J Appl Phycol (2010) 22:195–202
DOI 10.1007/s10811-009-9442-9

# Carpospore early development and callus-like tissue induction of *Chrysymenia wrightii* (Rhodymeniaceae, Rhodophyta) under laboratory conditions

**Fengjuan Zhao · Ziguo Zhao · Aihua Wang · Jidong Liu · Delin Duan**

Received: 13 February 2009 / Revised and accepted: 8 April 2009 / Published online: 20 May 2009


**Abstract** Morphological and culture studies of germlings derived from carpospores of *Chrysymenia wrightii* (Harvey) Yamada were carried out under various treatments combining temperature and irradiance. Basal, main, and tip branches were applied for inducing callus-like tissue. Focus was on how carpospores develop into germlings, how callus-like tissues are induced from explants, and how temperature and irradiance affect carpospore germination and discoid crust growth. Results show that carpospore development can be divided into three stages: division stage, discoid crust stage, and erect juvenile germling stage. Discoid crusts, even more than ten, might coalesce into a big discoid crust, and then developed into germlings. Filamentous fronds, formed on the rims of discoid crusts, exhibited in self-existence or co-existence form with germlings, could form spherical tufts if cultured separately. Filamentous callus-like tissues appeared on the tip branches after 13 days. PES is suitable for filament induction and culture, and filaments have potential use in germplasm preservation and vegetative propagation. Temperature (10, 15, 20, 25°C) and irradiance (8 and 36 μmol photons $m^{-2}$ $s^{-1}$) significantly influenced carpospore germination rate and discoid crust diameter. Carpospores germinated normally under 36 μmol photons $m^{-2}$ $s^{-1}$, 15~25°C, and maximum growth of discoid crusts was at 25°C, 36 μmol photons $m^{-2}$ $s^{-1}$; 10°C and 8 μmol photons $m^{-2}$ $s^{-1}$ did not favor carpospore germination or discoid crust growth.

**Keywords** Callus-like tissue · Carpospores · *Chrysymenia wrightii* · Development · Filamentous fronds · Rhodophyta

## Introduction

The red alga *Chrysymenia wrightii* (Harvey) Yamada, belonging to Florideophyceae, Rhodymeniales, and Rhodymeniaceae, is distributed along the coasts of China, Japan, Korea, and Russia (Tseng 1983; Yoshida et al. 1990; Perestenko 1994; Lee and Kang 2001), generally on lower intertidal rocks (Lee and Kurogi 1972). This alga is also a non-native species on the Atlantic coast of Europe (Bárbara et al. 2008) and is valued recently from points of view of invasive aspect (Williams and Smith 2007). With biological importance (Peña and Bárbara 2008), and high protein content and commercial carrageenan production, *C. wrightii* has potential economic value related to agar production and natural bioactive substance extraction (Xu et al. 2003, 2004; Zhang et al. 2005). Most economic harvest now comes from wild stock, but seedling culture is desirable (Glenn et al. 1996), requiring improved under-

Zhao FJ, Zhao ZG, and Wang AH are equal contribution to the work.

F. Zhao
Shandong Key Laboratory of Eco-environmental Science for Yellow River Delta, Binzhou University, Binzhou 256600, China

Z. Zhao
Department of Life Science, Binzhou University, Binzhou 256600, China

A. Wang
School of Life Science, Qingdao Agriculture University, Qingdao 266109, China

F. Zhao · A. Wang · J. Liu · D. Duan (✉)
Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
e-mail: dlduan@ms.qdio.ac.cn

standing of reproduction processes and improved culture techniques.

The *C. wrightii* life cycle features two diploid life stages and gametophytic generation, which followed the Polysiphonia pattern cycle of Florideophyceae. Usually, spore recruitment is usual in seaweed reproduction, and feasible seedling nursery cultivation is proven for *Gracilaria* culture (Levy et al. 1990; Destombe et al. 1993). However, detailed information about *C. wrightii* carpospore development is limited, restricting practical application of seedling nursery techniques.

Callus-like tissue can be used to induce thalli and undifferentiated filament clumps from algal explants and there has been much micro-propagation of some seaweed vegetation (Shao et al. 2004; Wang et al. 2006a; Kumar et al. 2007; Yin et al. 2007; Zhang et al. 2007). Such alternative seedling nursery techniques show promise because spore recruitment lead to lengthy seedling culture. However, there are no known reports regarding to occurrence and induction of *C. wrightii* filaments.

Our work had several aims: to track morphological changes of carpospores during early development, to analyze temperature and irradiance affects on germination and early growth, and to understand callus-like tissue induction and culture, which should assist *C. wrightii* seedling production from carpospores and filaments.

## Materials and methods

### Treatment and pre-culture of fronds for carpospore release and settlement

On July 3rd, 2005, mature *Chrysymenia wrightii* with cystocarps were collected from the intertidal zone of Taiping Bay, Qingdao (36°03′N, 120°20′E). Cystocarpic fronds were selected, brushed in filtered seawater and wiped with moistened cotton tissues to remove the epiphytes. Fronds were immersed in 1.5% KI for 10 min (Wang et al. 2006b) and rinsed thoroughly with sterilized seawater.

Rinsed fronds were excised into pieces (c. 1 cm long) and transferred to 16 Petri dishes (60 mm×15 mm) containing cover slips (three to four pieces per Petri dish). Several longer frond pieces (c. 3 cm long) were cultured in three beakers, each containing a glass slide wound up with nylon line. Frond segments in Petri dishes and beakers were pre-cultured in cultivation chambers using 18°C, 10± 2 μmol photons $m^{-2}$ $s^{-1}$, and 10 light/14 dark (L/D) photoperiod. Culture medium was filtered boiled seawater enriched with 0.2 $mmol.L^{-1}$ of $NO_3^-$-N ($KNO_3$) and 0.02 $mmol.L^{-1}$ of $PO_4^-$-P ($KH_2PO_4$), refreshed every 3 days.

Carpospores released 24–72 h after treatment and settled on immersed cover slips and nylon lines. When density reached 30–50 carpospores per microscopic field (magnification,

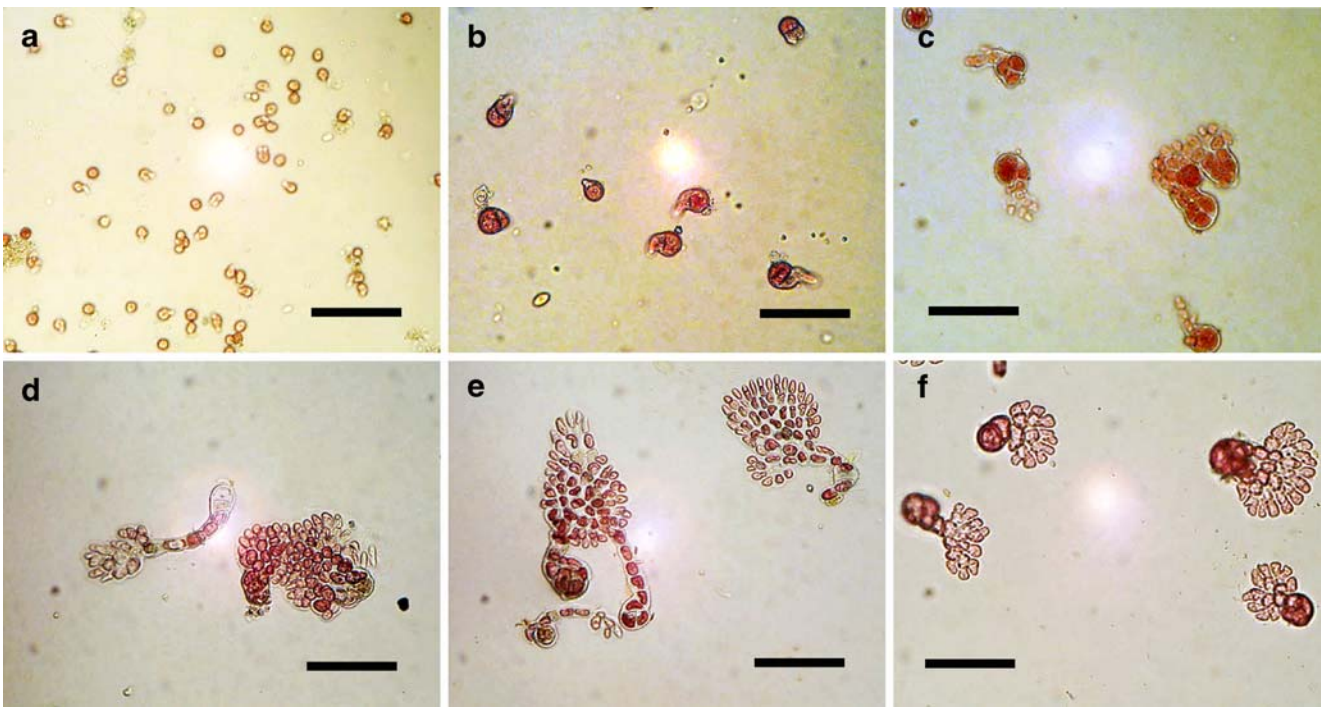


**Fig. 1** *Chrysymenia wrightii* carpospore germination **a** fresh released carpospores, *bar*=160 μm; **b** the first transverse cell division, *bar*=80 μm; **c** subsequent carpospore cell divisions, *bar*=60 μm; **d** and **e** long handle-like structures of few developing fronds, *bar*=66 μm; **f** Short handle-like and radiate structures of most developing fronds, *bar*=60 μm

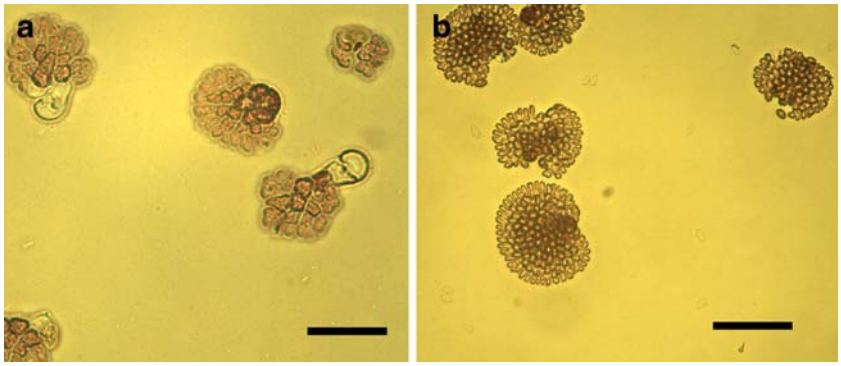


**Fig. 2** *Chrysymenia wrightii* development from carpospores to discoid crusts **a** extending radiate structures and disintegrating handle-like structures, *bar*=50 μm; **b** radiate structure approaches near discoid structure, *bar*=100 μm

10×10), algal frond segments were removed. Carpospores in Petri dishes were cultivated under different treatments described below. The beakers were cultured using 18°C, 30± 2 μmol photons $m^{-2}$ $s^{-1}$, and 12L/12D photoperiod.

## Morphological changes of carpospores during early development

Under 25°C, 36 μmol photons $m^{-2}$ $s^{-1}$, and 12L/12D photoperiod, the process from the earliest spore development to formation of erect juvenile germlings was observed using a light microscope (Nikon Eclipse 50i, Japan and Zeiss, Axioplan, Germany) and recorded with a digital microscopic camera (Photometrics Cool Snap, Germany).

## Temperature and irradiance effects on carpospores

Released carpospores in Petri dishes were cultured in controlled incubators (GXZ-260A, Ningbo, China). Temperature treatments were performed at 10, 15, 20, and 25°C with 36 μmol photons $m^{-2}$ $s^{-1}$ and 8 μmol photons $m^{-2}$ $s^{-1}$ controlled by cool-white fluorescent tubes (Philips,

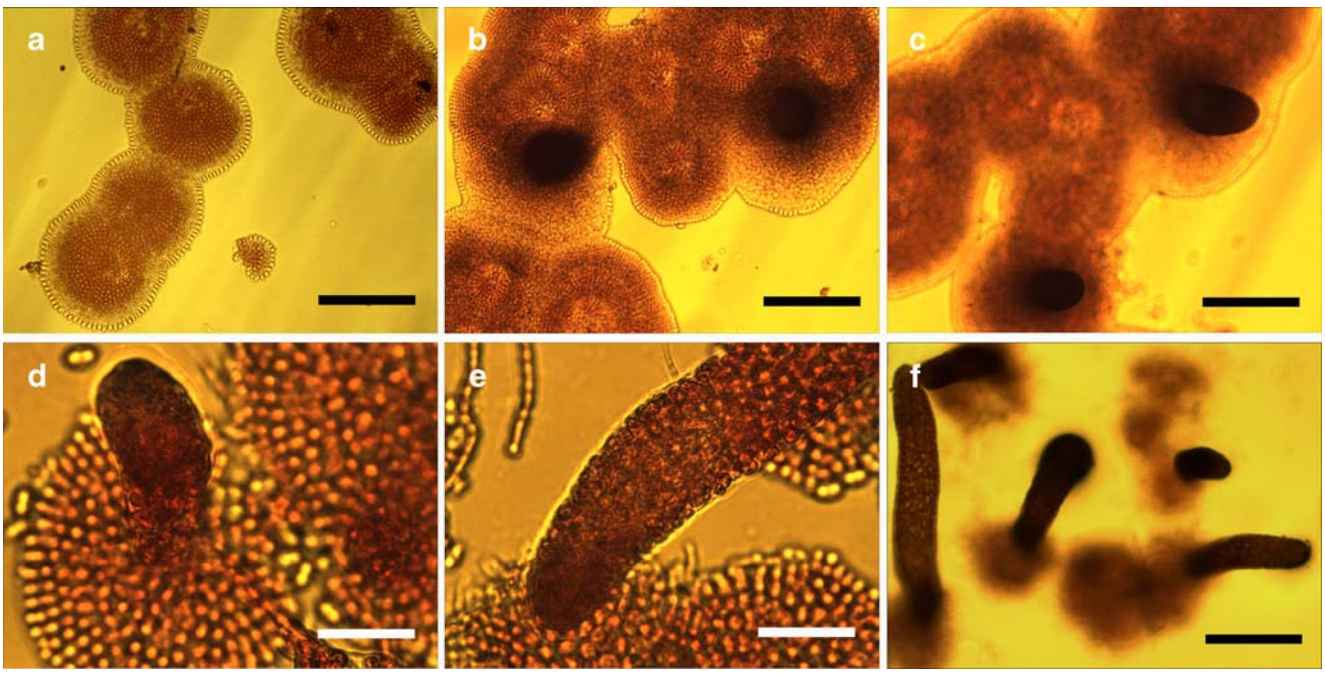


**Fig. 3** Discoid crusts derived from carpospores form juvenile germlings **a** discoid crusts have pigmented cells located in the center and transparent colloid around the disk, *bar*=167 μm; **b** coalescence of discoid crusts, *bar*=200 μm; **c** erect juvenile germlings appear from the surface of discoid crusts, *bar*=200 μm; **d** magnification of juvenile germling, *bar*=100 μm; **e** side view of germlings after 3 weeks, *bar*=100 μm; **f** germlings cultured for 7 weeks, showing increase in length, *bar*=200 μm


**Fig. 4** *Chrysymenia wrightii* germlings on the nylon string in beaker under indoor conditions **a** overhead view; **b** lateral view

Shanghai). Photon flux densities were measured using a quantum photometer (LI-COR, LI-250) and 12 L/12D culture photoperiod.

After 5 days, carpospore germination rates were determined in ten random microscopic fields (magnification, 10×10). Discolored and dying carpospores were not considered in this process. After 4 weeks under each culture condition, diameters of 50 randomly chosen discoid crusts in microscopic fields (magnification, 10×10) were measured by a micrometer fixed in the microscope eyepiece.

### Culture of filamentous fronds

Some massive filaments on the rims of discoid crusts were removed and cultured separately in conical flasks containing sterile PES-enriched seawater (Wang et al. 1994) in 18°C, 10 μmol photons $m^{-2}$ $s^{-1}$, and 12L/12D photoperiod. The conical flasks were shaken three times per day during culture.

### Induction and culture of callus-like tissues

After pretreatment, basal, main and tip parts of the *C. wrightii* were cut into explants (0.5~1 cm) and cultured separately in cylindrical vessels containing sterilized PES-enriched seawater in 18°C, 10 μmol photons $m^{-2}$ $s^{-1}$, and 12L/12D photoperiod.

Every vessel contained the same type of two explants, and one repetition was utilized. Culture medium was refreshed every 3 days. The vessels were shaken three times per day during the culture.

### Data analysis

One-way ANOVA was performed and Tukey's multiple comparison tests were used to compare germination rates of carpospores and diameters of discoid crusts grown under various temperature treatments. Student's *t* test was used to compare germination rates of carpospores and diameters of discoid crusts under high and low photon flux density treatments. Statistical analyses were carried out using SPSS with significance level set at $P<0.01$. Data are expressed as mean ± S.E.

## Results

### Development of juvenile germlings from carpospores

Carpospores of *Chrysymenia wrightii* that settled on immersed cover slips had mean diameter of 22 μm (Fig. 1a). Early carpospore development could be characterized as having three stages: division stage, discoid crust stage, and juvenile germling stage.

About 40% of carpospores began to germinate within 2 h of release and settlement (Fig. 1b). After initial cell transverse division (Fig. 1b) and various cell divisions, germinated carpospores developed into multi-cellular juvenile fronds (Fig. 1c) with radiate structures in the distal ends. A few fronds had long handle-like structures (Fig. 1d, e) and peculiar shapes (Fig. 1e); most fronds had short handle-like structures (Fig. 1f).

Handle-like structures of juvenile fronds usually faded and disintegrated gradually (Fig. 2a). Radiate structures extended all around, eventually transformed into small discoid crusts (Fig. 2b). Ten days after beginning culture with 36 μmol photons $m^{-2}$ $s^{-1}$, 25°C, and 12 L/12D, some germinative carpospores developed into tetrasporophytic discoid crusts.

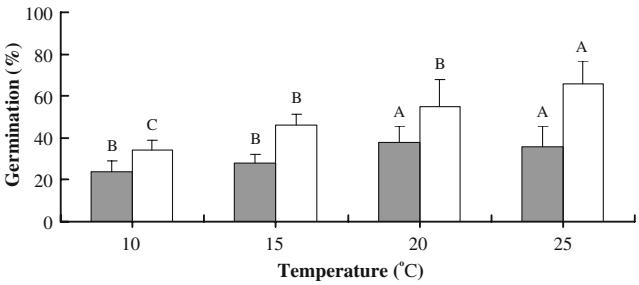


**Fig. 5** Temperature effect on germination rates with 8 (*filled*) and 36 (*empty*) μmol photons $m^{-2}$ $s^{-1}$. Values are means ± S.E. Statistically significant differences (ANOVA, Tukey) are indicated by capital letter superscripts ($P<0.01$)

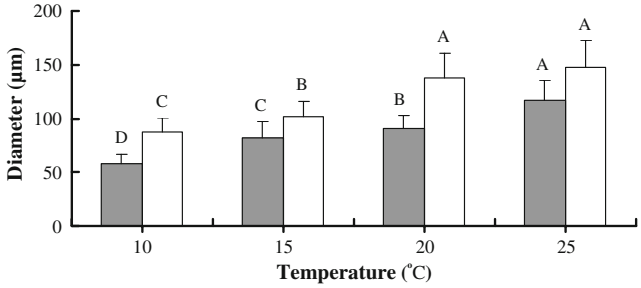


**Fig. 6** Temperature effect on discoid crust diameters with 8 (*filled*) and 36 (*empty*) μmol photons $m^{-2}$ $s^{-1}$. Values are means ± S.E. Statistically significant differences (ANOVA, Tukey) are indicated by capital letter superscripts ($P<0.01$)

Under all culture conditions, tetrasporophytic discoid crusts appeared three weeks after culture started.

Coalescence of two or more discoid crusts was observed in the discoid crust stage of *C. wrightii* (Fig. 3a and b). About 20–30 days after settlement central areas of discoid crusts arched slightly and apophyses appeared gradually from basal parts due to rapid multiplication of meristem (Fig. 3b and c). Apophyses eventually developed into erect axes and erect tetrasporophytic germlings appeared (Fig. 3d and e).

After 7–8 weeks, juvenile germlings (300–500 μm long) were visible on cover slips without magnification with 36 μmol photons $m^{-2}$ $s^{-1}$ at 20°C or 25°C (Fig. 3f). Several juvenile germlings that derived from carpospores settled on nylon lines and grew indoors for over 14 weeks, developing frond lengths of 1–3 cm (Fig. 4a and b).

## Temperature and irradiance effects on carpospore germination

Temperature (10, 15, 20, 25°C) significantly influenced carpospore germination rate under both high and low photon flux density, as determined 5 days after settlement of carpospores (ANOVA: low photon flux density, $F=8.733$, $P<0.01$; high photon flux density, $F=22.33$, $P<0.01$; Fig. 5). Carpospore germination rate increased as temperature increased from 10 to 25°C. Maximum germination rate was 66% with 36 μmol photons $m^{-2}$ $s^{-1}$ and 25°C. With low photon flux density (8 μmol photons $m^{-2}$ $s^{-1}$), germination rate differed significantly between the high temperature section (20°C, 25°C) and the low temperature section (10°C, 15°C; Tukey's test, $P<0.01$). There was no significant difference in germination rate within temperature sections. With high photon flux density (36 μmol photons $m^{-2}$ $s^{-1}$) there was no significant difference in germination rate under 15°C, 46% and 20°C, 52%.

Irradiance significantly affected carpospore germination rate at each testing temperature (Student's $t$ test, $P<0.01$). High photon flux density favored carpospore germination (Fig. 5).

## Temperature and irradiance effects on discoid crust growth

After 4 weeks culture under various conditions, discoid crust diameters were compared (Fig. 6). Temperatures significantly affected discoid crust diameters (ANOVA: low photon flux density, $F=150.64$, $P<0.01$; high photon flux density, $F=113.65$, $P<0.01$). With low photon flux density (8 μmol photons $m^{-2}$ $s^{-1}$), discoid crust diameters differed significantly (Tukey's test, $P<0.01$) under every testing temperature (min, 58 μm, 10°C; max, 116.9 μm, 25°C). Under high photon flux density (36 μmol photons $m^{-2}$ $s^{-1}$) there was no significant difference in diameters under 20°C (137.6 μm) and 25°C (148.2 μm).

Irradiance significantly affected discoid crust growth (Student's $t$ test, $P<0.01$) (Fig. 6). Discoid crust diameters were smaller under low photon flux density than under high photon flux density at each testing temperature.

## Newly observed phenomenon during early development

Shortly after the discoid crust stage occurred under conditions of 36 μmol photons $m^{-2}$ $s^{-1}$, 20°C and a

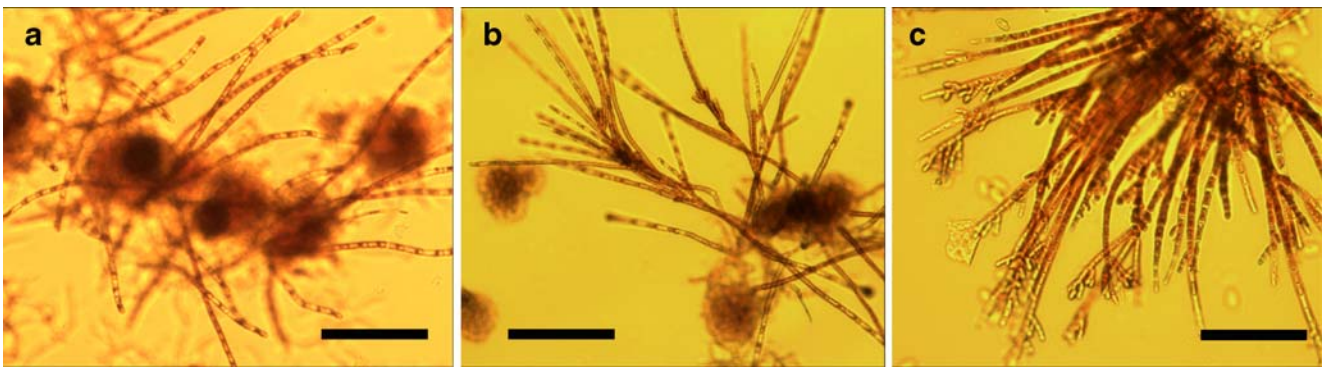


**Fig. 7** New development phenomenon of filamentous fronds in *Chrysymenia wrightii* **a** co-existence of normal germlings and eramose filamentous fronds composed of a row of cells, *bar*=100 μm; **b** ramose filamentous fronds, *bar*=100 μm; **c** developing ramose filamentous fronds cluster on discoid crusts without juvenile germlings, bar=100 μm

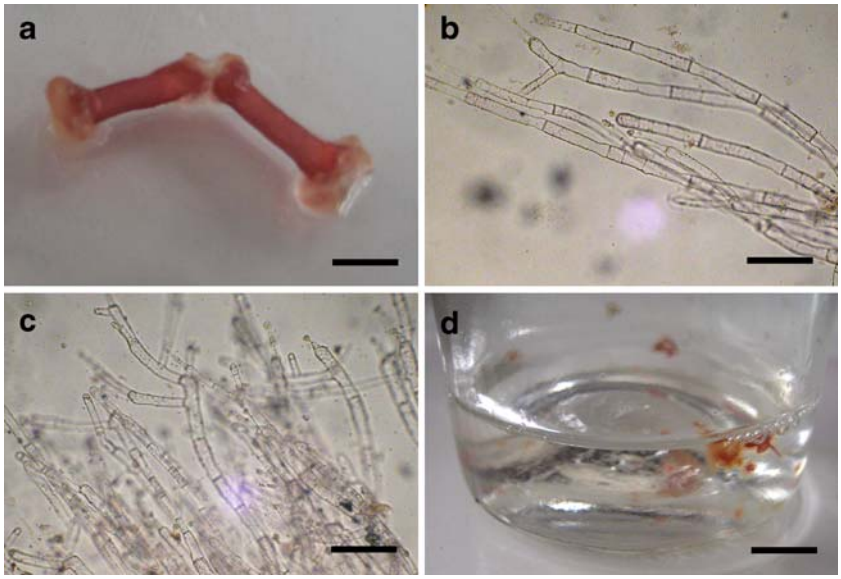


**Fig. 8** Callus-like tissues induction and culture from the explants **a** callus-like tissues appear from both sides of the tip branches, *bar*=0.3 cm; **b** eramose filamentous fronds, *bar*=30 μm; **c** ramose filamentous fronds, *bar*=30 μm; **d** tufts of filamentous fronds from explant under suspension culture, *bar*=1 cm

photoperiod of 12L/12D photoperiod, one or more uniseriate filaments (c. 6–7 μm diameter) appeared on the rims of about 10% of the discoid crusts (Fig. 7a). As development progressed, filaments appeared on most discoid crusts at all temperature grads with 36 μmol photons $m^{-2}$ $s^{-1}$ irradiation. Generally, filaments were more numerous as temperature increased, but few filaments appeared at 8 μmol photons $m^{-2}$ $s^{-1}$. Whether or not filaments were ramose in distal ends, filaments coexisted with juvenile germlings on the discoid crusts (Fig. 7a and b) or clustered on discoid crusts that lacked juvenile germlings (Fig. 7c).

Callus-like tissue induction and culture

Thirteen days after culture began, callus-like tissues were observed on the tip branches, appearing as pink colored filaments on both sides of the explants (Fig. 8a). Eramose and ramose filaments were observed (Fig. 8b and c). After culture for 15 and 17 days, callus-like tissues were also observed on the both sides of explants from basal and main branches of the thalli. Tufts formed 7 days after filaments were cultured in PES culture medium (Fig. 8d).

About 14 days after culture began, cultured filaments that separated from discoid crusts formed tufts in conical flasks containing sterile PES-enriched seawater (Fig. 9). The tufts partially dispersed through intense shaking. Due to nutritional limitations, filament growth gradually slowed as tuft diameters increased, but growth accelerated when filaments were separated and re-cultured. No transformation from filaments to discoid crusts or germlings was observed in the culture.

## Discussion

Deciphering the mechanism of carpospores development and germination is difficult due to complex interactions involving environmental factors, gene transcriptions and physiological and biochemical processes. In our study, not all carpospores germinated and germination rates varied with culture conditions. Carpospores that did not germinate turned from red color to green color and then gradually became pale. Sporeling development of *C. wrightii* recorded here has atypical primary cell division stage, discoid crust stage, and juvenile germling stage similar to other red algae (Ogata et al. 1972; Chen and Ren 1985, 1987).

*Chrysymenia wrightii* matures in summer times, indicating that high temperature favors germination and colonial growth. Thus, it was not surprising to find maximum

**Fig. 9** The tufts of filamentous frond from discoid crusts under suspension culture

germination rate for carpospores and discoid crust diameter at relatively higher temperatures. With 36 μmol photons $m^{-2}$ $s^{-1}$ irradiance, germination rates reached 46% at 15°C and 52% at 20°C, agreeing with previous work on *Gracilaria* (Chen and Ren 1987). Further study using more irradiance gradients is needed to confirm optimal carpospore germination conditions.

On the whole, discoid crust diameters increased with temperature under certain irradiance conditions. However, at 2 weeks, there was no significant difference between treatments of 20°C and 25°C with 36 μmol photons $m^{-2}$ $s^{-1}$, perhaps due to spatial restriction or nutrition supply priority of erect juvenile germlings.

Coalescence of five to eight discoid crusts was regularly found during the experiment (Fig. 3b and c). Occasionally, a coalesced discoid crust including more than ten discoid crusts occurred under high temperature and high photon flux density. Similar phenomena, involving even more than 50 discoid crusts were reported in red alga *Gracilaria verrucosa* (Jones 1956; Komiyama and Sasamoto 1957; Ren and Chen 1986). Such coalescence enhances spore settlement and adhesion of erect juvenile germlings, facilitating seaweed adaptation to marine inhabitation environment (Harper 1985; Kain and Destombe 1995; Santelices et al. 1996).

Erect juvenile germlings formed on single or coalesced discoid crusts, but only four to five erect juvenile germlings formed on relatively large crusts composed of five to eight disks. Whether or not coalesced discoid crusts produce erect juvenile germlings might depend on crust diameter just before coalescence (Santelices et al. 2004). No obvious differences appeared in the development of erect juvenile germlings from single or coalesced discoid crusts.

Filamentous fronds of red algae are usually composed of cells with high meristematic ability and rapid vegetative reproduction (Wang and Shen 1993; Shao et al. 2004; Wang et al. 2006a). It is noteworthy that filaments occurred during early discoid crust development and explant induction in *C. wrightii*. Filamentous fronds characteristics of *C. wrightii* are similar to filaments noted in *Grateloupia filicina* (Chiang 1993; Chen 1999) and *Gracilaria asiatica* (Zhao et al. 2006).

Our study shows that the sort of unialgal culture used for macroalgae such as *Halymenia sinensis*(Wang et al. 2006a), *Grateloupia turuturu* (Shao et al. 2004) and *Porphyra yezoensis* (Wang and Shen 1993)is also practical in preserving germplasms with filaments for *C. wrightii*. If filamentous fronds can grow via unialgal laboratory culture and can differentiate into erect juvenile germlings, those fronds will be useful for establishing vegetative clones and for preserving germplasm of economically valuable macroalgae. Further study is needed to elucidate the differentiation conditions and mechanisms from filament to juvenile germlings that affect seedling production in *C. wrightii*.

**Acknowledgments** This work was supported by the Shandong Agriculture Seedstocks Project and the Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX2-YW-N-47-02; granted to DL Duan). Thanks are kindly given to anonymous reviewers for comments and suggestions and Mr. Donald Sturge for English revision.

## References

Bárbara I, Lee SY, Peña V, Díaz P, Cremades J, Oak JH, Choi HG (2008) *Chrysymenia wrightii* (Rhodymeniales, Rhodophyta)—a new non-native species for the European Atlantic coast. Aquat Invasions 3:367–375. doi:10.3391/ai.2008.3.4.2

Chen YC (1999) The ultrastructure of *Grateloupia filicina* (Halymeniaceae, Rhodophyta) pit plugs. Bot Mar 42:531–538. doi:10.1515/BOT.1999.060

Chen MQ, Ren GZ (1985) The development process of sporelings of *Gracilaria verrucosa* (Hudson) Papenfuss. Oceanol Limnol Sin 16:181–187, in Chinese with English abstract

Chen MQ, Ren GZ (1987) The effect of temperature on the early growth and development of sporelings of *Gracilaria asiatica* Zhang and Xia. Oceanol Limnol Sin 18:301–308, in Chinese with English abstract

Chiang YM (1993) The developmental sequence of the marine red alga *Grateloupia filicina* in culture. Korean J Phycol 8:231–237

Destombe C, Godin J, Nocher M, Richard S, Valero M (1993) Differences in response between haploid and diploid isomorphic phases of *Gracilaria verrucosa* (Rhodophyta, Gigartinales) exposed to artificial environmental conditions. Hydrobiologia 260–261:131–137. doi:10.1007/BF00049011

Glenn EP, Moore D, Fitzsimmons K, Azevedo C (1996) Spore culture of the edible red seaweed, *Gracilaria parvispora* (Rhodophyta). Aquaculture 142:59–74. doi:10.1016/0044-8486(95)01249-4

Harper JL (1985) Modules, branches and the capture of resources. In: Jachson JBC, Buss LW, Cook RC (eds) Population biology and evolution of clonal organisms. Yale Univ. Press, New Haven, Connecticut, pp 1–33

Jones WE (1956) Effect of spore coalescence in the early development of *Gracilaria verrucosa* (Hudson) Papenfuss. Nature 178:426–427. doi:10.1038/178426b0

Kain JM, Destombe C (1995) A review of the life history, reproduction and phenonogy of *Gracilaria*. J Appl Phycol 7:269–281. doi:10.1007/BF00003997

Komiyama T, Sasamoto M (1957) Studies on the propagation of *Gracilaria verrusoca* (Hudson) Papenfuss I. On the settling of the spores and development of the early stage. Rep Invest Ariake Sea 4:25–34

Kumar GR, Reddy CRK, Jha B (2007) Callus induction and thallus regeneration from callus of phycocolloid yielding seaweeds from the Indian coast. J Appl Phycol 19:15–25. doi:10.1007/s10811-006-9104-0

Lee Y, Kang S (2001) A catalogue of the seaweeds in Korea. Cheju National University Press, Jeju, pp. [8], 1-662

Lee IK, Kurogi M (1972) Ecological observation of the members of Rhodymeniales in Hokkaido. Proceedings of the 7th International Seaweed Symposium, Sapporo. p. 131-134

Levy I, Beer S, Friedlander M (1990) Strain selection in *Gracilaria* spp. 2. Selection for high and low temperature resistance in *G. verrucosa* sporelings. J Appl Phycol 2:163–171. doi:10.1007/BF00023378

Ogata E, Marsui T, Nakamura H (1972) The life cycle of *Gracilaria verrucosa* (Rhodophyceae, Gigartinales) in vitro. Phycologia 11:75–80

Peña V, Bárbara I (2008) Biological importance of an Atlantic European maërl bed off Benencia Island (northwest Iberian Peninsula). Bot Mar 51:493–505. doi:10.1515/BOT.2008.057

Perestenko LP (1994) Red algae of the far-eastern seas of Russia. Komarov Botanical Institute, Russian Academy of Sciences, St. Petersburg, pp 1–330

Ren GZ, Chen MQ (1986) On the "coalescence" phenomena during the disc stage of young sporeling in *Gracilaria*. Mar Sci 10:49–50, in Chinese with English abstract

Santelices B, Correa JA, Isabel M (1996) Sporeling coalescence and intraclonal variation in *Gracilaria chilensis* (Gracilariales, Rhodophyta). J Phycol 32:313–322. doi:10.1111/j.0022-3646.1996.00313.x

Santelices B, Hormazabal M, Correa J, Flores V (2004) The fate of overgrown germlings in coalescing Rhodophyta. Phycologia 43:346–352

Shao KS, Wang JX, Zhou BC (2004) Production and application of filaments of *Grateloupia turuturu* (Halymeniaceae, Rhodophyta). J Appl Phycol 16:431–437. doi:10.1007/s10811-004-5503-2

Tseng CK (1983) Common seaweeds of China. Science, Beijing, China, pp 118–119

Wang SJ, Shen HS (1993) The rapid culture and breeding of free living filament clone of *Porphyra yezoensis*. J Shanghai Fish Univ 2:1–5, in Chinese with English abstract

Wang SJ, He PM, Yan XH (eds) (1994) Biotechnology of Seaweeds. Shanghai Scientific and Technical Publishers, Shanghai (in Chinese)

Wang AH, Shuai L, Duan DL (2006a) Filaments induction of *Halymenia sinensis* (Halymeniaceae, Rhodophyta). Bot Mar 49:352–354. doi:10.1515/BOT.2006.043

Wang AH, Wang JC, Duan DL (2006b) Early development of *Chondrus ocellatus* Holm (Gigartinaceae, Rhodophyta). Chin J Oceanol Limnol 24:129–133. doi:10.1007/BF02842811

Williams SL, Smith JE (2007) A global review of the distribution, taxonomy, and impacts of introduced seaweeds. Annu Rev Ecol Evol Syst 38:327–359. doi:10.1146/annurev.ecolsys.38.091206.095543

Xu XL, Fan X, Han LJ (2003) Anti-tumor activity and immunocompetence of macroalgae from Shandong sea area. Mar Sci 27:44–48, in Chinese with English abstract

Xu XL, Fan X, Song FH (2004) Study on bioactivity of Chinese marine alga extracts. Oceanol Limnol Sin 35:55–63, in Chinese with English abstract

Yin MY, Hu XY, Tseng CK (2007) Filament formation and differentiation in seven species of red algae. Bot Mar 50:113–118. doi:10.1515/BOT.2007.013

Yoshida T, Nakajima Y, Nakata Y (1990) Check-list of marine algae of Japan (revised in 1990). Jap J Phycol 38:269–320

Zhang Y, Feng Y, Li XM, Wang BG (2005) Acetylcholinesterase inhibitory screening for extractions of seaweeds. Oceanol Limnol Sin 36:459–464, in Chinese with English abstract

Zhang ZY, Wei HX, Han YX, Wang GS (2007) Induction and seedling incubation of filaments from crusts of sea weed *Grateloupia filicina*. J Dalian Fish Univ 22:164–169, in Chinese with English abstract

Zhao FJ, Wang AH, Liu JD, Duan DL (2006) New phenomenon during the early development of sporelings in *Gracilaria asiatica* Chang et Xia (Gracilariaceae, Rhodophyta). Chin J Oceanol Limnol 24:364–369. doi:10.1007/BF02842851