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THE HISTOGENESIS OF CALLUS IN COFFEA CANEPHORA STEM EXPLANTS AND THE DISCOVERY OF EARLY EMBRYOID INITIATION

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SUMMARY

In stem explants of *Coffea canephora* all parenchymatic tissues between epidermis and vascular cambium were found to be capable of being transformed into callus tissue, the cortex being the most active layer in this respect.

The initial callus cells enlarge and divide periclinally. As a result, the epidermis is ruptured so that the callus emerges. The possible rols of the parenchymatic tissues of the stele in the formation of callus occasionally observed on the cut surfaces of the explants has not been further investigated.

After 14 days of incubation proembryo-like structures could already be observed in the zone of callus formation.

1. INTRODUCTION

The application of in vitro techniques in the improvement of coffee has been discussed in a review of MONACO et al. (1977). In 1970 STARITSKY reported embryoid and plantlet development in *Coffea canephora* callus tissue. As a follow up of this work he developed techniques for the clonal mass propagation of *C. canephora* in vitro (STARITSKY 1980). The genetic uniformity of the young trees derived in this way still has to be established. Genetic variability already may find its origin in the parent tissue (e.g. endomitotic polyploids) from which the callus intermediate in the regeneration process arises. In his review SYBENGA (1960) refers to a publication of LELIVELD (1939) who found mitotic irregularities in root tip cells of *C. canephora* seedlings. Above-ground parts have not been investigated.

The study of the histogenesis of callus in *C. canephora* stem explants is the first step to reveal the nature of the embryoids. The initiation and development of the callus tissue was observed by means of light and scanning electron microscopy.

2. MATERIALS AND METHODS

The material originated from trees of *Coffea canephora* Pierre ex Froehner clone SA 158, growing in the greenhouses of the Department of Tropical Crop Science, Wageningen. Non-suberised parts of orthotropic shoots were sterilised in a 1% sodium hypochlorite solution for 10 minutes followed by two rinsings with sterilised water. The internodes were cut into transverse sections c. 1.5 cm long. To reduce the harmful effect of toxic oxidation products of phenolic exudates the stem segments were, consecutively, rinsed in a sterile L-cysteine-HCl solution (100 mg/l), 96% ethanol and sterile water.

The stem segments were placed upside down in culture tubes (160 × 22 × 1 mm) containing 15 ml agar nutrient medium. The tubes were closed by cotton wool plugs, sealed with parafilm and placed in a dark incubator at 28 °C.

The nutrient medium consisted of MS (Murashige & Skoog) inorganic salts, 1 mg/l thiamin, 10 mg/l L-cysteine-HCl, 2 mg/l glycine, 1 mg/l adenine, 100 mg/l meso-inositol. 100 mg/l casein hydrolysate, 30 g/l saccharose, 8 g/l agar (Difco) and as growth regulators 5 mg/l IBA + 1 mg/l BA. The culture tubes with medium were autoclaved at 110 °C for 15 minutes.

After an incubation period of 0, 1, 2, 3, 5, 7, 14, 21 or 26 days the explants were removed from the tubes and fixed in 10% acrolein at 0 °C, dehydrated and embedded in glycol-metacrylate polymer, according to the methods described by FEDER & O'BRIEN (1968). The plastic blocks were sectioned with a steel knife on a standard rotary microtome at 6 μm. The sections were stained following the periodic acid-Schiff (PAS) staining procedure.

SEM photographs were taken with a Cambridge Stereoscan Mark 2a from fresh and uncoated material.

3. RESULTS

A cross-section of a normal young, green internode shows the following zonation (*photographs 1* and *2*):

a. an epidermal layer with a thin cuticle;
b. a cortex with at its periphery small parenchymatic cells, in its middle several layers of large, thin-walled parenchymatic cells, and at its inner side, confined by an endodermoid layer in the form of a starch sheath, again a few layers of smaller cells;
c. the stele, consisting of pericyclic fibres, phloem, vascular cambium, xylem and pith parenchyma (see also VAROUSSIEAU 1940, METCALFE & CHALK 1950).

Explants incubated for a few days retain the structure as described above.

After the sixth day of incubation the stem explants show local swellings, and changes can be observed in the anatomy of the cortex where parenchyma cells enlarge and begin to divide periclinally. *Photograph 3* shows that this activity is not restricted to any particular place but may occur in the outer, middle as well as the inner layers of the cortex. The resulting callus initials can easily be distin-

guished from the normal cells because they are larger and more vacuolized, and have only a thin layer of cytoplasm. The cells originating from these callus initials are meristematic and contribute to the formation of a rather homogeneous callus tissue whose cells are elongated and arranged in long, radial rows. The callus tissue is spongy because of the presence of air-filled intercellular spaces.

In older stages the formation of callus initials is no longer restricted to the parenchymatic cortex layers but also other layers may become locally meristematic. Stretching and division of cells can be seen in the starch sheath (*Photograph 4*) and in the parenchyma cells centripetally of the pericyclic fibres (*Photographs 5* and *6*). The latter activity causes the rupture and fragmentation of the cylinder of pericyclic fibres. Callus formation in the epidermal layer, the pericyclic fibres, the vascular cambium, the xylem and the pith was never observed in the present experiments. Continued periclinal divisions and the radial stretching of the callus cells in the cortex cause the rupture of the epidermis and the emergence of the callus cells from 9 to 10 days of incubation onwards. Proembryo-like structures, showing a distinct similarity to zygotic proembryos of *Coffea* as described by MOENS (1964), may be seen after 14 days of incubation. Occasionally, callus tissue was observed at the cut ends of the explants. The importance for its initiation of, respectively, the parenchymatic tissues of the cortex and of the stele has not, however, been investigated.

4. CONCLUSIONS

In the present experiments the formation of callus initials was found to take place in all parenchymatic tissues between the epidermis and the vascular cambium, the role of the cortex layers being preponderant. No callus initials were observed in the epidermis, the pericyclic fibres, the vascular cambium, the xylem, or the pith parenchyma. The epidermis and the sheath of the pericyclic fibres do not show the capacity to increase in circumference and become broken up.

The early formation of proembyoids may explain the explosive appearance of embryoids of the same size after an incubation period of 16 weeks (STARITSKY 1980). Possibly the isolation of single cells from their neighbours (*Photograph 5* and *7*) is favourable for proembryoid formation after which further initiation is temporarily surpressed. However, only further microscopic research could support this assumption.

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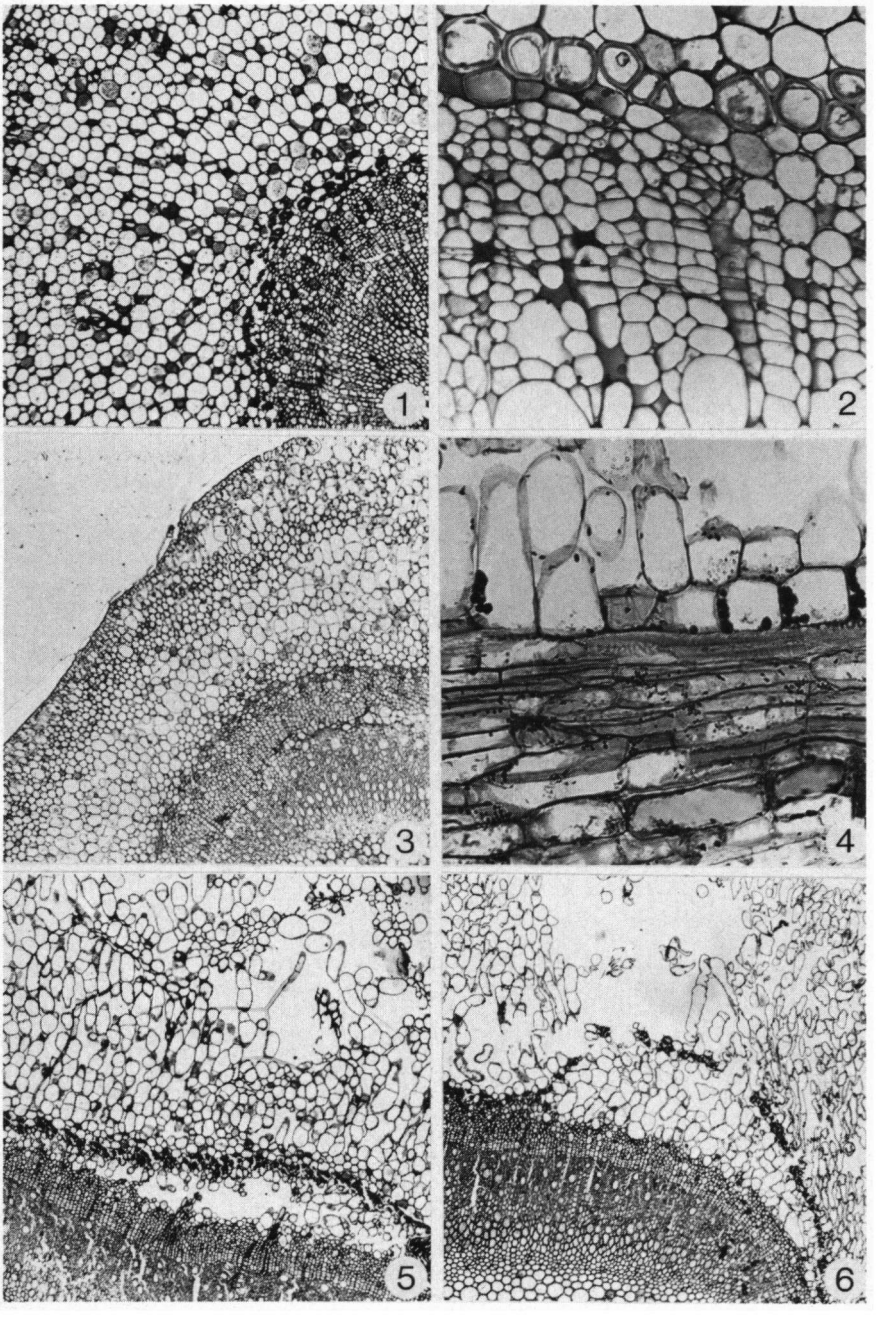
Photogr. 1. *Coffea canephora.* Cross section of a normal, young stem internode; Photogr. 2. Detail of the endodermoid layer, pericyclic fibres, phloem, vascular cambium and xylem; Photogr. 3. Cross sections of a stem explant after 7 days incubation showing callus initiation in the cortical layers; Photogr. 4. Detail of a longitudinal section of a 26 days old stem explant with stretching and division of the starch sheath; Photogr. 5 and 6. Cross sections of stem explants after 14 and 21 days of incubation showing callus formation in the tissue underlying the pericyclic fibres.
Photogr. 7. *Coffea canephora.* Detail showing ruptured epidermal layer with underlying callus tissue; Photogr. 8, 9 and 10. SEM photographs of a starting rupture, ruptured epidermis and emerging callus tissue respectively; Photogr. 11 and 12. Proembryo-like cell groups in callus tissue of a 14 days-old stem explant.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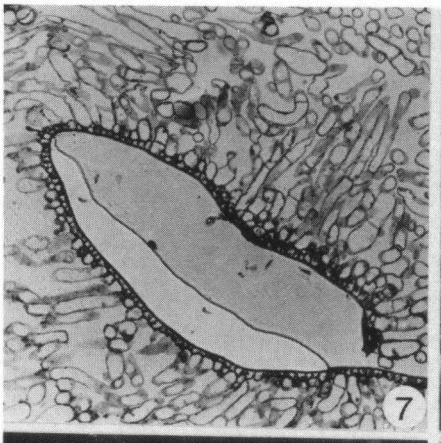
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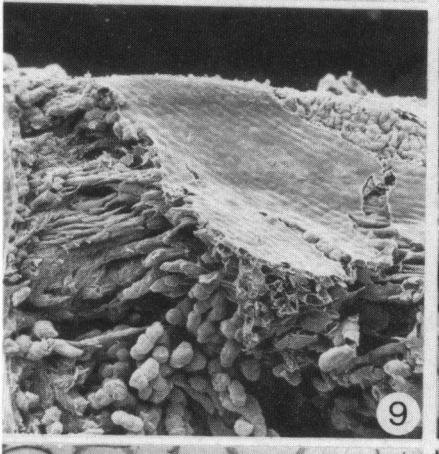
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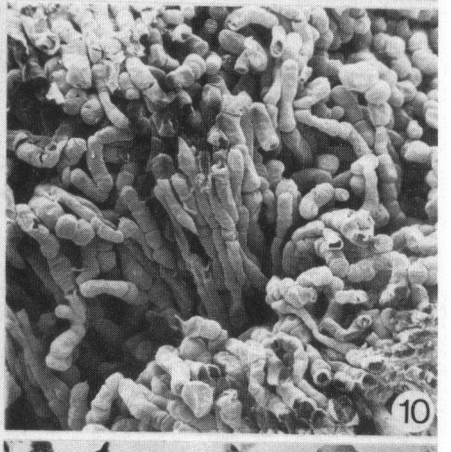
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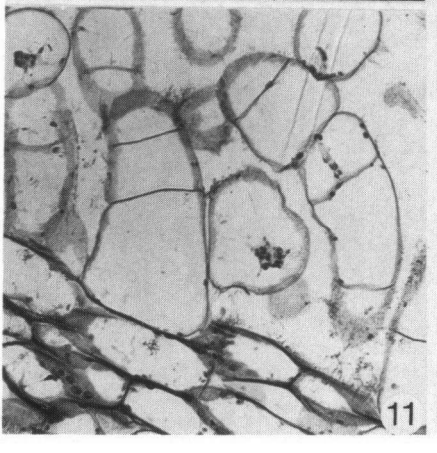
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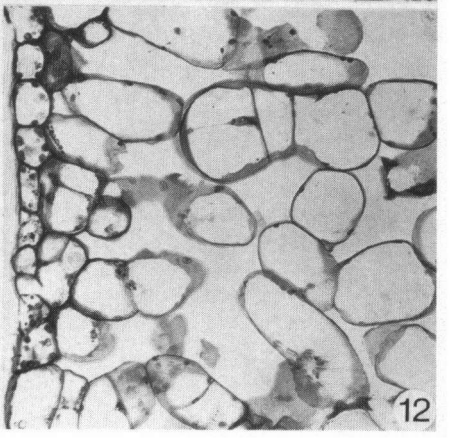
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