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Short Scientific Reports

Coconut Plantlets from Leaf Tissue Cultures*

The coconut (*Cocos nucifera* L.) is a cross pollinated palm grown in many of the tropical countries for nut and oil. Due to its long juvenile phase, high heterozygosity and exclusively seed propagated nature, application of conventional breeding methods for crop improvement is time consuming and difficult. In view of this, the naturally occurring palms with exceptionally high yield and other attributes cannot be utilised for large scale exploitation.

Tissue culture work on this crop has been in progress at many laboratories for a long time. Recently, Branton and Blake (1983) reported success in producing a clonal plantlet from root callus cultures. Pannetier and Buffard–Morel (1982) could induce somatic embryos in the leaf callus. However, differentiation from these is yet to be achieved. The inconsistency of results is due to the differences in genotype, physiological maturity of the explant, season and time of collection of the source material, etc. The secrecy regarding details of medium was also immense due to its commercial potential.

Coconut tissue culture work was started at the Central Plantation Crops Research Institute in late 70's. The work started with leaf cultures from seedlings since January 1982, have now produced for the first time, clonal plantlets through direct somatic embryogenesis from leaf explant. For this, the lowermost 10 cm segment of tender leaves, from two year old nursery grown seedlings of West Coast Tall variety of coconut was used as the source material. The tender leaf was surface sterilized by flaming after dipping in 70 per cent alcohol for 2–3 seconds. This sterilised leaf was cut into 5 mm long explants which were then placed on sterile culture medium.

A broad spectrum experiment (De Fossard, 1976) with modifications to suit this crop was adopted. Ingredients of the media were divided into 8 groups with a total of 22 subgroups (Table I). The 23 group stocks and 2 blanks: A_{1-3}, B_{1-3}, C_{1-3}, D_{1-3}, E_{1-5}, F_{1-6}, G and H, were prepared. These group stocks were mixed in all combinations so that one subgroup from each group will go into one combination of the medium. Agar and activated charcoal (to avoid chemical killing of the explant) were incorporated into all the 2430 combinations after

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Table I. *Levels of ions and compounds in the groups and subgroups of stock solutions used*

Group	Group ions/Compounds	Subgroups					
		1	2	3	4	5	6
A	K	L	M	M			
	Na	O	L	L			
	NH_4	H	H	H			
	NO_3	M	L	H			
	SO_4	M	L	H			
	PO_4	H	M	M			
	I	M	M	H			
	Cl	M	H	L			
B	Ca	L	M	H			
	Mg	M	L	H			
C	Mn	L	M	H			
	Zn	L	M	H			
	BO_3	M	M	L			
	Mo	M	M	H			
	Cu	M	L	H			
	Co	M	H	H			
	Ni	O	O	L			
	V	L	O	L			
D	myo-Inositol	H	H	H			
	Thiamine HCl	M	M	H			
	Pyridoxine HCl	M	L	H			
	Nicotinic acid	L	L	M			
	Glycine	M	H	L			
	Casein Hydrolysate	M	L	L			
	HCHO	O	L	O			
E	NAA	O	L	M	M	H	
	IAA	O	L	M	M	O	
	IPA	O	O	O	L	O	
F	Kinetin	O	L	L	M	M	H
	2iP	O	O	O	L	L	L
	BAP	O	O	L	O	M	H
G	Fe-EDTA	M					
H	Sucrose	M					

L: low, M: medium, H: high, O: nil

adjusting the pH to 5.8. After melting the agar, 15 ml each was dispensed into 25 × 150 mm Borosil culture tubes and autoclaved at 121°C for 15 minutes. After the pressure has come down, the tubes were shaken, three times at 10 mts intervals before agar has solidified, to distribute the charcoal evenly in the medium. All the cultures were incubated at 29±1°C at a photoperiod of 16h light from white fluorescent lamps.

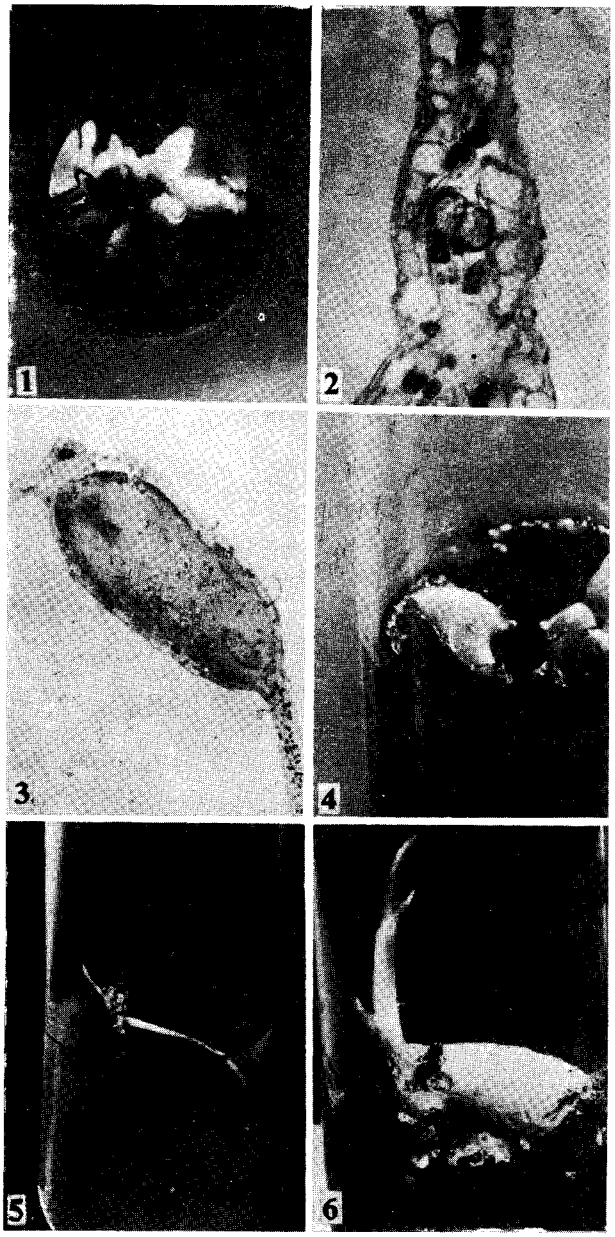

Fig. 1. Leaf explant with few maturing embryoids attached. Fig. 2. Section of the explant after 22 days of incubation showing two embryoids at an early stage of development. Fig. 3. CS of a single embryoid with vascularisation at the two poles still attached to the leaf explant. Fig. 4. A single embryoid with shoot pole root pole and rudimentary haustorium. Fig. 5. Germinating embryoid with shoot and primary root. Fig. 6. Plantlet with scale leaves and secondary roots.

Towards the second half of 1983, we could induce somatic embryogenesis with an incubation period of 16 weeks in 12 per cent of the explants. This time was gradually brought down to 3 weeks by adjusting Mg:K ratio and optimising the hormone level. Upto 48 embryoids were found to develop from a single leaf explant (Fig. 1). These embryoids at the end of 45–60 days maturation period got detached from the parent tissue. They were found to increase in number by a process of adventitious budding from the existing ones. Histological studies showed their origin to be directly from the vascular tissue (Figs. 2 and 3). There was no callus formation at any stage of this development. The haustorial part of the embryoid was suppressed in the course of the development giving a tripolar structure to the embryoid (Fig. 4).

Work since September 1983 was concentrated mainly to find out the requirements of the embryoids to 'germinate' into normal plantlets. Withdrawal of NH_4^+, HCHO and casein hydrolysate and simultaneous increase in auxin cytokinin ratio through several transfers (each time auxin was reduced to 75% and cytokinin increased to 125%) has resulted in the development of shoot (having green scale leaves with sheathing leaf base), and primary root with root-cap along with a primordial haustorium (Figs. 5 and 6). Further refinements in this medium are in progress.

Since there was no callus formation and also because of the fact that the auxin 2, 4–D was avoided throughout this experiment, it is expected that the plantlets produced through this procedure will be exact replicas of the source palm.

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REFERENCES

BRANTON, R. and BLAKE J. 1983. A lovely clone of coconut. *New Scientist.* **98: 554–557.**

FOSSARD De R. A. 1976. *Tissue Culture for Plant Propagators.* **pp. 132–149. The University of New England Printery, Australia.**

PANNETIER, C. and BUFFARD-MOREL, J. 1982. First results of somatic embryo production from leaf tissue of coconut, *Cocos nucifera* **L.** *Oleagineux.* **37: 349–354.**