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# Propagation of *Gracilaria edulis* (Gmelin) Silva by reproductive method

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ABSTRACT

*Gracilaria edulis* was successfully cultured by the reproductive propagation method in the Gulf of Mannar, during November-March 1991-'92 and 1992-'93. Healthy young plants were harvested twice during 1993 within 135 days of culture period. Nursery rearing for a brief period was done for the carpospores to grow upto the germling stage in enriched seawater before transplanting to the sea. The plant attained maximum length of 34 cm (average length 21.55 ± 7.30 cm) and a fresh weight of 12.430 kg on harvest. The quantitative estimation of agar showed an average yield of 14.57 % with gelling temperature of 48.8°C, melting temperature of 85.0°C and gel strength of 98.6 g/cm$^2$.

## Introduction

Red algae, which comprise the largest group of marine algae include the major agarophytes and carragenophytes. The main product of carbon fixation in the red algae are the storage compound, floridean starch and cell wall polysaccharides otherwise called agar - agar and carrageenan. Approximately 5,000 t of agar are produced annually in the world from about 30,000 t *Gracilaria* harvested from the natural stock. The countries engaged are Chile, Argentina, Brazil and South Africa (Chiang, 1981). Demand of *Gracilaria* has increased significantly over the last ten years and this has led to overharvesting of the natural stock in several geographical areas (Smith *et al.*, 1984; Wang *et al.*, 1984; Santelices and Ugarate, 1987). The decline of the natural beds of *Gracilaria* in recent years has prompted the development of several restoration techniques.

Farming of *Gracilaria* is a reliable method of increasing volume and quality. Several methods have been adopted to culture the plant. It may be from the spores or from the vegetative fragments. Vegetative method of propagation involves bottom planting or farming in open water, pond culture, raceway culture and tank culture (Pizarro and Barrales, 1986; Santelices *et al.*, 1984; Shang, 1976). *Gracilaria* has very high regenerative capacity and hence

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vegetative propagation by cuttings is presently done in net, ropes and ponds in inshore waters. However, nursery produced seedlings from spores is better for cultivation (Trono, 1987).

Experimental cultivation of *Gracilaria edulis* has been carried out in India by vegetative propagation (Rao, 1973, 1974; Krishnamurthy *et al.*, 1975; Chennubhotla *et al.*, 1978). Limited work has been carried out in India and elsewhere in the reproductive propagation of *Gracilaria* by using spores (Krishnamurthy *et al.*, 1969; Reeta, 1990, 1992; Reeta and Kaliaperumal, 1991; Charles, 1992; Oza *et al* ., 1994; Gleen *et al.*, 1996 and Alveal *et al.*, 1997). The earlier attempts to culture *Garcilaria edulis* from spores in India have been either a failure (Charles 1992) or met with limited success (Reeta and Kaliaperumal, 1991). Subsequently, further modifications have been made in the culture techniques with a view to improving their efficacy and the present paper embodies the results of such a study.

## Materials and methods

Healthy cystocarpic plants of *G. edulis* were collected from the nearshore area of the Gulf of Mannar near Thonithurai (9° 16' N and 79° E) and transported to the laboratory. A few healthy cystocarpic plants (approximately 100 g fresh weight) were selected, washed thoroughly in running seawater followed by sterilised seawater. Visible epiphytes were removed by brushing the plants before these were kept for spore output. Substrata such as polypropylene straw wound on a rectangular PVC frame, glass slides and circular cement blocks (9 cm dia) were placed in a 250 l fibreglass tank containing sterilised seawater. The cystocarpic plants were spread on the nylon mesh, 20-25 cm above the substrata. This arrangement was to give an uniform distribution of spores on the substrata. After 24 hrs of spore output, the plants were removed and the substrata along with the attached spores were transferred to the culture room. They were maintained under controlled conditions (temperature 23-25°C, light intensity 1000 lux and photoperiod 16:8 h L:D cycle) in enriched seawater (Walne, 1974). Culture medium was changed at weekly intervals. Regular observations under microscope were made on the growth of spores settled on glass slides and using an ocular micrometer the diameter of the spores were noted in mm. The spores were allowed to grow for 17 days in the laboratory till the erect frond developed from the parenchymatous disc of the dividing spores or the central medulla. Subsequently the germlings on the polypropylene straw and cement blocks were transplanted to the natural environment (Gulf of Mannar near CMFRI jetty and Thonithurai) during the favourable period for further growth. During 1991-'92, the spores liberated on cement blocks were tied on the coir rope vertically in the water column within a depth of 1.0 to 1.5 m (Fig. 1). During 1992-'93, the spores liberated on polypropylene straw were transplanted in the sea horizontally twisting in the hooks of barbed wire which were tied to four casurina poles in an area of 2 x 2 square metre (Fig. 2). In the Gulf of Mannar seaweed culture is not possible during April-September due to rough weather. Hence the culture period was selected from November to March during 1991-'92 and 1992-'93. Observations were made on the growth of the plants on different days of culture period.

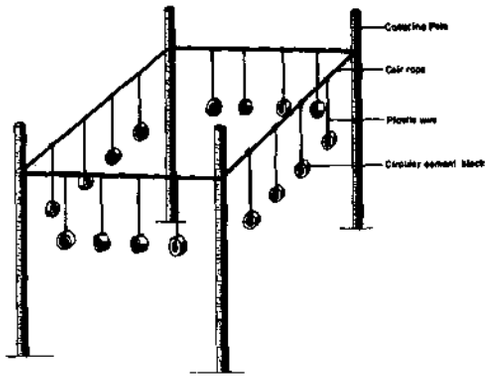

Fig. 1. Experimental culture method of *G. edulis* during 1991-'92.

Analyses for nutrients such as nitrite, nitrate, phosphate, silicate, dissolved oxygen and salinity of seawater were made by the standard method (Parson *et al.*, 1985). Meteorological data such as rainfall and average atmospheric temperature were recorded during the culture period.

## Results and discussion

Regular observations were made on the growth of carpospores maintained in the controlled environment. The spores are microscopic in nature with a size of 0.019 mm during liberation, got attached to the substratum by secreting a mucilagenous substance and started dividing to form an uniform parenchymatous disc or the holdfast. The central medulla or the holdfast was brighter in colour which further divided to form the pseudoparenchymatous cells or the erect frond (Fig. 3&4). The crop growth rate increased gradually till the 17th day and attained an average diameter of 0.230 ± 0.013 mm (Table 1). At this stage all the spores developed the erect frond and the germlings along with the substrata were transplanted to the natural environment and tied in the sea at a depth of 1.0 to 1.5 m. Visible size germlings appeared after 25 to 30 days of transplantation.

During 1991-'92, the growth rate was 0.76 ± 0.28 cm on the 34$^{th}$ day of transplantation but reached a length of 1.3 to 8.1 cm on 88$^{th}$ day of transplantation (mean length 2.91 ± 1.21 cm, n=93). Further observations could not be made due to complete predation of the crop. The epiphytes such as *Enteromorpha compressa, E. intestinalis, Ulva lactuca, U. reticulata, Laurencia obtusa, Padina boergesinii, Lyngbya majuscula* and epifauna such as gastropods, barnacles, brittle stars and hydroid colonies were found along with the cultured plants.

During 1992-'93 the growth rate was much faster being 5.29 ± 2.19 cm after 37 days of transplantation. The plants attained maximum size range of 11-34 cm (mean length 21.55 ± 7.30 cm, n=11) on the 74$^{th}$ day of transplantation. Since

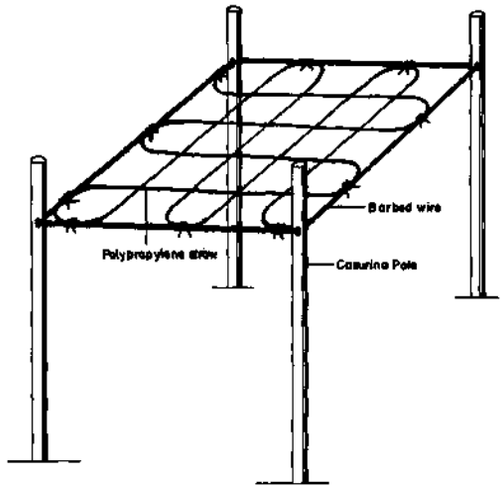


Fig. 2. Experimental culture method of *G. edulis* during 1992-'93.

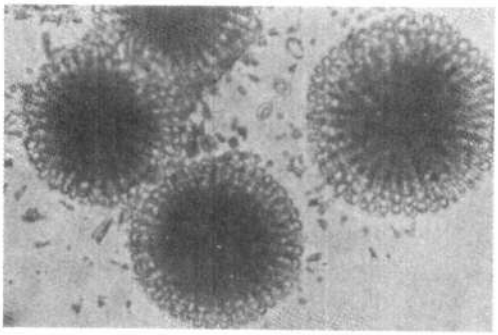

Fig. 3. Growing spores of *G. edulis*.

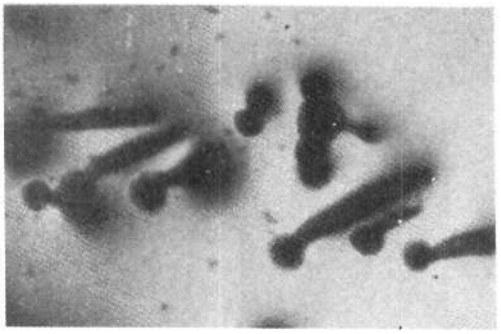

Fig. 5. Growing plants of *G. edulis* on polypropylene straw.

there was every chance of the plant being dislodged by wave action, the first harvest was made at this stage leaving behind the holdfast along with 1 cm basal portion of the plants. A total quantity of 2.78 kg fresh weight of plants was harvested. The growth rate was much faster after pruning and attained size range of 13.5-33.5 cm (mean length 24.05 ± 5.07 cm, n=11) after 44 days of pruning. The epiphytic growth was very less compared to the previous year. The epiphytes found attached to the plants were *Ulva lactuca* and *Enteromorpha compressa*. The second harvest was made at this stage and it yielded 9.65 kg of fresh *Gracilaria edulis* (Figs. 5&6). Work could not be continued further due to rough weather from April . Conditions for the experimental culture were different in 1991-'92 and 1992-'93. In 1992-'93 the improvement made in the culture techniques might have enhanced the growth rate by reducing the effect of predation and by avoiding self-shading of crops. During 1991-'92 the spores were liberated on both sides of the cement blocks hung in the water column and this might have attracted more predators. As the substratum was hard and rocky, attachment of green algal motile spores

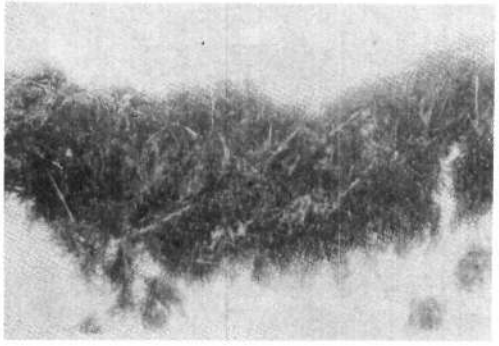

Fig. 4. Young germlings of *G. edulis* 17 days after spore liberation.

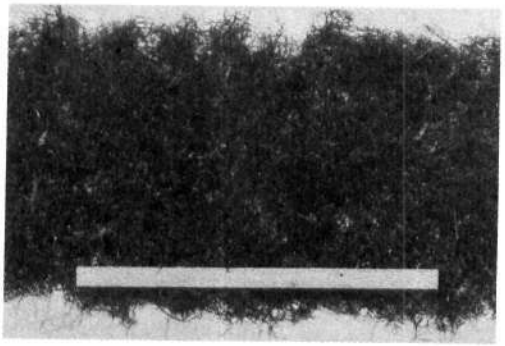

Fig. 6. Harvested plants of *G. edulis* grown from spores.

TABLE 1. *Growth of spores of* G. edulis *during nursery rearing*

| Days after spore output | Diameter of spores in mm ± SD | Crop growth rate mm/day |
|---|---|---|
| 0 | 0.019 ± 0.001 | 0 |
| 1 | 0.020 ± 0.002 | 0.001 |
| 3 | 0.027 ± 0.003 | 0.004 |
| 4 | 0.032 ± 0.000 | 0.005 |
| 6 | 0.058 ± 0.009 | 0.013 |
| 8 | 0.069 ± 0.006 | 0.006 |
| 10 | 0.096 ± 0.012 | 0.014 |
| 12 | 0.117 ± 0.014 | 0.011 |
| 13 | 0.151 ± 0.013 | 0.034 |
| 15 | 0.170 ± 0.24 | 0.010 |
| 17 | 0.230 ± 0.013 | 0.030 |

and gastropod shells was intense. The low growth rate might have been due to the non-availability of direct and uniform sunlight. In contrast, during 1992-'93, the germlings were transplanted horizontally in the surface water without exposing the plants to air even during the lowest low tides and this ensured maximum direct and uniform sunlight.

Meteorological data such as rainfall and average atmospheric temperature were compared for both the years (Figs. 7 & 8). Total monthly rainfall was more during 1992-'93 and constituted 77 % of the annual rainfall. The total rainfall during the culture period was 522.8 mm in 1991-'92 and 689.7 mm in 1992-'93. The lower salinity of seawater (Table 2) brought about by excess rainfall might have been favourable for the growth of the plants. Average atmospheric temperature was found to be less during 1992-'93 (Fig. 8).

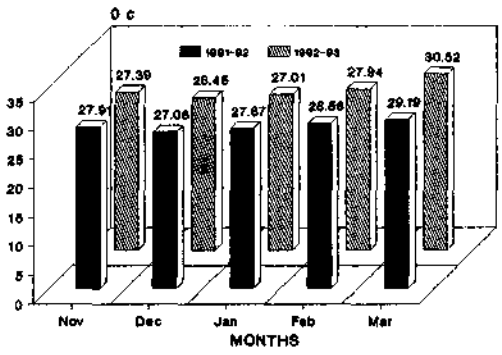


Fig. 7. Average atmospheric temperature during November-March 1991-'92 and 1992-'93.

Tables 2 and 3 present the values of dissolved oxygen, salinity and nutrients such as nitrite, nitrate, phosphate and silicate of seawater for the year 1991-'92 and 1992-'93 respectively. It was observed that the dissolved oxygen content of seawater did not show much variation between the years. The salinity was found to be relatively less (28.8-32.6 ppt) during 1992-'93 than (30.4 - 35.2 ppt) during 1991-'92. Nutrient contents of seawater showed irregular variations between the years and these do not appear to be limiting factors for plant growth in the natural environment.

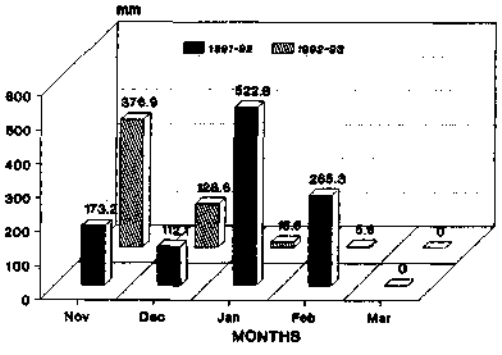


Fig. 8. Average rainfall during November-March, 1991-'92 and 1992-'93.

TABLE 2. *Growth and hydrological conditions in the experimental culture of* G. edulis *during 1991-'92*

| Days | Size (cm) | Nutrient contents (μ g at / l) | | | | DO | Salinity |
|---|---|---|---|---|---|---|---|
| | | Nitrate | Nitrite | Phosphate | Silicate | ml/1 | (ppt) |
| 34 | 0.4-1.6 | 1.88 | 0.06 | 0.15 | 14.0 | 4.17 | 30.4 |
| 42 | 0.2-1.7 | 1.00 | 0.08 | 0.20 | 32.0 | 4.06 | 32.0 |
| 50 | 0.3-1.8 | 1.00 | 0.06 | 0.18 | 29.0 | 6.63 | 35.2 |
| 57 | 0.2-2.7 | 1.00 | 0.06 | 0.18 | 29.0 | 5.67 | 35.2 |
| 66 | 0.6-6.5 | 1.75 | 0.06 | 0.15 | 19.0 | 4.41 | 32.0 |
| 74 | 0.6-4.8 | 1.63 | 0.06 | 0.10 | 20.0 | 6.39 | 31.2 |
| 81 | 0.8-5.2 | 1.50 | 0.07 | 0.15 | 19.0 | 2.60 | 32.0 |
| 88 | 1.3-8.1 | 1.00 | 0.04 | 0.03 | 29.0 | 5.97 | 32.3 |

Qualitative and quantitative analysis of agar showed an average yield of 14.5 %, gelling temperature of 48.8°C, melting temperature of 85.0°C and gel strength of 98.69 g/cm$^2$, which are comparable with the yield and quality of natural stock (Chennubhotla *et al.*, 1977). The total quantity of plants harvested during 1992-'93 culture period was 12.43 kg grown from the spores of approximately 100g of mother reproductive plants within 135 days culture period. The plants harvested from the culture sites were either young or sterile. The above observations indicate that lower salinity consequent to high rainfall and lower atmospheric temperature favour the growth of *Gracilaria edulis*. Brief nursery rearing of carpospores in enriched seawater in controlled environment and firm attachment of the spores to the substrata increase the survival rate of the spores before transplanting to the sea and this could reduce the loss of germlings by wave action. The advantage of this culture method over fragment culture is the very less requirement of mother reproductive plants, which could liberate enoromous spores, each of which will grow to a new plant (Alveal *et al.*,1997). The liberation of spores in

TABLE 3. *Growth and hydrological conditions in the experimental culture of* G.edulis *during 1992-'93*

| Days | Size (cm) | Nutrient contents (μ g at / l) | | | | DO | Salinity |
|---|---|---|---|---|---|---|---|
| | | Nitrate | Nitrite | Phosphate | Silicate | ml/1 | (ppt) |
| 37 | 02.5-08.0 | 1.37 | 0.01 | 0.01 | 18.0 | 5.61 | 28.8 |
| 50 | 08.0-15.0 | 1.25 | 0.02 | 0.05 | 10.0 | 6.09 | 30.4 |
| 57 | 08.4-15.5 | 2.13 | 0.17 | 0.03 | 20.0 | 5.82 | 32.2 |
| 74* | 11.0-34.0 | 0.05 | 0.04 | 0.08 | 16.5 | 2.86 | 29.6 |
| 84 | 07.5-14.0 | 0.25 | 0.04 | 0.08 | 11.0 | 5.88 | 32.0 |
| 93 | 07.0-15.0 | 1.50 | 0.17 | 0.05 | 11.0 | 4.05 | 30.6 |
| 99 | 15.0-25.0 | 0.38 | 0.02 | 0.18 | 17.0 | 6.32 | 32.0 |
| 118 | 13.5-33.5 | 0.50 | 0.02 | 0.13 | 12.5 | 4.87 | 32.6 |

* First harvest was made 74 days after transplantation.

controlled conditions will reduce the mortality percentage unlike in natural environment either due to wave action or due to predation by other organisms. It has been reported earlier that yield and quality of agar varied in *Gracilaria edulis* at different growth stage (Lignell and Pedersen, 1989). When the plants are harvested from the natural environment, they may belong to different growth phases and the yield and quality of agar may vary. But in the present culture method uniform size of plants of similar growth phase can be obtained during harvest and critical period of harvest can be made when the quantitative and qualitative yield of agar is maximum. The results of the project coincide with those reported by Doty and Fisher (1987) and Santelices and Doty (1989) for the *Gracilaria* species in Penang, Malayasia. Spore culture of *Garcilaria* is not as expensive as the vegetative method of cultivation. The equipment needed for this method is simple and can easily be utilised by the fishermen cooperative (Alveal *et al.*, 1997).

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