

IN VITRO CALLUS INITIATION, FORMATION AND EVALUATION OF SOME DATE PALM (*Phoenix dactylifera* L.) CULTIVARS.

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ABSTRACT

Uniform Shoot-tip explants of some female date palm cultivars (Sewy, Zaghoul, Samany, Sakouty and Malakaby) were cultured on modified Murashige and Skoog medium. Different concentrations of auxins *i.e* 2,4-Dichlorophenoxy acetic acid (2,4-D); Naphthalene acetic acid (NAA) and α -Naphthoxy acetic acid (NOA) were tested.

Data indicated that culturing of shoot-tips as explants on modified Murashige and Skoog medium is mainly recommended. Also, callus initiation of different date palm cvs were maximized by using (100 mg/L 2,4-D). However, the treatments of (10 mg/L 2,4-D + 10 mg/L NOA + 10 mg/L NAA) induced the best growth parameters (number of explant, percentage of callus formation and average value of callus formation). Meanwhile, Sewy cultivar had less swelling and callus formation compared with the other cultivars.

INTRODUCTION

Palm crop improvement has been slow due to their long-lived nature growth habit and lack to an adequate methods of propagation by vegetative means. However, some date palms produces off-shoots by the stimulation of a few basal axillary buds from the trunk in the early stages of the palm's life. The number of off-shoots produced by a date palm is primarily a varietal characteristic and their numbers ranged from 5 to 25 off-shoots, but due to the strong apical dominance it doesn't eventually grow in some cultivars. Therefore this method have reduced considerably the expansion of the existing palm groves.

Plantlets were successfully produced from shoot-tip explants of different date palm cultivars (El-Hennawy *et al.*, 1983; Beaus chesne *et al.*, 1986 and Wongkaew *et al.*, 1991). Monocotyledons react differently when considering callus indication generally being less likely to form callus tissue than dicotyledons, it is often only necessary to add some auxins as the hormonal stimulant for callus induction (Tisserat *et al.*, 1979, Tisserat 1983, Zaid and Tisserat, 1983a, EL-Hammady *et al.*, 1999, and El-Sharabasy, 2000). This study was planned to evaluate the ability of the tested cultivars in order to find out the superior treatments that induce the highest swelling callus initiation and callus formation.

MATERIALS AND METHODS

This study was carried out in the laboratory of Biotechnology Division, The Central Laboratory of Date Palm Research and Development and Shora Technology Lab. during the period between 2002-2004.

Plant material:

Young off-shoots, 10 – 15 cm diameter, 4-6 kg in weight were detached from the tested mature date palm trees (*Phoenix dactylifera* L.). "Sewy" cv. grown in El-Badrashin region, Giza governorate, Zaghloul and Sammany cvs. grown in Edco region, El-Behera governorate. In addition, Sakkouty and Malakaby cvs. grown at Abo El-Reesh in Aswan were used as the plant material. Off-shoots were handled very carefully during the removal of the apical tissues of stem (shoot-tip). The explant was soaked in a cold anti-oxidant solution (100 mg/L ascorbic acid and 150 mg/L citric acid) and kept in a refrigerator for about 2 hours prior surface sterilization. Explant was surface sterilized by using ethanol solution (70%) for 1 – 2 min then rinsed once with sterile distilled water and transferred to 50% Clorox (2.5% sodium hypochlorite) solution containing two drops of Tween-20 for 20 min.

Nutrient media:

The explants were cultured on modified Murashige and Skoog medium supplemented with 100 mg/L inositol, 100 mg/L glutamine, 1.5 g/L activated charcoal, 30 g/L sucrose, 3.0 mg/L isopentenyladenin(2iP) and 6.5 g/L Difco-Bacto agar. The pH of the medium was adjusted to 5.7, and then autoclaved at 121 °C under 15 lb/in² for 15 min.

Environmental conditions:

The cultured explants were incubated under 24 hrs of dark with average temperature of 27±1°C. Different experiments were conducted as follows:

1. Callus initiation:

Shoot-tip explants of different date palm cultivars were cultured on modified Murashige and Skoog medium (1962) supplemented with different growth regulators as follows:

- | | |
|--|------------------|
| 1- 100 mg/L 2,4-D | 2- 10 mg/L 2,4-D |
| 3- 100 mg/L NAA | 4- 10 mg/L NAA |
| 5- 100 mg/L NOA | 6- 10 mg/L NOA |
| 7- 10 mg/L 2,4-D + 10 mg/L NAA + 10 mg/L NOA | |

Where: 2,4-D= (2,4-Dichlorophenoxy acetic acid)
NAA= (Naphthalene acetic acid)
NOA= (α -Naphthoxy acetic acid)
2iP= (isopentenyladenine)

To determine the best treatments that induce the highest swelling and callus initiation of different cultivars.

2. Callus formation:

The developed callus from shoot-tip of different tested cultivars were transferred on MS medium supplemented with the same treatments and concentrations mentioned before to enhance the highest number of callus formed explants , percentage of callus formation and average values (g) of callus formation.

Data and calculations:

In all experiments, each treatment consisted of 6 replicates each of them contains one explant. Data were recorded as (number of swelling explant, percentage of callus development and volume of callus) , the experiments were designed as a complete randomized. The obtained data was statistically analyzed according to Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

1- Callus initiation:

Data presented in Table (1) and Photo (1) indicate that different types of auxins at different concentrations showed highly significant increase in number of explants of Sewy, Sammany and Zaghloul cultivars, respectively as compared with both Malakaby and Sakkouty cultivars. These results indicate also that the highest percentage of swelling and average value of swelling occurred in Sewy cultivar followed by Sammany in comparison to the other cultivars used. Meanwhile, Table (1) disclose that the higher concentration (100 mg/L) of 2,4-D, NAA and NOA were most pronounced on both number of swelling explants and its percentage in relation to the other concentrations used. However, using 100 mg/L of 2,4-D gave the highest significant increase among swelling followed by both (100 mg/L NAA) and (100 mg/L NOA) then (10 mg/L 2,4-D + 10 mg/L NAA and 10 mg/L NOA), respectively as compared with the other concentrations used. Moreover, these results reflect that treating date palm cultivars with the higher auxin concentration (100 mg/L 2,4-D, NAA and NOA) as well as, Sewy cultivar combined with 10 mg/L NOA resulted in the highest significant values for number of swelling in relation to the other interactions.

It appears from Table (2) and Photo (2) that both Zaghloul and Sakkouty cultivars showed highly significant increase in percentage of callus initiation followed by Sammany cultivar then Sewy cultivar as compared with Malakaby cultivar in a descending order, as affected by different auxins types and concentrations. However, significant increase of average value of callus initiation was obtained when Sammany cultivar was used. On the other hand, number of swelling did not show any statistical differences when different cultivars were used. Moreover, Table (2) disclose that using (100 mg/L 2,4-D) enhanced significantly number of explant and percentage of callus initiation followed by using (100 mg/L NAA) then using (100 mg/L NOA) as compared with the other concentrations used. However, significant increase of average value of callus initiation was obtained when using (10 mg/L 2,4-D + 10 mg/L NAA + 10 mg/L NOA). Moreover, Table (2) shows that using (100

mg/L 2,4-D) of both Zaghoul and Sakkouty cultivars as well as using (100 mg/L NAA) of Sammany and Sewy cultivars significantly increased number of swelling and percentage of callus initiation in relation to all other interactions. However, significant increase of average value of callus initiation was obtained when both Zaghoul and Sammany cultivars as well as Sakkouty cultivar were cultured on medium supplemented with (10 mg/L 2,4-D + 10 mg/L NAA + 10 mg/L NOA) were used as compared with the other combinations used. These results are in agreement with those of Zaled (1987), Tisserat *et al.* (1979). They found that good callus growth occurred from explants of date palm cultured on medium containing on 100 mg/L 2,4-D or NAA. In addition, Al-Ghamdi (1993), who found that good callus initiation and callus growth were obtained from MS salt media supplemented with (100 mg/L 2,4-D and 3 mg/L 2iP).

2- Callus formation:

Table (3) and Photo (3) indicates that different types of auxins at different concentrations enhanced significantly the increase in number of callus and percentage of callus formation of Sewy cultivar followed by Sammany then Sakkouty and Zaghoul as compared with Malakaby cultivar in a descending order.

Table (1): Effect of different auxins (2,4-D, NAA and NOA) concentrations (10, 100 mg/l) on swelling stage of different date palm (*Phoenix dactylifera* L.) cultivars after 6 weeks.

Parameters	Percentage of swelling (%)										Average value of swelling (g)							
	No. of swelling explants					Mean					Mean							
Cultivars	Zaghloul	Sammany	Sewy	Malakaby	Sakoty	Mean	Zaghloul	Sammany	Sewy	Malakaby	Sakoty	Mean	Zaghloul	Sammany	Sewy	Malakaby	Sakoty	Mean
100 2,4-D	6.00 A	6.00 A	5.00 AB	5.00 AB	5.00 AB	5.40 A	100.0 A	100.0 A	83.30 C	83.30 C	83.30 C	89.98 A	3.33 AB	3.50 A	2.33 E	2.66 D	3.00 C	2.96 A
10 2,4-D	5.00 AB	6.00 A	4.00 BC	4.00 BC	3.00 C	4.40 BC	83.30 C	100.0 A	66.63 D	66.63 D	50.00 E	73.34 D	2.33 E	1.83 GH	1.33 J	1.66 HI	1.50 IJ	1.75 E
100 NAA	6.00 A	5.00 AB	6.00 A	5.00 AB	5.00 AB	5.40 A	100.0 A	83.30 C	100.0 A	83.30 C	83.30 C	89.98 A	2.66 D	2.16 EF	3.33 AB	3.00 C	2.33 E	2.68 E
10 NAA	4.00 BC	4.00 BC	5.00 AB	3.00 C	4.00 BC	4.00 C	66.63 D	66.63 D	83.30 C	50.00 E	66.63 D	66.67 F	1.50 IJ	1.33 AB	2.16 EF	1.66 HI	1.83 GH	1.70 E
100 NOA	5.00 AB	6.00 A	6.00 A	4.00 BC	5.00 AB	5.20 A	100.0 A	100.0 A	100.0 A	66.63 D	83.30 C	87.66 B	2.33 E	3.16 BC	3.00 C	2.00 FG	2.66 D	2.63 B
10 NOA	4.00 BC	5.00 AB	6.00 A	4.00 BC	4.00 BC	4.60 B	66.63 D	83.30 C	100.0 A	66.63 D	66.63 D	76.68 C	1.60 I	2.16 EF	2.16 EF	1.83 GH	1.83 GH	1.92 D
10 2,4-D + 10 NAA + 10 NOA	4.00 BC	4.00 BC	5.00 AB	4.00 BC	4.00 BC	4.20 BC	66.63 D	66.63 D	83.30 C	66.63 D	66.63 D	70.02 E	1.00 FG	2.16 EF	3.00 C	2.33 E	2.16 EF	2.33 C
Mean	4.86 A	5.14 A	5.29 A	4.14 B	4.29 B		81.66 C	85.71 B	88.09 A	69.06 E	71.43 D		2.24 C	2.33 B	2.47 A	2.16 C	2.19 C	

Means Followed by the same letter (s) with each parameter are not statistically different at 1% level.

Table (2): Effect of different auxins (2,4-D, NAA and NOA) concentrations (10, 100 mg/l) on callus initiation of different cultivars of date palm (*Phoenix dactylifera* L.) after 6 weeks.

Parameters Cultivars Concentration (mg/L)	No. of Callus formed explants					Percentage of swelling (%)					Average value of swelling (g)							
	Zaghloul	Sammany	Sewy	Malakaby	Sakoty	Mean	Zaghloul	Sammany	Sewy	Malakaby	Sakoty	Mean	Zaghloul	Sammany	Sewy	Malakaby	Sakoty	Mean
100 2,4-D	4.00	3.00	3.00	3.00	4.00	3.40	66.70	50.00	50.00	50.00	66.70	56.68	0.83	0.66	0.50	0.83	0.83	0.73
	A	AB	AB	AB	A	A	A	B	B	B	A	A	F	G	H	F	F	DE
10 2,4-D	2.00	2.00	1.00	1.00	2.00	1.60	33.30	33.30	16.70	33.30	26.66	1.16	1.03	0.66	0.66	1.00	1.00	0.90
	BC	BC	C	C	BC	D	C	C	D	C	E	D	E	E	G	E	E	C
100 NAA	3.00	4.00	4.00	2.00	3.00	3.20	50.00	66.70	66.70	33.30	53.34	0.66	0.66	0.83	0.83	0.50	0.83	0.70
	AB	A	A	BC	AB	AB	B	A	A	C	B	B	G	G	F	H	F	E
10 NAA	2.00	2.00	1.00	1.00	1.00	1.40	33.30	33.30	16.70	16.70	23.34	0.83	1.00	0.66	0.66	0.66	0.66	0.76
	BC	BC	C	C	C	D	C	C	D	D	F	F	E	G	G	G	G	D
100 NOA	3.00	2.00	3.00	3.00	3.00	2.80	50.00	33.30	50.00	50.00	46.66	0.50	0.83	0.83	0.66	0.83	0.83	0.73
	AB	BC	AB	AB	AB	B	B	C	B	B	C	C	H	F	F	G	F	DE
10 NOA	2.00	2.00	3.00	3.00	3.00	2.60	33.30	33.40	50.00	50.00	43.34	1.03	1.33	1.50	1.33	1.33	1.33	1.31
	BC	BC	AB	AB	AB	C	C	C	B	B	D	E	C	B	C	C	C	B
10 2,4-D + 10 NAA + 10 NOA	3.00	3.00	2.00	2.00	3.00	2.60	50.00	50.00	33.00	50.00	46.66	2.00	1.93	1.33	1.33	2.00	2.00	1.72
	AB	AB	BC	BC	AB	C	B	B	C	B	C	C	A	A	C	C	A	A
Mean	2.71	2.57	2.43	2.14	2.71		45.23	42.86	40.49	38.10	45.24		1.00	1.06	0.90	0.80	1.07	
	A	A	A	A	A		A	B	C	D	A		B	A	C	D	A	

Means Followed by the same letter (s) with each parameter are not statistically different at 1% level .

Table (3): Effect of different auxins (2,4-D, NAA and NOA) concentrations (10, 100 mg/ l) on callus formation of different cultivars of date palm (*Phoenix dactylifera* L.) after 6 weeks.

Parameters	No. of Callus formed explants					Percentage of callus formation (%)					Average value of callus formation (g)							
	Zaghloul	Sammany	Sewy	Malakaby	Sakoty	Mean	Zaghloul	Sammany	Sewy	Malakaby	Sakoty	Mean	Zaghloul	Sammany	Sewy	Malakaby	Sakoty	Mean
100 2,4-D	9.00 A	10.00 A	8.00 A	9.00 A	8.00 A	8.80 B	75.00 C	83.30 B	66.67 E	75.00 C	66.70 E	73.33 B	2.91 C-F	3.00 B-E	2.41 HI	2.66 F-H	2.41 HI	2.67 B
10 2,4-D	7.00 A	8.00 A	6.00 A	6.00 A	6.00 A	6.60 D	58.30 F	66.70 E	49.67 G	50.00 G	50.00 G	54.93 D	2.00 J	2.33 I	1.66 K-M	1.83 J-I	1.58 LM	1.88 D
100 NAA	6.00 A	9.00 A	10.00 A	8.00 A	9.00 A	8.80 B	66.70 E	75.00 C	83.30 B	70.00 D	75.00 C	74.00 B	2.50 GI	2.50 GI	3.15 A-C	2.25 I	2.66 F-H	2.61 B
10 NAA	6.00 A	6.00 A	7.00 A	5.00 A	6.00 A	6.00 DE	50.00 G	50.00 G	58.30 F	41.70 H	50.00 G	50.00 E	1.83 J-I	1.91 JK	2.00 J	1.41 MN	1.75 J-L	1.78 D
100 NOA	7.00 A	8.00 A	9.00 A	6.00 A	8.00 A	7.60 C	58.20 F	66.70 E	75.00 C	50.00 G	66.70 E	63.32 C	2.00 J	2.33 I	2.83 A-F	1.75 J-L	2.52 G-L	2.29 C
10 NOA	5.00 A	6.00 A	7.00 A	4.00 A	6.00 A	5.60 E	41.70 H	50.00 G	58.30 F	33.30 I	50.00 G	46.66 F	1.16 N	1.75 J-L	2.00 J	1.23 N	1.91 JK	1.61 E
10 2,4-D + 10 NAA + 10 NOA	10.00 A	10.00 A	11.00 A	9.00 A	10.00 A	10.00 A	83.30 B	83.30 B	91.70 A	75.00 C	83.30 B	83.32 A	3.16 A-C	3.25 AB	3.33 A	2.75 E-G	3.08 A-D	3.11 A
Mean	7.43 C	8.14 AB	8.25 A	6.71 D	7.57 BC		61.89 D	67.86 B	68.99 A	56.43 E	63.10 C		2.22 B	2.44 A	2.28 A	1.98 C	2.27 B	

Means Followed by the same letter (s) with each parameter are not statistically different at 1% level .

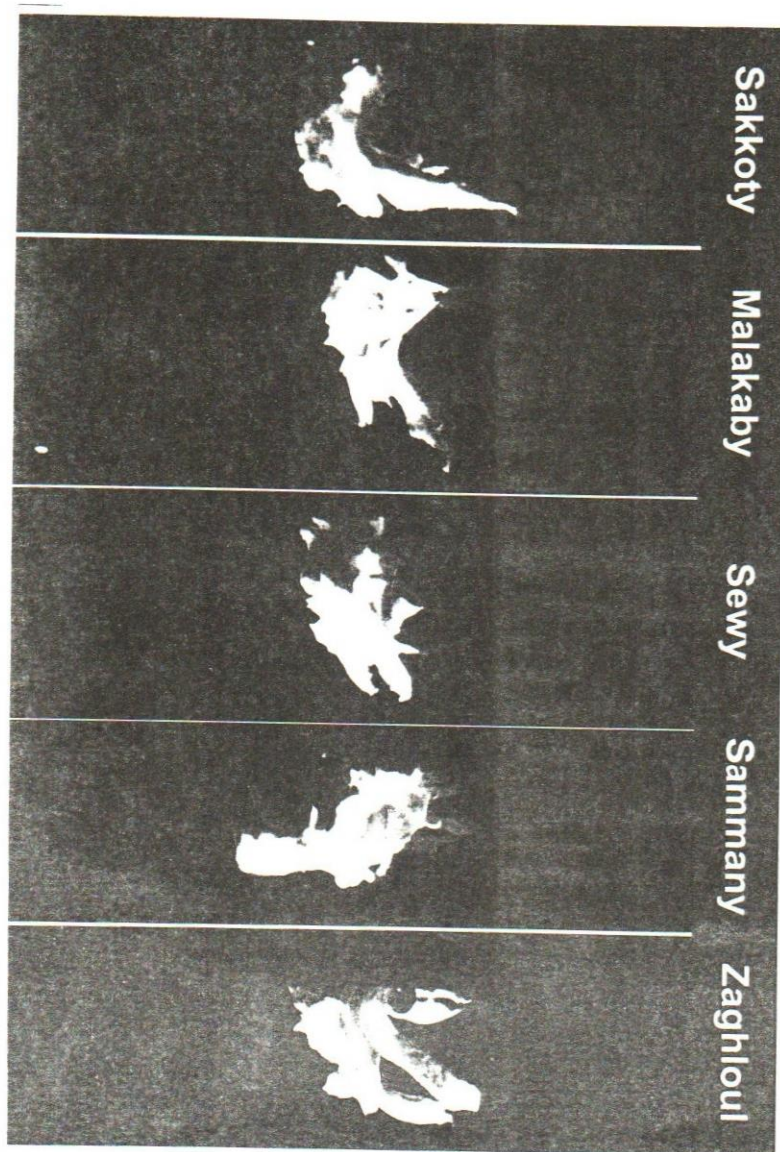


Photo (1): Effect of different auxins (2,4-D, NAA and NOA) concentrations (10 , 100 mg) on swelling stage of different date palm (*Phoenix dactylifera* L.) cultivars after 6 weeks.

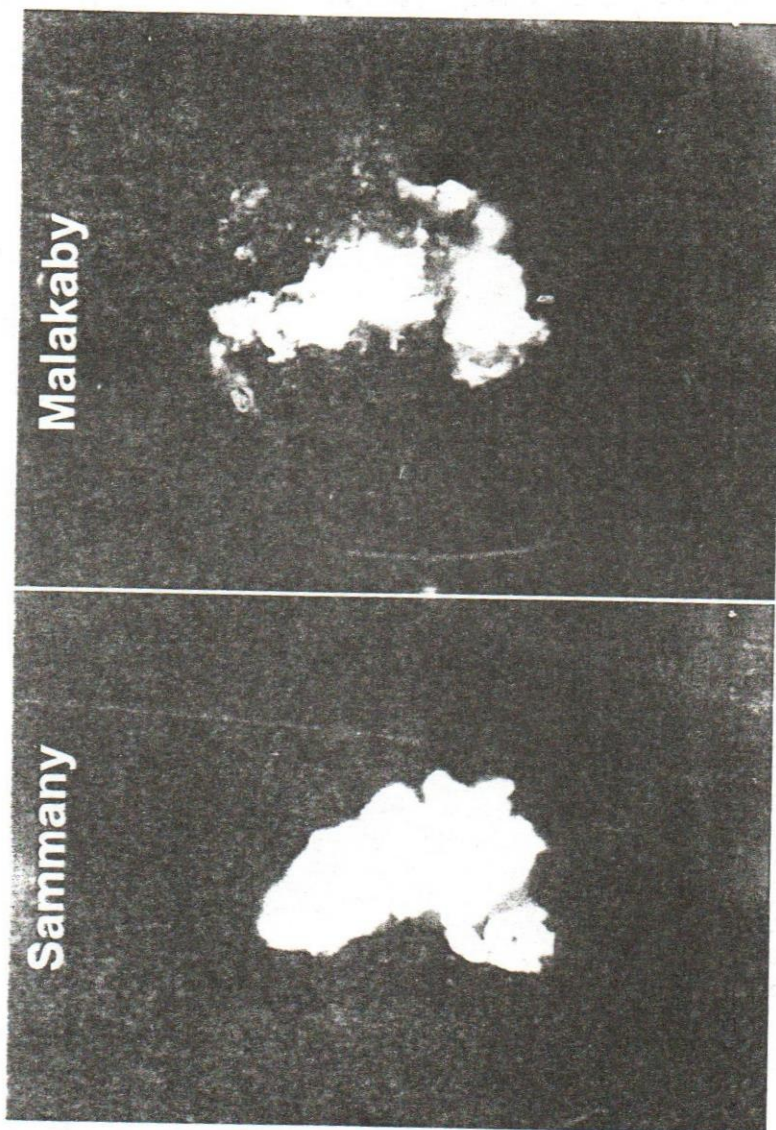


Photo (2): Effect of different auxins (2,4-D, NAA and NOA) concentrations (10 , 100 mg) on callus initiation of different cultivars of date palm (*Phoenix dactylifera* L.) after 6 weeks.

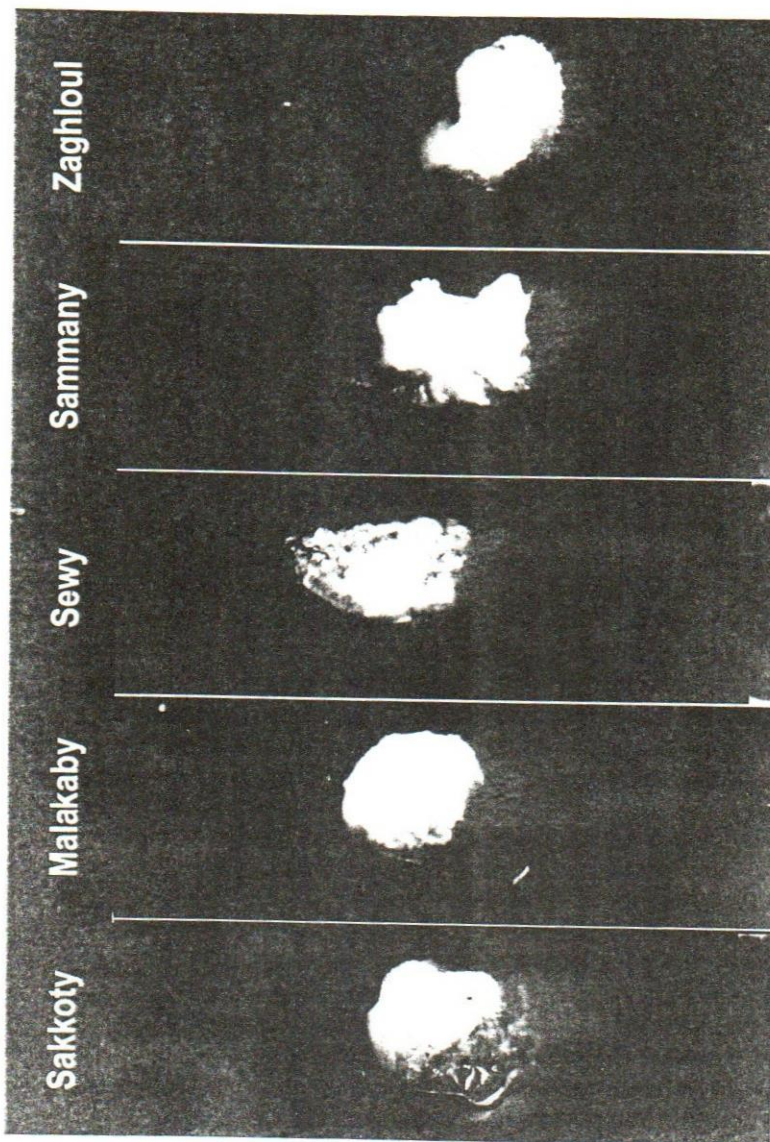


Photo (3): Effect of different auxins (2,4-D, NAA and NOA) concentrations (10 , 100 mg) on callus formation of Sammany and Malakaby cultivars of date palm (*Phoenix dactylifera* L.) after 6 weeks.

However, the highest average value of callus formation occurred when both Sammany and Sewy cultivars were used. Meanwhile, Table (3) reflected that using combined treatment with (10 mg/L 2,4-D + 10 mg/L NAA and 10 mg/L NOA) enhanced significantly all parameters under study (number of callus, percentage of callus formation and average value of callus formation) in relation to the other concentrations used. However, Table (3) reflects that number of callus didn't record statistical response to different interactions under study. Meanwhile, significant increase of percentage of callus formation and average value of callus formation were obtained when Sewy cultivar was cultured on medium supplemented with (10 mg/L 2,4-D + 10 mg/L NAA and 10 mg/L NOA). Such finding was mentioned by El-Hammady *et al.* (1999), reported that the highest percentage of embryogenic callus initiation and callus size of date palm was achieved on MS medium plus NAA at 20 or 50 mg/L and 2.0 mg/L 2iP, also, Nazek *et al.* (1993), reported that embryogenic callus of date palm were induced on medium supplemented with either 100 mg/L 2,4-D or 20 mg/L NAA.

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تنشئة وتقييم الكالس معمليا لبعض أصناف النخيل

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*المعمل المركزي لأبحاث وتطوير النخيل - مركز البحوث الزراعية - الجيزة
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أجري هذا البحث بالمعمل المركزي لأبحاث النخيل بمركز البحوث الزراعية بالجيزة خلال الفترة من ٢٠٠٢ إلى ٢٠٠٣م بهدف دراسة تأثير أنواع مختلفة من الأوكسينات على تطور وتنشئة الجزء النباتي لبعض أصناف النخيل النامية داخل الأنابيب حيث تم فصل القمة النامية ونميت على بيئة موراشيخ وسكوج المعدلة ، وتم دراسة تأثير أنواع مختلفة من الأوكسينات مثل نفتالين أسيتيك أسيد ، نفتوكسي أسيتيك أسيد ، ٢، ٤ داي كلوروفينوكسي أسيتيك أسيد بتركيزات (١٠ ، ١٠٠ ملجم/لتر) لكل منهم على حدة كذلك تم دراسة مخاليط من الأوكسينات الثلاثة بتركيز (١٠ ملجم/لتر).

ووجد أن زراعة القمة النامية على بيئة موراشيخ وسكوج المعدلة أعطت أفضل النتائج كما شجع استخدام ١٠٠ ملجم/لتر من ٢ ، ٤ داي كلوروفينوكسي أسيتيك أسيد نشوء وتطور الكالوس لجميع أصناف البلح تحت الدراسة وكانت النسبة المئوية للكالوس وحجمه أفضل ما يمكن باستخدام مخلوط من نفتالين أسيتيك أسيد + ٢، ٤ داي كلوروفينوكسي أسيتيك أسيد + نفتوكسي أسيتيك أسيد بتركيز ١٠ ملجم/لتر. ووجد أن الصنف السيوي أعطى أحسن نتائج حيث كانت نسبة الكالوس عالية للبيئة المزودة بـ ١٠ ملجم/لتر نفتالين أسيتيك أسيد + ١٠ ملجم/لتر نفتوكسي أسيتيك أسيد + ١٠ ملجم/لتر ٢، ٤ داي كلوروفينوكسي أسيتيك أسيد بصورة معنوية مقارنة بالأصناف الأخرى تحت الدراسة.