

Pharmacy, UWIST, Cardiff, CFI 3NU, U. K. in devising the apparatus for plant tissue culture.

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NODAL ORGANIZATION IN THE FAMILY ACANTHACEAE

NODAL anatomy of the family Acanthaceae belonging to the two subfamilies, viz., Thunbergioideae and Acanthoideae has been investigated. The opposite, decussate phyllotaxy observable in the majority of the taxa of the family can be confirmed by the transection of the node of *Thunbergia erecta* of Thunbergioideae which shows a unilacunar, unitrace condition (Fig. 1 A). As illustrated in Fig. 1 B-J, the representatives of the subfamily Acanthoideae which include

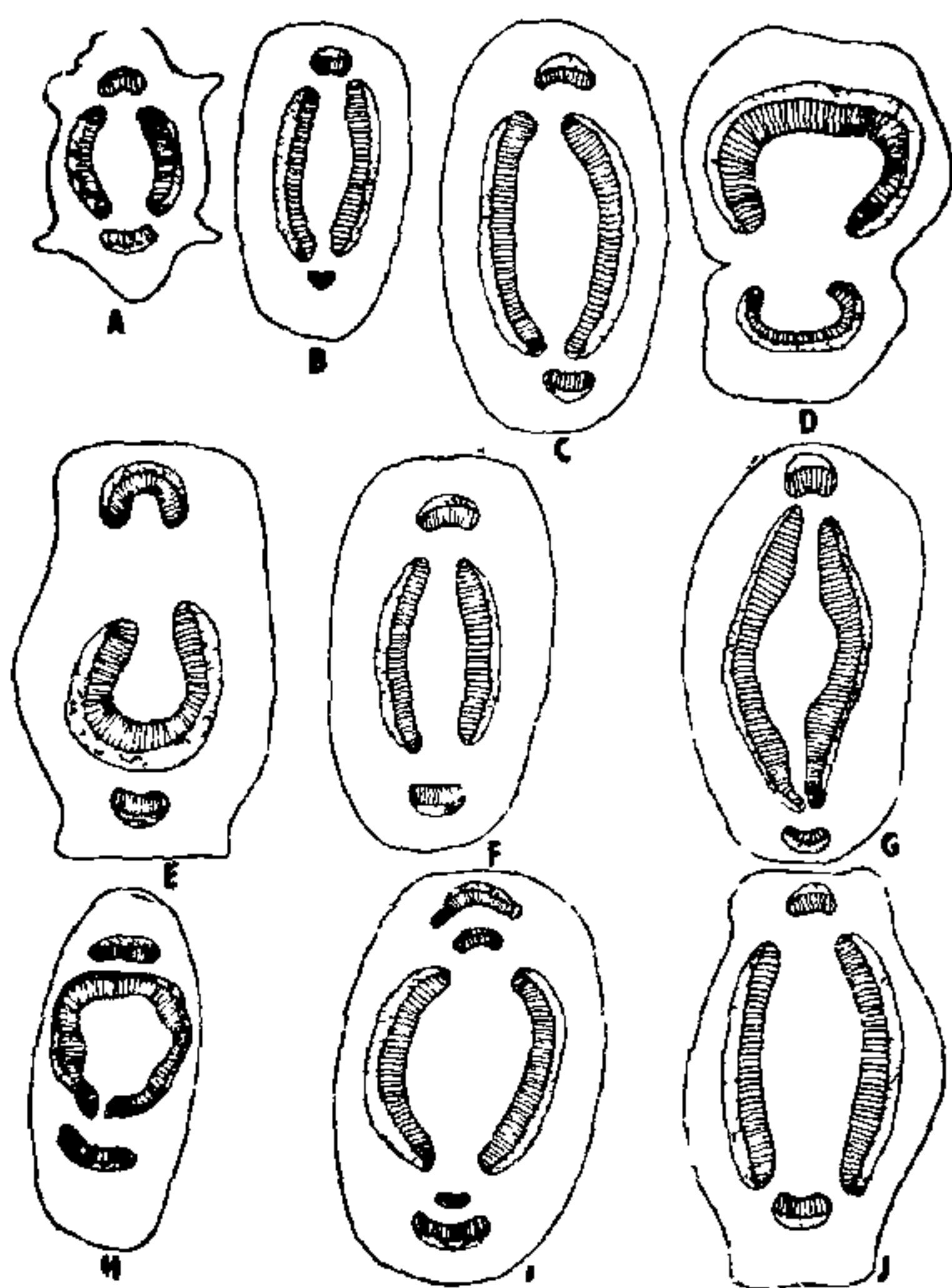


FIG. 1 A-J. Acanthaceae—Nodal Organization. Illustrations of transverse sections of the stems at the nodal regions of the members of the subfamilies Thunbergioideae (A) and Acanthoideae (B-J) exhibiting the distribution of vascular tissues. A, *Thunbergia erecta*; B, *Hygrophila polysperma*; C, *Daedalacanthus nervosus*; D, *Barleria prionitis*; E, *Crossandra infundibuliformis*; F, *Andrographis echioides*; G, *Adhatoda vasica*; H, *Justicia betonica*; I, *J. gendarussa*; J, *Peristrophe bicalyculata*. All $\times 40$.

Hygrophila polysperma (tribe Hygrophilae), *Daedalacanthus nervosus* (Ruellieae), *Barleria prionitis* (Barle-riaceae), *Crossandra infundibuliformis* (Andrographideae), *Adhatoda vasica*, *Justicia betonica*, *J. gendarussa*, and *Peristrophe bicalyculata*, possess uniformly unilacunar condition. This single trace enters the petiole, thus giving rise to single-stranded condition in the leaf. The marked similarity of the nodal type also gives the clue to the uniformity of the characters of the family.

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SOME CYTOCHEMICAL OBSERVATIONS ON THE GIANT CELLS IN HUMAN PLACENTA

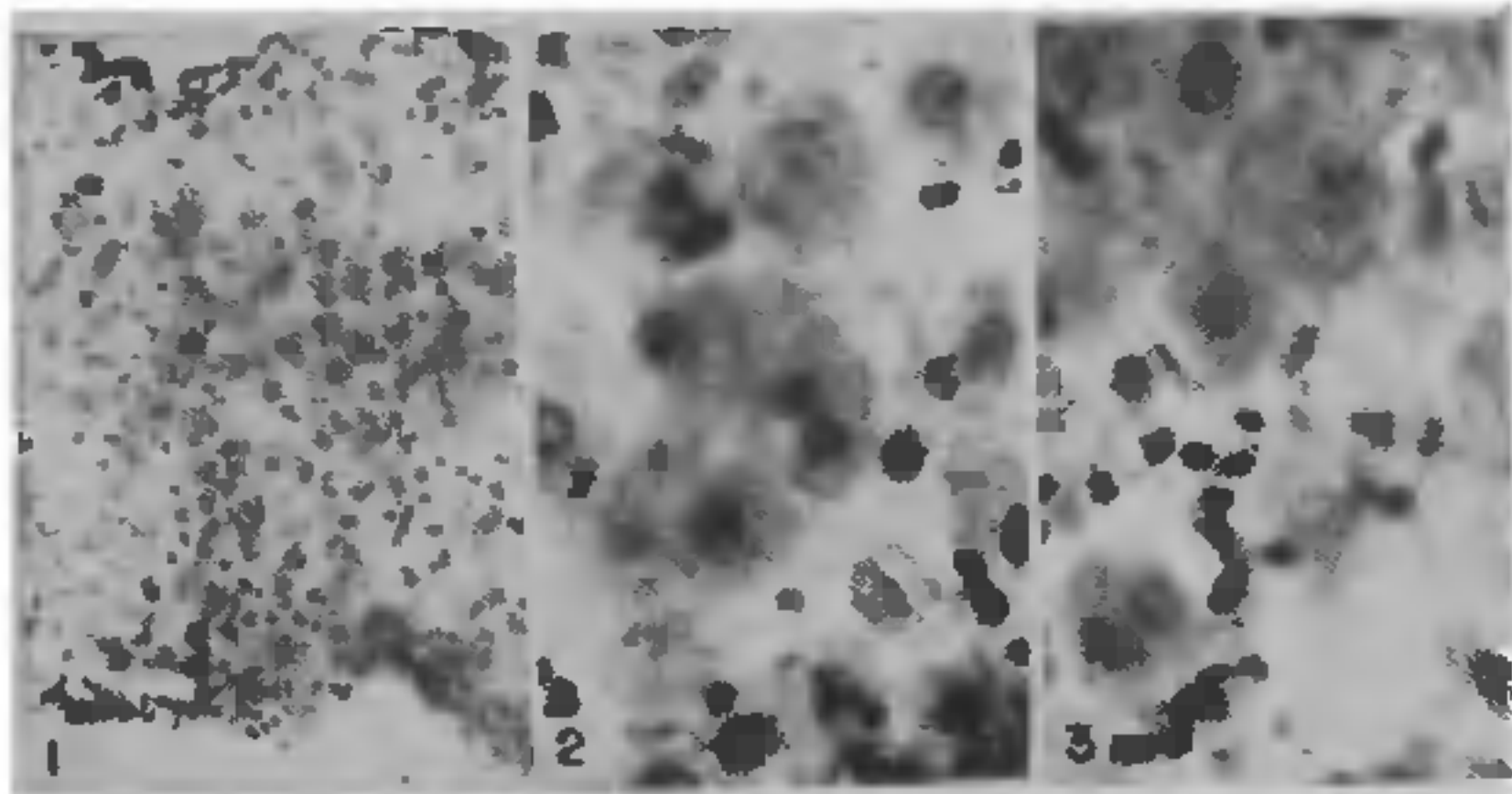
ALTHOUGH placental giant cells have been observed in a wide variety of mammals, such as bats, primates, carnivores and ungulates, their functions have not been clearly understood so far. They have been assigned various functions such as glycogen storage¹, transport of iron from mother to foetus^{2,3}, enzyme action on capillary walls⁴ phagocytic activity⁵ and endocrine function^{6,7}.

During the course of a detailed histochemical study of the human placenta at different stages of pregnancy the present authors noted a sudden and enormous increase in the population of giant cells in the human placenta during early pregnancy after which their number dropped equally suddenly. This spectacular rise and fall in the number of these cells prompted the present authors to undertake a detailed cytochemical examination of the giant cells of the human placenta with a view to understanding their possible functions.

Human placenta at different stages of gestation was obtained from normal pregnant women either after medical termination of pregnancy or after full term delivery. Each placenta was cut into numerous small pieces and fixed in neutral formalin, calcium acetate-formalin, Rossman's fixative and Carnoy's fixative. The tissues were processed in the usual manner and sectioned at 5 to 8 μ thickness after embedding in paraffin. Apart from routine staining by Ehrlich's haematoxyline and eosin, selected sections of suitably fixed pieces of each placenta were stained by periodic acid-Schiff procedure⁸ (PAS), Heidenhain's azan⁹, Cason's modifications of Mallory azan procedure¹⁰, aldehyde fuchsin-PAS-orange G (AF-PAS-OG)¹¹, methylene blue¹², methyl green-pyronin¹³, Bauer-Feulgen technique⁸. Some sections in each series were subjected to staining procedures for the detec-

tion of different kinds of mucopolysaccharides¹⁴⁻¹⁶. The above mentioned staining procedures include not only the techniques adopted for identifying different kinds of basophilia and mucins but also those which have been employed to demonstrate the different types of gonadotrophs in the adenohypophysis of mammals.

The giant cells, which occur on the maternal side of the deciduo-placental junction in human placenta (Fig. 1) reach their maximum population during 6 to 10 weeks of pregnancy. In routine histological preparations (haematoxylin-eosin), the giant cells exhibit a dark staining eosinophilic cytoplasm containing one or two vesicular nuclei (Fig. 2) each with a well defined nucleolus and flake-like chromatin granules, located subjacent the nuclear membrane. In sections stained by PAS the giant cells take a dark purple stain (Fig. 3) indicating the presence of large amounts of



FIGS. 1-3. Fig. 1. Part of the deciduo-placental at 8 week's gestation to show the large number of giant cells (haematoxylin-eosin). $\times 80$. Fig. 2. A part of figure 1 magnified to show the giant cells with vesicular nuclei. $\times 360$. Fig. 3. Part of the deciduo-placental junction of human placenta at 8 week's gestation (PAS-Weigert haematoxylin staining). Note the PAS-positive cytoplasm. $\times 360$.

mucopolysaccharides in them. Apart from the presence of moderate quantities of glycogen as demonstrated by Bauer-Feulgen procedure, the cells also contain large quantities of saliva-resistant PAS-positive material. Methylene blue staining gave intense basophilia. Similarly, considerable amounts of RNA was localized as revealed by methyl green-pyronin procedure. The elevated protein synthesis as revealed by localization of large amounts of RNA associated with high basophilia and occurrence of large quantities of saliva-resistant PAS-positive material, is an almost certain indication of synthetic activity of glycoprotein complexes. Cason's modification of Mallory azan procedure, sequential staining in AF-PAS-OG and aniline blue-PAS-OG, Halmi's method and its modification by Adam¹⁷ by adding performic acid as an oxidizing agent have been widely employed to demonstrate the different types of gonadotrophs in the adenohypophysis of mammals. These techniques,

which have been employed for the giant cells in the present investigation, have revealed that these cells react nearly like the gonadotrophs of the adenohypophysis of mammals. Phenylhydrozine-PAS¹⁸ reaction, alcian blue (pH 2.5)¹⁰ and staining by azure A (pH 3.0)²⁰ revealed the presence of sialomucins.

Previous work has revealed that HCG contains (in addition to glycoproteins) hexose, hexosamine and sialic acid. The present investigations have, therefore, revealed that, apart from storing glycogen, the giant cells are also in some way concerned in the production of HCG. It is interesting here to note that urinary excretion of HCG reaches maximum values between the 8th and 12th weeks of gestation²¹⁻²³. This is precisely the period when the population of giant cells also reaches its maximum values. The disappearance of giant cells from the utero-placental junction also synchronizes with the sudden fall in the urinary excretion of HCG. This is an added circumstantial evidence which suggests that the giant cells in human placenta may be involved in the production of HCG.

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"SUBSTRATE DECOCTION" A NEW TECHNIQUE TO ISOLATE THE MYXOMYCETES FROM DEAD PLANT MATERIALS

MYXOMYCETES in general do show neither substrate (host) specificity nor nutritional selectivity. However, some members seem to prefer a distinct type of substrate¹. Olive^{2,3} stated that initiation of fruiting in some protostelids can be correlated with the type of nutrition. Broadly speaking, nutrition of the Myxomycetes is of a 'HOLOZOIC' type.

By using a variety of laboratory techniques plasmodia or fructifications of Myxomycetes have been obtained from a variety of materials: rainwater⁴, debris⁵, or the atmosphere⁶ by exposing agar plates or coated slides to wind from which isolation can later be made. During the last 15-20 years, several authors have tried to isolate and culture Myxomycetes with the help of the 'moist chamber' technique and using bacteria, corn meal, lactose and yeast extract as food for growing plasmodia^{2-4,7-10}.

The present note reports the successful isolation of Myxomycetes by using a 'Substrate Decoction' nutrient solution.

Dead and half decayed plant material (leaves and twigs), collected at random from Aurangabad City, were used in this study. After keeping aside 4-5 pieces of each sample as a source of inoculum, the remaining material was used for making the decoction. Batches of 500 g plant material mixed with five liters of tap water in beakers, were autoclaved for 20 min at 15 lb/inch square.

Moist chambers were prepared by using Whatman filter paper No. 1, and sterile tap water. The 'substrate decoction' (5 ml) along with 15 ml of sterile tap water was poured into each moist chamber, inoculated with 4-5 pieces of the inoculum. All the inoculated moist chambers were kept undisturbed at 24 ± 1° C and away from the direct sunlight.

On the 4th day 5 ml of 'substrate decoction' diluted with 15 ml of sterile tap water was added to each chamber. Subsequent moistening was done on the 7th and 8th days and later on at 3 to 4 days interval according to the situation of chambers and the organisms growing in it. In some moist chambers plasmodia were conspicuous on the 8th day. The stock 'substrate decoction' showed growth of several bacteria, and other microscopic organisms. Hence, it was realised that special feeding to plasmodia is not required.

With the use of 'substrate decoction' the following 9 species (of 7 genera) were isolated:

- (1) *Dictyostelium* sp.
- (2) *Licea* sp.
- (3) *Cribraria violacea*
- (4) *Perichaena depressa*
- (5) *Physarella oblonga*
- (6) *Didymium crustaceum*
- (7) *D. squamulosum*
- (8) *D. dubium*
- (9) *Physarum cinereum*.

The number of genera and species isolated by this method is large as compared to the reports of previous authors.

In no case were fructifications observed before the 15th day. In some cases it took nearly two months. Plasmodia were observed in 56% of the moist chambers but fruiting was observed only in 35% of the chambers. Failure of fruiting in the case of the remaining 21% of the moist chambers, in which plasmodia were observed, may be due to pH of the medium, temperature, or photoperiodic effect. In general, it can be said that 'substrate decoction' prepared from the mixture of different substrates serves as a very favourable broad range medium (food), for the isolation of Myxomycetes. The 'substrate decoction' contains several organic materials in a dissolved state and in the form of small particulate suspension which favour the growth of bacteria and other micro-organisms and provides food for the growing plasmodia. The 'substrate decoction' contains also several inorganic salts required by the Myxomycetes themselves and the bacteria.

In this technique there is no need to supply external material as food. Also the method is more economical and easier.

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