HIGH FREQUENCY SOMATIC EMBRYO PRODUCTION AND MATURATION INTO PLANTLETS IN DATE PALM (*PHOENIX DACTYLIFERA* L.) THROUGH SUSPENSION CULTURE

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Abstract

A highly efficient somatic embryo production and maturation procedure has been developed to regenerate plantlets from date palm (*Phoenix dactylifera* L.). This procedure involves the acceleration of differentiation through manipulation of MS salt strength and growth regulators . Embryogenic calli were initiated from shoot tip and leaf primordial of date palm on MS medium supplemented with 5mg/l 2,4-D +3mg/l 2iP + 1mg/l IAA +1.5g/l activated charcoal (AC) . Friable callus were subcultured on liquid culture medium with different strength of MS formula, different concentrations of 2,4-D and different type of cytokinin . Liquid culture medium with half strength of MS formula containing 0.5 mg/l 2,4-D enhanced fresh weight of embryogenic callus (5 to 6 fold approximately/month), number of pro-embryo(globular and juvenile)and number of mature embryos . BA was superior to Kin or 2iP in promoting callus growth, pro-embryo (globular and juvenile) while Kin was superior to BA or 2iP in promoting number of mature embryos. Conversion frequency into normal plantlet was improved gradually with increasing the concentration of GA_3 in solid cultured medium to 0.4 mg/l.

Key words: *Phoenix dacty lifera*, suspension culture , pro-embryo (globular), maturation, germination

INTRODUCTION

Date palm, *Phoenix dactylifera* L., is one of the oldest fruit trees in the world and is mentioned in the Holy Qur'an and Bible. Date palm is one of the most important fruit trees in the Middle East and in the Saharan and Sub-Saharan regions of Africa. In some areas, this is the only tree which provides food, shelter and fuel to the communities. Dates are not only a staple food but are also an important export cash crop. (Zaid and Hegarty 2006) .

The multiplication of date palm was traditionally achieved by seeds and by off-shoots. However, these methods are improved by biotechnological methods such as somatic embryogenesis and organogenesis (El Hadrami *et al.*, 1998). Previous studies have shown that maturation and germination of somatic embryos can be influenced by various in vitro factors, including auxin and cytokinin concentration (Iraqui and Tremblay, 2001, Gonzalez *et al.*, 2001, Corredoira *et al.*, 2003).

The use of liquid media for regerenation of date palm has been reported by several authors (Sharma, *et al.* 1986, Daguin and letouze 1988, Bhaskaran and Smith 1992) Fki *et al.*, 2003 and Zouine and El Hadrami (2007). This technique enables the production of individualized embryos in synchronous growth, with both root and shoot poles, compared to the routine process with polyembryonic cultures and compulsory rooting treatment. (De Touchet *et al.* (1991) In the case of date palm, several works have been published describing some culture media for somatic embryogenesis (El Hadrami *et al.,* 1995, Masmoudi *et al.,* 1999, El Bellaj, 2000, Al- Khayri, 2001, 2002, Fki *et al.*, 2003, Zouine and El Hadrami, 2004, Gadalla, *et al.,*2004). However, few works deal with the effects of several culture media combinations on some physiological parameters during the multiplication and maturation of somatic embryos. In most case, 2,4-D and BAP have been used to support growth of somatic embryos (Ammirato, 1983).

In numerous systems, in spite of the high number of somatic embryos produced, problems with a lack or a low frequency of embryo conversion into plants occurred. To stimulate embryo conversion, and to improve the efficiency of plant regeneration, Giberellic acid (GA_3) is frequently employed in media used for somatic embryo conversion (Kim *et al.* 1997), and a significant stimulatory effect of this phytohormone was proved in cultures of *Sesamum indicum* (Xu *et al.* 1997) and *P. ginseng* (Yang and Choi 2000).

The present study aims to investigate the effect of various strengths of MS (2 MS,MS and Half strength medium), different concentrations of 2,4-D (0.0, 0.5 and 1.0 mg/l) and different type of cytokinin (Kin,2iP and BA) add to liquid culture medium on callus growth (Fresh weight), number of pro-embryos (globular and juvenile) and number of mature embryos, and different concentrations of GA_3 to stimulate embryo conversion, and to improve the efficiency of plant regeneration, of Khalas cv.

MATERIALS AND METHODS

This study was carried out in the Central Laboratory of Development of Date Palm Research at Giza, Egypt during the period from 2006– 2007.

Plant material:

The propagation process started with the selection of healthy offshoots from mother date palm (*Phoenix dactylifera* L.) of the cultivar Khalas grown in Suadia Arabia. Selected young offshoots of 5-7 kg in weight and about 50-70 cm in height were carefully separated from adult date palm.

Sterilization of plant material

Explants were surface sterilized under aseptic conditions. At first, they were immersed in 70% ethanol for 30 sec. then immersed in 0.5 g/l mercuric chloride ($HgCl_2$) for 5 min. and thoroughly washed with sterilized distilled water. After that, additional outer leaves primordial were removed from the sterilized explants. These explants were then exposed to double surface sterilization by commercial Clorox (5.25 % Sodium hypochlorite).The first one by 50% Clorox for 25 min then thoroughly washed with sterilized water. The second one was by 50% Clorox for 25 min and then they were washed with sterilized distilled water for three times. Some surrounding leaf primordial were carefully removed and shoot tip explants with 4-6 leaf primordial were sliced longitudinally into 4 sections and inoculated onto culture medium

Culture medium:-

Murashige and skoog medium (MS 1962) was used in this investigation. Culture medium was supplemented with 5mg/l 2,4-D , 3mg /l 2iP , 1 mg /l IAA ,40g/l sucrose, 100 mg/l glutamine and 1.5 g/l activated charcoal (AC) solidified with 6g/l Agar Agar. Prepared medium was adjusted to pH 5.7 ± 0.1 and distributed to small jars (200 ml), each one contain 35 ml of prepared medium and then autoclaved at 121 ^oC and 1.5cm /ins^2 for 25 min. Sterilized shoot tip slices were cultured on previous medium for thirty two weeks with regular transfer to fresh culture medium of the same supplements every four weeks to form callus (establishment stage). Culture jars were incubated at 25 ±2 ^oC under total darkness.

Establishment of embrogenic suspension culture:

Friable callus resulted from establishment stage was divided into 0.5 g /jar and cultured on 2 MS,MS and Half strength liquid medium supplemented with 0.0, 0.5 and 1.0mg/l 2,4-D in addition of activated charcoal at 1 g /l ,40g/l sucrose and 100 mg/l glutamine .Prepared medium was adjusted to pH 5.2 ± 0.1 , Cultured jars containing 50.0ml/ jar were incubated on a rotary shaker (120 rpm) at 25 ± 2^oC under a 16/8 (light/ dark) photoperiod at a 25 µE $m^{-2}s^{-1}$ photon lux for eight weeks (four weeks interval) . For growth measurement, the content of suspension aggregates from each flask (jar) were weighed at the end of subculture period after draining excess media on filter paper .The growth measurements were repeated 3 times with 2-3- jar per replicate .Callus growth or (Fresh weight), (globular and juvenile or pro embryos) and number of mature embryos was the data obtained in this stage.

Effect of different type of cytokinin

In this experiment, the following media were used:

Half strength MS +0.5 mg /l 2,4-D + 1.0 mg /l kin + 3.0 gm AC

Half strength MS +0.5 mg /l 2,4-D + 1.0 mg /l 2iP + 3.0 gm AC

Half strength MS +0.5 mg /l 2,4-D + 1.0 mg /l BA + 3.0 gm AC

This experiment was conducted to investigate the effect of cytokinin on callus growth or Fresh weight, (globular and juvenile or number of pro-embryos) and number of mature embryos.

In general, remained callus and pro- embryos were reculturing on Fresh liquid medium containing half strength MS + 0.5 mg /l 2,4-D + 1.0 mg /l BA + 0.5 gm AC to form mature embryos and to increase suspension aggregates or on solid culture medium with the same supplements to multiplication or complete their growth and development .

Germination stage (embryo growth and development):

In this stage, somatic embryos formed on previous stage was cultured on MS medium supplemented with 0.1mg/l NAA +0.05mg/l BA, 1g/l AC+40 g/l sucrose (Hassan *et al.*, 2007) in addition to different concentrations of GA_3 0.0, 0.1, 0.2 and 0.4 for four months (four weeks interval) and incubated at 25±2 °C and light intensity 1000 lux***.

MS + 0.0(control) + 1g/l AC+40 g/l sucrose

MS + **0.0** GA3 + 0.1mg/l NAA +0.05mg/l BA + 1g/l AC+40 g/l sucrose

MS + **0.1** GA_3 +0.1mg/l NAA +0.05mg/l BA + 1g/l AC+40 g/l sucrose

MS + **0.2** GA_3 +0.1mg/l NAA +0.05mg/l BA +1g/l AC+40 g/l sucrose

MS + **0.4** GA_3 +0.1mg/l NAA +0.05mg/l BA +1g/l AC+40 g/l sucrose

After four month the following data were recorded:

1- Percentage of germinated embryos.

2- Percentage of hyperhydric and browned embryos. Hyperhydrecity

Statistical analysis:

Data obtained were subjected to the analysis of variances of randomized complete design as recommended by (Sendecor and Cochran 1980). LSD at 5% level of significance was used to compare between means according to (Steel 1960).

RESULTS

Effect of MS salt strength and different concentrations of 2,4-D on fresh weight (callus growth) of date palm Khalas cv. after 2 months.

Data in Table (1) revealed, mean value of callus fresh weight as affected by MS salt strength and 2,4-D concentrations. The addition of 0.5 or 1 mg/l 2,4-D improved fresh weight compared with control which gave the lowest significant value of callus fresh weight. The effect of MS salt strength on callus fresh weight as in Table (1) reflected that, No significant differences was noticed between medium containing

half MS salt strength or full MS salt strength which were produced the highest significant values (5.9 or 5.05 gm respectively). While the lowest significant value of callus fresh weight was observed by using medium containing 2 MS salt strength (2.97 gm).The interaction between 2,4-D concentrations and MS salt strength showed that, The highest significant value of callus fresh weight (7.5 gm) was observed when friable callus of Khalas cv. was cultured on half MS salt strength with 0.5 mg /l 2,4-D followed by the addition of 1 mg/l 2,4-D to medium containing half MS salt strength.

Table 1. Effect of MS salt strength and 2, 4-D concentration on callus fresh weight of date palm Khalas cv. after 2 months.

2,4-D con.	2 MS (B)	MS	1\2MS	Mean
0.0 mg/l	1.90	3.75	3.90	3.18 B
0.5mg/l (A)	3.30	5.90	7.50	5.567 A
1.0 mg/l	3.70	5.50	6.34	5.18 A
Mean	2.967 C	5.050 B	5.9 BA	

LSD at 0.05 % A = 0.4709 B =0.4709 AB = 0.8155

Data in Table (2) showed that, No significant differences could be noticed between the number of pro-embryos resulted by using 0.5 or 1 mg/l 2,4-D which produced the highest significant values. While the lowest significant value of pro-embryo number was observed by using medium without 2,4-D.The number of pro-embryos formation was affected significantly by MS salt strength. Increasing the MS salt strength from half MS salt strength to 2MS salt strength in culture medium was significantly decreased the mean value of formed pro-embryos. The highest mean value of pro-embryos (51.33) was observed when half MS salt strength was used. While the lowest significant value in this respect was observed when 2 MS salt strength was used (20.67). The interaction between 2,4-D concentrations and MS salt strength showed that, The highest significant value of pro-embryos number (85.0) was observed when Khalas cv. friable callus was cultured on half MS salt strength with 0.5 mg /l 2,4-D followed by the addition of 1 mg/l 2,4-D to medium containing half MS salt strength.

Table 2. Effect of MS salt strength and 2, 4-D concentration on pro-embryos of date palm Khalas cv. after 2 months.

2,4-D con.	2MS (B)	MS	1/2MS	Mean
0.0 mg/l (A)	4	6	9	6.33 B
0.5 mg/l	25	41	85	50.33 A
1.0 mg/l	33	51	60	48.0 A
Mean	20.67 C	32.67 B	51.33 A	

L.S.D. at 0.05% A = 4.195 B = 4.195 AB = 7.267

Data in Table (3) indicated that, mean of mature embryos was affected significantly by different concentrations of 2,4-D. Increasing the 2,4-D concentrations from 0.0, 0.5 to 1.0mg/l significantly decreased the mean value of formed mature embryos.

The highest significant value of mature embryos (42.0) was observed by using medium supplemented with 0.5mg/l 2, 4-D followed by the addition of 1 mg/l 2,4-D to culture medium (15.33). While the lowest significant value of mature embryos number was observed by using medium without 2,4-D.

Regarding to the effect of MS salt strength, data showed that, using half strength or full strength of MS formula produced the highest significant value of mature embryos number without significant difference (29.0 or 27.0 respectively). While the lowest significant value of mature embryos were noticed when using medium containing 2 MS salt strength (7.0). The interaction between 2.4-D concentrations and MS salt strength in Table (3) showed that, the highest significant value of mature embryos (69.0) was observed when friable callus of Khalas cv. was cultured on medium containing half MS salt strength with 0.5 mg /l 2, 4-D followed by using full MS salt strength (50.0)

Table 3. Effect of MS salt strength and 2, 4-D concentration on mature embryos through suspension culture of date palm Khalas cv. after 2 months.

2,4-D con.	2MS (B)	MS	1/2MS	Mean
0.0 mg/l (A)	3.0	9.0	5.0	5.66 C
0.5mg/l	7.0	50.0	69.0	42.0 A
1.0 mg/l	11.0	22.0	13.0	15.33 B
Mean	7.0 B	27.0 A	29.0 A	

L.S.D at 0.05% A=5.768 B=5.768 AB=9.991

Data in Table (4) showed that the highest significant value of callus fresh weight (7 gm) was observed by the addition of 1 mg/l BA to culture medium, followed by the addition of 1 mg/l KIN without significant difference in between (respectively). No significant difference was noticed between using KIN or 2iP in culture medium. Pro embryos number was highly by using BA or KIN compared with 2iP which produced the lowest value in this respect. From the same Table data showed that KIN in culture medium produced the highest value of mature embryos number without differing with BA, while the lowest value was noticed using 2iP in culture medium.

Data in Table (5) and Fig (11 - 16) showed that, the highest significant percentage of germinated embryos (44%) was observed when mature embryos were cultured on medium supplemented with (0.4) mg/l GA_3 followed by the addition of 0.2 or 0.1 mg/l GA_3 or 0.0 GA_3 to medium (37%,33% and 25% respectively) with significant difference among them. While the lowest significant percentage of germinated embryos (17%) was observed when culture on medium free of plant growth regulators.

From the same Table data showed that, the percentage of hyperhydric embryos as affected by GA_3 concentrations .Increasing the concentrations of GA_3 in culture

medium from 0.0 to 0.4 mg/l significantly decreased the percentage of hyperhydric embryos from (83%) to (56%).Embryos that did not germinate became brown or hyperhydric .

Table 4. Effect of different cytokinins and 2,4-D on callus growth, embryos number, pro and mature through suspension culture of date palm cv. Khalas after 2 months.

Cytokinins con. and 2,4-D	Callus growth	No. of Pro-embryos	No. of Mature embryo
1.0 mg/l 2iP+ 0.5mg/l 2,4-D	4.0gm B	20.0 B	8.0 B
1.0mg/l BA+ 0.5 mg/l 2,4-D	7.0gm A	47.0 A	14.0 AB
1.0mg/l KIN+ 0.5 mg/l 2,4-D	4.9gm AB	32.0 AB	25.0 A
L.S.D at 0.05 %	2.42	24.04	15.21

Table 5. Effect of different concentration of GA_3 on embryos germination percentage of date palm cv.Khalas after 4 months.

GA3 con. (mg/l) A	Germination	Hyperhydrecity and browning
0.0 Control	17.0 E	83.0 A
0.0 GA_3	25.0 D	75.0 B
0.1 GA_3	33.0 C	67.0 C
0.2 GA_3	37.0 B	63.0 D
0.4 GA_3	44.0 A	56.0 E
L.S.D at 0.05 %	1.786	2.729

DISCUSSION

Effect of MS salt strength:-

Regarding the effect of MS salt strength, using half strength of MS media was superior to full MS or 2MS. In this result are in line with those reported by Zouine and El Hadrami (2007), (Fki et al., 2003).they found that half MS salt strength was the most preferable strength compared with full and double MS strength .

Effect of 2,4-D:-

The addition of 2,4-D to culture media in different concentrations was enhanced callus fresh weight, number of pro-embryos and mature embryos compared with control medium. These results are in line with those reported by (Fki et al., 2003) who found that in date palm cultivar (Deglet Nour), the subculture of embryogenic suspension in a fresh medium with low amounts of 2,4-D (1 mg/l) resulted in the differentiation of a large number of somatic embryos (from 10 to 200 embryos per month per 100 mg fresh weight of embryogenic calli) However, the role of 2,4-D to induce and to maintain the embryogenic capacity, depends of explant and this role is not yet better understand (Zimmerman, 1993). Zouine and El Hadrami (2007) reported that the liquid medium containing 0.1 mg /l of 2,4-D was beneficial for somatic embryo production.

The growth regulator 2,4-D is used extensively in plant tissue culture to induce somatic embryo formation. This growth regulator was originally used by Reinert (1959) and Halperin (1964) in the first experiments on somatic embryogenesis in carrot. Halperin and Wetherel (1964) were the first to recognize the true importance of 2,4-D in the process of induction of somatic embryos. A review by Kohlenbach (1978) describing the general factors that are necessary to promote somatic embryogenesis refers to 2,4-D as a preferred synthetic auxin for the induction of somatic embryo development. A later review by Evans *et al.* (1981). Summarized the literature of somatic embryogenesis and found that, in 57.7% of the reports, 2,4-D was used in the initial stage of dicot tissue culture to induce the formation of somatic embryos and in all of the cases surveyed in which monocot regeneration occurs. De Touchet, *et al.* (1991)reported that using PGR-free liquid medium –fully developed somatic embryos showing both a gemmule and a radicle, where obtained at a very low frequency, they did not germinate and became necrotic following their transfer to a solidified medium .

Effect of Cytokinin

From the previous results it could be concluded that adding different cytokinin (BA, KIN and 2iP) to liquid culture medium of date palm somatic embryos of cultivars under investigation stimulated its growth and development compared with cytokinin-free medium and BA was favorable for most parameters.

Merkle (1995) observed that the presence of cytokinins in the medium may result in the formation of multiple apices in somatic embryo, resulting in structures that are different to characterize as embryoenic or organogenic, on the basis of their appearance alone. The increase of the number of embryoids may be the consequence of the stimulation of multiple shoot formation in oil palm plant somatic embryo by long-term culture on medium with cytokinin . BA was the most effective cytokinin in promoting shoot numbers, leaves number and also length of formed shoots of date palm compared with kinetin and 2iP Gadalla, *et al.* (2004).

Aberlence-Bertossi *et al.*, (1999) found that for oil palm the addition of BA to the culture medium resulted in a significant increase in the number of somatic embryos on solid medium during the regeneration process and the addition of 6-benzyladenin during the development of somatic embryos was found to induce shoot apex differentiation and thus increased germination rates, by up to 70%.

The supply of 0.05 mg/ l BAP on the germination medium could be useful in terms of germination percentage of somatic embryos of date palm. Zouine and El Hadrami (2007). The somatic embryos that not germinate were deteriorated and became brown or hyperhydric. It is known that hyperhydricity phenomenon is major problem for a number of species cultured in liquid or in solid media, especially when the media were enriched with cytokinins (Piatczak et al., 2005). Trigiano (1997) indicated that in *Acanthopanax koreanum* nakai, cytokinin (BAP, Zeatin and/or Kinetin)

treatment suppressed both maturation and germination of somatic embryos, while stimulating secondary embryogenesis. On the other hand, Belal *et al.* (1997) found that shoot growth and development of date palm was observed on growth regulator-free medium.

Saker *et al.* (1998) found that 2iP is more effective than either kinetin or BA in shoot proliferation of date palm after callus formation phase.

Effect of Gibberellic acid (GA_3):

In numerous systems, in spite of the high number of somatic embryos produced, problems with a lack or a low frequency of embryo conversion into plants occurred. To stimulate embryo conversion, and to improve the efficiency of plant regeneration, a number of different strategies were tested.

Data in this investigation showed the beneficial effect of GA_3 on growth and development of date palm somatic embryos during germination stage. GA_3 at different concentrations increased gradually the percentage of germinated embryos compared with control medium which produced the lowest significant values.

Increasing GA_3 in germination media increased gradually the percentage of germinated embryos, these results are associated with those reported by (Hassan *et al.*, 2007) stated that, GA_3 added to germination medium at different concentrations in date palm enhanced the conversion frequencies to shoot and roots. (Kim *et al.* 1997) whose recorded that gibberellic acid (GA_3) is frequently employed in media used for somatic embryo conversion, and a significant stimulatory effect of this phytohormone was proved in cultures of *Sesamum indicum* (Xu *et al.* 1997) and *P. ginseng* (Yang and Choi 2000). (Hutchinson *et al.* 1997) it is believed that GA_3 is especially necessary in cultures of somatic embryos, which undergo dormancy. (Choi *et al.*, 1999. Sunandakumari *et al.*, 2005) reported that maturation of somatic embryos of *Euphorbia nivulia* Buch-HAM at different stage, especially at the early stage, took place in the presence of GA_3. Half strength MS medium enriched with 2-89 µM GA_3 facilitated maturation of 78 % embryos, whose cotyledons became dark green. The promoting effect of GA_3 on embryo maturation may be due to the synthesis of new gene products required for the completion of embryo development. Gibberellins (GA_3) are generally responsible for plant cell expansion and elongation, but their role in embryo development is not well understood. Gibberellins have been reported to both increase and decrease SE in plants depending on plant species (Rademacher 2000, Rudus *et al*, 2000). Contributing to variability in response to GA_3 are differences in biological effects on SE at different steps in the process, such as embryogenic tissue induction or embryo development and maturation.

Data under investigation showed that increasing the concentrations of GA_3 in germination medium decreased the percentage of browning and hyperhydric embryos this is may be due to increasing the percentage of germination caused by GA3.

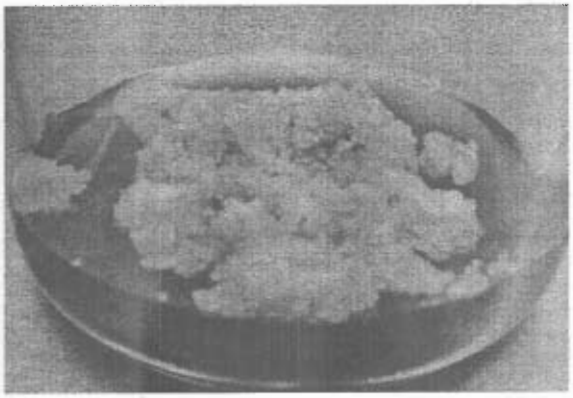
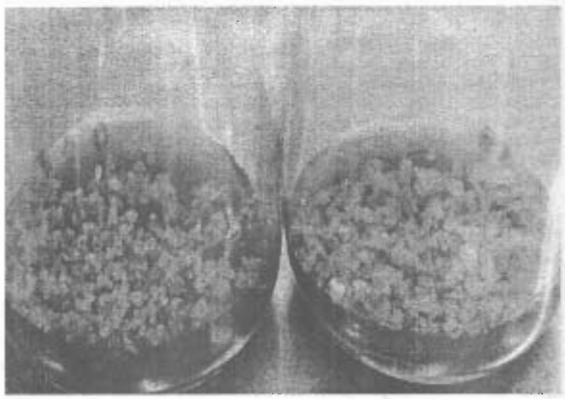


Figure1



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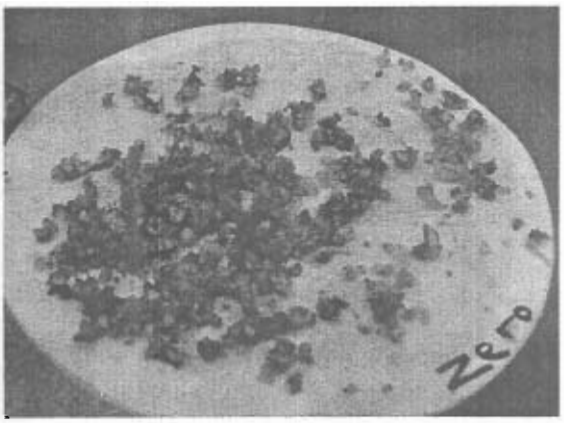

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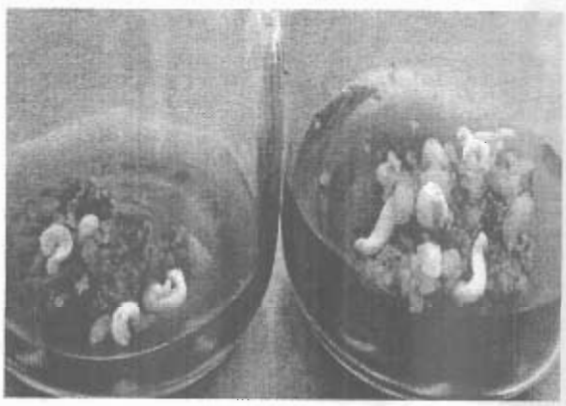

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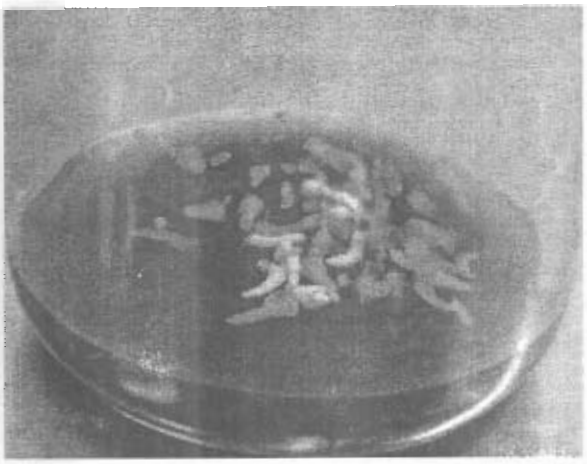

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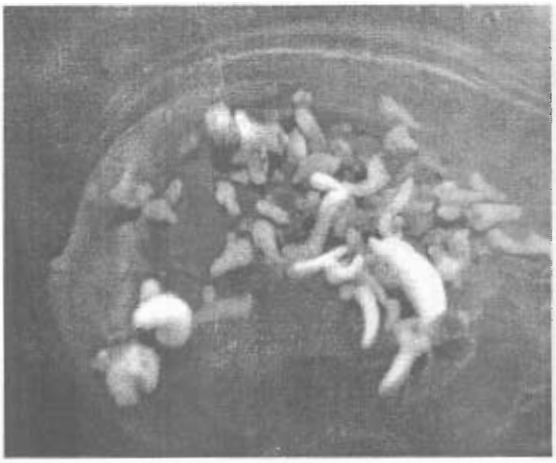

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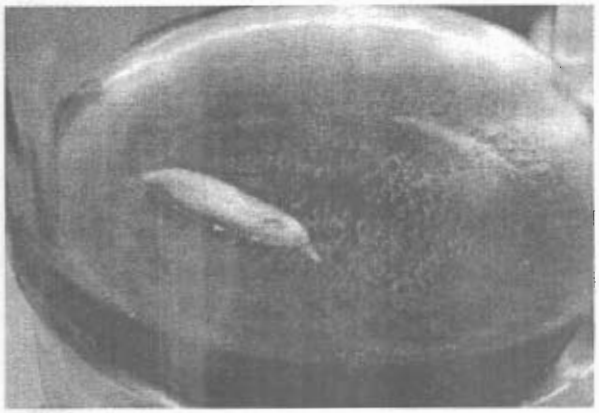

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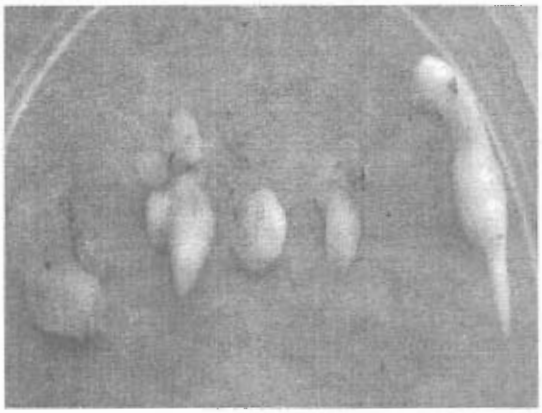

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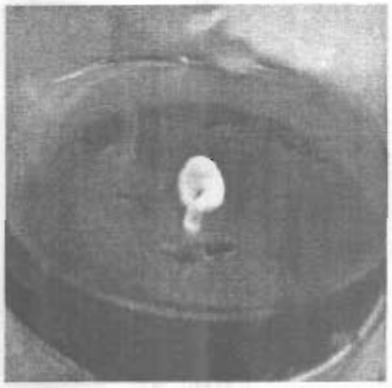

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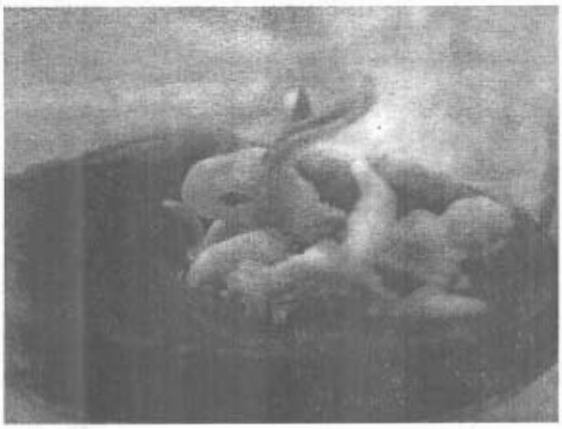

Figure 14

Figure 15

Date palm regeneration from embryogenic suspension culture.

1- Embryogenic callus with friable nodules. (Figure 1)

2- Synchronous maturation in hormone free liquid medium. (Figure 2- 3)

3- Pro and mature embryos on liquid medium (Figure 4).

4- Pro and mature embryos on solid medium. (Figure 5- 6).

5- Desiccation of mature embryos. (Figure 7 – 10).

6- Germination stage (embryo growth and development) and Browning (Figure 11 -- 15).

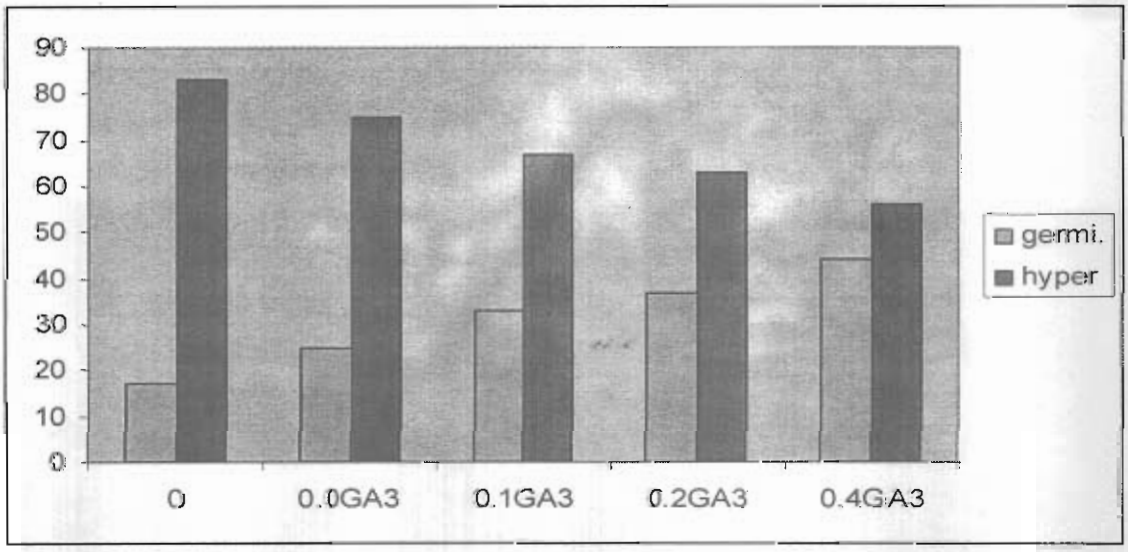

Fig 16. Effect of different concentration of GA_3 on embryos germination percentage of date palm cv. Khalas after 4 months.

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الإكثار السريع لإنتاج وإنضاج الأجنة الجسمية لنخيل البلح من خلال المعلقات الخلوية

عز الدين جاد الله جاد الله

المعمل المركزي للأبحاث وتطوير نخيل البلح – مركز البحوث الزراعية – الجيزة

أجرى هذا البحث بغرض الحصول على معدل إكثار عالي من الاجنه الجسمية لصنف نخيل البلح الخلاص.

تم الحصول على الكالوس من صنف الخلاص قيد الدراسة بزراعة أجزاء القمة النامية على بيئة MS المحتوية على ٥ مجم/لتر 2,4-D ؛٣ مجم/لتر 2iP ؛ ١ مجم/ لتر IAA ؛ ١,٥ جم/لتر فحم نشط لمدة ٣٢ أسبوع مع النقل المنتظم للأجزاء النباتية كل ٤أسابيع. تم زراعة أجزاء من الكالوس الجنيني (٠,٥) جم لكل جار و الناتج من المرحلة السابقة على بيئة سائله تحتوى علي تركيزات مختلفة من أملاح MS وتركيزات مختلفة من 2,4-D وأنواع مختلفة من السيتوكينين. وقد أظهرت النتائج أن البيئة السائلة المحتوية علي نصف قوة الأملاح والمحتوية علي ٠,٥ مجم/لتر 2,4-D شجعت الحصول على أعلى قيم لنمو الكالوس (الوزن الطازج) من خمس إلي ست مرات تقريبا كل شهر وكذلك عدد مبادئ الاجنه وعدد الأجنة الناضجة.

وقد حقق ال BA اعلي معدل للقيم من الوزن الطازج للكالوس وعدد مبادئ الاجنه مقارنة بال kin،2iP بينما حقق الكينتين اعلى معدل للقيم من عدد الاجنه الناضجه مقارنة بال BA ،2iP. كما زاد معدل إنبات الاجنه (التحول) إلي نبيتات تدريجيا بزيادة تركيز الجبريلين إلي ٠,٤ مجم /لتر.