The effect of IAA and other auxins on the growth of date palm (*Phoenix dactylifera* L.) shoot tips and callus

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Abstract

Shoot tips and callus of some cultivars of date palms (*Phoenix dactylifera* L.) were cultured on MS (Murashige and Skoog) and GM5 (Gambourge B5) media by using auxin IAA mainly and other auxins NAA, 2,4-D and IBA.

The results showed a significant superiority of cultivar Hillawi than CVS. Zahdi, Barhee, Braim and Khasab for growth value and dry weight of shoot tip segments. There is a significant superiority of auxin IAA in MS medium than both IAA and 2,4-D in GM5 for growth value and dry weight for shoot tip segments of CV. Hillawi, and significant superiority of auxin IAA at level 100mg/L than auxin NAA (100mg/L) for growth value and dry weight of shoot tip segments of cv. Hillawi , and also a significant superiority of auxin IAA (10mg/L) than IAA at levels (0, 25, 100mg/L) for growth value an dry weight of both shoot tips segments and leaves primordia of cv. Sayer. Finally there is a significant superiority of auxin IAA (10mg/L) than auxins NAA, IBA and 2,4-D at the same levels for growth value of embryogenic callus of cvs. Barhee and Hillawi whereas the superiority was for both IAA, NAA, than the rest auxins for embryo length for cvs. Barhee and Hillawi.

Introduction

Date palm *phoenix dactylifera* L. is the important tree of which man has been interested since the ancient ages especially in Iraq; the success of its agriculture is due to its tolerance to high temperature and salinity [Saker *et al.,* 1997]. The continuous decreasing in numbers of date palm trees in Basrah by many reasons enhancing to find an other way for propagation of date palm, this way is tissue culture technique.

The first atempt was begun by Shroeder (1970) who used shoot tips and axillary buds of date palm as explants. Tisserat (1979, 1981) obtained date palm plants by somatic embryogenesis from the culturing of shoot tips and auxillary buds on MS (Murashige and Skoog) medium supplemented with 100mg/L (2,4-D) .

Sharma *et al.*, (1984, 1986) also obtained callus from shoot tips and axillary buds on MS medium involving the change of NaH_2PO_4. $2H_2O$ from 170mg to 200mg/L. Mater (1986) obtained a yellowish nodular callus after 6-8 months of subculturing of initial callus from shoot tips of cultivars: Hillawi and Barhee on MS medium supplemented with BA (Benzyl adenine) and Kinetin (2mg/L each) , 2,4-D (100mg/L) to initiate callus and then NAA (Naphthalene acetic acid) 0.1mg/L instead of 2,4-D to form somatic embryos.

Abo El-Nil (1986) reported the initiation of callus from shoot tips and axillary buds on MS medium supplemented with 2,ip (2, Isopentenyl adenine) and NAA.

Al'utbi (1998) obtained callus for many local cultivars from shoot tips and axillary buds on MS medium supplemented with 2,4-D (100mg/L), Kinetin, Benzyl adenine (2mg/L for each) then substitution of 2,4-D by low levels of NAA enhancing the formation of somatic embryos then plantlets.

Omar (1988a) observed white grainy callus initiation from leaf segments in nutrient medium enriched with 3mg/2ip and 100mg/NAA.

The aim of this study is to explore the effect of IAA (Indole acetic acid) as natural auxin on shoot tip and callus of date palm In Vitro in addition to (2,4-D) and (NAA) those were believed to have blemish of carcinogenic for the first and weakness for the latter.

Preparation of explants:-

Date palm offshoots (2-3 years aged) cvs. Hillawi, Sayer, Zahdi, Khasab, Braim and Barhee were brought from Abu-Alkhaseeb orchards and dissected acropetally, shoot tips were excised and trimmed to shoot tip disc (8 x 3mm) preserved in antioxidant solution (150mg/L citric acid + 100mg/L Ascorbic acid) , then sterilized in infestant solution of sodium hypochlorite NaOCl 20% with two drops of tween-20 for 20 minutes washing with sterilized distilled water three times and culturing on fresh medium; all operations were performed in laminar air flow.

Preparation of medium :

A) MS medium according to (Murashige and Skoog, 1962).

B) Gambourge B5 medium (Gambourge and Eveligh, 1968).

Both types of media were supplemented with the following materials (mg/L), Sucrose (30000), NaH_2PO_4 (170), Meso-Inositol (100), thiamine-HCl (0.5), activated charcoal (3000), Agar (8000), Kinetin (3), Benzyl adenine (BA) (1):

Auxins were added according to statistical experiments requirements as follows:-

1) Auxins IAA (100mg/L) was added to MS medium for the growth of shoot tips of different varieties of date palm.
2) IAA, 2,4-D (100mg/L) were added to MS and GM5 media for the growth of shoot tips of cv. Hillawi.

[MS medium + auxin IAA], [MS medium + auxin 2,4-D], [Gambourge B5 medium + auxin IAA], [Gambourge B5 medium + auxin 2,4-D].

3) Auxins, NAA , IAA (100mg/L for each) were added to MS and GM5 media for the growth of shoot tips cv. Hillawi in combinations: [MS medium + Auxin NAA] , [MS medium + auxin IAA] [Gambourge B5 + Auxin NAA], [Gambourge B5 + Auxin IAA] .

4) IAA was added at level (0, 25, 50, 100) mg to MS medium for the growth of shoot tips and leaf primordia of cv. Sayer.
5) Auxins IAA, IBA, NAA, 2,4-D were added to MS medium for the growth of embryogenic callus and somatic embryos cvs. Barhee and Hillawi.

pH was adjusted at 5.7, Agar was added. After heating to 95°C the solution was cooled and dispensed in containers (test tubes and conical flasks). These containers were closed with cotton and aluminum foil , then they were autoclaved at 121°C and 1.05kg/cm^2 for 20 minutes.

C) Subculture of callus :

Callus was subcultured at periods of 60 days continuously on MS medium supplemented with 2,4-D or NAA (100mg/L) in addition to benzyl adenin (1mg/L) and kinetin (3mg/L).

d) All contents of MS medium are used for the preparation of a medium for somatic embryos formation; except auxins IAA, 2,4-D, NAA were removed with their concentration (100mg/L) and only NAA (1mg/L), kinetin (3mg/L) and BA (1mg/L) were added instead of them.

Growth value was calculated by the following formula:

$$\text{Growth value} = \frac{\text{Final fresh weight}}{\text{Initial fresh weight}}$$

(Almehdi, 1976)

All experiments were performed according to C.R.D. (Complete Randomized Design) (5-10 replicates) and the results were analyzed according RLSD. (AL-Rawi and Khalafullah, 1980).

Results

1- Effect of the addition of auxin IAA on the growth of shoot tips cvs. Hillawi, Braim, Khasab, Barhee and Zahdi to compare their responses.

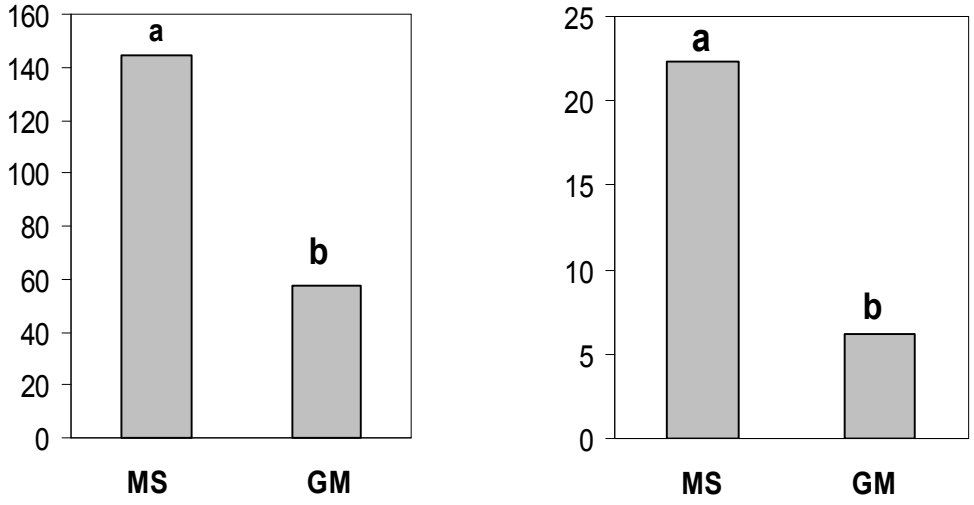
The results showed (Table 1) that significant superiority of cv. Hillawi in growth value (5.18) than all other treatments: Zahdi, Barhee, Braim, Khasab (2.75, 2.13, 1.87, 1.40), respectively, but there is no significant differences among the rest cultivars, also there is a significant superiority of cv. Hillawi in dry weight (62mg) than all other treatments: Barhee, Braim, Khasab and Zahdi (29mg, 17.40mg, 12.40mg and 35.40mg), respectively, and there is no significant difference among the rest treatments.

Table 1: Effect of response of five cultivars of date palm for the growth of shoot tips segments (300 gm as mean) on MS medium containing 100mg/L auxin IAA (after two months-culture period)

Treatments (five cultivars of date palm)					
Measurement	Hillawi	Braim	Khasab	Barhee	Zahdi
Growth value	5.18 *a	1.87 b	1.40 b	2.13 b	2.75 b
Dry weight (mg)	62.0 a	17.40 b	12.40 b	29.00 b	35.40 b

*Values with the same letter are not significantly different by RLSD analysis ($p \leq 0.05$).

2- Effect of the addition of auxins IAA to MS and GM5 media on the growth of shoot tip cv. Hillawi. The results showed (Fig. 1) a significant superiority at $p \leq 0.05$ of treatment [IAA in MS medium] in growth value (22.36) than the treatment [IAA in GM5 medium] (6.17), there is also a significant superiority at $p \leq 0.05$ of treatment [IAA in MS medium] in dry weight (144.80 mg) than the treatment [IAA in GM5 medium] 57.80mg.



(A) **(B)**

Fig. 1: Effect of the addition of auxin IAA to MS and GM5 media on the growth of shoot tip segments (300mg as mean) cv. Hillawi (after two months-culture period) A- growth value B- dry weight

The similar letters indicate that there is no significant difference by RLSD analysis ($p \leq 0.05$).

3- Effect of the addition of auxin IAA, NAA to MS medium on the growth of shoot tip segments cv. Hillawi.

Results showed a significant superiority of treatment [IAA, 100mg/L] than treatment [NAA, 100mg/L] for growth value and dry weight (table 2).

Table 2: Effect of the addition of IAA , NAA to MS medium on the shoot tip segments (300mg as mean) cv. Hillawi (after two months-culture period)

Measurements	Treatment 100mg/L	
	IAA	NAA
Growth value	21.99 a	5.00 *b
Dry weight (mg)	171.30 a	45.55 b

*Values with the same letter are not significantly different by RLSD analysis ($p \leq 0.05$).

4- Effect of the addition of different levels of auxin IAA on both shoot tip segments and leaves primordia of cv. Sayer.

A- Shoot tip segments:-

Results showed [Table 3] a significant superiority of treatment (IAA, 10mg/L) for growth value (20. 73) than treatments (0, 25, 100mg) for growth values (6.59, 11.93, 8.19) respectively, and there is no significant difference among the rest treatments, also there is a significant superiority of treatment (10mg/L) for dry weight (143.75mg/L) than treatments (0, 25, 100mg/L) (63.75, 89, 46 mg), respectively.

B- Leaves primordia:-

There is a significant superiority of treatment (IAA, 10mg/L) for growth value (31.88) than the treatments (0, 25, 100) (17.75, 10.53, 15.36) and for dry weight (207.75mg/L) than other treatments (123, 5, 112.25mg/L), respectively.

The results showed that the response of leaves primordia was better than that of shoot tips.

Table 3: Effect of addition of IAA (0.0, 10, 25, 100) mg/L on the growth of shoot tip segments (300 mg as mean) cv. Hillawi (after two months-culture period)

Measurements		Treatment IAA mg/L			
		0.0	10	25	100
Shoot tips	Growth value	8.19 b*	20.73 a	11.93 b	6.59 b
	dry weight (mg)	63.75 b	143.75 a	89.00 b	46.00 b

* Values with the same letter are not significantly different by RLSD analysis ($p \leq 0.05$).

Table 4: Effect of addition of IAA (0.0, 10, 25, 100) mg/L on the growth of Leaves primordia (300 mg as mean) cv. Hillawi (after two months-cultur period

Measurements		Treatment IAA mg/L			
		0.0	10	25	100
Leaves primordia	Growth value	17.75 b*	31.88 a	10.53 b	15.36 b
	dry weight (mg)	123.00 b	207.75 a	65.00 b	112.25 b

* Values with the same letter are not significantly different by RLSD analysis ($p \leq 0.05$).

5-A- Effect of the addition of auxins 2,4-D, IBA, NAA, IAA on the growth of embryogence callus and somatic embryos cv. Barhee.

There is a significant superiority at $p \leq 0.05$ of treatment IAA (10mg/L) for growth value (165.03) than treatments 2,4-D, IBA, NAA at the same ;level for each (10mg/L) for growth values (58.77, 34.52, 57.62) respectively.

There is also a significant superiority at $p \leq 0.05$ of treatments (IAA, NAA 10mg/L for each) for embryo length (12.58)mm, (12-16mm) respectively than the treatments (IBA,2,4-D 10mg/L for each) (7.86mm) (10.44mm) (Table 4).

B- Effect of the addition of auxins IAA, NAA, IBA, 2,4-D on the growth of embryogenic callus and somatic embryos cv. Hillawi.

Results showed [Table 6] a significant superiority of treatment IAA (10mg/L) for growth value [5.36] than treatments [IBA, NAA, 2,4-D] at levels 10mg/L [1.79, 2.44, 2.6], respectively.

There is also a significant superiority of treatments IAA, NAA (10mg/L) for embryo length 8.20, 8.73mm, respectively, than treatment IBA (10mg/L) for embryo length (4.35mm) but there is no significant difference among embryo number means.

Table 5:Effect of the addition of the auxins: NAA, 2,4-D, IAA, IBA on the growth of embryogenic callus cv. Barhee (after two months-culture period)

Measurements	Treatment 10 mg/L			
	NAA	2,4-D	IAA	IBA
Growth value	34.5 *b	58.77 b	165.03 a	57.62 b
Embryo length (mm)	12.585 a	10.44 b	12.16 a	7.86 c

* Value with the same letter is significantly different by RLSD analysis ($p \leq 0.05$).

Table 6: Effect of the addition of the auxins: NAA , 2,4-D, IAA, IBA on the growth of embryogenic callus cv. Hillawi (after two months-culture period)

Measurements	Treatment 10 mg/L			
	NAA	2,4-D	IAA	IBA
Growth value	2.60 b	2.44 b	5.36 *a	1.79 b
Embryo length (mm)	8.73 a	7.85 b	8.20 a	4.35 b

* Values with the same letter are not significantly different by RLSD analysis $p \leq 0.05$.

Discussion

According to our results, it is easily to classify that cv. Hillawi was higher in response to tissue culture than Barhee, Zahdi, Braim and Khasab; Our finding conform with those investigators working with other cultivars. Omar (1988b) found aclear differences in responses between other cultivars such as Khustawi, Sayer , Zahdi, Braim and Ashrasi.

Al'utbi (2000) reported a weak response of cv. Gantar to *In vitro* culture.

The superiority of MS medium than GM5 medium in the presence of auxin IAA may be related to constitutions levels and quantities of media salts, thus MS salts contain high level of nitrogen, potassium and ammonia than GM5 salts.

Although Zaid and Tisserat (1983) found a little effect of auxin IAA on the growth response of shoot development; however, this is not a sharp conclusion to exclude auxin IAA role absolutely in shoot growth response, keeping in mind that IAA is a natural plant growth hormone, moreover, Black and Edelman (1980) stated that IAA has a wide range of activities than other auxins; one of these activities that the synthetic auxins do not have a polar transport as IAA. The more effect of IAA than NAA in our current investigation may be to its nature as a plant growth hormone or to the weakness of NAA in its effect. Tisserat (1979) referred to the weakness of NAA in its effect .

The results which have been obtained along a large number of researches showed a large scale of variation among many auxins used; however some prefer 100mg 2,4-D for the development of shoot tip (Tisserat, 1979), others regard 10mg/L NAA is useful for the initiation of callus from shoot tip (Mater, 1986). Alutbi (1998) used 10mg/L NAA for embryogenic callus, whereas Omar (1988b) obtained callus by subculturing ovule segments on medium containing 10mg/L 2,4-D. Khan *et al*. (1982) used a medium containing IAA and NAA to obtain leaf-like structures on the surface

of primordia leaf tissues. Our finding 10mg/L IAA gives a significant increase in the development of shoot tips disagreed with (Zaid and Tisserat, 1983) for IAA role in the development of shoot tips. Some authors obtained better results by the corporation of IAA with other auxins NAA, NOA, 2,4-D, IBA (Dass *et al.*, 1989 ; Beau cheane, 1082).

Our findings conform with the works of other investigators for the superiority of leaf primordia than shoot tip segments in their development (Vermendi and Navarro, 1997), while other investigators also obtained notable growth from leaf primordia segments (Brachesne, Zaid, Rhiss, 1986 ; Shroeder, 1970 ; Omar, 1988a).

The results showed a good effect of IAA (10mg/L) among other auxins (IBA, 2,4-D, NAA), for the growth of embryogenic callus may be due to its natural characteristic feature as plant growth regulator which made a balance between endo and exogenic auxin in the level of 10mg/L to enhance the division of cells or increasing the osmotic content consequently, causing the increase in fresh weight.

The effect of NAA in causing an increase in embryo length may be due to the elongation of root pole.

Some researches point out the effect of NAA on rooting activity (Tisserat, 1982 ; Zaid and Tisserat, 1983; Mater, 1986 ; Omar, 1988a ; Dass *et al*., 1989; Alutbi, 1998).

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الخلاصة

تمت زراعة اطراف الفروع والكالس الجنيني لبعض اصناف نخلة التمر *Phoenix dactylifera* L. على الوسطين الزرعيين موراشيجي وسكوك وكامبورج B5 وباستعمال الاوكسين IAA (اندول حامض الخليك) بصورة رئيسة واوكسينات اخرى مثل NAA نفثالين حامض الخليك وIBA اندول حامض البيوتيريك و 2,4-D داي كلوروفينوكسي حامض الخليك، فاظهرت النتائج تفوقاً معنوياً لصنف الحلاوي على بقية الاصناف : الزهدي ، البرحي، البريم والخصاب في قيمة النمو وكذلك تفوق معنوي للاوكسين IAA في وسط MS على كل من IAA، 2,4-D في وسط GMB5 في قيمة النمو والوزن الجاف بالنسبة لقطع اطراف الفروع لصنف الحلاوي، وتفوق معنوي للاوكسين IAA تركيز ١٠٠ ملغم/لتر على الاوكسين NAA تركيز ١٠٠ملغم/لتر بالنسبة لقيمة النمو والوزن الجاف لاطراف الفروع لصنف الحلاوي، وكذلك تفوق معنوي للاوكسين IAA تركيز ١٠ ملغم/لتر على التراكيز (١٠٠، ٢٥، ٠) ملغم/لتر في قيمة النمو والوزن الجاف لكلا قطع اطراف الفروع وبادئات الاوراق لصنف الساير واخيراً تفوق معنوي للاوكسين IAA بتركيز ١٠ ملغم/لتر على الاوكسينات NAA، IBA، 2,4-D بنفس التركيز لقيمة النمو للكالس الجنيني لصنفي البرحي والحلاوي بينما كان التفوق المعنوي لكلا الاوكسنين NAA ، IAA على بقية الاوكسينات بالنسبة لطول الجنين لصنفي البرحي والحلاوي.