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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 substrate[1]. 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: rainwater[4], debris[5], or the atmosphere[6] 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 decocition' (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 supension 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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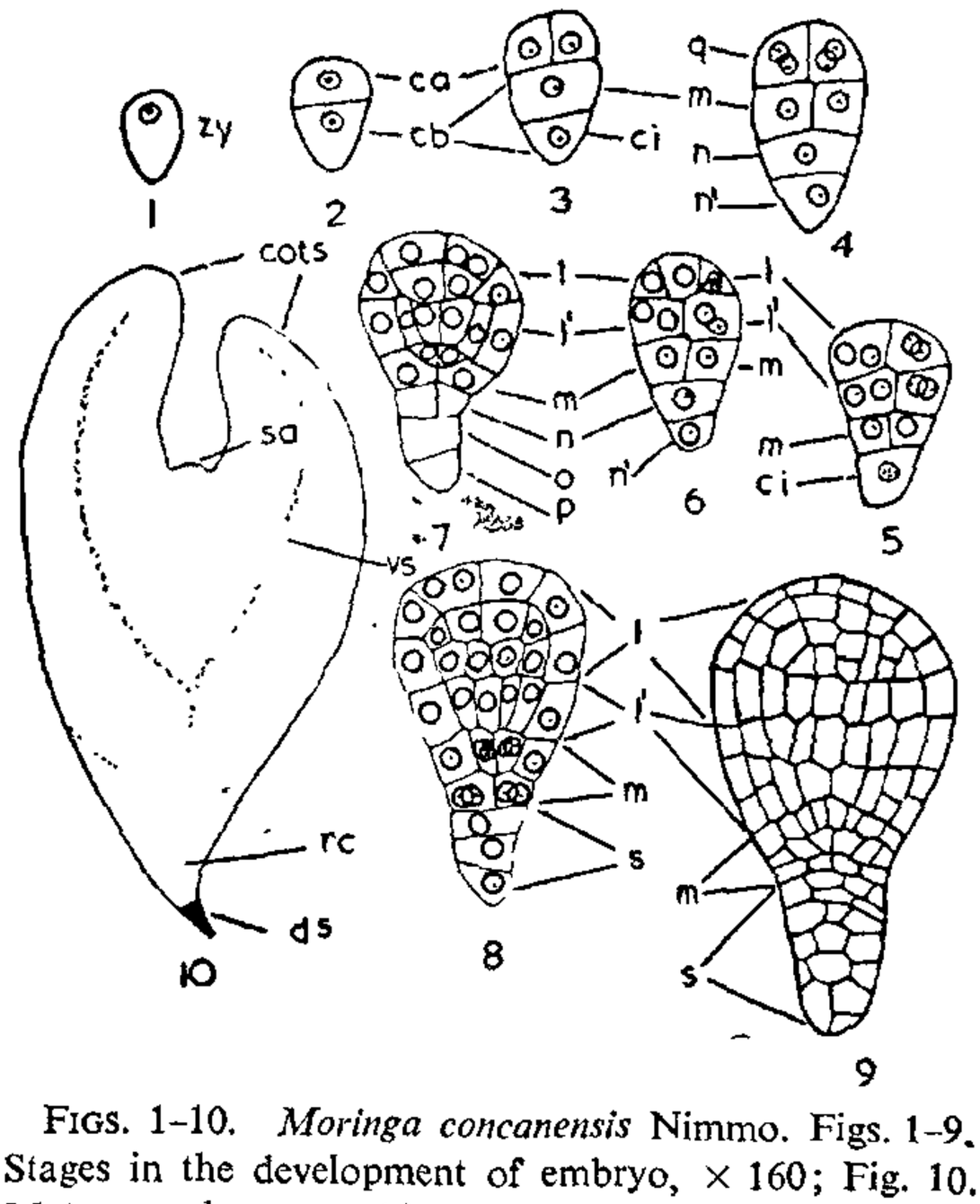
EMBRYO DEVELOPMENT IN *MORINGA CONCANENSIS* NIMMO.

As there has been no report of the development of embryo in *Moringa concanensis* Nimmo, a study of it was undertaken and the observations are recorded in this report.

The zygote undergoes a period of rest and divides, when the fruit is about 6 cms long, transversely to produce the terminal cell *ca* and the basal cell *cb* (Figs. 1, 2). Both the derivativies of the zygote divide further in such a way to organise a T-shaped proembryo comprising 4 cells disposed in three tiers *ca*, *m* and *ci* (Fig. 3). The two cells of *ca* (Fig. 3) now divide vertically forming a quadrant *q*, while *m* divides vertically (Figs. 4, 5) and *ci* transversely giving rise to *n* and *n'* (Fig. 4). At the third cell generation (Fig. 4) the proembryo comprises eight cells disposed in 4 tiers. Figure 6 represents the proembryo with 5 tiers ofc ells, that is, the two terminal tiers *l* and *l'* of four cells each, the next *m* with two juxtaposed cells and the lower two tiers *n* and *n'* of one cell each. In each cell of the terminal tier *l* an oblique wall is laid down to form an inner and an outer cell. The former undergoes further division in transverse and longitudinal planes over and again forming the plumule, while the latter constitutes the initial for the cotyledons (Figs. 7, 8, 9). Following the divisions in the tier *l*, the cells of the tier *l'* undergo periclinal divisions to demarcate dermatogen, periblem and plerome (Figs. 7, 8). Further divisions in! ' result in the differentiation of hypocotyl and the radicle (Figs. 8, 9).

Further divisions in *m* result in root apex and root cap. The derivatives of *ci* divide rather irregularly forming the suspensor of 2 to 3 seriate cells (Figs. 7, 8, 9). The mature embryo is fleshy and has massive cotyledons, discernible shoot apex, short hypocotyl, root cap and disintegrating cells of the suspensor (Fig. 10).



FIGS. 1–10. *Moringa concanensis* Nimmo. Figs. 1–9. Stages in the development of embryo, × 160; Fig. 10. Mature embryo, × 10.

(*cots*—cotyledons; *dsc*—disintegrating cells of the suspensor; *rc*—root cap; *sa*—stem apex; *vs*—vascular supply, *zy*—zygote).

From the above the embryogeny conforms to the Myosurus variation of the Onagrad type of Johansen[2] (1950) or Megarchetype IV in the First Period of Souéges (*vide* Crété[1], 1963); this is at variance with the Asterad type reported for *Moringa oleifera*, where the embryo was without a suspensor, as reported by Narayana[3] (1962).

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