

Tissue Culture of Stem Explants of Coconut (*Cocos nucifera* L.)

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Summary. — The authors have studied the conditions of induction of callus from the tissue of adult coconut stems. The aim is to find a method of vegetative propagation of the coconut by tissue culture. Induction of callus was obtained on nutritive media of simple composition, in the presence of coconut water, at 31 °C and in the dark. The importance of the simultaneous presence of the ammonium and nitrate ions has also been shown. Cellular division produced cells of the embroid type. It now remains to study conditions for culturing these calli and to find out whether the embroid cells can give rise to seedlings.

INTRODUCTION

The coconut (*Cocos nucifera* L.) is amongst the top five most important oil-producing crops in the world. Since coconut plantations are restricted by climate to the lowlands in tropical areas [1] it is unlikely that the output of coconut products could be increased substantially by enlarging the area of plantations. On the other hand there is evidence [4] that productivity of the average plantation could be increased considerably by planting only high-yielding stock. This is not easily achieved in practice since planting material must at present be raised from seed which is extremely variable due to the outbreeding habit of *Cocos*. Moreover, improvement by breeding is very slow since several years must elapse before yield potential can be assessed.

A method of vegetative propagation for the coconut is thus a highly desirable objective. It would enable clones to be propagated from elite palms already in existence, or from the F₁ hybrids now being produced as well as from strains selected by the plant breeder for high-yield and disease-resistance. Although the coconut cannot normally be propagated vegetatively, occasional palms with suckers at the base have been observed, branched palms can be rooted by air-layering, and coconut offsets from the inflorescence can be separated and apparently rooted [3], but none of these effects can be induced at will. Thus, in searching for a method of vegetative propagation, tissue culture techniques are a possibility since they have been shown to be effective in the propagation of numerous plant species [9], including the monocotyledonous species sugar cane [7], rice [11] and pineapple [2]. One method of tissue culture propagation involves the initiation of callus on the mother explant, sub-culturing and multiplication of the callus, followed by differentiation of the callus to give plantlets. This paper describes the first of these steps using coconut stem tissue.

A series of experiments were carried out with the aim of inducing callus on coconut stem explants, and data are given from a factorial experiment which shows the important effects of temperature, coconut water and nitrogen source on growth of this tissue. In the final section the anatomical changes over the first four weeks of incubation are described.

MATERIALS AND METHODS

Stem explants were prepared from the sub-apical tissue of mature trees of *Cocos nucifera* L. var. Malayan Dwarf. Sterile cores were taken with a cork borer and cut into discs giving explants 3 mm in diameter and 2 mm thick. Each disc was placed on a filter paper bridge in a borosilicate glass tube (75 × 25 mm) containing liquid nutrient medium.

The constituents of the basic medium are shown in table I. Coconut water was taken from mature green coconuts. After adjusting the pH of the medium to 5.7, it was sterilised by dispensing through a Millipore 'Swinnex' filter into sterile tubes. The cultures were incubated in the dark throughout, since this had been shown in preliminary work to produce better callus initiation than incubation in the light.

TABLE I. — The constituents of the basic medium
(Composants du milieu de base)

	mM		μM
NaH ₂ PO ₄ · 2 H ₂ O	0.8	Fe EDTA	100
KCl	6.0	H ₃ BO ₃	500
CaCl ₂ · 2 H ₂ O	2.4	MnSO ₄ · 4 H ₂ O	500
MgSO ₄ · 7 H ₂ O	1.0	ZnSO ₄ · 7 H ₂ O	30
		KI	25
		Na ₂ MoO ₄ · 4 H ₂ O	1
Sucrose	20 g/l	CoCl ₂ · 6 H ₂ O	0.1
		CuSO ₄ · 5 H ₂ O	0.1

The data collected were fresh and dry weight per explant, which was analysed statistically, and a visual assessment of surface proliferation (callus). A subjective ranking for cell proliferation was made on a scale from 0 (no cell proliferation) to 5 (thick cell proliferation) but this was not suitable for statistical analysis.

In the experiment described, temperatures of 28, 31 and 34° were combined in a factorial experiment with 2 levels of coconut water (nil and 15 p. 100) and 3 sources of nitrogen (8 mM NH₄Cl, 8 mM NaNO₃ or 4 mM NH₄Cl + 4 mM NaNO₃). The basic nutrient medium, modified from those of Heller [8] and Murashige and Skoog [10], is shown in table I.

The explants described in the final section were grown on the same basic medium with sucrose increased to 40 g/l and KI to 50 μM, and with the addition of NH₄Cl 8 mM, NaNO₃ 8 mM, l-glutamine 16 mM,

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γ -amino butyric acid 16 mM, l-cysteine 4×10^{-7} M, 2,4-D 10^{-7} M, kinetin 5×10^{-6} M and meso-inositol 5.6×10^{-7} M. This medium was used because concurrent experiments in this laboratory showed the advantages of a defined medium, of which further details have now been published [5].

RESULTS

1. — The effects of temperature, coconut water and nitrogen source.

All three factors were of considerable importance for the growth of the explants (Fig. 1, 2). The omission of coconut water inhibited growth (Fig. 1) and gave a high degree of browning. There was slight proliferation of the explants (Fig. 2) chiefly in the presence of $\text{NH}_4^+ + \text{NO}_3^-$ at 31 and 34°, and in the presence of NO_3^- alone at 34°.

When coconut water was present considerably more growth occurred in all treatments (Fig. 1) and there was much less browning of the explants. Callus production was always greater with $\text{NH}_4^+ + \text{NO}_3^-$, regardless of the temperature (Fig. 2), but all sources of nitrogen gave proliferation at 31° which was always more favourable than either 28 or 34°, especially in the presence of NH_4^+ alone.

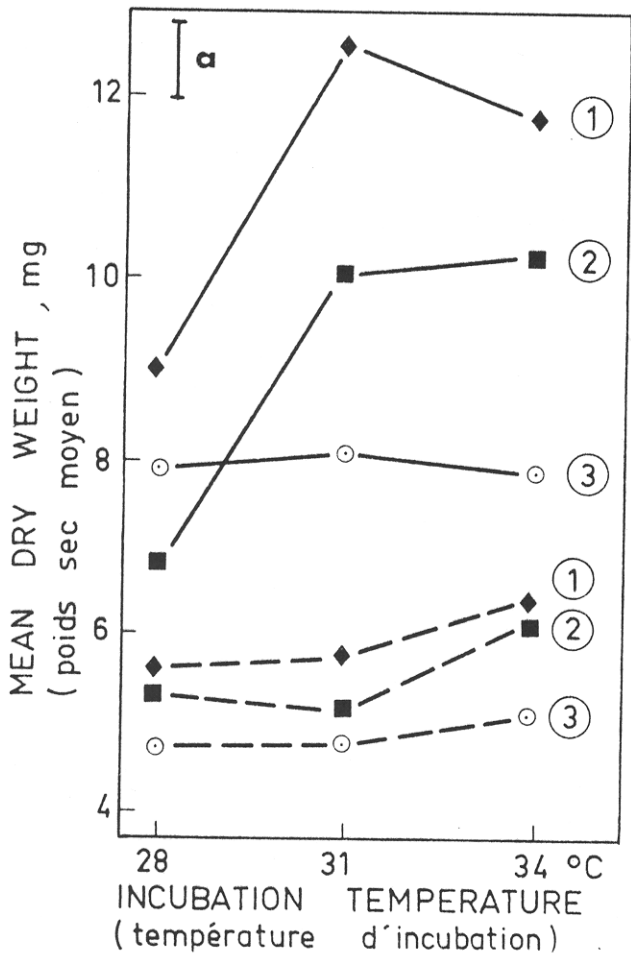


FIG. 1. — The effects of temperature and nitrogen source on dry weight in the presence and absence of coconut water (Effets de la température et de la source d'azote sur le poids sec en présence et en l'absence d'eau de coco); — With (avec), — — no (sans) coconut water (eau de coco); (a): Lsd (moindre écart type) 1: $\text{NH}_4^+ + \text{NO}_3^-$; 2: NO_3^- ; 3: NH_4^+ .

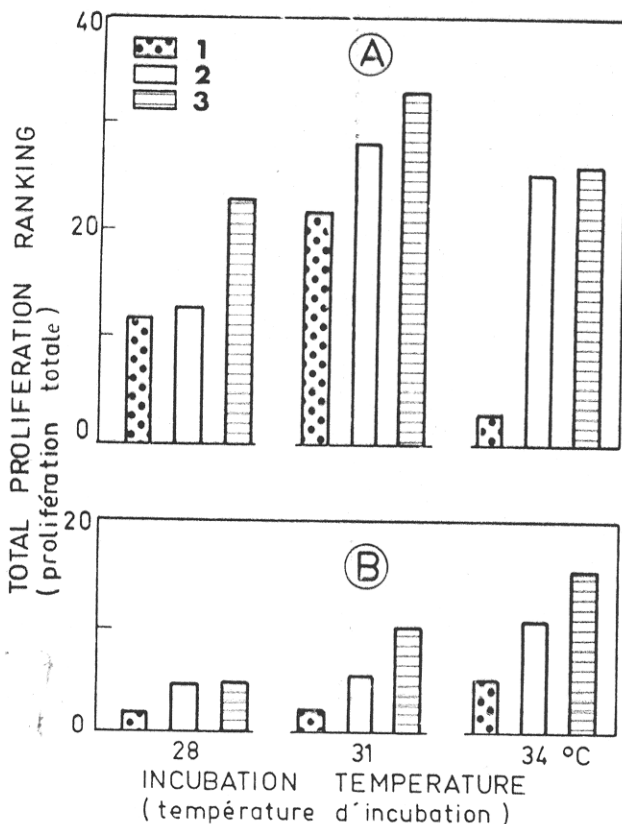


FIG. 2. — The effects of temperature and nitrogen source on proliferation in the presence and absence of coconut water (Effets de la température et de la source d'azote sur la prolifération, en présence et en l'absence d'eau de coco); A: with (avec), B: no (sans) coconut water (eau de coco); 1: 8 mM NH_4Cl ; 2: 8 mM NaNO_3 ; 3: 4 mM NH_4Cl + 4 mM NaNO_3 .

2. — Anatomical observations of explants grown on a defined medium.

After 4 weeks of incubation on a defined medium the explants appeared healthy with little or no browning. The fresh weight increased rapidly at the beginning of the culture period (Fig. 3), indicating

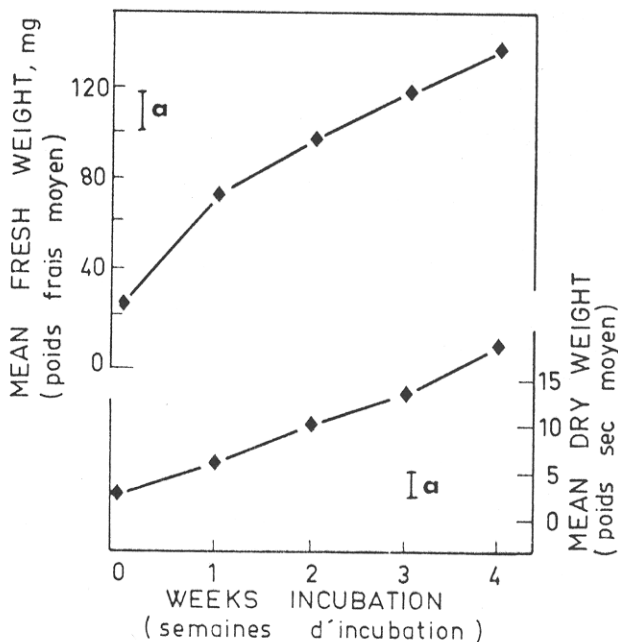
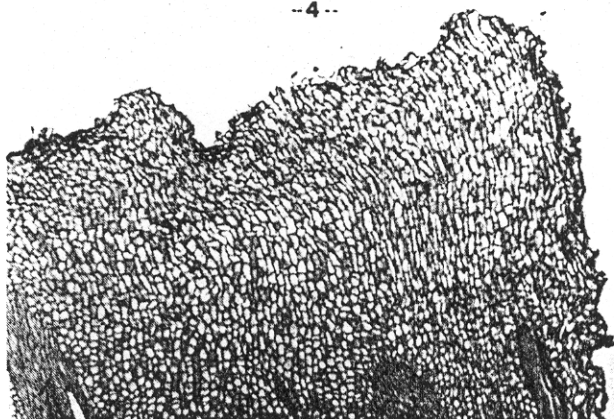
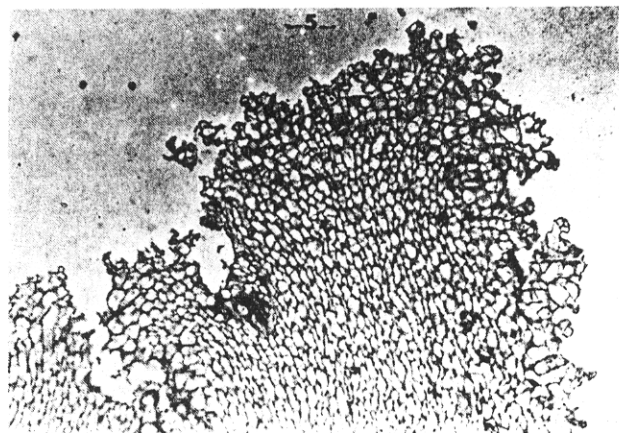


FIG. 3. — Fresh and dry weight increase on a defined medium (Augmentation du poids sec et du poids frais sur un milieu défini) — mg. (a): Lsd (moindre écart type).

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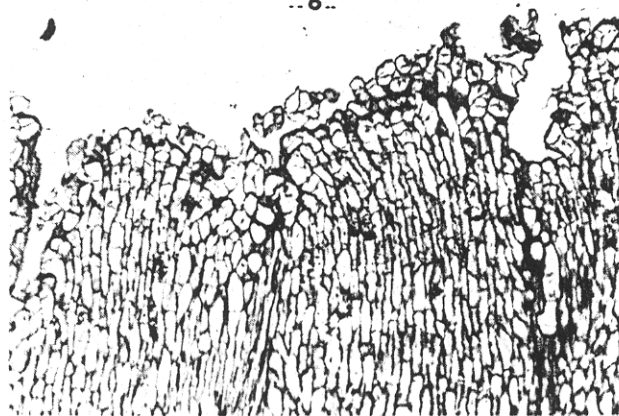
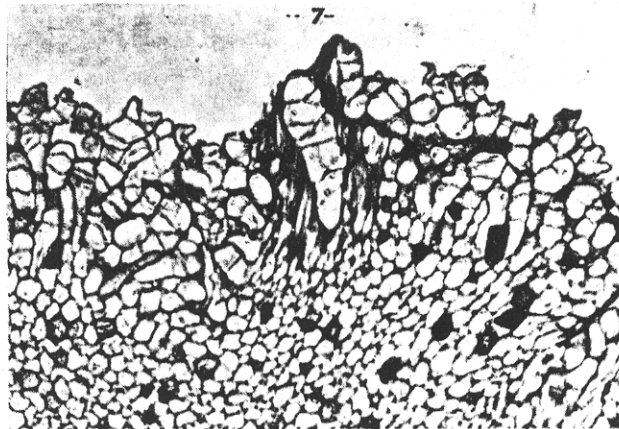


FIG. 4, 5, 6. — L. S. coconut stem after 1, 2, 3 weeks in culture (Section longitudinale du stipe du cocotier après 1, 2, 3 semaines de culture);

(fig. 4, 5 : $\times 37,5$; fig. 6 : $\times 50$).

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considerable water uptake and cell expansion during this time, whereas the dry weight increase was almost linear throughout the four weeks. Anatomical sections of the explants showed that by the end of the first week of incubation, the entire explant had increased in size, mainly by way of cell enlargement, but that some cell division had also taken place. There was very little proliferation on the surface of the explant at this time (Fig. 4) but by the end of the second week considerable expansion and some cell divisions in the surface layers had occurred giving an irregular surface to the top of the explant (Fig. 5). By the end of the third week cell division was occurring throughout the explant, with divisions in all directions, sometimes yielding cells of a cambium-like type (Fig. 6) or of an unusually large size (Fig. 7). At the end of the fourth week this proliferation was even more pronounced.

These observations indicate that growth and cell division of coconut stem tissue can start within the first week after inoculation. Cell divisions occurred throughout the explant, but especially on the surface yielding a dense mass of callus with cells of varying types. Some cells (Fig. 6) appear to be similar to those described by Gautheret [6] in Jerusalem artichoke, whereas others (Fig. 7) appeared to resemble pro-embryos or embryoids which seemed to have arisen from the internal division of one large cell, as has been observed in several species, including carrot [12].

CONCLUSIONS

Callus induction on coconut stem tissue has been achieved on nutrient media of relatively simple composition with known components. Cultures grown at a pH of 5.7 and at a temperature of 31 °C on a medium containing mineral salts with both ammonium and nitrate, a high iodine level, a few amino acids, an auxin, kinetin and 4 p. 100 sucrose, and in the presence of coconut water, showed virtually no browning indicating that optimum conditions can eliminate this form of deterioration. Further development must now concentrate on the sub-culturing of the callus and an investigation into whether the embryoid-type cells have the potentiality to regenerate plantlets.

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FIG. 7. — T. S. coconut stem after 3 weeks in culture (Section transversale du stipe du cocotier après 3 semaines de culture); ($\times 75$).

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RÉSUMÉ

La culture de tissus d'explantats de stipe de cocotier (*Cocos nucifera* L.).

PIMCHAI APAVATJRUT et J. BLAKE, *Oléagineux*, 1977, **32**, n° 6, p. 267-271.

Les auteurs ont étudié les conditions de l'induction de cals à partir de tissus de stipe de cocotiers adultes. L'objectif est de trouver une méthode de propagation végétative du cocotier par culture de tissus. L'induction des cals a pu être obtenue sur des milieux nutritifs de composition simple, en présence d'eau de coco, à 31 °C dans le noir. L'importance de la présence des ions ammonium et nitrates ensemble a également été montrée. Les divisions cellulaires ont donné naissance à des cellules de type embryôide. Il reste à étudier les conditions de culture de ces cals et à savoir si les cellules embryôides pourront régénérer des plantules.

Mots clés : Cocotier, Propagation végétative, Culture de tissus, Eau de coco, Azote.

RESUMEN

Cultivo de tejidos de explanta de estipe de cocotero (*Cocos nucifera* L.).

PIMCHAI APAVATJRUT y J. BLAKE, *Oléagineux*, 1977, **32**, n° 6, p. 267-271.

Los autores estudiaron las condiciones de la inducción de callos a partir de tejidos de estipe de cocoteros adultos, a fin de descubrir un método de propagación vegetativa del cocotero por el cultivo de tejidos. Se obtuvo la inducción de los callos en medios nutritivos de composición simple, en presencia de agua de coco, a 31°, en la oscuridad. Asimismo se demostró la importancia de la presencia conjunta de los iones amonio y nitrato. Las divisiones celulares dieron origen a células de tipo embryôide. Ahora queda por estudiar las condiciones de cultivo de tales callos, y por saber si las células embroides pueden regenerar plántulas.

La culture de tissus d'explantats de stipe de cocotier (*Cocos nucifera* L.)

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INTRODUCTION

Le cocotier (*Cocos nucifera* L.) fait partie des cinq cultures oléagineuses les plus importantes au monde. Les plantations de cocotiers étant limitées aux basses plaines des régions tropicales à cause des exigences climatiques [1], on ne peut espérer augmenter très sensiblement la production totale par l'extension des zones de plantation. Par contre, on a montré [4] que la production d'une plantation moyenne pouvait être considérablement augmentée si on plantait uniquement un matériel végétal à haut rendement.

En pratique, ce but n'est pas facilement accessible car ce matériel doit être produit actuellement à partir de la graine, laquelle est elle-même très variable en raison de l'exogamie habituelle du cocotier. Par ailleurs, l'amélioration par croisements est très longue car il faut attendre plusieurs années avant de pouvoir évaluer le potentiel de production.

C'est donc un objectif hautement souhaitable que de trouver une méthode de propagation végétative pour le cocotier. Ceci permettrait de propager des clones à partir des meilleurs palmiers existants, ou d'hybrides F1 actuellement produits,

ou de variétés choisies par le sélectionneur pour leur haut rendement ou leur résistance aux maladies. Bien qu'il soit normalement impossible de propager le cocotier par voie végétative, on observe de temps en temps un cocotier avec des surgeons à la base du stipe ; des palmiers ramifiés peuvent prendre racine par marcottage aérien, et on trouve des rejetons de cocotiers produits par l'inflorescence, apparemment séparés et enracinés [3], toutefois aucun de ces résultats ne peut être produit expérimentalement.

La technique de culture de tissus est une possibilité dans la recherche d'une méthode de propagation végétative puisqu'elle a été trouvée efficace pour la propagation de nombreuses espèces végétales [9], et en particulier plusieurs espèces monocotylédones dont la canne à sucre [7], le riz [11] et l'ananas [2].

Une des méthodes de propagation par culture de tissus comporte les étapes suivantes : initiation de cals sur l'explantat maternel, sous-culture et multiplication de cals, suivies de la différenciation des cals pour obtenir des plantules. Cet article décrit la première de ces étapes, avec utilisation de tissu de stipe de cocotier.

Une série d'expériences a été réalisée dans le but d'inclure des cals sur des explantats de stipe de cocotier et nous exposons les données d'une expérience factorielle qui montre l'importance de la température, de l'eau de coco et de la source d'azote sur la croissance de ce tissu. Dans la dernière partie sont décrits les changements anatomiques survenus au cours des quatre premières semaines d'incubation.

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