Studies on *Gelidiella acerosa* (FORSSKÅL) FELDMANN et HAMEL V : Germination of tetraspores and nuclear changes of the germinating spores

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It has been observed that the spore becomes multinucleate before the formation of germ-tube and only one functional nucleus passes into the germ-tube, while the rest are left in the body of the original spore. Hence it has been suggested that the "*Gelidium*-type" of germination can also be characterized by the multinucleate condition before the formation of the characteristic germ-tube.

The method of germination of spores has been reported for *Gelidiella acerosa* (FORSSKÅL) FELDMANN et HAMEL by CHIHARA and KAMURA.[1] The germination of the spore is typically "*Gelidium*-type" in which the spore contents move into a protuberance formed at one side of the 'empty' spore and this protuberance is then cut off by a wall to form the germ-tube. This germ-tube then develops into the germling. The present study revealed a difference in certain aspects of germination of tetraspores of the alga. Also the behaviour of the nuclei during germination is not known in this alga, though it has been reported for *Gelidium* (BOILLO,[2] KANEKO[3]).

Methods

The fertile ramulus with mature tetraspores of the freshly collected plant was placed on a slide, which in turn is put in a bowl of filtered pasteurized seawater to which antibiotic mixture was added to reduce bacterial activity.

All spore cultures have been initiated at Veraval, the place of collection, where they were kept at the north window. They were then transferred to Bhavnagar and kept in culture room maintained at 26 ± 1° under continuous illumination of 1000–

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1200 lux. No aeration was provided. Pasteurized seawater was prepared by filtering fresh seawater through the filter paper and then heat upto 70° once a day for successive 3 days (PROVASOLI[4]).

The stock solution of antibiotic mixture was made by dissolving the following 3 antibiotics in 100 ml of distilled water ; crystalline penicilline G. potassium I. P. (1,000,000 units) 50 mg, chloromycetin (chloramphenicol) 100 mg, streptomycin sulfate (equivalent to 1 g streptomycin base) 100 mg. One ml of the mixture was added to 100 ml of culture solution or pasteurized seawater. Of the culture solutions tried, Erd Schreiber solution as given by PROVASOLI[4] gave satisfactory results.

Germinating spores were fixed by 3 : 1 acetic : alcohol and by formalin-acetic-alcohol (WESTBROOK[5]) and stained by Heidenhein's haematoxylin for the observation of nuclei.

Observations

Sporeling : Liberation of spores was observed within few hours of trasnferring the fertile ramulus into the pasteurized seawater. Initially the liberated spores were ovoid or ovate elongate, with rounded ends. Immediately after their liberation they were observed to round off into a spherical shape measuring 21.4-24.9 μ in diameter. They looked as light-pinkish masses on either side of the ramulus on the slides in the cultures. Each spore begins to germinate by putting forth an outgrowth to one side of the spore (Fig. 1, B). After this finger shaped protuberance reaches approximately the diameter of the spore, the spore contents flow into it. This outgrowth, here after referred to as the germ-tube, is cut off from the body of the original spore by a cross wall perpendicular to the long axis of the germ-tube. It has also been observed that the germ-tube elongates between the transverse wall and the original spore. The contents may fill up the whole germ-tube or may occupy only its apical portion (Fig. 1,C).

The apical portion of the germ-tube, cut off from the rest of the spore, divides by a curved longitudinal wall to form two unequal daughter cells, one fusiform and the other elongated bowl-shaped (Fig. 1, D). The second division is transverse in both the cells, further divisions being more in the elongate bowl-shaped cells than in the other (Fig. 1, F). In some cases (Fig. 1, E) the first division is also transverse and after a row of 3-4 cells is formed, they divide longitudinally into two groups of unequal cells. Thus whatever be the first division, longitudinal or transverse, ultimately two groups of cells seperated along the long axis are formed (Fig. 1,M-N). At 6-8 celled stage, a cell is formed from the distal end of the fusiform portion. This is the rhizoidal initial which grows out into a long hyaline rhizoid (Fig 1, I-K). At this stage the

original hyaline spore wall can be seen attached to the dome-shaped part of sporeling. The sporeling continues to grow to form a multicellular structure. At this stage, the group of cells in the sporeling evident at the earlier stages are not distinguishable (Fig. 1, O). Also the original spore wall and the rhizoids disappear. From one end of this cellular mass presumably from the side opposite to the rhizoid, an apical cell is differentiated (Fig. 1, P, Q). The apical cell of the sporeling starts segmentation cutting a cell below. This cell by two vertical divisions produces two pericentral cells and a central cell. As the growth of the sporelin continues, the outer most layer of cells elongates and forms the surface layer of cells of the sporeling (Fig. 1, W).

The germling now measuring about 150 μ long is upright with the apical cell at its acute apex. The upright germling may be described as the primary shoot as it forms the erect primary axis. From the basal part of this primary shoot an initial of the secondary shoot was observed to develop (Fig. 1, W). Further growth of this initial was not observed. Thus it has been observed that the primary shoot formed from the sporeling is vertical. After the shedding of the primary rhizoid by the sporeling, no further formation of rhizoids was observed in the cultures. In some cases, the germling tends to creep along the substratum in the cultures. In one such case, one or two laterally situated initials was observed, but they did not develop beyond this stage (Fig. 1, U).

The development of primary shoot in the laboratory cultures stopped after 3 months.

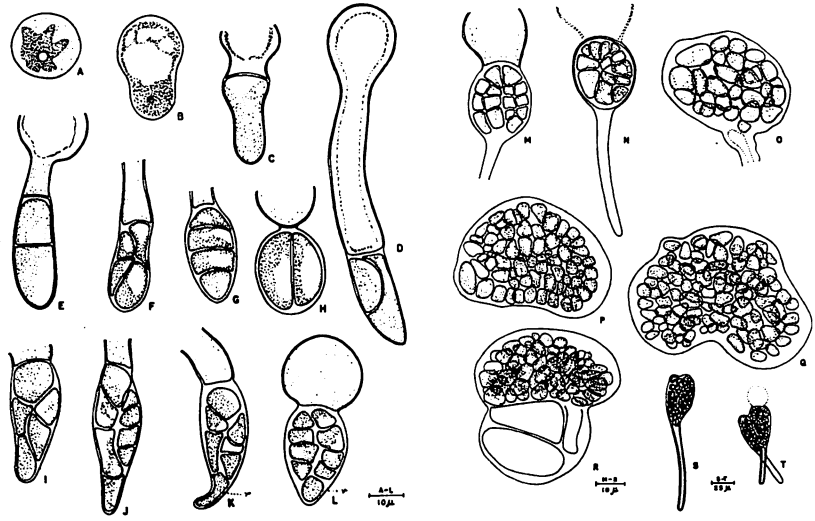

Fig. 1

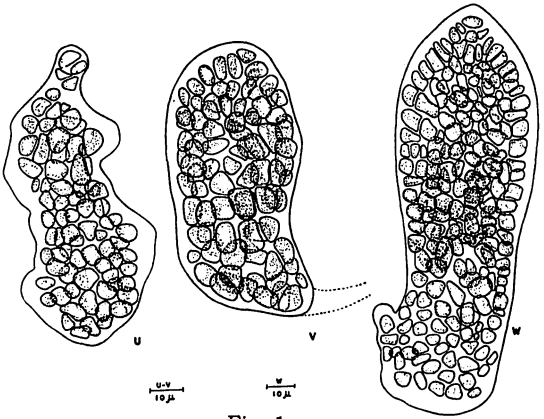

Fig. 1

EXPLANATIONS OF FIGURES

Fig. 1 : Germination of tetraspores in *Gelidiella acerosa* (FORSSKÅL) FELDMANN et HAMEL

A. Tetraspore before commencement of germination
B. Formation of protuberance into which the protoplasmic contents are moving(3-4 hours after being attached).
C. Cutting off of a germ-tube by wall at right angles to the protuberance.
D. First division of the germ-tube by formation of a curved vertical wall to form two unequal cells.
E. First division of the germ-tube by a transverse wall.
F. Second division of the two unequal cells formed in D by a transverse wall.
G. Second division of cells formed in E by a transverse wall to form a row of cells.
H. An abnormal case of vertical wall formation during the first division to form equal cells in the germ-tube.
I-L. Further divisions in the sporeling and the formation of rhizoidal initial (r)
M-N. The young sporeling with two distinct groups of cells and the primary rhizoid.
O. Formation of a multicellular body without differentiation into groups of cells.
P-Q. Two cases of formation of a apical cell from the cellular mass.
R. Abnormal sporeling where the old spore cell contents have divided.
S-T. Formation of apical cell when the two cell masses are clearly evident in Japanese alga (from CHIHARA and KAMURA 1963)
U. Sporeling creeping (on the slide) in the culture. Note the central cell and two pericentral cells.
V. Sporeling 30 days old.
W. Sporeling after 54 days of culture. Note the central cell and two pericentral cells.

Nuclear changes during spore germination : The spore is uninucleate at the time of liberation. Before the formation of the characteristic protuberance during the spore germination, the nucleus divides to form 4-6 nuclei (Fig. 2, A). Thus by the time the germ-tube is formed, the spore becomes multinucleate (Fig. 2, B). When the cytoplasm flows into the protuberance, only one functional nucleus moves along with it (Plate 1. a), while the rest of them remain behind in the original spore. Thus the original spore is not completely 'empty' as thought earlier, but still contains few nuclei and a little amount of cytoplasm (Plate 1. b ; Fig. 2. D, E). In a rare case it has been observed that the single nucleus of the spore moves along with the cytoplasm into the germ-tube without going further division (Fig. 2. C). The single functional nucleus of the germ-tube divides repeatedly accompanied by wall-formation (Fig. 2. F, C). The nuclei left in the original spore gradually degenerate. This kind of division has been uniformly found during spore germination.

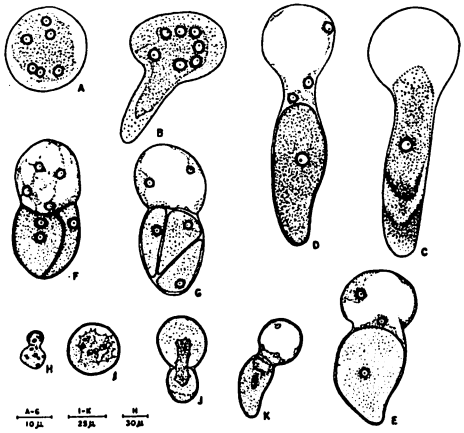

Fig .2.

EXPLANATIONS OF FIGURES

Fig. 2 : Behaviour of nucleus during tetraspore germination in *Gelidiella acerosa* (FORSSKÅL) FELDMANN et HAMEL

A. Tetraspore with 6 nuclei before the formation of germ-tube.

B. Germ-tube formation after the spore has become multinucleate.

C. Abnormal case of undivided spore nucleus flowing with the cytoplasm into the germ-tube

D - E. Germ-tube, which is cut off by a wall from the rest of the spore with one nucleus. Note the remaining nuclei formed earlier left behind in the original spore

F. Formation of curved wall to form two unequal cells with simultaneous division of nuclei. Note the nucleus of the larger cell has divided prior to wall formation.
G. Three uninucleate cells in the germ-tube.
H. Large nucleus moving into the germ-tube in *Gelidium latifolium* (from BOILLOT, 1963).
I - K. Tetraspore germination in *Gelidium vagum*. (KANEKO, 1966)
I. Spore with four nuclei.
J. Germ-tube formation with one nucleus.
K. Side view of metaphase of the first nuclear division in the initial cell and the four nuclei in the original spore.

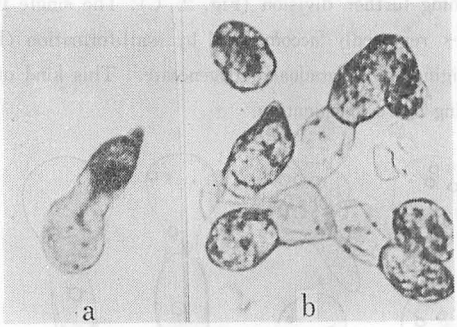

Plate 1.

Explanation of *plate* 1.

Behaviour of nucleus during germination of tetraspores of *Gelidiella acerosa* (FORSSKÅL) FELDMANN et HAMEL
a. Germination of the tetraspores showing the flowing of the cytoplasm into the protuberance with one nucleus. Note the nuclei left in the sporec cavity.
b. Germinating tetraspores showing the tetraspores showing the single nucleus in the germ-tube and the multinucleate original spore.

Discussion

The germination of tetraspores was studied by FELDMANN[6] in *Gelidiella pannosa* and by CHIHARA and KAMURA[1] in *Gelidiella acerosa*. The latter authors state that the mode of germination in *Gelidiella* is of "*Gelidium*-type" as designated by CHEMIN.[7] My observations on the alga under investigation agree with those of the earlier authors but with some differences in details.

The important difference observed in the present study from that of the previous authors is regarding the origin of the apical cell of the sporeling. In the case of the Japanese alga, the apical cell is formed very early in the development from the

fusiform part of the sporeling (Fig. 1, S, T), while in the alga under study the apical cell does not develop until a big mass of cells is formed. Due to the delay the formation of the apical cell, it was become difficult to locate exactly the part of the original two halves of the sporeling, from which the apical cell has been differentiated. This delayed formation of the apical cell from a discoid or irregular mass of cells of the sporeling is a very significant feautre of the germination pattern of the Indian alga.

The work of BOILLOT[2] and KANEKO[3] in *Gelidium*, and the authors observations in *Gelidiella* now have shown that the spore nucleus divides before the protoplasm flows into the germtube.

BOILLOT[2] reports that during germination of the tetraspore in *Gelidium latifolium* the spore nucleus divides to form one big and one small nucleus, and finally the spore contains 6–8 nuclei. But Kaneko[3] did not observe the division of the spore nucleus into a big and a small nucleus and also he observed only 4 nuclei formed in the spore before the protoplasm flowed into the germ-tube. In the present observation in *Gelidiella acerosa* it was found that the spore nucleus divides to form 6–8 nuclei of uniform size and only one functional nucleus moves with the flowing cytoplasm into the germ-tube. No further divisions of the nuclei left in the original spore have been observed in the present alga as reported for *Gelidium vagum* by KANEKO.[3] Thus division of the spore nucleus before the formation of the germ-tube can also be considered as another important feature of the "*Gelidium*-type" of germination, in which after the formation of a germ-tube and flowing of cytoplasm into it, wall formation takes place.

SUMMARY

The germination of tetraspores of *Gelidiella acerosa* (FORSSKÅL) FELDMANN et HAMEL is typically "Gelidium-type" in which spore contents move into a protuberance formed at one side of the 'empty' spore and this protuberance is then cut off by a wall to form the germ-tube. The germ tube then develops into the germling The apical cell of the germling is differentiated. very late in the development of the sporeling, when it is a discoid irregular mass of cells.

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