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Tissue Culture of Kappaphycus Alvarezii (Doty) Doty ex. Silva for Improved Callus Development & Plantlet Regeneration

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Abstracts

Kappaphycus alvarezii (Doty) Doty ex. Silva, red seaweed is one of the best sources of kappa carragenan and has been globally introduced in many maritime countries for its commercial applications. Cultivation of this seaweed requires a large number of propagules with desirable traits which include high growth rates and resistance to diseases. *Kappaphycus alvarezii* cultivated in the farming area of Purunabandha and Gokharkuda of Ganjam district, Odisha was used as explants in the study of regeneration of new plants. Provasoli's enriched seawater (PES) medium was used as the basal medium. For clonal multiplication through in vitro culture using explants of the red alga K. alvarezii, attempt was made to use different percentages (5, 10, 15, 20%)coconut water (CW)to the culture medium as a cost effective method for callus induction and plantlet regenerations. High frequency callusing was induced (71±5. 8%)on Provasoli Enriched Medium supplemented with BA 1mg/L+NAA 2. 5mg/L. With supplementation of coconut water (CW) in the medium containing phytohormones, maximum callusing (80+4. 9%) was observed with BA 1mg/L+NAA 2. 5mg/L+20% CW and minimum regeneration of plantlets (35±3. 5%) was observed with medium, phytohormone and coconut water (PES+2-4-D 2. 5mgl/l+10% CW). The

stimulating action was also observed in CW containing medium for expediating the time required for callusing and regeneration of plantlets.

Key words- Kappaphycus alvarezii, coconut water, callus induction, phytohormone, micropropagation.

Introduction

Studies on tissue culture of seaweeds have been carried out since 1980 with the main aim to devise strategies for micropropagation and genetic improvement of economically useful species and for *in vitro* production of phycocolloids [1, 2&3]. Callus formation is reported for the first time in *Sargassum polycystum* [4]. Production of callus from algal organism is a relatively new field and lies far behind that of land plants[5, 6]. Callus provides the starting point for many of the techniques of genetic manipulation of plants, in particular the induction of somaclonal (protoclonal) variation, somatic hybridization and its transformation. Protoplasts have been produced from a number of species of multicellular marine algae and plants have been regenerated from protoplasts of Chlorophyta, Phaeophyta and Rhodophyta[7, 5, 6, 1]. Isolation of protoplasts from agar yielding *Gracilaria tikvahiae* and *Gracilaria lameniformis* is reported for the first time in 1986[6]. The objective of present study is to develop a reproducible and efficient regeneration system for production of uniform, elite and improved plantlets for large scale production to conserve the carrageenophyte for commercial exploitations.

Materials and Methods

The seaweed brought from the sea needs to be acclimatized and maintained in a clear and controlled environment of the aquarium placed in the laboratory. Seaweeds was maintained in the aquarium of 30 cm wide, 45 cm height, &45 cm long. Healthy one of five cm size pieces of the thallus near the apical region were cut and cleaned several times in sterilized sea water and soaked in 70% (v/v) ethanol for 1 min and then immersed in solution containing 1% (v/v) Sodium hypochlorite and 5-10 drops of Tween -20 for 20 minute. After surface sterilization, they were rinsed six times with sterile distilled water. Under Laminar air flow cabinet, explants were excised and cultured in the PES basal medium containing 3, 0% sucrose (w/v), 0. 7% agar and different concentrated combination of plant growth regulators like 2-4 – Dichlorophenoxy acetic acid (2-4-D), 6-Benzylamino purine (BAP), N6-Benzyladenine (BA), Indole 3 Acetic Acid (IAA) and Napthalene Acetic Acid (NAA). The pH of the medium was adjusted to 5. 8 before autoclaving at 121°C for 20 minute. The stock culture was sub-cultured at 20-30 days intervals.

Culture conditions

Stock cultures and explants were initially incubated in darkness in a culture chamber at 25°C±2°C. Subsequently explants were incubated under a 16:8 hour (light/dark)

photoperiod with light supplied by cool –white fluorescent lighting at an intensity of 60 ME $m^{-2}s^{-1}$ at a constant temperature of 25±2°C.

Result and Discussion

The percentage of calli that regenerated increased with concentration of CW upto 20% supplementation with PES medium. with concentration <15%, however callus induction was negligible. percentage of callus increased significantly with concentration of CW 15 and 20% supplementation with PES medium.

Table 1 shows the Callus Induction in PES Medium Supplemented with different combination of Phyto-hormones &coconut water (CW). Each treatment was done with 10 replicates. When the medium was supplemented with 2-4-D (1 mg to 2. 5 mg /L), the time of plantlet generation decreases with increase in concentration and percentage of plantlet increases with increase in concentration of BA except at 1&1. 5 mg/L where no plantlet generated. Better results obtained (30±3. 1%) with 2-4-D at 2. 5 mg/L. With addition of 15-20%CW, the generation time decreases and percentage of callus induction increases. (42±3. 3% at 20% CW and 35% at 15% CW).

When the medium was supplemented with IAA (1 mg to 2. 5 mg /L), the time of plantlet generation decreases with increase in concentration and percentage of plantlet increases with increase in concentration of IAA except at 1&1. 5 mg/L where no plantlet generated. Better results obtained (40±4. 94%) with IAA at 2. 5 mg/L. With addition of 15-20%CW, the generation time decreases and percentage of callus induction increases. (45+4. 2% at 15% CW and 48+4. 7% at 20% CW). When the medium was supplemented with IBA (1 mg to 2. 5 mg /L), the time of plantlet generation decreases with increase in concentration and percentage of plantlet increases with increase in concentration of IBA except at 1&1. 5 mg/L where no plantlet generated. Better results obtained (35±4. 0%) with IBA at 2. 5 mg/L. With addition of 15-20%CW, the generation time decreases and percentage of callus induction increases (40+4. 3% at 15% CW and 46+4. 2% at 20% CW). When the medium was supplemented withBAP (0. 5 -2. 0 mg/L)& IBA (1 mg to 2. 5 mg /L), the time of plantlet generation decreases with increase in concentration and percentage of plantlet increases with increase in concentration of IBA upto 1. 0 mg BAP &1. 5 mgIBA beyond that it decreases. Better results obtained (70±3. 5%) with PES+BAP 1mg/l+IBA1. 5mgl/l. With addition of 15-20%CW, the generation time decreases and percentage of callus induction increases (75+3. 1% at PES+BAP 2mg/l+IBA2. 5mgl/l+15%CW and 78+4. 4% at PES+BAP 2mg/l+IBA2. 5mgl/l+20% CW).

Table-3 shows Plantlet regeneration from the Callus under the influence of different combinations of phytohormones supplemented with Provasoli medium &coconut water (CW). When the medium was supplemented with BA (1 mg to 2. 5 mg /L), the time of plantlet generation decreases with increase in concentration and percentage of plantlet increases with increase in concentration of BA. Better results obtained (40±2. 5%) with BA at 2. 5 mg/L. With addition of 15-20%CW, the generation time decreases and percentage of callus induction increases (43±2. 6% at 15% CW and 46±2. 4% at 20% CW). When the medium was supplemented with

NAA (1 mg to 2. 5 mg /L), the time of plantlet generation decreases with increase in concentration and percentage of plantlet increases with increase in concentration of NAA. Better results obtained (30±3. 8%) with NAA at 2. 5 mg/L. With addition of 15-20%CW, the generation time decreases and percentage of callus induction increases (38±3. 2% at 15% CW and 40±3. 6% at 20% CW). When the medium was supplemented withBA (1. 0 mg/L)& NAA (1 mg & 2. 5 mg /L), the time of plantlet generation decreases with increase in concentration and percentage of plantlet increases with increase in concentration. Better results obtained (71±5. 8%) with PES+BA 1mg/L+NAA 2. 5mg/L. With addition of 15-20%CW, the generation time decreases and percentage of callus induction increases (75+5. 1% at PES+BA 1mg/L+NAA 2. 5mg/L+15% CW and 80+4. 9% at PES+BA 1mg/L+NAA 2. 5mg/L+20% CW).

The expediated callus proliferation and plantlet development with CW addition would maximize potential use of this tissue culture system as a method for rapid propagation of Kappaphycus alvarezii.

Table-1. Callus Induction in PES Medium Supplemented with different combination of Phyto-hormones &coconut water (CW)

Treatment Medium	No of Explant	Days of Callus Induction	Average Rate % of Callus Induction in Basic Medium
PES (Control)	10	30	7
PES+5 % CW	10	NC	-
PES+10%CW	10	NC	-
PES+15%CW	10	23	9
PES+20%CW	10	20	10
PES+2-4-D 1mgl/l	10	NC	-
PES+2-4-D 1. 5mgl/l	10	NC	-
PES+2-4-D 2. 0mgl/l	10	31	25±2. 2
PES+2-4-D 2. 5mgl/l	10	26	30±3. 1
PES+2-4-D 2. 5mgl/l+15% CW	10	25	35±3. 5
PES+2-4-D 2. 5mgl/l+20% CW	10	20	42±3. 3
PES+IAA 1mgl/l	10	NC	-
PES+IAA 1. 5mgl/l	10	NC	-
PES+IAA 2. 0mgl/l	10	22	30±2. 2
PES+IAA 2. 5mgl/l	10	20	40±4. 94
PES+IAA 2. 5mgl/l+15%CW	10	16	45+4. 2
PES+IAA 2. 5mgl/l+20% CW	10	14	48+4. 7

Table-2. Callus Induction in PES Medium Supplemented with different combination of Phyto-hormones &coconut water (CW)

Treatment Medium	No of Explant	Days of Callus Induction	Average Rate % of Callus Induction in Basic Medium
PES+BAP 0mg/l+IBA1. 0mg/l	10	NC	-
PES+BAP 0 mg/l+IBA1. 5mgl/l	10	NC	-
PES+BAP omg/l+IBA2mgl/l	10	24	20+3. 54
PES+BAP omg/l+IBA2. 5mgl/l	10	23	35±4. 0
PES+BAP omg/l+IBA2. 5mgl/l+15% CW	10	20	40+4. 3
PES+BAP omg/l+IBA2. 5mgl/l+20% CW	10	18	46+4. 2
PES+BAP 0. 5mg/l+IBA1mgl/l	10	19	60±5. 1
PES+BAP 1mg/l+IBA1. 5mgl/l	10	20	70±3. 5
PES+BAP 1. 5mg/l+IBA2mgl/l	10	18	30+2. 5
PES+BAP 2mg/l+IBA2. 5mgl/l	10	16	25±1. 8
PES+BAP 2mg/l+IBA2. 5mgl/l+15%CW	10	14	75+3. 1
PES+BAP 2mg/l+IBA2. 5mgl/l+20% CW	10	14	78+4. 4

Table-3. Plantlet regeneration from the Callus under the influence of different combinations of phytohormones supplemented with Provasoli medium &coconut water (CW)

Medium	Day of regeneration of plantlets	% of Plantlets Induction
PES+BA 1mg/L	60	20±1. 6
PES+BA 1. 5mg/L	65	25±1. 8
PES+BA 2mg/L	50	30±2. 1
PES+BA 2. 5mg/L	45	40±2. 5
PES+BA 2. 5mg/L+15% CW	42	43±2. 6
PES+BA 2. 5mg/L+20% CW	38	46±2. 4
PES+NAA 1mg/L	80	18±2. 1
PES+NAA 1. 5mg/L	84	20±1. 8
PES+NAA 2mg/L	90	25±2. 7
PES+NAA 2. 5mg/L	75	30±3. 8
PES+NAA 2. 5mg/L+15% CW	65	38±3. 2
PES+NAA 2. 5mg/L+20% CW	60	40±3. 6
PES+BA 1mg/L+NAA 1mg/L	45	50±4. 1
PES+BA 1mg/L+NAA 2. 5mg/L	40	71±5. 8
PES+BA 1mg/L+NAA 2. 5mg/L+15% CW	30	75+5. 1
PES+BA 1mg/L+NAA 2. 5mg/L+20% CW	28	80+4. 9

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