Histological Studies on Cultured Cells of Cassia Senna

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Very nearly all callus cultures are derived from tissues composed of vacuolated cells. Two major types of vacuolated cells are involved: those of the vascular ambium which may already be in a state of active division and variety of parenchyma cells which are quiescent and have to be induced to divide. Rapid division of tissue leads to the callus from which large masses of highly vacuolated cells are formed.

Materials and Methods:

Fresh tissues from callus cultures (13 months growth) were mounted in glycerol and chloralhydrate, and viewed under the transmitted light microscope.

The procedure adopted for the preparation of the callus tissue for ultra-structural examination was according to table 1. Approximately 500*u* sections were cut using LKB ultratome III and viewed using AEI Transmission Electron Microscope 6 after staining the sections with uranyl acetate and lead citrate.

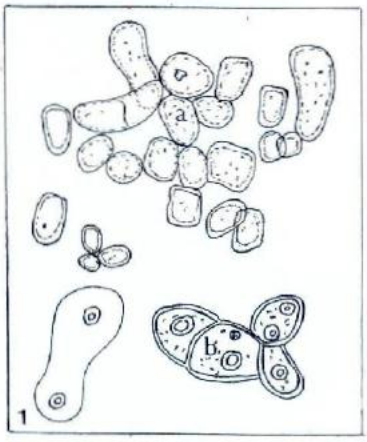
TABLE 1

EMBEDDING PROCEDURE

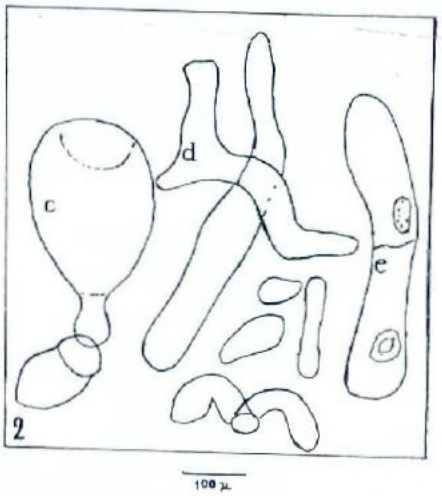
		Temp.	*Time*
Pre-fixative	Glutaraldehyde 3% M/10 Phosphate Buffer pH 7.4	0 C	2 hrs
Wash	M/10 Phosphate Buffer pH 7.4	0 C	21 hrs
Post-fixative	Milloning phosphate Buffered Osmium 1% pH 7.4	0 C	2 hrs
Wash	50% Alcohol	0 C	15 mins
Dehydration	a. 70% alcohol	0 C	20 mins
	b. 90% alcohol	0 C	15 mins
	c. 100% ethanol	0 C	30 mins
	d. 100% ethanol	Room	40 mins
Infiltration	a. Propylene oxide	Room	35 mins
	b. 50/50 P. Oxide/ Araldite	Room	24 hrs
Curing	37 C in an oven		24 hrs
	60 C in an oven		72 hrs

Observations:

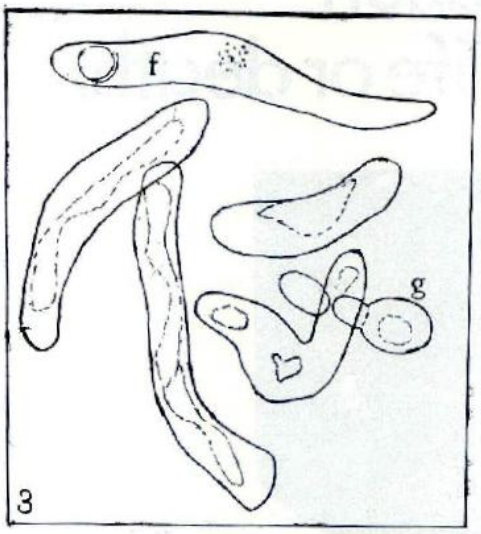
Callus tissues of *Cassia senna* are composed of a high proportion of vacuolated cells. Cells studied under the light microscope were found to be largely thin-walled isodiametric parenchymatous cells varying from 50*u* to 540*u* in diameter; size and form depending largely on the location of the cell in callus from which it was derived. Apart from the sizes, there was little variation in appearance of cells. Diagrams 1,2 and 3 illustrate the general structure of cells as viewed under light microscope both in low and high magnifications. Range of cell form and structure is also illustrated in these diagrams. The vacuoles are enlarged and take a large proportion of cell volume. With continued development cells become larger and more highly vacuolated, thus achieving a typical parenchymatous appearance (Rai et al 1974). They are often markedly elongated and characteristically shaped (Diagram 2). Others originating from the central region of the callus are usually small, compact and bearing a spherical profile. They are relatively uniform in their sizes (Diagram 1). Cells originating from the outer surface of the callus are large, elongated and of varied shapes and structures. They are sometimes seen containing cytoplasmic material and sometimes they are empty. Some cells are similar in appearance to budding yeast cells.



a. Cells are small and uniform in their sizes, compact and sometimes overlapping, granules thinly scattered within the cells.
b. Cells forming a chain, visible nucleus, single cell with two nuclei occasionally seen.



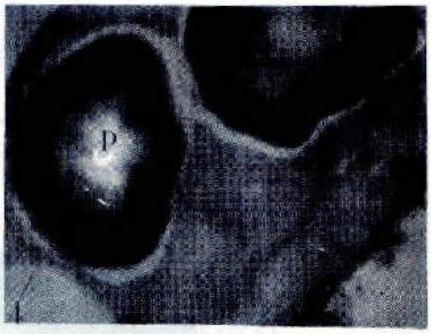
c. Cells with a "yeast-budding" profile.
d. Cell with a curious shape (slipper-like), invariably present in cell cultures in small number.
e. Division of the cell appears almost complete, divided by a septum.



f. Elongated cells, nucleus usually in the corner end, granules scattered and sometimes aggregated.
g. Cells with a "yeast-budding" profile.

There is little structural variation in callus cells of *Cassia senna* with the cells of other cultures grown in our tissue culture laboratories and examined microscopically, e.g. *Parthenicissus tricuspidata, Dacus carota* etc., It appears that environmental factors may have more effect upon the shape and form of cells *in vitro* rather than their origin.

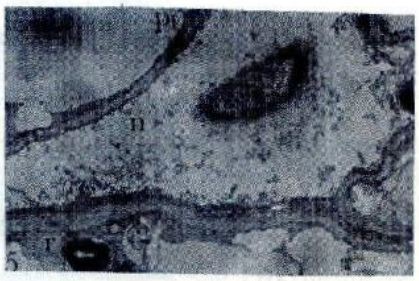
Photograph 4,5 and 6 illustrate the ultrastructural features of cells of *Cassia senna*. Callus cells are highly vacuolated, usually having only one large central vacuole surrounded by a thin layer of cytoplasm. The nucleus is embedded in *ectoplasm* and organelles tend to congregate around it. Nucleoli are clearly visible in the cells undergoing high mitotic activity. Plastids are in abundance. They are devoid of starch grains. Plastids were generally scattered within the cell but occasionally may be found in tight cluster. The final stages of cell expansion involve utilization of most of the starch and therefore senescent cells are usually devoid of it. The small vacuoles near the nucleus do not contain osmophilic material. Rough endoplasmic reticulum and ribosomes are frequently present. On one side of the cell wall the plasmalemma may be very wavy, whilst on the other side the plasma membrane forms an even line. Nucleus assumes a more rounded profile but sometimes an irregular shape was observed too. The nucleiolar material is loosely packed. The nucleus contains a large central electron transparent region with granular material, sometimes cytoplasmic strands traverse the central vacuole. This type of structure suggests that cells are in the process of division and multiplication. Mitochondria are present and some contain electron-dense granules which are larger than ribosomes. Cytoplasmic and membranous protrusions were observed but rarely. Occasionally, some vacuoles were seen to contain dense substances. These unidentified electron-dense bodies were also found in the cytoplasm. These dense materials may perhaps be interpreted as the deposits of secondary products. This interpretation is basically indirect and based on assumption.



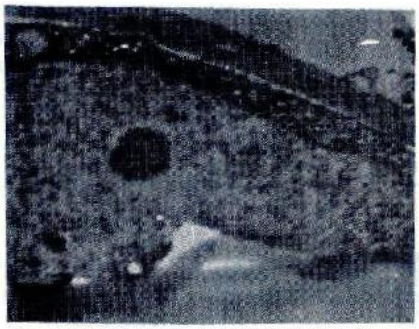
Showing large plastids (devoid of starch grains) and a smooth and regular cell wall 7,000.

Abbreviations:

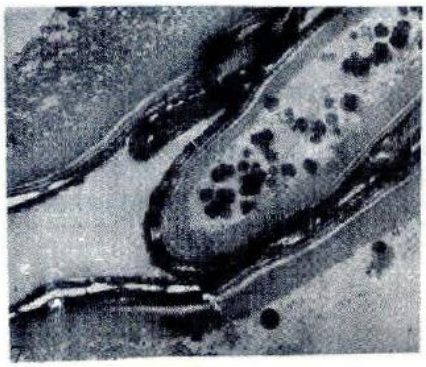
n–nucleus
ne–nucleolus
cw–cell wall
v–vacuole
p–plastid
pi–proteinaceous inclusions
r–ribosome



Cytoplasmic details of a cell showing enlarged nucleous with an embedded nucleoli. Thin peripheral cytoplasmic layer contains proteinaceous inclusions. Ribosomes are centred in the periphery of the nucleus. 12,500.



Detail of the cell showing a single vacuole. The nucleus is filled with fine granule like structures. 10,200.



Vacuole containing electron-dense bodies, perhaps the deposits of secondary products (anthraquinones) 25,000

REFERENCE

P.P. Rai, T.D. Turner and S.L. Greensmith, 1974, Journa lof Pharmacy and Pharmacology, 26, 722–726.