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Gibberellic acid and 2,4-D as growth regulators in laboratory culture of seaweeds

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The growth regulatory effects of gibberellic acid (GA) and 2,4-D (2,4-dichlorophenoxy acetic acid) on a green alga, *Ulva fasciata* and two species of red algae, *Gracilaria corticata* and *Hypnea valentiae* were studied. For a culture period of 30 days, the thallus segments showed varying growth rates on pretreatment with 6 to 20 mg/l of the above growth substances. These compounds stimulated growth and increased wet weight of the algae. At higher concentrations (16-20mg/l) 2,4-D was growth inhibitory, whereas, GA showed significantly higher growth at 16-20mg/l concentrations. It is concluded with the present study that plant hormones like GA and 2,4-D could be used for enhancement of algal production in culture practices at different concentrations.

In view of the increasing demand for seaweeds, it has become imperative to culture them, as the natural stock is being heavily exploited throughout the world, and demand for algal products exceed supply. Besides increasing the total supply, seaweed culture is also attractive in that it has the capacity of providing high quality raw material. Use of growth promoting substances or hormones to improve the biomass production, in lagoons and brackishwater areas is one of the various modern techniques in algal culture. Many studies have tried the effect of different growth regulating substances like natural auxins, gibberellins, kinetin and other synthetic phytohormones like 2,4-D and NAA (naphthalene acetic acid) on several seaweeds[1-10]. The present study was carried out to investigate the effect of growth regulators, GA and 2,4-D on the growth of thalli of three selected species of seaweeds of economic value, growing luxuriantly along the Kerala coast, by laboratory culture experiments.

Materials and Methods

The seaweeds selected for the study were *Ulva fasciata* Delile (Chlorophyceae),*Hypnea valentiae* (Turn) Mont (Rhodophyceae) and *Gracilaria corticata* J Ag. (Rhodophyceae). They were collected from Thiruvananthapuram and Kozhikode districts , during June-July months during south-west monsoon in 1988. The substances tested for their growth regulatory properties were GA and 2,4-D (Sigma, USA). The selected specimens were thoroughly washed and transported in ice-box with seawater to the laboratory. It was aerated and maintained in fresh filtered seawater as stock culture.

The culture studies were done according to the cut-piece' method[11] for *Gracilaria corticata* and *Hypnea valentiae*. Cut-pieces of 20 mm and 30 mm were taken respectively for the above two species. For *Ulva fasciata*, pieces of thallus 10×10 mm size were cut from the middle region of uniform sized plants.

The growth substance solutions were prepared at concentrations of 6, 8, 10, 12, 16 and 20 mg/l in sterilized seawater. These concentrations were selected[2] after preliminary experiments with concentrations in the range of 0.1 to 10 mg/l. Pretreatment was done for 24 h in the test concentrations for the three species and controls were maintained without pretreatment. The thalli pieces were then transferred to sterile petri-dishes of 90 mm diameter containing 50 ml of modified Erdshrieber culture medium[12] in triplicates. The salinity and *p*H of the medium were maintained at 30 ppt and 7.5 respectively.

The cultures were kept on illuminated racks fitted with fluorescent lamps of 40 W, at room temperature ranging from 26° to 28°C. The light intensity ranged between 1000 to 1500 lux. Photoperiod was maintained for 12:12 LD. The culture medium was renewed on alternate days and the experiment was run for 30 days, since maximum growth occurs during the first 30 days during the culture period. Experiments were repeated for more reliability of the results.

Linear growth measurements of `cut-pieces' of *Gracilaria corticata* and *Hypnea valentiae*, from treatments as well as controls were taken to the nearest millimeter on every five days. As the thallus of *Ulva fasciata* is uniformly two layered and thin, it is possible to record areal measurements as an index of growth. These measurements were also taken at 5 day intervals.

Results

The growth effects of GA and 2,4-D on cut-pieces of

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G.corticata is given in Table 1A. GA in concentrations ranging from 6 to 20 mg/l showed a progressive increase in length over the control. At 20 mg/l, maximum increase in length and fresh-weight over the initial i e: 20.6 mm and 33.69 mg were obtained, whereas, control showed 16.19 mm and 15.5 mg increase only. 2,4-D was less effective beyond 16 mg/l, and at 10 mg/l, 17.5 mm and 26.9 mg increase in length and weight were recorded over the initial with 2,4-D. In the course of the experiment, all the fragments developed holdfasts for attachment and a number of proliferations ranging from 4 to 6 numbers were also observed. Throughout the experimental period, all the fragments were in vegetative state.

Table 1B presents the data on growth of thallus of *Hypnea valentiae* after pre-treatment with GA and 2,4-D. During the culture period, mean length and fresh-weight of thallus segments in control increased 11.8 mm and 52.78 mg over the initial, whereas, on treatment with GA at 20 mg/l an increase in mean length and fresh weight of 23.5 mm and 88.86 mg was observed. At lower concentrations also, slight increase in length and fresh weight were recorded. Application of 2,4-D resulted in

Table 1—Growth measurements of *Gracilaria corticata* (A),*Hypnea valentiae*(B) and *Ulva fasciata(C)* on treatment with Gibberellic Acid and 2,4-D in 30 days.

	Control	GA Cone (mg/l)						2,4- D (mg/l)					
	--	6	8	10	12	16	20	6	8	10	12	16	20
						(A) *Gracilaria corticata*							
Initial length(mm)	20	20	20	20	20	20	20	20	20	20	20	20	20
Final length (mm)	36.2	36.4	38.1	38.6	38.9	39.3	40.2	36.8	37.3	37.5	37.2	36.0	35.1
	±2.7	±2.0	±0.8	±0.8	±1.4	±1.3	±1.7	±1.1	±1.6	±1.1	±1.4	±1.3	±1.5
Increase in length (mm)	16.2	16.4	18.1	18.6	18.9	19.3	20.2	16.8	17.3	17.5	17.2	16.0	15.1
Initial	21.8	19.6	20.8	21.0	20.0	20.7	22.8	23.0	24.6	22.8	24.8	23.4	28.8
weight(mg)	±2.0	±1.7	±1.7	±1.3	±2.8	±1.5	±1.6	±1.3	±1.8	±1.5	±1.8	±2.2	±2.3
Final	37.2	34.2	40.1	44.2	46.7	49.8	56.5	42.5	48.8	49.7	49.5	44.2	46.5
weight(mg)	±2.1	±2.3	±1.8	±1.9	±2.3	±2.1	±2.1	±1.5	±1.4	±1.3	±1.8	±1.9	±2.5
Increase in weight (mg)	15.4	14.5	19.3	23.2	26.7	29.1	33.7	19.5	24.2	26.9	24.7	20.8	17.7
No of proliferations	4	5	4	5	6	6	6	5	6	5	6	6	5
						(B) *Hypnea valentiae*							
Initial length (mm)	30	30	30	30	30	30	30	30	30	30	30	30	30
Final length (mm)	41.6	41.5	43.0	43.3	47.8	50.3	53.5	46.3	46.5	47.5	51.3	43.5	41.8
	±2.7	±1.5	±1.7	±1.5	±4.5	±3.4	±3.1	±3.4	±4.7	±1.0	±1.6	±1.0	±1.6
Increase in length (mm)	11.6	11.5	13.0	13.3	17.8	20.3	23.5	16.3	16.5	17.5	21.3	13.5	11.8
Initial weight (mg)	72.4	69.0	67.9	73.0	66.0	71.9	70.7	71.0	66.4	61.7	65.7	68.6	68.3
	±2.5	±1.8	±1.8	±1.3	±3.2	±2.8	±2.5	±3.1	±2.6	±1.5	±1.9	±1.8	±1.7
Final weight (mg)	125.1	117.7	120.8	133.7	144.8	158.6	159.6	133.3	134.5	139.0	139.8	130.7	120.1
	±2.3	±1.2	±1.7	±1.5	±3.4	±2.4	±2.6	±3.1	±2.8	±1.7	±1.8	±2.1	±1.9
Increase in weight (mg)	52.7	48.7	52.9	60.7	78.8	86.7	88.9	62.3	68.1	77.3	74.1	62.1	51.8
						(C) *Ulva fasciata*							
Initial area(mm^2)	100	100	100	100	100	100	100	100	100	100	100	100	100
Final surface area(mm^2)	109.8	121.0	126.5	132.3	133.3	140.3	154.7	118.3	126.0	132.8	113.0	101.5	92.3
	±2.1	±2.4	±2.1	±3.0	±2.8	±2.6	±2.3	±3.0	±5.1	±4.9	±3.4	±2.0	±4.5
Increase in surface area (mm^2)	9.8	21.0	26.5	32.3	33.3	40.3	54.7	18.3	26.0	32.8	13.0	1.5	0.33
Initial weight (mg)	15.8	15.2	16.9	15.3	15.5	18.1	18.2	16.2	16.9	16.8	15.6	16.2	17.0
	±2.4	±2.5	±1.9	±2.9	±1.9	±2.5	±3.0	±2.0	±2.5	±3.2	±2.8	±2.1	±3.4
Final weight (mg)	27.2	30.3	34.2	32.0	32.9	39.9	41.0	33.2	34.9	35.2	30.3	26.5	27.0
	±1.9	±2.4	±2.1	±2.2	±1.9	±2.4	±2.5	±1.8	±2.5	±3.3	±2.5	±1.9	±3.2
Increase in weight (mg)	11.4	15.1	17.3	16.7	17.4	21.8	22.8	17.0	18.0	18.4	14.7	10.3	10.0

relatively lower growth rate. The maximum increase was at 12 mg/l i e: 21.3 mm and 74.09 mg in mean length and weight respectively, which was almost equal to the control.

The results of pretreatment of *Ulva fasciata* segments with GA and 2,4-D are given in Table 1C. The mean increase in surface area and fresh weight for control was 9.83 mm^2 and 11.43 mg respectively. The pieces of *U. fasciata* responded significantly at all the six concentrations of GA used. At 20 mg/l which showed maximum growth measurements, increase in mean surface area and fresh weight was 54.67 mm^2 and 22.76 mg respectively. The 2,4-D was less effective for *U. fasciata*. At 10 mg/l, maximum growth was obtained which was 32.8 mm^2 of surface area and 18.4 mg fresh weight over the initial. At 16 mg/l and 20 mg/l, growth was retarded when compared to control. Table 2 shows statistical significance in growth rate at different treatment levels of 2,4-D and GA using ANOVA.

Discussion

The results indicate that both the growth substances used, 2,4-D and GA increased the biomass for all the three species studied, at various concentrations ranging from 6 to 20 mg/l. Also it was observed that *G.corticata*, *H.valentiae* and *U.fasciata*, on pre-treatment with GA resulted in statistically significant growth at higher concentrations (16-20 mg/l). The higher growth rates can be attributed to the growth stimulating effect of GA as reported earlier[11]. The difference in percentage increase in growth measurements for *G.corticata* and *H.valentiae*, where *G.corticata* was showing more growth can be attributed to the differential absorption of GA and 2,4-D on pretreatment with difference in their cell structure. It was observed in earlier studies[11] that GA was growth promoting at higher concentrations ranging from 10 to 20 mg/l.

The surface area measurement of *U.fasciata* has also shown an enhancement in growth by application of GA. It was reported that there is significant increase in surface area and fresh weight due to the exogenous application of GA in *U.rigida*[8]. Maximum increase in growth by GA in elongation of sporelings of *U.lactuca* was recorded as 0.1 mg/l and not lethal over a wide range of concentrations[3]. Present results show that GA was growth enhancing at higher concentrations (16-20 mg/l).The data on the effect of various concentrations of 2,4-D on the three species of seaweeds indicate that, while, treatment with lower concentrations progressively stimulated growth rate as indicated by length, surface area and fresh weight measurements, treatment with higher concentrations (16-20 mg/l), was not showing significant growth or even inhibitory.[5]. In the present observations, significant growth was obtained at 10 to 12 mg/l concentrations of 2,4-D in 30 days. This is in contrast with some earlier reports showing growth enhancement after 55 days to 70 days for *G.corticata*[2]. Some workers have reported that the effect of applied gibberellin accelerates the growth already in progress and it lasts only for 30 to 40 days[9]. Taking this into consideration, the present experiments had run for 30 days.

For concluding, it may be true that the exogenous auxins and gibberellins probably act in conjunction with the hormones native to the algae, thereby promoting the growth rate. It is possible that the seaweeds selected for the present study may also contain these hormones and that addition of those in growth medium augments this supply, thereby increasing the growth. Though, growth has augmented by 2,4-D and GA at various concentrations, we cannot be certain that algae normally employ such compounds to regulate their processes. It is observed in the present study, that different species of seaweeds respond to growth regulators in different ways and the concentrations used also affects the results as reported by other workers[11].

Table 2—Significance (ANOVA) between treatments as well as replicates for *Gracilaria corticata*, *Hypnea valentiae* and *Ulva fasciata*

Source	D.F	Sum.Sqr	Mean Sqr	F.value	Remarks
		Gracilaria corticata			
Treat	12	144.813	12.068	96.04	Hi.Sig(1%)
Replic	5	564.031	12.806	89.48	Hi.Sig(1%)
Error	60	7.539	0.126		
		Hypnea valentiae			
Treat	12	360.98	30.080	25.18	Hi.Sig(1%)
Replic	5	856.88	71.380	43.44	Hi.Sig(1%)
Error	60	71.69	1.195		
		Ulva fasciata			
Treat	12	13318.56	1109.88	37.24	Hi.Sig(1%)
Replic	5	88197.19	7639.44	591.82	Hi.Sig(1%)
Source	60	1788.31	29.81		

The use of algae in investigations of the action of plant growth substances has not been fully exploited. Some algae show a remarkable response in a comparatively short-term. With proper precautions and adequate consideration of experimental errors, there are many advantages to be gained by the present type of studies on lower level plants like seaweeds, uncomplicated by the complex structures of higher plants which needs more sophisticated methodologies to study growth processes.

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