Scientia Marina 74(2)
June 2010, 297-303, Barcelona (Spain)
ISSN: 0214-8358
doi: 10.3989/scimar.2010.74n2297

Life history, morphological variability and growth rates of the life phases of *Gracilaria tenuistipitata* (Rhodophyta: Gracilariales) *in vitro*

JOSÉ BONOMI BARUFI, EURICO CABRAL DE OLIVEIRA,
ESTELA MARIA PLASTINO and MARIANA CABRAL DE OLIVEIRA

Instituto de Biociências da Universidade de São Paulo, R. do Matão, 277, travessa 14. Cidade Universitária, São Paulo, 05508-900 SP, Brazil. E-mail: jose.bonomi@usp.br

SUMMARY: *Gracilaria tenuistipitata*, a species of commercial interest, is becoming a model organism for studies on red algal physiology and molecular biology as it can be grown easily *in vitro* under a broad range of conditions. Most of the experiments carried out around the world have been based on a tetrasporophytic clone isolated in our laboratory from a specimen collected in China. Here we describe the life history of this species, give anatomic details of the reproductive structures, illustrate the morphological variability of tetraspore progeny and compare the growth rate of gametophytic and sporophytic thalli. Tetrasporophytic branches showed higher growth rates than gametophytic branches.

Keywords: *Gracilaria tenuistipitata*, life history, reproduction, tetraspore progeny, Rhodophyta, Gracilariales.

RESUMEN: Ciclo de vida, variabilidad morfológica y crecimiento de las diferentes generaciones de *Gracilaria tenuistipitata* (Gracilariales, Rhodophyta) *in vitro*. – *Gracilaria tenuistipitata*, una especie de interés comercial, se está convirtiendo en un organismo modelo para estudios en fisiología y biología molecular de algas rojas debido a sus facilidades de crecimiento *in vitro* bajo una gran variedad de condiciones. La mayoría de los experimentos llevados a cabo a nivel mundial se han basado en un clon tetrasporofítico aislado en nuestro laboratorio, a partir de un espécimen recolectado en China. En este trabajo, se describe el ciclo de vida de esta especie, se dan detalles anatómicos de sus estructuras reproductoras, se ilustra la variabilidad morfológica de la progenie de tetrasporas y se compara el crecimiento de talos gametofíticos y tetrasporofíticos. Talos tetrasporofíticos presentaron tasas de crecimiento más elevadas que los talos gametofíticos.

Palabras clave: *Gracilaria tenuistipitata*, ciclo vital, reproducción, progenie de tetrasporas, Rhodophyta, Gracilariales.

INTRODUCTION

The genus *Gracilaria* (Rhodophyta: Gracilariales) has been intensively investigated due to its economic importance, high diversity and taxonomic problems (e.g. Bellorin *et al.*, 2002). Among the many species recognised in the genus, *Gracilaria tenuistipitata* C.F. Chang *et* B.M. Xia, a species originally described from China, has been a favourite alga for different types of investigations because it is easily kept *in vitro* under a broad range of growing conditions (e.g. Macchiavello *et al.*, 1998; Carnicas *et al.*, 1999; Hagopian *et al.*, 2004). In addition to the academic interest, *G. tenuistipitata* is cultivated in China as a source of agar and as fodder in abalone (*Haliotis* sp.) farms (Chiang, 1981; Lin and Liao, 1999).

Most studies on *G. tenuistipitata* have used a clone of a tetrasporophytic lineage isolated in our laboratory. Nevertheless, there is very little information on the life history of this species, or informa-

tion on its morphology. Moreover, the growth rate and the performance of the gametophytic generation have never been mentioned in the literature.

Here we describe the life history of *G. tenuistipitata*, completed *in vitro*, show the morphological variability of specimens derived from tetraspore progeny and compare the growth rates of the sporophytic and gametophytic phases.

MATERIALS AND METHODS

Algal material

The material used in our experiments is derived from a tetrasporophytic branch of a specimen collected in Haikou, Hainan Island, China, by E.C. Oliveira in 1990. This material was isolated by J. Macchiavello and has been kept in unialgal culture (photoperiod of 14 h, 25ºC and 30±10 µmol photons m^{-2} s^{-1}) in the *Gracilaria* germoplasm bank at the "Edison J. de Paula" laboratory, University of São Paulo.

Culture conditions

The standard culture conditions are: sterile seawater adjusted to 21 psu and enriched with von Stosch growing medium (Ursi and Plastino, 2001), 14h light: 10h dark, 24±1°C, 70±5 µmol photons m^{-2} s^{-1} (Osram 40W day light fluorescent tubes). Cultures were bubbled with compressed air for 30 min h^{-1}. The medium was renewed and thalli were observed weekly.

Life history

Tips of a tetrasporophytic specimen from our germoplasm bank were incubated in the standard culture conditions in Erlenmeyer vials with 500 ml of medium. Tetrasporangia were noticed after five weeks. Ten branches bearing tetrasporangia were transferred to Petri dishes with sterile seawater and observed under a stereomicroscope for the presence of released tetraspores. Tetraspores were transferred with a Pasteur pipette to four Petri dishes with 50 ml of enriched seawater until plantlets of 2-3 mm were produced. Thirty five plantlets were detached from the bottom of the plates and cultivated in Erlenmeyer flasks with 200 ml of the growing medium until cystocarps became visible to the naked eye. Branches with cystocarps were isolated and kept in the growing medium until carpospores were released. Carpospores were isolated and grown in the same conditions described for tetraspores. Fertile material was hand-sectioned and stained with 1% aqueous aniline blue to study the reproductive structures. Voucher specimens were deposited in the herbarium of the Biosciences Institute, University of São Paulo (SPF) numbered as SPF 56193 (fertile tetrasporophytes), SPF 56194 (female gametophytes with cystocarps) and SPF 56195 (fertile male gametophytes).

Growth rates

Apical branches (length 1 cm, total fresh weight ca. 1 mg) of female gametophytes and of unfertile tetrasporophytes (n=4) were cultivated for 6 weeks in 400 ml of enriched seawater in the standard conditions described above. The biomass was recorded weekly. Branches were gently blotted dry and weighted weekly on an analytical balance Mettler – Toledo AE 200S. Growth rates (GR) were assessed weekly according to Lignell and Pedersén (1989). A two-way analysis of variance was performed using the software STATISTICA 7.0 (StatSoft, Inc.), considering the time and life-history stages as independent variables and the growth rates as dependent variables. This analysis was followed by a Newman-Keuls a posteriori test.

RESULTS

The life history of *Gracilaria tenuistipitata* is of the *Polysiphonia*-type and was completed in 135 days. Tetrasporophytic branches cultivated in standard culture conditions produced tetrasporangia in ca. 20 days (Fig. 1A). Tetrasporangia are scattered on the cortex in small numbers (Fig. 1B), and are produced from the most external subcortical cells. They are ovoid, measure 24-33 x 45-48 µm and are divided according to a decussate pattern (Fig. 1C). Released tetraspores sunk, attached to the bottom of the vial, produced a cell wall and started the germination process. Around 15 days later, an erect cylindrical axis developed from a basal disk. Erect axes were initially unbranched, but later became sparsely branched. Secondary erect axes were formed from the disc-like base. At the age of 45 days, cystocarps and spermatangial conceptacles could be observed.

The morphology of gametophytes at the age of 53 days is shown in Figure 2. The differences in size

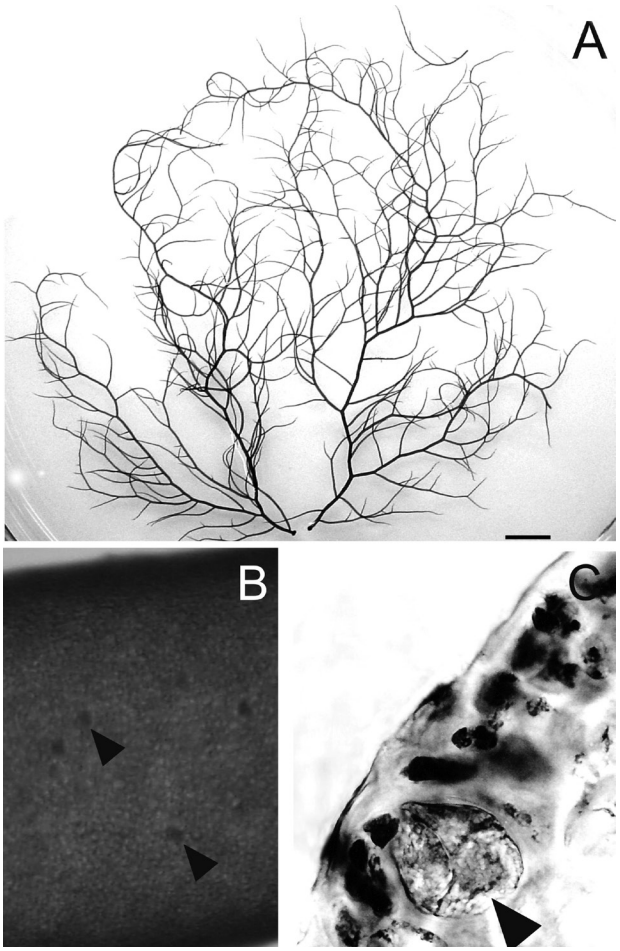

FIG. 1. – *Gracilaria tenuistipitata*. Habit and anatomy of tetrasporophytes. (A) Branches from the parental specimen. (B) Detail of a tetrasporophytic thallus with tetrasporangia frontal view (arrowheads). (C) Mature decussate divided tetrasporangium. Scale bars: A = 1 cm; B = 90 μm; C = 10 μm.

and branching of the tetraspore progeny, kept under the same conditions, are obvious, and there is large morphological variability resulting from the segregation process in the tetrasporangia.

Female gametophyte thalli kept together with male ones developed cystocarps, which are dome-shaped, with a light constriction at the base, and measure ca. 300 μm in diameter near the base when mature (Fig. 3A-B). Carpospores are released through a conspicuous ostiole at the tip of the cystocarp (Fig. 3C). The gonimoblasts are organized in a single gonimolobe; the pericarp has 7-10 layers of anticlinal rows (Figs. 3C, 3E); connecting filaments between the gonimoblasts and the pericarp are present at the cystocarpic base (Fig. 3D-E); carposporangia are produced in short series at the tips of the gonimoblast rows (Fig. 3C-E); a star shaped fusion cell can be seen at the base of the carposporophyte (Fig. 3F).

Released carpospores germinated in the same pattern as tetraspores (*Dumontia*-type) (Chemin, 1937). The resulting tetrasporophytes took around 20 days to become fertile and showed similar morphology to the stock plants, without much variation like that observed for gametophytes.

Male gametophytes could be easily detected under a stereomicroscope due to the presence of lighter spots under light transmitted from below (Fig. 4A). Spermatangial conceptacles (37 to 46 μm) are densely distributed all over the male specimens (Fig. 4B). In cross sections they appear as small depressions

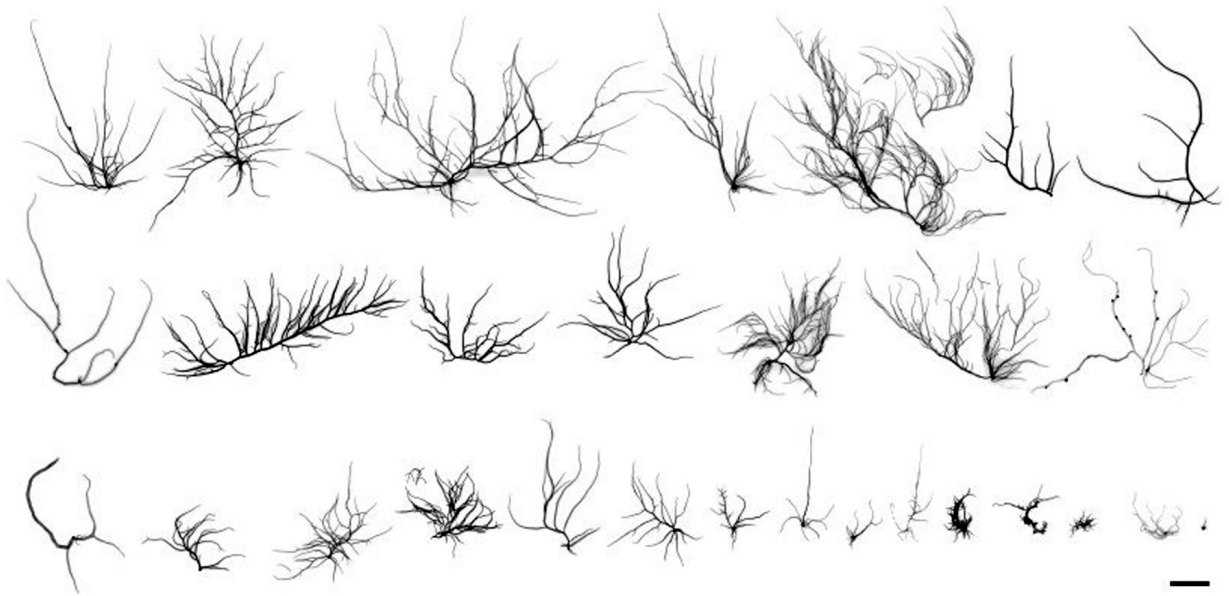

FIG. 2. – *Gracilaria tenuistipitata*. Gametophytes at the age of 53 days, originated from tetraspores and cultivated in the same conditions, showing a large variability in thalli morphology. Scale bar = 1 cm.

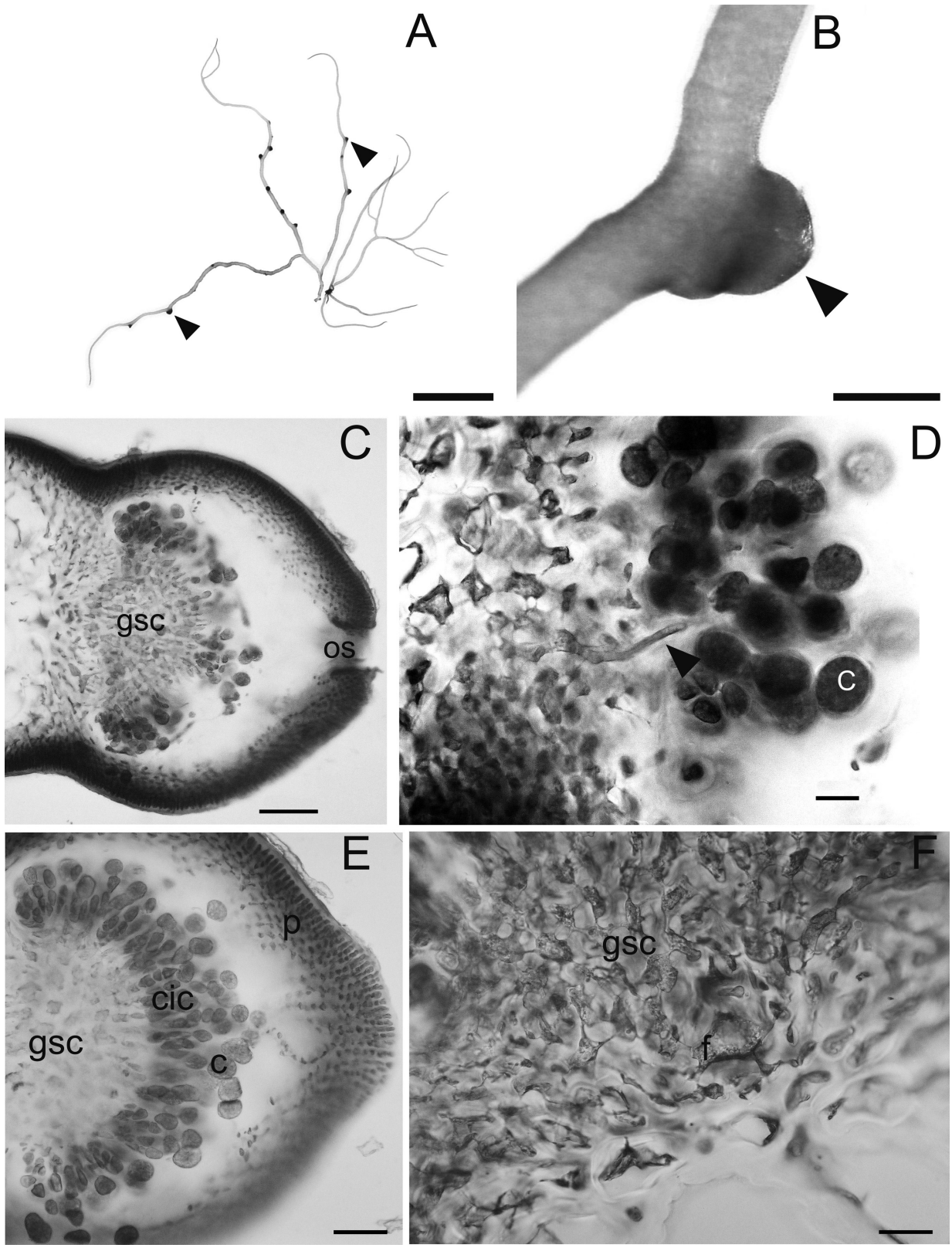

FIG. 3. – *Gracilaria tenuistipitata*. Cystocarpic plants. (A) Habit of a plant with cystocarps (arrowheads). (B) Mature cystocarp (arrowhead). (C) Median vertical section of a cystocarp, showing constricted base, gonimoblast cells (gsc) and ostiole (os). (D) Median vertical section of a cystocarp, showing the nutritive tubular cell (arrowhead) and carposporangia (c). (E) Cystocarp in transversal section, showing gonimoblast cells (gsc), carposporangium initial cells (cic), carposporangia (c) and the pericarp (p). (F) Median vertical section of a cystocarp showing the fusion cell (f) and gonimoblast cells (gsc). Scale bars: A = 2 cm; B = 500 μm; C = 100 μm; D = 20 μm; E = 50 μm; F = 20 μm.

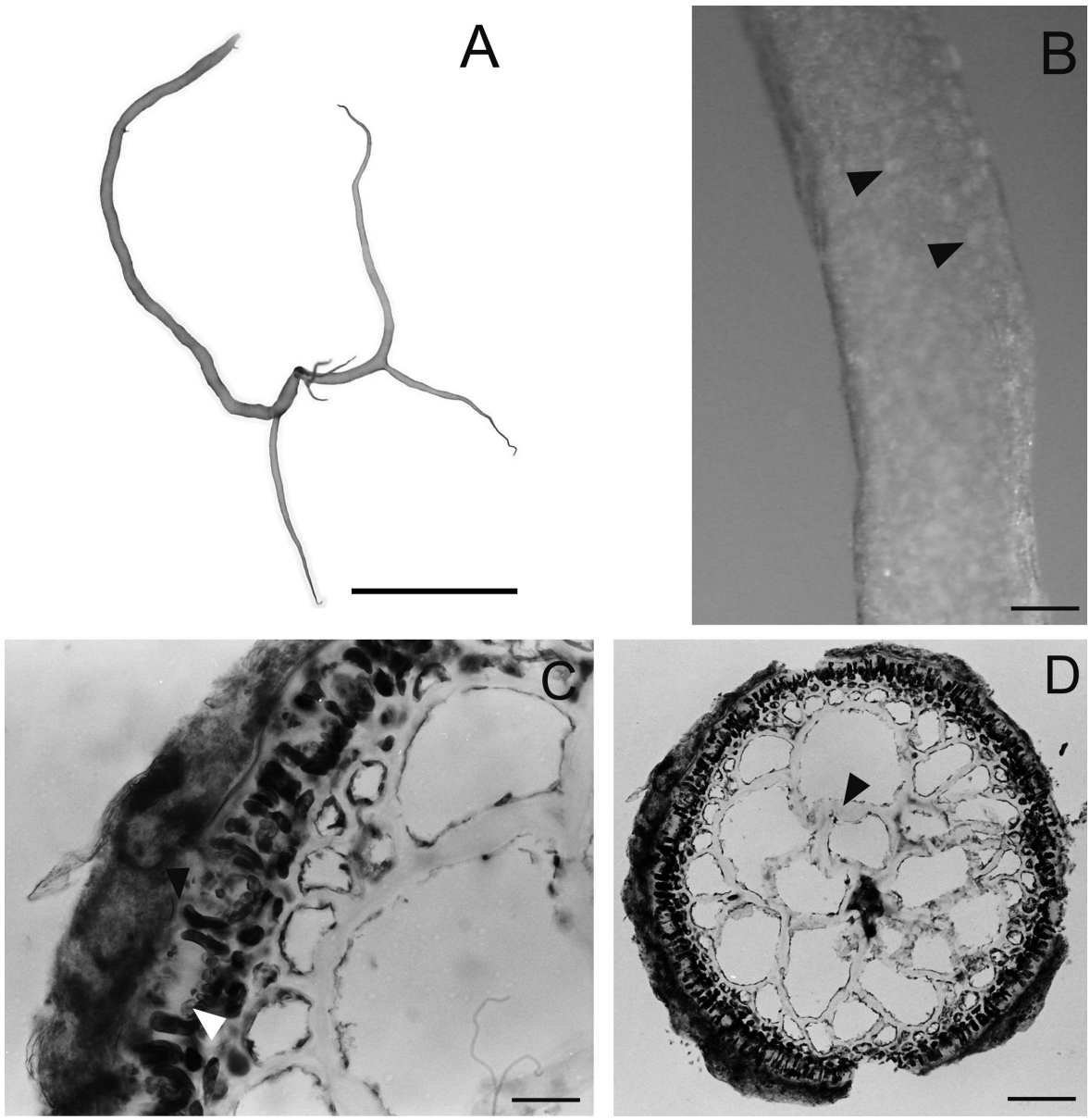

FIG. 4. – *Gracilaria tenuistipitata*. Male gametophytes. (A) Habit of a fertile plant. (B) Spermatangial conceptacles (arrowheads). (C) *Textorii* type spermatangial conceptacles containing columnar cells (black arrowhead) and spermatangia (white arrowhead). (D) Cross section from a thallus showing thickened cell walls (arrowhead) and macrocystiated cellular arrangement. Scale bars: A = 1 cm; B = 250 μm; C = 15 μm; D = 100 μm.

in the cortical region and are of the "*textorii*-type". The conceptacles are bordered by elongated flanking cells (Fig. 4C-D). Thallus anatomy shows a macrocystiated arrangement of cells (Fig. 4D).

The growth rates were significantly influenced by time and life history phases ($F=13.154$; $p<0.01$ and $F=76.021$; $p<0.01$, respectively). Tetrasporophytic branches showed higher growth rates than gametophytic ones (24.01 ± 0.68%FW.day^{-1} for tetrasporophytes and 21.1 ± 0.89 for gametophytes). Absolute values of growth rates ranged from 6.36 to 27.7% FW.day^{-1} (gametophytes) and from 5.88 to 35.5% FW.day^{-1} (tetrasporophytes). Growth rates decreased over time, regardless of the life-history phase. After three weeks, the growth rates started to decrease, and reached the lowest values after six weeks (Fig. 5).

DISCUSSION

Since the isolation of *G. tenuistipitata* in our laboratory this species has been taken to other laboratories in the world and is becoming a "model

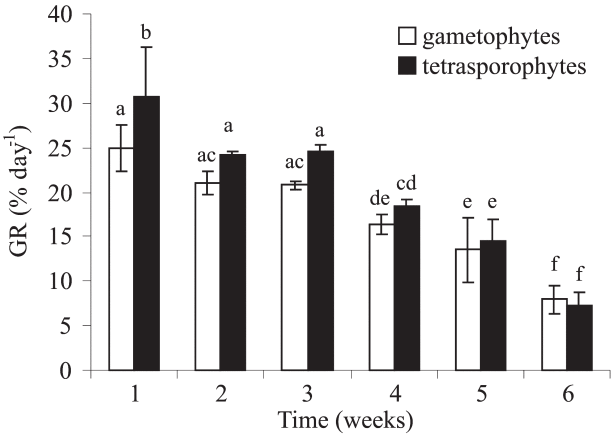

FIG. 5. – Growth rate (GR) of tetrasporophytes and female gametophytes of *Gracilaria tenuistipitata* over six weeks of cultivation. Bars signify standard deviations. N=4. The different letters over bars mean statistical differences observed after the Newman-Keuls *a posteriori* test.

organism" for different kinds of investigations (e.g. Israel *et al.*, 1999; Mercado *et al.*, 2001). Nevertheless, we have not found reports concerning its *in vitro* life history or studies on the performance of the gametophytic phase.

This is the first record of male gametophytes of *G. tenuistipitata* obtained *in vitro*. The only description of male structures for *G. tenuistipitata* is the one given by Chang and Xia (1976) based on specimens from natural populations. Bellorin (2002) interpreted the structures described by these authors as being of the *textorii*-type, which agrees with our observations.

The first life history of a *Gracilaria* sp. was completed by Ogata *et al.* (1972) with material from Japan identified as *G. verrucosa* (Hudson) Papenfuss, a name that was later abandoned. Since then the life histories of a few other species have been completed *in vitro* (McLachlan and Edelstein, 1977; Bird *et al.*, 1977; Guimarães *et al.*, 1999; Oliveira and Plastino, 1984; Plastino and Oliveira, 1988; Yamamoto, 1991; Costa and Plastino, 2001). This study confirms the hypothesis that *G. tenuistipitata* also has a "*Polysiphonia*-type" life history as described for other species, although deviations have been reported in some species (Plastino and Oliveira, 1984; Kain and Destombe, 1995).

It is well known that species of red algae of commercial interest which are propagated vegetatively are mostly infertile and seem to lose the capacity to reproduce sexually (Bird *et al.*, 1986). Therefore, it is worth noting that this *G. tenuistipitata* has kept its potential to form reproductive structures intact and completed its life history after being replicated vegetatively in our lab for more than 15 years. The failure of Oliveira and Plastino (1984) to complete the life history of some *Gracilaria* spp. may be due to the lack of optimal conditions for triggering the formation of reproductive cells rather than the loss of reproduction potential.

The life history of *G. tenuistipitata* was completed in about 4.5 month, which is a short period when compared to *G. foliifera* (Forsskål) Børgesen (around 8 months, McLachlan and Edelstein, 1977), *G. debilis* (Forsskål) Børgesen (5 months), *Gracilaria* sp. (9 months) (Oliveira and Plastino, 1984) and *G. domingensis* (Kützing) Sonder ex Dickie (6 months) (Guimarães *et al.*, 1999). The time necessary to complete the life history of *G. tenuistipitata* was similar to wild strains of *G. birdiae* Plastino and Oliveira, which completed the *in vitro* life history in 4 months (Costa and Plastino, 2001). However, as mentioned by other authors, the time needed to complete a life history depends on different factors, such as temperature, nutrients and the amount of photosynthetic active radiation, and so should not be taken exclusively as a specific attribute.

Tetraspore progeny yielded a range of morphologically different individuals that had not yet been seen for a *Gracilaria* species, although they had been registered for other red algae, such as *Kappaphycus alvarezii* (Doty) Doty ex P.C. Silva (Paula *et al.*, 1999). Morphological differences between gametophytic and tetrasporophytic generations were observed in some *Gracilaria* spp., and the gametophytic phase was usually smaller than the sporophytic phase (Oliveira and Plastino, 1984; Kain and Destombe, 1995). This large variability restricts the possibility of describing taxonomical varieties based on gross morphology, as is the case of *G. tenuistipitata* var. *liui* Zhang et Xia. However, this again shows that it is a very effective method for producing cultivars for commercial farming, as has been demonstrated for other seaweeds, including for instance, *G. tikvahiae* polyploids and mutants (Patwary and van der Meer, 1983), *G. chilensis* C.J. Bird, McLachlan and E.C. Oliveira intra-clonal strains (Santelices and Varela, 1993) and *K. alvarezii* tetraspore progeny (Paula *et al.*, 1999).

The growth rates of tetrasporophytes were higher than those of female gametophytes but mostly in the first week. Considering the following weeks, growth rates were similar comparing both phases. The relative performance of the two phases varies with the species. Santelices and Varela (1995) observed

higher growth rates in fertile female gametophytes in comparison to fertile tetrasporic plants of *G. chilensis.* Ursi and Plastino (2001) observed higher growth rates of female gametophytes of *G. birdiae* compared with male gametophytes and tetrasporophytes, whereas Barufi (2004) obtained the opposite results for the same species. This indicates that the performance of gametophytes is a matter of genetic diversity. Furthermore, the differences with respect to growth rates can be related to the different nutritional needs of the reproductive stages, which are advantageous for the survival of the species in heterogeneous environments (Ursi and Plastino, 2001).

The results presented in this work on the life history and on the variability of tetraspore progeny give further evidence that *G. tenuistipitata* is a model organism among the florideophyte red algae and are also relevant for strain selection for commercial cultivation of this species.

ACKNOWLEDGEMENTS

This study was supported by The National Council for Scientific and Technological Development (CNPq) (scholarships and research project 470417/2006-4) and the Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES) for a scholarship to J.B. Barufi. We thank Rosário Petti for technical assistance and Eugenia Marquez Garrido for the review of the Spanish abstract.

REFERENCES

Barufi, J.B. – 2004. *Efeitos da luz na reprodução, no crescimento e no conteúdo pigmentar de* Gracilaria birdiae *(Gracilariales, Rhodophyta)*. MSc. thesis, Univ. S. Paulo.

Bellorin, A.M. – 2002. *Sistemática e filogenia molecular de algas gracilarióides (Gracilariaceae, Rhodophyta)*. Ph. D. thesis, Univ. S. Paulo.

Bellorin, A.M., M.C. Oliveira and E.C. Oliveira. – 2002. Phylogeny and systematics of the marine algal family Gracilariaceae (Gracilariales, Rhodophyta) based on small subunit rDNA and its sequences of Atlantic and Pacific species. *J. Phycol.,* 38: 551-563.

Bird, C.J., J. McLachlan and D. Grund. – 1977. Studies on *Gracilaria*. 5. *In vitro* life history of *Gracilaria* sp. from the Maritime Provinces. *Can. J. Bot.,* 55: 1282-1290.

Bird, C.J., J. McLachlan and E.C. Oliveira. – 1986. *Gracilaria chilensis* sp. nov. (Rhodophyta, Gigartinales), from Pacific South America. *Can. J. Bot.,* 64: 2928-2934.

Carnicas, E., C. Jiménez and F.X. Niell. – 1999. Effects of irradiance on the pigment composition of *Gracilaria tenuistipitata* var. *liui* Zhang et Xia. *J. Photochem. Photobiol. B. Biol.,* 50: 149-158.

Chang, C.F. and B.M. Xia. – 1976. Studies on Chinese species of *Gracilaria*. *Stud. Mar. Sin.,* 12: 91-163 + 2 pl.

Chemin, E. – 1937. Le développement des spores chez les Rhodophycées. *Rev. Gen. Bot.,* 49: 205-234.

Chiang, Y.M. – 1981. Cultivation of *Gracilaria* (Rhodophyta, Gigartinales) in Taiwan. *Proc. Int. Seaweed Symp.,* 10: 569-574.

Costa, V.L. and E.M. Plastino. – 2001. Histórico de vida de espécimens selvagens e variantes cromáticas de *Gracilaria* sp. (Gracilariales, Rhodophyta). *Rev. Bras. Bot.,* 4: 483-490.

Guimarães, M., E.M. Plastino and E.C. Oliveira. – 1999. Life history, reproduction and growth of *Gracilaria domingensis* (Gracilariales, Rhodophyta) from Brazil. *Bot. Mar.,* 42: 481-486.

Hagopian, J.C., M. Reis, J.P. Kitajima, D. Bhattacharya and M.C. Oliveira. – 2004. Comparative analysis of the complete plastid genome sequence of the red alga *Gracilaria tenuistipitata* var. *liui* provides insights on the evolution of rhodoplasts and their relationship to other plastids. *J. Mol. Evol.,* 59: 464-477.

Israel, A., M. Martínez-Goss and M. Friedlander. – 1999. Effect of salinity and pH on growth and agar yield of *Gracilaria tenuistipitata* var. *liui* in laboratory and outdoor cultivation. *J. Appl. Phycol.,* 11: 543-549.

Kain, J.M. and C. Destombe. – 1995. A review of the life history, reproduction and phenology of *Gracilaria*. *J. Appl. Phycol.,* 7: 269-281.

Lignell, A. and M. Pedersén. – 1989. Agar composition as a function of morphology and growth rate. Studies on some morphological strains of *Gracilaria secundata* and *Gracilaria verrucosa* (Rhodophyta). *Bot. Mar.,* 32: 219-227.

Lin, M.C. and C.M. Liao. – 1999. 65Zn(II) accumulation in the soft tissue and shell of abalone *Haliotis diversicolor* supertexta via the alga *Gracilaria tenuistipitata* var. *liui* and the ambient water. *Aquaculture,* 178: 89-101.

Macchiavello, J., E.J. Paula and E.C. Oliveira. – 1998. Growth rate responses of five commercial strains of *Gracilaria* (Rhodophyta, Gracilariales) to temperature and light. *J. World Aquac. Soc.,* 29: 259-266.

McLachlan, J. and T. Edelstein. – 1977. Life-history and culture of *Gracilaria foliifera* (Rhodophyta) from South Devon. *J. Mar. Biol. Assoc. U.K.,* 57: 577-586.

Mercado, J.M., P. Sánchez, R. Carmona and F.X. Niell. – 2001. Limited acclimation of photosynthesis to blue light in the seaweed *Gracilaria tenuistipitata*. *Physiol. Plant.,* 114: 491-498.

Ogata, E., T. Matsui and H. Nakamura. – 1972. The life cycle of *Gracilaria verrucosa* (Rhodophyceae, Gigartinales) *in vitro*. *Phycologia,* 11: 75-85.

Oliveira, E.C. and E.M. Plastino. – 1984. The life-history of some species of *Gracilaria* (Rhodophyta) from Brazil. *Jpn. J. Phycol. (Sôrui),* 32: 203-208.

Patwary, M.U. and J.P. van Der Meer. – 1983. Growth experiments on morphological mutants of *Gracilaria tikvahiae* (Rhodophyceae). *Can. J. Bot.,* 61: 1654-1659.

Paula, E.J., R.T.L. Pereira and M. Ohno. – 1999. Strain selection in *Kappaphycus alvarezii* var. *alvarezii* (Solieriaceae, Rhodophyta) using tetraspore progeny. *J. Appl. Phycol*., 11: 111-121.

Plastino, E.M. and E.C. Oliveira. – 1984. On the life-history of *Gracilaria* (Rhodophyta) from Brazil. *Jpn. J. Phycol. (Sôrui,)* 32: 203-208.

Plastino, E.M. and E.C. Oliveira. – 1988. Deviations in the life-history of *Gracilaria* sp. (Rhodophyta, Gigartinales), from Coquimbo, Chile, under different culture conditions. *Hydrobiologia,* 164: 67-74.

Santelices, B. and D. Varela. – 1993. Intra-clonal variation in the red seaweed *Gracilaria chilensis. Mar. Biol*., 116: 543-552.

Santelices, B. and D. Varela. – 1995. Regenerative capacity of *Gracilaria* fragments: effects of size, reproductive state and position along the axis. *J. Appl. Phycol.,* 7: 501-506.

Ursi, S. and E.M. Plastino. – 2001. Crescimento *in vitro* de linhagens de coloração vermelha e verde clara de *Gracilaria* sp. (Gracilariales, Rhodophyta) em dois meios de cultura: análise de diferentes estádios reprodutivos. *Rev. Bras. Bot.,* 4: 585-592.

Yamamoto, H. – 1991. Life history of *Gracilaria salicornia* (C.Ag.) Dawson (Gracilariales, Rhodophyta) *in vitro*. *Jpn. J. Phycol. (Sôrui),* 39: 55-56.

Scient. ed.: E. Ballesteros.
Received March 25, 2009. Accepted July 15, 2009.
Published online March 1, 2010.