

SOMATIC EMBRYOGENESIS AND PLANT REGENERATION OF SOME EGYPTIAN SEMI-DRY DATE PALM CULTIVARS: 1- SOMATIC EMBRYOGENESIS

Ali, A. S.

Central Laboratory for Research and Development of date palm.

ABSTRACT

This study performed, at Central Laboratory for Research and Development of Date palm at Giza during the period from 2000-2002, to produce a new date palm plants instead of oldest, diseased, affected by red (Indian) palm weevil trees and protect an important semi-dry date palm cultivars from extinction, using tissue culture techniques. Rapid production with maintain the original off-shoots of some semi-dry date cultivars (Amry and Aglany) in Sharkia Governorate was the main target of this study.

The results of this investigation the highest showed significant values of browning percentage observed in the leaf primordial explants of date palm Amry cv. extended in the subsequent cultures for 32 weeks. Callus formation was superior by using Amry cv., shoot-tip explants and culturing explants for 32 weeks. Embryogenic callus formation significantly affected by using different growth regulators containing-media. MB medium produced the highest significant value of embryogenic callus formation and also Amry cv., culture period for 32 weeks were superior in this respect. Embryogenic callus of Amry cv. produced the highest significant value of embryo formation after 12 weeks. M1, M3 and M7 were the most effective media to produce highest significant value of embryo formation.

INTRODUCTION

Date palm, *Phoenix dactylifera* L., belongs to Order: Palmales, Family: Palmaceae (Areaceae) is one of the oldest fruit trees in the world and is mentioned in Quran and Bible. Dates cultivars were classified according to the available heat units requirement into soft, semi dry and dry. Heat units were calculated in relation to temperature above zero or 18°C during the period from May 1st to the end of October, but the later is more realistic and accepted by most researchers. In addition, generally determined the areas which have heat units from 2100 for soft dates cultivars and the areas from 3600- 4700 for semi dry and dry ones (Hussein *et al.*, 1979). Sharkia governorate is one of the most important areas in Egypt for producing semi-dry especially Amry and Aglany cultivars.

Propagation of most species in the palm family is dependent on seed germination and development (Kiem, 1958). Seed-propagated palms do not bear true to type due to heterozygosity. Conventional vegetative propagation made through off-shoot. Unfortunately, relatively few off-shoots are produced during a date palm's life time and mostly during the juvenile life-cycle (Barrett, 1973). Therefore, tissue culture micropropagation, has been employed to aid in the clonal propagation of numerous plant species (De Fossard, 1976). The number of date palm plantations throughout Sharkia is continuously decreased as a result of diseases infections and reduction in agricultural land and practices, as well as the growth habit of the date palm. All these factors necessitate the tissue culture as an alternative means for propagation of the date palm. Therefore, this

study was carried out to find out the most suitable treatments for the vegetative propagation and production of date palm (semi-dry cultivars) via somatic embryogenesis by using tissue culture techniques. The first study included browning phenomenon, callus formation and differentiation of some Egyptian semi-dry date palm namely Amry and Aglany as affected by explant type and culture period. The study also included the effect of plant growth regulators on callus differentiation and embryos formation.

MATERIALS AND METHODS

This study was conducted in the Central Laboratory for Research and Development of Date palm at Giza during the period from 2000 to 2002.

2.1. Plant material:

The propagation process was started with the selection of healthy off-shoots from mother date palm trees of semi-dry cultivars (Amry and Aglany) which obtained from palms grown at Sharkia governorate.

The young off-shoots of 2-4 years, ranging in weight from 5-7 kg and about 50-80 cm in length were used as a source of explants (shoot tip and leaf primordia).

2.2. Explant preparation and sterilization:

The selected young off-shoots were carefully transferred to the laboratory after separation from mother tree in April and then prepared by removing the adventitious roots, fibrous sheath and leaves by knife. Removing leaves from off-shoots were continued until the white soft leaves nearer the apical meristem had appeared. The apical meristem plus few leaf primordia were used as explant material. Explants were soaked in a running tap water for 2 hours and soaked in anti-oxidant solution of ascorbic (100 mg/l) and citric acid (150 mg/l) for 30 min. to avoid culture browning.

Explants were surface sterilized under aseptic conditions by using ethyl alcohol (70%) for one min. followed by immersion in 0.5 g/L mercuric chloride (HgCl_2) for 5 min. and then rinsed one-time with sterile distilled water and transferred to double surface sterilization by commercial Clorox (5.25% sodium hypochlorite NaOCl) supplemented with two drops of Tween-20 per 100 ml solution, the first one by 40% Clorox for 15 min and thoroughly washed with sterilized distilled water for one time and the second by 60% Clorox for 25 min and then washed with sterilized distilled water for three times. Under aseptic conditions, outer soft leaves were removed to obtain a shoot tip (shoot tip 5-10 mm in length, composed of the apical meristem and 4-6 leaf primordia), cut longitudinally into 4 sections and inoculated onto culture medium). Also soft leaf primordia were cut longitudinally into two or three parts. These explants were used as an initial explant material for indirect embryogenesis.

2.3. Nutrient medium:

The basal nutrient medium in these experiments was Murashige and Skoog (1962) medium including vitamins (0.1 mg/l thiamine HCl + 0.5 mg/l nicotinic acid + 0.5 mg/l pyridoxin). After preparation of the medium, pH was adjusted to 5.7 ± 0.1 before the addition of agar (6.0 g/l). The culture medium was distributed into culture jars (200 ml). Each jar contained 35 ml of the prepared medium and was capped with polypropylene closure and autoclaved at 121°C at 15 lb/in^2 for 20 min. all cultured jars were incubated at controlled room temperature ($27 \pm 2^\circ\text{C}$ and total darkness).

To achieve the aim of this work, the following experiments were conducted:-

EXP 1. Effect of explant type and date of culture on browning and callus formation:

In this experiment, the effect of explant type (shoot tip and leaf primordia) on browning and callus formation of Egyptian semi-dry date palm (Amry and Aglany) was studied. Sterilized shoot tip (S.T.) and leaf primordia (L.P.) explant were cultured on MS basal medium supplemented with 100 mg/l 2,4-dichlorophenoxy acetic acid (2,4-D), 3 mg/l iso-pentenyl adenine (2iP), 40 mg/l adenine sulphate, 170 mg/l $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 200 mg/l glutamine, 3 g/l activated charcoal, 30 g/l sucrose and 6 g/l agar.

The culture medium was distributed (35 ml) into small jars (200 ml). Cultured jars were incubated at $27 \pm 2^\circ\text{C}$ under total darkness condition through 32 weeks. Browning and callus percentage were recorded.

EXP 2. Effect of plant growth regulators on embryogenic callus

The calli produced from the previous step were transferred to the following media to test its ability to change compact callus to embryogenic callus)

M0- MS without plant growth regulators (Control)

MA-MS+10 mg/l 2,4-D +3mg/l 2iP

MB-MS+10 mg/l 2,4-D +3 mg/l 2iP+ 5mg/l naphthalene acetic acid (NAA) + 5mg/l indole acetic acid (IAA)

MC-MS+10 mg/l 2,4-D + 3 mg/l 2iP+5 mg/l naphthoxy acetic acid (NOA)+5mg/l IAA+

MD-MS+ 1 mg/l indole buteric acid (IBA)+ 1mg/l benzyl adenine (BA).

The embryogenic callus formation percentage was recorded after 16 weeks for all semi-dry cultivars tested. (two semi-dry cultivars tested).

EXP 3. Maturation of somatic embryos

In this experiment, embryogenic callus which resulted from date palm shoot tip for Egyptian semi-dry cultivars (Amry and Aglany from Sharkia governorate) were used as a source of plant material. Embryogenic callus were divided into pieces of approximately (1x1 cm) and cultured on the following media: 0-MS (control) 1-MS+0.1mg/l (NAA) 2-MS+0.1mg/l NAA+0.1 mg/l Kinetin (kin). 3-MS+0.1mg/l NAA+ 0.05 mg/l benzyladenine (BA) 4-MS+0.1mg/l NAA+0.1 mg/l 2iP. 5-MS+0.1mg/l NAA + 0.1mg/l BA ribozide. 6-MS+0.2mg/l Kin. 7-MS+0.4 mg/l 2iP and 8-MS+ 0.2 mg/l BA ribozide.

These media were selected for this experiment after obtaining promising results from several preliminary experiments. For each cultivars under investigation each treatment included three replicates (small jars). Each jar contained about 1x1 cm. Embryogenic callus (friable callus). All cultures jar were incubated in total darkness at 27±2°C for 12 weeks with subculturing to corresponding fresh medium every month and average number of embryos was recorded after 12 weeks.

Statistical analysis:

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

RESULTS AND DISCUSSION

Effect of date palm cultivar, type of explants and culture period on browning percentage:

Data in Table 1 revealed the browning percentage as effected by cultivars (Amry and Aglany), type of explant (shoot-tip and leaf primordia) and culture period (18, 24 and 32 weeks).

Regarding to the effect of cultivar, data cleared that Amry cv. produced excessive significant value of browning percentage (100%) compared with Aglany cv. (92.7%).

Browning degree was significantly affected by explant type. The highest significant value of browning percentage was noticed with leaf primordia explant (98.6%), this value was reduced significantly to (94%) with shoot tip explants.

Table (1): Effect of explant type and culture period on browning of some Egyptian Semi-dry date palm cultivars (Amry and Aglany).

Cultivars (A)	Type of explant (B)	Culture period (C)			Mean
		18 weeks	24 weeks	32 weeks	
Amry	ST	100 a	100 a	100 a	100 a
	LP	100 a	100 a	100 a	100 a
	Mean	100 a	100 a	100 a	100 a
Aglany	ST	76.0 d	92.0 c	96.0 b	88.0
	LP	96.0 b	96.0 b	100 a	97.3
	Mean	86.0 d	94.0 b	98.0 b	92.7 b
		18 weeks	24 weeks	32 weeks	Mean
	ST	88.0 d	96.0 c	98.0 b	94.0 b
	LP	98.0 b	98.0 b	100 a	98.7 a
	Mean	93.0 c	97.0 b	99.0 a	

L.S.D. at 0.05%

A = 0.4586
AC = 0.6485

B = 0.5616
BC = 0.7942

AB = 0.7947
ABC = 1.123

C = 0.4586

The interaction between date palm cultivars and type of explant reflected that, excessive browning was produced by using shoot tip and leaf primordia of Amry cv. (100%), while less significant percentage was observed with shoot tip of Aglany cv. followed by leaf primordia of Aglany cv. (92.7%).

Concerning the effect of culture period, the results revealed that browning percentage progress to the highest significant value when subculture period extended to 32 weeks (8 weeks intervals) as the percentage was (99%). This percentage was reduced significantly to 97.0 and 93.0% when explants cultured for 24 and 18 weeks respectively with significant difference inbetween.

The interaction between cultivar and culture period showed that the highest significant value of browning percentage was observed with Amry cv. during all cultured period under investigation (100%) followed by Aglany cv. cultured for 32 weeks (98%), while the lowest significant percentage was noticed when Aglany cv. cultured for 18 weeks (86%).

Referring to the interaction between type of explant and subculture-period. The results indicated that the highest significant value of browning percentage was noticed by using leaf primordia explants after 32 weeks (100%), while the lowest significant percentage was observed when using shoot tip explants for 18 weeks. Using shoot tip explants for 32 weeks, leaf primordia for 18 and 24 weeks produced the same percentage of browning (98%).

Our results on semi-dry cultivars of date palm show that browning percentage affected significantly by cultivar, type of explant and culture period Amry cv., leaf primordia explants and culture period for 32 weeks produced excessive browning. These results were agreement with those reported by Gadalla (2003) who postulated that shoot tip explants of some dry cultivars of date palm were found to give less browning than leaf primordia explants, Bakry (1994) who pointed out that total phenolic compounds, leaf primordia of date palm showed high content, while shoot-tips and sub-shoot tips exhibited less level, Abo El-Saoud (1999) who mentioned that leaf primordia was found to give more browning than shoot tip and sub-shoot tip of date palm. Also, El-Shafey *et al.* (1999) found that shoot tip had a significant reduced mean value of the number of explant tissue forming browning compared with sub-shoot tip and leaf primordia.

Al-Dawayati (2000) mentioned that callus browning degree values of date palm cv. Sewi increased significantly by increasing subculture (24 and 32 weeks) period. The callus browning degree values was significantly low after 8 and 16 weeks in culture.

Callus formation

Effect of explant type and culture period of some Egyptian semi-dry date palm namely Amry and Aglany on callus formation percentage is shown in Table (2). Data reveal the callus formation percentage as affected by culture period and type of explant of date palm

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Table (2): Effect of explant type and culture period on callus formation of some Egyptian Semi-dry date palm cultivars (Amry and Aglany).

Cultivars (A)	Type of Explant (B)	Culture period (C)			Mean
		18 weeks	24 weeks	32 weeks	
Amry	ST	100 a	100 a	100 a	100 a
	LP	85.7 c	85.7 c	85.7 c	85.7 c
	Mean	92.85 a	92.85 a	92.85 a	92.85 a
Aglany	ST	92.0 b	92.0 b	92.0 b	92.0 b
	LP	66.7 e	66.7 e	70.8 d	68.1
	Mean	79.35 c	79.35 c	81.4 b	80.05 b
		18 weeks	24 weeks	32 weeks	Mean
	ST	96.0 a	96.0 a	96.0 a	96.0 a
	LP	76.2 c	76.2 c	78.25 b	76.88 b
	Mean	86.1 b	86.1 b	87.125 a	

L.S.D at 0.05%

A= 0.2949

B= 0.3612

AB=0.5108

C= 0.2949

AC = 0.4171

BC=0.5108

ABC= 0.7224

Regarding to the effect of date palm cultivars on callus formation, data showed that the highest significant value of callus formation percentage was noticed with Amry cv. (92.75) compared with Aglany cv. as the value of callus formation percentage was (80.05%).

Respecting to the effect of explant type, data cleared that, the shoot tip explants were superior in producing the highest significant value of callus formation percentage (96.0%) compared with the percentage obtained with leaf primordia explants (76.88%) which produced the lowest significant value of callus formation.

The interaction between date palm cultivar and type of explant indicated that the highest significant value of callus formation percentage was observed by using shoot tip explant of Amry (100%) followed by shoot tips of Aglany cv. (92%) and Amry cv. when using leaf primordia explant (85.7%) with significant difference while, the lowest significant value of callus formation percentage was observed by using leaf primordia explant of Aglany (68.1%).

Regarding to the culture period, data clearly showed that the highest significant value of callus formation percentage was observed when culture period extended to 32 weeks (87.12%) while the lowest significant value of callus formation percentage was observed when explant cultured for 18 and 24 weeks (86.1%).

Referring to the effect of interaction between date palm cultivar and culture period, data revealed that the highest significant values of callus formation percentage was observed with Amry cv. after 18, 24 and 32 weeks as the results were the same (92.85%), followed by Aglany cv. after 32 weeks (81.4%), while the lowest significant value of callus formation percentage was noticed with Aglany cv. after 18 and 24 weeks as the percentage was the same (79.35%).

Table (3): Effect of different media and culture period on embryogenic callus formation of some Egyptian Semi-dry date palm cultivars (Amry and Aglany).

Media (A)	Culture period (B)										Mean
	4 Weeks		8 Weeks		12 Weeks		16 Weeks		Mean		
	(C) Amry	Aglany	Amry	Aglany	Amry	Aglany	Amry	Aglany	Amry	Aglany	
M0	4.1	0.0	8.2	0.0	12.5	4.1	25.0	8.2	12.45 h	3.075 e	7.76% e
MA	58.3	37.5	66.7	50.0	79.2	58.3	83.3	70.8	71.88 c	54.15 g	63.01% d
MB	66.7	54.2	75	66.7	83.3	75.0	95.6	87.5	80.15 a	70.85 c	75.50% a
MC	54.2	50.0	62.5	62.5	70.8	70.8	79.2	79.2	66.67 d	65.625 e	66.15% c
MD	62.5	45.8	70.8	54.2	79.2	62.5	87.5	75.0	75.0 b	59.375 f	67.19% b
Mean	49.16	37.5	56.64	46.68	65	54.14	74.12	64.14	61.23 a	50.62 b	55.92%
Mean	43.33% d		51.66% c		59.57% b		69.12% a				

Media	4 Weeks	8 Weeks	12 Weeks	16 Weeks
M0	2.05 p	4.1 o	8.3 n	16.6 m
MA	47.9 l	58.35 i	68.75 f	77.5 d
MB	60.45 h	70.85 e	79.15 c	91.55 a
MC	52.1 k	62.5 g	70.8 e	79.2 c
MD	54.15 j	62.5 g	70.85 e	81.25 b

L.S.D at 0.05

A = 0.01336

B = 0.1889

AB = 0.2671

C = 0.2112

AC = 0.2986

BC = 0.4223

ABC = 0.5973

Respecting to interaction between type of explants and culture period, the results cleared that the highest significant value of callus formation percentage was noticed by using shoot tip explants cultured for 18, 24 or 32 weeks which produced the same highest significant values of callus formation percentage (96%). This percentage reduced significantly to 78.25% with leaf primordia explants cultured for 32 weeks. Leaf primordia explants cultured for 18 and 24 weeks produced the same lowest significant percentage of callus formation (76.2%).

From results mentioned before, it could be concluded that genotype, type of explant and culture period affected significantly callus formation percentage of date palm Amry cv. was the most affective genotype to produce the highest significant value compared with Aglany cv. Shoot tip explants were superior compared with leaf primordial for callus formation percentage. These results was in line with those reported by Gadalla (2003) who recorded that Gondiola c.v. was the most effective dry genotype of date palm to produced the highest significant value of callus formation percentage (81.64%) compared with other dry genotype under investigation. Hornung *et al.* (2000) found that callus formation of date palm is dependent on the genotype use.

Callus differentiation

Data in Table (3) reveal the embryogenic callus formation as affected by different plant growth regulators containing-media and culture period of date palm cultivars.

Regarding to the effect of date palm cultivars on embryogenic callus, data show that Amry cv. was superlative for producing embryogenic callus (61.23%) compared with Aglany cv. which produced the lowest significant value of embryogenic callus formation percentage (50.52).

Referring to the effect of different plant growth regulators data in Table (3) showed that MB have ability to develop compact callus to embryogenic callus with the highest significant percentage (75.58%) compared with other growth regulators containing-media, followed by MD, MC and MA as the percentages were 67.19, 66.15 and 63.01% respectively with significant difference among the treatments. Only 7.76% of cultured compact callus succeeded to develop embryogenic callus by using medium free from growth regulators (M0).

The interaction between different cultivars and culture media reflected that compact callus of Amry cv. cultured on MB produced the highest significant percentage of embryogenic callus formation (81.15%) followed by Amry cv. cultured on MD (75.0%), Amry cv. cultured on MA and Aglany cultured on MB produced the same percentage of embryogenic callus formation (72%). Only 3.08% from cultured compact callus of Aglany cv. converted into embryogenic callus when cultured on MS free growth regulators (M0).

Regarding to the culture period, data reflected that, the highest significant value of embryogenic callus percentage was observed when compact callus of two cultivars cultured for 16 weeks (69.12%), followed by 12 weeks (59.57%) and 8 weeks (51.66%) with significant descending difference.

Respecting to the interaction between different cultivar and culture period on embryogenic callus, data clearly show that, the highest significant value of embryogenic callus percentage was observed when compact callus of Amry cv. was cultured for 16 weeks (74.12%). This percentage was reduced significantly to 64.14% when compact callus of Aglany cv. cultured for 16 weeks. While the lowest significant value of embryogenic callus percentage was noticed when compact callus of Aglany cv. was cultured for 4 weeks (37.5%). In addition compact callus of Amry cv. cultured for 12 weeks and compact callus of Aglany cv. cultured for 16 weeks produced the nearest values of embryogenic callus formation (65.0 and 64.14%, respectively).

The interaction between culture period and different media component reflected that using MB to culture compact callus for 16 weeks was the most effective treatment to produce the highest significant value of embryogenic callus (91.55%) followed by using MD (81.25%) and MC (79.2%) for 16 weeks and MB for 12 weeks (79.15%). While the lowest significant value of embryogenic callus percentage was observed when compact callus of date palm cvs cultured on M0 medium free from growth regulators with all culture periods under investigation.

The results revealed that adding different growth regulators (2,4-D, NAA, NOA) and 2iP) to embryogenic callus medium of date palm produced the highest significant values of embryogenic callus formation compared with control medium (without growth regulators) and the presence of NAA or NOA were most effective in this respect.

Similarly, Mater (1986) obtained nodular embryogenic callus of date palm on medium containing high level of 2,4-D or NAA. Sudhersan *et al.* (1993) used MS medium supplemented with NAA, NOA, 2iP and kinetin for embryogenic callus formation of date palm cv. Barhee.

Madhuri and Shankar (1998) used MS medium supplemented with growth regulators (BA, NAA, and others) additives as embryogenic medium for date palm.

Treatment with auxin at high concentration is needed to cause differentiation and to elicit totipotency. Various types of auxins have been used for this purpose, viz., the nature auxin IAA and the synthetic ones NAA, NOA and 2,4-D. The later is the most efficient and the most commonly used auxins for the promotion of somatic embryogenesis in plant tissue cultures. A positive correlation between the level of added auxin and the level of methylation in DNA. Among the auxins, 2,4-D could reach the highest intracellular concentration as free auxin, and is also the most efficient in promoting hypermethylation and in morphogentic activity. If, instead, auxin is retained in the medium, the embryogenic progression stops somewhere before globular stage. The point of arrest is more or less typical of each cell line. Therefore, there will be plant lines which proliferate showing various stages from undifferentiated cell to globular embryos and others showing various stages from undifferentiated cells and PEM (pro- embryogenic masses). It should be noted that auxin affects differentiated of an explant by inducing de-differentiation on one hand and formation of embryo primordia (PEM) on the other. This apparent contradiction can be understood if one considers that once PEM are formed. They became insensitive to auxin.

Auxin sensitivity is then regained at a later stage (post-globular) when the embryos, in the presence of auxin, will stop their differentiative programme and revert back to unorganized tissue (Purohit, 1999). He mentioned that induction of embryogenic state in differentiated explants after requires extensive proliferation through unorganized callus cycles, death or disruption of surrounding explant cells, and/or high levels of a synthetic auxin such as 2,4-D or picloram. Plasmolysis of explant cells was also shown to enhance somatic embryogenesis. These factors are presumed to alter the epigenetic state of cells, and may be related in their ability to disrupt the cell-cell interactions requirement to maintain coordinated patterns of development. Starting with a cultured of embryogenic cells in the form of proliferation requires, the initiation of bipolar differentiation to produce cotyledons, shoot apex and root pole, and then, in sequence, maturation of embryos, germination or conversion to plantlets.

El-Bellaj *et al.* (2000) found that IAA oxidase activity in embryogenic calli was three fold that of non-embryogenic calli. IAA-oxidase activity in embryogenic calli was higher than that of ionic oxidases, while no such difference was detected in non-embryogenic calli. IAA-oxidase, as well as endogenous IAA, may therefore have a crucial role in date palm somatic embryogenesis.

Effect of culture media with different components on embryos formation:

Embryogenic callus of Amry and Aglany cvs. were cultured in different media with different supplements M0, M1, M2, M3, M4, M5, M6, M7 and M8) to investigate its effects on embryo formation.

Data in Table (4) reveal the embryo formation as affected by semi-dry cultivars and culture media.

Referring to the effect of two cultivars data in Table (4) clearly show that Amry cv. produced the highest significant value of embryo number (6.48) compared with Aglany cv. (5.53 embryo/explant).

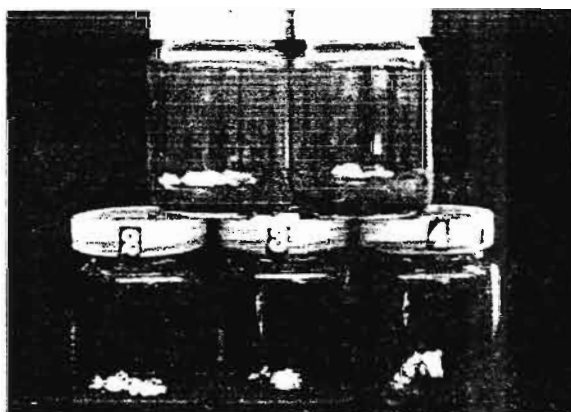
Respecting to the effect of culture media, presented data clearly show that culture media No. M1, M7 and M3 were the most effective culture media to produce the highest significant value of embryos number as the mean values were (7.5, 7.2 and 7.15, respectively) followed by medium No. M0 (medium-free supplements) and No. M6 without significant difference among them as the mean values were 6.85, 6.60 embryo/explant respectively while the lowest significant value was observed when embryogenic callus cultured on M2 media (3.4 embryo/explant). Other culture media produced intermediate number of embryos ranging from the highest and the lowest significant values. The interaction between cultivars and culture media showed that the highest significant value of embryos number/explant was noticed when Amry cv. embryogenic callus cultured on M7 (8.3 embryo/explant), this value reduced to 8.0, 8.0, 7.3 embryo/explant) when Amry cv. embryogenic callus cultured on M1, M6 and M0 respectively and to 7.0 embryo/explant when Aglany cv. embryonic callus cultured on M1, M3 without significant differences among them while the lowest significant value of this parameter was observed when Aglany cv. Embryonic callus cultured on M2 (3.1 embryo/explant).

1- Callus formation

(Aglany) (Amry)



2- Embryogenic callus (Amry)



2- Embryos Formation (Amry)



Fig 1

Table (4) : Effect of media supplemented with different plant growth regulators on embryos formation (number of embryos) of date palm genotype (Amry and Aglany) after 12 weeks.

Medium (B)	Cultivars (A)		
	Amry	Aglany	Mean
M0	7.3 abc	6.4 bcd	6.85 ab
M1	8.0 ab	7.0 abc	7.50a
M2	3.7 f	3.1 f	3.40e
M3	7.3 abc	7.0 abc	7.15a
M4	5.7 cd	6.0 cd	5.85 b
M5	5.0 de	5.0 de	5.00 cd
M6	8.0 ab	5.2 de	6.60 ab
M7	8.3 a	6.1 cd	7.20 a
M8	5.0 de	4.0 ef	4.50 de
Mean	6.478 a	5.533 b	

L.S.D at 0.05%

A=0.5665 B=1.202

AB=1.699

From results mentioned before it could be noticed that using MS medium + 0.1 mg/l NAA, MS medium + 0.1 mg/l NAA + 0.05 mg/l BA, MS medium + 0.4 mg/l 2iP and MS medium-free growth regulators produced the highest significant values of embryos formation.

In this respect, Tisserat (1979 a&b, 1981a&b), Sharma *et al.* (1984), Falcon and Marcheschi (1988), Kackar *et al.* (1989) and Shakib *et al.* (1994). mentioned that somatic embryos and viable plantlets were induced on MS medium without growth regulators.

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تكوين الأجنة الجسدية وإنتاج النباتات لأصناف النخيل المصرية النصف جافة:

١- تكوين الأجنة الجسدية

عبد الحليم سيف الدين على

المعمل المركزي للأبحاث وتطوير نخيل البلح - مركز البحوث الزراعية .

أجريت هذه الدراسة بالمعمل المركزي لأبحاث وتطوير نخيل البلح في الفترة من ٢٠٠٠ إلى ٢٠٠٢ بهدف الإكثار السريع للأصناف النصف جافة من نخيل البلح (العموي والعجلاني) بمحافظة الشرقية وذلك لحمايتها من الانقراض وإحلال وتجديد النباتات المريضة والمسننة والمصابة بسوسة النخيل الحمراء.

وفي هذا البحث تم عمل الإكثار السريع لتلك الأصناف النصف جافة عن طريق تكوين الأجنة الجسمية وأجرى هذا البحث لدراسة تأثير نوع الجزء النباتي وعدد مرات النقل على التلون البني وعلى تكوين الكالس وكذلك لدراسة تأثير نوع البيئة على تكوين الكالس الجنيني وتكوين الأجنة الجسمية وقد تم الحصول على أعلى نسبة من التلون البني عند استخدام الصنف العمري ومبادئ الأوراق الأولية وبزيادة مرات النقل (حتى ٣٢ أسبوع).

تكونت أعلى نسبة من الكالس عند استخدام صنف العمري والقمة النامية وكذلك بزيادة مرات النقل (حتى ٣٢ أسبوع).

تكونت على البيئة MB أعلى نسبة من الكالس الجنيني مع الصنف العمري وبزيادة مرات النقل (حتى ١٦ أسبوع).

تكونت على البيئات M7, M3, M1 أكبر عدد من الأجنة الناضجة مقارنة بباقي البيئات الأخرى وكذلك الصنف العمري مقارنة بالصنف العجلاني.