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CHAPTER 10

Clonal Propagation: Palms

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I. INTRODUCTION

The Arecaceae (Palmae) is a family of woody perennial monocots and consists of about 200 genera and 2500 species (Corner, 1966; McCurrach, 1960; Tomlinson, 1961). Palms are common to the tropical regions of the world and occur to a lesser extent in the subtropical regions (e.g., *Phoenix dactylifera* L., date palm). Palms have had numerous agricultural and economic uses since the dawn of history and constitute the second most important plant family to humans aside from the Gramineae. The importance of the palm as a multiuse plant employed in subsistence agriculture has diminished in the last 200 years. Today, palm crops provide edible and nonedible commodities, often produced through large-scale plantation systems, that are consumed both domestically and internationally.

Improvement in palm crops has traditionally been slow due to their long-lived nature, growth habit, habitat, and absence in many species of an adequate vegetative propagation method. Many species, such as the coconut palm (*Cocos nucifera* L.) and oil palm (*Elaeis guineensis* Jacq.), can be propagated only by seed. Progeny resulting from seed usually exhibit heterozygosity. Also, propagation of palms through suckering, branching, or teratological events is limited.

Micropropagation offers an alternative for the rapid clonal production of palms. Since the 1970s, several reports on palm tissue culture have appeared in the literature (Ammar and Benbadis, 1977; Corley *et al.*, 1977; de Guzman *et al.*, 1979; Eeuwens and Blake, 1977; Poulain *et al.*, 1979; Rabéchault *et al.*, 1970; Reuveni, 1979; Reynolds and Murashige, 1979; Tisserat, 1979, 1981). Free-living palms have been obtained from embryogenic callus in oil (Corley *et al.*, 1977) and date palms (Tisserat, 1979, 1981). Date palm plantlets derived from rooted shoot tips and lateral buds have also been transferred to soil (Poulain *et al.*, 1979; Tisserat, 1981). The following information is based on research by the author since 1977 on rapid propagation of date palms.

II. MATERIALS AND METHODS

Methods and procedures used to obtain clonal and zygotic tissue cultures from adult, offshoot, and seedling date palms have been previously described (Tisserat, 1979, 1981). A detailed step-by-step procedure is offered to outline the protocol employed to obtain plantlets from a variety of palm explant sources. Nutrient media employed to establish and maintain palm cultures and plantlets *in vitro* are summarized in Table I.

A. Procedure Used to Obtain Plantlets from Shoot Tip and/or Lateral Bud Callus

1. Dissect offshoots, seedlings, or trees, using a hatchet or serrated knife when appropriate. Remove leaves acropetally, exposing the lateral buds at the axil of each leaf. Shoot tips are removed from the shoot terminal after all mature leaves are peeled away. Store buds and tips in cold antioxidant solution (150 mg/liter citric acid and 100 mg/liter absorbic acid). Keep explants in the refrigerator at 0°C until the surface sterilization procedure is performed.
2. Trim the outermost leaves of the buds and tips to obtain explants that are 0.5 cm^2.
3. Sterilize the explants by wrapping them in cheesecloth to prevent loss during handling procedures in a 25 × 150-mm culture tube. Sterilize them

TABLE I

Palm Tissue Culture Media

Components	Media types (mg/liter)				
	Callus production	Plantlet germination	Shoot tip	Shoot proliferation	Adventitious rooting
Inorganic salts					
Murashige and Skoog (MS) (1962)	+	+	+	+	+
Carbohydrate source					
Sucrose	30,000	30,000	30,000	30,000	30,000
Vitamin sources					
Meso-inositol dihydrate	100	100	100	100	100
Thiamine·HCl	0.4	0.4	0.4	0.4	0.4
Complex addenda					
Phytagar	8,000	8,000	8,000		8,000
Charcoal, activated neutralized	3,000	3,000	3,000		
Phytohormones					
2,4-Dichlorophenoxyacetic acid (2,4-D)	100		10		
N^6-(Δ^2-isopentyl)adenine (2iP)	3			10	
α-Naphthaleneacetic acid (NAA)				0.1	0.1

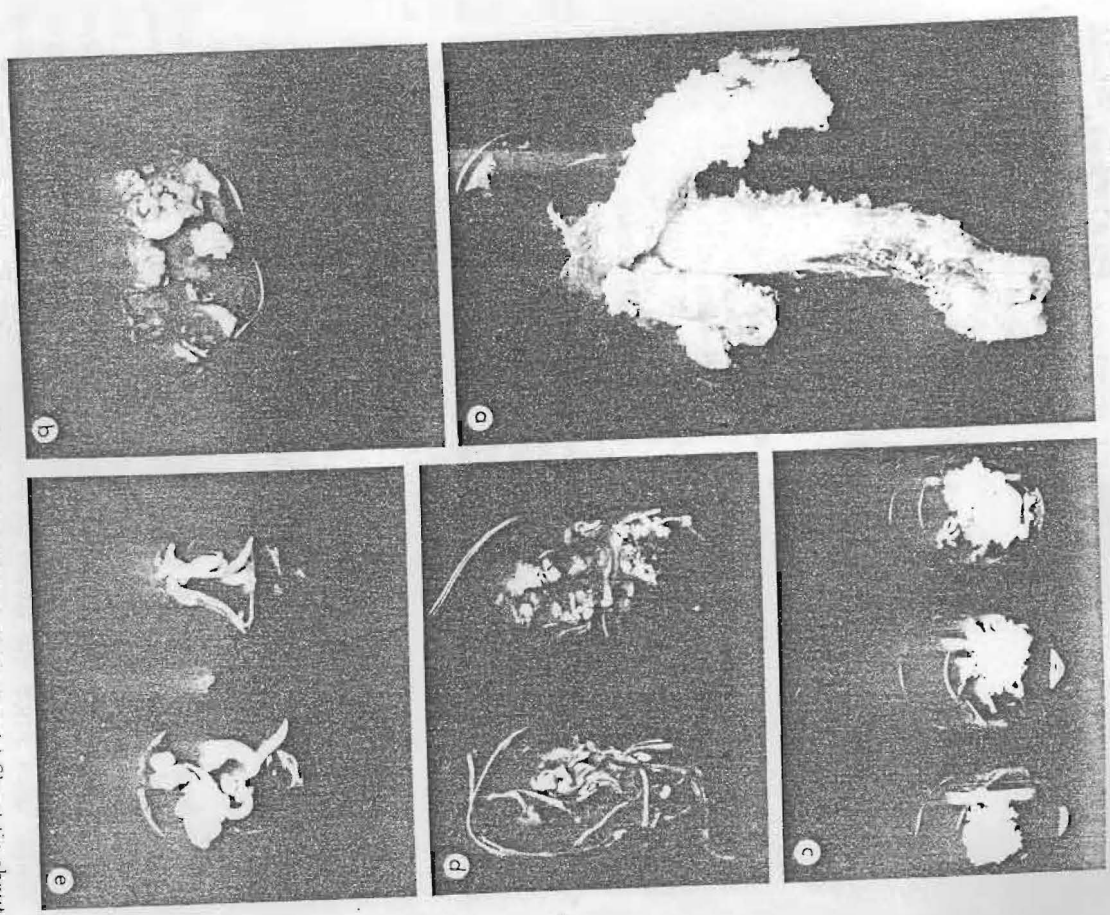

Fig. 1. Asexual embryogenesis in date palms from cultured shoot tips. (a) Shoot tip about 4 weeks old cultured on a medium containing 100 mg/liter 2,4-dichlorophenoxyacetic acid (2,4-D), 3 mg/liter N^6(Δ^2-isopentyl)adenine (2iP), and 0.3% charcoal. (b) Initiation of nodular callus from leaves. (c) Friable callus producing asexual embryos on media devoid of hormones. (d) Production of asexual embryos and plantlets from callus. (e) Recultured isolated plantlets.

in 2.6% sodium hypochlorite solution (containing 1 drop of Tween-20 per 100 ml solution) for 15 min. Dislodge air bubbles from the tissues by periodic agitation of the tube. Pour off the bleach solution and rinse three times with sterile water. Remove the explants and transfer them aseptically to the sterile Petri dish (15 × 150 mm in diameter).

4. Remove additional leaves from the shoot tip and bud explants to obtain a culture that is 1–3 mm^2 in size. A 10-sec dip of this explant into bleach solution may reduce contaminates prior to planting.

5. Plant the explant on the surface of callus production agar medium, as described in Table I.

6. Reculture the explants at 8-week intervals. Callus initiation is evident after two to three culture passages (Fig. 1a,b).

7. When white, friable, nodular callus is prominent, subculture 1-cm^2 pieces to plantlet germination media (Table I) and incubate the cultures at 28°C under a 16-hr photoperiod of 50 foot-candle (fc) intensity. Asexual embryos and green plantlets will usually become apparent within 2–4 weeks in culture (Fig. 1b–e).

8. To enhance adventitious root formation, reculture the seedling with the primary root trimmed to 1–2 cm in length to adventitious rooting medium (Table I). Continue the reculturing procedure every 8 weeks for one to three culture passages until the plantlets reach a length of 10 cm, with two to three leaves, and possesses an adventitious root system (Fig. 2a).

9. Transfer of plantlets to free-living conditions: Plantlets are carefully removed from the agar medium without damage to the root system, and are soaked in distilled water for 15 min to avoid dehydration and remove excess adhering media. The plantlets are then rinsed three times with distilled water, sprayed with 0.5% benolate (du Pont Co., Wilmington, Delaware) fungicide solution, and transferred to a soil medium. The soil medium consists of sterile peat moss and vermiculite in a 1:1 (v/v) ratio. Plantlets are planted in either 3-in.-diameter plastic pots or jiffy peat pots and enclosed within a transparent tent composed of two interlocking clear polystyrene tumblers.

10. Administer weekly applications of 0.5% benolate to the foliage to minimize fungal growth. Water the pots every day with distilled water and once a week with one-fourth strength Hoagland's solution during the first 2 months of development. Incubate the plants initially in an environmentally controlled chamber under 800 fc light intensity, 16-hr photoperiod at 28°C for 2 weeks. Transfer the plants to a shaded greenhouse. Gradually acclimate the plantlets to the greenhouse humidity conditions by punching holes in the plastic cover. After 2 months, the covers may be removed and the plant treated as a normal palm seedling (Fig. 2b).

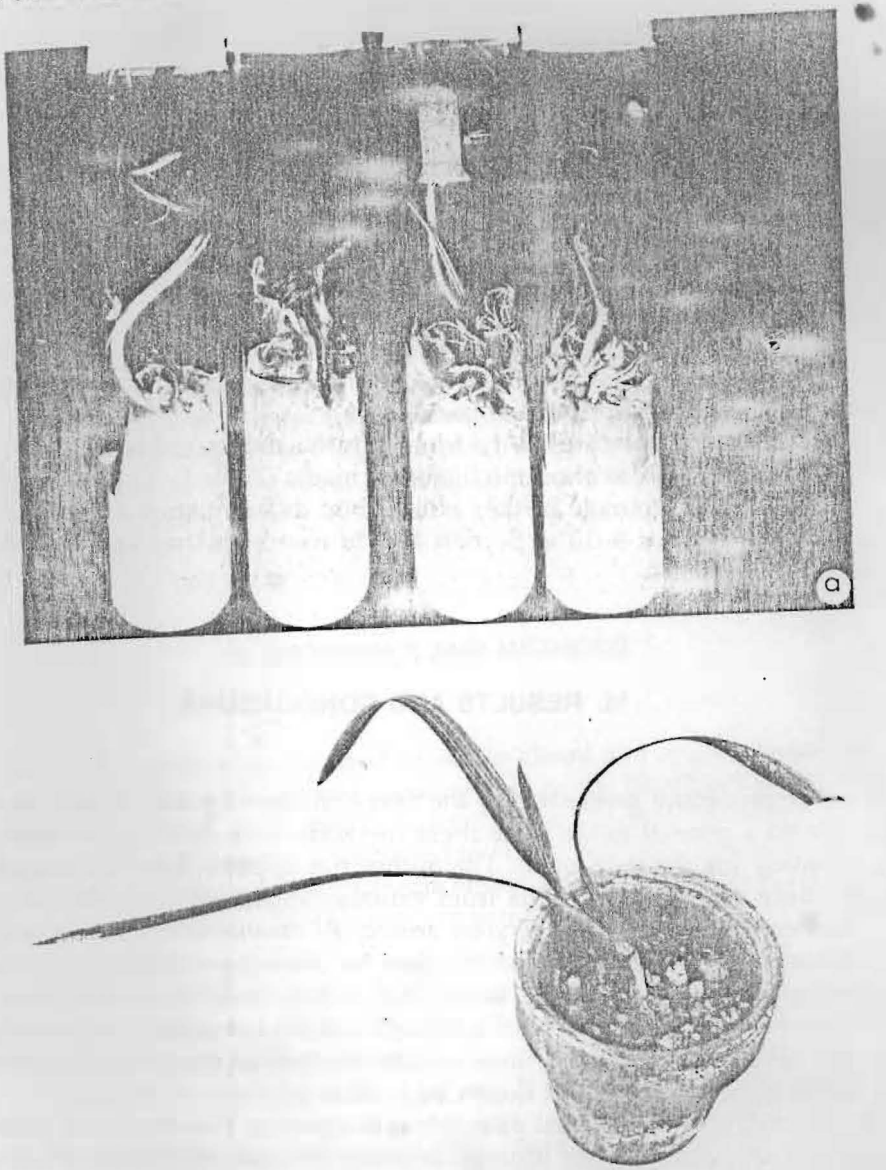

Fig. 2. Plantlets obtained from callus. (a) Plantlets producing adventitious roots in media containing 0.1 mg/liter α-naphthaleneacetic acid (NAA). Note that the second and fourth cultures from the left are also producing axillary bud outgrowths. (b) Free-living date palm plantlet.

B. Procedure Used to Propagate Palms by Rooting Shoot Tips

1. Repeat the protocol for excision and planting of shoot tips and/or lateral bud explants as described in steps 1–4 in Section II,A.
2. Plant the explants on the surface of shoot tip media as described in Table I.
3. Incubate the cultures under 50 fc intensity of a 16-hr photoperiod at 28°C in an environmental chamber.
4. The cultures will initiate leaves and increase considerably in size within the next 4–6 weeks in culture. Reculture the explant to fresh medium at the end of the 8-week culture passage.
5. Axillary shoot outgrowths from cultured shoots and buds can be obtained by transfer to shoot proliferation media (Table I). Divide the shoot structures to encourage further axillary bud differentiation as desired.
6. Follow steps 8–10 in Section II,A to root buds and tips and obtain free-living palms.

III. RESULTS AND CONCLUSIONS

The procedure presented for the tissue culture of palms should be considered a general guide since these methods were developed almost exclusively for the date palm. The author has applied these techniques to produce embryogenic callus from various explants of a number of palm species (*Phoenix reclinata, Erythea armata, P. canariensis, P. pusilla,* and *P. sylvestris*). The tissue of choice in palms for obtaining embryogenic callus is either the actively growing lateral bud (when available) or the shoot tip (Tisserat, 1981). Older tissues and organs do not respond well in culture, and callus produced from them usually has limited morphogenetic potential (e.g., mature leaf and flower bud callus produces roots only).

Browning of the original palm tissue is common. However, this problem can be minimized either through inclusion of adsorbents such as charcoal or frequent reculturing of small-size explants.

Each piece of embryogenic date palm callus contains thousands of proembryos. Subdivision of this callus to media devoid of hormones will yield hundreds of visible asexual embryos and plantlets within a few weeks (Tisserat, 1981). Successful transfer of plantlets to soil depends on potting plantlets with adequate photosynthetic shoot and adventitious root systems. Production of plantlets from offshoots initiated on cultured shoot

tips and buds is a less well-developed technique compared to the asexual embryogenesis process. The parameters involved in maximizing offshoot initiation *in vitro* still remain to be explored. However, plantlets produced by the organogenesis process should be clonal and produced with less risk of genetic aberrance than callus-derived plantlets (Tisserat, 1981).

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