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Mass culture of *Undaria* gametophyte clones and their use in sporeling culture

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Abstract

Undaria pinnatifida (Harv.) Sur. is one of the three main seaweed species under commercial cultivation in China. In the mid-1990s the annual production was about 20 000 tons dry. The supply of healthy sporelings is key to the success of commercial cultivation of *Undaria*. Previous studies demonstrated that instead of the zoospore collection method, sporelings can be cultured through the use of gametophyte clones. This paper reports the experimental results on mass culture of clones and sporeling raising in commercial scale. Light had an obvious effect on growth of gametophyte clones. Under an irradiance of 80 μmol m^{-2} s^{-1} and favorable temperature of 22–25 °C, mean daily growth rate may reach as high as 37%. Several celled gametophyte fragments were sprayed onto the palm rope frame. Gametogenesis occurred after 4–6 days. Juvenile sporeling growth experiments showed that nitrate and phosphate concentrations of 2.9 10^{-4} mol l^{-1} and 1.7 10^{-5} mol l^{-1} were sufficient to enable the sporelings to maintain a high daily growth rate. Sporelings can reach a length of 1 cm in a month. Since 1997, extension of the clone technique has been carried out in Shandong Province. Large-scale production of sporelings for commercial cultivation of 14 and 31 hectares in 1997 and 1998 had been conducted successfully.

Introduction

Commercial cultivation of *Undaria* began in China only in the last decade. Today, *Undaria* production ranks third, after *Laminaria* and *Porphyra*, in the seaweed cultivation industry in China, where *Undaria pinnnatifida* (Harv.) Sur. is the main species under cultivation concentrated in two northern provinces, Liaoning and Shandong. The annual production in the mid-nineties was about 20 000 tons dry weight (Wu, 1998), five times the pre-1980 figures. The per hectare yield is generally around 11 tons dry weight.

All the sporelings used for cultivation were cultured in a greenhouse with ambient temperature. The key step in the zoospore collection sporeling culture technique developed in the mid-eighties is to collect zoospores in the summertime. The mature sporophylls are kept in a dark moist container for several hours to induce mass discharge of zoospores which attach themselves onto ropes and give rise to male and female gametophytes. As soon as the temperature decreases to 20–22 °C, discharge of female and male gametes takes place and consequently zygotes are formed. After some 3 months of being cultured in the greenhouse, the sporelings grow up to about 1 cm in length and can be moved to the open sea in late autumn. Long term cultivation in greenhouse is very expensive and laborious and the sporelings are inevitably attacked by diseases caused by certain kinds of saprophytic bacteria (Wu et al., 1979) and alginic acid decomposing bacteria (Chen et al., 1984). The rot disease due to alginic acid decomposing bacteria e.g. *Pseudomonas* causes decay of the holdfast by enzymatic action of alginase, finally resulting in the detachment and loss of the sporelings. The disease is serious, sometimes destroying virtually the entire crop. Therefore, a new method of culturing sporelings by the use of gametophyte clones was developed (Pang & Wu, 1996). This paper reports the extension work of culturing sporelings by the use of gametophyte clones in 2 sporeling culture stations.

In general, the gametophyte clone method includes four steps:

1. Isolation of single male and female gametophytes and induction of gametophyte clones.
2. Mass culture of male and female gametophyte clones.
3. Sporophyte induction and sporeling culture in greenhouse.
4. Sporeling culture in the open sea.

Materials and methods

Collection of zoospores, formation of gametophytes and induction of clones

Sporophyll from selected strain No. 10 with mature sporangial sori was thoroughly sterilized by immersion in a 2% (w/v) sodium hypochorite seawater solution for 10 min, washed, and then subjected to partial drying at 15 °C for 2–4 h to induce mass discharge of zoospores. The dense zoospore suspension was diluted until several zoospores could be seen per field under microscope (100×); slides were then put into the dilute suspension to allow the zoospores to attach. The attached zoospores were cultured under 20 °C in Provasoli enriched seawater (PES) (Provasoli, 1968) at irradiance of 16 μmol m^{-2} s^{-1}. Gametophytes formed after 3 d. Male and female gametophytes were picked up one by one by sterilized glass pipette. The isolated unialgal gametophytes grew into small, visible clones after a month's culture. These gametophyte clones were then propagated vegetatively in bigger containers.

Effect of light on the growth of gametophyte clones

Male and female gametophyte clones were cut by a blender into several celled small fragments, washed thoroughly several times with sterilized seawater, and then cultured separately in 20 l cylindrical glass bottles with inner diameter of 45 cm. The culture room was air-conditioned to maintain a temperature of 25±0.5 °C. Cool white fluorescent tubes (40w) were used as light source with a light period L:D of 12:12 and the distance between the bottle and the light source was adjusted so that the exterior surface of the bottle could receive different irradiances. Six treatments of 20, 40, 60, 80, 100, and 120 μmol m^{-2} s^{-1} were set up. Provasoli enriched seawater (PES) was used as culture medium and was renewed every seven days. The medium was bubbled with filtered air to provide sufficient oxygen and carbon dioxide. The transmitted lights and the weight increments were measured periodically during the experiment.

Commercial scale cultivation of sporelings and disease control

After a period of growth, the gametophyte clones grew up to small balls of 0.5–2 mm diameter. Male and female clones were mixed by the ratio of 4:1, and then cut into several celled fragments by a blender. The suspension was then sprayed onto palm rope frames and kept motionless for several days to allow the gametophytes to attach on the substratum. The culture was kept in ambient temperature ranging from 20 to 15 °C in a greenhouse. Seawater was enriched with NO_3-N and PO_4-P to concentrations of 2×10^{-1} mol m^{-3} and 2×10^{-2} mol m^{-3}, respectively, and was renewed every 4–5 days. Light was carefully controlled at about 80–100 μmol m^{-2} s^{-1}.

In 1997, a commercial scale sporeling cultivation experiment was conducted at Old Stone Man Sporeling Culture Station in Qingdao. A total of 315 palm rope frames (50 cm × 100 cm) were used for the first crop cultivation experiment (Li et al., 1998). In 1998, 300 frames were used for the second crop cultivation experiment in Longxudao Sporeling Culture Station, Rongcheng City.

It has been proved that long term cultivation in greenhouse may cause the detachment of sporelings. The experiment was, therefore, designed to shorten the period of sporeling culture by the use of gametophyte clones instead of the routine method of zoospore collection. In order to control the multiplication of alginic acid decomposing bacteria, clones were cultured in the above mentioned seawater media supplemented with chloromycetin at concentration of 1 g l^{-1}.

Results and discussion

Mass culture of gametophyte clones

Experiments showed that clones grew fast vegetatively under 25±0.5 °C. Growth rate declined sharply above 25 °C. Male and female gametes could be formed and discharged only in a temperature range of 5–22 °C.

Table 1 shows that on day 6 the average daily growth rates reached as high as 37.1% and 38.6% under irradiances of 80 and 100 μmol m^{-2} s^{-1}. The

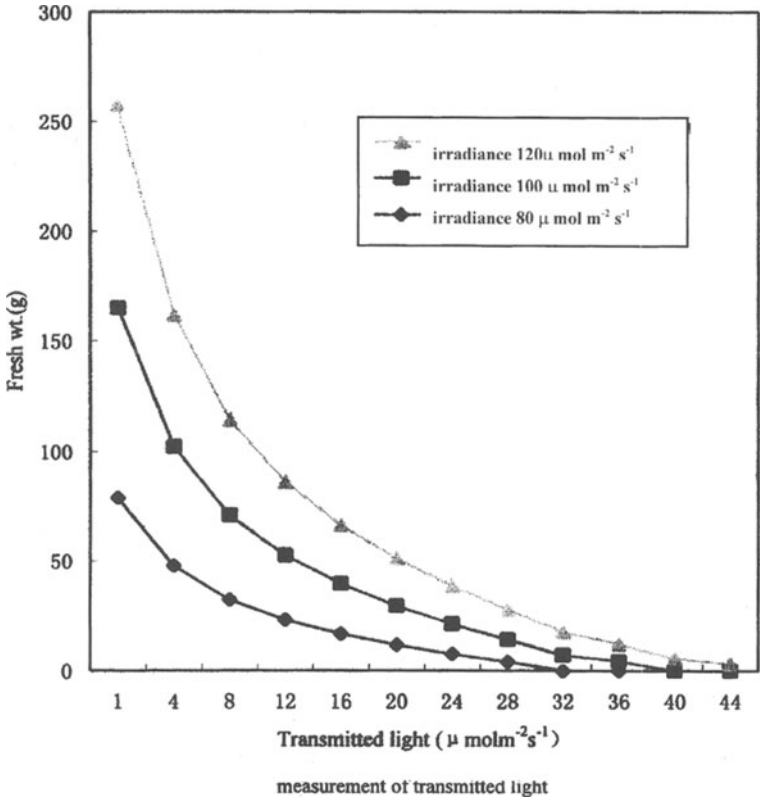

Figure 1. Detection of growth of *Undaria pinnatifida* gametophyte clones through the measurement of transmitted light.

growth rates declined sharply after day 13. On day 13 the growth rates approached 11.0 and 12.8, 3.5 and 6 times higher than that on day 27. This means that fresh weight of clones can be doubled in 3 d during the first 6 d. It is therefore suggested that clones should be harvested on day 6 to day 13. Experimental result of Ms. Hu Xiaoyan and L. Popoba in 1997 (pers. comm.) showed a photosynthetic rate of 33.9 μmol O_2 g^{-1} h^{-1} under irradiance of 80 μmol m^{-2} s^{-1} in *Undaria pinnatifida* gametophyte clones. Such a high rate coincides with the high increment in fresh weight shown in this experiment. This very high growth rate makes clone culture on commercial scale possible.

A better understanding of the relationship between the increment of fresh weight and the decline of transmitted light led us to believe that transmitted light can be used as an index to estimate the fresh weight of clones in the bottle. Figure 1 was plotted so that one may find the fresh weight through the measurement of transmitted light. For example under irradiance of 100 μmol m^{-2} s^{-1}, if the transmitted light is 4 μmol m^{-2} s^{-1}, the clones in the bottle should be 54 g or so. This makes the estimation easier and diminishes the possibility of contamination.

Commercial scale cultivation of sporelings

Experimental results showed that male and female gametes will be formed 5–6 d after blender cutting, consequently sporophytes will appear the next day. Therefore, it is believed wounding promotes the formation of oogonium and antheridium. Similar to that of *Laminaria*, eggs discharge only after a period of darkness. Sperms will release immediately after the discharge of eggs and fertilization takes place afterwards (Wu et al., 1979). One day after fertilization, two celled sporophytes appeared. Mitosis took place in darkness too, once every 24 h. Three days after fertilization, seven celled sporophytes were formed. Juvenile sporeling growth experiments showed that nitrate and phosphate concentration of 2.9×10^{-4} mol l^{-1} and 1.7×10^{-5} mol l^{-1} were sufficient to enable the sporelings to maintain a high growth rate. Under such favorable conditions, sporelings grow fast in 1 month to 1 cm in length, which is the right size for

Table 1. Growth of gametophyte clones of *Undaria pinnatifida* under different light regimes

Irradiance ($\mu molm^{-2}s^{-1}$)	Day 6		Day 13		Day 20		Day 27	
	Fresh wt.(g)	Average daily growth rate (%) after last measurement	Fresh wt.(g)	Average daily growth rate (%) after last measurement	Fresh wt.(g)	Average daily growth rate (%) after last measurement	Fresh wt.(g)	Average daily growth rate (%) after last measurement
20	23.3	22.2	34.1	6.6	50.6	6.9	68.5	5.0
40	31.3	36.2	48.2	7.8	69.9	6.5	91.2	4.3
60	29.9	33.2	48.4	8.9	70.7	6.6	91.1	4.1
80	32.2	37.1	57.0	11.0	84.6	6.9	102.7	3.1
100	33.1	38.6	62.9	12.8	88.0	5.7	101.0	2.1
120	30.8	34.6	59.5	13.3	81.2	5.2	95.2	2.5

10 g of clones were inoculated to each bottle in the beginning of experiment.

their transplantation to the open sea. After transplantation, the daily growth rate in length of the young sporophytes was measured and calculated to be 15%. Since 1997, extension of the clone technique has been carried out in Shandong Province. Large scale production of sporelings for commercial cultivation of 14 and 31 hectares in 1997 and 1998 has been conducted successfully.

Contamination of clone culture by alginic acid decomposing bacteria and long term cultivation of sporelings in the greenhouse are believed to be the cause of the detachment disease of sporelings. The addition of chloromycetin to the clone culture medium showed obvious inhibitory effect on these bacteria. Moreover, the use of the clone method of raising sporelings shortened their culture period from 3 months to 1 month. This resulted in more effective control of the detachment disease. Since 1997, extension of the disease control technique has been carried out in the Old Stone Man Station in Qingdao and Longxudao, and large scale production of healthy sporelings for commercial use has been achieved successfully.

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