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Full Length Research Paper

Regeneration of germlings and seedlings development from cauline leaves of *Sargassum thunbergii*

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A method of producing artificial *Sargassum thunbergii* seedlings is urgently desired to meet the increasing demand of raw materials used for aquaculture and for the sake of environmental protection. This is the first report of attempt to study the vegetative propagation of this species using cauline leaves under laboratory conditions. On average, 45.75% of the excised leaves survived and one leaf could produce several new individuals. Adventitious burgeons grew into branches about 2 mm in length after 3 months of culture. The new individuals were cutoff and could be used as seedlings for raft culture and seabed restoration after a further culture to elongate enough for hand-planting.

Key words: *Sargassum thunbergii,* artificial seedling, brown seaweed, cauline leaves.

INTRODUCTION

Sargassum spp. have drawn lot of attentions for its active substances, such as antitumor polysaccharide (Fujihara et al., 1984; Zhuang et al., 1995; Jo et al., 2005), anticoagulative polysaccharide (Athukorala et al., 2006), anthelmintic components (Lee and Min, 1970), as well as peroxynitrite-scavenging and reactive oxygen scavenging constituents (Seo et al., 2004; Park et al., 2005). *Sargassum thunbergii* (Mert.) O. Kuntze, named rat-tail alga in China after its morphological characteristics, is becoming increasingly valuable as a resource of diet for aquaculture, especially with regards to feeding sea cucumber (Liu et al., 2004). During the past ten years, wild stocks of the species have been subject to intense harvesting by local inhabitants. With increasingly high levels of harvesting, some places of natural stands of *S. thunbergii* have collapsed (Zou et al., 2005). Recently, sexual breeding of *S. thunbergii* have been achieved under laboratory conditions (Wang et al., 2006). However, availability of a production technique for artificial seedling breeding is needed and it would be advantageous over the current method for large-scale breeding.

Knowledge regarding the biology of *S. thunbergii* is exceedingly limited and this precludes the development of seedling cultivation techniques aimed at alleviating the negative impact of indiscriminate harvesting currently affecting the wild stocks. Under natural conditions, vegetative propagation from holdfasts is an important method sustaining the population (Yoshida et al., 1999). Practices have proved that seedlings with holdfasts grow faster and have lower morality than those without, and this is the key reason makes *S. thunbergii* cultivation different from other commercial algae, such as *Gracilaria lemaneiformis*, which can be successfully cultured using any parts of its vegetative filaments (Hurtado-Ponce et al., 1992; Nelson et al., 2001; Ryder et al., 2004). Studies reveal that zygotic embryos of *Sargassum* for making seedlings are only formed seasonally (Tokuda et al, 1987; Nanba and Okuda, 1992; Sun et al., 2007). Tissue culture of several other species of *Sargassum* has been conducted. Spontaneous formation and development of adventitious embryos from the cauline leaves of *S. macrocarpum* are observed in laboratory culture. Swellings from the single leaf of *S. macrocarpum* became cylindrical protuberances and grew into 'daughter' thalli, which detach from their 'mother' thalli and develop into individual thalli exhibiting the same morphological processes as zygotic embryos (Yoshida et al., 1999) However, factors induce adventitious embryos in *S. macrocarpum* are still uncertain and the process is complicated and time consuming (Yoshida et al., 1999). In the present study, we report the regeneration of germlings developed from cauline leaves of *S. thunbergii* and their

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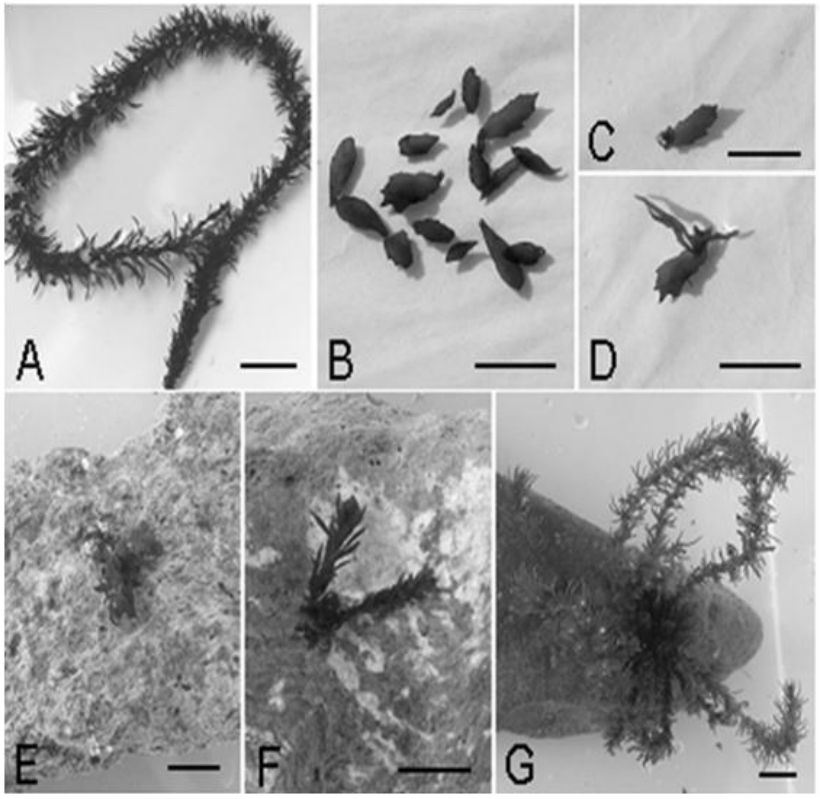

Figure 1. Seedling regeneration of *S. thunbergii from the* cauline leaves under laboratory conditions: (A) cauline branches obtained from the wild, (B) leaves used for the regeneration, (C) a newly developed germlings from the cauline leaves, 3 months after the culture, (D) the developing germlings on the leave, 4 months after the culture, (E) an individual attaching to the stone after 1 month of field culture; (F) a *S. thunbergii* plant with several newly-formed cauline branches after 2 months of field culture, (G) a mature *S. thunbergii* plant after 3 months of field culture (Bar, 1 cm).

availability as artificial seedlings.

MATERIALS AND METHODS

Samples were collected between August and November 2005 from the intertidal zone (35.35°N, 119.30°E) of the Second Bathing Beach, Qingdao, China using a pump placed 2 m depth under the water surface, and filtered with plankton nets (200 μm net and 20 μm net nested inside), then, autoclaved and prepared into enriched seawater with ES (enriched seawater) (Mclachlan, 1979). In the laboratory, intact plants were isolated, washed several times with sterile seawater, sterilized with 1% sodium hypochlorite for 2 min, then rinsed with autoclaved seawater and placed into a sterile aquarium (d=40 cm, h=30 cm) containing enriched seawater, and maintained at 15°C, with illumination of 50 photons m-2s-1 provided by cool-white fluorescent tubes and with a photonperiod of 12:12 h. The branches of *S. thunbergii* fronds were cut (2 to 3 cm in length) and rinsed with antibiotic enriched seawater solution made of ampicillin, penicillin, rifampicin, nystatin (0.2mg ml^{-1} each) andof GeO (0.1g ml^{-1}) for about 6 h. The leaves were cutoff using a surgeon knife (sigma) and cultured in flasks (5 L) containing autoclaved enriched seawater (1:80, w/w), which were bubbled 24 h in the incubator (Ningbo Jiangnan, GXZ-430ABC, Ningbo, China). Ten flasks were included in the treatment and culture media were weekly changed.

RESULTS AND DISCUSSION

The percentage of the survived excised leaves from *S. thunbergii* plant was 45.75% (n=201) and regeneration of adventitious embryos was observed (Figure 1). Longer cauline branches (Figure 1A) were selected and cut into segments for sterilization, then glossy and intact leaves were cutoff using a surgeon knife (Figure 1B). After 3 months of culture, semispherical swellings, about 2 mm in diameter, arose from the petioles end of the cauline leaves and became cylindrical protuberances (Figure 1C). Each protuberance developed into a new germling on the mother plant leaf (Figure 1D). New germlings were easily detached from the mother plant by scraping with forceps. The morphological characteristics of these germlings were quite similar to those developed from zygotic embryos with a few small cauline leaves (Figure 1D). Within several weeks of detachment, rhizoids emerged at the basal part of the germlings and they attached to the culture substances

(Figure 1E). The cauline leaves, which began to produce adventitious embryos, were gradually covered with one or several of newly developed germlings. The growth of each germling was very slow. It took 3 to 5 months to grow big enough for the convenience of manual operation. The germlings could be stored in culture vessels for about half a year under the culture conditions described previously with the medium being renewed weekly. After several months in laboratory culture, germlings were taken out of the vessels and cultured outdoor with the substances, such as stones (Figure 1E). Rhizoids developed fast at the basal part of the germlings and they attached to the culture substances and leaves were produced consecutively at the top of the axis of each plant (Figure 1F). After another 3 months of culture, the length of the largest cauline branch was more than 10 cm (Figure1G). The primary branches showed rapid elongation in the field.

Their daily growth rate was about 0.5 cm/day from early March to late June. At that time, the germlings had three to thirteen cauline branches (Figure 1G).

All plants began to mature after 4 months of field culture. They were all, males or females, the same as their matrixes indicating that all new germlings were clones. Vegetative propagation of the genus *Chondrus* (Chen and Taylor, 1978), *Gracilaria* (Goldstein, 1973; Santelices and Varela, 1995) and *Eucheuma* (Doty, 1987) through thallus fragmentation is well known and it has allowed a rapid expansion in farming of these species all over the world, suggesting that vegetative propagation is a good method of generating propagules for large scale cultivation. However, there is an exceedingly limited amount of information regarding the regulatory effect of physical factors such as temperature, light, etc on the regeneration processes of *S. thunbergii.* In this study, incubation at 15°C under 50 photons·m^{-2} · s^{-1} and 12:12 h LD illumination were suitable conditions used in the seedling breeding as they showed high burgeon ratio and fast growth rate of the germlings. Considering the sustenance of the resource, it is becoming increasingly apparent that the present status imposes a severe pressure on the wild stocks of *S. thunbergii*, and the available information indicates that some stands have already been abandoned by fishermen as a result of over-exploitation. From the current observations, it is clear that the leaf-origin germlings of *S. thunbergii* can grow and mature the same as the cauline branches in the wild and, therefore, can be useful for artificial seedling production. Also, the results indicate that the produced seedlings could be stored for a long term in the laboratory without loss of growth activity and vegetative regeneration capability.

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