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II- *IN VITRO* PROPAGATION OF DATE PALM (*Phoenix dactylifera* L.) cv. "ZAGHLOUL" BY SOMATIC EMBRYOGENESIS

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ABSTRACT

The present investigation was carried out during three successive years (1999- 2001), in order to study the effect of the cytokinin (BA) to auxin (NAA) ratio in culture medium (Murashige and Skoog, 1962) on somatic embryogenesis 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 callus formation percentage as compared with the leaf primordia explant.
2- MS (1962) medium supplemented with 3.0 $mg1^{-1}$ 2 ip + 30.0 $mg1^{-1}$ NAA, the callus formation percentage was significantly the highest (100% in shoot tip explant and 50.0% in leaf primordia explant).
3- Embryogenesis percentage was significantly the highest (87.5%) on MS (1962) medium supplemented with 3.0 $mg1^{-1}$ B A + 1.0 $mg1^{-1}$ NAA. On the same medium, average number of germinated embyoids per callus was significantly the highest (2.75).
4- MS medium supplemented with 3.0 $mg1^{-1}$ NAA in medium produced the highest rooting percentage (100 %), greatest average roots number per shoot (12.0) and the longest roots (10.0 cm).
5- Finally, 70% of the obtained plants of date palm cv. "Zaghloul" were successfully transplanted to soil.

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 generally propagated by using offshoots which produced in low number through the tree whole life time. Thus, rapid propagation of date palm through tissue culture is the most promising techniques for production of sufficient numbers of date palm offshoots with higher quality and yields (Moursy and Sakr, 1996).

Callus was obtained from various date palm tissues that included, leaf primordia, inflorescence, ovule, shoot tip, axillary buds and roots (Reuveni, 1979; Sharma *et al*., 1991; Abo El-Nil, 1986; Kachar *et al*., 1989; and Nazeri *et al*., 1993).

Embryogenic callus has been obtained from shoot tips and buds excised from offshoots (Mater, 1986; Sharma *et al*., 1980, 1984 and 1986; Tisserat, 1979 a and b, and 1982; and Zaid and Tisserat, 1983).

Objectives of the present study

1. Developing of an efficient and reliable protocol for micropropagation of date palm “Zaghloul” cv. using somatic embryogenesis (embryoid formation) derived from callus.
2. Growing of embryoids to proliferated shoots.
3. Rooting of proliferated shoots.
4. Transplanting of plants to soil.
5. Production of uniformity plants of date palm “Zaghloul” cv.

MATERIALS AND METHODS

The present investigation was conducted during three successive years (19 99 – 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 cytokinin (BA) to auxin (NAA) ratio in culture medium (Murashige and Skoog, 1962) on somatic embryogenesis in the callus cultures was studied in this work.

1. Callus Formation

1.1. 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 bottoms of old leaves and the fibrous sheath were peeled off starting from the base and continued upwards. Removing the bottoms 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).

1.2. Sterilization

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

1.3. Culture Media

The aseptic shoot tip and leaf primordia explants were cultured in glass test tubes (25 x 180 mm), filled with 15 ml (each) Murashige and Skoog (1962) medium supplemented with various concentrations of plant growth

regulators included 2 ip [N6 – (2 – Isopentenyl) – adenine] at 0.0 , 2.0 , 3.0 and 4.0 $mg1^{-1}$ either alone or combined with NAA (α - Naphthalen acetic acid) at 00.0 , 20.0 , 30.0 and 40.0 mgl^{-1}. MS (1962) medium was used at full strength plus 30 gl^{-1} sucrose and 8 gl^{-1} agar. The PH of the media was adjusted to 5.7 before adding agar (Difco Bacto-agar, 8000 $mg1^{-1}$). One explant cultured in test tube. The culture tubes closed with cotton, capped with aluminum foil, and sterilized in an autoclave at 121 ^{o}C for 20 min, then left to cool and harden for 24 hrs before being used.

The combinations between 2ip and NAA concentrations in MS culture media (callus formation media) were represented by 16 combinations as indicated in Table (1) and took the combination code from C_1 to C_{16}.

1.4. Culture Conditions

The cultures were incubated in complete darkness under temperature of 27 $\pm$ 2 ^{0}C. Callus formation was evaluated after 8 weeks from culturing date by the use of callus formation percentage as an index which calculated as follows:

$$\text{Callus formation \%} = \frac{\text{No. of cultured tubes with callus formation}}{\text{Total no. of cultured tubes}} \text{ x } 100$$

Callus formation index = fresh weight of the formated callus per explant: $\geq$ 100 mg.

2. Embryogenesis

2.1. Culture Media

The obtained callus was used as a tissue source for induction of embryoids (somatic embryogenesis). This callus aseptically transferred to test tubes (25 x180 mm), filled with 15ml (each) MS medium supplemented with plant growth regulators including BA (N6 – Benzyladenine) at 0.0 , 1.0 , 2.0 and 3.0 mgl^{-1} either alone or combined with NAA at 0.0 , 1.0 , 2.0 and 3.0 mgl^{-1} for embryogenesis and embryoids germination. The germinated embryoids were individually transferred to the same medium composition for subsequent growth and development. The PH of the media was adjusted to 5.7 before adding agar. The culture tubes closed with cotton, capped with aluminum foil and sterilized in an autoclave at 121 ^{o}C for 20 min, then left to cool and harden for 24 hrs before being used. One callus (100 mg) cultured in test tube.

The combinations between BA and NAA concentrations in MS culture media (embryogenic media) were represented by 16 combinations as indicated in Table (2) and took the combination code from C_1 to C_{16}.

2.2. Culture Conditions

The cultures were incubated at 27 $\pm$ 2 ^{0}C with 16 hrs light from fluorescent lamps (2 lamps per shelf), followed by 8 hrs dark periods.

The embryogenesis induction was evaluated after one subculture (8 weeks) by the use of embryogenesis percentage which calculated as follows:

$$\text{Embryogenesis \%} = \frac{\text{No. of cultured tubes with formated embryoids}}{\text{Total no. of cultured tubes}} \times 100$$

3. Rooting of Proliferated Shoots

3.1. Culture Media

The main objective of these experiments was to determine the best auxin (NAA) concentration on the rooting of proliferated shoots of date palm "Zaghloul" cv. Uniform proliferated shoots ($\geq$ 4 cm in length) derived from direct organogenesis and somatic embryogenesis were transferred to glass test tubes (25 x 180 mm) filled with 15 ml (each) MS medium supplemented with NAA at 0.0 , 1.0 , 2.0, 3.0 and 4.0 $mg1^{-1}$ plus 30 $g1^{-1}$ sucrose and 8 gl^{-1} agar. The PH of the media was adjusted to 5.7 before adding agar. One proliferated shoot cultured in test tube. The culture tubes closed with cotton, capped with aluminum foil, and sterilized in an autoclave at 121 ^{o}C for 20 min, then left to cool and harden for 24 hrs before being used. Rooting percentage, average number of roots per shoot and average root length per shoot were recorded after 8 weeks of culture. The rooting percentage calculated as follows:

$$\text{Rooting percentage} = \frac{\text{No. of cultured tubes with rooted shoots}}{\text{Total no. of cultured tubes}} \times 100$$

3.2. Culture Conditions

The cultures were maintained for 8 weeks in a 16/8 hrs light/dark cycle at 27 $\pm$ 2 ^{o}C with fluorescent light.

4. Statistical Analysis

In all experiments, each treatment consisted of three replicates with eight tubes for each in a completely randomized design. One explant (for callus formation) or callus (for embryoid formation) or proliferated shoot (derived from axillary bud and embryoid for rooting) cultured in test tube and the statistical procedures were applied according to Steel and Torrie (1980).

5. Transplanting of the Plants to Soil

The obtained plantlets (derived from direct organogenesis and somatic embryogenesis) of date palm cv. "Zaghloul" were potted (when reaching 5 – 7 cm in height) in a sterilized vermiculite for one month. Irrigation was carried out every four days with nutrient solution (1/2 MS medium without sucrose). The plants (when reaching 10 – 15 cm in height) were then transferred to plastic pots containing a mixture of 1 vermiculite: 1 peat moss and acclimatized rapidly for 3 months under intermittent mist in greenhouse. Observations on survival and growth were recorded.

RESULTS AND DISCUSSION

1. Effect of 2ip and NAA combinations on callus formation percentage

Data concerning the effect of 2 ip and NAA combinations on callus formation percentage from shoot tip and leaf primordia explants, are listed in Table (1). The results indicated that, callus formation percentage from shoot tip explant was significantly the highest (100%) on 3.0 mgl^{-1} 2ip + 30.0 mgl^{-1} NAA combination (C_{11}). On the contrary, the lowest percentage (37.5%) was resulted in 4.0 mgl^{-1} 2ip + 20.0 (C_{14}) or 40.0 (C_{16}) mgl^{-1} NAA combination. On

the other hand, the data showed no callus formation 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 mgl^{-1} 2ip + 20.0 (C_2) or 30.0 (C_3) or 40.0 (C_4) mgl^{-1} NAA combination, and on 0.0 mgl^{-1} NAA + 2.0 (C_5) or 3.0 (C_9) or 4.0 (C_{13}) mgl^{-1} 2 ip combination.

Table (1): Effect of 2ip and NAA combinations on callus formation[z] percentages of explant types in date palm cv. "Zaghloul" cultures.

Combination Code	Growth regulators in $mg1^{-1}$		Explant types	
	2ip	NAA	Shoot tip	Leaf primordia
C_1	0.0	00.0	00.0 F*	00.0 E
C_2	0.0	20.0	00.0 F	00.0 E
C_3	0.0	30.0	00.0 F	00.0 E
C_4	0.0	40.0	00.0 F	00.0 E
C_5	2.0	00.0	00.0 F	00.0 E
C_6	2.0	20.0	87.5 AB	37.5 B
C_7	2.0	30.0	75.0 BC	25.0 C
AAC_8	2.0	40.0	50.0 DE	12.5 D
C_9	3.0	00.0	00.0 F	00.0 E
C_{10}	3.0	20.0	75.0 BC	25.0 C
C_{11}	3.0	30.0	100.0 A	50.0 A
C_{12}	3.0	40.0	62.5 CD	12.5 D
C_{13}	4.0	00.0	00.0 F	00.0 E
C_{14}	4.0	20.0	37.5 E	00.0 E
C_{15}	4.0	30.0	50.0 DE	12.5 D
C_{16}	4.0	40.0	37.5 E	00.0 E
L.S.D. 0.05			14.7	10.395

[z] The produced callus was white and friable.

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

Table (1) indicated that, the callus formation percentage from leaf primordia explant was significantly the highest (50.0%) on 3.0 mgl^{-1} 2ip + 30.0 mgl^{-1} NAA combination (C_{11}). In contrast, the lowest percentage (12.5%) was resulted in 40.0 mgl^{-1} NAA + 2.0 (C_8) or 3.0 (C_{12}) mgl^{-1} 2ip combination, and in 4.0 $mg1^{-1}$ 2ip + 30.0 $mg1^{-1}$ NAA combination (C_{15}).

On the other side, the data showed no callus formation 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 mgl^{-1} 2 ip + 20.0 (C_2) or 30.0 (C_3) or 40.0 (C_4) mgl^{-1} NAA combination, on 0.0 mgl^{-1} NAA + 2.0 (C_5) or 3.0 (C_9) or 4.0 (C_{13}) $mg1^{-1}$ 2ip combination, and on 4.0 $mg1^{-1}$ 2ip + 20.0 (C_{14}) or 40.0 (C_{16}) mgl^{-1} NAA combination.

2. Effect of BA and NAA combinations on embryogenesis percentage

In respect to the effect of BA and NAA combinations on embryogenesis percentage from callus, the results in Table (2) indicated that, embryogenesis percentage was significantly the highest (87.5%) on 3.0 $mg1^{-1}$ BA + 1.0 $mg1^{-1}$ NAA combination (C_8). On the contrary, the lowest percentage (12.5%) was resulted in 1.0 $mg1^{-1}$ BA + 2.0 $mg1^{-1}$ NAA

combination (C_{10}), and in 3.0 $mg1^{-1}$ NAA + 1.0 (C_{14}) or 2.0 (C_{15}) or 3.0 (C_{16}) $mg1^{-1}$ BA combination. On the other hand, the data showed no embryogenesis 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 + 1.0 (C_2) or 2.0 (C_3) or 3.0 (C_4) $mg1^{-1}$ BA combination, and on 0.0 $mg1^{-1}$ BA + 1.0 (C_5) or 2.0 (C_9) or 3.0 (C_{13}) $mg1^{-1}$ NAA combination.

3. Effect of BA and NAA combinations on average number of germinated embryoids

Data concerning the effect of BA and NAA combinations on average number of germinated embryoids per callus, are listed in Table (2). The results indicated that, average number of germinated embryoids per callus was significantly the highest (2.75) on 3.0 $mg1^{-1}$ BA + 1.0 $mg1^{-1}$ NAA combination (C_8). In contrast, the lowest average (0.709) was resulted in 1.0 $mg1^{-1}$ BA + 1.0 $mg1^{-1}$ NAA combination (C_6), and in 2.0 $mg1^{-1}$ BA + 2.0 $mg1^{-1}$ NAA combination (C_{11}). On the other hand, the data showed no embryogenesis 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 + 1.0 (C_2) or 2.0 (C_3) or 3.0 (C_4) $mg1^{-1}$ BA combination, and on 0.0 $mg1^{-1}$ BA + 1.0 (C_5) or 2.0 (C_9) or 3.0 (C_{13}) $mg1^{-1}$ NAA combination.

Table(2): Effect of BA and NAA combinations on embryogenesis[z] percentages and average number of germinated embryoids per callus of date palm cv. "Zaghloul" cultures.

Combination Code	Growth regulators in $mg1^{-1}$		Embryogenesis (%)	Average number of germinated embryoids/callus
	BA	NAA		
C_1	0.0	0.0	00.0 F*	0.000F
C_2	1.0	0.0	00.0 F	0.000F
C_3	2.0	0.0	00.0 F	0.000F
C_4	3.0	0.0	00.0 F	0.000F
C_5	0.0	1.0	00.0 F	0.000F
C_6	1.0	1.0	25.0 D	0.709D
C_7	2.0	1.0	50.0 C	1.375C
C_8	3.0	1.0	87.5 A	2.750A
C_9	0.0	2.0	00.0 F	0.000F
C_{10}	1.0	2.0	12.5 E	0.250E
C_{11}	2.0	2.0	25.0 D	0.709D
C_{12}	3.0	2.0	75.0 B	2.416B
C_{13}	0.0	3.0	00.0 F	0.000F
C_{14}	1.0	3.0	12.5 E	0.250E
C_{15}	2.0	3.0	12.5 E	0.250E
C_{16}	3.0	3.0	12.5 E	0.250E
L.S.D. 0.05			11.622	0.00537

[z]Only the nodular callus produced viable somatic embryos.

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

From the overall results it is evident that micropropagation of date palm "Zaghloul" cv. could be achieved successfully through the somatic embryogenesis from callus. These results are in agreement with those

reported by Veramendi and Navarro (1997); Sakr *et al.*, (1998); Sharon and Shankar (1998); Ahmed (1999) and El-Hammady *et al*., (1999).

Moreover, shoot tip explant was the best plant material for callus formation in date palm cv. "Zaghloul". These findings were assured by Khan *et al*., (1983); Tisserat (1983); Al-Maari and Al- Ghamdi (1995); Quraishi *et al*., (1997) and El-Shafey *et al*., (1999). Who found that shoot tip explant was the best material for callus formation and regeneration of date palm cvs.

It is worthy to mention that callus of date palm "Zaghloul" cv. could be produced by culturing shoot tip and leaf primordia explants on media containing a relatively moderate concentration of auxin (NAA) and low concentration of cytokinin (2 ip). These results are in accordance with those found by Tisserat (1979 a and b) and Abo El- Nil (1986).

The highest percentage of callus formation was obtained on MS (1962) medium supplemented with 3.0 $mg1^{-1}$ 2 ip + 30.0 $mg1^{-1}$ NAA. These results were in complete agreement with those reported by El- Hammady *et al*., (1999). Who found that the highest percentage of callus formation in date palm "Sewy" cv. was achieved on MS medium plus NAA at 50 $mg1^{-1}$ and 2.0 $mg1^{-1}$ 2 ip.

These findings partially agreed with those obtained by Sharon and Shankar (1998). Who found that callus of date palm "Yakubi" cv. was initiated on modified MS medium supplemented with 2,4– D (50 $mg1^{-1}$) + Kinetin (1.0 $mg1^{-1}$) + 2 ip (0.5 $mg1^{-1}$). In addition, Ahmed (1999) reported that callus of "Zaghloul" cv. was formed on MS medium supplemented with 100 $mg1^{-1}$ 2 , 4- D + 3 $mg1^{-1}$ 2ip.

On the other hand, these findings disagreed with those reported by Sakr *et al*., (1998). Who found that callus of "Zaghloul" cv. was proliferated onto MS medium supplemented with 10 $mg1^{-1}$ 2 ,4– D +3 $g1^{-1}$ activated charcoal (AC).

The results indicated that embryogenesis percentage was the highest on MS medium supplemented with 3.0 $mg1^{-1}$ BA + 1.0 $mg1^{-1}$ NAA. These findings partially agreed with those reported by Sakr *et al*., (1998). Who found that shoot proliferation after a phase of callus formation in date palm "Zaghloul" cv. was confined to MS medium containing 3 $mg1^{-1}$ 2ip + 0.1 $mg1^{-1}$ NAA + 3 $g1^{-1}$ AC. In addition, Sharon and Shankar (1998) found that embryogenic medium for callus of "Yakubi" cv. was MS medium + BA (2 $mg1^{-1}$) + NAA (0.5 $mg1^{-1}$) + NOA (0.5 $mg1^{-1}$). Moreover, Ahmed (1999) reported that embryogenic medium for callus of "Zaghloul" cv. was MS medium supplemented with 0.1 $mg1^{-1}$ NAA + 0.1 $mg1^{-1}$ 2ip. Recently, El-Hammady *et al*., (1999) reported that somatic embryogenesis and embryos germination in date palm " Sewy" cv. were obvious on modified MS medium plus 0.1 $mg1^{-1}$ NAA.

However, these results disagreed with those obtained by Zaid and Tisserat (1983) induced embryogenic callus of date palm on MS medium containing 3 $mg1^{-1}$ 2ip + 100 $mg1^{-1}$ 2, 4 – D. In addition, Shakib *et al*., (1994) reported that somatic embryos of date palm variety "Estamaran" were formed on medium without growth regulators.

The results also indicated that the highest average number of germinated embryoids per callus was obtained on MS medium supplemented

with 3.0 $mg1^{-1}$ BA + 1.0 $mg1^{-1}$ NAA. These findings partially agreed with those reported by El-Hammady *et al*., (1999). Who found that the highest average number of germinated embryos of date palm "Sewy" cv. was recorded from callus proliferated on MS medium with 2.0 $mg1^{-1}$ 2 ip + 50 $mg1^{-1}$ NAA.

4. Rooting of Proliferated Shoots

Data in Table (3) illustrated the effect of five concentrations of NAA in MS medium on rooting percentage, average number of roots per shoot and average root length per shoot.

The results revealed that NAA at 3.0 or 2.0 or 4.0 or 1.0 $mg1^{-1}$ significantly increased rooting percentage than control treatment (0.0 $mg1^{-1}$ NAA). The highest rooting percentage (100 %) was recorded by NAA at 3.0 $mg1^{-1}$, while the lowest rooting percentage (37.5 %) was obtained in control treatment (0.0 $mg1^{-1}$ NAA). In addition, the highest significant average number of roots per shoot (12.0) was recorded by NAA at 3.0 $mg1^{-1}$, while the lowest number (3.0) was obtained in control treatment (0.0 $mg1^{-1}$ NAA).
Data also, indicated that NAA at 3.0 $mg1^{-1}$ resulted in the highest significant average root length per shoot (10.0 cm). On the contrary, 0.0 $mg1^{-1}$ NAA (control treatment) produced the lowest average root length (1.0 cm).

Table (3): Effect of five concentrations of NAA on rooting of proliferated shoots of date palm cv. "Zaghloul" cultures.

NAA ($mg1^{-1}$)	Rooting (%)	Average number of roots/proliferated shoot	Average root length (cm)
0.0	37.5 D*	3.0 D	1.00 D
1.0	62.5 C	5.2 C	3.20 C
2.0	87.5 AB	8.0 B	4.64 B
3.0	100.0 A	12.0 A	10.00 A
4.0	75.0 BC	7.0 BC	3.40 C
L.S.D. $_{0.05}$	20.34	1.84	0.862

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

Results in Table (3) revealed that 3.0 $mg1^{-1}$ NAA produced the highest rooting percentage, greatest average roots number per shoot and the longest roots. These findings partially agreed with those obtained by Belal and El-Deeb (1997). Who reported that rooted shoots were produced on MS medium supplemented with 3.0 $mg1^{-1}$ NAA and 0.5 $mg1^{-1}$ kinetin.
Contradictory results were reported by Tisserat (1983), Zaid and Tisserat (1983), Tisserat (1984), Wonghaew *et al*., (1991) and Awad (1999). Who found that optimum adventitious rooting obtained on MS medium containing 0.1 $mg1^{-1}$ NAA. In addition, Anjarne and Zaid (1993) reported that rooted shoots were produced on MS medium supplemented with 5.0 $mg1^{-1}$ NAA.

5. Transplanting of the Obtained Plants to Soil

The date palm cv. "Zaghloul" plants were transferred to greenhouse conditions after acclimatization for three months. These plants survived with a well-developed adventitious root system and 2-3 erect leaves. Finally, 70% of date palm plants of "Zaghloul" cv. were successfully transplanted to soil.

These findings agreed with those obtained by Tisserat (1984) who reported that plantlets of date palm were transferred to pots containing 1:1 peat moss: vermiculite. Plantlets of 10 –15 cm length with 2–3 leaves were transferred to soil with surival rates close to 100%. In addition, Al-Jibouri *et al*., (1988) studied transfer of the *in vitro* regenerated date palms to the soil and reported that date plants derived from tissue culture, showed the highest success rate (up to 78.9%) for transfer to free living conditions. However, Shakib *et al*., (1994) reported that the plantlets of date palm were transferred to soil in the greenhouse when they were 10 – 15 cm tall. In this concept, Quraishi *et al*., (1997) reported that the survival *ex vitro* was 70 – 80% when well- rooted plants of date palm 8 – 12 cm in length were used. In addition, Sharon and Shankar (1998) reported that plantlets of date palm were successfully transferred to pots containing a mixture (1:1) of vermiculite and peat moss. Recently, Ahmed (1999) obtained the highest percentage of survival (90%) after three months from planting of date palm "Zaghloul" cv. plants in greenhouse.

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٢- الإكثار الدقيق لنخيل البلح صنف "الزغلول" عن طريق تكوين الأجنة الجسمية

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أجرى هذا البحث خلال ثلاث سنوات متتالية (١٩٩٩ – ٢٠٠١) بغرض الإكثار المعملى الدقيق لنخيل البلح صنف "الزغلول" باستخدام تقنية زراعة الأنسجة وذلك بدراسة تأثير نسبة السيتوكينين (بنزايل أدنين) إلى الأكسين (نفثالين حمض الخليك) فى بيئة الزراعة (مورا شيج وسكوج ١٩٦٢) على تكوين الأجنة الجسمية فى مزارع الأنسجة.

ويمكن تلخيص النتائج الرئيسية لهذه الدراسة فى النقاط التالية:

1- أثبت استخدام منفصل القمة النامية أثبت تفوقه فى النسبة المئوية لتكوين الكالس مقارنة باستخدام منفصل منشأ الورقة.

2- أدى استخدام بيئة مورا شيج وسكوج (١٩٦٢) مضاف إليها ٣ ملجرام فى اللتر ٢ أيزو بنتانيل أدنين + ٣٠ ملجرام فى اللتر نفثالين حمض الخليك إلى الحصول على أعلى نسبة مئوية لتكوين الكالس وذلك بصورة جوهرية (١٠٠% بالنسبة لمنفصل القمة النامية و٥٠% بالنسبة لمنفصل منشأ الورقة).

3- أدى إستخدام بيئة موراشيج وسكوج (١٩٦٢) مضاف إليها ٣ ملجرام فى اللتر بنزايل أدنين + ١ ملجرام فى اللتر نفثالين حمض الخليك إلى الحصول على أعلى نسبة مئوية لتكوين الأجنة الجسمية وذلك بصورة جوهرية(٨٧,٥ %) . كما أدى استخدام نفس البيئة إلى الحصول على أعلى متوسط لعدد الأجنة النابتة بالنسبة للكالس الواحد وذلك بصورة جوهرية (٢,٧٥).

4- أدت إضافة نفثالين حمض الخليك بتركيز ٣ملجرام فى اللتر إلى بيئة موراشيج وسكوج (١٩٦٢) إلى الحصول على أعلى نسبة مئوية للتجذير (١٠٠%) وأكبر متوسط لعدد الجذور بالنسبة لكل فرخ (١٢) وأطول الجذور (١٠سم).

5- أظهر نقل ٧٠% من النباتات الناتجة لنخيل البلح صنف "الزغلول" إلى التربة نسبة عالية من النجاح.