

Plantlet regeneration from root segments of Date palm tree (*Phoenix dactylifera* L. cv. Barhee) producing by *in vitro* culture

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Abstract. This study was conducted during two seasons growth (2010/2011) in order to induction of root segments taken from plantlets producing by *in vitro* culture on growth and differentiation to produce direct and indirect shoots by organogenesis method. The results showed the ability of root segments to produce direct shoots when cultured by MS medium supplied with 2.0 mg.l⁻¹ BA and 0.2 mg.l⁻¹ NAA. This treatment was a high significant in the percentage of response for direct shoot induction and number of shoots/explant comparison with other treatments reached 41.67 % and 4.6, respectively. Also, these results showed the possibility of root segments on callus induction when cultured in MS medium supplemented with 40 mg.l⁻¹ 2,4-D and 3 mg.l⁻¹ 2ip. This callus formation gave a high significant rate of response to producing the indirect shoots when this callus is re-cultured at the first time in MS medium supplied with 0.5 mg.l⁻¹ BA and 0.1 mg.l⁻¹ NAA, which reached to 53.34 %. Also, the MS medium supplied with the same concentration of BA recorded the highest rate of proliferation reached 5.2 shoot/100 mg callus.

Key Words: Date palm, *in vitro*, organogenesis, proliferation, root segment, shoot.

Introduction. The Date palm (*Phoenix dactylifera* L.) is an important source of high quality food for humans and animals (Al-Baker 1972). There are more than 600 cultivars of Date palm planted in Iraq. The Barhee cultivar is the best of Date palm varieties that the Iraqi grower would like to cultivate and propagate. The propagation method by offshoots is traditional method of vegetative propagation common of the Date palm. This cultivar is characterized by low production of offshoot numbers and the difficulty of getting them for expensive prices. Many researchers turn to the propagation of Date palm by tissue culture technique to overcome their difficult (Muter 1991). Some researchers used the shoot tips and axillary buds as explants for micro propagation of Date palm tree (Hassan 1987; Al-Khalifa 2007; Ibrahim 2008). Many of researchers got positive results when they used the other explants such as root segments because the most plant organs have the capacity to grow if there are optimum conditions of growth media and environmental factors (Al-Taha 2008). Smith & Thomas (1973) were able of callus induction from roots of coconut seedlings cultured *in vitro*. Smith (1975) showed the possibility of stimulating adventitious buds to form plantlets from induction callus of date palm roots by *in vitro*. Due to the fact that the most of the Date palm trees cv. Barhee in Iraq are old ages, passed the stage of producing offshoots, they are in small number and endangered, the current study was conducted in order to use other explants such as root segments for the purpose of propagation of date palm trees *in vitro* rather than vegetative explants.

Material and Method

The root segments (1 cm length) were obtained from plantlets of Date palm (*Phoenix dactylifera* L. cv. Barhee) produced in *in vitro* culture. These root segments were horizontally cultured directly in MS medium (Figure 1, A).

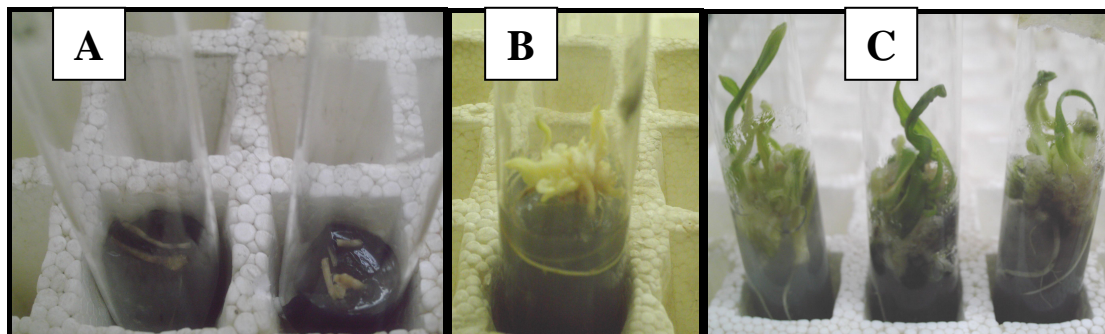


Figure 1. Direct shoots induction from root segments cultured in MS medium supplied with 2.0 mg.l^{-1} BA and 0.2 mg.l^{-1} NAA after 12 weeks from culturing. A - root segment culture; B - direct shoots induction; C - growth and developed shoots.

Effect of different concentrations of BA on direct shoot formation. The root segments in this experiment cultured in MS medium (Murashige & Skoog 1962) with addition of 30 gm.l^{-1} sucrose, 7.0 gm.l^{-1} agar, 3.0 gm.l^{-1} activated charcoal and some vitamins at 1.0 mg.l^{-1} concentration for each one of them. This medium was supplemented with different concentrations of BA (0.5 , 1.0 or 2.0 mg.l^{-1}) and 0.2 mg.l^{-1} NAA for each treatment as well as control treatment (MS medium without hormone). These cultures were incubated at a temperature of $27 \pm 1^\circ\text{C}$ and light intensity below 1000 Lux provided by white fluorescent lamps for 16 h. Every treatment in the experiment was replicated 12 times. The data was recorded after 12 weeks from culturing as following:

- the percentage of response of root segment on direct shoot formation;
- number of direct shoots/explant.

Effect of different concentrations of 2,4-D on direct callus induction. The root segments were cultured in the same medium (just like previous) supplied with different concentrations of 2,4-D (10 , 20 or 40 mg.l^{-1}) and 3.0 mg.l^{-1} Zip for each treatment, as well as control treatment. These cultures were incubated at a temperature of $27 \pm 1^\circ\text{C}$ in the dark. This experiment was performed in 12 replicates. The response percentage of root segment on callus induction was recorded after 12 weeks from culturing.

Effect of different concentrations of BA on indirect shoot formation. Amount of callus (100 mg weight) was cultured in the same medium (just like previous) supplemented with different concentrations of BA (0.1 , 0.5 , 1.0 or 2.0 mg.l^{-1}) and 0.1 mg.l^{-1} NAA for each treatment, as well as control treatment. These were cultured in the same experimental conditions as it shown in figure 1. The number of replicates of this experiment was 15. The data was recorded after 16 weeks from culturing as following:

- the percentage of response of callus mass on indirect shoot formation;
- number of indirect shoots/explant.

Statistical design and analysis. Completely randomized design was used. The data was subjected to the analysis of variance and mean values were compared using revised LSD at 5 % (Snedecor & Cochran 1986).

Results and Discussion

Effect of different concentrations of BA on direct shoot formation. Table 1 show that root segments gave the best significant response for direct shoot formation and number of shoot/explant when was cultured in MS medium supplemented with 2.0 mg.l⁻¹ BA and 0.2 mg.l⁻¹ NAA in comparison with other treatments, and reached 41.67 % and 4.6 shoots/explant, respectively (Figure 1). But, the root segment cultured in MS medium without hormone (control treatment) did not give any response for direct shoot formation. Also, table 1 show that multiplication and induction of shoots increased with increasing BA concentration. This result is in accordance with Nagori & Purohit (2004). The buds and shoots induction from root segment cultured in MS medium depends on meristematic and active cells. This root segment produces high hormone rates such as cytokinins and gibberellins. These rates of cytokinin induced shoot formation from root segment (Smith & Thomas 1973; Van Staden & Smith 1978). Moreover, the growth regulators added to medium play an important role in the growth and differentiation of explants (Tisserat 1984).

Table 1

Effect of different concentrations of BA with 0.2 mg.l⁻¹ NAA on a response percentage of root segments for direct shoot formation and number of shoots/explant after 12 weeks from culturing

BA concentration (mg.l ⁻¹)	Response of root segment for direct shoot formation	Number of shoots/explant
0.0 (without hormone)	0.00 c	0.0 d
0.5	8.34 c	2.4 c
1.0	25.00 b	3.2 b
2.0	41.67 a	4.6 a

Effect of different concentrations of 2,4-D on direct callus induction. Figure 2 show that root segment cultured in MS medium supplied with 40 mg.l⁻¹ 2,4-D and 3.0 mg.l⁻¹ 2ip induced small mass of the white yellowish callus after 12 weeks in the dark.

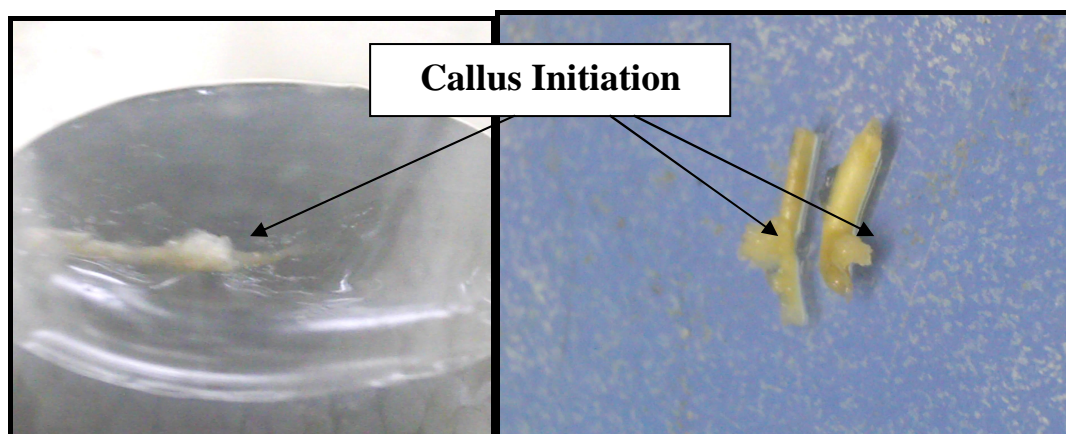


Figure 2. Callus induction from root segments cultured in MS medium supplied with 40 mg.l⁻¹ 2,4-D and 3 mg.l⁻¹ 2ip after 12 weeks from culturing.

This treatment in comparison with other treatments gave a significantly high response percentage of root segments on callus induction, reaching 41.67 % (Table 2). When the root segment was cultured in MS medium without hormone did not give any response to callus induction. The callus induction from root segment was present in different regions of segment except the cutting zone. This callus was produced from active meristematic tissue on segment such as adventitious buds. These results (Table 2) illustrated the importance of balance between auxins and cytokinins on callus induction and

differentiation. The correlation between callus induction and increasing of auxin concentration (2,4-D) in MS medium was positive. The auxins play an important role in cell induction to division and growth because they are stimulating respiration enzymes production to provide energy for cell division (Al-Maari 1995).

Table 2

Effect of different concentrations of 2,4-D with 3.0 mg.l⁻¹ 2ip on a response percentage of root segment for callus induction after 12 weeks from culturing

<i>2,4-D concentration (mg.l⁻¹)</i>	<i>Response of root segment for callus induction</i>
0.0 (without hormone)	0.00 c
10	8.34 bc
20	16.67 b
40	41.67 a

Effect of different concentrations of BA on indirect shoot formation. Table 3 and figure 3 shows that callus (100 mg weight) cultured in MS medium supplemented with 0.5 mg.l⁻¹ BA and 0.1 mg.l⁻¹ NAA gave a highly significant percentage response of callus for indirect shoot formation (53.34 %) and number of shoots/100 mg callus (5.2) in comparison with other treatments.

Table 3

Effect of different concentrations of BA with 0.1 mg.l⁻¹ NAA on a response percentage of root segments for direct shoot formation and number of shoots/100 mg callus after 16 weeks from culturing

<i>BA concentration (mg.l⁻¹)</i>	<i>Response of callus (100 mg weight) for indirect shoot formation</i>	<i>Number of shoots/100 mg callus</i>
0.0 (without hormone)	0.00 c	0.0 c
0.1	0.00 c	0.0 c
0.5	53.34 a	5.2 a
1.0	26.67 b	3.4 b
2.0	20.00 b	3.0 b

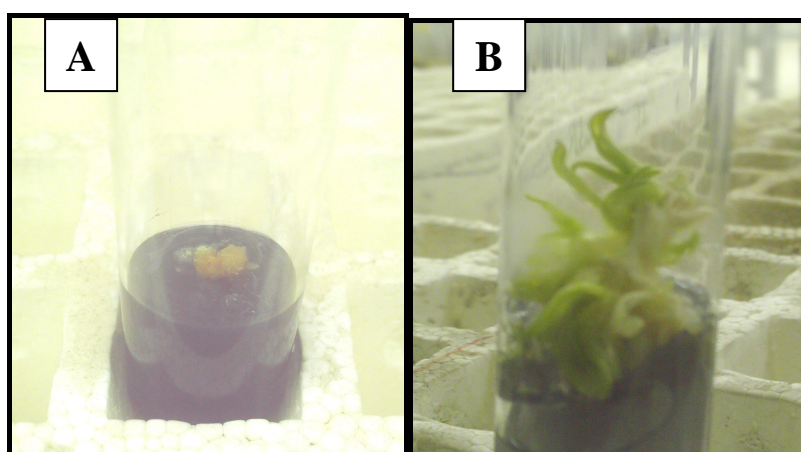


Figure 3. Indirect shoots induction from callus (100 mg weight) cultured in MS medium supplied with 0.5 mg.l⁻¹ BA and 0.1 mg.l⁻¹ NAA after 16 weeks from culturing. A- callus culture; B - indirect shoots induction.

The BA concentration of 0.5 mg.l⁻¹ may be perfect concentration to induce indirect shoots from callus culture. The direct and indirect shoots produced from callus depend on the

interaction between auxins and cytokinins and their concentration in MS medium (Skoog 1971; Auge 1984; Gabr & Tisserat 1985; Tisserat 1979; Amin 2001). Cytokinins were stimulated shoot formation increasing DNA multiplication and cell division, when the auxins stimulate cell division and enlargement (Auge 1984). Also, the BA induces callus growth because it prevents auxin-oxidation, which leads to an increase in the endogenous auxin in explant (George & Sherrington 1984). The obtained results are in accordance with Al-Taha (2008). The same author was found that callus formed root tip of Local orange (*Citrus sinensis*) cultured in MS medium supplemented with 7.0 mg.l⁻¹ BA gave indirect shoots.

Conclusions. We are concluded from the present study that root segments of Date palm plantlet have ability of growth and differentiation for direct and indirect shoots formation when they are cultured in the right medium and concentration of plant growth regulators, according to the nature of growth.

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