

**A Report on the Culture of Embryos of Dwarf Coconut,  
*Cocos nucifera* L var *nana* In vitro**

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The possibility of culturing excised embryos of coconut, *Cocos nucifera* L. var. *nana* *in vitro* has been investigated. Embryos were excised from nuts harvested 10-11 months after pollination. Incubation of these embryos in a liquid medium (a modification of Y3 medium, Eeuwens, 1978) at 30-32°C and under a 12hr photoperiod of low light intensity ( $2.0\mu\text{Em}^{-2}\text{s}^{-1}$ ) resulted in about 62% of the cultured embryos developing into seedlings. A high concentration of sucrose stimulated haustorium development and subsequent root initiation. A significant retardation in growth of the embryos and complete cessation of haustorium development and root initiation occurred in the absence of activated charcoal. Four month old seedlings were transplanted successfully into soil. It is concluded that activated charcoal and a high concentration of sucrose are two important factors contributing to successful growth of coconut embryos *in vitro*.

Attempts were also made to preserve the coconut embryos in culture in a dormant state. A special survival medium which suppresses the growth of the embryos has been developed. 32% of the embryos incubated in the survival medium for a period of 5 months developed into seedlings when transferred to the development medium.

## INTRODUCTION

The technique of *in vitro* embryo culture is employed in many laboratories to raise plants from either undeveloped or non-germinating embryos resulting from incompatible crosses (De Guzman et al, 1971; Gosal & Bajaj, 1983; Thomas & Pratt, 1981). This technique now appears to be also useful in laboratory studies with crop species, including the cumbersome perennials like coconut. Investigations on trace element deficiencies with coconut seedlings were carried out using the sand culture technique (Nathaneal, 1961). A major problem in this procedure is the absorption of significant quantities of trace elements from the endosperm, by the seedling during germination and the possible translocation of these minerals within the tissue. This problem can be overcome by using seedlings developed from embryos *in vitro* as the experimental material. The use of this material has the added advantage that a large number of treatments can be tested with many replicates, under uniform conditions in a laboratory/glasshouse. These seedlings may also be used in studies on host-parasite relationship of diseases such as lethal yellowing, metabolic disorders such as tapering and in germination studies of coconut. Another useful application of *in vitro* techniques will be to eliminate diseased stocks from germplasm material and also to cut down transport costs.

De Guzman, in 1971 reported successful plant development from embryos of the makapuno type of coconut (homozygous recessive embryo which does not grow *in planta*). The first attempts of embryo culture of non-makapuno type of coconut were those of Cutter & Wilson (1954) and Abraham & Thomas (1962). Fisher & Tsai in 1978 also reported growth in cultured embryos but complete plant regeneration has not been very successful. Iyer (1981) reported the development of seedlings from embryos of West Coast Tall coconuts but these had a high rate of mortality on transplanting to soil. The present paper reports some of the observations on *in vitro* culture of embryos of the dwarf coconuts of Sri Lanka.

## MATERIALS AND METHODS

The three colour forms of the dwarf coconut, *Cocos nucifera* var *nana*, namely *pumila*, *regia* and *eburnea* were chosen as the source of experimental material. Embryos were excised from the kernel of mature nuts (10 to 11 months after pollination) and disinfected by immersing in 2% (w/v) calcium hypochlorite for 5 min and washing in five changes of sterile distilled water. Sterile embryos (100 per experiment) were then cultured in a pre-sterilized ( $1.1 \text{ kg cm}^{-2}$  for 15 min) liquid medium. The culture medium described by Eeuwens (1978) was used with modifications. 0.09M sucrose and activated charcoal (0.25% w/v) were incorporated into the medium and the pH was adjusted to 6.0. Cultures were incubated at a temperature of about 30 to 32° C under low light intensity and subjected to occasional mild agitation.

## RESULTS & DISCUSSION

Coconut embryos cultured in the above medium germinated rapidly with no apparent dormant period. About 62% of the cultured embryos developed into young seedlings consisting of about four scale leaves and two photosynthetic leaves in a short period of 4 months (Fig. 1).

The root system at this stage consisted of 1 to 2 primary roots with many laterals and a small developing haustorium.

The presence of charcoal (0.25–1.0% w/v) in the culture medium was observed to be absolutely essential for seedling development (Table 1). Further, the characteristic browning of embryonic tissues in culture has never been observed in the presence of charcoal. A significant retardation in growth of the embryos and complete cessation of haustorium development and root initiation occurred when charcoal was omitted from the medium (Fig. 2). The exact mechanism by which activated charcoal stimulates embryo growth is not understood. However, as has been suggested by several other workers (Constantin et al, 1977; Martineau et al, 1981; Wang & Huang, 1976) the beneficial effect of charcoal may be attributed to the removal of inhibitors and perhaps excessive quantities of both tissue-produced and exogenously supplied growth regulators from the culture medium by adsorption.

The study also revealed the importance of a high concentration of sucrose for the initiation of roots in embryos of coconut cultured *in vitro* (Table 2). When concentration of sucrose in the medium was increased from 0.09M to 0.18M a substantial increase in size of the haustorium occurred leading to rapid development of the root system, in a short period of 6–8 weeks (Figs. 3, 4). The failure to develop a root system in embryos

*Embryo culture of dwarf coconut*

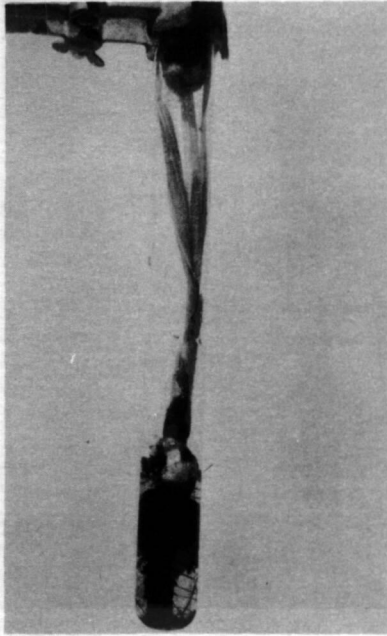


Figure 1

Fig. 1 A 4-month old seedling growing in liquid medium supplemented with 0.25% activated charcoal and 0.09M sucrose.



Figure 2

Fig. 2 An 11-month old seedling developed in the medium described in Fig. 1 but minus activated charcoal. Note stunted growth, degenerated haustorium and complete absence of roots.

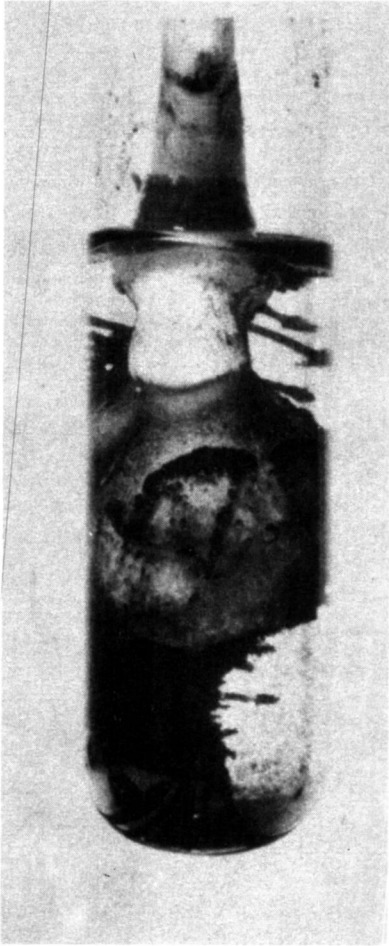


Figure 3

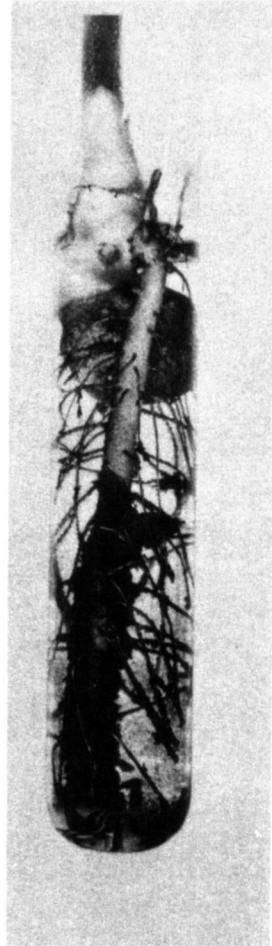


Figure 4

Fig. 3,4 2-month old seedlings developing in the medium supplemented with 0.18M sucrose. Note the enlarged haustorium and the developing root system.

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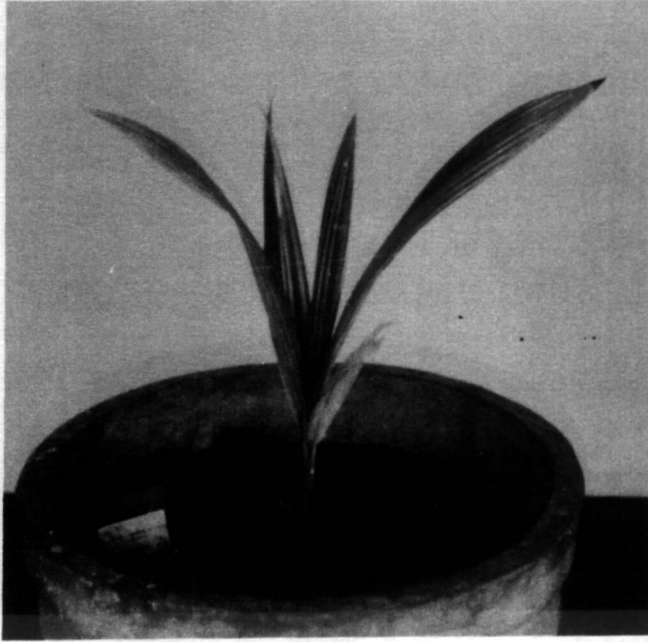


Figure 5

Fig. 5 Seedling illustrated in Fig. 1, 4 months after transplanting into soil.

cultured *in vitro* (occasional and/or due to treatment effect) was always observed to be associated with extremely poor development of the haustorium (Fig. 2). These results indicate that haustorium development may well be a pre-requisite for rapid root initiation in cultured embryos and that sucrose is one important factor contributing to the induction of haustorium development *in vitro*. Thus it appears that under appropriate culture conditions, excised embryos of coconut tend to follow closely the *in vivo* developmental pattern of the seedling. De Guzman et al (1971) also emphasised the importance of high concentration of sucrose for root initiation in makapuno embryos cultured *in vitro*. Four-month old seedlings were transplanted (after removal of the haustorium) into pre-sterilized soil and exposed to humid conditions under a low light intensity of about 6000 lux. A majority of the seedlings died of a common microbial infection, after about 5 weeks in soil. The survivors were exposed to the ordinary atmospheric conditions (Fig 5) after about 8 weeks in the humidity chamber.

Table 1. *Effect of activated charcoal on growth of coconut embryos cultured in vitro.*

Percent activated charcoal (w/v) in culture medium	Av. shoot length, mm.	Av. number of leaves (including scale leaves)	Av. number of primary roots	Av. number of secondary roots.
0	1	0.1	0	0
0.25	23.3	2.5	1.0	1.1
1	17.8	2.0	0.9	0.9
2	2.8	0.4	0.1	0.3
Significance	***	***	*	NS-
C.D.	9.83	0.13	0.14	0.10
C of V	77.3 %	26.97 %	29.25 %	23.34 %

\*\*\* P = 0.001, \* P = 0.05

Embryos were cultured in complete medium containing different concentrations of activated charcoal and 0.09M sucrose. Measurements were recorded after 10–11 weeks in culture.

Table 2. *Effect of sucrose on growth of coconut embryos cultured in vitro.*

Concentration of sucrose in culture medium	Av. shoot length, mm	Av. number of leaves (including scale leaves)	Av. number of primary roots	Av. number of secondary roots
0	0.5	0.1	0.0	0
0.09M	24.8	2.5	1.0	1.4
0.18M	30.5	2.8	2.0	21.6
Significance	***	***	***	***
C.D.	6.57	0.64	0.43	0.25
C of V	37.47 %	30.12 %	31.27 %	39.93 %

\*\*\* P = 0.001

Embryos were incubated in complete medium supplemented with different concentrations of sucrose and 0.25 % activated charcoal. Measurements were recorded after 10 weeks in culture.

### *Embryo culture of dwarf coconut*

The feasibility of preserving the coconut germplasm by suppressing their growth in a special survival medium was also investigated. Excised embryos remained dormant or grew very slowly when incubated in the survival medium. No signs of deterioration or discoloration were seen even after 11 months in culture. However, growth resumed quickly when embryos incubated in this medium for 5 months were transferred to the development medium and 32% of these embryos developed into seedlings. This indicates that growth may be arrested and embryos maintained in a 'dormant' state for a period of 5 months without affecting their potential to develop into seedlings. The maximum limit of the duration of induced dormancy was not determined. The technique facilitates the transport of germplasm from overseas and their preservation for short periods.

Regeneration *in vitro* of vigorously growing seedlings from excised embryos of the three colour forms of the *nana* variety is now possible. The embryos can also be induced to undergo 'dormancy' in culture and then regenerate plants on transfer to appropriate culture media. There were clear indications that the seedlings were capable of withstanding the stress of the new environment. The survival rate may be increased by acclimatising seedlings to the changes in moisture stress and light intensity and to pesticides by a preparatory step, prior to transplanting.

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