CHAPTER VI

IN VITRO STUDIES ON *ADIANTUM TRAPEZIFORME* L.

GAMETOPHYTES AND GAMETOPHYTIC CALLUS

IN VITRO STUDIES ON ADIANTUM TRAPEZIFORME L. GAMETOPHYTES AND GAMETOPHYTIC CALLUS

Adiantum is probably one of the most popular of all ferns on account of its wide distribution, medicinal uses and ornamental foliage (Figs. 71,72). Still, it is little understood from morphological and phylogenetical points of view (Nayar, 1962). Since fern prothalli are sensitive to the environmental changes, numerous examples of morphological plasticity of fern gametophytes to altered cultural conditions have been reported (Mohr, 1962; Atkinson and Stokey, 1964; Miller, 1968; Nayar and Kaur, 1969, 1971). The fern prothallus was, however, characteristic in its structure, form and development in each major taxonomic group.

The work described in this chapter was aimed to study the morphology of spore, conditions favourable for its germination and the pattern of prothallial development. Callus tissue was also initiated from the prothalli and attempts were made to induce morphogenetic changes in the callus.

Experiment No. 6-1 : Spore and its germination

Spores were acetolysed by following Erdtman's method (1954) as described in Chapter II, Materials and Methods,

Fig. 71. Herbarium specimen of Adiantum trapeziforme L.

Fig. 72. Metaphase of leaftip of Adiantum trapeziforme L. (2n = 58) (1500X)


HERBARIUM
No. MK. 5
Family Pteridaceae
Genus Adiantum
Species
Local Name
Locality Baroda.
Remarks
Date : July 70
Collected by
Det. - G. Panigrahi.

Fig.71

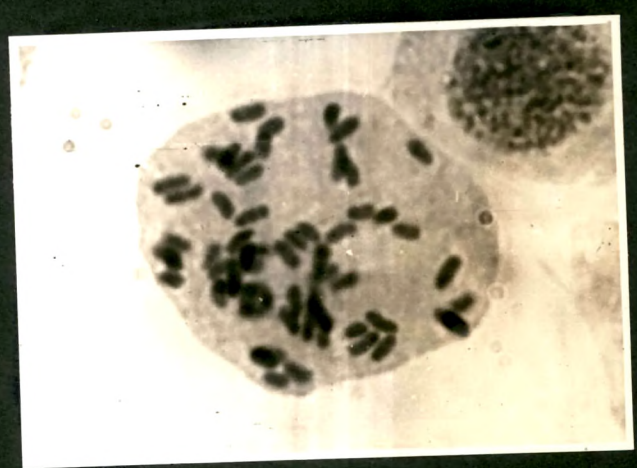

Fig.72

8,B. The spores were mounted in glycerine jelly and their morphological features observed.

The spores were yellowish in colour and tetrahedral with clear triradiate mark. They had distinct intine and smooth exine (Fig. 73A,B).

The spores collected from fertile fronds were sterilized as described in Chapter II, Materials and Methods 3, C. The sterilized spore suspension was then uniformly spread on agar slants in test tubes containing Knudson's medium (Table 2). Three different pH (4.5, 5.5, and 6.5) of the media were tested to determine optimal pH for spore germination. Similarly, varying agar concentrations (0.5%, 0.8% and 1.0%) were used to solidify the medium. Observations were made with stereomicroscope to ensure spore germination in culture tubes which were incubated at 26±2°C in continuous light.

It was seen after four weeks that the formation of gametophytes was maximum on medium with pH 5.5 when compared with other pH levels tested. It was further noticed that the softer the culture medium, the better the spore germination. Moreover, freshly collected spores invariably gave higher germination than the spores preserved for various lengths of time. Hence, for subsequent studies of prothallial development the pH of

Fig. 73. Stages of prothallial development of Adiantum trapeziforme L.
(A) and (B) spore (1500X)
(C) and (D) spore-germination (450X)
(E) and (F) 4-5 cell filament (150X)
(H) and (G) prothallial plate formation (100X)
(I) Adult prothallus (24X)
(J) Antheridium (450X)
(K) Archegonium (450X)

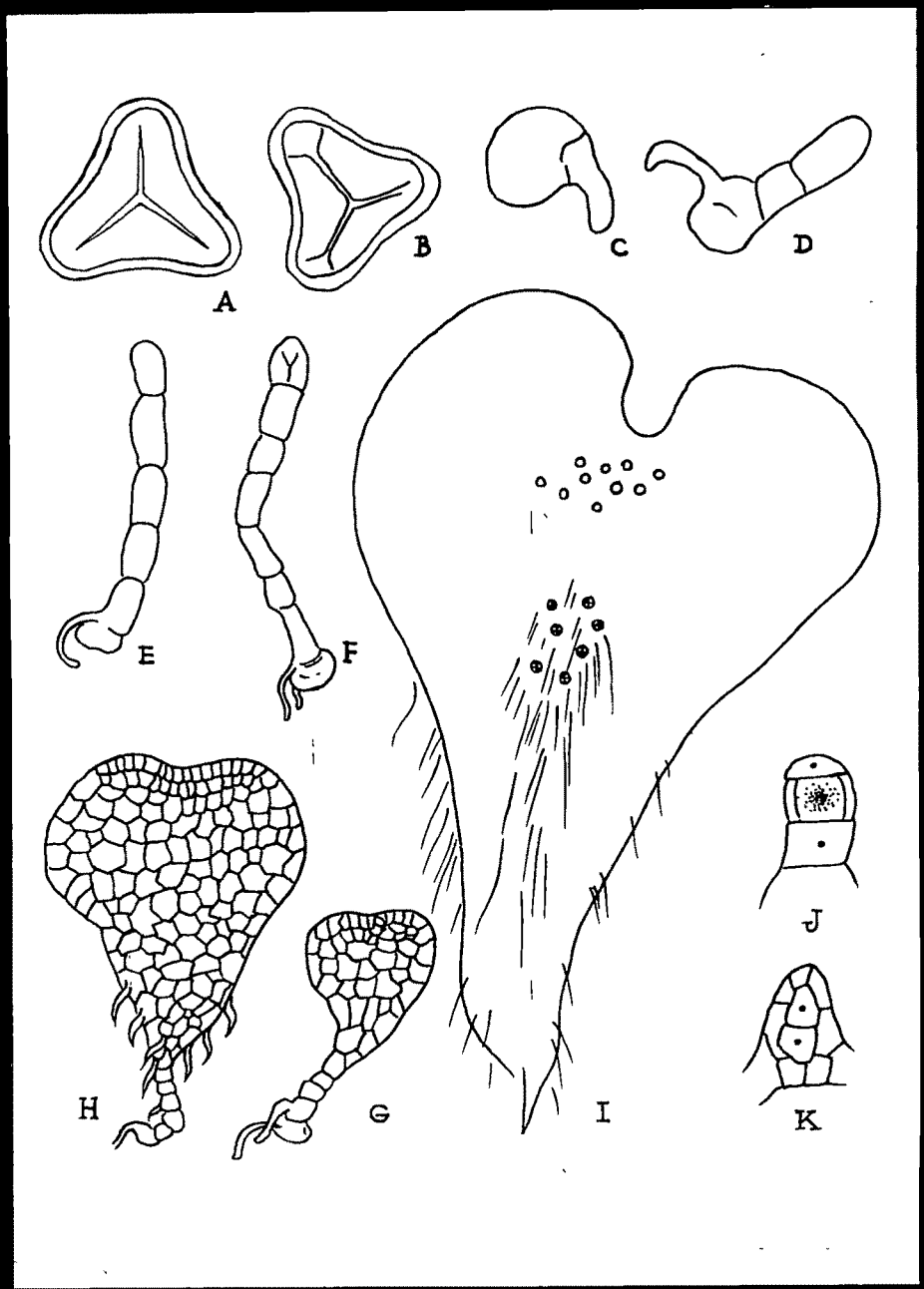

Fig.73

the Knudson's medium was adjusted to 5.5 and the medium was solidified with 0.5% Difco Bacto agar.

Experiment No. 6-2 : Development and regeneration of prothallus

Under cultural conditions provided the spores germinated after 7-9 days. The germination started with the exine rupturing along the triradiate mark and the intine protruding out as a germ papilla, which soon developed chloroplasts (Fig. 73C). At this stage a rhizoid was produced (Fig. 73D). With the division of the germ papilla, 3 to 4 barrel shaped cells containing dense chloroplasts were produced resulting in a 4-5 celled germ-filament (Fig. 73E). After this stage, the terminal cell divided by walls oblique to the long axis of the filament and a meristematic apical cell was established (Fig. 73F). By anticlinal divisions of this cell, one cell thick layer of obovate prothallial plate was produced (Fig. 73G). Soon the apex of the thallus became notched (Fig. 73H) and later the prothallus assumed typical cordate shape (Fig. 73I). By this time some more rhizoids were formed which at first were colourless but gradually turned dark brown to black.

Regeneration

During the above development, it was observed that when the prothalli became cordate, few of the posterior cells from the germ-filament gave rise to new prothallial filaments (Fig. 74). Formation of new prothalli was quite rapid and soon a prothallial colony assuming rosette appearance was formed (Fig.75). The regenerated prothalli grew not only on the ventral surface and from the margins but also formed dense growth on the dorsal surface of the parent prothallus. The gametophytes could be separated quite easily from such a prothallial colony.

Sex Organs

Antheridia were produced when the prothalli were 6 to 8 weeks old. They appeared earlier on Knudson's medium containing 2% sucrose - the medium which supported vigorous growth of the prothalli. On medium with lower (1%) sucrose level, antheridia developed at a later stage. The antheridium was of typical Leptosporangiate type consisting of three cells - a basal cell, a central androgonial cell and a terminal cell which later on cut off an opercular cell (Fig. 73J). The cell next to the opercular cell became ring-shaped surrounding the apical portion of the androgonial cell.

Fig. 74. Photomicrograph of formation of new filaments from prothallus (60X)

Fig. 75. Prothallial colony

Fig.74

Fig.75

After the antheridial formation, the midrib began to develop behind the meristem in the median plane of the symmetrically cordate prothallus. At this time the archegonia made their appearance on the thickened midrib (Fig. 76). Each archegonium was fairly uniform in structure with an axial row of three cells surrounded by a jacket of one layer of cells (Fig. 73K).

Experiment No. 6-3 : Effect of sucrose concentrations on prothalli

30 ml of Knudson's medium containing different levels of sucrose - 1%, 2% or 4%, were separately inoculated with 4 week old prothalli developed on Knudson's medium containing 1% sucrose. Culture flasks were incubated at 26±2°C in continuous light (300 Lux).

After incubation for 8 weeks, it was noticed that prothalli growing on Knudson's medium containing 4% sucrose became quite thick and on their dorsal surface profuse hairs developed (Fig. 77). The formation of hairs had been earlier found to be the first external indication of the initiation of apogamy (Chapter V: Expt. 5-3). Prothalli growing on 2% sucrose too showed this change, but after 12 weeks of incubation. On

Fig. 76. Photomicrograph of mature prothallus with sex organs (24X)

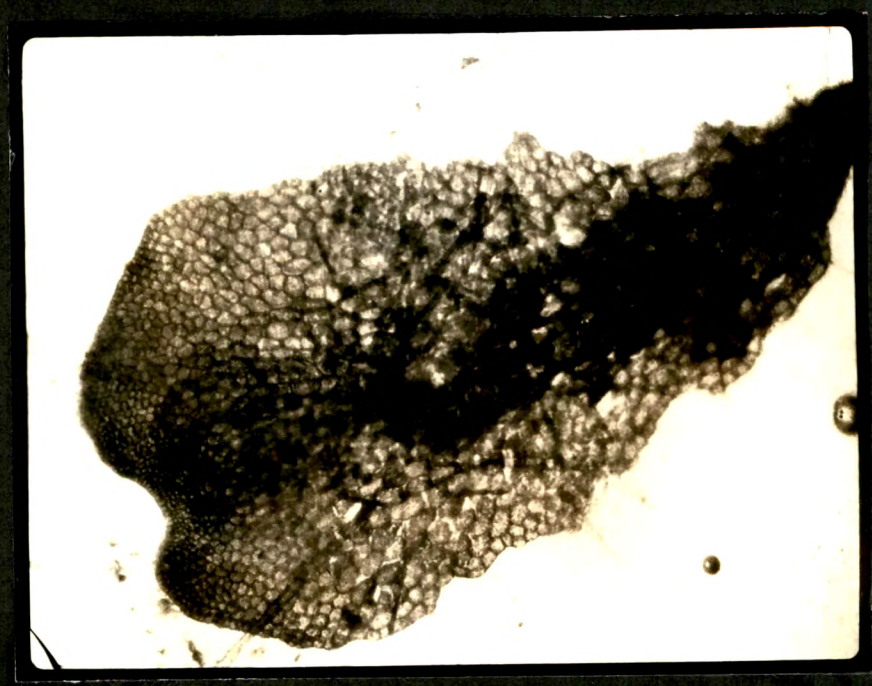

Fig.76

Fig. 77. Prothalli grown on Knudson's medium containing 4% sucrose showing hair formation

Incubation: 8 weeks in light at 26±2°C

Fig. 78. Prothalli grown on Knudson's medium containing 4% sucrose showing apogamous shoot formation

Incubation: 12 weeks in light at 26±2°C

Fig.77

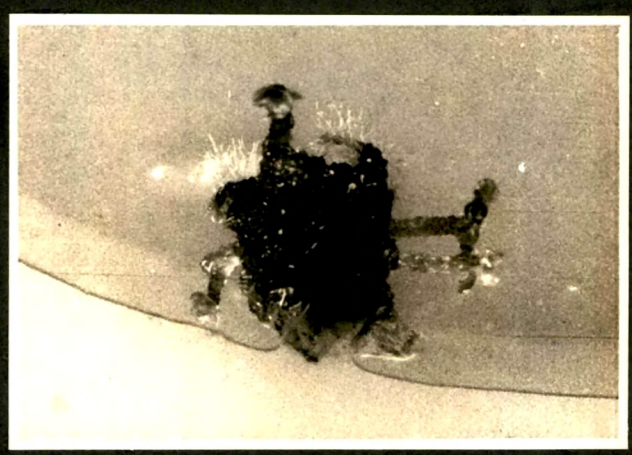

Fig.78

1% sucrose containing Knudson's medium, on the other hand, the prothalli remained quite thin. Distinct sporophytic shoots appeared in 4% sucrose medium after incubation for 12 weeks (Fig. 78). Clearly, increased sugar concentrations invariably led to morphogenetic changes in the gametophyte, even in those cases in which apogamy does not naturally occur.

Experiment No. 6-4 : Regeneration of gametophytes from prothallial cells

4 weeks old prothalli grown on Knudson's medium containing 1% sucrose were immersed in 10 ml of sterile distilled water and were crushed very gently by hand. The resultant suspension was then filtered through a nylon mesh of 300 μ pore size. 2 ml aliquots of the filtrate were plated on 15 ml of Knudson's medium containing 2% sucrose in petri dishes (see Chapter II, Materials and Methods, 6). The plates were incubated at 26$\pm$2°C in continuous light.

The filtrate when examined microscopically showed the presence of free cells and cell-aggregates consisting of upto 4-5 cells. Within four weeks prothalli developed from these plated cells and at the end of six to eight weeks, these prothalli became mature.

Experiment No. 6-5: Initiation of callus on the prothalli

30 ml of Knudson's media containing 2% sucrose and supplemented with and without 10% coconut milk along with 1.0 mg/l or 2.0 mg/l 2,4-D were inoculated with sterilized (1 ml) spore suspension. 4, 6 and 8 weeks old prothalli from Knudson's medium containing 1% sucrose were also inoculated separately on above said media. The culture flasks were incubated at 26±2°C in continuous light.

The callus initiated on 6 and 8 weeks old prothalli after incubation for 4 weeks (Fig. 79). The callus initiation was more rapid on the prothalli transferred to Knudson's medium containing 2% sucrose and supplemented with 10% coconut milk and 2 mg/l 2,4-D. The callus developed on the prothallus where prothallial cells came in close contact with the medium. No callus initiation occurred on germinating spores and also on 4 week old prothalli in any of the media tested. Callus developed also on 6 and 8 weeks old prothalli on Knudson's media containing 2.0 mg/l 2,4-D and 1.0 mg/l 2,4-D which lacked coconut milk; however, it initiated only after prolonged period of incubation. In both the cases the callus was deep green in colour. It showed the presence of scattered tracheids when squashed.

Experiment No. 6-6 : Differentiation of callus

30 ml of Knudson's basal media with and without 2% and 4% sucrose were inoculated separately with callus pieces about 1.0 cm in diameter. Culture flasks were incubated in the environmental chamber adjusted at 25±1°C with constant illumination of 5000 Lux. 4 replicate flasks were incubated also in complete darkness.

After incubation for 4 weeks, apogamously developed leaves were noticed on callus transferred to 4% sucrose containing medium (Fig. 80). On the basal medium, on the other hand, gametophytes were observed after 6 weeks in light (Fig. 81). However, neither sporophytic nor gametophytic forms differentiated on the callus incubated in the dark.

DISCUSSION

For the germination of spores suitable pH (5.5) and agar concentration (0.5%) were determined (Expt. 6-1). The development of prothallus was of the Adiantum type. There was profuse regeneration of gametophytes from very young prothalli, which resulted into rosette of colonies (Expt. 6-2). Sucrose concentration in the medium was

Fig. 79. Prothalli grown on Knudson's medium containing 10% CM, 2% sucrose and 2.0 mg/l 2,4-D showing callus initiation

Incubation: 4 weeks in light at 26±2°C

Fig. 80. Callus grown on Knudson's medium containing 4% sucrose (no auxin) showing apogamous shoot (S) formation

Incubation: 4 weeks in light at 26±2°C

Fig. 81. Callus grown on Knudson's medium (no sucrose and no auxin) showing regeneration of gametophytes (G)

Incubation: 8 weeks in light at 26±2°C

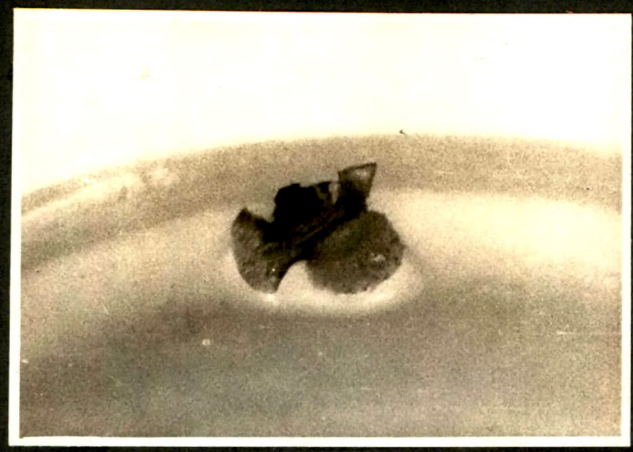

Fig.79

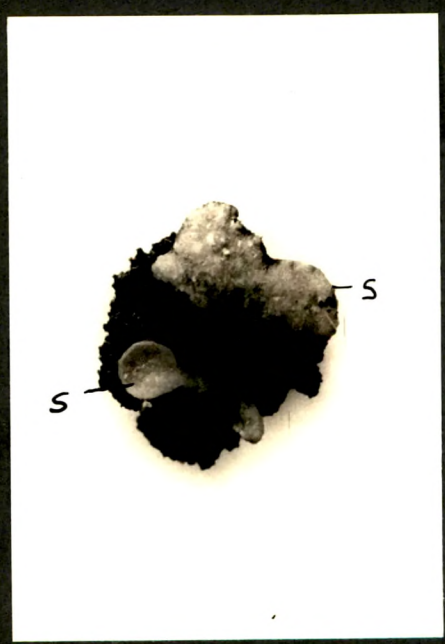

Fig.80

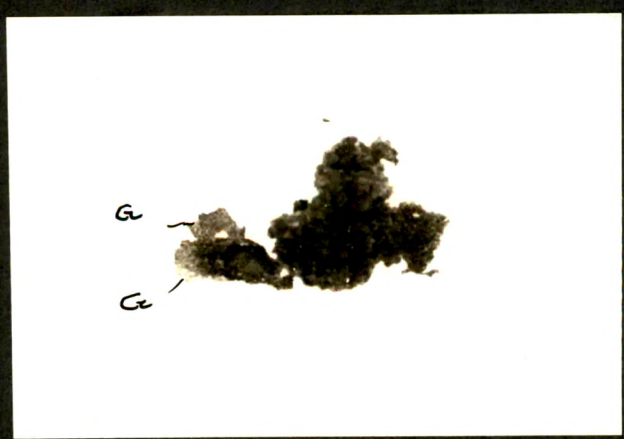

Fig.81

responsible for variation in prothallial morphology; the higher concentration (4%) resulting in the production of apogamous shoots (Expt. 6-3).

Free cells and aggregates of few cells obtained from young prothalli by gentle mechanical maceration, displayed the ability to regenerate into gametophytes (Expt. 6-4). Callus developed only on 6 to 8 week old prothalli supplied with 2% sucrose and 2 mg/l 2,4-D along with 10% CM (Expt. 6-5). The callus proved promising material for morphogenetic studies. It could be differentiated into sporophyte or gametophyte depending upon the presence or absence of sucrose in the medium (Expt. 6-6).