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Sporulation in *Gracilaria crassa* Harvey ex. J.Agardh at different environmental factors

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ABSTRACT.

Effect of environmental factors such as exposure to air and desiccation, salinity, temperature, light and photoperiod on tetraspore shedding in the red alga *Gracilaria crassa* was studied under laboratory condition. In the tetrasporic thalli exposed to air in shade and sun for different durations, maximum quantity of spores were liberated from the thalli in submerged condition in control experiment. Spore output was observed at salinity ranging from 10 to 40‰ with peak output at 30‰. Spore discharge was found at temperature ranging from 20 to 40° C with maximum quantity of spore liberation at 25°C. Spore shedding was recorded at light intensity ranging from 10 to 100 μ $Em^{-2}S^{-1}$ with peak discharge at 20 μ $Em^{-2}S^{-1}$. Maximum spore release occurred in long day condition at 12 : 12 LD cycle.

Introduction

Now the red algae *Gelidiella acerosa, Gracilaria edulis, G.crassa* and *G. foliifera* are used as raw material for the production of agar by Indian seaweed industries. *Gelidiella acerosa* is used for manufacture of bacteriological grade agar and *Gracilaria* spp for food grade agar. Whenever there is scarcity for *Gracilaria edulis, G. crassa* is commercially exploited from Mandapam area of southern Tamilnadu coast. It is collected since 1983 from Gulf of Mannar islands and Mandapam coast. Information on environmental factors influencing spore release in *G. crassa* will be very useful to cultivate this species by reproductive propagation method and also to conserve the natural stock to use as seed material.

Studies were made on the effect of environmental factors on spore liberation in *Gracilaria corticata, G.edulis* and *G.textorii* growing at Mandapam and Visakhapatnam coast (Subbarangaiah *et. al.*, 1975; Umamaheswara Rao, 1976; Subbarangaiah, 1978 ; Shoba, 1985; Umamaheswara Rao and Subbarangaiah, 1985). As no information is available on this aspect of study in *Gracilaria crassa,* the present study was undertaken. Results obtained on the effect of environmental factors such as exposure to air (desiccation), salinity, temperature, light and photoperiod on tetraspore output in this red alga are presented in this paper. Data collected on the shape and size of tetraspores and environmental and hydrological parameters from the collection locality are also given.

Materials and Methods

The tetraspore plants of *Gracilaria crassa* Harvey ex J.Agardh growing in the intertidal and subtidal areas at Thonithurai were collected during the period November, 1997 to October, 1999 They were thoroughly washed in the seawater at the collection site and transported to the laboratory in plastic bucket containing seawater. Small clumps with good tetrasporargial sori were selected and washed thoroughly several times with sterile seawater. Then they were placed in petridishes of 5 cm diameter filled with 20ml of sterile seawater. The experimental sets were illuminated by a fluorescent cool white tube light at 20 μ E^{-2} S^{-1} for 8 hours during the day time from 9 A.M. to 5 P.M. except in the case of temperature and light experiments. The tetraspores liberated were counted daily after 24 hours to study the effect of different environmental factors on tetraspore shedding.

The tetraspores liberated in the petridishes were counted by preparing a spore suspension of known volume and using a plankton counting chamber following the method of Kaliaperumal and Umamaheswara Rao (1982) and Umamaheswara Rao and Kaliaperumal (1983). The degenerating spores were not counted. The average values of two counts and the total volume of spore suspension were taken into account for computing the spore output. When the spore shedding was less, the counting of spores was made by keeping the petridishes on a transparent grid sheet under monocular microscope. The spore output is finally expressed as number of spores/g fr.wt. Ten experiments were conducted for each factor.

For studying the influence of desiccation or exposure to air, tetrasporic thallus of *G.crassa* were exposed to air in shade and in the sun. The materials were blotted using blotting paper to remove the water on their surface before exposure. The materials exposed to air in shade upto 2 hours (15 minutes interval upto 1 hour and then ½ hour interval) and upto 1 hour (5,10,15,30,45 and 60 minutes) in the sun were transferred to petridishes containing sterile seawater. Controls (0 minute exposure) were also maintained in all these experiments. During the time of conducting these experiments, the temperature in the shade was 30° ± 2°C and the relative humidity varied from 48 to 61%. In the open air, the temperature was 33 ± 2° C and the relative humidity varied from 41 to 46%.

A stock solution of 100‰ salinity was prepared by adding common salt to the seawater collected from the inshore area and sterilizing it. The salinity was determined using a salinometer (Atago Hand Refractometer). The lower grades were prepared from the stock solution by adding requisite quantity of distilled water. The tetraspore output was estimated at 0,10,20,30,40,50,60,70 and 80‰ salinities. The influence of 9 different temperatures 0, 10, 15, 20, 25, 30, 35, 40 and 45°C on tetraspore liberation was studied by maintaining the petridishes with fruiting materials for 24 hours in a temperature controlled dark incubator or refrigerator.

Light intensity experiments were conducted at 0,10,20,30,40,60,80 and 100μ Em^{-2} S^{-1}. For studying the effect of photoperiod on tetraspore shedding, petridishes with fruiting materials were subjected to 0 : 20, 4 : 20, 8 : 16, 12 : 12, 16 : 8, 20 : 4 and 24 : 0 light and dark cycles by keeping them in separate light and dark chambers. These experiments at seven different light and dark regimes were conducted at 20 μ Em^{-2} S^{-1}

The shape and size of tetraspores were studied by taking random samples from the spore suspension in different months of the years. The shape of spores was observed under monocular microscope and the size was

measured using ocular micrometer. Data on atmospheric temperature, seawater temperature, salinity and light intensity were collected from the collection site to correlate the results with the environmental conditions existing in the field and to know the optimal conditions required for maximum spore output. The atmospheric and seawater temperature were measured using a thermometer (Jennson Delux). The light intensity in the intertidal and subtidal region at the collection locality was measured using an underwater lux meter (EMCON).

Results

Changes observed in the tetraspore liberation in controls (0 minute exposure) and at different periods of exposure to air in the shade and in the sun are shown in Fig.1. These experiments were conducted not only to study the effect of exposure during low tides and the resultant desiccation of plants on spore production, but also to know the variations of spore release in shade as well as in areas directly exposed to the sun. In the fruiting materials exposed to shade, sporulation was seen upto 120 minutes with maximum liberation of tetraspores in control and decline in spore output was found from 15 minutes to 120 minutes exposure (Fig 1. A). Changes observed in spore output were more marked in the fruiting materials exposed to sun for even short periods of 5, 10 and 15 minutes. Tetraspore output was observed from the thalli exposed upto 15 minutes. Tetraspore output was observed from the thalli exposed upto 15 minutes with maximum shedding of spores in 0 minutes exposure (Fig.1 B).

Data collected on the effect of salinity on tetraspore output showed marked variations at different salinities of seawater tested. Spore liberation was observed from 10 to 40‰ with peak output of spores at 30‰ (Fig.2). In the experiments conducted on

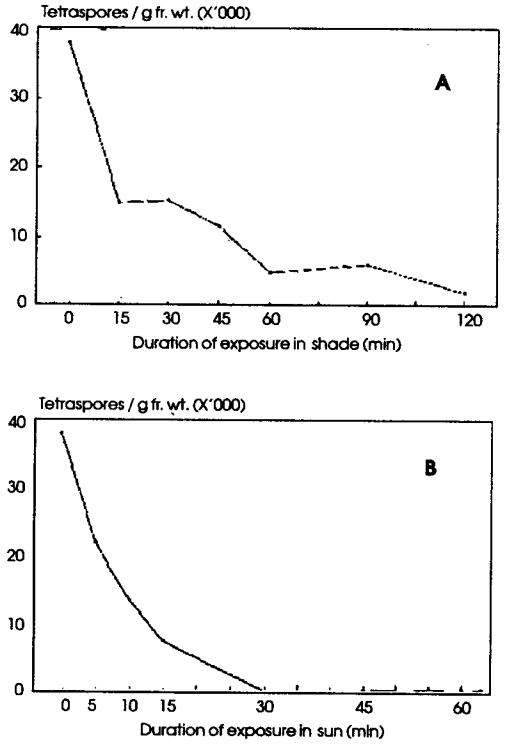

Fig.1. *Effect of exposure in shade and in sun on tetraspore output of* Gracilaria crassa

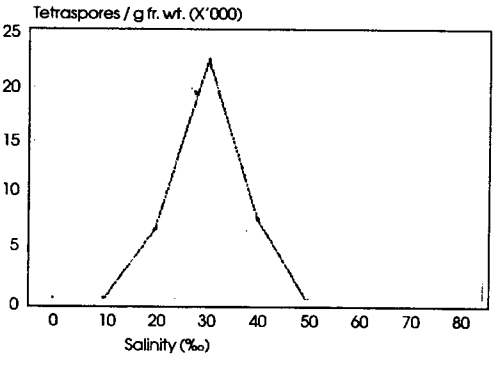

Fig.2. *Effect of salinity on tetraspore output of* Gracilaria crassa

The shape and size of tetraspores in *G. crassa* in this investigation almost agree with the shape and size of species recorded earlier in *G. crassa* (Shyam Sunder *et.al.*, 1991) *G. verrucosa* (Oza and Krishnamurthy, 1967) and *G. corticata* (Oza, 1976; Mohan Joseph and Krishnamurthy, 1977).

The present study reveals that submerged condition of the alga, light intensity of 10-20 μ Em^{-2} S^{-1} and water temperature of 20-30 °C are suitable for maximum shedding of tetraspores in *G. crassa.* These experimental findings closely agree with the hydrological and environmental condition existing in the intertidal and subtidal regions of Mandapam coast.

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