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I- *IN VITRO* PROPAGATION OF DATE PALM (*Phoenix dactylifera* L.) cv. "ZAGHLOUL" BY DIRECT ORGANOGENESIS

El- Sabrout, M.B.
Pomology Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

ABSTRACT

The present investigation was carried out during three successive years (1999- 2001), in order to study the effect of the cytokinin (Kin) to auxin (NAA) ratio in culture medium (Murashige and Skoog, 1962) as well as the explant type on the direct organogenesis in date palm cv. "Zaghloul" cultures.

The main results can be summarized in the following points:

1- Shoot tip explant proved to be the best in terms of budding percentage and number of developed buds per explant as compared with the leaf primordia explant.

2- Using the MS medium supplemented with 10.0 $mg1^{-1}$ Kin + 1.0 $mg1^{-1}$ NAA, the budding percentage was significantly the highest (100 % in shoot tip explant and 87.5% in leaf primordia explant). Also, average number of developed buds per explant was significantly the highest (2.12 per shoot tip explant and 1.25 per leaf primordia explant).

3- Shoot multiplication, average number of proliferated shoots per axillary bud explant and average length of proliferated shoot were superior, during four successive subcultures.

4- The 1st , 2nd , 3rd and 4th subculutres, averaged number and length of proliferated shoots per axillary bud explant was significantly the highest such number was 3.5, 4.0, 4.0 and 4.62 and average length of proliferated shoot was (4.83 cm, 5.62 cm, 6.25 cm and 6.52 cm, respectively).

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the most important fruit crops in Egypt. The total number of female date palms is about 8,945,304 palms and the total production of date fruit attained about 905, 953 tons according to the statistics of the Ministry of Agriculture and Land Reclamation, Cairo, 1999.

Date palm is vegetatively propagated by offshoots to maintain a unique genetic makeup of the variety. Seed propagation was not appropriate for commercial production due to the dioecious nature and high genetic heterozygosity (Anon, 1982).

The obtaining of date palm offshoots in a sufficient number for commercial propagation through the conventional rooting on the mother palm is a major problem since some cultivars produce a few number of offshoots. Therefore, the introduction of new cultivars or increasing the existing date acreage with clonal varieties would be slow if limited to propagation by offshoots. Moreover, the possible distribution of disease or insect – infested

offshoots is always a potential problem (Schroeder, 1970 and Tisserat, 1979 a and b).

In the recent years, tissue culture techniques are considered the most promising means for rapid clonal propagation of date palm varieties (El-Hennawy and Wally, 1980; Abo El-Nil *et al.*, 1986; and Belal and El-Deeb (1997).

Objectives of the current study

1- Developing an efficient and reliable protocol for micropropagation of date palm cv. “Zaghloul” using axillary bud proliferation (direct organogenesis).
2- Growing of axillary buds to proliferated shoots.
3- Shoot multiplication.
4- Production of uniformity plants of date palm cv. “Zaghloul” .

MATERIALS AND METHODS

The present investigation was carried out through three successive years (1999 – 2001), in order to study the possibility of using tissue culture technique for rapid and economical micropropagation of the desirable cultivar of date palm (Zaghloul cv.). The effect of the cytokinin (Kin.) to auxin (NAA) ratio in culture medium (Murashige and Skoog, 1962) as well as the explant type on the direct organogenesis in the explant cultures was studied in this work.

1. Plant Material

Offshoots of about 10 –15 cm in diameter, 2-3 kgs in weight were detached in February (1999) from adult date palm “Zaghloul” cv. grown in a private orchard located at Rasheed region, El-Behera Governorate.

The greater part of their leaves was cut off, the hard bottom of old leaves and the fibrous sheath were peeled off starting from the base and continued upwards. Removing the bottom of the old leaves was continued until the white soft tissues nearer to the heart of the offshoot appeared. By reaching the soft tissues, the offshoot was handled very carefully during the removal of the apical tissue of stem 8-10 cm in length and 3-4 cm in diameter (a sheathing leaf base was still enclosing the heart which included the apical meristem and several leaf primordia).

2. Sterilization

The apical tissue of stem was soaked in cold anti-oxidant solution (150 $mg1^{-1}$ ascorbic acid and 100 $mg1^{-1}$ citric acid) and kept in a refrigerator at 5 ^{o}C for 2 hrs.

The apical tissue of stem was placed in Erlenmeyer flask (1 litre) and surface disinfected using ethyl alchol 70% for 5 min., followed by sodium hypochlorite (Na O Cl) solution at 3% for 20 min. Surface sterilization of the apical tissue was carried out in a laminar flow hood. The sheathing leaf base that enclosed the heart was removed and the heart was resterilized using Na

O Cl solution at 1.5% for 10 min, then rinsed three times with distilled sterile anti-oxidant solution. Shoot tip explant ranged from 0.5 – 1.0 cm in length. The top portions of the removed leaf primordia were discarded while the bases were used as leaf primordia explants which were 0.3 – 0.5 cm in length.

3. Culture Media

Murashige and Skoog (1962) (MS) medium was used at full strength plus 30g1^{-1} sucrose and 8 g1^{-1} agar for culturing two types of explants (shoot tip and leaf primordia). The aseptic explants were cultured in glass test tubes (25 x 150 mm) filled with 10 ml (each) MS medium supplemented with various concentrations of plant growth regulators included kinetin (Kin.) (6 – Furfuryl amino purine) at 0.0, 5.0, 10.0 and 20.0 mg1^{-1} alone or combined with NAA (α - Naphthalen acetic acid) at 0.0, 0.5,1.0 and 2.0 mg1^{-1}. The test tubes were covered with cotton and capped with aluminum foil.

The pH of the media was adjusted to 5.7 using Na OH and HCl before adding agar (Difco Bacto-agar). The media sterilized in an autoclave at 121 ^{o}C for 20 min., then left to cool and harden for 24 hrs before being used.

4. Culture Conditions

The cultures were incubated at 27 $\pm$ 2 ^{o}C, under 16 hrs light period from fluorescent lamps (2 lamps per shelf), followed by 8 hrs dark period. Budding percentage calculated as follows:

$$\text{Budding percentage} = \frac{\text{Number of explants that formed distinctive buds}}{\text{Total number of explants}} \times 100$$

The budding percentage and number of buds for each type of explants were recorded after 6 weeks of explant culture. The resultant buds (axillary buds) were used as a mother stock explants for the shoot multiplication experiments.

5. Shoot Multiplication

5.1 Culture Media

The axillary buds that were produced from the shoot tip and leaf primordia cultures (the former experiments) were excised under aseptic conditions and transferred (individually) to 100 ml Erlenmeyer flasks, filled with 25 ml (each) MS medium supplemented with combinations of plant growth regulators included Kin and NAA at the same concentrations mentioned above, 3% sucrose and 0.8% agar. The pH of the media was adjusted to 5.7 before adding agar. The Erlenmeyer flasks covered with cotton and capped with aluminum foil. The media autoclaved at 121°C for 20 min, then left to cool and harden for 24 hrs before being used. Routine subculture of axillary buds was carried out every 6 weeks up to four subcultures. At the end of each subculture (after 6 weeks), the proliferated shoots were individually separated and transferred to a rooting medium.

5.2 Culture Conditions

The cultures were maintained at 27 $\pm$ 2 ^{o}C, under fluorescent light on a 16/8 hrs light/dark cycle. Both the average number of new proliferated

shoots (axillary shoots) that produced by each axillary bud (explant) and the average shoot length (cm) were recorded at the end of each subculture (6 weeks).

The proliferation rate = No. of proliferated shoots per explant in axillary bud cultures at the end of each subculture.

The resultant shoots (new proliferated shoots) were used as a mother cultures for the subsequent rooting experiments.

6. Statistical Analysis

In all experiments, each treatment consisted of three replicates with eight explants for each in a completely randomized design. One explant or axillary bud cultured in test tube or in Erlenmeyer flask and the statistical procedures were applied according to Steel and Torrie (1980).

The combinations between kinetin (Kin) and NAA concentrations in MS culture media were represented by 16 combinations as indicated in Tables (1 to 3) and took the combination code from C_1 to C_{16}.

RESULTS AND DISCUSSION

1- Effect of Kin and NAA combinations on budding percentage

Data concerning the effect of Kin and NAA combinations on budding percentage from shoot tip and leaf primordia explants, are listed in Table (1). The results indicated that, budding percentage from shoot tip explant was significantly the highest (100%) on 10.0 $mg1^{-1}$ Kin + 1.0 $mg1^{-1}$ NAA combination (C_{11}). On the contrary, the lowest percentage (37.5%) was resulted in 5.0 $mg1^{-1}$ kin + 0.0 $mg1^{-1}$ NAA and 20.0 $mg1^{-1}$ kin + 2.0 $mg1^{-1}$ NAA combinations (C_2) and (C_{16}), respectively. On the other hand, the data showed no budding occurred (0.00%) on the basal medium without the addition of growth regulators (C_1). Also, a similar result was observed (0.00%) on 0.0 $mg1^{-1}$ kin + 0.5 (C_5) or 1.0 (C_9) or 2.0 (C_{13}) $mg1^{-1}$ NAA combination.

Table (1) indicated that, the budding percentage from leaf primordia explant was significantly the highest (87.5%) on 10.0 $mg1^{-1}$ Kin + 1.0 $mg1^{-1}$ NAA combination (C_{11}). In contrast, the lowest percentage (12.5%) was resulted in 20.0 $mg1^{-1}$ kin + 2.0 $mg1^{-1}$ NAA combination (C_{16}).

On the other side, the data showed no budding occurred (0.00%) on the basal medium without the addition of growth regulators (C_1). Also, a similar result was observed (0.00%) on 0.0 $mg1^{-1}$ NAA + 5.0 (C_2) or 10.0 (C_3) or 20.0 (C_4) $mg1^{-1}$ kin, and on 0.0 $mg1^{-1}$ kin + 0.5 (C_5) or 1.0 (C_9) or 2.0 (C_{13}) $mg1^{-1}$ NAA combination.

2- Effect of kin and NAA combinations on average number of developed buds per explant

In respect to the effect of Kin and NAA combinations on average number of developed buds per shoot tip and leaf primordia explants, the results in Table (1) indicated that, average number of developed buds per shoot tip explant was significantly the highest (2.12) on 10.0 $mg1^{-1}$ Kin + 1.0 $mg1^{-1}$ NAA combination (C_{11}). On the contrary, the lowest average number (0.37) was resulted in 5.0 $mg1^{-1}$ Kin + 0.0 $mg1^{-1}$ NAA, 20.0 $mg1^{-1}$ Kin + 0.0

mg1^{-1} NAA and 20.0 mg1^{-1} Kin + 2.0 mg1^{-1} NAA combinations (C_2), (C_4) and (C_{16}), respectively.

On the other hand, the data showed no budding occurred (0.00 %) on the basal medium without the addition of growth regulators (C_1). Also, a similar result was observed (0.00%) on 0.0 mg1^{-1} Kin + 0.5 (C_5) or 1.0 (C_9) or 2.0 (C_{13}) mg1^{-1} NAA combination.

Results in Table (1) indicated that, average number of developed buds per leaf primordia explant was significantly the highest (1.25) on 10.0 mg1^{-1} Kin + 1.0 mg1^{-1} NAA combination (C_{11}). On the contrary, the lowest average number (0.5) was recorded with 20.0 mg1^{-1} Kin + 0.5 mg1^{-1} NAA and 5.0 mg1^{-1} Kin + 2.0 mg1^{-1} NAA combinations (C_8) and (C_{14}), respectively.

On the other hand, the results showed no budding occurred (0.00%) on the basal medium without the addition of growth regulators (C_1). Also, a similar result was observed (0.00%) on 0.0 mg1^{-1} NAA + 5.0 (C_2) or 10.0 (C_3) or 20.0 (C_4) mg1^{-1} Kin, on 0.0 mg1^{-1} Kin + 0.5 (C_5) or 1.0 (C_9) or 2.0 (C_{13}) mg1^{-1} NAA, and on 20.0 mg1^{-1} Kin + 2.0 mg1^{-1} NAA combination.

Table (1): Effect of Kin and NAA combinations on budding percentage and average number of developed buds per explant type of date palm cv. "Zaghloul" cultures.

Combination code	Growth regulators in mg1^{-1}		Explant types			
			Shoot tip		Leaf primordia	
	Kin	NAA	Budding (%)	Av.number of buds/explant	Budding (%)	Av. number of buds/explant
C_1	0.0	0.0	00.0 F*	0.00 J	0.00 E	0.00 E
C_2	5.0	0.0	37.5 E	0.37 I	00.0 E	0.00 E
C_3	10.0	0.0	75.0 BC	0.62 G	00.0 E	0.00 E
C_4	20.0	0.0	50.0 DE	0.37 I	00.0 E	0.00 E
C_5	0.0	0.5	00.0 F	0.00 J	00.0 E	0.00 E
C_6	5.0	0.5	62.5 CD	1.00 F	50.0 CD	1.00 B
C_7	10.0	0.5	87.5 AB	1.62 C	75.0 AB	1.00 B
C_8	20.0	0.5	75.0 BC	1.12 E	62.5 BC	0.50 D
C_9	0.0	1.0	00.0 F	0.00 J	00.0 E	0.00 E
C_{10}	5.0	1.0	75.0 BC	1.75 B	62.5 BC	0.75 C
C_{11}	10.0	1.0	100.0 A	2.12 A	87.5 A	1.25 A
C_{12}	20.0	1.0	87.5 AB	1.37 D	50.0 CD	0.62 CD
C_{13}	0.0	2.0	00.0 F	0.00 J	00.0 E	0.00 E
C_{14}	5.0	2.0	62.5 CD	0.62 G	50.0 CD	0.50 D
C_{15}	10.0	2.0	50.0 DE	0.50 H	37.5 D	0.75 C
C_{16}	20.0	2.0	37.5 E	0.37 I	12.5 E	0.00 E
L. S. D. 0.05			17.24	0.031	14.7	0.211

*** Values followed by the same letters are not significantly different at the 0.05 level of probability (in the same column).**

3- Effect of Kin and NAA combinations on average number of proliferated shoots per axillary bud explant

Regarding the effect of Kin and NAA combinations on average number of proliferated shoots per axillary bud explant through four subsequent subcultures, data are listed in Table (2). The results indicated that, in the first subculture, average number of proliferated shoots per axillary bud explant was significantly the highest (3.5) on 10.0 mg1^{-1} Kin +1.0 mg1^{-1}

NAA combination (C_{11}). On the contrary, the lowest average number (0.25) was achieved in 0.0 mg1^{-1} Kin +0.5 mg1^{-1} NAA combination (C_5).

On the other hand, the data showed no proliferated shoots occurred (0.00) on the basal medium without the addition of growth regulators (C_1). Also, a similar result was observed (0.00) on 0.0 mg1^{-1} Kin + 2.0 mg1^{-1} NAA combination (C_{13}).

Results in Table (2) cleared that, in the second subculture average number of proliferated shoots per axillary bud explant was significantly the highest (4.0) on 10.0 mg1^{-1} Kin + 1.0 mg1^{-1} NAA combination (C_{11}). In contrast, the lowest average number (0.25) was resulted in 0.0 mg1^{-1} Kin + 0.5 (C_5) or 2.0 (C_{13}) mg1^{-1} NAA combination.

On the other side, the results showed no proliferated shoots occurred (0.00) on the basal medium without the addition of growth regulators (C_1). Similar trend was noticed in the 3rd and 4th subcultures.

Data in Table (2), also, indicated that, in the third subculture, average number of proliferated shoots per axillary bud explant was significantly the highest (4.0) on 10.0 mg1^{-1} Kin + 1.0 mg1^{-1} NAA combination (C_{11}). On the contrary, the lowest average number (0.37) was obvious in 0.0 mg1^{-1} Kin + 2.0 mg1^{-1} NAA combination (C_{13}).

Results in Table (2) revealed that, in the fourth subculture, average number of proliferated shoots per axillary bud explant was significantly the highest (4.62) on 10.0 mg1^{-1} Kin + 1.0 mg1^{-1} NAA combination (C_{11}). In contrast, the lowest average number (0.62) was achieved with 0.0 mg1^{-1} Kin + 0.5 mg1^{-1} NAA combination (C_5).

Table (2): Effect of Kin and NAA combinations on average number of proliferated shoots per axillary bud explant of date palm cv. "Zaghloul" cultures.

Combination code	Growth regulators in mg1^{-1}		1st subculture	2nd subculture	3rd subculture	4th subculture
	Kin	NAA				
C_1	0.0	0.0	0.00 I	0.00 M	0.00 M	0.00 K
C_2	5.0	0.0	0.50 FG	0.50 J	0.87 H	1.00 I
C_3	10.0	0.0	0.62 F	0.87 H	1.00 G	1.40 H
C_4	20.0	0.0	0.50 FG	0.62 I	0.62 J	0.67 J
C_5	0.0	0.5	0.25 H	0.25 L	0.50 K	0.62 J
C_6	5.0	0.5	1.12 D	1.62 E	2.37 D	2.62 E
C_7	10.0	0.5	1.37 C	1.87 C	2.27 E	2.80 D
C_8	20.0	0.5	1.00 DE	1.25 F	2.25 E	3.00 C
C_9	0.0	1.0	0.37 GH	0.37 K	0.75 I	1.00 I
C_{10}	5.0	1.0	1.12 D	1.75 D	2.75 C	2.90 CD
C_{11}	10.0	1.0	3.50 A	4.00 A	4.00 A	4.62 A
C_{12}	20.0	1.0	2.00 B	2.25 B	3.00 B	3.80 B
C_{13}	0.0	2.0	0.00 I	0.25 L	0.37 L	1.33 H
C_{14}	5.0	2.0	0.62 F	0.87 H	1.00 G	2.17 F
C_{15}	10.0	2.0	0.87 E	1.00 G	1.87 F	2.60 E
C_{16}	20.0	2.0	0.37 GH	0.50 J	0.87 H	1.67 G
L.S.D. 0.05			0.240	0.093	0.072	0.137

*** Values followed by the same letters are not significantly different at the 0.05 level of probability (in the same column).**

3- Effect of kin and NAA combinations on average length of proliferated shoot

As for the effect of Kin and NAA combinations on average length of proliferated shoot through four subsequent subcultures, data are listed in Table (3). The results indicated that, in the first subculture, average length of proliferated shoot was significantly the highest (4.83 cm) on 10.0 $mg1^{-1}$ Kin + 1.0 $mg1^{-1}$ NAA combination (C_{11}). On the contrary, the lowest average length (0.76 cm) was noticed in 20.0 $mg1^{-1}$ Kin + 2.0 $mg1^{-1}$ NAA combination (C_{16}).

On the other hand, the results showed no proliferated shoots occurred (0.00) on the basal medium without the addition of growth regulators (C_1). Also, a similar result was observed (0.00) on 0.0 $mg1^{-1}$ Kin + 2.0 $mg1^{-1}$ NAA combination (C_{13}).

Table (3) indicated that, in the second subculture, average length of proliferated shoot was significantly the highest (5.62 cm) on 10.0 $mg1^{-1}$ Kin + 1.0 $mg1^{-1}$ NAA combination (C_{11}). On the contrary, the lowest average length (0.70 cm) was resulted in 0.0 $mg1^{-1}$ Kin + 2.0 $mg1^{-1}$ NAA combination (C_{13}).

On the other hand, the results showed no proliferated shoots occurred (0.00) on the basal medium without the addition of growth regulators (C_1). The same trend was obvious in the 3rd and 4th subcultures.

Data in Table (3) indicated that, in the third subculture, average length of proliferated shoot was significantly the highest (6.25 cm) on 10.0 $mg1^{-1}$ Kin + 1.0 $mg1^{-1}$ NAA combination (C_{11}). In contrast, the lowest average length (0.75 cm) was resulted in 0.0 $mg1^{-1}$ Kin + 2.0 $mg1^{-1}$ NAA combination (C_{13}).

Table (3): Effect of Kin and NAA combinations on average length (cm) of proliferated shoot of date palm cv. "Zaghloul" cultures.

Combination code	Growth regulators in $mg1^{-1}$		1st subculture	2nd subculture	3rd subculture	4th subculture
	Kin	NAA				
C_1	0.0	0.0	0.00 K*	0.00 L	0.00 K	0.00 M
C_2	5.0	0.0	1.50 G	1.50 I	2.00 G	2.20 I
C_3	10.0	0.0	2.70 D	2.85 E	3.05 F	3.12 F
C_4	20.0	0.0	1.20 H	1.55 I	1.80 H	2.00 J
C_5	0.0	0.5	1.00 I	1.00 J	1.60 I	2.30 HI
C_6	5.0	0.5	3.20 C	4.00 D	4.28 D	4.36 D
C_7	10.0	0.5	4.15 B	4.95 B	5.20 C	5.50 C
C_8	20.0	0.5	1.20 H	1.95 GH	2.15 G	2.32 HI
C_9	0.0	1.0	1.65 G	1.80 H	2.00 G	2.40 H
C_{10}	5.0	1.0	4.20 B	4.60 C	5.60 B	5.70 B
C_{11}	10.0	1.0	4.83 A	5.62 A	6.25 A	6.52 A
C_{12}	20.0	1.0	1.50 G	2.00 G	2.00 G	2.56 G
C_{13}	0.0	2.0	0.00 K	0.70 K	0.75 J	0.86 L
C_{14}	5.0	2.0	2.25 E	2.45 F	3.40 E	3.83 E
C_{15}	10.0	2.0	2.00 F	2.48 F	3.10 F	3.16 F
C_{16}	20.0	2.0	0.76 J	1.00 J	1.50 I	1.80 K
L. S. D. 0.05			0.156	0.156	0.164	0.138

*** Values followed by the same letters are not significantly different at the 0.05 level of probability (in the same column).**

The results in Table (3) revealed that, in the fourth subculture, average length of proliferated shoot was significantly the highest (6.52 cm) on 10.0 $mg1^{-1}$ Kin + 1.0 $mg1^{-1}$ NAA combination (C_{11}). On the contrary, the lowest average length (0.86 cm) was resulted in 0.0 $mg1^{-1}$ Kin + 2.0 $mg1^{-1}$ NAA combination (C_{13}).

From the overall results it is evident that micropropagation of date palm "Zaghloul" cv. could be achieved successfully through the direct development of axillary buds from either shoot tip or leaf primordia explant as a starting material. These findings are in agreement with those reported by Rhiss *et al.*, (1979); Tisserat (1984); Belal and El-Deeb (1997) and Awad (1999).

Moreover, shoot tip explant proved to be the best type of explants for *in vitro* date palm "Zaghloul" cv. since it gave the highest budding percentage and number of developed buds per explant. These results are in harmony with those found by Rhiss *et al.*, (1979) and Tisserat (1984). In addition. Awad (1999) reported that shoot tip explant of date palm "Sewey" cv. proved to be the best in terms of budding percentage and number of developed buds per explant compared with the leaf primordia explant.

The obtained results indicated that the combination of cytokinin (Kin) and auxin (NAA) appeared to be essential for axillary bud development and shoot proliferation in date palm cv. "Zaghloul" cultures.

It is worthy to mention that bud development and shoot proliferation of date palm "Zaghloul" cv. was accomplished on MS (1962) medium supplemented with 10.0 $mg1^{-1}$ kin combined with 1.0 $mg1^{-1}$ NAA.

These findings were in complete agreement with those reported by El- Hennawey and Wally (1980). Who found that for clonal multiplication using the off- shoots shoot tip disces. Bud differentiation *in vitro* took place in Murashige and Skoog (1962) inorganic salt mixture with the addition of 1.0 $mg1^{-1}$ of both NAA and Kinetin. Moreover, Abo El-Nil *et al.*, (1986) found that organogenesis was regulated by the ratio of cytokinin to auxin rather than by the concentration of each compound individually. Also, Wongkaew *et al.*, (1991) obtained the proliferated shoots from shoot apex tissues when were cultured on the Murashige and Skoog (1962) medium, supplemented with 10.0 $mg1^{-1}$ Kinetin and 1.0 $mg1^{-1}$ NAA.

On the other hand, these findings partially agreed with those reported by Zaid and Tisserat (1983). They obtained the developed shoots from shoot tips when were cultured on the Murashige and Skoog (1962) medium containing 10.0 $mg1^{-1}$ NAA. Furthermore, Gabr and Tisserat (1985) obtained the optimum shoot tip development and axillary budding from shoot tip explants when were cultured on the Murashige and Skoog (1962) medium, supplemented with 1.0 $mg1^{-1}$ NAA and 15.0 $mg1^{-1}$ BA or 2 ip. In addition, Belal and El-Deeb (1997) found that shoot proliferation was best on the Murashige and Skoog (1962) medium, supplemented with IAA (0.5 $mg1^{-1}$) + NAA (0.5 $mg1^{-1}$) + 10.0 $mg1^{-1}$ BAP.

Recently, Awad (1999) reported that bud development and shoot proliferation were superior on the Murashig and Skoog (1962) medium plus 1.0 $mg1^{-1}$ (2ip + Kin) and 0.5 $mg1^{-1}$ NAA. The highest length of proliferated

shoots was obtained on the Murashige and Skoog (1962) medium, contained 0.5 $mg1^{-1}$ (2ip + Kin) and 0.5 $mg1^{-1}$ NAA.

The best results for shoot elongation of date palm "Zaghloul" cv. were obtained on MS medium containing 10.0 $mg1^{-1}$ kin combined with 1.0 $mg1^{-1}$ NAA in the 1st , 2nd , 3rd and 4th subcultures. These findings are in accordance with those found by Poulain *et al.*, (1979) and El-Hennawy *et al.*, (1983).

On the other hand, Shaheen and Said (1986), and Belal and El-Deeb (1997) achieved axillary shoot proliferation of date palm cvs. Sefi, Zaghloul and Sammani on MS medium supplemented with NAA at ranges from 0.1 – 3.0 $mg1^{-1}$ and 2ip or BA at 5.0-15.0 $mg1^{-1}$.

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١- الإكثار الدقيق لنخيل البلح صنف "الزغلول" عن طريق التكوين المباشر للأعضاء

محمد بدر الصبروت
قسم الفاكهة – كلية الزراعة – جامعة الإسكندرية – الإسكندرية – مصر

أجرى هذا البحث خلال ثلاث سنوات متتالية (١٩٩٩- ٢٠٠١) بغرض الإكثار المعملى الدقيق لنخيل البلح صنف "الزغلول" بإستخدام تقنية زراعة الأنسجة وذلك بدراسة تأثير نسبة السيتوكينين (كينتين) إلى الأكسين (نفثالين حمض الخليك) فى بيئة الزراعة (موراشيج وسكوج ١٩٦٢) وأيضا دراسة تأثير نوع المنفصل النباتى على التكوين المباشر للأعضاء فى مزارع الأنسجة.
ويمكن تلخيص النتائج الرئيسية لهذه الدراسة فى النقاط التالية:

1. إن استخدام منفصل القمة النامية أثبت تفوقه فى النسبة المئوية لتكوين البراعم وعدد البراعم المتكونة بالنسبة لكل منفصل مقارنة باستخدام منفصل منشأ الورقة.
2. أدى استخدام بيئة موراشيج وسكوج (١٩٦٢) مضاف إليها ١٠ملجرام فى اللتر كينتين + ١ملجرام فى اللتر نفثالين حمض الخليك إلى الحصول على أعلى نسبة مئوية لتكوين البراعم وذلك بصورة جوهرية (١٠٠% بالنسبة لمنفصل القمة النامية و ٨٧,٥% بالنسبة لمنفصل منشأ الورقة وأيضا متوسط عدد البراعم المتكونة لكل منفصل كان الأعلى بصورة جوهرية (٢,١٢بالنسبة لمنفصل القمة النامية و ١,٢٥ بالنسبة لمنفصل منشأ الورقة).
3. كما أدى استخدام نفس البيئة السابقة إلى الحصول على أعلى قيم لكل من تعدد الأفرخ ومتوسط عدد الأفرخ المتكونة بالنسبة لكل منفصل برعم أبطى ومتوسط طول الفرخ المتكون وذلك خلال أربعة زراعات (نقلات) متتالية.
4. ففى النقلة الأولى والثانية والثالثة والرابعة فإن متوسط عدد الأفرخ المتكونة بالنسبة لكل منفصل برعم أبطى كان الأعلى بصورة جوهرية (٣,٥ ، ٤,٠ ، ٤,٠ ، ٤,٦٢ على التوالى) وكان متوسط طول الفرخ المتكون الأعلى بصورة جوهرية (٤,٨٣سم ، ٥,٦٢سم ، ٦,٢٥سم ، ٦,٥٢سم على التوالى).