EMBRYOID FORMATION IN CALLUS TISSUES OF COFFEE

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SUMMARY

The isolation and subculturing of callus tissues from three coffee species is described. In callus tissues of one species, i.e. *Coffea canephora* Pierre ex Froehner ('Robusta' coffee), embryoid and plantlet formation was observed.

1. INTRODUCTION

Experiments of the last few years have shown that embryoids are readily formed in callus tissues of many plants. However, reports from woody plants are scarce (WOLTER 1968). Therefore the isolation of a coffee callus tissue and the induction of embryoids in such tissue is of scientific importance, especially in the field of plant morphogenesis. Besides, there may be practical applications in coffee propagation and breeding.

2. MATERIALS AND METHODS

The material was derived from clones of three coffee species, i.e., *Coffea arabica* L., *Coffea canephora* Pierre ex Froehner ("Robusta" coffee) and *Coffea liberica* Bull ex Hiern. All the clones are growing in the greenhouses of the Department of Tropical Crop Husbandry at Wageningen. The methods used for the sterilization, isolation and subculturing of the plant tissues were described in detail by GAUTHERET (1959).

After sterilization by immersion in a saturated solution of calcium hypochlorite for 15 minutes to 2 hours, stem pieces (c. $1\frac{1}{2}$ cm long) were cut from young plagiotropic and orthotropic shoots. These stem pieces were placed upside down in an agar nutrient medium. Modifications were used of the media developed by Heller (in GAUTHERET 1959) and LINSMAIER & SKOOG (1965).

The explants and subcultured callus tissues were placed in the dark at 28 °C or in a growing cabinet in which a 12 hour light period (Philips fluorescence tubes TLF 33, t = 30 °C) alternated with a 12 hour dark period (t = 25 °C).

3. RESULTS

3.1. Isolation and subculturing of callus tissue

The experiments in 1967, 1968 and the beginning of 1969 with "Arabica" and "Robusta" material proved that:

1. Only explants from the soft green internodes of the apical part of vigorously growing orthotropic shoots form an abundant callus tissue suitable for subculturing.

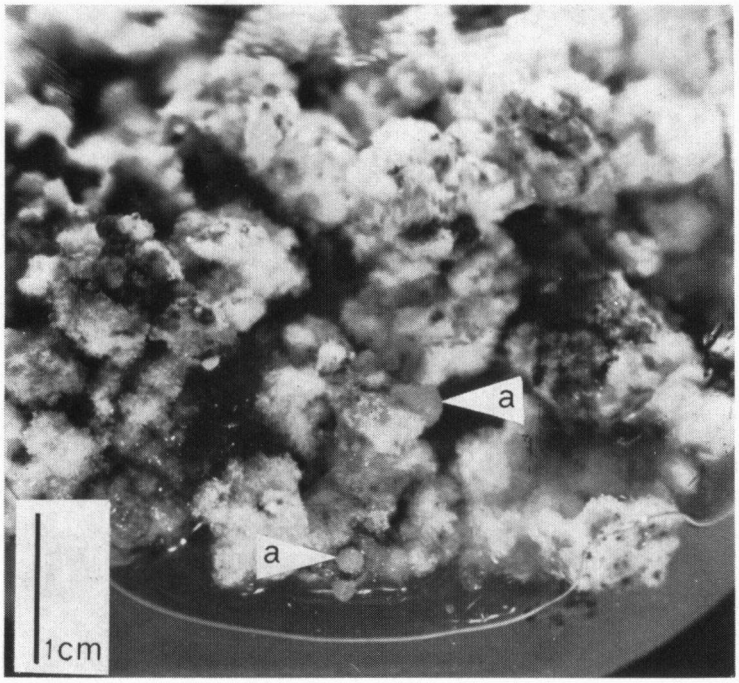

Fig. 1. Spongy type of callus tissue (first transfer). a. Clusters of globular embryoids.

2. Sterilization longer than 15–20 minutes and damage of the outside layers of the explants should be avoided.
3. Modifications of Heller's medium are not suitable for coffee callus cultures.
4. Initial callus growth is only achieved in the dark.

The result of other treatments was an excessive darkening of the explants, the nutrient media and the subcultured callus tissues. The dark substance seems to be poisonous for the living tissues and only very slowly growing callus tissue can be isolated.

If the above mentioned criteria are considered, an abundant, fast growing callus tissue can easily be obtained within a few months. The introduction of a new coffee species (*C. liberica*) into the experiments proved the value of this

Table 1. Composition of modified LINSMAIER & SKOOG (1965) medium.

Constituents	mg/l	constituents	mg/l	constituents	mg/l
NH_4NO_3	1650	H_3BO_3	6.2	Sucrose	30,000
KNO_3	1900	$MnSO_4 . 4\ H_2O$	22.3	Agar agar	10,000
$CaCl_2 . 2\ H_2O$	440	$ZnSO_4 . 7\ H_2O$	8.6	Thiamine HCl	1.0
$MgSO_4 . 7\ H_2O$	370	KI	0.8	L-Cysteine HCl	10
KH_2PO_4	170	$Na_2MoO_4 . 2\ H_2O$	0.25	Inositol (Meso)	100
Na_2EDTA	37.3	$CuSO_4 . 5\ H_2O$	0.025	Kinetin	0.1
$FeSO_4 . 7\ H_2O$	27.8	$CoCl_2 . 6\ H_2O$	0.025	2,4-D	0.1
				or NAA (K-salt)	1.0

statement. Only 24 explants were needed for the formation of a fast growing callus tissue.

A favourable and simple Linsmaier and Skoog modification for callus tissue of the three coffee species is given in *table 1*.

Two types of callus tissue are formed. The first type has a white spongy appearance, caused by air-filled spaces between the cells. The cells are elongated and arranged in long filaments (*fig. 1*). The second type of tissue is more compact (*fig. 2*). The cells are isodiametric and under the microscope the callus looks like parenchyma tissue. Both tissues are homogeneous but transition is possible.

3.2. Embryoid formation

Embryoid formation until now has only been observed in callus tissue of "Robusta" coffee. From the compact type of tissue, isolated cells or cell-clusters fall down on the uncovered surface of the nutrient medium. These cells differentiate and form conglomerates of multicellular yellowish globules (*fig. 2*). After elongation and development of two leaf primordia at one end the globules are transformed into embryoids (*fig. 3*). If the culture tubes or bottles are placed in the light the leaf primordia become green. The embryoids develop into small isolated plantlets with leaves, hypocotyl and primary root (*fig. 4*).

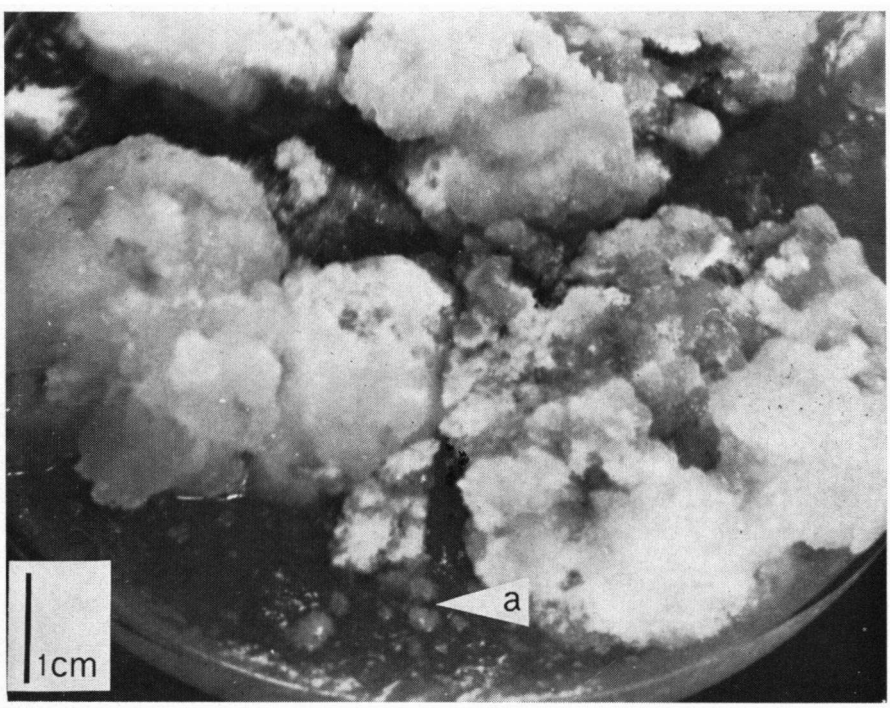

Fig. 2. Compact type of callus tissue (fourth transfer). a. Clusters of globular embryoids on uncovered agar medium.

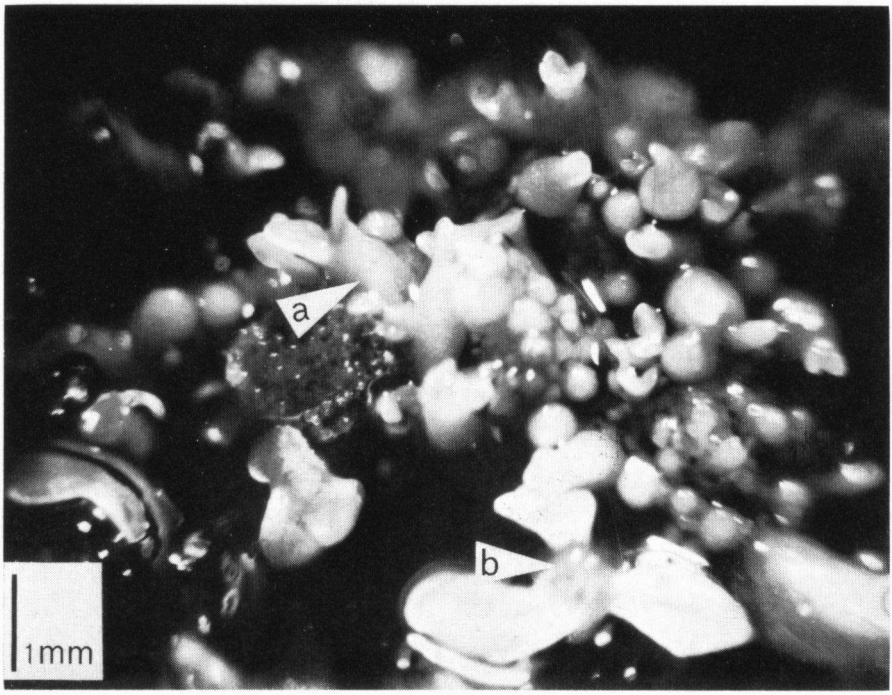

Fig. 3. Embryoid development. a. Embryoid with two leaf primordia. b. Plantlet with three 'cotyledons'.

These embryoids were observed in first generation and in fourth generation callus tissues.

4. DISCUSSION

The experiments have shown the paramount importance of the nature of the starting material. Only the soft green internodes at the top of vigorous orthotropic shoots are suitable for the isolation of a fast growing callus tissue. The subcultured coffee callus tissues are susceptible to changes in temperature. After a rather short period – when the dark growing cabinet was out of order for a week – at a temperature of about 20 °C all the callus tissues ceased growing. None of them recovered after repair of the cabinet and new isolations had to be made. The same is true for temperature rises. When a photograph is made, heat of the light kills tissues and especially green plantlets.

The globular embryoids are only formed at the periphery of callus tissue or isolated at uncovered medium (*figs. 1, 2* and *5*). Perhaps cell isolation is a necessity for the formation of embryoids. On the other hand, if a cluster of globules is formed they propagate fast. The presence of other embryoids or plantlets seems to have a stimulating effect on the formation of new embryoids (*cf.* KONAR & NATARAJA 1969).

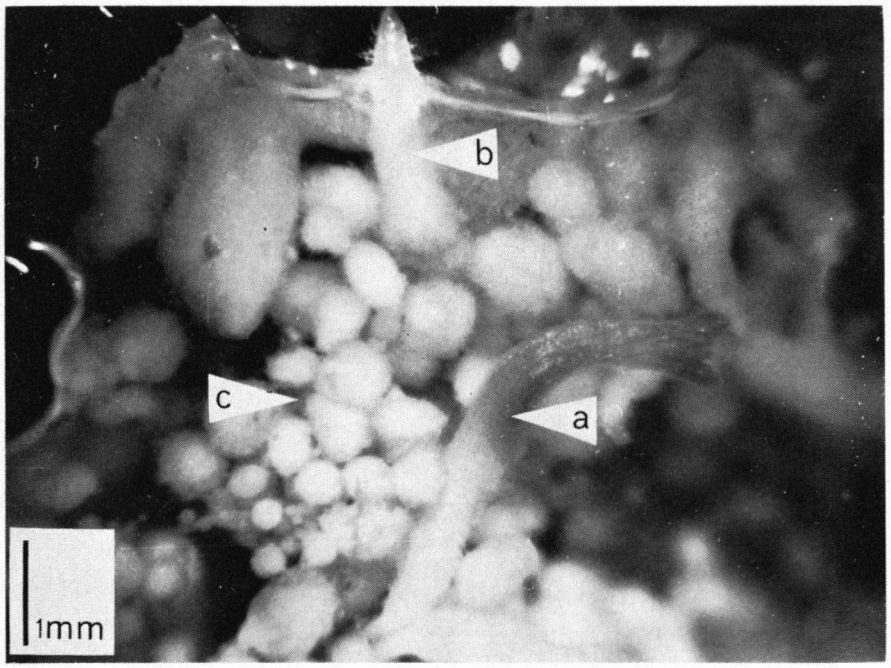

Fig. 4. Embryoid and plantlet development. a. Small plantlet with cotyledons, hypocotyl and primary root. b. Embryoid with developing primary root. c. Globular embryoids.

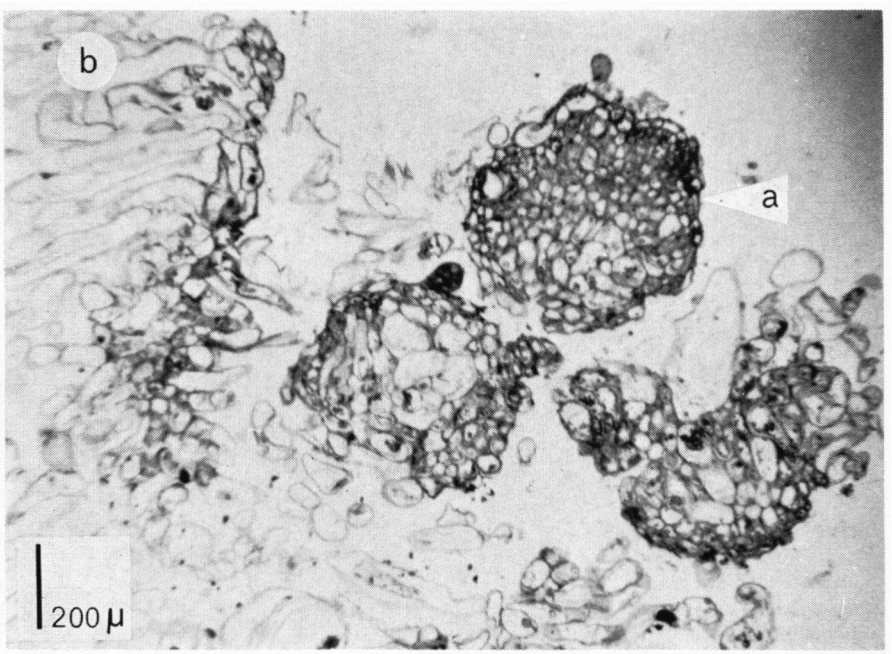

Fig. 5. Globular embryoids developing at the periphery of callus tissue. a. Globular tissue embryoid. b. Filamentous callus.

The formation of large plantlets (1–2 cm long) on the medium without dedifferentiation is rather unique (*cf.* WILMAR & HELLENDOORN 1968).

Further experiments are in preparation, for instance the use of suspension cultures and the isolation and rearing of the plantlets.

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