TETRASPOROGENESIS IN *Gelidium floridanum* (GELIDIALES, RHODOPHYTA): CYTOCHEMICAL STUDY

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Gelidium is a genus of red algae with a very wide geographic range. It is an economically valuable seaweed used in agar production. The taxonomy of red algae is based on the complexity of reproductive structures and life histories. *Gelidium* has a triphasic, isomorphic life history. One of these phases is a tetrasporophyte that undergoes meiosis in its reproductive areas to create haploid tetraspores that are cruciate. A spore is a specialised cell that can reproduce a new plant, and sporangium is a structure within which the tetraspore arises. Tetrasporangia produce tetraspores that develop into the haploid gametophytic generations (1).

Tetrasporic plants *G. floridanum* W. R. Taylor were collected on the interditial zone at Sambaqui beach, Florianópolis-SC. Small fertile branches with stichidia were fixed with PFA in 0,1M phosphate buffer. The material was dehydrated in ethanol series, infiltrated and included on historesin. Transverse sections were prepared with manual microtome and tungsten knife. To determine basic chemical composition on tetraspores, we used histochemical methods like toluidine blue (TB-O) for acidic polysaccharides (2), alcian blue (AB) for sulphate acidic polysaccharides (3), periodic acid Schiff (PAS) for neutral polysaccharides and coomassie brilliant blue (CBB) for total proteins (4).

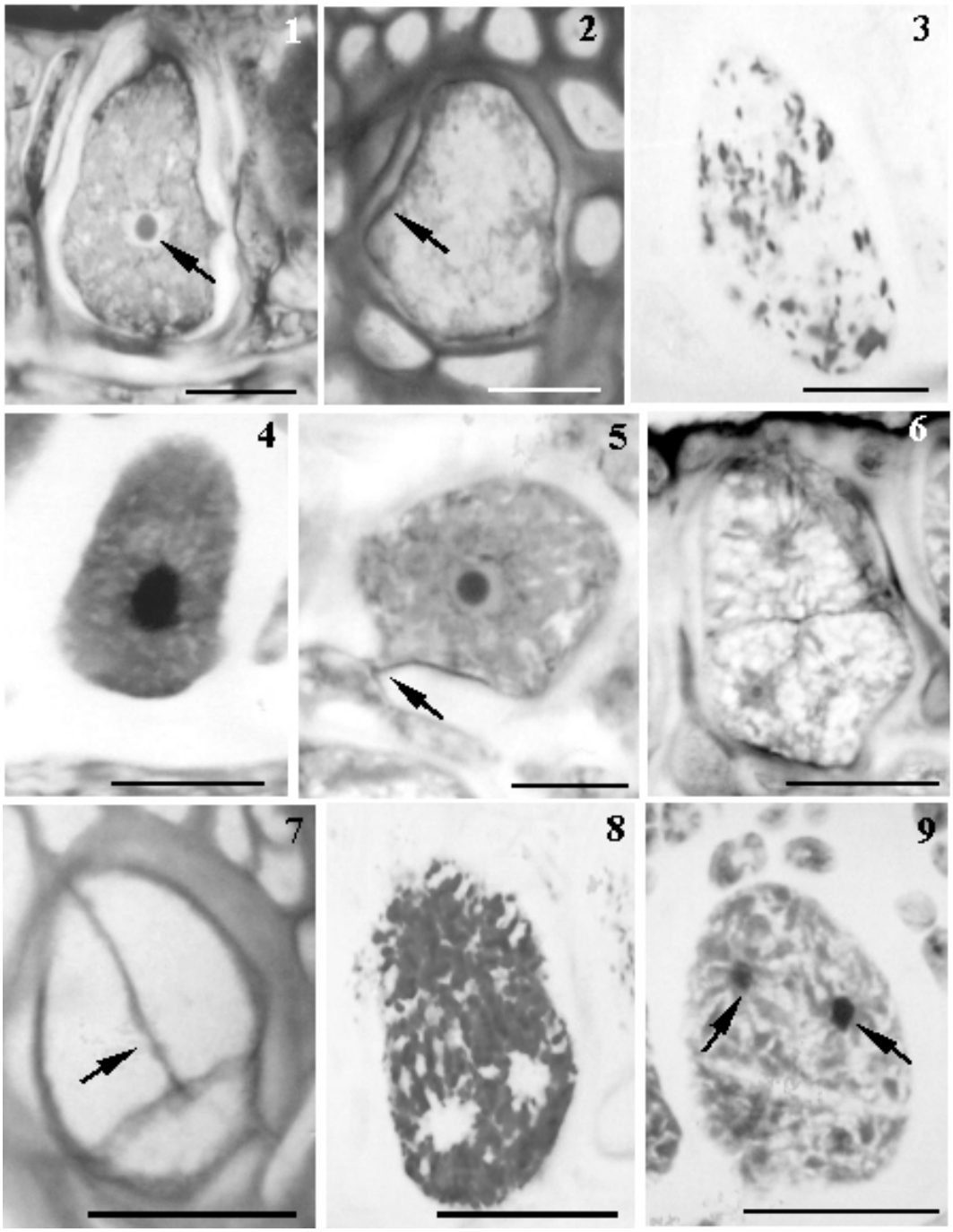
Tetrasporocytes are formed by the division of some cortical cells, each one giving rise to a stalk cell and a terminal tetrasporocyte. The young uninucleate tetrasporocyte elongates rapidly and increases on cytoplasm content. On this stage, cytoplasm of young tetrasporocytes stained with TB-O showed a large nucleus with an evident nucleolus and an orthochromatic reaction opposite to the metachromatic reaction seen in all extracellular material and cell walls, where the acidic polysaccharides were located (Fig. 1). When stained with AB we could notice the presence of sulphate acidic polysaccharides in extracellular material and cell walls as identified with TB-O stains (Fig. 2). With PAS, we did not see an intensive reaction because young tetrasporocytes are beginning the starch grains synthesis (Fig. 3). With CBB the young tetrasporocytes showed an intensive reaction on cytoplasm, which determinate the high content of proteins because of the elevated metabolic activity associated with the formation of new organelles (Fig.4-5).

Once differentiated, a young tetrasporangium gives rise to four uninucleate, equal-sized tetraspores after meiosis division. Each tetraspore is released, germinates and gives rise to a gametophytic phase. As meiotic nuclear divisions occur quite rapidly, no detailed observations were made, therefore we only analysed the direct citochemical changes on cytoplasm. In mature tetrasporangium, when stained by TB-O we saw a reduction of the orthochromatic reaction on cytoplasm, and the metachromatic reaction remains intensive on extracellular material and cell walls, indicating the acidic polysaccharides contents (Fig. 6), as we could also see with AB (Fig. 7). In this stage, large quantities of starch grains were observed in the cytoplasm with intense PAS reaction. These starch grains are probably a crucial reserve for germination (Fig.8). When stained with CBB, the matured tetrasporangium did not present an increase on protein content (Fig. 9)

This result shows the progressive synthesis of reserve material, specially starch grains, during terasporangium development. This guarantees that tetraspores will be able to germinate, and give rise to a gametophitic phase, which allows the continuation of the plant cycle.

References:

[1] M. Guiry, Biology of red algae, 14 (1990) 347-376.
[2] C. Ravetto, Journal Histochemical and Cytochemical, 12 (1964) 44-45.
[3] N. Feder and T. P. O'Brien, America Journal of Botany, 55 (1968) 123-142.
[4] P. Gahan, Plant Histochemistry and Cytochemistry, (1984).



Figs. 1-5. Young tetrasporocytes. Scale bar = 10μm. Fig. 1. Tetrasporocyte stained with TB-O. Observe the central nucleus (arrow). Fig. 2. Tetrasporocyte stained with AB, note the positive reaction on cell wall (arrow). Fig. 3. Tetrasporocyte stained with PAS, shows a small quantity of starch grains on cytoplasm. Fig 4-5. Stained with CBB. Fig. 4. Note the hight proteins content on cytoplasm. Fig. 5. Pit connection between tetrasporocyte and stalk cell (arrow). Figs. 6-9. Mature tetrasporangia. Scale bar = 20 μm. Fig 6. TB stain: tetrasporangium after meiosis. Fig. 7. AB stain: cell wall on cruciate tetrasporangium (arrow). 8. High content of starch grains on cytoplasm, stained with PAS. Fig. 9. Evident nucleus on tetraspores inside the tetrasporangium (arrow), and cytoplasm with regular quantity of proteins.