CURRENT STATE OF THE ART OF THE USE OF TISSUE CULTURE TECHNIQUES IN VEGETATIVE PROPAGATION OF COCONUT IN SRI LANKA

By

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Abstract

Culture of coconut tissues derived from the tender leaf, inflorescence and immature embryo was attempted with a view to develop methodologies for vegetative propagation of coconut. Tender leaf tissues produced somatic embryos and some neoformations without any callus intermediate. These somatic embryos did not develop any further but produced callus which was easily subcultured. Callus was also obtained from inflorescence explants for micropropagation. Large numbers of somatic embryos can be produced from the immature embryo callus. Callus induction from immature embryo is highly dependent on stage of development of the embryo but was rather rare. Mature zygotic embryos of coconut have been germinated in vitro and transferred to soil successfully. In addition, methods have been developed for explanting and culture of embryos in the field and for short term preservation of mature zygotic embryos. The feasibility of using the embryo culture technqiue for screening drought-tolerant coconuts is also being investigated.

Introduction

The in vitro culture of coconut, aimed at cloning elite palms has a long history dating back to the 1970's. Although there are records of plantlet production from coconut tissues, plant regeneration is inconsistent due to recalcitrant nature of the tissue. Most work in this regard has concentrated on two types of explants: the leaf and the inflorescence. In this laboratory, full time tissue culture research was commenced about 5 years ago and our main objective is to develop technologies for clonal propagation of

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coconut. This paper describes the progress of work carried out using tender leaf, inflorescence and the zygotic embryo of coconut.

Materials and Methods

The sources of explants were tender leaves, tender inflorescences and immature nuts of Cocos nucifera L var typica. Three culture media were used, Eeuwens (1976), Tuyen and Guzman's (1983) and medium no. 72 developed in this laboratory, following the de Fossard's (1976) method. All three media contained 2-2.5g activated charcoal per litre culture medium and were either liquid or solidified with 0.8% agar. Aseptically dissected materials were placed on culture media and incubated initially in the dark at 26-28°C.

Observations

Culture of leaf tissues

Leaf tissues excised from the cabbage of the palm and culture in medium no. 72 produced globular bodies in 50% of the cultures. Some produced neoformations. The globules were creamy white, bipolar and resembled the immature zygotic embryos of coconut. Low 2,4-D and activated charcoal were essential for globule formation. The explants never produced callus in culture. Rarely, direct rhizogenesis occurred from the main vascular strands. Globule formation in the leaf tissues explanted from seedlings was consistent but the explants from bearing palms were totally unresponsive. further, explants derived from seedlings also responded differently, depending on the developmental maturity of the explant. Leaf tissues explanted from typica and three colour forms of variety nana produced globular structures irrespective of their genotypic differences.

Our attempts to achieve sustained growth and germination of globular embryo-like bodies were unsuccessful. Sporadic germination of globular bodies

occurred in a few cultures but complete plant developement was not possible. Adventive root formation and haustorium development were rather common. Development of a haustorium from the globular body further indicates that it is an embryo. However, when the globules were cultured in germination meium of the mature zygotic embryo, a creamy white, compact fast growing callus was produced. Callus was successfully subcultured. This project was supported by a grant from USAID.

Culture of inflorescence explants of coconut

The advantages of using the inflorescence are that it has numerous meristamatic points on it and the inflorescence can be removed without destroying the donor plant.

Floral meristem explants derived from -3 to -12 inflorescence (the youngest open inflorescence on the palm being taken as 0)were cultured in the medium of Tuyen and Guzman (1983). Callus formation occurred from tissues excised from -5, -6 and -7 inflorescences. Cold pre-treatment ($4^{o}C$, 24-48h) enhanced callusing. Occasionally, roots developed from the callus but no embryogenesis was observed.

Cultured of immature embryos of coconut

The immature embryos of coconut appear to be the most promising source of explants for micropropagation but very little experimental work has been carried out, for the obvious reason that the cloning of elite material is not possible by this method.

1-2mm diameter embryos were excised from 6-7 months old nuts and cultured in medium no. 72 and incubated in the dark. Embryos produced whitish, compact highly embryogenic callus tissues in the presence of 12-20 um 2,4-D. About 50% of callus cultures produced globular embryos when transferred to 8 um 2,4-D. Embryos in 22% of these cultures

germinated and produced shoots 6mm long when 6-Benzylamino purine and kinetin (10um each) were incorporated into the culture medium. Stage of development of the embryo, the orientation of the explant on the culture medium and genotype were some important factors influencing callus induction.

Methods have also been developed for in vitro germination of mature coconut zygotic embryos, short term preservation of embryos to facilitate germplasm transport and for field explanting of coocnut embryos. Germination of a local non germinating variety of coconut, dikiri is also now possible by in vitro culture.

As regards the use of developed technologies in the coconut industry, so far no effort has been made to use the techniques in germplasm collection methods such as field explanting, trnsport and in quarantine measure.

In conclusion, no method has yet been developed for vegetative propagation of coconut. For vegetative propagation research, immature embryo of coconut seems to be the best explant. Stage of development of the explant however, is critical. The morphogenetic competence of the explant is present for a brief period. A deeper understanding of the internal situation of the explant through collaborative research may perhaps help develop a sustainable method for vegetative propagation of coconut.

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