Plant regeneration in date palm (*Phoenix dactylifera*) cv. Khalas overcoming four different fungus contaminations by using biological control

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ABSTRACT

In the present investigation, date palm plant regeneration procedure overcoming four different fungus contamination using biological control was described. Three different concentration treatments of actinomycetes culture filtrate (5, 10 and 15%) from two isolates (Streptomyces bobilii and Streptomyces grisiobrunneus) were used to study their effect in overcoming four different fungi which contaminate in vitro date palm i.e. Fusarium moniliforme, Rhizoctonia solani, Macrophomina phaseolina and Alternaria alternata. Medium supplemented with S. bobilii surpassed medium supplemented with S. grisiobrunneus in regeneration characteristic values. Actinomycetes 10 % treatment proved to be the best for enhancing both survived explant and callus induction percentage, while 15% treatment proved to be the best for enhancing regenerated shoots per callus. Herein, we report improved regeneration system for Khalas cultivar, which is strongly needed for successful genetic transformation, overcoming fungi contamination destructiveness.

Key words: *Date palm, Khalas, Regeneration, Contamination, Biological control.*

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) of the family *Arecaceae* not only is a key plantation crop but also a major fruit crop in most Arab countries (El-Juhany, 2010). The top ten producing countries were Egypt, Saudi Arabia, Iran, United Arab Emirates, Pakistan, Algeria, Sudan, Oman, Libya, and Tunisia (Kader and Hussein, 2009). Date palm has an environmental impact as well as economic importance, since it affords a main source of income for local farmers and linked industries. Also, Dates are a highly nutritious source for sugar, minerals, and vitamins and have been considered as an antiatherogenic nutrient (Al-Shahib and Marshall, 2003). "Khalas" is one of the most well-known palm cultivars in Saudi Arabia, as it has moderate sweetness level, and thus suits most people. Genetic engineering could help in improving numerous traits in date palms. The application of genetic engineering techniques in date palm requires the availability of a more advanced regeneration system. Date palm has recalcitrant nature to regeneration/ transformation. Also, date palm *in vitro* regeneration has prolonged procedures that extend more than one year which is also coupled with contamination, not only due to

date palm long term cultures, but also explant borne contamination. *In vitro* contamination in date palm appears to be a common problem with approximately 30 % of *in vitro* cultures loss (Veramendi and Navarro, 1997). Therefore, it is necessary to develop an *in vitro* protocol in order to overcome contamination complications, thus improving regeneration and transformation. Date palms under the Egyptian conditions are subjected to infection with different diseases caused by many soil-borne pathogenic fungi which may cause considerable losses (Baraka *et al*., 2011), whereas, the most virulent fungus was *Fusarium moniliforme* (Arafat *et al.*, 2012). Also, several fungi were evidenced as causal pathogens in Egypt, i.e. *Alternaria sp.; Macrophomina phaseolina;* and *Rhizoctonia solani* (El- Zawahry *et al.*, 2000; Sarhan, 2001; El-Morsi, 2004 and El Deeb *et al.*, 2007). The use of biocontrol agents have become commonly accepted as a "natural agent" and of a potential in replacing the use of chemicals and antibiotic for inhibiting the pathogenic organisms (Yuhana, 2010). Therefore, their application has revealed the potential as an effective strategy to reduce either chemicals or antibiotics in controlling the pathogenic agents which contaminate date palm *in vitro* cultures. Arafat *et al.* (2012), firstly showed that actinomycetes had the highest efficacy *in vitro* against soil-borne pathogenic fungi of date palms. The aim of the present investigation was to enhance date palm, cv. Khalas, regeneration characteristics *via* overcoming fungi contamination destructiveness. Herein, we report a reliable protocol using actinomycetes to overcome pathogenic fungi that cause contamination in date palm *in vitro* cultures.

MATERIALS AND METHODS

Two different actinomycetes treatments with three different concentrations and four different fungal treatments had been used to study their effects on regeneration characteristics, i.e. survived explant percentage (%), callus induction percentage (%) and regenerated shoots per callus and research was conducted as following:

Materials and explants

Outer leaves of 3-4 year-old offshoots of the date palm, cv. Khalas, were removed exposing shoot tip region, which was excised and placed in a chilled antioxidant solution mainly containing 150 mg/l of both ascorbic acid and citric acid to prevent browning. Shoot tip tissue (about 8 cm long) was surface sterilized with 70% ethanol for 1 min, then followed by 15 min with 30% (v/v) commercial Clorox (5.25% Sodium hypochlorite) supplemented with few drops of Tween 20. Tissues were then rinsed four times with sterile distilled water and placed in the sterile antioxidant solution. Subsequently, tissues surrounding the shoot tips were aseptically removed to expose the shoot tip terminal (about 1cm long), which was longitudinally cut into four pieces (5-mm long approx.). *Streptomyces bobilii* and *Streptomyces grisiobrunneus* isolated from soil by Dr. Abd El-Rahman Metwally, Central laboratory of Date Palm Researches and Development, Plant Protection Department, Agricultural Research Centre, Giza, Egypt. Each isolate were then subsequently grown on starch medium which mainly contains starch 20.0 g/l, K_2HPO_4 1 g/l, KNO_3 2 g/l, $MgSO_4$ 0.5 g/l, $CaCO_3$ 3 g/l, NaCl 100 g/l and 1 ml from trace element in solution which consists of 0.1 g/ml $FeSO_4.7H_2O$, 0.1 g/ml $MnCl_2.4H_2O$ and 0.1 g/ml $ZnSO_4.7H_2O$ for four weeks at 35°C, then filtered aseptically by

sterile Wat.1 double filter paper. Filtrates were received in sterilized beakers and added aseptically at three concentrations (5%, 10% and 15 %) to the different tissue culture media at different stages under investigation.

Callus induction

Explants were cultured individually onto callus induction medium (CIM) basically containing Murashige and Skoog salts and vitamins (Murashige and Skoog 1962) supplemented with 0.2 g L-glutamine, 30 g sucrose, 1 g charcoal, 6 g agar and 100 mg/l 2,4-Dichlorophenoxyacetic acid (2,4-D), 3 mg/l 6-(γ,γ-Dimethylallylamino) purine (2-ip). Subsequently, autoclaved medium was inoculated by 1 ml spore suspension from different tested fungus treatments, i.e. *Fusarium moniliforme, Rhizoctonia solani, Macrophomina phaseolina, Alternaria alternata* at concentration of 100 spore/ml. Cultured explants were then incubated at dark for three weeks from culturing at temperature of 27°C, then subcultured every three week intervals onto fresh CIM medium for six months.

Somatic embryogenesis

Induced calli were subcultured onto embryogenesis induction medium (EIM) containing Murashige and Skoog salts and vitamins, 0.2g L-glutamine, 30 g sucrose, 1 g charcoal, 6g agar and supplemented with 0.1 mg/l naphthalene acetic acid (NAA). Cultures were incubated for three weeks from subculturing at temperature of 27°C, and kept under white fluorescent light under 16 h /8 h light/dark cycle, then subcultured every three week intervals onto fresh EIM medium for three extra months.

Plant regeneration

Embryogenic callus induced were then subcultured onto shoot regeneration medium (SRM) which contains Murashige and Skoog medium salts and vitamins, 0.2 g L-glutamine, 30 g sucrose, 1 g charcoal, 6 g agar and supplemented with 0.1 mg/l NAA and 0.05 mg/l benzyl adenine (BA). Callus were incubated for three weeks from subculturing at temperature of 27°C, and kept under white fluorescent light under 16 h /8 h light/dark cycle, then subcultured every three- week intervals onto fresh SRM medium for three more months.

Rooting and acclimatization

Subsequently, regenerated shoots were subcultured onto rooting medium (RM) containing Murashige and Skoog salts and vitamins, 0.2 g L-glutamine, 30 g sucrose, 1 g charcoal, 6 g agar and supplemented with 0.1 mg/l NAA and 0.1 mg/l indole acetic acid (IAA). Regenerated shoots were then incubated for three weeks from subculturing at temperature of 27°C, and kept under white fluorescent light under 16 h /8 h light/dark cycle. Obtained rooted plantlets were then transferred into pots and successfully established in the greenhouse.

Statistical analysis

Data obtained were exposed to the proper statistical analysis of completely randomized design described by Snedecor and Cochran (1969), in three replicates. Means obtained were differentiated by using Duncan's new multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

To improve the distinctive date palm cultivar "Khalas" *via* biotechnology applications, it is critical to establish an efficient regeneration system defeating destructive contamination problems. This protocol will play an important role in opening the door widely for genetic transformation technology applications. The main aim of this work was to establish a reliable efficient regeneration system for cultivar Khalas using the antagonistic effects of two actinomycetes treatments, its concentrations and four fungus treatments causing *in vitro* contamination in date palm cultures.

Callus initiation and plant regeneration

It was evidently confirmed that improving plant regeneration characteristics *via* overcoming fungi contamination destructiveness is strongly needed for successful plant regeneration/transformation protocol in date palm.

I. Effect of actinomycetes

Results in Fig (1) represent the effect of the two actinomycetes treatments (*S. bobilii* and *S. grisiobrunneus*) on regeneration characteristics, i.e. survived explant percentage (%), callus induction percentage (%) and regenerated shoots/callus. It was indicated that *S. bobilii* had a slight increase than *S. grisiobrunneus* in survived explant percentages. On the contrary, callus induction percentage and regenerated shoots/callus were mostly alike across the two actinomycetes tested treatments. Regeneration characteristics values were in agreement with those recorded by Eshraghi *et al*. (2005) and Al-Khayri (2010).

II. Actinomycetes concentration effect

Data presented in Fig. (2) exhibit the effect of three actinomycetes concentration treatments (5, 10 and 15 %) on regeneration characteristics; i.e. survived explant percentage, callus induction percentage and regenerated shoots/callus. Data shows that increasing actinomycetes concentration treatment up to 10 %, survived explant and callus induction percentages were increased. On the contrary, by increasing concentration from 10% to 15%, survived explant and callus induction percentages decreased. However, regenerated shoots/callus increased by increasing actinomycetes concentration treatment up to 15%. It was concluded that actinomycetes concentration treatment acts in a specific manner against diversified tested fungal contaminations wherein the appropriate concentration treatment differs among different regeneration stages and, in turn, affects probably regeneration characteristics. These values obtained on date palm regeneration characteristics were most alike with those obtained by Bhaskaran and Smith (1992), Aslam *et al.* (2011), Al-Khayri (2011) and Khierallah *et al.* (2015).

III. Fungus effects

Results in Fig. (3) demonstrated the effect of studied fungi, i.e. *Fusarium moniliforme, Rhizoctonia solani*, *Macrophomina phaseolina* and *Alternaria alternata* on regeneration characteristics. Moreover, it indicated that plant regeneration characteristics responded variedly among different tested fungi. Survived explant percentage, callus induction percentage and regenerated shoots/callus were affected among the studied fungus treatments, whereas, *Alternaria alternata* had a tendency of superiority and achieved the highest values in regeneration characteristics, *i.e.* percentage of survived explant and callus induction

characteristics surpassing *Fusarium moniliforme*, *Rhizoctonia solani* and *Macrophomina phaseolina*, respectively. On the contrary, *Macrophomina phaseolina* scored the highest value of regenerated shoots/callus, while *Alternaria alternata* scored the lowest values. The differences among the studied fungus treatments in regeneration characteristics may be due to the virulent effect which differed among tested fungi treatments, which in turn could affect cell growth and differentiation process which in turn affect different regeneration characteristics. Regeneration characteristics values obtained were in agreement with those obtained by Othmani *et al.* (2009), Hassan and Taha (2012) and Boufis *et al.* (2014).

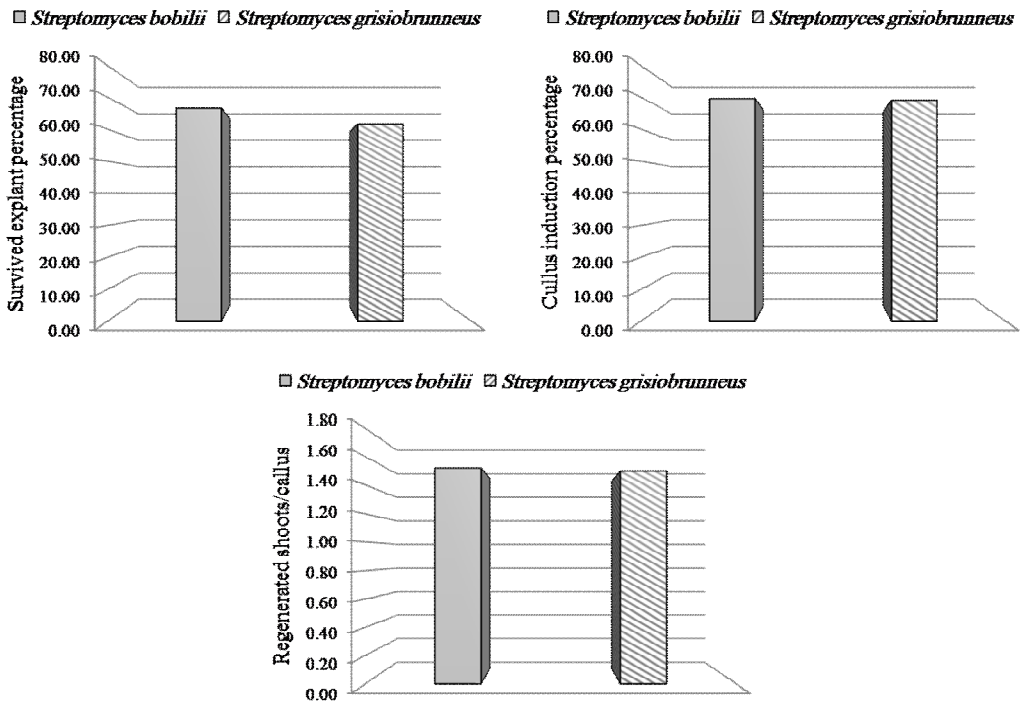

Fig. (1): Effect of actinomycetes on plant regeneration characteristics.

IV. Actinomycetes X actinomycetes concentrations interaction effect

Data presented in Table (1) show the effect of interaction between actinomycetes treatments **(***Streptomyces bobilii* and *Streptomyces grisiobrunneus*) and actinomycetes treatment concentrations (5, 10, and 15 %) on regeneration characteristics. Table (4) reveals that there was a slight significant response in percentage of survived explant and percentage of callus induction as affected by the combination between actinomycetes treatment and its concentration treatments. Survived explant percentage recorded the highest values when cultured on either *Streptomyces bobilii* or *Streptomyces grisiobrunneus* at 10 % treatment which was the most suitable treatment, while more or less treatment concentrations produced less value. It was noticed from Table (4) that there was a significant effect on number of shoots per callus as affected by the interaction between actinomycetes treatments and its concentration treatments. Highest values were scored when cultured on 15 % treatment under both actinomycetes tested treatments. On the contrary, *Streptomyces bobilii* or *Streptomyces grisiobrunneus* scored the lowest shoots per callus values when cultured at 5% treatment. It could be suggested that the differences in cell differentiation sensitivity to actinomycetes treatments and its concentration is probably due to the effect of antagonistic function which control in turn fungi and plant cell growth interactions. Results obtained in regeneration characteristics were in agreement with those achieved by Eke *et al.* (2005), Al-Khayri (2011) and Ibrahim *et al.* (2012).

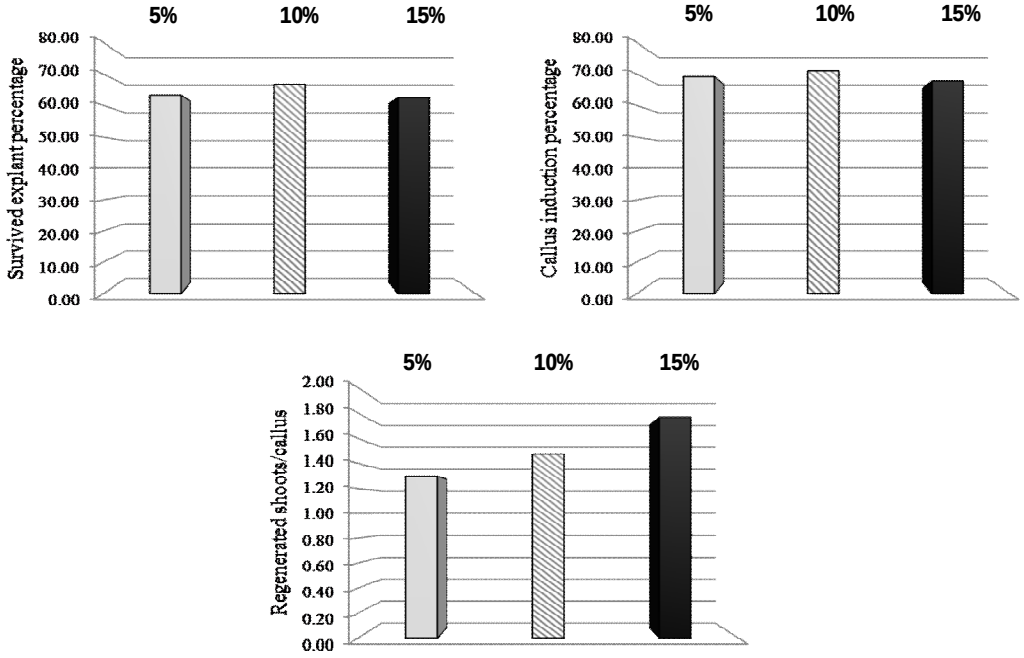

Fig. (2): Effect of actinomycetes concentrations on plant regeneration characteristics.

Table (1): Effect of interaction between actinomycetes and its concentration on regeneration characteristics.

Actinomycetes	Actinomycetes concentration		
	5 %	10 %	15 %
	1. Survived explant percentage (%)		
Streptomyces bobilii	65.00 AB	68.33 A	66.67 AB
Streptomyces grisiobrunneus	61.67 AB	65.00 AB	58.33 B
	2. Callus induction percentage (%)		
Streptomyces bobilii	69.44 AB	71.67 A	67.78 B
Streptomyces grisiobrunneus	68.89 AB	70.56 AB	67.78 B
	3. Regenerated shoots/callus		
Streptomyces bobilii	1.33 C	1.49 B	1.75 A
Streptomyces grisiobrunneus	1.26 C	1.45 B	1.79 A

Means followed by different capital letters are significantly different at P= 0.05 according to Duncan's multiple range test.

V. Actinomycetes × fungi interaction effect

Table (2) showed the interaction between the studied actinomycetes treatments and fungus treatments. Regeneration characteristics as measured by survived explant percentage (%), callus induction percentage (%) and regenerated shoots/callus were affected significantly at both actinomycetes treatments (*S. bobilii* and *S. grisiobrunneus*) among the four tested fungus treatments as shown in Table (1). *S. bobilii* and *S. grisiobrunneus* treatments with fungus treatment (*Alternaria alternata*) scored the highest values of regeneration characteristics as measured by survived explant percentage (%) and callus induction percentage (%), while *S. bobilii* and *S. grisiobrunneus* treatments scored the lowest values with *Macrophomina phaseolina* treatments. On the other hand, *S. bobilii* and *S. grisiobrunneus* treatments with fungus treatments (*Rhizoctonia solani and Macrophomina phaseolina*) scored the highest regeneration characteristics values as measured by regenerated shoots/callus, while *S. bobilii* and *S. grisiobrunneus* treatments scored the lowest values with *Alternaria alternata* treatment. The antagonism influence differences were observed on regeneration characteristics *via* different interaction treatments may be due to the presence of different *in situ* biochemical and physiological interactions in both plant and fungus on the cellular level, which may affect fungi growth and in turn cell differentiation manners. Similar regeneration characteristics values were reported on date palm by Hassan and Taha (2012) and Mazri (2014).

Table (2): Effect of interaction between actinomycetes and fungi on regeneration characteristics.

Actinomycetes	Fungi			
	Fusarium moniliforme	*Rhizoctonia solani*	*Macrophomina phaseolina*	*Alternaria alternata*
	1. Survived explant percentage (%)			
S. bobilii	75.56 B	55.56 CD	46.67 D	88.89 A
S. grisiobrunneus	62.22 C	51.11 D	46.67 D	86.67 A
	2. Callus induction percentage (%)			
S. bobilii	88.89 B	55.55 C	40.74 D	93.33 A
S. grisiobrunneus	85.93 B	56.29 C	41.48 D	92.59 A
	3. Regenerated shoots/callus			
S. bobilii	1.36 BC	1.70 A	1.76 A	1.27 C
S. grisiobrunneus	1.47 B	1.70 A	1.72 A	1.10 D

Means followed by different capital letters are significantly different at P= 0.05 according to Duncan's multiple range test.

VI. Actinomycetes concentrations × fungi interaction effect

Regeneration characteristics, i.e. survived explant percentage (%), callus induction percentage (%) and regenerated shoots/callus obtained were affected significantly (Table 3) by the interaction between fungus treatments and actinomycetes concentration treatments. Actinomycetes treatment concentration of 5 % with *Fusarium moniliforme* fungi treatment scored its highest survived explant percentage values. On the contrary, both 10 and 15 % treatment concentration scored its highest survived explant percentage values with *Rhizoctonia solani* treatment and also *Alternaria alternata* treatments showed the same attitude, while *Macrophomina phaseolina* fungus treatment responded best at 5 and 10 % concentration treatment. Data recorded in Table (3) confirmed that 10% concentration treatment scored the highest callus induction percentage values with *Fusarium moniliforme* and *Rhizoctonia solani* fungus treatments. On the contrary, 5 % concentration treatment achieved the highest callus induction percentage values with *Macrophomina phaseolina* fungus treatment. On the other hand, both concentration treatments of 10 and 15 % with *Alternaria alternata* fungus treatment responded best. In contrary, regeneration characteristics as measured by regenerated shoots/callus (Table 3) demonstrated that 15 % concentration treatment achieved the highest response with all four tested fungus treatments. It could be suggested that these differences in regeneration characteristics response to actinomycetes treatment × fungi treatment concentrations interaction may owe to the fungi genetic variation which in turn act to affect whole regeneration process. Regeneration characteristics data recorded are in the same trend of those recorded by Mazri, (2012) and Al-Khayri and Ibraheem (2014).

Table (3): Effect of interaction between actinomycetes concentration and fungi on regeneration characteristics.

Actinomycetes concentration	Fungi			
	Fusarium moniliforme	Rhizoctonia solani	Macrophomina phaseolina	Alternaria alternata
	1. Survived explant percentage (%)			
5 %	73.33 BC	46.67 EF	50.00 EF	83.33 AB
10 %	70.00 C	56.67 DE	50.00 EF	90.00 A
15 %	63.33 CD	56.67 DE	40.00 F	90.00 A
	2. Callus induction percentage (%)			
5 %	88.89 B	54.44 D	43.33 E	90.00 B
10 %	90.00 B	57.78 D	42.22 E	94.44 A
15 %	83.33 C	55.55 D	37.78 F	94.44 A
	3. Regenerated shoots/callus			
5 %	1.18 F	1.47 D	1.39 DE	1.12 F
10 %	1.39 DE	1.67 C	1.67 C	1.16 F
15 %	1.68 C	1.95 B	2.16 A	1.27 EF

Means followed by different capital letters are significantly different at P=0.05 according to Duncan's multiple range test.

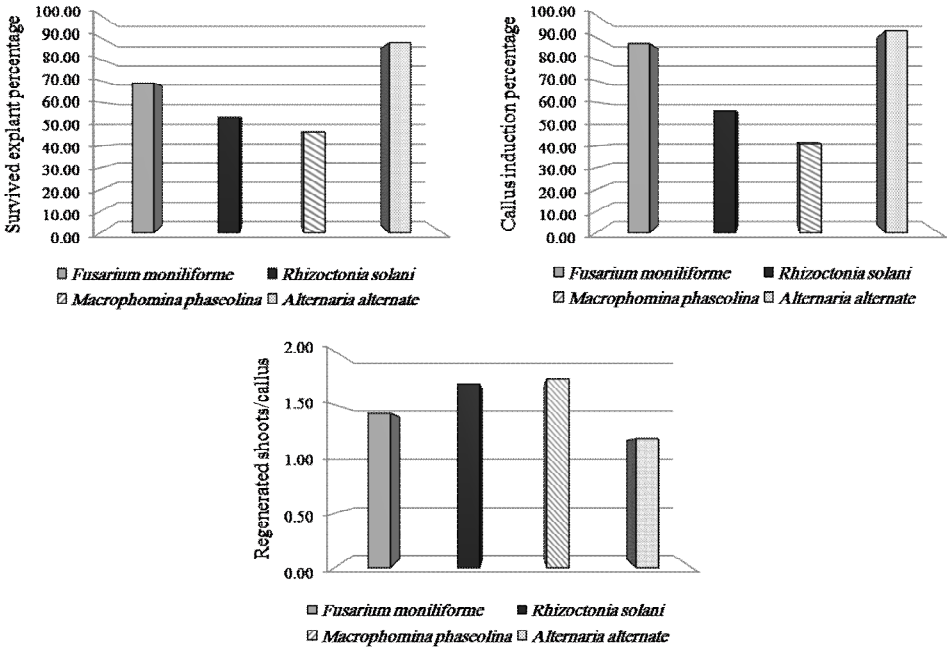

Fig. (3): Effect of fungi on plant regeneration characteristics.

VII. Actinomycetes × concentrations × fungi interaction effect

Effect of interactions between actinomycetes, concentrations and fungi on plant regeneration characteristics were presented in Fig (4) and (5). The highest value of regeneration characteristics as measured by survived explant percentage (%) for *Fusarium moniliforme* was scored its highest values with treatment *S. bobilii* at 10 % treatment, also, highest values for *Rhizoctonia solani* was achieved with treatment *S. bobilii* but at 15 % treatment. Moreover, the highest values of survived explant percentage (%) for *Macrophomina phaseolina* was attained with treatment *S. bobilii* and *S. grisiobrunneus* at 5 and 10 % treatments; respectively. In addition, survived explant percentage (%) for *Alternaria alternata* achieved its highest values with treatment *S. bobilii* and *S. grisiobrunneus* but at 10 and 15 % treatments; respectively. *S. bobilii* had no tendency in superiority over *S. grisiobrunneus* through plant callus induction percentage (%), whereas, *Fusarium moniliforme* treatment reached its highest value with *S. bobilii* at 10 % treatment, while *Rhizoctonia solani* and *S. grisiobrunneus* at 10 % scored its highest values. Furthermore, *S. bobilii* and *S. grisiobrunneus* treatments with *Macrophomina phaseolina* scored its highest values at 5 and 10 % treatments; respectively, while *S. bobilii* and *S. grisiobrunneus* treatments with *Alternaria alternata* achieved its highest values at 10 and 15 % treatments; respectively. Also, data presented in Fig. (4) indicate that regeneration characteristics as measured by Regenerated shoots/callus were affected significantly by the interactions effects between actinomycetes, actinomycetes concentrations and fungus treatments. High concentration treatment (15 %) from *S. grisiobrunneus* was the most suitable treatment for *Fusarium moniliforme*, *Rhizoctonia solani* and *Macrophomina phaseolina*. In contrast, *S. bobilii* at 15 % treatment scored the highest values with *Alternaria alternata*. Therefore, it could be concluded that actinomycetes elevated concentration is considered to be the optimum treatment which in turn plays a vital role towards defeating fungal contamination in each step and therefore leads to best regeneration characteristics motivation and stimulation improvements.

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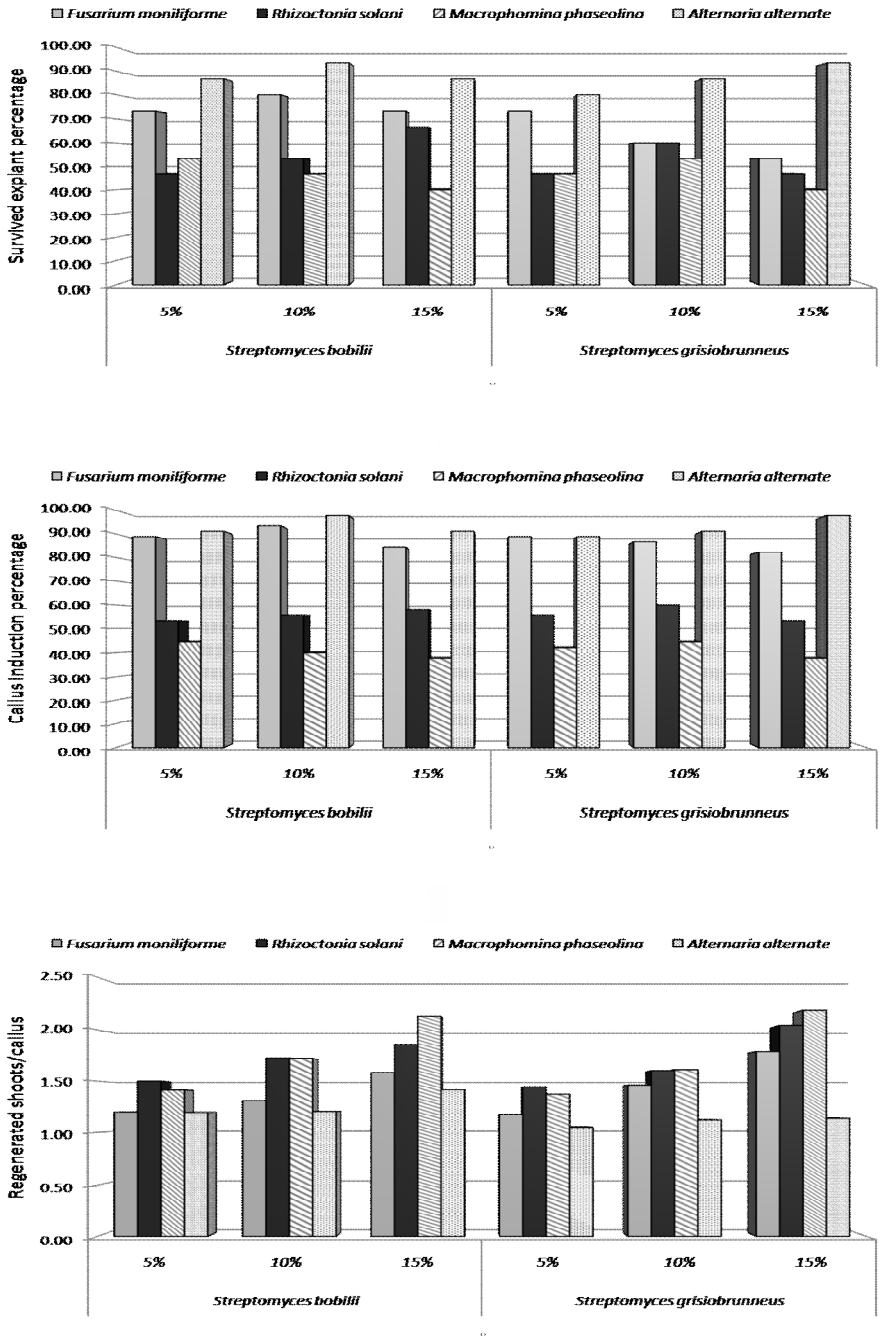

Fig.(4): Effect of actinomycetes, concentrations and fungi interaction on regeneration characteristics.

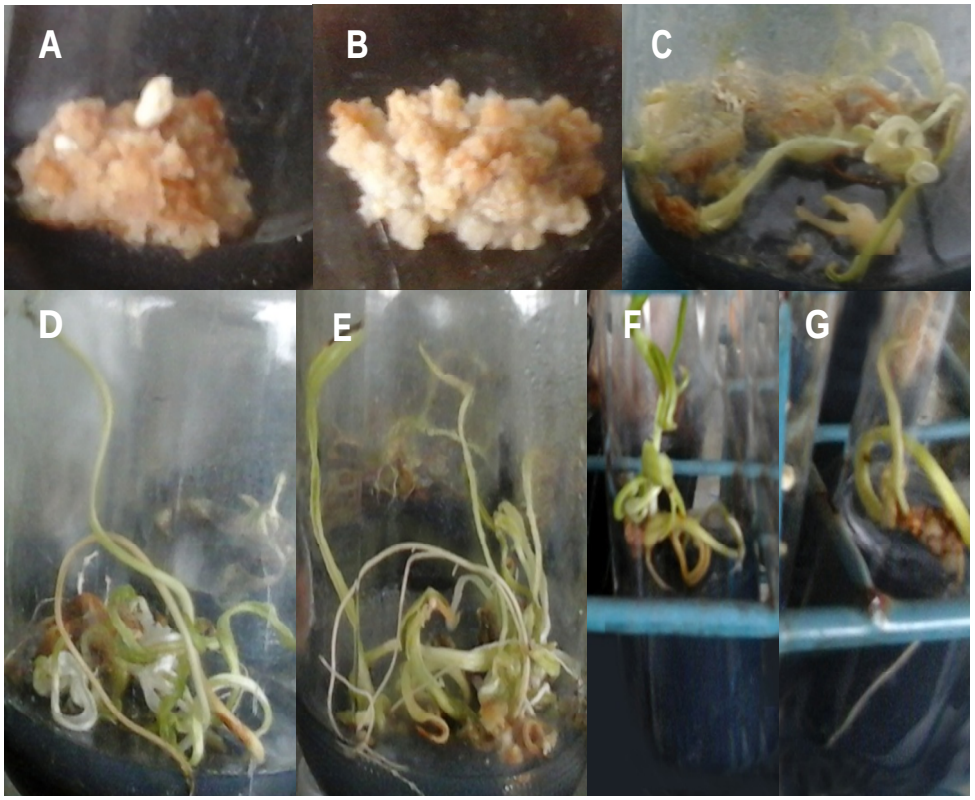

Fig. (5): Plant regeneration in date palm cv. Khalas: a. Khalas cultivar calli growing on embryogenesis induction medium (EIM). b. Khalas calli showing embryogenesis on (EIM) medium. c. Primordial shoot initiation on regeneration medium (SRM). d. Calli showing multiple shoot regeneration on SRM medium. e. Shoots elongation and multiple primordial shoots on SRM medium. f. Khalas plantlet grown onto rooting medium (RM). G. Rooted Khalas plantlet onto RM medium.

REFERENCES

Al-Khayri, J. M. (2010). Somatic embryogenesis of date palm (Phoenix dactylifera L.) improved by coconut water. Biotechnol., 9: 477-484.

Al-Khayri, J. M. (2011). Basal salt requirements differ according to culture stage and cultivar in date palm somatic embryogenesis. Am J. Biochem. Biotech., 7: 32-42.

Al-Khayri, J. M. and Ibraheem Y. (2014). *In vitro* selection of abiotic stress tolerant date palm (*Phoenix dactylifera* L.): A review. Emir. J. Food Agric., 26(11): 921-933.

Al-Shahib, W. and Marshall R. J. (2003). The fruit of the date palm: its possible use as the best food for the future? Int'l. J. Food Sci. Nutr., 54(4): 247-59.

Arafat, K. H.; Mohamad M. and Elsharabasy S. (2012). Biological control of date palm root rots disease using Egyptian isolates of Streptomycetes. Res. J. Agric. Biol. Sci., 8(2): 224-230.

Aslam, J.; Khan S. A.; Cheruth A. J.; Mujib A. and Sharma M. P. (2011) Somatic

embryogenesis, scanning electron microscopy, histology and biochemical analysis at different developing stages of embryogenesis in six date palm (*Phoenix dactylifera* L.) cultivars. Saudi J. Biol. Sci., 18: 369-380.

Baraka, M. A.; Fatma M. Radwan and Arafat K. H. (2011). Survey and identification of major fungi causing root rot on date palm and their relative importance in Egypt. J. Biol. Chem. Environ. Sci., 6(2): 319-337.

Bhaskaran, S. and Smith R. H. (1992). Somatic embryogenesis from shoot tip and immature inflorescence of *Phoenix dactylifera* cv. Barhee. Plant Cell Rep., 12: 22-25.

Boufis, N.; Khelifi Slaoui M.; Djillali Z.; Zaoui D. and Morsli A.; Bernards M. A.; Makhzum A. and Khelifi L. (2014). Effects of growth regulators and types of culture media on somatic embryogenesis in date palm (*Phoenix dactylifera* L. cv. Degla Beida). Scient. Hort., 172: 135-142.

Duncan, D. B. (1955). Multiple range and multiple "F-test". Biometrics, 11: 1-42.

Eke, C. R.; Akomeah P. and Asemota O. (2005). Somatic embryogenesis of date palm (*Phoenix dactylifera* L.) from apical meristem tissues from "Zebia" and "Loko" landraces. Afr. J. Biotech., 4: 244-246.

El-Deeb, H. M.; Lashin, S. M. and Arab, Y. A. (2007). Distribution and pathogenesis of date palm fungi in Egypt. Acta Hort., 736:421-429.

El-Juhany, L. I. (2010). Degradation of date palm trees and date production in Arab countries: Causes and potential rehabilitation. Aust. J. Basic and Appl. Sci., 4(8): 3998-4010.

El-Morsi, M. A. (2004). Studies on certain fungal diseases of date palm offshoots in New Valley, Egypt. Ph.D. Thesis, Fac. Agric., Assiut Univ., pp, 110.

El-Zawahry, Aida, M.; El-Morsi, M. A. and Abd-Elrazik, A. A. (2000). Occurrence of fungal diseases on date palm trees and off-shoots in New Valley governorate and their biological control. Assiut J. Agric. Sci., 31(3): 189-212.

Eshraghi, P.; Zaghami R. and Mirabdulbaghi M. (2005). Somatic embryogenesis in two Iranian date palm cultivars. Afr. J. Biotech., 4: 1309-1312.

Hassan, M. M. and Taha R. A. (2012). Callogenesis, somatic embryogenesis and regeneration of date palm *Phoenix dactylifera* L. cultivars affected by carbohydrate sources. Int'l. J. Agric. Res., 7: 231-242.

Ibrahim, I. A.; Hassan M. M. and Taha R. A. (2012). Partial desiccation improves plant regeneration of date palm *in vitro* cultures. Wudpecker J. Agric. Res., 1: 208-214.

Kader, A. A. and Hussein A. M. (2009). Harvesting and post-harvest handling of dates, International Center for Agricultural Research in the Dry Areas (ICARDA). Aleppo. Syria., 4: 1-15.

Khierallah, H. S. M.; Al-Hamdany M. H. S.; Abdulkareem A. A. and Saleh F. F. (2015). Influence of sucrose and pacloburtazol on callus growth and somatic embryogenesis in date palm cv. Bream. Int'l., J. Curr. Res. Aca. Rev., 1: 270-276.

Mazri, M. A. (2012). Effect of liquid media and *in vitro* pre-acclimatization stage on shoot elongation and acclimatization of date palm (*Phoenix dactylifera* L.) cv. Najda. J. Orna. Hort. Plants, 2: 225-231.

Mazri, M. A, (2014). Effects of plant growth regulators and carbon source on shoot proliferation and regeneration in date palm (*Phoenix dactylifera* L.) '16-bis'. J. Hort. Sci. Biotech., 89: 415-422.

Murashige, T. and Skoog F. (1962). A revised medium for rapid growth and

bioassays with tobacco tissue cultures. Physiol. Planta., 15: 473-497.

Othmani, A.; Bayoudh C.; Drira N.; Marrakchi M. and Trif M. (2009). Somatic embryogenesis and plant regeneration in date palm *Phoenix dactylifera* L., cv. Boufeggous is significantly improved by fie chopping and partial desiccation of embryogenic callus. Plant Cell Tissue Organ Cult., 97: 71-79.

Sarhan, A. R. T. (2001). A study on the fungi causing decline of date palm trees in middle of Iraq. 2nd Int'l. Conf. Date Palm, 25-27 March, Al Ain, UAE, pp, 424-430.

Snedecor, G. W. and Cochran W. G. (1969). Statistical Methods (6th Ed.), Iowa State University Press, Ames, USA.

Veramendi, J. and Navarro L. (1997). Influence of explant sources of adult date palm (*Phoenix dactylifer*a L.) on embryogenic callus formation. J. Hort. Sci., 72: 665-671.

Yuhana, M. (2010). Biocontrol agents in aquaculture: Production and their application. J. Akuakultur Indonesia, 9(1): 16-20.

الملخص العربي

نظام لاستيلاد النباتات في نخيل البلح صنف خلاص يتغلب على اربعة فطريات مختلفة مسببة للتلوث باستخدام المقاومة الحيوية

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في الدراسة الحالية، تم وصف نظام لاستيلاد النباتات يتغلب على اربعة فطريات تسبب التلوث باستخدام المقاومة الحيوية.تم استخدام ثلاثة معاملات من تركيزات مختلفة (٥، ١٠، ١٥ %) من راشح النمو لعزلتين من الاكتينوميسيتس (*Streptomyces bobilii* و *Streptomyces grisiobrunneus*) لدراسة تأثيرهم فى التغلب على اربعة من الفطريات المختلفة (*Fusarium moniliforme*، *Rhizoctonia solani*، *Macrophomina phaseolina* و *Alternaria alternata*) والتي تحدث التلوث فى مزارع انسجة نخيل البلح. تفوقت البيئة المزودة بـ *S. bobilii* على البيئة المزودة بـ *Streptomyces grisiobrunneus* في قيم صفات الاستيلاد. اثبتت معاملة الاكتينوميسيتس (١٠ %) انها الأفضل لتعزيز القيم المئوية لنجاة المنفصل النباتي ولأستحثاث الكالس، بينما اثبتت المعاملة (١٥ %) انها الافضل لتعزيز قيم النباتات المستولدة لكل كالس. وفي هذه الدراسة، تم استحداث نظام لاعادة الاستيلاد في صنف خلاص، والمؤثر فى نجاح النقل الجيني، يتغلب على التأثير المدمر للتلوث الفطري.