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Somatic embryogenesis from shoot tip and immature inflorescence of *Phoenix dactylifera* cv. Barhee

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Summary. A method of clonal propagation *via* somatic embryogenesis of date palm, cultivar Barhee, which has potential for large scale commercial application as well as for developmental studies on embryos is described. Cultures were initiated from shoot tip and immature inflorescence explants, both of which were capable of development into embryogenic callus. When the embryogenic callus was cultured in liquid suspension on a rotary shaker, hundreds of embryos developed from milligram quantities of callus in a fairly synchronous manner. Scanning electron microscopy showed globular, heart-shaped and torpedo-shaped embryos. Green leaves emerged from a white cotyledonary sheath.

Key words: *Phoenix dactylifera* - Somatic embryogenesis

Introduction

Phoenix dactylifera cv. Barhee is an elite variety of date palm, clonal propagation of which is much desired in the date palm growing regions of the world. The conventional method of propagation by separating the vegetative offshoots is slow and yields only a few offshoots from a parent plant. Induction of vegetative bud development from shoot tip or axillary bud explants in culture is another method of propagation (Tisserat 1984; Bouguedoura et al. 1990); however, the number of buds produced per explant is again limited. In a species where clonal propagation is the norm rather than the exception, a more efficient method of cloning would be highly desirable. Somatic embryogenesis has been obtained from excised embryos (Ammar and Benbadis 1977; Reynolds and Murashige 1979) and somatic tissues of the cultivars 'Halawy' (Tisserat 1979) and 'Khadravi' (Dass et al. 1989; Kackar et al. 1989; Sharma et al. 1984). Plantlet production was not synchronous, however, in these methods. A more efficient method of propagation in the cultivar Barhee is described in this paper.

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Materials and Methods

Plant material. Offshoots of *Phoenix dactylifera* cv. Barhee, of approximately 3 to 4 ft. in height were obtained by surface freight from Indio, California. The mature leaves were removed acropetally using a saw and tapestry knife. The shoot tip consisting of the apical meristem, its surrounding leaves and the subtending meristematic tissue was removed with a clean knife and placed in an antioxidant solution made up of 150 mg citric acid and 100 mg ascorbic acid per liter of water (Reynolds and Murashige 1979). The inflorescence, if present in the axil of the offshoot, was removed with its protective sheath (spathe) intact and refrigerated at 4°C until used.

Tissue culture. Shoot tips, cut into 2 to 3 cm pieces for easy handling, were surface sterilized in 1% sodium hypochlorite solution containing 1 drop of Tween 20 per 100 ml. After three rinses in sterile water the pieces were transferred to sterile petri dishes and cut into pieces of approximately 5x5 mm each.

The inflorescence, after rinsing the spathe with 70% ethanol, was removed from the sheath and the spikelets were cut into 6 to 8 cm-

long pieces. They were stored in the antioxidant solution until sterilization with sodium hypochlorite solution.

Table 1. Composition of media used for culture

	1	2	3	4
MS SALTS +				
Na H_2PO_4;H_2O	170*	170	170	--
Adenine sulfate	40	40	40	--
Thiamine-HCl	0.4	0.4	0.4	0.25
Pyridoxine-HCl	--	--	--	0.25
Nicotinic acid	--	--	--	1.3
Ca-pantothenate	--	--	--	0.25
Biotin	--	--	--	0.004
Myoinositol	--	--	--	50
Glycine	--	--	--	7.7
2,4-D.	100	--	--	--
Isopentenyl adenine	1	--	--	--
Sucrose	30,000	30,000	30,000	40,000
Difco agar	8,000	8,000	--	--
Gelrite	--	--	--	1,500
Activated charcoal	3,000	3,000	--	--

*** In milligrams per liter**

The immature flower buds were separated from the rachilla. All explants were dipped in fresh 1% sodium hypochlorite solution before placing on callus induction medium (Table 1, medium 1) in 22 x 150 mm culture tubes. The cultures were maintained at room temperature either in the dark or light with a light intensity of 28 μE m^{-2} s^{-1} with a photoperiod of 16 hrs. After 3 to 4 months , the explants were transferred to fresh medium 1 and incubated for another 2 to 3 months under the same culture conditions as before.

At this time the cultures were visually selected and explants that had turned tan, contaminated or ceased growth were discarded. Those that were white in color, had increased in size and showed indications of callus formation were selected for further subculture. These tissues were transferred to medium 2 (Table 1) in culture tubes, incubated under the same conditions as before and subcultured to fresh medium at 2 to 3 month intervals until a white friable callus formed (Fig. 1).

Two hundred milligrams of this friable callus were transferred aseptically to 25 ml of medium 3 (Table 1) in 50 ml culture flasks. The flasks were incubated for one month on rotary shakers at 150 rpm at room temperature and a light intensity of 28 μE m^{-2} s^{-1}, provided by Sylvania Grolux fluorescent lamps with a photoperiod of 16 hrs. After one month, 2 ml portions of the suspension were transferred to 25 ml of fresh medium 3 in 50 ml culture flasks. Incubation at 150 rpm was continued for another 3 weeks.

The medium was then decanted off and the embryos were placed on medium 2 in Falcon Petri dishes. Cultures were incubated for one month under the same light, temperature and photoperiod as mentioned earlier, or in the dark.

Plantlets that developed on this medium were transferred to medium 4 in individual culture tubes and placed in the light for leaf expansion and root elongation. Plantlets then were transferred to vermiculite when 3 to 4 leaves had emerged from the cotyledonary sheath and the roots had elongated.

Each plantlet was washed in running tap water to remove adhering agar, sprayed with 0.5% aqueous benomyl solution and planted in sterile vermiculite moistened with one-half strength Hoagland's solution. The shoot portion of the plantlets was kept covered with beakers for a few days to maintain humidity, and the vermiculite was moistened sparingly with half-strength Hoagland's solution, until the plants were well established.

Histology. The friable callus on medium 2 was fixed in 4% paraformaldehyde and 0.2% glutaraldehyde in 25 mM phosphate buffer, pH 7.2; post-fixed in 1% osmium tetroxide and embedded in araldite 502-EMBED 812 embedding medium (Mollenhauer 1964). Sections of 1 μm thickness were cut on a Reichert Jung ultramicrotome and stained with 1% toluidine blue for light microscopy.

Scanning electron microscopy. The friable callus and the embryos from suspension culture were fixed in 3% glutaraldehyde in100 mM phosphate buffer, pH 7.3 at 4°C, dehydrated in alcohol, critical point dried and mounted on aluminium stubs coated with a layer of gold approximately 20 nm thick in a cold sputtering system. Electron micrographs were taken with a JEOL TSM-25 S11 scanning electron microscope.

Results and Discussion

Callus induction in this cultivar was extremely slow. Formation of callus from the apical meristem required a minimum of 6 months. The tender leaf bases surrounding the meristem enlarged soon after culture initiation but these tissues did not form embryogenic callus. Immature floral buds showed various responses. Some enlarged in size and produced root-like structures from the carpels. Others turned tan and died. But several of them enlarged and formed callus from the carpels which subsequently produced embryogenic callus indistinguishable from the embryogenic callus from the shoot tip. The response of cultures in the light was not significantly different from those in the dark

in terms of callus development. Embryogenic callus was white, had a granular appearance (Fig. 1) and was friable. Histological examination of this callus revealed several globular proembryonic masses (Fig. 2). A core of actively dividing cells was present in the callus which seemed to be capable of forming more proembryonic clusters as indicated by its ability to proliferate in culture. Some cultures were maintained for more than three years without losing their embryogenic potential.

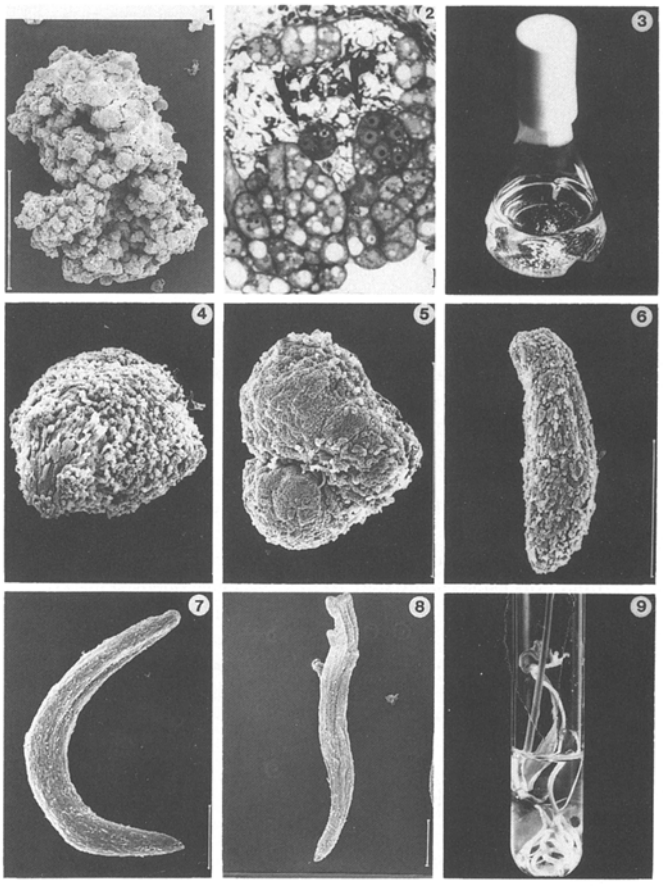

Fig.1. Scanning electron micrograph (SEM) of friable embryogenic callus. Bar=1 mm.
Fig.2. Light microscopy of embryogenic callus showing multicellular proembryonic masses (arrows). Bar=10 μm.
Fig.3. Embryogenic callus in suspension
Figs. 4-7. SEMs of developing somatic embryos. Bar=1 mm.
Fig.8. SEM of an embryo with distinct root-shoot axis. Bar=1 mm.
Fig.9. Plantlet

Upon transfer of the friable callus to suspension culture (Fig. 3), the proembryonic masses developed into embryos after passing through several distinct stages of embryo development as shown in Fig. 4-8. Within 3 weeks, hundreds of embryos could be obtained from the suspension culture. These embryos had to be subcultured after one month to promote further growth. Approximately 1000 embryos could be obtained from 200 mg embryogenic callus cultured per flask. When plated on solid medium, 40% of these embryos developed into normal plantlets (Fig. 9) with a leaf emerging through a white cotyledonary sheath and a long wiry root from the opposite end. These could be transferred to soil. The remaining embryos either aborted or turned out to be plants with only a shoot or root, but not both.

The heart- and torpedo-shaped embryos observed were not analogous to similar structures in the somatic embryogenesis of dicotyledonous plants. The significance of these stages in somatic embryogenesis in date palm is not clear. The formation of embryos of distinct developmental stages will lend this method suitable for studies on developmental embryogenesis or for encapsulation of embryos for storage or shipment.

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