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DIRECT ORGANOGENESIS OF STEM SHOOT TIP CULTURE OF DATE PALM (*Phoenix dactylifera*, L) ZAGHLOUL CV.AS AFFECTED BY CYTOKINENS AND AUXINS

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ABSTRACT

Date palm (phoenix dactylifera, L)Zaghloul cv.shoot tip cultures as affected by cytokinens and auxins .were studied aming to stimulating shoots formation from offshoot explant tips.

Shoot tip explant failded completely to form additional shoots during the first culturing periods up to 12 weeks overall auxins treatments. Explants were able to produce shoots when celtured on MS media supplemented with either of BA or 2ip at the different concentreations used especially at the 3rd culturing period;18 weeks. The interaction treatments showed an additive effect in this respect.

INTRODUCTION

Date palm (*Phoenix dactylifera,* L ; Palmaceae, Arecaceae) micropropagation has been initiated to produce plantlets from economically important cultivars such as Zaghloul and others. It has been considerable interest in developing tissue culture micropropagation techniques to obtain large number of plantlets and reduce the dependence on offshoots propagation. Schroeder (1970) conducted an early experiment to study the micropropagation of date palm tissue culture technique consisted of excising apical meristem tissue from the growing point of the young offshoot (2-3 years old) and utilized sometimes, the entire lateral bud with or without a segment of leaf tissue. He exposed the apical bud-portion of the offshoot by removing the leaf bases to the white succulent heart tissues. The heart was then surface sterilized in 10% Chlorox for 15-30 minutes or covered with alcohol and flamed. All subsequent steps then processed in a sterile transfer room. Tisserat (1979b) using shoot tips excised from young offshoots (2-4 years old) which were cut from seedlings date palm (*Phoenix dactylifera,* L) grown in India and California. He reported that shoot tips consisted of the apical meristem and soft inner leaves were 5 mm in length and can be used in micropropagation of date palm. The outer portion of the leaf bases was removed and the apical meristem approximately 5 mm^3 was cut and used for propagation. Shoot tips and meristem tissue were removed from offshoot after the leaves were peeled away.Hervan *et al.* (1991) reported that the highest rate of callus formation was obtained with shoot tips. While, callus was not obtained from the area below the shoot tips. Bakry (1994) found that shoot tip in date palm tissue culture was preferred other explants of sub-shoot tip and leaf primordium for stimulating best type of callus production (more granular texture).

The present investigation aimed to study the effects of cytokinins and auxins on the number and percentages of established shoot tip explants which produced axillary buds of date palm zaghloul cv during different culturing period.

MATERIALS AND METHODS

Direct organogenesis method was used for the vegetative propagation using only the shoot tip explant of Zaghloul date palm culture aiming to enhance shoot growth and induce axillary budding. One hundred offshoots are used in this experiment as a source of the explants obtand from Faculty of Agric.Cario Univ. Egypt.

The media of Murashig and Skoog ; MS 1962 as given in Table (I) was used as basal nutrient media using gelrite as a gelling agent at 1.5g/l. The pH was adjusted at 5.7-5.8 prior to the addition of gelrite.

Two experiments were carried out in the laboratories of the Agric Botany Dept, Faculty of Agric, Mansoura Univ, Egypt for direct organogenesis method to investigate the effect of either of different cytokinins or auxins concentrations on production of axillary buds from the established shoot tip explants.

Table (I): Composition of basal nutrient medium of Murashig and Skoog; MS (1962).

To make up liter of MS medium ml	mg/liter medium	g/liter S.S.	Constituents	Stock solution S.S.
20	1650	82.5	NH_4NO_3	A
20	1900	95.0	KNO_3	B
5	6.2	1.24	H_3BO_3	C
	170	0.05	KH_2PO_4	
	0.83	34.0	KI	
	0.25	0.005	$Na_2MoO_42H_2O$	
	0.025	0.166	$CoCl_22H_2O$	
5	440	88.00	$CaCl_22H_2O$	D
5	370	74.00	$MgSO_47H_2O$	E
	22.3	1.72	$MnSO_44H_2O$	
	8.6	4.46	$ZnSO_47H_2O$	
	0.025	0.005	$CuSO_45H_2O$	
5	37.25	7.45	Na_2EDTA	F
	27.85	5.57	$FeSO_47H_2O$	
5		0.2	Thiamine HCl	G
		0.1	Nicotinic acid	
		0.1	Pyridoxine HCl	
		0.4	Glycin	

Stock solution F: is made differentially from the others.
To prepare stock solution F: dissolve each constituents in 200 ml distilled water; heat Na_2EDTA solution; with continuous stirring add Fe SO_47H_2O solution when cool dilute to 1000 ml with distilled water

Experiment I:

The basal nutrient medium supplanted with 100 mg meysoinositol + 0.4 mg/l thiamine-HCl + 30 g/l sucrose + 2g activated charcoal + 1.5g/l gelrite was used either with or without addition of the different cytokinin levels (mg/l) as follows:

1- MS alone (control)
2- MS + 5.0 BA
3- MS + 10.0 BA
4- MS + 5.0 2iP
5- MS + 10.0 2iP
6- MS + 5BA+ 5.0 2iP
7- MS + 5BA+ 10.0 2iP
8- MS + 10BA+ 5 2iP

Each treatment was replicated 6 times (6 culturing jars; 250 ml.). The nutrient media for each treatment was distributed into culture jars, each one contained 35 ml of the specific prepared medium.

The culture jars were immediately capped with polypropulin closure and autoclaved at 121oC, 15/1bs/inch2 for 20 minutes.

Sterilized shoot tip explants were cultured on the specific medium and incubated at 25-27c^{o} for 16/18 hrs day/night condition using white fluorescent tubes giving intensity of about 1500 Lux. The incubation was took place for 6 months with 4-sub-culturing, 6 weeks intervals. At each sub-culturing date, all survived plants were transferred and recultured on the same fresh specific media and the number as well as percentages of shoot tip explants which produced axillary buds were recorded.

Experiment II:

MS basal nutrient media supplemented with 100 mg/l meyoinositol + 0.4 mg/l thiamine – HCl + 30 g/l sucrose + 2g/l activated charcoal + 1.5 g/l gelrite with or without addition of auxins were used to examined their effects on the percentage of shoot tip explants which produced axillary buds and the total number of additional axillary buds which recorded at each sub-culturing date. The investigated different treatments auxin concentrations (mg/l) are presented as follows:

1- MS alone (control)
2- MS + 5.0 NAA
3- MS + 10.0 NAA
4- MS + 5.0 IBA
5- MS + 10.0 IBA
6- MS + 5 NAA+ 5.0 IBA
7- MS + 5 IBA+ 10.0 NAA
8- MS + 10 IBA+ 5 NAA

The culture medium of each treatment were distributed in the culture Jars as mentioned before, each treatment was replicated 6 times (6 jars) and each of them contained one established shoot tip explant.

The explants were repeatedly subcultured for four times at six weeks intervals into corresponding fresh specific basal nutrient media. All cultured jars were incubated at 27-28c^{o} under artificial light 16/8 hrs (day/night) using white fluorescent lamps giving about 1500 Lux intensity as previously mentioned.

RESULTS AND DISCUSSION

Effects of cytokinens

Data in Table II show that all shoot tip explants failed completely to form additional axillary buds during the first culturing periods up to 12 weeks. Explants were able to produce axillary buds when cultured on MS medium supplemented with either of BA or 2iP at each concentrations used and at the 3rd culturing period (18 weeks). The increasing percentage was a concentration dependent. More axillary buds were produced at the interactions treatments. The high value was recorded at the treatment MS + 5 BA + 10 2iP and MS + 10 BA + 5 2 iP.

Table II: Effects of MS basal nutrient media supplemented with or without different concentrations of cytokinins on the number and percentage of established shoot tip explants which produced axillary buds (A.B) of date palm; (*Phoenix dactylifera,* L) Zaghloul cv during different culturing period.

Treatments mg/l	Culturing period (weeks)									
	6		12		18		24		Mean	
	A.B No.	%	A.B No.	%	A.B No.	%	A.B No.	%	A.B No.	%
MS alone (control)	-	0	-	0	-	0	-	0	-e	0
MS + 5.0 BA	-	0	-	0	1	16.6	2	33.3	0.75c	12.50
MS+10 BA	-	0	-	0	1	16.6	2	33.3	0.75c	12.50
MS+5.0 2iP	-	0	-	0	1	16.6	2	33.3	0.75c	12.50
MS+10 2iP	-	0	-	0	2	33.33	3	50	1.25b	20.80
MS + 5.0 BA+5 2iP	-	0	-	0	2	33.33	3	50	1.25b	20.80
MS + 5.0 BA+10 2iP	-	0	-	0	3	50.0	3	50	069a	25.00
MS + 10 BA+5 2iP	-	0	-	0	1	16.6	1	16.6	0.5d	8.30
Mean	-	0	-	0	1.4B	20.8*B*	2.00A	33.3*A*	0.69	14.24

Means in the same column or row having different superscripts are significantly differ at P≤0.05

However, less increase were recorded with MS + 10 BA+ 5 2 iP treatment similar to that noticed with either of them alone at 5 mg/l. MS alone failed to produce any axillary buds till the end of the experimental period. In this context, Gabr and Tisserat (1985) mentioned that formation of axillary shoots in date palm was infrequent (5-10 % of cultures). Inoculation of cytokinins seemed to stimulate more lateral buds out growth from tips than occurred in its absence. Such tips produced only one or two axillary buds initially after 6-24 weeks in culture. These tips produced more buds, averaging about 1-3 per transfer.

On the other hand, Zaid and Tisserat (1983a and b) suggested that inoculation of cytokinins in the culture nutrient medium of date palm did not improve shoot development from tip explant. According to Smith and Murashige (1970) the addition of exogenous cytokinin to the nutrient medium was unnecessary and even inhibitory to meristem development.

It was suggested that palm meristem may either synthesis an adequate cytokinin supply or does not requires exogenous cytokinin like, growth regulators for its initial development.

Effects of auxins

Data in Table III reveal that shoot tip explants failed completely to produced any additional axillary buds during the first (12 weeks) for all treatment. At 18 weeks, the explants were able to produce axillary buds when cultured on MS medium supplemented with different concentration of auxins (NAA, IBA).

(1) Direct organogenesis of stem shoot Tip of date palm (*Phoenix dactylifera,* L) by cytokinens.
(2) Direct organogenesis of stem shoot Tip of date palm (*Phoenix dactylifera,* L) by auxins.

There are an additive effect when different auxins used in combination with each others on axillary buds formation. The high value was recorded for (NAA) levels (5 and 10 mg/l) at the jars of MS + 10 NAA and MS + 5 IBA + 10 NAA and MS + 5 NAA while, this percentage was increased to 50% at the end of the fourth subculture (24 weeks) with the additional axillary buds.

Table III: Effects of MS basal nutrient media supplemented with or without different concentrations of auxins on the number and percentage of established shoot tip explants which produced axillary buds (A.B) of date palm (*Phoenix dactylifera,* L) Zaghloul cv during different culturing period.

Treatments mg/l	Culturing period (weeks)									
	6		12		18		24		Mean	
	A.B No.	%	A.B No.	%	A.B No.	%	A.B No.	%	A.B No.	%
MS alone (control)	-	0	-	0	-	0	-	0	-d	0d
MS + 5.0 NAA	-	0	-	0	2	33.3	3	50	1.25a	20.8a
MS+10 NAA	-	0	-	0	2	33.3	3	50	1.25a	20.8a
MS+5 IBA	-	0	-	0	1	16.6	2	33.3	0.75b	12.50b
MS+10 IBA	-	0	-	0	2	33.3	3	50	1.25a	20.8a
MS + 5 NAA + 5 IBA	-	0	-	0	1	16.6	2	33.3	0.75b	12.50b
MS +5 IBA + 10 NAA	-	0	-	0	2	33.3	3	50	1.25a	20.8a
MS + 10 IBA+5 NAA	-	0	-	0	1	16.6	1	16.6	0.5c	8.32c
Mean	-	0	-	0	1.38B	22.88*B*	2.13A	35.4*A*	0.88	14.56

Means in the same column or row having different superscripts are significantly differ at P≤0.05

IBA alone gave low results for shoot tip explant which able to produce additional axillary buds reached to about 16.6% (one jar) in the second (18 weeks) culture period. The percentage increased to 33.3% with raising in number of additional axillary buds to two axillary bud at the end of the fourth subculturing period (24 weeks). The low value was recorded in jars of MS + 5 NAA + 10 IBA which produced about 16.6% with one additional axillary buds. MS alone failed to produce axillary buds till the end of experimental period. Tisserat (1982) mentioned that about 30-40 of the asexual plantlets of date palm cultured on nutrinet media containing 0.1 mg/l NAA produced both proliferated adventitious roots and axillary shoot out growths. One or two additional shoots were usually produced from well rooted vigorous plantlets.

Zaid and Tisserat (1983a&b) reported that better date palm shoot tip developed was occurred with the nutrient media contained 10 or 100 mg/l NAA. Multiple shoots occurred in about 10% of the cultures planted on nutrient media devoid of charcoal containing 0.1 mg/l NAA and at some what lower frequency from tips cultures on charcoal containing media (10 mg/l NAA). Gabr and Tisserat (1985) reported that shoot tips of several palm species cultured on MS media containing NAA was developed into plantlets with well developed leaves and adventitious roots in 2-6 months from planting.

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تأثير الأكسينات والسيتوكينينات على زراعة القمة الطرفية بالطريقة المباشرة

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هدفت تجارب البحث إلى دراسة أثر بعض السيتوكينينات والأكسينات خلال مراحل الاكثار الدقيق للفسائل لنخيل البلح (صنف زغلول) بالطريقة المباشرةعلى تكوين النبتات المأخوذ ة من القمم الطرفية لفسائل. 7و قد أوضحت النتائج عجز القمم الطرفية ، كأجزاء نباتية مستخدمة فى هذه الطريقة ، عن تكوين براعم جانبية خلال فترة التحضين الأولى حتى 12 اسبوع، وقد تمكنت من النشاط بعد تلك الفترة بدرجات متباينة تبعاً لنوع الهرمون و تركيزه ولقد وأظهرت معاملات التداخل بين السيتوكينيات المستخدمة والأكسينات تأثيرات إضافية فى هذا الشأن . .