EFFECT OF EXPLANTS AND INCUBATION CONDITIONS ON GROWTH OF THE *IN VITRO* CULTURED TISSUES OF TWO DATE PLAM CULTIVARS.

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ABSTRACT

The present investigation was carried out in the laboratories of Pomology Department, Faculty of Agriculture, Alexandria University during the 1997 and 1998 in order to study the effect of explant types and incubation conditions on growth of the cultured tissues of two date palm (*Phoenix ductylifera L.*) cultivars. The obtained results showed that, inflorescence explants were significantly, superior over the apical tip or primary leaf tissues regarding the mean length and diameter of the growing tissues, however, there were no significant differences among all cultured tissues regarding their color or viability. The studied traits were not significantly affected by the incubation conditions. Moreover, the interaction between the two factors had no significant effect.

INTRODUCTION

The date palm (*Phoenix ductylifera L.*) is one of the oldest cultivated tree crops. The earlier Known records in Iraq show that its cultured was probably established as early as 3000 B.C. The date palm also has been in Egypt since prehistoric times, but its culture did not become important there until somewhat later than Iraq. From western Iran across Arabia and North Africa, dates have been a staple food for native population. The date palm is one of many examples of tree crops that benefit immediately from applications of the recent biotechnologies of plant tissue culture. Therefore, the main purpose of the present work aims at studying the effect of explants and incubation conditions on the growth of the cultured tissues of two date palm cultivars (Zaghloul and Samany).

MATERIALS AND METHODS

This experiment was conducted during 1997 and 1998 seasons. Two Egyption date palm cultivars namely; Zaghloul and Samany were used. The used explants were apical tip, primary leaves and immature

inflorescences. The basal salts of Murashige & Skoog (MS), in addition to certain addifives such as C.W (50ml / L) casein hydrolyate (20m/L), carbon source (30 ml/L glucose) and agar (7g / L) were used. As for the growth substances, 3 mg / L cytokinin (Kinetin for Zaghloul and 6-Benzyl adenine BA for Samary), 10mg / L auxin (2,4-D for Zaghloul and NAA for Samany) were used. Concerning the disinfection and pre-culture operations, 5% (V/V) liquld soap solution for 5 min., 70% (V/V) ethyl alcohol for 5 min., 2.6% (W/V) sodium hypochlorite for 15 min., antioxidant solution (150 mg/L citric acid + 100 mg/L ascorbic acid) for 20 min., and rinsed 3 times with distilled water for 5 minates. Day light and darkness at room temperature ($25 \pm 1\ ^0C$) and 95 % relative humidity (RH) were the incubation conditions.

Medium was prepared and dispensed in jars (20 ml in each), tissues were disinfected and placed immediately in aseptic conditions, then incubated at suitable temperature and relaive humidity. The treatments of this experiment were as shown in table (A).

Cultured tissues were noticed durinally each one week and contominated or dried tissues were removed,. Tissues size (length & diameter), colour and viability were measured as growth prameters after 8 week of culture. Dimensions of tissue size were measured in mm and tabulated. The date concerning the colour of tissues, have been determined according to colour chart (Fig.A). Viabiltity of the growing tissues was determined on the basis of the data in Table (B)

Table (A): Treatments of the experiment.

NO	Zaghloul cv.	Samany cv.
1	Day light & Apical tip.	Day light& Apical tip.
2	Day light& Primary leaves.	Day light& Primary leaves.
3	Day light& Inflorescences.	Day light& Inflorescences.
4	Darkness& Apical tip.	Darkness& Apical tip.
5	Darkness& Primary leaves.	Darkness& Primary leaves.
6	Darkness & Inflorescences.	Darkness& Inflorescences.

Table (B): Degrees of tissue viability after culturing.

Rank (socur)	Viability period after culturing
2	4 weeks growing after culturing
4	5 weeks growing after culturing
6	6 weeks growing after culturing
8	7 weeks growing after culturing
0	8 weeks growing after culturing

Reculture:

After 8 weeks, the growing tissues were recultured on fresh medium with the same composition. Immature inflorescence cultures (4 mm diameter) which separated from its branch, complete growing apical tip or divided tissues (2-4 mm in diameter) and complete growing primary leaf tissues (2 mm in both dimensions) were recultured after disinfection. Cultures were incubated under the same conditions.

Eight weeks later, the remaining immature inflorescence growing were recultured (for Zaghloul) and subcultured (for Samany). Four tissues of Zaghloul and 50 tissues of Samany were recultured and incubated at day light conditions. Samany tissues (8 tissues) which still growing were divided into 16 tissues and subcultured after another 8 weeks and incubated at day light condition.

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

RESULTS AND DISCUSSION

Length of growing tissues:

The data presented in Table (1) represent the effect of the studied factors (explants and incubation conditions) and their possible interaction on the mean length of the growing tissues of the two cultivars. The obtained results revealed that incubation conditions (day light or darkness) had no significant effect on the mean length of the growing tissues of both cultivars. In addifion, the twin interaction (explants X incubation conditions) also showed no significant effect. In the meantime, explants factors showed a significant effect on the mean length of the growing tissues of the two cultivars.

Concerning this scop, the growing inflorescence tissues were significantly longer than the other growing tissues for each cultivars in different incubation conditions. The recorded values were 4.20, 4.00 and 10.89 mm for apical tip. primary leaves and inflorescence respectively, for zaghloul cultivar. The corresponding values for samany were 4.47, 4.23 and 11.52 mm, respectively. Moreover, there were no significant differences between apical tip and primary leaves growing tissues of both cultivars.

The present results, also indicated that flower primordia attached to rachilla branch gave rise to nodule of callus, while apical tip and primary leaves as well increased slightly in size without inducing callus or organs. The obtained results agreed with these of Tisserat (1981), Sharma *et al*. (1984), Omar (1988), Del-Rosario (1991) and Verdiel *et al*. (1994). However, such results disagreed with those of Staritsky (1970), Rabechault and Martin (1976) and Harven *et al.* (1991).

Diameter of growing tissues:

In regard to explants factor, data of Table (2) indicated that inflorescence growing tissues had significantly larger diameter than that of the other growing tissues (apical tip or primary leaves) for both cultivars. The tobulated values of the mean diameter were 3.25, 2.27, and 2.25 mm for inflorescence, apical tip and primary leaves, respecively, for Zaghloul cultivar. The corresponding values-in the same order – for Samany were 3.18, 2.41 and 2.13 mm. It is worth mentioning that, there were no significant differences between mean diameter of apical tip and primary leaves growing tissues were found for Zaghloul or Samany cultivars.

Similar results were earlier reported by Dodds (1983), Daikh and Demarly (1987), Shaheen (1990), Besse *et al.* (1992) and Bakry (1994).

Viability and Colour of growing tissues:

The obtained results revealed that, almost all cultured tissues continued their active growth till the 8^{th} week after culturing. According to the ranking given in Table (B) included in materials and methods, the range of viability scour was 8.20- 9.00 point for all treatments, so the differences were not singnificant.

Statistical analysis of the obtained data indicaed that these were no significant effects for either the explant types or the incubation condition or their interaction on the colour of the growing tissues for Zaghloul or Samany cultivars. Generally, the colour degrees of the growing tissues ranged between 3.44 – 4.56 colour points or from creamy dark to creamy light or white according to a colour chort.

Reculture of growing tissues:

As for the results of the reculture growing tissues of both cultivars. Generally, the obtained results indicated that mass (noduls) of callus can

be obtained by using inflorescence explants, however, apical tip and primary leaf tissues failed and were not differentiated for Zaghloul or Samany cultivars. Callus growing tissues successed to still growing to 150 days for Zaghloul and 200 days for Samany. In the meantime, large number of cultures and subcultures can be obtained using few explants (by divided grew tissues and subcultured). Samany cultures had best observations compared to those of Zaghloul concerning numbers, colour and size of the growing tissues and the period from culture to the end of subcultures.

The results indicated that the activity of growing tissues was decreased gradually, this could be due to increasing of oxidative enzyme, activity. Such conclusion may be confirmed by the findings of AL-Bakir *et al*. (1989) and Booij *et al*. (1993) who found that polyphenol oxidase (PPO) and peroxidase (POD) activities were determined in the meristematic tissues which used in tissue cuthere of Khestawi cultivar, monthly throughout a whole year. POD and PPO were observed at various levels in the examined explants.

In addition, observations of this experiment indicated that there was a relationship between both of green colour and vigorous growing tissues and day light incubation conditions. It can be due to the influence of sun light on chllorophyll content in surface layer, then photosynthesis and growh of callus.

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Table (1): Effect of explants and incubation condtions on the mean length of the growing tissues (mm).

Cultivar	Incubation condition	* Explants			Mean (mm)
		A.t	P.L	In.	
Zaghloul	Day light.	4.29	4.00	10.67	6.70
	Darkness.	4.12	4.00	11.11	6.71
	Mean (mm)	4.20	4.00	10.89	__
Samany	Day light.	4.38	4.14	11.11	6.84
	Darkness.	4.56	4.31	12.00	6.86
	Mean (mm)	4.47	4.23	11.52	__

* A.t = Apical tip, P.L = Primary leaves and In. = Inflorescence.

L.S.D (0.05)	Explants		Incubation condition		Exp. X Incub,	
	Zaghloul	Samany	Zaghloul	Samany	Zaghloul	Samany
	0.40	0.75	N.S	N.S	N.S	N.S

Table (2): Effect of explans and incubation conditions on the mean diameter of the growing tissues (mm).

The used Cultivars	Incubation Conditions	*Explants			Mean (mm)
		A.t	P.I	In.	
Zaghloul	Day light	2.43	2.29	3.06	2.63
	Drkness	2.13	2.21	3.44	2.64
	Mean (mm)	2.27	2.25	3.25	__
Samany	Day light	2.44	2.21	3.17	2.65
	Darkness	2.39	2.06	3.19	2.54
	Mean (mm)	2.41	2.13	3.18	-

A.t. = Apical tip P.I = Primary leaves In. = Inflorescence

L.S.D (0.05)	Explant		Incubation conditions		Interaction	
	Zaghloul	Samany	Zaghloul	Samany	Zaghloul	Samany
	0.43	0.54	NS	N.S	N.S	N.S

الملخص العربى

تأثير الأجزاء النباتية وظروف التحضين على نمو الأنسجة النباتية المنزرعة بزراعات الأنسجة لصنفين من أصناف البلح.

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أجريت هذه الدراسة على صنفين أصناف البلح الطرية وهى الزغلـول والـسمانى، بهدف التعرف على تأثير كل من الأجزاء النباتية المستخدمة فى الاكثار الدقيق وكذلك ظروف التحضين، والتفاعل بين هذين العاملين على الأنسجة الناميـة والمنزرعـة تحـت ظـروف زراعات الأنسجة على نمو هذه الأجزاء النباتية.

ولقد أوضحت نتائج التحليل الاحصائى للبيانات المتحصل عليها ما يلى:

1 – أن النموات الناتجه من أجزاء مأخوذة من النورة الزهرية كانت اكبـر حجمـا (طـولا واتساعا) عن مثيلاتها المأخوذة من القمة الطرفيه أو تلك المأخوذة من بادرات الأوراق. ومع ذلك فإنه لم تلاحظ فروقات جوهرية بين الأنسجة المنزرعة فيمـا يتعلـق بلونهـا وحيويتها.

2 – أن الصفات قيد الدراسة لم تتأثر جوهريا بعامل التحضين (ضوء النهار أو الإظلام)، كما أن التفاعل بين هذين العاملين لم يظهر أيه تأثيرات جوهرية.