Notes on Embryo Culture of Palms

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Embryo culture involves excising an embryo aseptically from the seed and removing it to a sterile nutrient medium for germination. An excellent review of all aspects of general plant embryo culture is covered by Narayanaswami and Norstog (1964). Embryo culture has proved useful for several reasons. Firstly, embryos that would abort if left to develop naturally in the fruit, or embryos resulting from interspecific hybridization where defective endosperms are common, may sometimes be excised at an early stage of development and cultured successfully (Hartman and Kester, 1968). Secondly, embryo culture may be used to circumvent lengthy germination due to physical and/or chemical inhibitors in the fruit and/or seed. Excised embryos are usually freed from these inhibitors and begin immediate growth. Thirdly, green pod culture of orchids is done exclusively by embryo culture (Hartmann and Kester, 1968).

Although much work has been done on embryo culture of other plants, little work has been done on the embryo culture of palms. Rabechault (1962) studied the effects of indoleacetic acid on *in vitro* cultures of *Elaeis guineensis* embryos, while Abraham and Thomas (1962) and Cutter and Wilson (1954) cultured *Cocos nucifera* embryos. Guzman and Rosario (1964) cultured embryos of the 'Makapuno' cultivar of coconut and Balaga and Guzman (1970) and Guzman, Rosario, and Eusebio (1970) investigated root and shoot development of the 'Makapuno' cultivar in response to varying compositions of rooting media. Embryo culture may prove valuable to palm enthusiasts for several reasons. Embryos resulting from interspecific or intergeneric hybridization in palms (as currently being undertaken by Merrill Wilcox at Gainesville, Florida) may be improperly nourished due to a defective endosperm. Perhaps this problem can be overcome using embryo culture. Some palms, such as *Arenga engleri*, *Orbignya* spp., and *Attalea* spp., are notorious for slow germination due to physical inhibitors in the fruit, e.g., a thick endocarp, or chemical inhibitors in the seed. Perhaps if the embryos were excised and removed from these inhibitors they would germinate readily. Also, collectors of palm seed, especially of rare species, may find embryo culture of benefit. Often collectors are stymied by lack of ripe fruit but perhaps it may be worthwhile to collect unripe fruit. Although difficult, it may be possible to culture immature embryos, as these have been cultured successfully in other plants (Maheshwari 1962, 1963; Narayanaswami and Norstog 1964).

The impetus for my own study comes from a desire to devise a method for rapid multiplication of common palms. Embryo culture might lead to rapid multiplication if multiple adventitious shoots or embryos could be stimulated to form from one embryo. Formation of multiple adventitious shoots is common in green pod culture of orchids and has been observed in other plants by Maheshwari (1962, 1963) and Narayanaswami and Norstog (1964). Other workers have attempted to devise methods for rapid multiplication of palms by vegetative means. Davis (1969) suggested that coconuts be prop-

agated by artificially splitting the growing point, by inducing the inflorescences to revert to vegetative shoots, or by inducing formation of bulbil-shoots on inflorescences. Success by such methods is rare and unpredictable.

Methods

Embryos of *Pritchardia kaalae* Rock and *Veitchia joannis* H. Wendl. were utilized. Embryos were aseptically excised and placed on a sterile, modified Vacin and Went medium for orchids (see Table 1). For one who lacks access to a laboratory, any of the commercial formulae available from orchid firms will suffice. The following procedures were then followed:

1. Fruit and instruments were cleansed in 95% ethyl alcohol.
2. Mesocarp and endocarp were removed using ordinary horticultural clippers.
3. Endosperm with embedded embryo was immersed for 2 seconds in 95% ethyl alcohol.
4. Embryo was excised (using a scalpel dipped in 95% ethyl alcohol and flamed) into a 5% Clorox solution for 5 minutes and then to a 1% Clorox solution for 1 minute.
5. Embryo was placed in culture tubes on a sterile medium and placed under a 40-watt Gro-Lux lamp at 85°F. Contamination will show after 48 hours.

If one has mastered aseptic excision, the Clorox baths may be eliminated. Working under a hood and filtered-airflow apparatus is desirable but not essential, for I have excised embryos while simply sitting at a desk in a draft-free room.

Nutrient requirements for sterile media are similar to requirements of other plants. However, as more work is done on nutrient composition, species-specific media will be developed. I did not include coconut water in my modified Vacin and Went formula, for I wanted to eliminate as many unknowns as possible and to arrive at a relatively simple medium. Coconut water did not prove to be essential for growth of fully developed embryos.

Embryo age is important when considering nutrient requirements. The developing embryo becomes more and more able to germinate and its cultural requirements become less complex as it develops. Embryo culture at early stages of embryonic development is difficult. Ironically, these stages hold the most

Table 1. Components of a modified Vacin and Went medium.

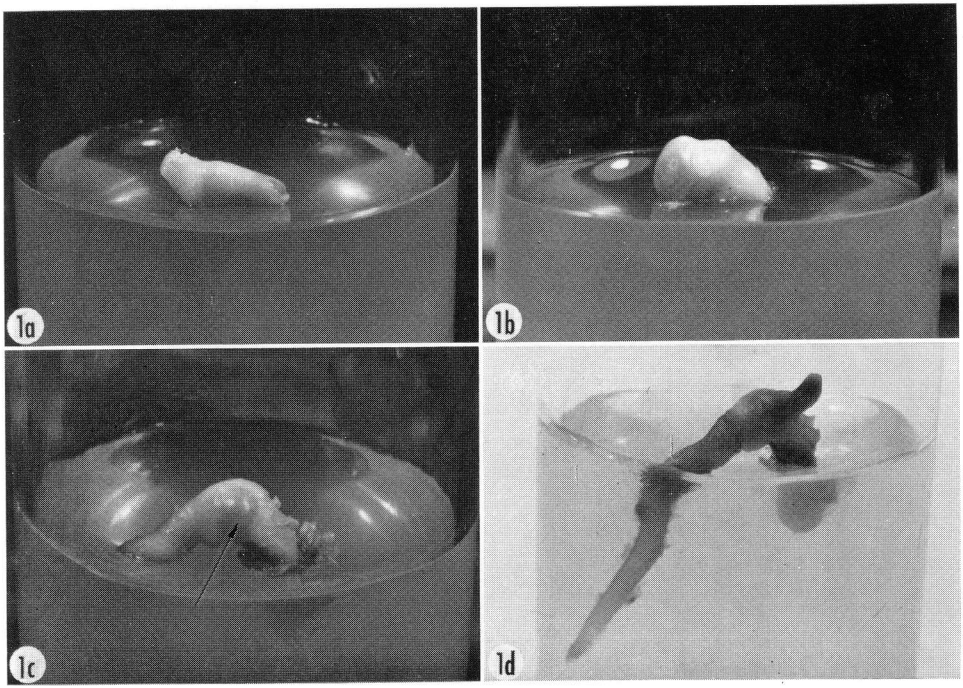
Components	Amount	
Tricalcium phosphate $Ca_3(PO_4)_2$	0.20	grams
Potassium nitrate KNO_3	0.525	grams
Monopotassium acid phosphate KH_2PO_4	0.25	grams
Magnesium sulfate $MgSO_4 \cdot 7H_2O$	0.25	grams
Ammonium sulfate $(NH_4)_2SO_4$	0.50	grams
Manganese sulfate $MnSO_4 \cdot H_2O$	0.0057	grams
Sucrose	20.00	grams
Agar	8.00	grams
EDTA (chelated iron)	5	ml/l
Water	850	ml
Coconut water (optional)	150	ml
Adjust pH to 5.8–6.0		

promise for formation of adventitious shoots. Attaining the correct nutrient balance of inorganic elements, sugars, vitamins, and organic complexes found in coconut water for culture of young embryos is difficult and may be species-specific. On the other hand, fully developed embryos need only inorganic elements and sugar, and as they grow, even the sugar may be eliminated.

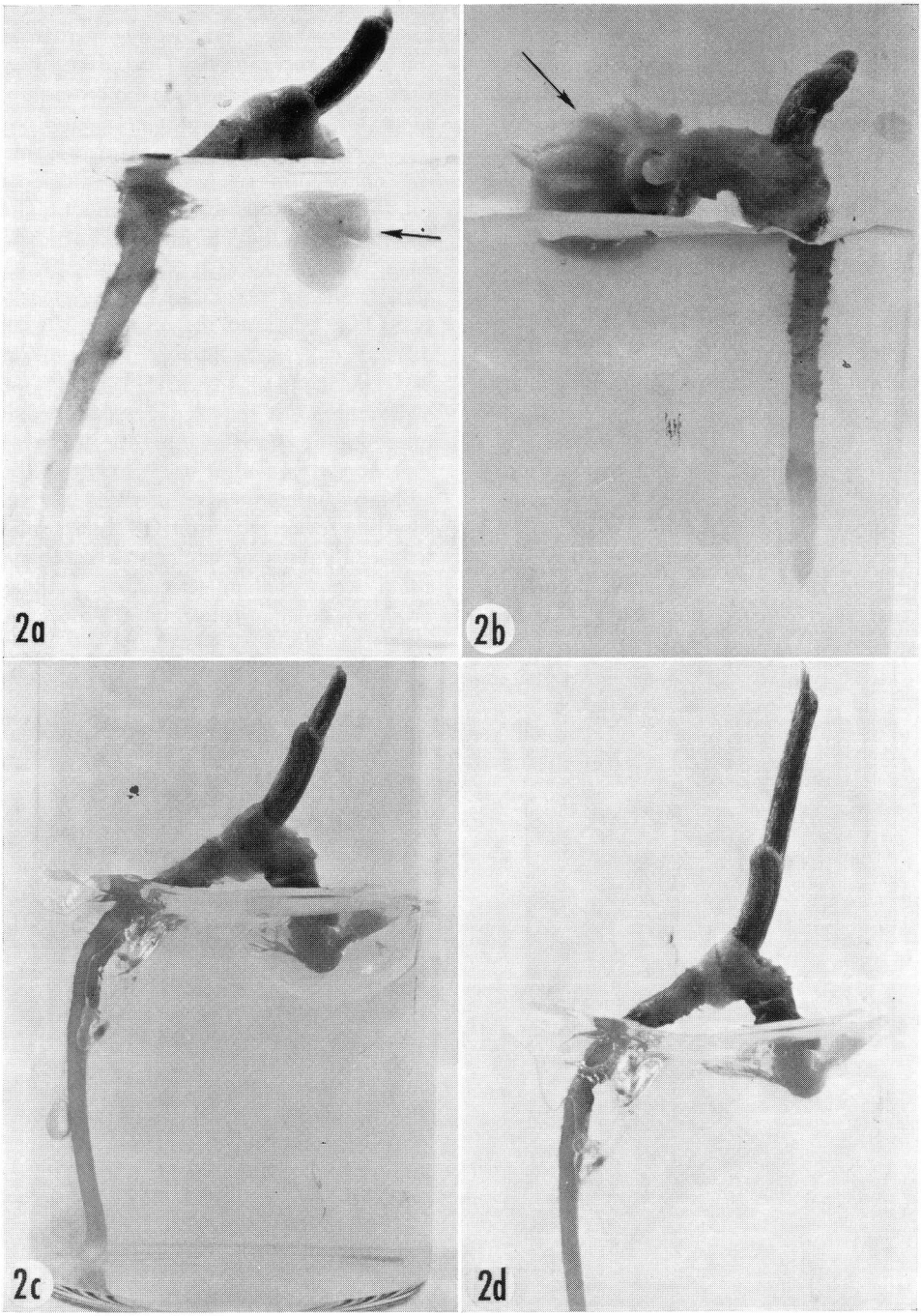
Observations

Figure 1a shows the embryo of *Pritchardia kaalae* after excision. Within 48 hours of excision, the embryo began to swell (Fig. 1b). This initial swelling is to exert the shoot and root primordium beyond the seed, enabling them to grow unhindered (Tomlinson 1961). Ten days after excision a well-developed root is visible as well as a green, domelike structure covering the shoot (arrow, Fig. 1c). Seventeen days after, the first green, sheathlike leaf has emerged from the domelike structure (Fig. 1d). Figures 2a and 2b (arrows) show development of the modified cotyledon or haustorium. This structure grows into the endosperm to assimilate nutrients for the developing root and shoot. The second sheathlike leaf appeared after 35 days (Fig. 2c). Fifty days after excision the third sheathlike leaf is emerging (Fig. 2d) and the seedling is well on the way to producing its first flat leaf.

Embryos of *Veitchia joannis* developed in the same manner as those of *Pritchardia kaalae*. Figure 3a shows the embryo immediately after excision. Swelling occurred after 48 hours and within 14 days of excision a root and shoot were evident (Fig. 3b). Figure 3c shows an emerging root and root cap.

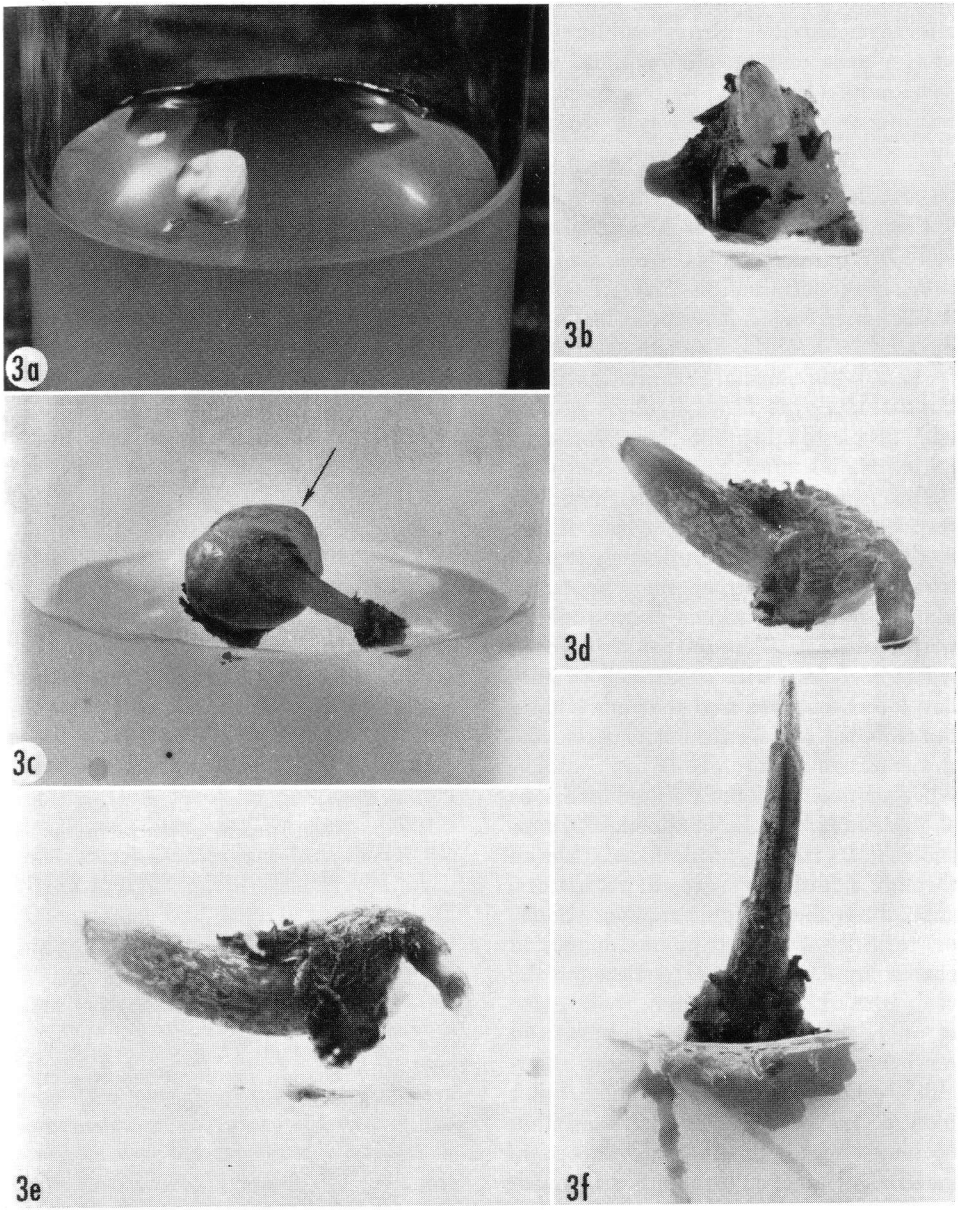


1. *Pritchardia kaalae*. a, embryo after excision; b, embryo 48 hours after excision; c, embryo 10 days after excision, showing root and area of future shoot (arrow); d, embryo 17 days after excision. Photos by Kheng Tuan Cheah.



2. *Pritchardia kaalae*. a,b, embryo showing haustorium (arrow); c, embryo 35 days after excision; d, embryo 50 days after excision. Photos by Kheng Tuan Cheah.

Notice the swelling on the upper right portion of the embryo (arrow). This is an area of future shoot growth. Figure 3d shows development 28 days after excision. Thirty-five days after excision the second sheathlike leaf has appeared (Fig. 3e). At this point I positioned the embryo so it was situated with its shoot in a vertical rather than horizontal position. At the same time I embedded



3. *Veitchia joannis*. a, embryo after excision; b, embryo 14 days after excision; c, embryo showing root, root cap, and area of future shoot (arrow); d, embryo 28 days after excision; e, embryo 35 days after excision; f, embryo 50 days after excision. Photos by Kheng Tuan Cheah.

the base of the embryo in the medium where before it was simply lying on the medium. This produced a marked increase in growth due to more root contact with the medium and subsequently more absorption of nutrients. Fifty days after excision the third sheathlike leaf was evident and secondary roots were developing (Fig. 3f). At this point the seedling was well on the way to the production of its first bifid leaf.

Conclusions

From preliminary results, I feel that embryo culture holds promise for shortening germination time. *In vitro* development of shoots took 14 to 21 days for *Pritchardia kaalae* and *Veitchia joannis*, while normal germination times are 30–45 days. More work is needed with species known for their slow germination.

It was found that cultures should be transflasked to fresh, sterile nutrient media every month until they attain true leaves. Then they may be transplanted into conventional potting mixes.

Other results showed that coconut water is not essential for growth of fully developed embryos and that embedding the embryo in the medium increased the growth rate.

Development of multiple adventitious shoots remains to be obtained. Perhaps successful results will be achieved through addition of growth regulators, subculture of embryonic roots or shoots, or refinement of techniques suggested earlier by Davis (1969). Perhaps the real breakthrough, though, will come through the use of tissue culture and meristem cloning as is common in orchids, chrysanthemums, and other plants. Staritsky (1970) showed that coconut and oil palms could be propagated by tissue culture but that much work is still needed.

Embryo culture can be interesting work for the palm enthusiast. It opens to view a phenomenon rarely seen, the actual development of a palm from embryo to seedling.

LITERATURE CITED

ABRAHAM, A. AND K. J. THOMAS. 1962. A note on the *in vitro* culture of excised coconut embryos. Indian Coconut J. 15: 84–88.

BALAGA, H. Y. AND E. V. DE GUZMAN. 1970. The growth and development of coconut "Makapuno" embryos *in vitro*. II. Increased root incidence and growth in response to media composition and to sequential culture from liquid to solid medium. Philipp. Agric. 53: 551–565.

CUTTER, V. AND K. WILSON. 1954. Effect of coconut endosperm and other growth stimulants upon the development *in vitro* of embryos of *Cocos nucifera*. Bot. Gaz. 115: 234–240.

DAVIS, T. A. 1969. Clonal propagation of the coconut. World Crops 21: 253–255.

GUZMAN, E. V. DE AND D. A. DEL ROSARIO. 1964. The growth and development of *Cocos nucifera* L. (Makapuno) embryos *in vitro*. Philipp. Agric. 48: 82–94.

GUZMAN, E. V. DE, A. G. DEL ROSARIO, AND E. C. EUSEBIO. 1970. The growth and development of coconut "Makapuno" embryo *in vitro*. III. Resumption of root growth in high sugar media. Philipp. Agric. 53: 566–579.

HARTMAN, H. AND D. KESTER. 1968. Plant propagation principles and practices. Prentice Hall, Inc. Englewood Cliffs, N.J.

MAHESHWARI, P. 1962. Plant embryology: a symposium. Council of Scientific and Industrial Research. Delhi, India.

———. 1963. Recent advances in the embryology of angiosperms. Int. Society of Plant Morphologists. University of Delhi, India.

NARAYANASWAMI, S. AND K. NORSTOG. 1964. Plant embryo culture. Bot. Rev. (Lancaster) 30: 587–628.

RABÉCHAULT, H. 1962. Recherches sur la culture "in vitro" des embryons de palmier à huile (*Elaeis guineensis* Jacq.) 1. Effets de l'acide β indolyl-acetique. Oléagineux 17: 757–764.

STARITSKY, G. 1970. Tissue culture of the oil palm (*Elaeis guineensis* Jacq.) as a tool for its vegetative propagation. Euphytica 19: 288–292.

TOMLINSON, P. B. 1961. Anatomy of the monocotyledons II. Palmae. Oxford University Press, London.