On the farm, border palms showed more pronounced symptoms than interior ones. Using fU5/rU3 primers, the typical DNA samples from this investigation yielded positive nPCR findings (Figure 2), indicating the presence of phytoplasma infection (Figure 2). The five date palm cultivars used in this study varied in how contagious they were., regardless of where on the farm they were located, some cultivars demonstrated resistance to or susceptibility to phytoplasma infection. Only one of the five cultivars, Magdoul, showed no symptoms, tested negative for phytoplasma infection by PCR, and showed no amplification, indicating that this cultivar is likely more resistant to or less vulnerable to phytoplasma infection. The severity of the symptoms varied across the other 4 cultivars (Khalas, Sokari, Barhi, and Zaghlul), but PCR results showed that all of them were infected. The extracted and cleaned nPCR products have been analyzed on a 1% agarose gel using an Invitrogen gel extraction kit.

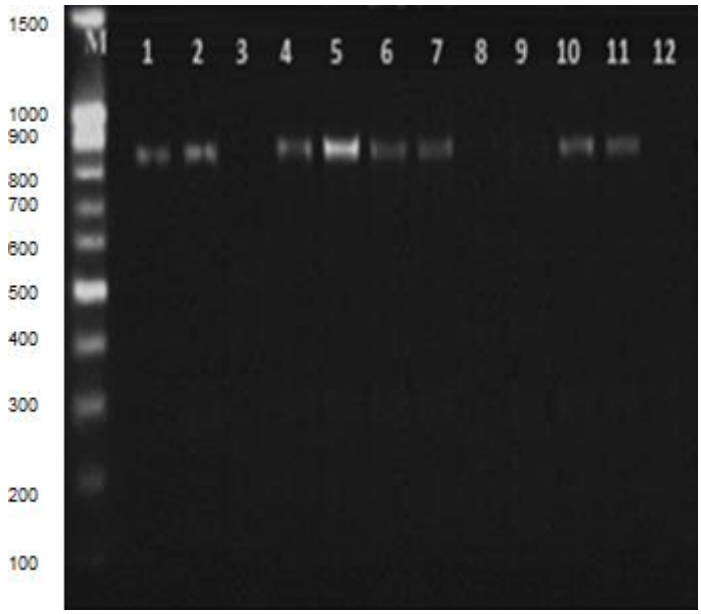

Fig. 2. Gel electrophoresis of nested PCR of primers fU5/rU3. Shown positive nPCR product of 880 bp. Lane1: +v positive control, Lanes 2, 3 Khalas cv., lanes 4, 5 Sokari cv, lans 6 , 7 Barhi cv, lans 8 , 9 Magdoul cv, and lanes 10 , 11 Zaghloul cv. and lane 12 −ve: healthy control, , M: 1Kb DNA marker (Invitrogen™, USA).

Metabolites produced by phytoplasma infection:

Chlorophyll a, b, and total chlorophyll concentrations were dramatically reduced in phytoplasma-infected plants, as indicated in Table 1, among other primary metabolites. Additionally, the chl a/b ratio dropped. Otherwise, soluble sugar was decreased while carotenoid was raised (Fig. 3). Proline, on the other hand, was more abundant in the plants with phytoplasma infection.

Antioxidant enzyme activity is affected by phytoplasma infection:
As can be seen in Table 2, the activities of all of the antioxidants, including peroxidase, polyphenol oxidase, catalase, and oxidase, have increased in the plants that have been infected with phytoplasma (Fig.4).

Table 1. Alteration in primary and secondary metabolites in phytoplasma-infected date palm cultivars.

Primary Metabolites	Healthy Plants (mg kg^{-1})	Infected Plants (mg kg^{-1})
Chl a	3.898 ± 0.028	1.201 ± 0.053
Chl b	4.136 ± 0.027	1.562 ± 0.313
Total chl	5.216 ± 0.125	1.221 ± 0.313
Carotenoids	3.122 ± 0.129	1.296 ± 0.131
Soluble sugar	2.799 ± 0.183	1.818 ± 0.338
Proline	0.765 ± 0.054	1.368 ± 0.121
Ascorbic acid	23.512 ± 1.58	14.723 ± 1.872

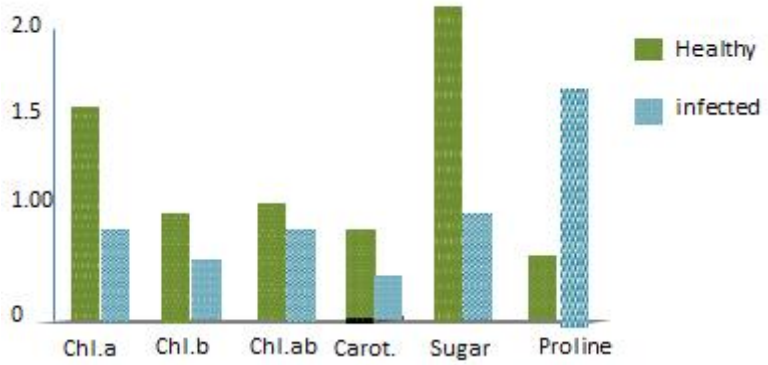

Fig.3. Metabolites produced by phytoplasma infection

Table 2: Antioxidant alterations in phytoplasma-infected date palm varieties.

Anti-oxidants	Healthy Plants (mg kg^{-1})	Infected Plants (mg kg^{-1})
Peroxidase	0.279 ± 0.052	1.153 ± 0.281
Poly-phenol oxidase	0.065 ± 0.035	0.186 ± 0.077
Catalase	0.79 ± 0.161	2.242 ± 0.633
Oxidase	0.290 ± 0.052	1.368 ± 0.182

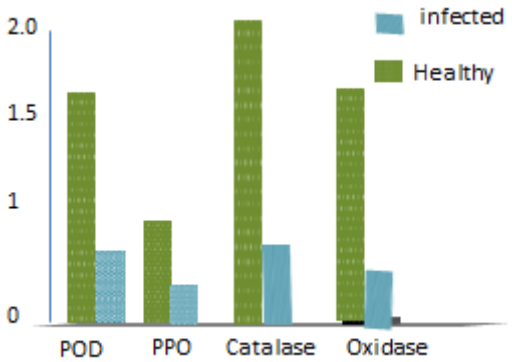

Fig. 4 . Antioxidant enzyme activity is affected by phytoplasma infection.

Production of date palm plants free phytoplasma using tissue culture technique:

When the resulting date palm plants from in vitro culture were propagated and all the samples were examined by nPCR, none of the created plants yielded positive results. In contrast to the phytoplasma-infected plants, the phytoplasma-free plants expanded quickly (Table 3 and Figure 5). According to the findings in Table 3, the in vitro culture could produce 100% healthy and clean plants. Overall, all plants examined were cleaned to 100% using tissue culture technology.

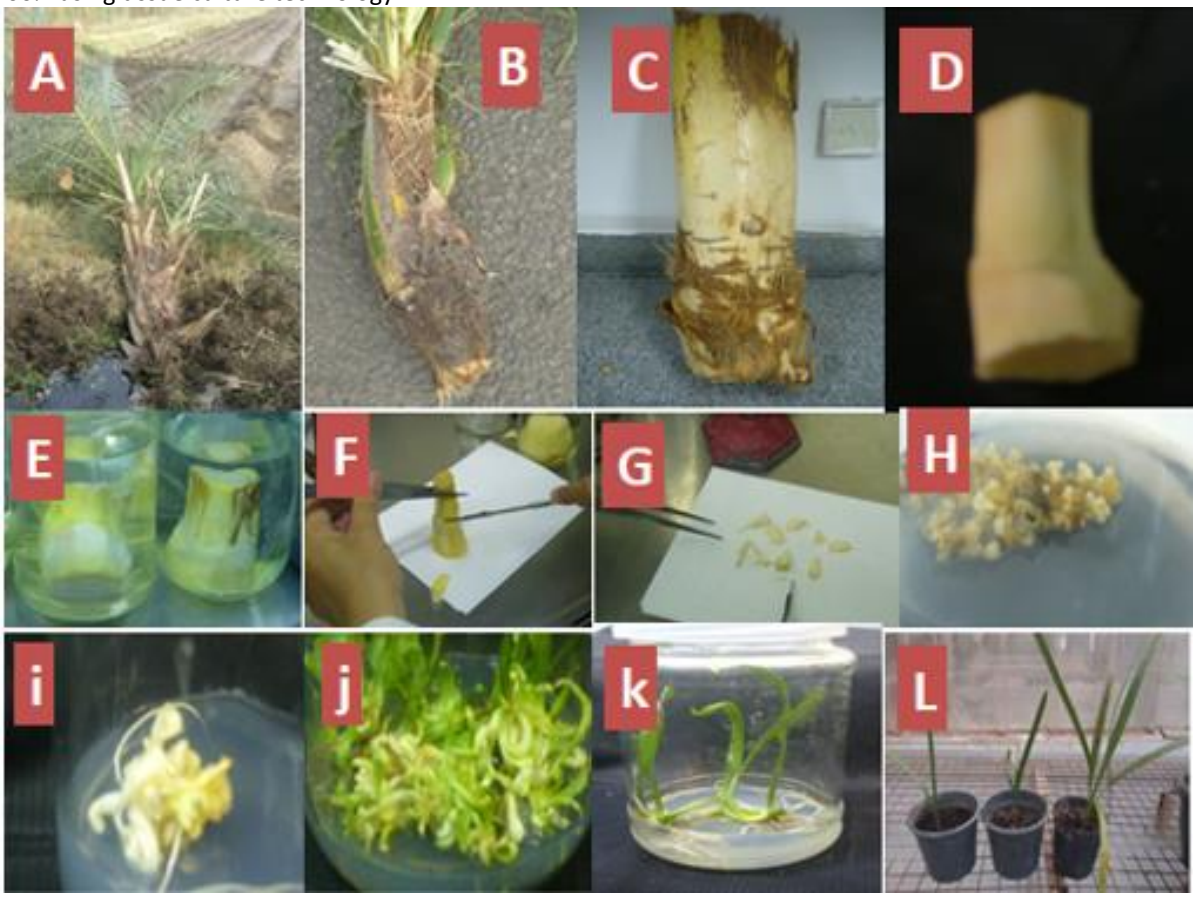

Fig. 5. Illustration of the different date palm regeneration stages via organogenesis. (A &B), offshoot of date palm source. (C), Offshoot preparation and removal of external leaves. (D&E), Offshoot shoot tip ready to be disinfected. (F&G), Explants from shoot tip offshoot at the starting stage. (H), Embryogenic callus induction. (I), Embryogenic callus proliferation. (J), shoot multiplication cultures. (K), Rooting of date palm plantlets and (L), acclimatized in vitro plants free phytoplasma.

Table 3. The health status of date palm plantlets after *in vitro* culture.

Cultivars	Plants tested	Positive plants	Negative plants
Barhi	3	00	3
Magdoul	3	00	3
Khalas	3	00	3
Sokari	3	00	3
Zaghloul	3	00	3

DISCUSSION

The most significant commercial crops in the area are probably infected by the wijam (phytoplasma), a dangerous and invasive disease in Egypt. The yearly production of dates has drastically declined, dropping from 128,807 tons in 2013 to 73,900 tons in 2015, according to agricultural statistics. Although the average production of dates per palm tree is thought to be around 48.0 kg, local cultivars have only averaged 41.82 kg of dates per palm tree up until 2013, compared to 20.38 kg per palm tree in 2016.

The current symptoms development of yellow streaking, stunting of new growths and fruit size reduction on the cultivated date palms have been investigated, such observations revealed a phytoplasma infection in the cultivated date palms in Giza governorate. These observations were similar to those that have been described previously for the infection of date palms with phytoplasma by (Cronje *et al.,* 2000; Al-Awadhi

et al., 2002; Abou-El-Einin, 2010; Alkhazindar, 2014). Representative samples were collected and immediately sent for diagnosis using nPCR. The preliminary results revealed the presence of 16Sr DNA phytoplasma infection, which was the basic of this study.

The amplified sequences were similar to one another and matched the 16S ribosomal RNA gene that had previously been described. The 5 date palm cultivars used in this study, regardless of where they were located on the farm, varied in their susceptibility to infection and tolerance to it. Only one of the five cultivars, Magdoul, displayed no symptoms, tested negative for PCR amplification, and exhibited no amplification, indicating that this cultivar is likely more resistant to or less vulnerable to phytoplasma infection. The symptom intensity of the other 4 cultivars (Barhi, Khalas, Sokari, and Zaghlul) varied although the PCR findings were 100% positive for infection.

Date palm micropropagation is a cost-effective strategy for rural development (Rajmohan, 2011; Reineke 2019., Quaglino *et al.* 2019; Jakovljevi *et al.,* 2020). Date palm micropropagation procedures have been established and successfully used in commercial production, it has been shown (Hoop, 2000). Micropropagation of date palms by means of somatic embryogenesis and organogenesis is contingent upon a few variables. These variables include browning of the tissue, explant source, explant age, explant size, used cultivars, light intensity and quality, temperature, pH of the medium, plant hormones, culture medium composition, and culture age. Browning of the tissue causes the tissue to become less viable for micropropagation. When compared to other crops, the efficiency of producing date palm plants utilising tissue culture was improved (Hazubska-Przybył *et al*., 2020; Mona M. Hassan, 2021). Additionally, date palm micropropagation is a promising technique that eliminates phytoplasma and other infections while reducing labour costs and time requirements. Additionally, growing date palm cultivars that are tolerant of or resistant to phytoplasma near the farm's boundaries will likely lessen or eliminate the phytoplasma transmission by leaf hopper insect vectors. Furthermore, to lessen the spread of the phytoplasma disease, affected palms should receive oxytetracycline antibiotic treatments along with other antibiotics. Future surveys must keep track of the spread of the Al-Wijam illness in Egypt, and further research is needed to characterise the 16S rDNA phytoplasma species that infect the palms and act as the disease's vector. A straightforward method for producing phytoplasma-free, healthy planting material from plants with advanced symptoms is in vitro culture of phytoplasma pathogen-infected date palm cultivars. Only tissue cultures can provide us with plants that are 100% free of phytoplasma and other diseases.

CONCLUSIONS

The findings of this study provide evidence that the Date Palm phytoplasma pathogen is present in Egypt and reveal the prevalence of the disease in the area that was investigated. These findings should serve as a wake-up call to begin looking for a remedy, a treatment, and the development of management programs. It is possible to remove phytoplasma and other infections using an identical strategy, which is the production of date palm free plants using the tissue culture technique. The research also shows that different date palm cultivars have different levels of vulnerability to phytoplasma infection. The researchers advise that tolerant date palm cultivars be planted around the perimeters of date palm plantations in order to prevent the illness from spreading. To find a solution to stop the spread of the date palm phytoplasma disease in the region, further physiological studies and additional molecular data are required. This is because understanding the means by which the disease is transmitted as well as its interactions in the vectors and host plants is necessary in order to do so.

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أنتاج شتلات نخيل البلح الخالية من الفيتوبلازما (الويجام) باستخدام زراعة الأنسجة في مصر

طارق السيد عبدالباسط* و خالد ابراهيم عواد صقر و احمد النبوي الشوربجي و أميرة مجاهد اسماعيل

معهد بحوث امراض النباتات، مركز البحوث الزراعية

*بريد المؤلف المراسل drtarekelsayed71@gmail.com

مرض ويجام (فيتوبلازما) من نخيل البلح هو تهديد اقتصادي لإنتاج نخيل البلح في مصر. تم تحديد أعراض المرض في مختلف بساتين النخيل المزروعة. تم استخدام تفاعل البلمرة المتسلسل لتحليل ما مجموعه عشر عينات من خمسة أصناف مختلفة من نخيل التمر. جاءت هذه العينات من الأشجار السليمة والمريضة. كشفت نتائج تجربة تفاعل البوليميراز المتسلسل المتداخل عن وجود جزء من الحمض النووي الريبي فيتوبلازما في الأشجار المصابة. علاوة على ذلك ، لم تظهر أي عدوى على أحد أصناف نخيل البلح (المجدول) ، بينما أظهرت الأنواع الأربعة الأخرى (خلاص وسكري وبارحي وزغلول) درجات متفاوتة من العدوى. تم تقليل محتوى الكلوروفيل أ ، ب ، والكلوروفيل الكلي بشكل كبير بسبب التغيرات البيوكيميائية. وفي الوقت نفسه ، زادت مستويات البرولين والكاروتين في النباتات المصابة بينما انخفضت مستويات حمض الأسكوربيك والسكر القابل للذوبان. زادت أنشطة الإنزيمات المضادة للأكسدة مثل بوليفينول أوكسيديز وبيروكسيديز وكاتلاز وأوكسيديز في الأشجار المصابة بالبلازما النباتية. تم عرض تقنية لتكوين الأعضاء في المختبر لنشر أصناف نخيل التمر. تمت زراعة ثقافات الأطراف الإنشائية المعزولة على وسائط التصلب المتعدد المكملة بـ 2,4-د 100 مجم/لتر و 1.5 جم/لتر من الفحم لمدة أربعة إلى تسعة أسابيع ، اعتمادا على الصنف. للسير الذاتية. استخدمت اصناف " مجدول "و" بارحي " ، وتجديد الكالس ، واستطالة النبتة ، والضرب على وسائط التصلب المتعدد التي تحتوي على 1 ملغم/لتر نا و 1.5 جم/لتر من الفحم المنشط.

الكلمات المفتاحية: نخيل التمر ، الفيتوبلازما ، انزيمات الاكسدة ، زراعة الانسجة