EFFECT OF CULTIVARS AND EXPLANTS ON THE GROWTH OF THE *IN VITRO* CULTURED TISSUES OF TWO DATE PALM CULTIVARS

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ABSTRACT

This study was conduced during 1997 and 1998, the obtained results indicated that, 1- Cultivar factor did not significantly affect the length of the growing tissue although, it significantly affected the diameter and color or the growing tissues as well.. 2- The length and the diameter of cultured tissues were greatly affected by the type of the explant; 3- BA as well as NAA significantly affected the length, diameter and color of the growing tissues.

INTRODUCTION

Date palm (*Phoenix dectylifera* L.) trees are essemtial integral components of farming systems in dry and semi-arid regions and can be produced equally well in small farm units or as larger scale commercial plantation units. The tremendous advantage of the tree is its resilience, its requirement for limited inputs, its term productivity and its multiple purpose attributes.

Date palm breeding is hampered by the long generation cycles trees, it usually takes more than 30 years to complete three backcrosses and to obtain the first offshoots from an intervarietal cross. To produce sufficient offshoots for testing in the field, other generations are required and if the breeding target is yield or friut quality even more time will be needed.

Biotechnology tools of tissue culure and genetic. engineering can now effectively speed up all of the above processes (improving the characteristics, reduce the periods of breeding programs and improving crop). Therefor this investigation aims to study the effect of explants, BA and NAA on the growth of cultures tissues of Zaghloul and Samany date palms.

MATERIALS AND METHODS

The present study was conducted during 1997 and 1998 seasons on Zaghloul and Samany date palm cultivars. The used explants were apical tip and immature inflorescences. The basal salts of Murashige and Skoog (MS), in addition to certain additives such as 30 gm sucrose, 30 ml C.W and 7 gm agar /L were used. As for the growth substances, BA (0,1&3 mg/L) and NAA (0,10&30 mg/L) were used. Concerning the disinfection and pre-culture operations, 5% (v/v) liquid soap solution for 5 min., 70 % (v/v) ethyl alcohol for 5 min., 2.6 % (W/V) sodium hydrochlorite for 15 min.; antioxidant solution (150 ml/L citric acid + 100 mg/L ascorbic acid) for 20 min and rinsed 3 times with distilled water for 5 min. Beside using 0.01% (W/V) potassium permanganate added to sodium hybochlorite and UV rays used for one hour in culture cabinet before culture operation beginning. The incubation conditions were day light, room temperature and 95% relative humidity.

Medium was prepared and dispensed in jars (20 ml in each), tissues were disinfected and placed immediately in aseptic conditions and incubated in polyethylene box (small tent). Contaminated and dried tissues were excluded and removed weekly.

Data were collected and tabulated for statistical analysis using Complete Randomize Design according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

The data representing the effect of cultivars, explants and growth substances on the length, diameter, viability and colour of the growing tissues are presented in Tables 1 to 6.

Length of the growing tissues:

The present results indicated that the growing inflorescence tissues were significantly longer than the growing apical tip tissues for both studied cultivars under studied levels of NAA and BA.

Regarding the effect of explants, BA and their interaction, the data indicated that BA at 3mg. /L ranked superior over the other two levels (1 and 0 mg/L). It was also found that the length of the growing tissues was siqnificantly increased with increasing the cytokinin level in the culture medium. Also, the length of inflorescence was siqnificantly greater than that of the apical tip. As for the interaction between the inflorescence

explant and BA at 3mg/L, it gave the highest mean length as compared with the other interaction types and the differences were significant. The lowest value was recorded for the interaction between BA at o mg/L and apical tip of explant, (Table 1). The results of this study are in line with those of sharma *et al*. (1980), Gupta et al. (1984), Calero *et al*. (1990) and Bhaskar and Subbash (1996), and disagreed with those of Agaton *et al* (1992) and EL-Hadrami *et al*. (1995).

As for the effect of NAA, the explants varied greatly in their response to different studied levels of NAA applied to the culture medium. High level (30 mg/L) was superior than the other levels (10 and 0 mg/L). The interaction between cultivars and explants greatly affected the length of the growing tissues. The mean length of Samany inflorescence growing tissues responded greatly as compared with the other cultured tissues followed by Zaghloul inflorescenc growing tissue, whereas no significant difference was found between Samany and Zaghloul apical tip growing tissues.

With respect of the interaction effect of cultivars, explans and NAA levels, it was found that cultivar, as well as the explant varied greatly in their response to the different levels of NAA. NAA at 30 mg/L gave the highest mean length for both cultivars when the apical tips were used as explants, whereas NAA at 10 mg/ occupied the second rank for Zaghloul and the third for Samany.Almost the same trend was found when the inflorescence was used as explants (Table 2). These results, generally, agreed with those of Khalil *et al*. (1983) and Bakry (1994), and disagreed with Eeuwens (1978), Lioret (1981) and Ahmed (1999).

As for the effect of the interaction between cultivars ,explants and the different BA levels (Table 3),the data displayed that the inflorescence used under 3 mg/L BA exhibited the highest value for both cultivars. When the apical tips were used as explants, the response of cultivar varied according to BA levels. Under BA at 3 mg/L, Zaghloul explant responded greatly than Samany. The same trend was noticed for BA at 1mg/L. The present results agreed with those of El-Hennawy and Wally (1978), Drira (1983), Omar (1988) and Saker *et al*. (1998).

Diameter of growing tissues:

With respect to cultivars, explants and their interaction, the results showed that Samany cultivar growing tissues were superior than those of Zaghloul regarding the mean diameter, and also the apical tip growing tissues had siqnificantly larger mean as compared with inflorescence

ones. The data listed in Table (4) displayed that Zaghloul inflorescence growing tissues had inferior mean diameter compared to other growing tissues.

Regarding the influence of interaction between explants and NAA, the data in Table (5) showed that there were three superior interactions; apical tip tissues cultured in presence 30 or 10 mg/L NAA and inflorescence tissues cultured on NAA-free medium, and three inferior interactions; apical tip tissues green in NAA – free medium and inflorescence tissues grew in medium contain 30 or 10 mg/L NAA. The difference between superior and inferior interaction was significant. These results agreed with those of Sugismura et al. (1987), Sugimura and Salavanta (1989), MajidiHervan et al. (1993) and Verdeil et al. (1994).

As for the effect of interaction between cultivars, explants and BA levels, Zaghloul apical tip tissues grew in presence of 3 mg/L BA had larger mean diameter as compared with other growing tissues but they did not significantly differ from those of Samany inflorescence tissues cultured on medium containing 10 or 30 mg/L BA. Samany apical lip tissues cultured on BA – free medium had inferiur mean diameter values as compared with other growing tissues but did not significantly differ from the growing tissues of Zaghloul inflorescence cultured on medium containing 0 or 3 mg/L BA. The present results agreed with those of EL-Hennawy and Wally (1978), Omar (1988) and Saker et al. (1998) and disagreed with Ahmed (1999).

Vaibility of growing tissues:

The present results showed that the differences among cultivars, explants and BA and NAA levels and their interactions were not statistically significant.

Colour of growing tissues:

The present results indicated that the colour of the growing tissues was significantly affected by cultivars and BA, while their interaction had no significant effect.

Data in Table (6) declared that Samany growing tissues had significantly the highest mean degree of colour as compared with Zaghloul ones. It means that colour of the growing tissues ranged from yellowish green for Samany to whitish green for Zaghloul. There were significant differences among tissues growing on medium containing

different levels of BA. It was found that BA had significant increasingly efffect on development of tissue colour.

Sub – and reculture of growing tissues:

Growing tissues were subcultured on fresh medium with the same composition and supplemented with 3 mg BA + 30 mg NAA per liter. Apical tip drived callus tissues of both cultivars were divided into 2-4 parts (2x2mm), and the flower primordia derived callus were separated from their branches and divided into 2 parts for each. Tissues were immersed in antioxidant solution, disinfected, rinsed with sterilized distelled water and horizontally subcultured on prepared medium in jars. Cultures were incubated under day light, room temperature and 95% relative humidity (80 cultured jars of apical tip callus, and 60 of flower primordia callus were subcultured in 24/5/1997 for Zaghloul and Samany cultivars). Contaminated and dried cultures were removed weekly.

After 6 weeks, apical tip tissues of both cultivars (50 jars for Zaghloul and 56 jars for Sameny) induced compact callus, embryogenic callus or shoot tissues with creamy light, whitish green or green colour. Also, flower primordia tissues (49 jars for Zaghloul and 38 jars for Samany) induced compact callus or embryogenic callus with creamy light or whitish green colour. Produced shoot tissues were 6-8mm in length and 4-6mm in diameter, and callus tissues (compact or embryogenic) were 6-9mm in diameter. These subcultures were observed for two weeks.

In 20/7/1997, second subculture was carried out in tubes using the same medium. Tissues were cleaned from dried layers or parts and the apical tip drived callus tissues were dissected into 2- 4 parts, shoot tissues were not dissected while callus drived from flower primordia were dissected. Cultures (120 tubes from opical tip and 65 tubes from flower primordia for Zaghloul and Samany) were incubated under the same described conditions.

After 4 weeks, the cultures were more contaminated and dried and the remained ones were prepared for 3rd reculture which carried out in 10/9/1997 for only growing tissues using the same medium but without growth substances to initiate root differentiation.

The observations indicated that tissues remained growing for 210 days then break off. It may be due to unsuitable used medium for 3rd

reculture. Finally, this experiment insured the obtaining large numbers of cultures using few explants.

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Table (1): Effect of BA and explants factors on the mean length of the growing tissues (mm) as an average for both Zaghloul and Samany studied cultivars.

Explants	BA levels (mg/L)			Mean (mm)
	0	**1**	**3**	
Apical tip	5.98	6.26	7.39	6.56
Inflorescence	10.65	12.09	12.87	11.88
Mean (mm)	8.47	9.32	10.23	--

L.S.D	Explants	BA	Exp X BA Interaction
(0.05)	0.24	0.30	0.42

Table (2): Effect of NAA factor, (cultivars X explants) and (cultivars X explants X NAA) interactions on the mean length of the growing tissues (mm).

Cultivars	NAA levels (mg/L)				Mean (mm)
Cultivar	Explant	0	10	30	
Zaghloul	Apical tip	6.03	6.81	6.92	.60
	Inflorescence	11.33	11.71	12.05	11.69
Samany	Apical tip	6.34	6.18	7.00	6.53
	inflorescence	12.03	12.10	12.05	12.06
Mean (mm)		8.93	9.20	9.51	--

L.S.D	NAA	Cultivars X Explants	Cultivars X Explants X NAA
(0.05)	0.30	0.32	0.59

Table (3): Effect of (Cultivars X explants X BA) interaction on the mean length of the growing tissues (mm). The values of mean length were at descendingly arrangement.

Interaction treatment			Mean (mm)*
Cultivar	**Explant**	**BA level**	
Samany	Inflorescence	3 mg/L	13.33 a
Zaghloul	"	"	12.43 b
Samany	"	1 mg/L	12.24 b
Zaghloul	"	"	11.93 b
Samany	"	O mg/L	10.70 c
Zaghloul	"	"	10.60 c
"	Apical tip	3 mg/L	7.65 d
Samany	"	"	7.11 d
Zaghloul	"	1 mg/L	6.35 e
Samany	"	0 mg/L	6.33 e
"	"	1 mg/L	6.18 ef
Zaghloul	"	0 mg/L	5.62 f

* Values with the same letters are not significantly differed.

Table (4): Effect of the interaction between two cultivars, two explant types and three levels of BA on the mean diameter of the growing tissues (mm).

Interaction treatment			Mean (mm)*
Cultivars	**Explants**	**BA (mg/L)**	
Zaghloul	Apical tip	3 mg/L	4.60 a
Samany	Inflorescence	l mg/L	4.38 ab
Samany	Inflorescence	3mg/L	4.20 abc
Samany	Apical tip	3mg/L	4.17 bc
Zaghloul	Apical tip	1 mg/L	4.12 bc
Samany	Apical tip	1 mg/L	4.10 bc
Samany	Inflorescence	0 mg/L	4.08 bc
Zaghloul	Apical tip	0 mg/L	3.88 cd
Zaghloul	Inflorescence	I mg/L	3.85 cd
Zaghloul	Inflorescence	3mg/L	3.67 de
Zaghloul	Inflorescence	0 mg/L	3.65 de
Samany	Apical tip	0 mg/L	3.39 e

* Values with the same letters are not significantly differed.

Table (5): Effect of the interaction between explants and NAA on the mean diameter of the growing tissues (mm).

Explant types	NAA levels (mg/L)			Mean (mm)
	0	10	30	
Apical tip	3.92	4.24	4.47	4.22
Inflorescence	4.36	3.73	3.83	3.97
Mean (mm)	4.14	3.99	4.15	--

L.S.D (0.05)	Explants	NAA	Interaction
	0.17	N.S	0.29

Table (6): Effect of cultivars and BA factors on the tissues colour.

Cultivars	BA levels (mg/L)			Mean (colour points)
	0	**1**	**3**	
Zaghloul	5.38	5.73	6.24	5.80
Samany	5.74	6.37	6.71	6.27
Mean (colour points)				

L.S.D (0.05)	Cultivars	BA	CvsXBA
	0.30	0.37	N.S

تأثير الصنف والأجزاء النباتية على نمو الأنسجة المنزرعة من صنفى نخيل البلح الزغلول والسمانى

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أجريت هذه الدراسة خلال عامى 97 ، 1998 على صنفين من اصناف البلح الطرية والمنزرعة فى مصر (الزغلول والسمانى) بهدف التعرف على تأثير كل من الصنف والجـزء النباتىالمنزرع على بيئات خاصة (زراعات الأنسجة) على نمو وتطور هذه الأجـزاء. ولقـد دلت النتائج المتحصل عليها على ما يلى :

1 – أن عامل الصنف لم يؤثر جوهريا على طول الجزء النامى ولو أنه قد أثر جوهريا علـى قطر ولون النسيج النامى ، 2 – قد تأثر الى حد بعيد كل من طول وقطـر النـسيج النـامى باختلاف الجزء المستخدم فى الاكثار ، 3 – وضح من النتائج أن كل من BA و NAA أثرت جوهريا على طول وقطر وكذلك لون الأنسجه الناميه.