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*Full Length Research Paper*

# Somatic embryogenesis in two Iranian date palm cultivars

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**Shoot tips were removed from 3 to 4 year-old offshoots of adult date palm (*Phoenix dactylifera* L. cv. Khanizi and Mordarsing) and were cultured on medium that consisted of Murashige and Skoog basal salts medium. After 12 months they were transferred to three different treatments of growth regulators. Five months later, cv. Khanizi produced embryogenic callus on a medium containing 453 µM 2,4-dichlorophenoxyacetic acid (2,4-D), 15 µM $N^2$-(2-isopentenyl)adenine (2ip), and 13 µM 6-benzyl amino purine. This callus was subcultured to another medium that was supplemented with 54 µM NAA and 148 µM 2ip. At this stage the embryos grew into plantlets. The cv. Mordarsing explants, on a medium containing 679 µM 2,4-D and 15 µM 2ip, produced embryogenic callus but the embryos remained in the globular stage.**

**Key words:** Callus, *in vitro* tissue culture, offshoot, *Phoenix dactylifera*, regenerant.

## INTRODUCTION

Date palm (*Phoenix dactylifera* L.), a monocotyledonous and dioecious species belonging to the Aracaceae family, is widely cultivated in arid regions of the Middle East and North Africa (Al-Khayri et al., 2001), and is one of the main export crops of Iran. Date palm can be propagated by seeds, which usually produces trees bearing fruits. Rooted offshoots are preferred for conventional propagation because they produce true-to-type trees with fruit quality identical to that of the mother tree (Al-Khayri et al., 2001; Bajaj, 1992). However, there are many problems associated with this system (Popenoe, 1973); the availability of offshoots is limited because the number produced by each palm tree is low, very erratic and cannot be successfully controlled (Al-Khayri et al., 2001; Veramendi et al., 1996). The offshoot must remain attached to its parent tree for a long time (2-3 years) until an adequate root system develops (Bajaj, 1992). In addition, the methods of exision is complicated and time consuming, and the percentage of offshoots successfully established in soil is highly variable (30 to 80%) (Al-Khayri et al., 2001; Veramendi et al., 1996).

Micropropagation provides an attractive alternative method for large-scale propagation and commercial production of date palm trees (Al-Khayri et al., 2001). This is especially true with somatic embryogenesis, which leads to the production of bipolar structures nominally capable of germination without separate shoot and root induction phases. Sometimes the distinction between structures derived by organogenesis and somatic embryogenesis can be blurred, especially when it is necessary to give shoot and/or root development treatments to the latter. For this reason it is necessary to present histological information at least once for each species, in order to verify that somatic embryos have

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**Abbreviations:** BAP, 6-Benzyl amino purine; cv., cultivar; 2,4-D, 2,4-dichlorophenoxyacetic acid; MS, Murashige and Skoog (1962) media; 2ip, N2-(2-isopentenyl) adenine.

**Table 1.** Three different treatments for embryogenic callus induction.

| Medium | 2,4-D (mg/l) | 2ip (mg/l) | BAP(mg/l) |
|---|---|---|---|
| 1 | 100 | 3 | - |
| 2 | 150 | 3 | - |
| 3 | 100 | 3 | 3 |

**Table 2.** Summary of growth regulator combinations on which callus induction and somatic embryogenesis from cultivars were observed.

| Plant growth regulator (µM) | Callus induction (%) | | Somatic embryo formation | | Somatic embryo maturation | |
|---|---|---|---|---|---|---|
| | Khanizi | Mordarsing | Khanizi | Mordarsing | Khanizi | Mordarsing |
| 2,4-D(453)+2ip(15) | 44 | 12 | - | - | - | - |
| 2,4-D(453)+2ip(15) | 0 | 2 | - | + | - | - |
| 2,4-D(453)+2ip(15)+BAP(13) | 11 | 0 | + | - | + | - |

been produced from an explant (Thorpe, 1995). Histological evidence has been presented in support of somatic embryogenesis for *Acanthopanax* (Gui et al., 1991), Castanea (Gonzalez et al., 1985), Elaeis (Schwendiman , J. et al. 1988), *Phoenix* (Tisserat et al., 1980).

## MATERIALS AND METHODS

### Explants preparation

Shoot tips were removed from 3 to 4 year-old offshoots of *P. dactylifera* cv. Khanizi and Mordarsing, which were collected from Minab in the south of Iran. The tips were surface disinfected in 70% ethanol for 1 min followed by 15 min immersion in a 2.6% sodium hypochlorite solution with 1 ml/l Tween 20 and rinsed 4 times with sterile distilled water. Shoot tip termini, sectioned longitudinally into four 5-mm long pieces were cultured individually on culture initiation medium.

### Culture initiation and callus induction

The culture medium consisted of MS (Murashige and Skoog, 1962) basal salt medium comprising of (per liter): 170 mg $NaH_2PO_4$, 125 mg myo-inositol, 200 mg glutamine, 1 mg nicotinic acid, 1 mg pyridoxine-HCl , 5 mg thiamine, 30 g sucrose and 7 g agar (Sigma), 453 µM 2,4-D, 15 µM 2ip, and 1.5 g activated charcoal (acid-washed, neutralized) (Sigma). The media were adjusted to pH 5.7. The cultures were incubated in darkness at 24±3°C for 12 months during which they were transferred at 1-month intervals to 3 different treatments of growth regulators according to Table 1. These cultures were incubated in darkness at 24±3°C for 5 months during which they were transferred at 1-month intervals after embryogenic callus was produced to another medium that contained MS basal salts (Murashige and Skoog, 1962) to which was added (per liter) 54 µM NAA, 148 µM 2ip, and 1.5 g activated charcoal. On this medium the callus develops into plantlets.

### Data analysis

The number of callus-forming explants, calli producing embryos was recorded at the end of each subculture phase. The results were assessed by a standard analysis of variance for a randomized complete block design (Kintzios et al., 1999).

## RESULTS AND DISCUSSION

The effects of the different growth regulator treatments on callus and somatic embryo induction are summarized in Table 2. After 5 months in three different treatments, both cv. Mordarsing and Khanizi on medium 1 produced callus but they were non-embryogenic. cv. Mordarsing on medium 2 and cv. Khanizi on medium 3 produced embryogenic callus. By viewing cv. Khanizi under a binocular microscope we observed different stages of embryogenic callus: globular, heart, torpedo and cotyledonary stage (Ramawat, 2003) (Figure 1a-d). On medium 2 no callus was formed. The other cultivar, Mordarsing, on medium 2 produced embryogenic callus but it stopped at the globular stage. (Figure 1e,f). Embryogenic callus (cv. Khanizi) that was transferred to a medium containing NAA and 2ip developed plantlets with a shoot and a root (Figure 2).

This study shows that the type of cultivar has a very significant effect on callus and somatic embryo induction. Also specific plant growth regulator (PGR) combinations at particular concentrations were effective in inducing a callus tissue on different cultivars of date palm (Table 3). The medium that produced embryogenic callus in cv. Khanizi had a different effect on another cultivar, cv. Mordarsing. There is not yet any general protocol for micropropagation of date palm (*P. dactylifera* L.) via

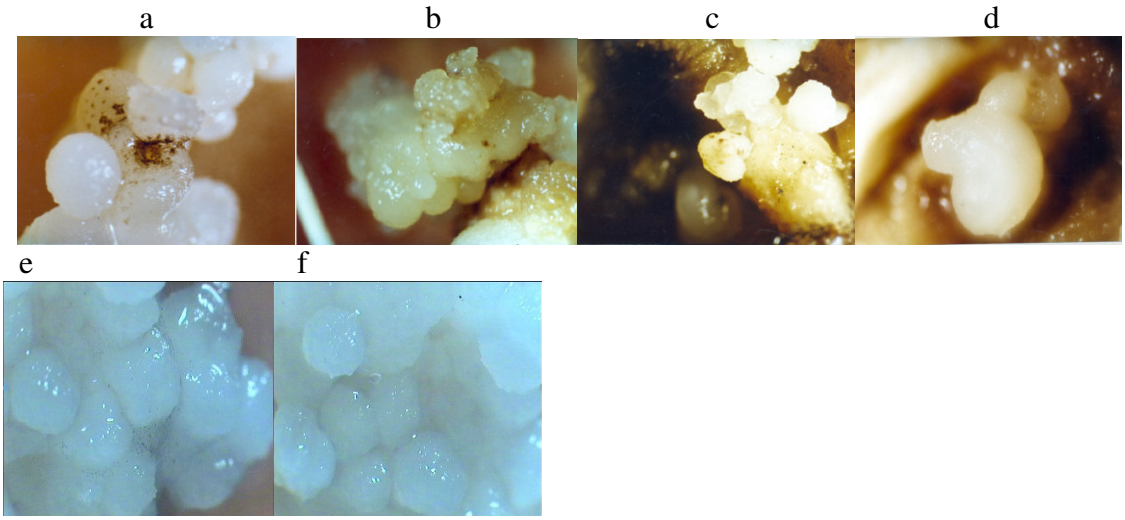


**Figure 1. (a-d)** Different stages of embryogenic callus derived from cv. Khanizi on medium supplemented with 453 µM 2,4-D, 15 µM 2ip and 13 µM BAP. **(e-f)** Globular stage of embryogenic callus from cv. Mordarsing on medium supplemented with 679 µM 2,4-D and 15 µM 2ip.

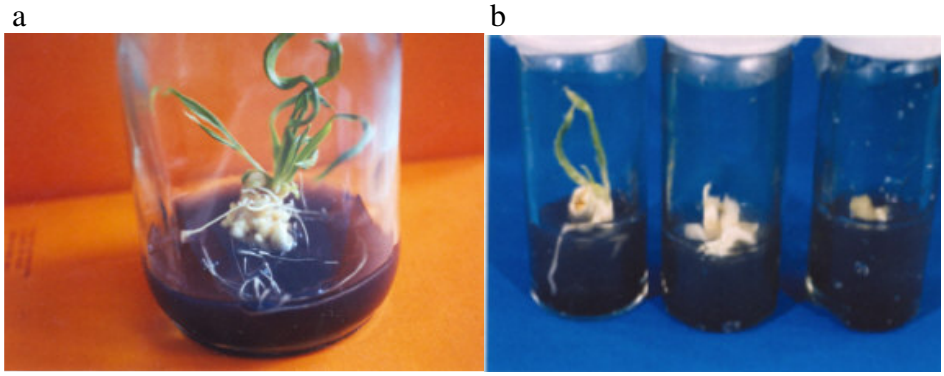


**Figure 2.** Plantlets derived from embryogenic callus of cv. Khanizi on medium supplemented with 54 µM NAA and 148 µM 2ip.

**Table 3.** Analysis of variance of callus induction production from two Iranian cultivars of date palm in response to different growth regulator treatments.

| Source of variation | df | Mean square |
|---|---|---|
| Growth regulator treatment (A) | 2 | 1255.5000*** |
| Cultivar type (B) | 1 | 840.500*** |
| AB | 2 | 441.500*** |
| Error | 10 | 3.467 |
| **Total** | **17** | |

somatic embryogenesis. The composition of medium and the time are different from cultivar to cultivar. Different media have been suggested by Al-Khayri et al. (2001), Veramendi et al. (1996), Bajaj (1992).

Several problems are still associated with the micropropagation of the date palm; probably the most important being explant contamination, browning, and response of cultured explants asexual embryogenesis initiation. The slow-growing nature of the date palm adds an additional problem, and so far no method is known to accelerate its growth to allow fast screening of the tissue culture-derived plants (Bajaj, 1992).

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