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Evaluation of Nutritional Component in Some Seaweeds from Rameshwaram coastal areas

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Abstract

The different concentration of ammonium nitrate, sucrose and spermidine enriched f/2 media was applied for growth and development of thallus of seaweeds. Amongst different concentration of nutrient enriched media, spermidine (100μM) enriched f/2 medium influences the maximum number of vegetative branches and a callus (disorganized cell mass that arose from the organized tissue of the explant), Although regeneration was observed in sucrose and ammonium nitrate supplemented enriched media but the speed of regeneration and growth was found to be slow compared to spermidine. The simultaneous formation of filamentous outgrowth was observed at the cut ends of explants. These filamentous outgrowths later developed several vegetative branches cultured in different concentration nutrient enriched media. The explants taken from the basal portion of the thallus regenerated maximum number of vegetative branches compared to the apical portion. The pattern of the growth of vegetative branches was found to be uniaxial and lactotype growth. A complete plantlets along with several branches was grown successfully in spermdine supplemented enriched f/2 medium after 1 months of culture. The color of plantlets was bushy and quite similar to that of field material in appearance.

Key words: Polyamines (PAs), Spermidine (spd), f/2, Enriched seawater (FSW), sucrose, ammonium nitrate, cystocarp.

Introduction

Marine algae commonly known as Seaweeds, found attached to the bottom in relatively shallow coastal water. They are found in rocky seashore areas, lagoons and reed areas of Indian Sub-continent. They are considered as the food supplement for 21st century as source for proteins, lipids, polysaccharides, mineral, vitamins and enzyme. In nature, there are about 900 species of green seaweeds including 4000 red species and 1500 brown species. Some 221 species of seaweed are utilized commercially. Of these, about145 species are used for food and 110 species for phycocolloid production (Agar, Algin, Carrageenan etc.). The nutrients like sucrose, ammonium nitrate and spermidine play an important role on the growth, development and callus formation. They have been labeled as a new class of growth substances and a type of plant growth substances and a type of plant growth regulator or hormonal second messenger (Galston and Kaur-Sawhney,1990; Lee and Chu, 1992; Tiburcio *et al*.,1993; Gasper *et al*.,1997; Soccianti *et al*., 2000; Tassoni *et al*., 2000). In marine algae, spermidines have been studied in relation to their occurrence within different algal groups (Hamana and Matsuzaki, 1982) and their involvement in cell division (Cohen *et al*., 1984). Interestingly, ammonium and nitrate are the major nitrogen sources of seaweeds in the natural habitat and are used as nourishment for seaweed in integrated multi-trophic aquaculture system. Several experiments have been conducted for several seaweed species on the interaction of ammonium and nitrate uptake. The utilization of ammonium and nitrate by seaweed varies among species and assimilation of these nutrients influences the growth. It has been observed that the presence of ammonium inhibits the nitrate uptake; thus ammonium uptake dominated nitrate uptake when exposed to a combination of NH4+ and NO_3 (Thomas and Harrison,1987). Interestingly, Sucrose is also the major photoassimilate transported from source to sink tissues in birch that is hydrolyzed into glucose and fructose to provide energy via respiration, while maltose is the predominant sugar in barley (Salisbury and Ross,1985; Lindqvist and Asp 2002). Recent evidence has, however, shown that in plants, sugars such as sucrose, glucose, and fructose function not only as substrates for growth but affect sugar sensing systems that initiate changes in gene expression and subsequent plant growth (Koch,1996). Sugar depletion, for example, up regulates genes for

photosynthesis, carbon remobilization and export, resulting in vegetative or shoot growth. In contrast, incubation of root systems in sugar solutions (i.e., sucrose or glucose) leads to the repression of photosynthetic genes, decreased rates of net photosynthesis and carbon remobilization in favor of enhanced root development (Koch, 1996; Martin *et al*.,1997).

The aim of this work was to study the influence of the addition of nutrients on the growth and development of vegetative branches along with embryoid structures on seaweeds. All these findings show great achievements in seaweed tissue culture work in our laboratory in order to select regenerate and propogate possible clones as seed stock for the commercial purpose.

Materials and Methods

In vitro culture studies

Collection of Sample

Three Field trips were under taken annually to different coastal areas of Rameshwaram. The samples were collected during low tide.

Rameshwaram coasts

The tide of Rameshwaram coast (9º14`N, 79º14`E) was high, having rocky seashore areas enriched with luxuriant growth of, *G.corticata* , *G. verrucosa, G. edulis* etc. The thallus of each species attached strongly on rocks and coral stones having minute initiation of young plants (Fig. 1).

Fig.1: Showing the point of collection of Seaweeds from Rameshwaram coastal area of Tamil Nadu, India.

Cleaning of thallus

The thallus of seaweeds was washed properly in the seawater for several times in the field with the help of fine brush to remove contaminated particles.

Transportation of thallus

Later the thallus of seaweeds wrapped in the absorbent cotton moistened with seawater, kept inside the ice box and transported to the laboratory in the A.C compartment.

Inside the laboratory, the healthy thallus was washed with filtered seawater regularly under laminar flow. The thallus was treated with a few drop of GeO_2 and maintained a stock Culture (Given below).

Maintenance of Stock culture

Composition of Germanium dioxide and Antibiotic mixture

Streptomycin sulphate	1gm
Penicillin-G	1gm
NaOH	4gm
GeO_2	250 mg

1gm streptomycin sulphate and Penicillin-G dissolved in 100ml of distilled water is added to make 1ml antibiotic mixture. To make 1 ml GeO2, 4gm of NaOH and 250 mg of GeO2 dissolved in 100ml of distilled water later boiled for a few minutes.

Culture modification

1gm of fresh thallus from Stock culture was taken and inoculated into triplicates containing marine media, f/2 enrichment seawater (Guillard and Ryther, 1962), enriched with different concentration (50µM and 100 µM) of nutrients like Ammonium Nitrate, Spermidine and Sucrose. The salinity was maintained at 20ppt and pH was adjusted to 7.8. Sterilization was done by autoclaving the media and glasswares at 120°C with 15 Ib/ $Inch^2$ pressure for 15 minutes.

The following method was employed during *In vitro* study :

a) f/2 enrichment seawater was also used as control medium.

b) Culture condition was maintained at 20°C in 12:12 L:D photoperiod.

c) Salinity was maintained at different range 25-30ppt.
d) pH of all media was adjusted to 7.8.
e) Harvesting was made at an interval of after 14 days.

Results and Discussion

Effect of different concentration of sucrose (50µm & 100 µm) enriched f/2 medium

The effect of different concentration of sucrose promotes the evaluation of growth and development of plantlets from the different surface of cut end. The detailed can be shown in Figure 2 a. In the present study, the treatment of different concentration of Sucrose was shown on the different types of edible seaweeds collected from different coastal areas of Rameshwaram, Tamil Nadu. The highest rate of Growth was observed in *Padina gymnospora* and *S. vulgare*, however the lowest growth rate was observed in *E.intestinalis,* which was similar as spermidine treatment, observed in 100µM Sucrose concentration. Explants of different types of seaweeds were cultivated in f/2 Enriched Seawater culture medium supplemented with Sucrose (50µM & 100 µM). All experiments were carried out in the presence of light.

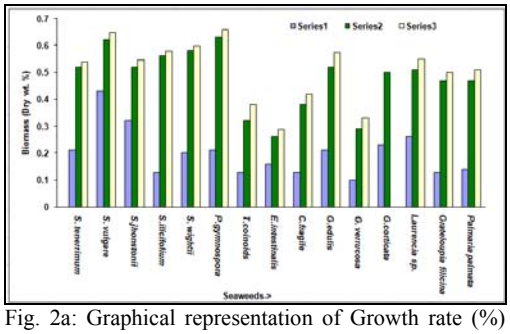

Fig. 2a: Graphical representation of Growth rate (%) among different types of seaweeds culture in f/2 Control and f/2 Plus Sucrose enriched Medium (Series 1= f/2 Control, Series 2 = 50µM, Series 3 = 100 µM).

Sucrose is an effective carbon source for the vegetative propagation of seaweeds liquid media. Except Sucrose, Mannose and galactose all had no effect on growth or morphogenesis of the explants. A decrease in the concentration of sucrose in PES culture medium reduced the morphogenetic capacity of the explants, which developed into compact cell masses. In higher plant, the effects of different concentration of sucrose were observed in the propagation & growth of *Dendrobium* sp second love (Orchidaceae) plants. It was found that an increase level of sucrose concentration caused an increase in the amount of total soluble carbohydrate in the absence of light. In higher plant, in the presence of light, 2% sucrose was significantly more effective than the other concentration used, while in the darkness 3% and 4% sucrose provided the highest value from average root length. Percival *et al*., (2005) evaluate the influence a factorial combination of six different sugars (galactose, rhamnose, sucrose, glucose, fructose, and maltose) on root and shoot growth, chlorophyll fluorescence, photosynthesis, and leaf chlorophyll and carotenoid concentrations of birch (*Betula pendula*) following transplanting. It was found that the highest increases in girth and in root, shoot, and leaf dry weight at the cessation of the experiment were recorded following applications of sucrose as a root drench at a concentration of 70 g/L. Application of the sugars tested in this investigation to root systems of birch following severe root pruning reduced mortality from 15% (controls) to Zero. However in the present study (i.e in lower plants), at maximum concentration of Sucrose (100µM), the stipe portion showed the initiation of minute cystocarp on its surface, which grown rapidly and attained the maturity. In contrast to decreases of sucrose concentration, the rate of growth of leaf, stipe and rhizoidal portion enhanced enough growth of plants. The use of stipe, leaf and rhizoidal portion of seaweed proved to be an alternative source of neutraceutical & food supplements. However, the exogenous carbon source provided by the culture medium was sufficiently enough to maintain plant growth in the dark.

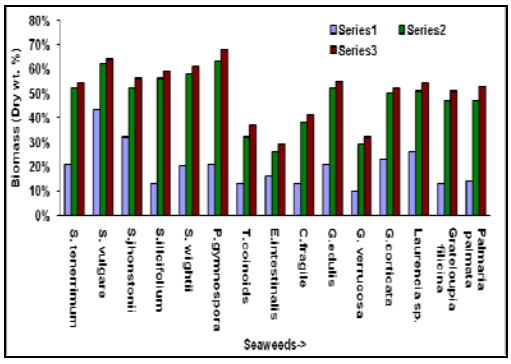

Fig. 2b: Graphical representation on Growth rate (%) of different concentration of Spermidine plus f/2 media among different types of seaweeds (Series 1= f/2 Control, Series 2 = 50µM, Series 3 = 100 µM).

Effect of different concentration of spermidine (50µm & 100 µm) enriched f/2 medium

The treatment of different concentration of spermidine enriched f/2 media, influenced growth and regeneration in explants cultured at different concentration (Figure 2b). Amongst all concentration used spermidine 100µM in f/2 media showed best results in *Padina gymnospora* in comparison to other macro algae (Seaweeds).In contrast to PES enriched 50µM concentration growth of formation of branches was slow even after 28 days of inoculation. However, in f/2 control media, the growth was too slow in *S. wightii, Ulva fasciata* and *Laurencia sp.* in comparison to 50 µM and 100 µM concentration medium. Most of the complete plant after attaining maturity, bleached in control medium. Amongst different types of plant material, in control medium, the maximum growth was observed in *G.verrucosa* respectively. The influence of spermidine enriched PES culture medium was tested on axenic *in vitro* cultures of carposporelings of *Grateloupia doryphora* & *G. corticata*. The spermidine (100µM) induced a callus, transformed the carposporelings into cell masses that produced shoots. However, the lower concentration was inhibitory, rare formation of carposporelings or resulted in the inhibition of the morphogenesis (i.e. shoot emission) in the cell mass.Yokoya, (2004) reported the effects of plant growth regulators on callus formation, growth and regeneration in axenic tissue cultures of *Gracilaria tenuistipitata* and *Gracilaria perplexa* (Gracilariales , Rhodophyta).

Spermidine (100µM) enriched f/2 culture medium followed a similar pattern to spermidine (50 µM) culture f/2 medium plantlets. During cultivation in media with PAs, the growth and development of carposporelings was enhanced. In f/2, successive cellular divisions were evident until the formation of one main axis after 14 days of release, whereas PAs increased cell division during carpospore development, produced cell masses, and induced morphogenesis. The cell masses were formed between 7 and 14 days after release. A most evident effect on cell division was observed in Spermidine, where cell masses were obtained after 7 days (Figure 2c). In some higher plants, the characteristics of spermidine binding to total solubilized protein from plant plasmalemma turned out to be similar to those previously found in plasma membrane vesicles which confirm the hypothesis that the specific polyamine interaction between spemidine and plasma membrane, occurs with the protein component of the membranes. If the spermidine binding protein is a polyamine receptor, this specific binding protein could activate the reaction chain responsible for the range of polyamine effects at cellular levels (Iglesias-Prieto *et al*., 1993). Overall study showed that the control f/2 medium enhanced lower growth than spermidine enriched f/2 medium.

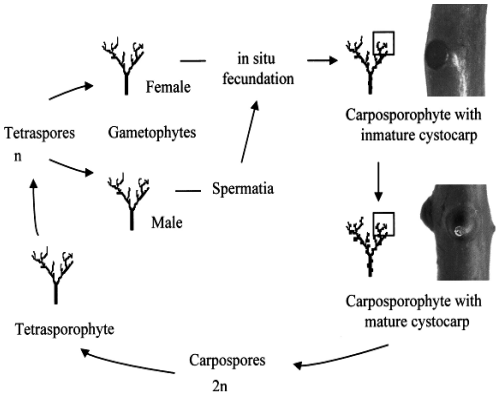

Fig.2c: Triphasic life cycle of *Gracilaria sp.*, showing the external morphology for the two stages of cystocarp maturation studied within the carposporophytic phase during *in vitro* culture.

Effect of different concentration of ammonium nitrate (50µm & 100 µm) enriched f/2 medium

The supplementation of ammonium nitrate in PES media influenced the growth and regeneration in holdfast, stipe and leaves. Amongst the different concentration of f/2 media used, ammonium nitrate 100µM enriched f/2 media showed the best results. The treatment of 100 µM ammonium Nitrate, plant showed maximum growth (%) in comparison to f/2 control and 50µM ammonium Nitrate medium. In f/2 control medium, the most of the plant bleached and retard their growth, the lowest growth was observed in *G. verrucosa*. In the treatment of 50µM, the maximum growth was observed in *S. vulgare*. With increase of ammonium nitrate concentration (i.e 100 µM), the plant grown vigorously and complete their life cycle. Besides, the treatment of 50 µM concentration of ammonium nitrate concentration, the maximum growth was shown in *P.gymnospora* and slow growth was observed in *T.conoids*. Thomas *et al*., (1985) reported the nitrogen uptake and growth of the germlings and mature thalii of *Fucus distichus*. Rao, (1945)

reported the life history of *Sargasum tenerrimum.* Fagerberg and Dawes, (1976) reported that mitosis occur at the active portion of surface cell of stipes in *Sargassum filipendula* after 3rd of inoculation. Alvaro *et al*., (1999) investigated the effect of salinity and pH on growth and agar yield of *Gracilaria tenuistipitata* in laboratory and outdoor cultivation. Friedlander,(1991) reported the effect of light and ammonium on growth, epiphytes and chemical constituents of *Gracilaria conferta* in outdoor cultures.

Nedumaran *et al*., (2009) reported the nutrient Relationships in the Seaweeds of Uppanar Estuary-South East Coast of India. They found nutrients like a nitrate, nitrite, phosphate and silicate varied from 7.75 to 23.75 µm; 0.55 to 11.25 µm; 0.10 to 2.78 µm and 23 to 150 µm respectively. Padhi *et al*.,(2010) reported the four seaweeds *Enteromorpha intestinalis, Chondrus crispus, Gracilaria verrucosa* and *Polysiphonia sertularioides* were examined for their efficiency in uptake of nitrate and ammonium to access the potentiality of these algae for removal of nutrients from aqua culture effluents. *Enteromorpha intestinalis* and *Gracilaria verrucosa* removed nitrate from the medium at considerable higher than those measured for *Chondrous crispus* and *Polysiphonia sertularioides*. At similar temperature and irradiance the Vmax of nitrate uptake *in E. intestinalis and G. verrucosa* are thrice that of *Chondrous crispus*. Similarly, Harrison et al. (1986) reported the nitrogen uptake kinetics in three year-classes of *Laminaria groenlanidca* (Laminariales: Phaeophyceae). Gordillo et al. (2001b) reported the Photosynthetic acclimation to photon irradiance and its relation to chlorophyll fluorescence and carbon assimilation in the tolerant green alga *Dunaliella viridis* (Dere *et al*.,2003). Bird, (1976) reported the simultaneous assimilation of ammonium and nitrate by *Gelidium nudifrons* (Gelidiales: Rhodophyta). Hiroyuki, (1991) reported the effects of nitrate supply on ammonium assimilations in the blade of *Laminaria japonica* (Phaeophyceae). Chapman *et al*., (1977) reported the seasonal growth in *Laminaria longicruris* relations with dissolved inorganic nutrients and intertidal reserves of nitrogen.

Andreas *et al*., (2009) reported the direct comparison of the performance of the seaweed biofilters, *Asparagopsis armata* and *Ulva rigida.* Ammonium and nitrate were simultaneously uptaken from the medium by the blade tissue segments. This indicates that nitrate supply promotes the assimilation and incorporation activities of ammonium in the blade of *L. japonica.* Thomas et al. (2003) reported the time courses of ammonium and nitrate uptake rates were determined for five intertidal macrophytes collected directly from the field (Brasco *et al*., 1982). When a 15-µM pulse of ammonium was added to the incubation medium, all three annuals and one of the two perennials, *Fucus gardneri*, showed enhanced ammonium uptake rates for the first 15 min of a 30-min exposure. This is the first report of non-linear nutrient depletion by plants grown in the field. There was no enhancement in the initial nitrate uptake rate. In fact, nitrate uptake had to be induced in the low intertidal *Gracilaria pacifica* by incubating it in 30 µM nitrate for 20 min. All species took up both ammonium and nitrate simultaneously.

In f/2 control media, from holdfast region, a group of leaves produced after 40 days whereas in f/2 enriched 100µM concentration of ammonium nitrate solution respectively. However similar growth was also observed in spermidine enriched f/2 control but the growth rate was slow. The production of number of leaves from the holdfast region also varied within the different media. In some cases, some epiphytic growth seen over the rhizoidal portion of thallus. The growth was significantly lower in *S. illcifolium* and *Grateloupia filicina* .The detailed results are summarized in figure 2d.

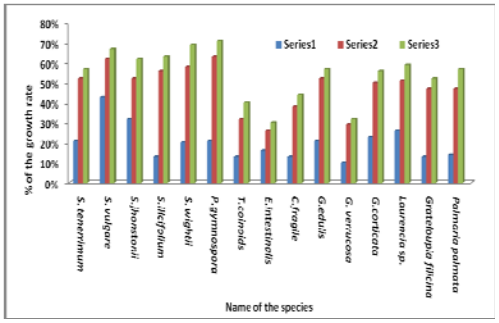

Figure 2d : Graphical representation of Growth rate (%) among different types of seaweeds culture in f/2 Control and f/2 Plus Ammonium Nitrate enriched Medium (Series 1= f/2 Control, Series 2 = 50µM, Series 3 = 100 µM).

Overall study showed that in control medium, the biomass production of seaweeds was lower than nutrient enriched media (ammonium nitrate, sucrose and spermidine enriched medium).

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