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A Branched Coconut Seedling in Tissue Culture[1]

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As a part of the research effort to combat lethal yellowing disease of palms, we have been working on methods for growing coconut (*Cocos nucifera* L.) seedlings in tissue culture (Fisher and Tsai, 1978). Our objective was to grow healthy germ-free coconut plants that could later be used for inoculation with the casual agent of lethal yellowing, presumably a mycoplasmalike organism. Such a germ-free system will be very useful in demonstrating whether a mycoplasmalike organism causes lethal yellowing and determining which insect, if any, transmits the disease.

While growing isolated coconut embryos in test tubes, we discovered that one out of approximately 238 embryos formed twin shoots as shown in the figure. This branched seedling indicates that one original growing point has divided into two separate growing points after five scale leaves were produced by the original embryonic axis. The two branches were unequal in size and number of leaves when the seedling became contaminated with mold after its transfer to fresh medium. Two genetically identical twin palm trees would have developed had this seedling continued to grow.

There have been reports of coconuts that branch above ground, usually as a result of injury (Davis, 1969; Ridley, 1907). In addition, the rare development of two or, even more rarely, three seedlings from one coconut has been observed. Such "twins" or "triplets" are usually derived from two or three separate seeds present in one nut (one fruit) instead of the usual one seed. Evidence for this is the fact that each seedling comes from a different "eye" of the coconut, indicating that two or three carpels of the female flower each produced an embryo. Each embryo forms its own separate endosperm (meat) as noted by Davis (1979; Fig. 5). Therefore, such "twins" or "triplets" are not genetically identical. They are equivalent to fraternal twins in man. On the other hand, polyembryony or the presence of more than one embryo per seed has been reported in palms. Such rare triplets contained within the endosperm of a single nut have been illustrated by Davis (1979; Fig. 5). They are presumably derived from a single fertilized egg cell and are, therefore, genetically identical.

The experimental induction of twin palm seedlings was attempted by Davis (1968) with limited success. He sliced the shoot tips of germinating coconut seeds with a razor and found that a small percentage formed two shoots, after recovering from the injury, similar to the branched embryo described here. It is possible that our branched embryo resulted from an injury unknowingly in-

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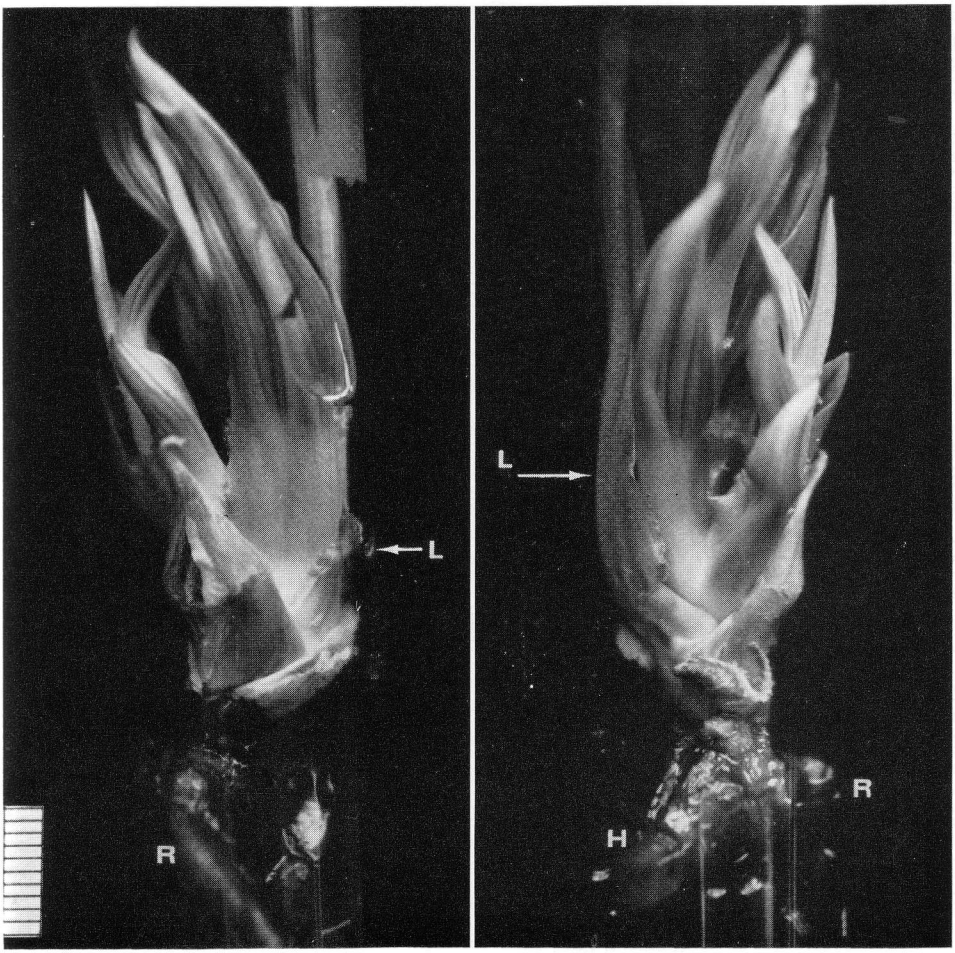

1. Branched coconut seedling growing on agar medium in test tube: two views, showing haustorium (H), root (R), and leaf immediately below the point of branching (L). Scale marked in millimeters.

flicted during the dissection of the embryo from the seed. If this is the case, then cutting the apex of the germ-free embryo might be a more efficient and successful method of inducing identical twin palms. Balaga (1975) carried out this procedure but with little successful induction of twin shoots. If refined, this method could be a new and significant tool for clonal propagation of coconuts and other palms.

An interesting side note is a recent observation made at Fairchild Tropical Garden. A newly germinated seed of *Rhyticocos amara* (Jacq.) Becc. (FTG #77-288) had three separate cotyledonary stalks arising from the same "eye." Each stalk bore a normal seedling apex. This indicates that either a single carpel had three separate embryos, or a single embryo divided to produce identical triplets. Multiple embryos may occur rarely in the date palm (*Phoenix dactylifera* L.) according to Dr. O. Reu-

veni who observed this phenomenon in Israel (Reuveni, personal communication).

For the present, branched or twin embryos are mainly a curiosity. However, the fact that they can occur naturally, even though extremely infrequently, does indicate that there is a potential for the cloning of palms by embryo multiplication. An annotated bibliography on embryo and tissue culture of palms is presented below for those interested in learning more about these techniques.

Literature Cited

Balaga, H. Y. 1975. Induction of branching in coconut. Kalikasan (Philipp. J. Biol.) 4: 135–140.

Davis, T. A. 1968. Difficulties in the genetic improvement of the coconut: a promising alternative method. Indian J. Genet. Plant Breed. 28A: 154–164.

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Fisher, J. B. and J. H. Tsai. 1978. In vitro growth of embryos and callus of coconut palm. In Vitro 14: 307–311.

Ridley, H. N. 1907. Branching in palms. Ann. Bot. (London) 83: 417–422.

Appendix

A selected, annotated bibliography on embryo and tissue culture of palms.

Ammar, S. and A. Benbadis. 1977. Multiplication végétative du palmier-dattier (*Phoenix dactylifera* L.) par la culture de [sic] tissues de jeunes plantes issues de semis. Compt. Rend. Hebd. Séances Acad. Sci. 284D: 1789–1792.

Callus and inflorescences produced from embryos; callus regenerated roots and shoots; some regenerated plants transferred to soil.

Apavatjrut, P. and J. Blake. 1977. Tissue culture of stem explants of coconut (*Cocos nucifera* L.) Oléagineaux 32: 267–271.

Callus produced from subapical bud explants; no subculturing or organogenesis.

Corley, R. H. V., J. N. Barrett, and L. H. Jones. 1977. Vegetative propagation of oil palm via tissue culture. Oil Palm News (Tropical Products Institute) 22: 2–7.

General view of production of clonal seedlings from callus and how these seedlings behave in the field; methods for plantlet production given in Jones, L. H. 1974. Oil Palm News 17: 1–8. First report of successful cloning of a palm on a commercial scale.

Eeuwens, C. J. and J. Blake. 1977. Culture of coconut and date palm tissue with a view to vegetative propagation. Acta Horticulturae 78: 277–286.

Callus produced from explants but no subcultures or organogenesis; young inflorescences produced roots and "shoot-like" structures.

Eeuwens, C. J. 1978. Effects of organic nutrients and hormones on growth and development of tissue explants from coconut (*Cocos nucifera*) and date (*Phoenix dactylifera*) palms cultured *in vitro*. Physiol. Pl. (Copenhagen) 42: 173–178.

This paper, together with an earlier one (Physiol. Pl. (Copenhagen) 36: 23–28, 1976), examines in detail the nutritional requirements of tissue explants in culture; roots produced from explants and subcultured.

Fisher, J. B. and J. H. Tsai. 1978. In vitro growth of embryos and callus of coconut palm. In Vitro 14: 307–311.

Embryos successfully cultured; a vigorous callus with an unusual chromosome number (aneuploid) did not form organs.

Guzman, E. V. de, A. G. del Rosario, and E. C. Eusebio. 1971. The growth and development of coconut "Makapuno" embryo *in vitro*. III. Resumption of root growth in high sucrose media. Philipp. Agric. 53: 566–579.

Third part in a series dealing with embryo culture of this variety which cannot normally be germinated (Part II. Philipp. Agric. 53: 551–564, 1971; Part I. Philipp. Agric. 53: 65–78, 1969; and an earlier paper: Philipp. Agric. 48: 82–94, 1964); these papers are the most complete studies of coconut embryo culture.

Hodel, D. 1977. Notes on embryo culture of palms. Principes 21: 103–108.

Embryos of *Pritchardia kaalae* and *Veitchia joannis* were successfully cultured on agar.

RABÉCHAULT, H., J. P. MARTIN, AND S. CAS. 1972. Recherches sur la culture des tissus de Palmier à huile (*Elaeis guineensis* Jacq.). Oléagineux 27: 531–534.

Callus derived from leaf bases, small roots and shoot buds differentiated from this callus, but plantlets not produced. Rabéchault and his French co-workers have produced a series of studies on culture of oil palm embryos, i.e. in Oléagineux 28: 333–340, 1973; 27: 249–254, 303–305, 1972; 25: 519–524, 1970; 20: 79–87, 1965; and in Compt. Rend. Hebd. Séances Acad. Sci. 270D 3067–3070, 1970. The most recent paper is in: Oléagineux 31: 159–163, 1976.

REUVENI, O. AND H. LILIEN-KIPNIS. 1974. Studies of the *in vitro* culture of date palm (*Phoenix dactylifera* L.) tissues and organs. Pamphlet No. 145. Agricultural Research Organization, Bet Dagan, Israel. 40 pp.

Final report on extensive studies on propagation of date palm, only embryos were successfully cultured.

SMITH, W. K. AND J. A. THOMAS. 1973. The isolation and *in vitro* cultivation of cells of *Elaeis guineensis*. Oléagineux 28: 123–127.

Vigorous callus derived from embryos; apical shoot meristem and roots in agar and liquid cultures; roots regenerated from callus were diploid although callus had many aneuploid cells; no shoots formed.

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

Excised parts of the apical bud produced leaves and roots, probably part of original apex. Small male inflorescences also expanded in culture.

TISSERAT, B. 1979. Tissue culture of the date palm. J. Heredity 70: (in press).

Embryoids derived from callus produced from either shoot tip explants or embryos; green shoots and roots formed in culture but not yet transplanted into soil.

WANG, P.-J. AND L.-C. HUANG. 1976. Beneficial effects of activated charcoal on plant tissue and organ cultures. In Vitro 12: 260–262.

Growth improved by adding charcoal to medium; embryos of *Hyophorbe lagenicaulis* (*Mascarena lagenicaulis*), *H. verschaffeltii* (*M. verschaffeltii*), and *Caryota urens* grown.

LETTERS

Editor, Principes
Dear Sir,

The question by Mr. Savage on the *Licuala* seeding habit (*Principes* 22: 142, 1978) may be an environmental one.

I remember looking for seeds in the *Licuala* forests near Cardwell in late December 1976 (early summer) but without success. Only days later I collected *Licuala* seeds in quantity in tropical rainforest south of Cooktown.

The fruiting licualas were scattered individuals in relatively open situations where light conditions were better than average. The non-fruiting ones in question grew in dense palm bogs in a relatively low rainfall area.

Licuala ramsayi (*L. muelleri*) can be grown outside in my area at 35° 30′ South where the mean winter temperature is 50°F. But here it needs a suitable microclimate and is not a palm for exposed positions.

As an indoor plant it is undemanding and outright beautiful.

Yours faithfully,
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