SPORES' GERMINATION OF *Gelidiun Floridanum* (GELIDIALES, RHODOPHYTA): CYTOCHEMICAL AND ULTRASTRUCTURAL STUDY

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Spores germination, be they tetraspores or carpospores, are known to follow well-established patterns of cell division at the order or even family level in the Rhodophyta. In the Gelidiales, germination starts by the production of a tube-like outgrow cell to where the spore contents migrate, remaining the original spore body as an apparently empty wall attached to the growing germeling. Although the sequence of cell division in the Gelidiales has been known since [1] and documented several times in the literature for different species, information regarding ultrastructure of spores and spore germination in red alga is limited to few works [2-3]. So far, we haven't found references on the cytochemical of the germination process.

Tetrasporophytic specimens of *G. floridanum* W. R. Taylor were incubated in Petri dishes containing sterile seawater to release tetraspores. For light microscopy, the slides with spores and germelings were fixed 4% paraformaldeheyde in phosphate buffer, dehydrated in ethanol series and embedded in historesin. After that, sections with 2 to 3 μm were cut with a tungsten knife. For cytochemical studies the sections were stained with 0,5% alcian blue (AB) [4] and 0,5% toluidine blue (AT-O) for locating acidic sulphated polysaccharides [5], while periodic acidic Schiff (PAS) reaction was used for neutral polysaccharides [6]. For transmission electron microscopy observations tetraspores and sporelings attached to glass slides were fixed overnight with 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) plus 0.2 M sucrose, the settled spores were removed from the slides and centrifuged. The pellet was post-fixed with 1% osmium tetroxide, dehydrated in a graded acetone series and embedded in Spurr's resin. The material was observed with a Zeiss EM900 TEM

Tetraspores are released without cell wall, enclosed in a transparent mucilage envelope, which may have a role in the initial attachment of the spore to the substrate. The released tetraspores have large starch reserves (Fig.1). Upon release and attachment the tetraspores undergo a period of rapid division. Photosynthesis alone is probably not capable of providing all energy and carbon needs of these rapidly dividing and enlarging cells, for what we suppose the starch reserves would also be required. The PAS methods puts clearly in evidence granules of cytoplasmic starch occupying big volumes in the non-germinating spores. These starch grains are used during the spore germination, but along this process new starch grains are also synthesised (Figs. 1-2). The positive reaction with PAS in the cell wall points to the presence of thick fibrils which represent possibly the sites where cellulose are localised. These fibrils are embedded in a disperse matrix that is positive with AB e and metachromatic with AT-O (Figs. 3-5). This matrix indicates the presence of acidic sulphated polysaccharides, the agar.

At the ultrastructural level it can be seen that the non-germinated spores are organised with the chloroplasts at the perinuclear region, and starch grains and dictyosomes at the periphery (Fig. 6).

Germination starts by apparent disorganisation of the spore contents. Soon after attachment the spore becomes polarised, with migration of the cytoplasm content to one pole. In this pole many dictyosomes are observed, probably involved with cell wall disruption (Fig. 7), while at the opposite pole we observe the formation of a vacuole. Germination process continues with the emission of a germ tube, which gradually lengthens while cellular organelles migrate into it. As the process of vacuolation increases, the vacuole pushes the chloroplasts and others organelles towards the germ tube (Fig. 8). Dictyosomes seem to play an important role in the formation of the vacuole and the new cell wall. When most of the protoplast has been pushed to the tube, a thin layer of cell wall is formed separating the new cylindrical cell from the body spore, and both cells remain linked by a pit connection.

References:

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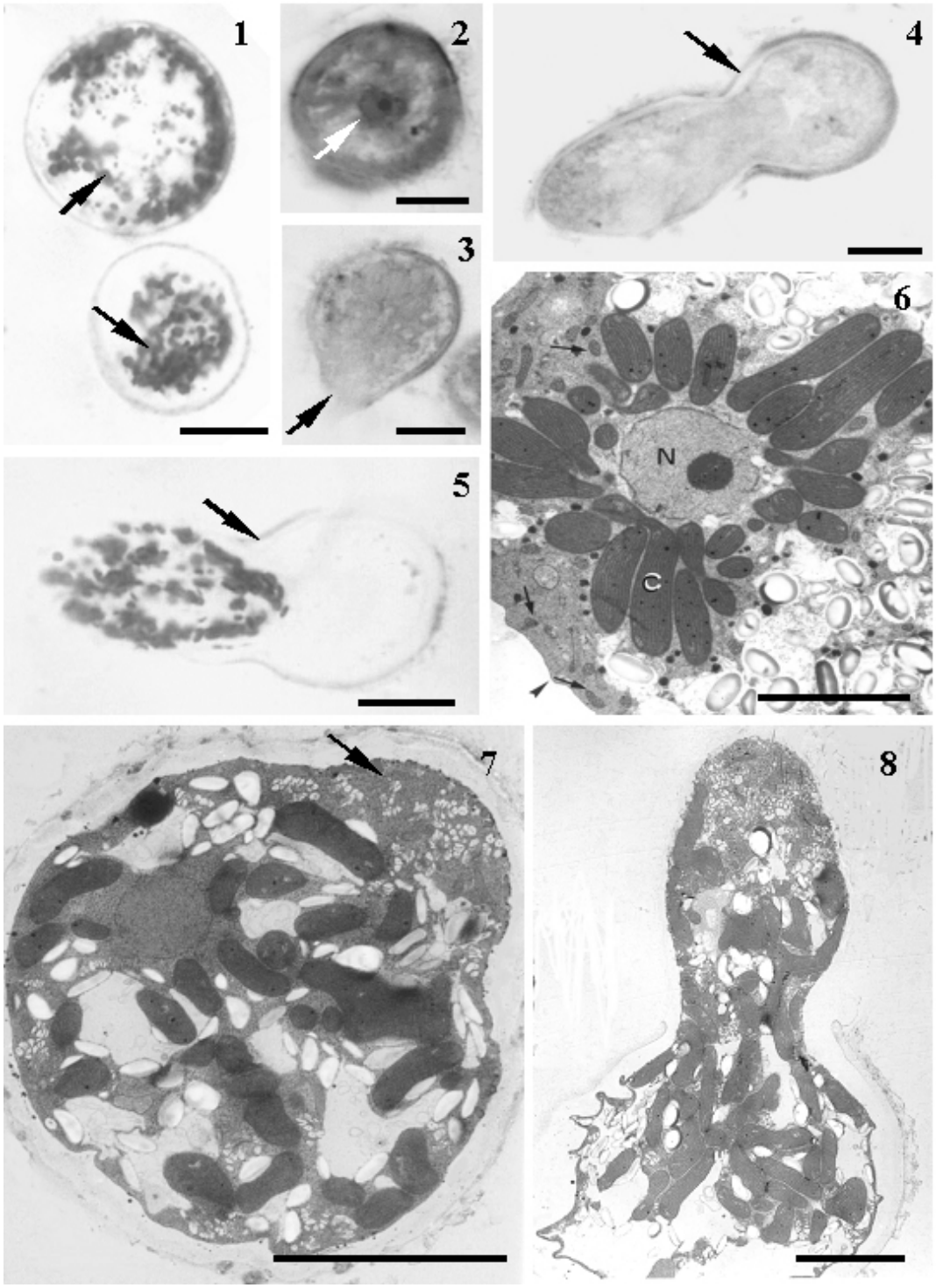

Figs. 1-5. Ligth micrographs of tetraspores of *G. floridanum.* Bar = 10μm. Fig. 1. Non-germinated spores with PAS. Starch grains stain red (arrows). Fig. 2. Non-germinated spore with AT-O. observe the metachromatic reaction in the cell wall and central nucleus (arrow). Fig. 3. Germinating spore with AT-O showing the initial formation of germe tube (arrow). Fig. 4. Germinating spore with AT-O. Cytoplasm contents can be observed into the germe tube. Cell wall stain metachromatically (arrow). Fig. 5. Germinating spore with PAS. Starch grains migration into germe tube and cell wall positive reaction (arrow). Figs. 6-8. Transmission electron micrographs. Bar = 20μm. Fig. 6. Non-germinated spore with chloroplasts around the nucleus. Fig. 7. Spore beginning the germination with dictyosomes at the tip of the protuberance (arrow). Fig. 8. Cell contents migration from spore to the germ tube. With dictyosomes at the tip.