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# PRODUCTION OF SOMATIC EMBRYOS FROM LEAF TISSUES OF COCONUT, COCOS NUCIFERA L.

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## INTRODUCTION

The coconut palm, Cocos nucifera L., generally cross-pollinated, is propagated only by seed.
Although a few rare phenomena such as branching, off-shoots, transformations of spathes or flowers into vegetative shoots have been reported (1,2), no routine vegetative propagation method has been worked out.
Tissue culture using fragments of stems and inflorescences has been described (3,4,5,6,7,8).
We present here our first results concerning the production of somatic embryos from young leaf tissues.

## MATERIALS AND METHODS

The starting material was obtained from the Malayan Dwarf Yellow x West African Tall hybrid (PB 121) produced by I.R.H.O.
Young leaves were taken without damaging the apex from i) nursery and 5 year-old plants ii) mature trees.
Pieces of leaflets were placed on media containing Eeuwens' salts(3), Morel's vitamins, 3% saccharose, 0,8% agar. Activated charcoal and auxins (2,4D or TCPP) at various concentrations were added to this basic medium. BA was used for embryogenesis. All cultures were incubated at 27°C.
For histological examinations tissue sections were stained by iron-hematoxylin.

## RESULTS

During the first weeks of culture, the explants grew in length and thickness. The growth was all the quicker as the explant was closer to the apex (that is less differentiated). The fragments looked healthy and no or few browning phenomena occured. Then, slight scar type proliferations appeared on damaged tissues (cuts, wounds). These proliferations did not exhibit any significant growth during the following weeks of culture.
Two or three months after the culture started, nodules appeared at the abaxial surface of the limb (fig.1). They arose near the vascular bundles as revealed by histological examinations; in a later stage clumps of meristematic cells were observed (fig.2).
About one month after the first nodules appeared, the calluses exhibited various aspects: most often they were small and compact with well defined shape (fig.3), sometimes they were softer with diffuse limits. The number of calluses increased rapidly on a donor explant, while their size increased relatively slowly. Most of the time, these calluses originated in several points of the explant; in addition, a large number of small calluses appeared on a single origin point.
Quantitative results were different according to the age of the donor tree. The proportion of explants bearing calluses was 70% for nursery plants, 50% for 5 year-old tree and 20% for mature trees. In the latter case, calluses appeared after at least 3 months of culture and they were less numerous on a donor fragment. This difference in performance seemed due mainly to the fact that the leaf fragments taken from a mature tree without damaging the apex were older than those isolated from young trees.
The calluses were isolated and transferred on a fresh medium. New formations, globular, pearly and white or pale-yellow in colour appeared on those isolated from nursery and 5 year-old tree tissues (fig.4).

They were identified as embryoids; their somatic embryo character was supported by histological examinations (fig.5). The first embryoids were observed 6 months after the explants were placed in culture. Multiplication of the embryoids was observed and a small mass made up of several embryoids was obtained (fig.6).

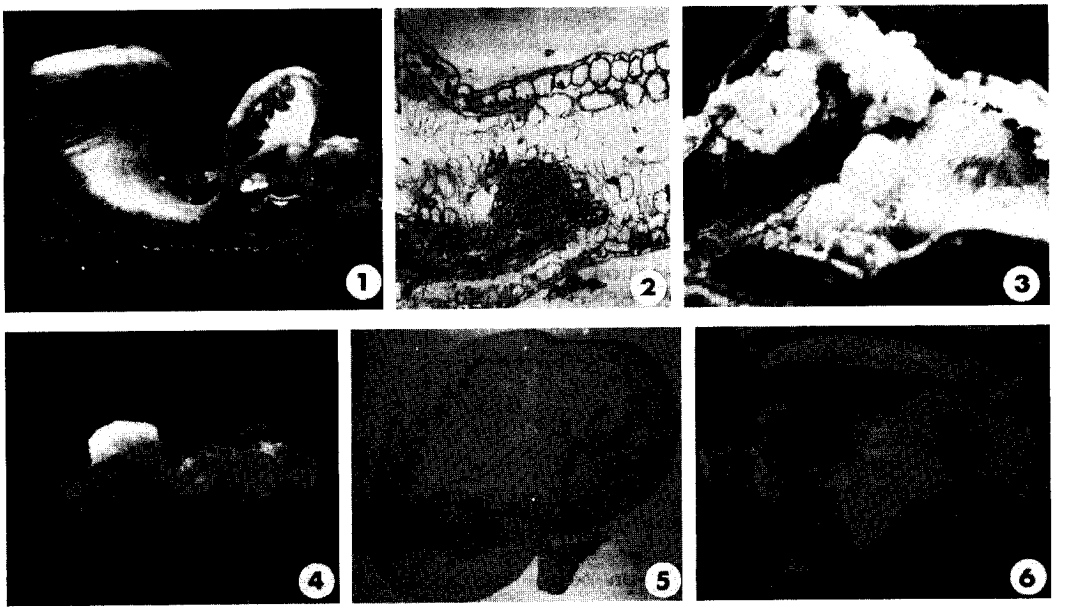


Fig.1. Leaf explant at day-60 of culture, nodules appearance. Fig.2. Section in a leaflet fragment. Note a meristematic clump near the vascular bundle. Fig.3. Leaf explant at day-90. Fig.4. Embryoid on isolated callus. Fig.5. Section in a young embryoid. Fig.6. Mass made up of several linked embryoids.

DISCUSSION

Our results show the induction of asexual embryogenesis from young leaf explants and the conversion of embryoids into plantlets.

The application of this tissue culture technique to vegetative propagation requires that the explants be derived from mature trees. However, promising results were obtained: embryogenesis was induced from 5 year-old tree tissues, callogenesis from mature tree explants was observed. In addition, the fact that no embryoid was yet obtained from mature tree may be related to the low proportion of calluses giving embryoids; the number of calluses appeared from mature tree might be not large enough to observe embryogenesis. Our work must concentrate on the optimisation of the culture conditions for callogenesis from adult tree explants and for the increase of embryogenic callus proportion.

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