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Propagation of Date Palms by Shoot Tip Cultures

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Abstract. **Shoot tips, 0.5 mm in length, of date palm (*Phoenix dactylifera* L.) were established on Murashige and Skoog (MS) inorganic salts and (per liter) 30 g sucrose, 0.4 mg thiamine · HCl, 100 mg myo-inositol, 10 mg naphthaleneacetic acid (NAA), 3 g charcoal, and 8 g agar. After 8 weeks, tips were proliferated through axillary bud outgrowths on a liquid medium devoid of charcoal containing 0.1 mg/liter NAA and 10 mg/liter 6-benzylamino purine (BA). These additional shoots then could be rooted by réculture to an agar medium devoid of BA containing 0.1 mg/liter NAA and successfully transferred to soil.**

Date palms are propagated commercially by rooting the limited number of basal offshoots produced by desirable trees. Micropropagation of date palms through tissue culture techniques would offset slow growth rates and limited vegetative propagation potential and would provide large numbers of desirable clones on demand. Palm tissue culture techniques currently have been restricted to the production of asexual plantlets via callus (1, 2, 4). This paper describes the production of free-living date palms through lateral bud multiplication *in vitro* using seedling shoot tips as the explant source. Commercial propagation of date palms would be performed using offshoots obtained from choice trees, necessitating modification of the techniques presented.

Shoot tips were dissected from 2-year-old seedlings grown in a 1 peat : 1 vermiculite mixture (v/v) in a greenhouse. The foliage and adventitious roots were decapitated, followed by acropetal removal of outward leaves to obtain a shoot tip which was 5 mm in length and about 3 mm in diameter. Tips were surface-sterilized in 2.63% NaOCl for 15 min. Additional leaves were removed and the apical meristem region with 3–5 leaf primordia was planted on nutrient medium. The basal medium consisted of MS salts (3) plus (per liter), 30 g sucrose, 100 mg myo-inositol, 0.4 mg thiamine · HCl, and 8 g Phytagar at pH 5.7. Twenty-five ml were distributed per 25 × 150 mm culture tubes and these were capped with enclosures (Bellco Kaputs). The medium was autoclaved at 1.1 kg/cm^2 and 121°C for 15 min. Explants and subsequent cultures were maintained at a constant 28° under a 16-hr photoperiod at 1.1 klx. Ten to 20 replicates were employed in each treatment. Cultures were transferred to fresh medium every 8 weeks. Liquid medium was employed in some cases. Cultures were agitated continuously at 70 rpm on a gyrotory shaker in 120-ml, polypropylene specimen containers.

Shoot establishment and proliferation. Additions of BA, indoleacetic acid, NAA, N^6-(Δ-isopentyl)adenine, 6-furfurylaminopurine, and 2,4-dichlorophenoxyacetic acid at 0, 0.1, 1.0, 10, and 100 mg/liter levels to basal medium supplemented with 0.3% charcoal were tested on excised shoot tips in preliminary work. Initial contamination rates varied from 20–40% after the first culture period. High auxin levels stimulated callus growth; low auxin levels aided in shoot differentiation. Addition of cytokinins at any level did not enhance shoot differentiation. Medium containing 10 mg/liter NAA only was found to be satisfactory for shoot tip establishment in culture based on culture survival and length of shoot growth. About 5–10% of the shoot tip cultures grown in this medium produced axillary bud outgrowths. Axillary bud outgrowths from tips cultured in other treatments were not observed. Adventitious root formation from tips was found to occur intermittently when tips were cultured on medium containing NAA; tips cultured in other treatments did not produce roots after 8 weeks in culture.

Table 1. Influence of NAA and BA on axillary budding after 16 weeks from shoot tip date palm explants and after 32 weeks from tip explants which initially were cultured 8 weeks on medium containing 0.3% charcoal and 10 mg/liter NAA and then were recultured to treatment medium.

Concn (mg/liter)		Axillary budding[z]	No. buds ± SE/
NAA	BA	(%)	culture
	16 weeks		
0.1	0	12.5	1.1 ± 0.6
	0.1	0	1.0 ± 0
	1.0	22.2	1.2 ± 0.8
	10.0	22.2	1.4 ± 0.9
1.0	0	12.5	1.2 ± 0.8
	0.1	0	1.0 ± 0
	1.0	20	1.2 ± 0.7
	10.0	0	1.0 ± 0
	32 weeks		
0.1			1.4 ± 0.8
	10.0 in agar		3.5 ± 2.4
	10.0 in liquid	20	
		27.3	

[z]10 to 15 replicates originally employed per treatment.

Various combinations and concentrations of NAA (0, 0.1, 1.0, and 10 mg/liter) and BA (0, 0.1, 1.0, and 10 mg/liter) were added to basal medium to test their effect on enhancing shoot proliferation from excised shoot tip explants. Shoot tips exhibited limited axillary bud formation on a wide variety of media (e.g., 0.1 mg/liter NAA with 1.0 or 10 mg/liter BA) (Table 1). Axillary budding was not observed from cultures on media containing 0 or 10 mg/liter NAA regardless of the BA levels. After 2 recultures (4 months), shoots usually grew too large to be recultured in the 25 × 150 mm culture tubes. Adventitious roots commonly developed in several cultures when cultured on media containing 0.1 or 1.0 mg/liter NAA with 0.0–1.0 mg/liter BA. Transfer of 15 established shoots obtained from explants initially cultured on medium containing charcoal and 10 mg/liter NAA to liquid or agar basal medium with 0.1 mg/liter NAA and 10 mg/liter BA in 120-ml specimen containers allowed for further shoot differentiation and proliferation. Motion in the liquid medium often fragmented the cultures; leaves in these cultures became succulent and enlarged considerably compared to those incubated on agar medium. Axillary shoots grew out from cultured shoots in this environment more frequently than from shoots cultured on agar medium (Table 1; Fig. 1).

Rooting and transplanting shoots to soil. Adventitious rooting was obtained readily after reculturing separated shoots to an agar nutrient medium containing 0.1 mg/liter NAA without charcoal, following 8–16

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Fig. 1. Axillary shoot proliferation from date palm shoot tip cultures on liquid medium containing 0.1 mg/liter NAA and 10 mg/liter BA. Culture is 32 weeks old.

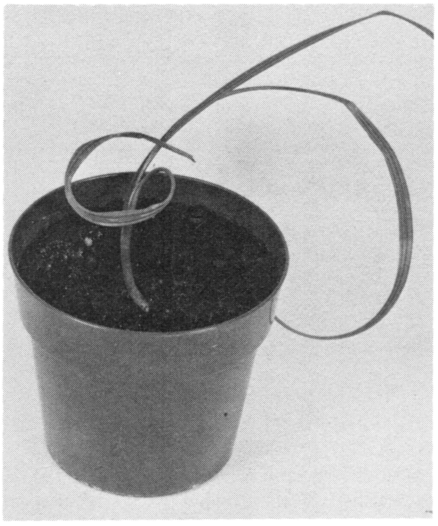

Fig. 2. Example of free-living, shoot-tip-derived plantlet. Plantlet is about 4 months old. Pot is 7 cm in height.

weeks in culture. Plantlets were rinsed in distilled water to remove residual sucrose and transplanted to pots containing 1 peat : 1 vermiculite mixture (v/v) in a shaded greenhouse. As with asexual palms derived from callus, the size of the plantlet was found to be the most important parameter for successful transplanting to soil (4) (Fig. 2). Plantlets that were 10–15 cm in length with 2–3 leaves and possessed a well-developed adventitious root system could invariably be transplanted successfully to soil with survival rates close to 100%.

Much variation was observed among shoot tip cultures for each treatment, reflecting the genetically heterozygous nature of date palm seedlings. Up to 20 shoots currently have been obtained from a single shoot culture after 6 months in culture; however, not all cultured shoots showed such proliferation *in vitro*. Most shoots did not produce additional shoots even after 6 months in culture although they developed into robust plantlets. Further refinement of this technique is necessary to maximize offshoot production *in vitro* so that a system for commercial application can be developed.

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Cultivation of Banana using Plantlets from Meristem Culture

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Abstract. **Plantlets orginating from adventitious buds of explants obtained from the decapitated shoot apex of a banana sucker established well under field conditions and gave rise to mature plants with uniform growth and normal yield of fruit. A total of one million pathogen-free plantlets for commercial planting was produced in 1983. Introduction of plantlets for commercial planting prevents the spreading of fusarial wilt of banana by planting materials to the disease-free orchards.**

A new race (race 4) of *Fusarium oxysporum* Schl. f. sp. *cubense* (E.F. Smith) Synder & Hansen, capable of causing wilt of Cavendish varieties of banana (*Musa sapientum* L.), appeared in the Southern part of Taiwan in 1967 (4). Since then it has become the most devastating disease of banana affecting more than 300 hectares of orchards annually in recent years. Seed corms obtained from wilt-infested areas are important sources of inoculum (3, 4). To prevent spreading of the disease to a disease-free orchard, it is necessary to obtain seed corms for planting from wilt-free areas. This is becoming increasingly difficult because of the widespread nature of the disease. A meristem culture technique was, therefore, developed for mass propagation of the pathogen-free banana plantlets for commercial planting. We report here the procedures of this technique and the performance of banana plants in the field using plantlets from meristem culture as planting material.

The method of Ma and Shii (1) was employed for inducing the formation of adventitious buds from the explant obtained from the decapitated shoot apex of sucker. About 5–10 adventitious buds proliferated from each explant after incubation for 6–8 weeks at 25°C (Fig. 1A). The medium used was that of Smith and Murashige (2) which contains Murashige and Skoog salt mixture plus (per liter) 0.4 mg thiamine-HCl, 100 mg L-tyrosine, 100 mg myo-inositol, 2 mg indole-3-acetic acid, 2 mg kinetin, 160 mg adenine sulphate, 30 g sucrose, and 8 g Difco Bacto agar. The pH of the medium was adjusted with 2N KOH to 5.8 before autoclaving. Repetitive dissection of the aggregated adventitious buds was made for increasing the bud population. The tissue with buds was cut longitudinally into smaller pieces, each consisting of 1–2 buds, and placed on the above medium. This sequence of grow-divide-grow was continued until a sufficient population of buds was obtained. It is estimated that the

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