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In vitro PROPAGATION OF EGYPTIAN DRY DATE PALM 1- EFFECT OF EXPLANT TYPE AND DATE OF CULTURE ON BROWNING PHENOMENON & CALLUS FORMATION AND PLANT GROWTH REGULATORS CONTAINING-MEDIUM ON EMBRYOGENIC CALLUS FORMATION

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ABSTRACT

The current study was undertaken from 1998 to 2002 on Egyptian dry date palm cvs.,Gondeila,Sakkoty ,Malakaby ,Bartamuda and Shamia. The aim was to study micropropagation of Egyptian dry date palm cultivars via somatic embryogenesis . The study included the type of explant and date of culture on the browning phenomenon and callus formation. The effect of a nutritional medium containing different plant growth regulators on embryogenic callus formation was studied. Shoot tip explant gave less browning than leaf primordia. The highest significant browning percentage was recorded with Sakkoty followed by Shamia and Malakaby cvs., while the lowest was obtained with Gondeila and Bartamuda. The highest significant browning percentage was observed when the explants were cultured in March followed by October, while the lowest was observed in December and February .

Gondeila produced the highest significant callus percentage

followed descendingly by Shamia , Malakaby and Bartamuda . The highest callus formation was recorded with shoot tip explant cultured in October or March .Compact callus of different dry date palm cultivars onto M2 medium (MS+ 10 mg /l 2,4-D+5 mg/l NAA +5 mg/l IAA +3mg/l 2iP) produced the highest significant percentage of embryogenic callus with Sakkoty (95.8%) and Malakaby (95%) cultivars as compared with Shamia (87.55), Gondeila (83.3%) and Bartamuda (60.13%) cultivars .

***Key words** :browning , callus, dry date palm, in vitro.*

1.INTRODUCTION

Date palm , *Phoenix dactylifera* L., belongs to the Order : Palmales, Family : Palmaceae (Areaceae) is one of the oldest fruit trees in the world and is mentioned in the Quran and Bible .Date cultivars are classified according to the available heat unit 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. Aswan is one of the most important areas in Egypt for producing dry cultivars (Hussein *et al.*, 1979).

Propagation of most species in the palm family depends on seed germination and development (Kiem,1958). Seed- propagated palms do not bear true to type fruits due to heterozygosity .Conventional vegetative propagation is made through off-shoots. Unfortunately, relatively few off- shoots are produced during a date palm 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 inherent advantage of tissue culture over field propagation is a greater plant production potential from a single plant tissue culture. Moreover this technique may offer a plausible method to produce large numbers of genetically uniform palms . Due to the

National Irrigation Projects (Aswan and high Dams) in Aswan area, the number of palms dropped from 2.5 millions in the beginning of this century to about 1.061189 million palms which represent about 12% of the total numbers of palms in Egypt(Hussein *et al.,* 1993). The number of date palm plantations throughout the world is continuously decreasing as a result of disease 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 seeks to find out the most suitable treatments for the vegetative propagation and production of date palm (dry cultivars)*via* somatic embryogenesis by using tissue culture techniques. The first part included browning phenomenon and callus formation and differentiation of some Egyptian dry date palms namely ,Gondeila, Sakkoty, malakaby, Bartamuda and Shamia as affected by explant type and date of culture. The study also included the effect of the nutritional medium containing plant growth regulators on callus differentiation .

2. MATERIALS AND METHODS

This study was carried out in the Central Laboratory for Research and Development of Date Palm at Giza during the period from 1998 to 2002.

2.1.Plant material

The propagation process started with the selection of healthy off-shoots from mother date palm trees of dry cultivars (Gondeila, Sakkoty, Malakaby, Bartamuda and Shamia) obtained from palms grown at Aswan.

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

2.2. Explant preparation and sterilization

The selected young shoots were carefully transferred to the laboratory after separation from the mother trees and then prepared

by removing the adventitious roots , fibrous sheath and leaves by knife. Removing leaves from the off-shoots was continued until the white soft leaves near the apical meristem had appeared. The apical meristem plus a few leaf primordia were used as explant material. Explants were soaked in a running tap water for 2 hours then soaked in sterile 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 alchohol (70%) for 1 min followed by immersion in 0.5 g/l mercuric chloride ($HgCl_2$) for 5 min and then rinsed once 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 the 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 + 0.5mg /l nicotinic acid + 0.5 mg /l pyrodoxin). 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 and 15 lb /in^2 for 20 min. All cultured jars were incubated at controlled room temperature (27 ±2 C° and total darkness) .

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

2.3.1.Effect of explant type and date of culture on browning and callus formation

In this experiment , the effect of explant (shoot tip or leaf primordia) and the date of culture (February , March, October and December) on browning and callus formation of Egyptian dry date palm (Gondeila , Sakkoty ,. Malakaby, Bartamuda and Shamia) were studied .Sterilized shoot tips (S. T.) and leaf primordia (L.P.) 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 $NaH_2PO_4.2H_2O$, 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±2°C under total darkness condition through 32weeks. Browning and callus percentages were recorded.

2.3.2. 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)

M1-MS+10 mg / l 2,4-D +3mg / l 2iP

M2-MS+10 mg/l 2,4 –D +3 mg / l 2iP+ 5mg/l naphthalene acetic acid (NAA) + 5mg/l indole acetic acid (IAA)

M3-MS+10 mg /l 2,4-D.+ 3 mg / l 2iP+5 mg/l naphthoxy acetic acid (NOA)+5mg /l IAA+ M4-MS+1mg/l indole buteric acid (IBA)+1mg/l benzyl adenine (BA).

The embryogenic callus formation percentage was recorded after four months for all dry cultivars tested.

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

3.RESULTS AND DISCUSSION

3.1.Browning

The effect of explant type and date of culture of some Egyptian

dry date palms namely Gondeila , Sakkoty , Malakaby , Bartamuda and Shamia on browning percentage is shown in Table (1). It is obvious that the shoot tip had the superior values (41.13 % browning) for reduced browning as compared with leaf primordia (47.76 % browning) explant. This may be due to the high concentration of endogenous auxin in the shoot tip explant.

Table (1): Effect of explant type and date (month) of culture on the browning percentage of date palm genotype (different cultivars) after 18 weeks.

Cultivars (C)	Type of explants(T)	Month of culture (M)				Mean
		February	March	October	December	
Gondeila	ST	0.0	92.3	41.7	4.2	34.6
	LP	0.0	93.3	65.0	2.2	40.1
	Mean	0.0	92.8	53.4	3.2	37.33 D
Sakkoty	ST	37.5	82.4	49.1	33.3	50.6
	LP	50.0	78.9	75.7	50.0	63.7
	Mean	43.8	80.7	62.4	41.7	57.11 A
Malakaby	ST	0.0	100.0	55.7	13.3	42.3
	LP	0.0	100.0	88.9	20.0	52.2
	Mean	0.0	100.0	72.3	16.7	47.24 BC
Bartamuda	ST	0.0	100.0	20.9	0.0	30.2
	LP	0.0	85.7	41.7	0.0	31.9
	Mean	0.0	92.9	31.3	0.0	31.04 DE
Shamia	ST	4.2	85.0	80.0	23.1	48.1
	LP	0.0	90.0	88.9	25.0	51.0
	Mean	2.1	87.5	84.5	24.1	49.53 B
	Mean of D	9.2	90.8	60.8	17.1	
		D	a	b	c	
	Mean of T	ST 41.13			LP 47.76	

L.S.D at 0.05 C=7.02 CT= NS T=NS TM= 8.88
M= 6.28 CM= 14.03 CTM=NS
ST=Shoot tip LP =leaf primordia

Explant	Feb	Mar	Oct	Dec	
Shoot-tip	8.3	91.94	49.48	14.78	41.13
Leaf primordia	10.0	89.58	72.04	19.44	47.76

The high auxin concentration delayed the initiation of phenol synthesis and reduced subsequent rate of observable browning. Browning of tissue and adjacent medium is assumed to be due to the oxidation of polyphenols and formation of quinones, which are highly reactive to the tissue. These substances may have a profound physiological effect on the cultured tissue. Several enzymes which are widely distributed in the plant oxidize phenols to quinones (Bakry 1994 and El-shafey *et al.* 1999). The present study also shows that browning percentage increased during March (90.8%) and October (60.8%) and decreased during February (9.2 %) and December (17.1%). In this respect, Al-Maarri and Al-Gahamdi (1995) postulated that Hillaly cv. collected monthly throughout the year, showed that explant browning and growth cessation predominated cultures initiated during the warm months. Explants established in November through March reduced browning .The highest significant browning percentage was observed in March with Malakaby (100%) followed by Bartamuda (92.9%) and Gondeila (92.9%) .

3.2.Callus formation and differentiation

3.2.1.Callus formation

A cell from any part of the plant like shoot tip, bud, leaf mesophyll cells , epidermis, cambium, anthers, pollen, fruit etc., when inoculated in a suitable medium under aseptic laboratory conditions can differentiate and multiply. This results into the formation of an amorphous mass of cells known as callus, which can be induced to re-differentiate on appropriate medium to develop embryoids which directly develop into plantlets, eventually giving rise to a whole viable plant . Callus production was enhanced by increasing auxin concentration , being the highest in date palm nutrient medium with 100 mg/l 2,4- D. Callus formation depends on the genotype used , nutrient medium, explant type and date of culture (Shakib *et al.* 1994, Purohit 1999 and Hornung *et al.* 2000). Data presented in Table (2) show the effect of explant (shoot tip and leaf primordia) and date of culture (February, March, October and December) on callus formation of Egyptian dry date palm namely Gondeila, Sakkoty, Malakaby, Bartamuda and Shamia .The results indicated that callus formation formed well with shoot tip explant (79.37 %) than leaf

primordia (60.67 %) when inoculated in date palm callus medium (Tisserat , 1979) .It may be due to high concentrations of endogenous auxin in shoot tip cells. Gondeila cv. produced the highest significant callus formation percentage (81.64 %) followed by Shamia (73.94 %). The lowest significant value of significant value of callus formation percentage resulted with Sakkoty (62.3 %), regardless of

Table (2): Effect of explant type and date (month) of culture on the callus formation percentage of date palm genotype after 18 weeks.

Cultivars (C)	Type of explants (T)	Month of Culture (M)				Mean
		February	March	October	December	
Gondeila	ST	86.0	92.3	100.0	79.2	89.4
	LP	70.0	86.7	90.0	48.9	73.9
	Mean	78.0	89.5	95.0	64.1	81.64 A
Sakkoty	ST	75.0	94.1	83.0	44.4	74.1
	LP	30.0	63.2	83.8	25.0	50.5
	Mean	52.5	78.7	83.4	34.7	62.30 D
Malakaby	ST	60.0	100.0	98.4	53.3	77.9
	LP	50.0	72.0	77.8	20.0	55.0
	Mean	55.0	86.0	88.1	36.7	66.44 C
Bartamuda	ST	66.7	83.3	79.1	66.7	74.0
	LP	50.0	81.0	59.2	40.0	57.6
	Mean	58.4	82.2	69.2	53.4	65.75 C
Shamia	ST	83.3	90.0	83.3	69.2	81.5
	LP	41.3	80.0	94.4	50.0	66.4
	Mean	62.3	85.0	88.9	59.6	73.94 B
	Mean of D	61.2	84.3	84.9	50.0	
		B	a	a	c	
	Mean of T	ST 79.37			LP 60.67	

L.S.D at 0.05

C= 1.06
T= 0.67
M= 0.95
CT= 1.50
TM= 1.34
CM= 2.12
CTM= 3.00

ST=shoot tip LP =leaf primordia

Explant	Feb	Mar	Oct	Dec	
Shoot tip	74.20	91.94	88.76	62.56	79.37
Leaf primordia	48.26	76.58	81.04	36.78	60.67

the explant used. The interaction between cultivars and type of explant was significant at 5% level .The highest significant callus formation percentage was observed with shoot tip explants of Gondeila cv. followed by Shamia and Malakaby cvs., while the lowest was recorded with leaf primordia explants of Sakkoty cv.

Referring to the effect of culture time, our data showed that the significant callus formation percentage was observed when explants were cultured in October and March without significant difference in between .The lowest significant callus formation percentage was observed with explants cultured in December .

3.2.2.Callus differentiation

Data presented in Table (3) show the effect of plant growth regulators containing MS nutrient medium on callus differentiation of dry date palm. Embryogenic callus was affected by plant growth regulators added to MS nutrient medium and genotype used. Compact callus of different dry cultivars cultured onto M2 medium (MS + 10 mg/l 2,4 D+ 5mg/l NAA + 3 mg/l 2iP) produced the highest significant percentage of embryogenic callus (84.35%) followed by M3 (73.36%) and M4 (72.93%) nutrient medium .

Table (3): Effect of different plant growth regulators containing media on the percentage of emberyogenic callus developed from (compact) callus of date palm genotypes through 4 months.

Cultivars (C)	Media (M)					Mean
	M0	M1	M2	M3	M4	
Gondeila	8.3	66.7	83.3	87.5	62.5	**61.66 C**
Sakkoty	12.5	54.2	95.8	91.5	83.3	**67.46 B**
Malakaby	25.0	91.7	95.0	83.3	66.6	**72.34 A**
Bartamuda	6.25	65.6	60.13	50.0	72.93	**50.98 E**
Shamia	12.5	50.0	87.5	54.5	79.2	**56.74 D**
Meen	12.91	65.64	84.35	73.36	72.93	
	D	C	A	B	B	

L.S.D at 0.05 C =1.38 M =1.38 C*M =2.19

There are different responses due to cultivars. Malakaby cv. achieved the highest significant mean value(72.34%) for embryogenic

callus formation from compact callus . The lowest significant mean value (50.98%) was observed with Bartamuda cv.The other cultivars(Sakkoty, Gondeila and Shamia) produced intermediate mean values of embryogenic callus (67.46, 61.66, 56.74% respectively) with significant differences amongst the previous cultivars. The interaction between type of medium containing plant growth regulators used and genotype was significant at 5% level .The highest significant mean values of embryogenic callus were observed from callus of Sakkoti and Malakaby cvs. cultured onto M2 medium as gave 95.8% and 95% respectively without a significant difference in between. In this respect Saker *et al.*(1998) proliferated callus culture of date palm on MS medium supplemented with 10 mg/l 2,4-D., 50 mg/l adenine sulfate , 170 mg/l NaH2PO4. Madhuri and Shankar (1998) obtained white friable callus from date palm Yakubi cultivar on MS medium supplemented with 50 mg/l kinetine and 1mg/l 2iP after 8-10 weeks of incubation in the dark .

A tissue explanted *in vitro* consists of several cell types .The explant is treated with growth regulators so that its cells (or , rather , a fraction of them capable of responding to hormonal stimuli) de-differentiate and start to proliferate . In the presence of an auxin, after several days (sometimes weeks) a population of small , compact cells emerges in this tissue which can be hand-picked or concentrated by a process of differential centrifugation through a Ficoll gradient. These small compact cells divide in an asymmetrical way and their daughter cells, sticking together, give rise to typical cell clumps which have been called proembryogenic masses or embryogenic clusters. From these proembryogenic masses, embryos develop upon dilution of cells and removal of hormones totipotency, once acquired, is a long - lasting capacity in the sense that a cell population that has acquired this property can be subcultivated in the presence of auxin for months and years and yet retain the capacity to generate embryos upon removal of the hormone(Purohit ,1999) .

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إكثار الأصناف الجافة المصرية لنخيل البلح باستخدام الزراعة النسيجية ١- تأثير نوع المستقطع النباتي وموعد الزراعة على التلون البني وتكوين الكالس،وتأثير نوع البيئة على تكوين الأجنة الجسمية

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ملخص

أجريت هذه الدراسة في السنوات من ١٩٩٨-٢٠٠٢ بهدف الإكثار السريع للأصناف الجافة من نخيل البلح بمحافظة أسوان وذلك لحمايتها من الإنقراض وإحلال وتجديد النباتات المريضة والمسنة وحتى يمكن الإستفادة من الأعداد الكثيرة الناتجة من الإكثار السريع فى زراعة الآراضى الجديدة بتوشكى وشرق العوينات.

تم فى هذا البحث عمل الإكثار السريع لتلك الأصناف الجافة عن طريق الأجنة الجسمية وأجرى هذا البحث لدراسة تأثير نوع المستقطع النباتى وموعد

الزراعة على التلون البنى وعلى تكوين الكالس ، ولدراسة تأثير نوع البيئة على تكوين الأجنة .

لم يلاحظ وجود فرق معنوى بين القمة النامية والوريقات الأولية على الرغم من أن القمة النامية كانت أقل فى التلون البنى . بينما سجل صنف السكوتى نسبة كبيرة فى التلون البنى يليه الشامية والملكابى . بينما سجل الجنديلة والبرتمودا أقل نسبة فى التلون البنى. حققت زراعة المستقطعات النباتية فى مارس وأكتوبر أعلى نسبة من التلون البنى بينما حققت الزراعة فى فبراير وديسمبر أقل نسبة من التلون البنى. و تحققت أعلى نسبة للتلون البنى عند زراعة المستقطعات النباتية سواء القمة النامية أو مبادئ الأوراق فى شهر مارس .

تحقق أعلى نسبة من الكالس عند زراعة القمة النامية مقارنة بالوريقات الأولية.

حقق صنف البرتمودا أعلى نسبة من الكالس بينما حقق صنف السكوتى أقل نسبة من الكالس. تكونت أعلى نسبة من الكالس عند زراعة المستقطعات النباتية فى أكتوبر يليه شهر مارس بينما أقل نسبة تكونت من الكالس كانت فى شهر ديسمبر .

تحققت عند زراعة الكالس المتماسك لمختلف الأصناف الجافة على البيئة M2 أعلى نسبة من الكالس الجنينى يليها البيئة M3 ثم M4 بينما تحققت أقل نسبة من الكالس الجنينى عند الزراعة على بيئة خالية من منظمات النمو .

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