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Management of Phytoplasma associated with sesame (*Sesamum indicum* L.) in Assiut governorate, Egypt

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Abstract

Phytoplasma associated with sesame can cause serious economic losses in sesame production in Assiut governorate, Upper Egypt. Various primary symptoms indicating phyllody disease were noted, including proliferation (witches' broom), yellowing, and the transformation of capsules into flowers, resulting in a significant decrease in sesame yield. Phyllody symptomatology and incidence were studied in three sesame varieties (Giza 32, Shandawel 3, and Sohag 1). Four irradiation treatments It was irradiated using Cobalt 60 at different doses (150, 200, 250, and 300 Gy of gamma rays). Data showed that Shandawel 3 was categorized as a moderately resistant cultivar, while Sohag 1 was grouped as resistant and Gize 32 was categorized as moderately susceptible. This study also revealed that all irradiation treatments (150, 200, 250, and 300 Gy) reduced the percentage of infected rate and disease severity caused by phytoplasma. The study recommends that using resistant varieties is an efficient and sustainable approach to controlling susceptibility to phytoplasmas in sesame.

Keywords: sesame, symptoms, Phytoplasma detection, varieties, irradiation.

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1. Introduction

Sesame (*Sesamum indicum* L.) of the Pedaliaceae family serves as an ancient oilseed crop. Sesame is considered to have both nutritional and medicinal values. Sesame plants suffer from several fungal, viral, bacterial, and phytoplasma diseases. Phytoplasmas are cell-wall-less bacteria that have very small genome sizes and are considered among the smallest self-replicating living organisms (Bertaccini et al., 2014). Phytoplasma can induce diverse types of symptoms in sesame plants. The unique symptoms induced by phytoplasma infection are stunting, altering the color of leaves from green to yellow, and the transformation of the floral parts into leafy structures containing no capsules or seeds. The most prevalent symptoms of sesame are phyllody and witch's broom. In phyllody symptoms, the flowers of the infected plants turn similar to leaves, while in witches 's broom symptoms, the leaves of the infected sesame plants tend to gather at the top or one side of the infected sesame plants, giving the appearance of witch's broom. Phytoplasma associated with sesame is recognized as a serious risk for the cultivation of sesame in several countries, including Egypt, producing yield losses of up to 33.9 percent in yearly output (Abraham et al., 1977). The phytoplasma are among the obligate pathogens of plants and exist in the phloem tissues of the infected host plants. phytoplasma are being transmitted mainly by insect vectors. The fact that phytoplasma cannot be cultivated on artificial mediums and can only be maintained in their plant hosts has made the research of phytoplasma highly arduous and complex. During the previous years, PCR has been utilized for the identification of a huge range of bacteria, including phytoplasma. Several approaches have been developed for the universal detection of phytoplasma. The main difference between these methods is the type of primers used in PCR reactions. The primers used in PCR to detect phytoplasma infecting plants are normally designed to amplify a specific region in highly conserved ribosomal (rDNA) genes. Nested-PCR assays can increase both the sensitivity and specificity of phytoplasma detection in plant samples. The aims of this study are to confirm the presence of phytoplasma in symptomatic sesame plants presenting evident phyllody symptoms by employing polymerase chain reaction (PCR), describe the main symptoms associated with the disease in Assiut governorate, and evaluate certain sesame varieties for their reaction to phytoplasma infection under greenhouse conditions. As well as the application of some cobalt irradiation treatments on sesame seeds to combat this destructive disease.

2. Materials and methods

2.1 Source of samples

The phytoplasma causal pathogen was isolated from symptomatic sesame plants showing typically symptoms of phytoplasma collected from different fields in Assiut Governorate, Egypt. All samples underwent DNA extraction, molecular detection, and identification at the Molecular Biology Unit, Assiut University, Egypt. The DNA extraction was stored at -20°C for subsequent tests.

2.2 Molecular identification of Phytoplasma

2.2.1 DNA extraction

The plant samples were disrupted using a T mortar and pestle (100 mg wet weight or 20 mg lyophilized tissue). Subsequently, 400 ml of Buffer AP1 and 4 ml of RNase A were added to each sample. After Vortex, the samples were incubated at 65°C for 10 minutes, and the tubes were rotated two or three times. 130 ml of Buffer P3 were added to each tube and incubated for five minutes.

2.2.2 PCR amplification, cloning and sequencing

Universal Primers for Detection of Phytoplasma (S54LP of SP_F 50-C ATG GAG GCC GAATTC ATG TTT AAA ATC AAA AAT AAT TTA-30 and S54LP of SP_R 50-GC AGGTCGACGGATCC TTA TTT TCA TCA TTT AAA GTT TTT-30) (Maejima et al., 2014) were used to confirm the presence of Phytoplasma in symptomatic sesame plants. The PCR outcomes were analyzed on a 1% agarose gel, stained with ethidium bromide, and observed under UV (Sambrook and Russell, 2001).

2.3 Plants inoculation with phytoplasma (Pathogenicity test)

Pathogenicity was carried out on sesame (Giza 32 cultivar). Sesame plant tissues exhibiting characteristic phytoplasma symptoms were collected and mashed in sterilized water with the use of a pestle and mortar, and then pressed through extremely fine muslin material. Mechanically inoculated with the freshly extracted sap using a syringe injection in the stems of plants at different ages (4, 6, 8, and 10 weeks after planting), four pots were used for each stage. Plants were washed with a mild stream of water immediately after inoculation to eliminate extra inoculum and put in insect-free cages for symptom development. The isolate proved its pathogenic capability in the pathogenicity test, as it produced typical symptoms of phytoplasma, including phyllody, stunting, yellowing, and witch's broom symptoms. Percentages of infection plants were recorded at the end of the growing season. The following equations were utilized to calculate the disease incidence:

$$\text{Percentage of infected plants} = \text{Number of diseased plants} / \text{total number of plants} \times 100$$

2.4 Response of certain sesame cultivars to phytoplasma infection

The response of three sesame cultivars (Giza 32, Shandawel 3, and Sohag 1) to phytoplasma disease was evaluated under greenhouse conditions by recording the incidence of phytoplasma infection (incidence percentage calculated on the basis of diseased plants over the total plants assessed), as mentioned above. Also, disease severity was recorded using a scale of 0–6. Incidence and disease severity were recorded according to Akhtar et al. (2013), where 0 = no phytoplasma infection (highly resistant), 1= 1–10 percent plant infected (resistant), 2= 10.1–30 percent plant infected (moderately resistant), 3= 30.1–50 percent plant infected (moderately susceptible), 4= 50.1–75 percent plant infected (susceptible), 5= more than 75.1 percent of plants infected (highly susceptible). Disease severity was converted into a percentage as follows:

$$Disease\ severity\ (\%) = \frac{(0A\ +\ 1B\ +\ 2C\ +\ 3D\ +\ 4E\ +5F)}{Maximum\ grade\ rate\ \times\ number\ of\ plants\ (5T)} \times 100$$

Where A, B, C, D, E, and F are the number of leaf plants corresponding to the numerical grades 1, 2, 3, 4, and 5, respectively, and 8 is the total number of plants multiplied by the maximum disease grade (5).

2.5 Management of Phytoplasma associated with sesame through irradiation

Four irradiation treatments (sesame seeds were irradiated using Cobalt 60 at different doses of 150, 200, 250, and 300 Gy of gamma rays) were evaluated under greenhouse conditions in 2022 for phytoplasma incidence.

2.5.1 Seed radiation treatments

A specified quantity (2 g per dosage) of dry, homogeneous, and healthy seeds of Giza 32 cultivar of sesame were exposed to radiation using a Co60 (Cobalt 60) gamma source with varying dosages (150, 200, 250, and 300 Gy) of gamma rays at Assiut University, Faculty of Science, Department of Nuclear Physics. Five pots were used as replicates, and each pot was seeded with eight disinfected sesame seeds. Sesame plants at age 8 weeks were challenged with phytoplasma, and data on disease incidence and disease severity were recorded after 3 weeks of challenging sesame plants with phytoplasma.

2.6 Statistical analysis

Data was analyzed to determine the relevance of variations among treatments with regard to phytoplasma disease severity. Once F-values were significant ($P<0.05$), means were compared using the least significant difference (LSD) test (Gomez & Gomez, 1984).

3. Results and Discussion

3.1 Samples source

Sesame diseased and healthy samples were obtained from Assuit Governorate, Egypt. All collected samples were showing clear symptoms of phytoplasma infection such as phyllody, green leaf like floral, proliferation and virescence except the healthy plant as shown in Figure (1).

3.2 Symptoms of phytoplasma on sesame plants

Different forms of phytoplasma symptoms were identified on sesame plants. phytoplasma disease symptoms found in the samples obtained from the field (Figure 1). The most recognizable signs of the illness are the transformation of floral parts into green leaf-like structures, followed by extensive vein cleaning in different floral sections. The ovary is replaced with elongated structures, nearly like a shoot. The calyx becomes polysepal, while the sepals become leaf-like and remain smaller in size. The phytoplasma flowers become actiomorphic in symmetry, and the corolla becomes polypetalous and deep green. The veins of the bloom become thick and fairly noticeable. The stamens keep their form but become flattened, exhibiting a predisposition to be leaf-like. The anthers turn green and contain aberrant pollen grains. The carpals are changed into a leaf fusion at the borders, and this false ovary enlarges and flattens, presenting a smooth feel and a wrinkled surface due to the thickening of capillary wall veins. Instead of ovules inside the ovary, there are little petiole-like outgrowths, which eventually expand and burst through the walls of the false ovary, giving small shoots (Figure 1). These branches continue to develop and generate new leaves and phytoplasma blooms. Plant diseases caused by the presence of phytoplasma often display a range of symptoms that are suggestive of changes in the normal balance of plant enzymes and hormones (Lee et al., 2000). Also, Youssef Sahar et al. (2018) observed that some symptoms in sesame are like phytoplasma symptoms such as grouping of branches of developing tissues, virescence, which is pigmentation of non-green flower parts to green, phyllody, formation of bunchy fibrous secondary roots, weakness of plants, reddening of leaves and stems, generalized yellowing, and phloem necrosis. Jomantiene et al. (1998) noticed similar symptoms in strawberries. Recently, the availability of four full-sequenced genomes has provided new chances for the establishment of effective control measures. As phytoplasmas lack the cell wall, their membrane proteins and released proteins work directly in the cytoplasm of the host plants and insects. Thus, the prediction of secreted proteins encoded in the phytoplasma genome is vital for understanding phytoplasma-host interactions. For example, SAP11, SAP54, and TENGU have been identified as phytoplasma effector proteins that regulate plant gene expression, resulting in a modification of plant morphology (MacLean et al. 2011; Sugio et al., 2011).

Figure 1: Phyllody symptom of sesame. A= A shoot showing internodes shortening with dense leaves (witches' broom). B= yellowing sometimes accompanied the disease. C= The capsules turn into flowers. D= Healthy sesame plant.

3.3 Molecular detection of sesame phytoplasma strains

PCR amplification was used for phytoplasma detection using the universal phytoplasma PCR primer P1/P7 (Figure 2). Nested primer R16F2n/R16R2 was used to confirm the infection with a product size range of about 1250 bp for all infected samples. This technique has been widely utilized for the detection of phytoplasma and is probably the most totally investigated. It recognizes all strains of phytoplasma, whereas no findings were obtained in health plants (Marzachi, 2004). The PCR approach readily separates plant and phytoplasma so that a screening of variations for the presence of phytoplasma might take place (Schneider et al., 1995). PCR has been widely used in the detection of many organisms, including viruses. The results have greatly contributed to determining the distribution and diversity of the phytoplasma in sesame plants.

3.4 Pathogenicity test

Results in Table (1) showed that the age of sesame plants affected the incidence of phytoplasma diseases. Whereas injecting the plants with phytoplasma after 8 weeks of cultivation resulted in a high infection rate in the plants with phytoplasma diseases. Injecting the plants in the 6th and 4th weeks gave a moderate phytoplasma infection. While injections of plants in the 10th week of cultivation gave the least percentage of infection with phytoplasma.

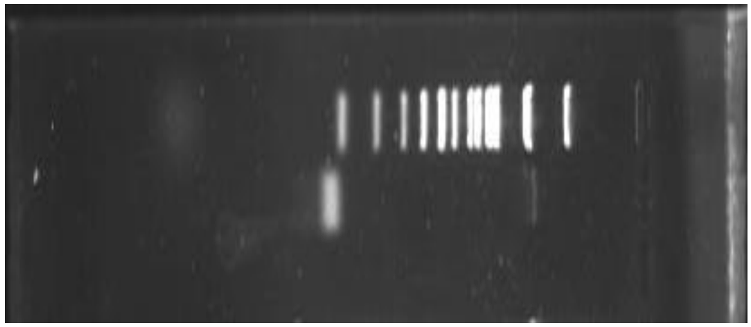

Figure 2: Gel electrophoresis of PCR product amplified using universal Phytoplamsa primers.

The findings revealed that the majority of the plants were infected between 8 and 9 weeks after seeding during the blooming season. Sesame plants infected before blossom initiation developed severe symptoms over the entire plant and demonstrated full sterility. This study, in accordance with Taye et al. (2019), found that the degree of the change of floral components into deformed structures was connected with the period of infection. However, plants infected during flowering exhibited severe symptoms on the top of the plants, occasionally followed by a few rudimentary blooms that released extremely small capsules with degenerated seeds. I also observed that sickness. Incidence progress in each treatment was a modest increase from 8 to 11 weeks after seeding. This study is in line with Akhtar et al. (2009), who found that the severity of the transformation of floral components into deformed structures was connected with the period of infection. However, plants infected during blooming exhibited severe symptoms on the upper part of the plants, occasionally followed by a few rudimentary blooms that delivered very little capsules, and yellowing was also apparent. The period of infection impacts the severity of the phyllody illness. Infections in the early stages of plant development had severe symptoms on the whole plant, and plants infected during blooming had severe symptoms on the top portion of the plant. Klein (1977) noted that plants infected early displayed signs such as stunting, loss in leaf size, and short internodes. It should be emphasized that the increased prevalence of the indirect symptoms in the research should be driven by the late seeding of the nursery beyond the regular sowing period.

Table 1: Infection percentage of sesame plants inoculated with phytoplasma.

Pathogen injection dates	Percentage of infection
4 weeks	30^{ab}
6 weeks	40^{ab}
8 weeks	60^{a}
10 weeks	15^{b}
L.S.D 5%	36.13

3.5 Response of certain sesame cultivars to phytoplasma infection

Data in Table (2) revealed that the lowest percentage of infection and disease severity in plants was observed in Sohag cultivars, as they showed disease incidence and disease severity of 7.5% and 2.5%, respectively, and could be categorized as tolerant cultivars. whereas the Giza 32 variety showed the highest percentage of infected plants, about 40%, and also showed the highest disease severity, so it could be categorized as moderately susceptible. Shandawel 3 cultivar also showed a small degree of resistance to phytoplasma infection as it developed disease incidence and disease severity up to 10% and 18.50%, respectively. These results indicate that sesame cultivars are different in their response to phytoplasma infection, and some sesame cultivars showed a degree of resistance to phytoplasma infection. Taye et al. (2019) discovered that sesame plants infected before blossom initiation exhibited severe symptoms on the entire plant and indicated full sterility. A lot of writers observed that the sesame crop was substantially impacted by phytoplasma disease in different regions (Singh et al., 2007; Win et al., 2010; Mahmoud, 2013). According to Akthar et al. (2013), from 133 sesame genotypes that were tested for two years, there were extremely significant variances in the degree ofPhyllody disease resistance achieved in the infection of all genotypes. Based on infection percentage values, none of the genotypes was classified as very resistant, while 7 were resistant, 9 were moderately resistant, 28 were tolerant, 33 were moderately susceptible, 23 were susceptible, and 33 were extremely vulnerable. The phytoplasma disease has a significant effect on lowering sesame output. The current investigation demonstrated that the tested sesame varieties have a distinct variation in the degree of resistance detected between the varieties and react differently (resistant, tolerant, and susceptible) against Phyllody disease. Sohag1 variety was somewhat resistant, while Shandwel 3 variety is tolerant, but Giza 32 variety was sensitive to phytoplasma illness. The use of resistant cultivars is recognized as an affordable and permanent technique for managing this phytoplasma disease. Resistant, tolerant, or immune plant varieties have so far been selected by phytoplasma inoculation, symptom observation, and variety selection (Jarausch et al., 2011), as well as by marker-assisted selection programs (Bisognin et al., 2009). Unfortunately, a limited range of plant species have shown resistance or tolerance to phytoplasmas.

Table 2: Response of certain sesame cultivars to phytoplasma infection under greenhouse conditions.

Cultivar	Percentage of infection (%)	Disease severity (%)
Shandawel 3	10.0^{b}	18.50^{ab}
Sohag 1	7.50^{b}	2.50^{b}
Giza 32	40.0^{a}	25.00^{a}
L.S.D 5%	12.967	19.005

3.6 Management of phytoplasma associated with sesame using cobalt seed irradiation

Four treatments by irradiation were applied to manage the sesame phytoplasma under a greenhouse in 2022. Data in Table (3) showed all treatments by irradiation (150, 200, 250, and 300 Gy) reduced phytoplasma infection. Seed treatment with 300 Gy as sesame plants treated with doase showed the lowest disease incidence and disease severity (2.50 and 19.50) was more effective in affecting the percentage of infected rate and disease severity, respectively, with phytoplasma, followed by 250 Gy (5.50 and 28.50) and 200 Gy (12.50 and 26.50), respectively. While treatment with 150 Gy had the least effect by rate (15 and 31.50), The study proved that cobalt irradiation could be a useful method to control phytoplasma associated with sesame plants under Assiut governorate conditions, and further studies are required to study the effect of cobalt irradiation under field conditions.

Table 3: Effect of cobalt seed irradiation on sesame phytoplasma infection under greenhouse.

Treatment	Infected plant (%)	Disease severity (%)
150 Gy	15.00[b]	31.50[a]
200 Gy	12.5[b]	26.50[a]
250 Gy	5.00[c]	28.50[a]
300 Gy	2.50[c]	19.50[a]
Control	40.0[a]	25.00[b]
L.S.D 5%	9.89	12.74

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