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Influence of growth regulators (2, 4-D and Kinetin) on vegetative and reproductive behaviour of some green algae

Gour Gopal Satpati, Camellia Nandi and Ruma Pal*

Phycology laboratory, Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Kolkata- 700019, West Bengal, India

***Corresponding author**: rpalcu@rediffmail.com

Abstract

A detailed study was done to understand the effect of phytohormones on morphology and growth performances of green filamentous algal taxa viz. *Pithophora mooreana*, *P. cleveana*, *P. roettleri*, *Cladophora glomerata*, *Spirogyra orientalis*, *Oedogonium hindustanense*, *O. mexicanum*, *O. lemmermannii*, *O. rufescens f. minuta* and *Trentepohlia torulosa in vitro*. Different proportion of 2, 4-Dichlorophenoxy acetic acid (2, 4-D) and kinetin (2:1 and 1:2 of 1mg/mL concentration) were applied in growth medium in laboratory condition. Drastic change in cell proliferation, cell enlargement and initiation of reproductive structures were observed. Both cell length and breadth were increased with time at high 2, 4-D and low kinetin ratio (2:1). The statistical analysis showed significant relationship of cell length and breadth under the variables 2:1 and 1:2 of *P. roettleri* ($p<0.05$). A significant result ($p<0.05$) was obtained in cell length of *O. lemmermannii* and cell breadth in *P. mooreana* under two variables, 2:1 and 1:2 hormone ratios. High 2, 4-D level induced formation of reproductive structures viz. sporangium, oogonium, akinetes etc whereas its low concentration does not show any major changes. The high kinetin induced rapid cell division which results cell proliferation and enlargement. The detail morphological and reproductive changes are reported in the present communication.

Key words: 2, 4- D, kinetin, morphogenesis, phytohormones, reproductive behaviour, statistical analysis

Introduction

Plant hormones are signalling molecules produced within the plant and occur in extremely low concentrations. Auxins are compounds that positively influence cell enlargement, bud formation and root initiation where as cytokinins (CK) influence cell division and shoot formation. The antagonistic and synergistic effect of these on plant growth and development was studied for a long time (Su *et al*., 2011). A very few studied were done about the role of auxin and cytokinin on growth and reproductive behaviour on algae. Cell growth and enlargement of *Chlorella vulgaris* was studied by Yin (1937). Davidson (1950) studied the effects of auxins on growth of *Fucus evanescence* and *Ascophyllum nodusum*. The green filamentous alga *Ulothrix* showed different developmental stages after exposure of auxin and gibberellic acid (Conrad *et al*., 1959). The coenocytic alga *Caulerpa sertularoides* showed different developmental phases after the exposure of auxin (Mishra and Kefford, 1969). Indole-3-acetic acid (IAA) also played an important role for growth and development of *Caulerpa prolifera* (Dawes, 1971). Stimulation of IAA, indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) on growth and vegetative survival of *Cladophora glomerata* was studied by Pandey and Agrawal (2013). The effect of gibberellic acid and 2, 4-dichlorophenoxyacetic acid (2, 4-D) on growth of *Ulva fasciata*, *Gracilaria corticata* and *Hypnea valentiae* were studied by Joseph and Chennubhotla (1999). The effect of 2, 4-D, gibberellic acid and kinetin on carposporelings of *Grateloupia* was studied *in vitro* (Garcia-Jimenez *et al*., 1998). The growth of two microalgae *Dunaliella* and *Haematococcus* were studied under different concentrations of 2, 4-D and kinetin (de Jesus Raposo and de Morais, 2013).

The objective of our work deals mainly the growth and development of the green algal genera by means of vegetative and reproductive behaviour for cell regeneration and biomass production.

Materials and methods

Algal genera studied

The study was performed using 10 filamentous green algal taxa collected from different fresh water habitat of Kolkata (West Bengal, India). These are *Pithophora mooreana*, *P. roettleri*, *P. cleveana*; *Oedogonium hindustanense*, *O. mexicanum*, *O. lemmermannii*, *O. rufescens f. minuta*; *Cladophora glomerata*; *Spirogyra orientalis* and *Trentepohlia torulosa*.

Experimental

Algae were found growing on a variety of substratum including rocks, soils, bark of trees or, epiphytic. The samples were scraped off the substratum and then transferred into the sample collecting bottles using a pair of forceps. The samples were then washed repeatedly to remove any debris that may be present and treated with antifungal and antibacterial solutions to remove any bacteria or fungal spores that may be contaminating the samples. All the samples are then established in culture in unialgal form. For maintenance and experimentation of microalgae the Bold Basal medium (Bold, 1949) was used. The flasks containing BBM for culture of green algae were then inoculated with the plant growth hormones: 2, 4-D and Kinetin in two sets. One set contained 2, 4-D and kinetin in the ratio of 1: 2 and in the other set it had 2: 1. The hormone concentration was used as 1mg/mL. The cultures were maintained in the culture room at 20±2ºC under 16:8 light-dark cycles. The periodic microscopic observation of each sample was done over a period of 40 days to observe changes in the growth and cultural behaviour of the cells.

Statistical analysis

The one way ANOVA analysis was done using XLSTAT statistical software version 2013 to analyze the variation of cell length and cell breadth under two different concentrations of hormones (2:1 and 1:2) as distinct variables and 95% confidence interval.

Results

Behaviour of green algae under the influence of hormones

Microscopic studies of the samples were done at regular intervals to observe any possible change in the growth patterns of the algae. A general increase in growth was observed for all the experimental genera. The observations of major vegetative and reproductive changes are presented in Table 1.

The cell length and breadth of the vegetative cells of all the genera increased with time in hormone treated culture. Among these *Pithophora mooreana* showed extensive growth with the enlargement of cells up to 1180.67 µ in length and 177.33 µ in breadth after 32 days of incubation. Variations in cell size in different algal samples were seen. A particular ratio of auxin (2, 4-D) and cytokinin (Kinetin) were used to proliferate the cells both in length as well as in diameter. In figs. 1-4, we observed that in respect to the control, extensive branching and cell elongation took place with time. The akinetes were induced in the experimental sets (2, 4-D: Kinetin= 2:1 and 1:2) of *Pithophora*.

The epiphytic *Oedogonium hindustanense* showed the development of the vegetative body with prominent holdfast and calyptras followed by oogonium (Figs. 1J-M). The two fold amount of 2, 4-D induced extensive branching in *Cladophora* (Figs. 1 Q-S) whereas excess kinetin induced the formation of swollen cells at the terminal end of the filaments (Figs. 2A-C). Cell elongation of epiphytic *Oedogonium mexicanum* on *Cladophora* was observed in the excess 2, 4-D (Figs. 2D-H) where as the cell diameter and length increased in excess kinetin (Figs. 2I-M). In both cases, oogonium was found within the filaments (Figs. 2D-M). A large number of akinetes (both intercalary and terminal) were found in *Pithophora roettleri* under high and low concentration of 2, 4-D and kinetin (Figs. 2N-U). The cell diameter from a young to mature filament in *Oedogonium lemmermannii* was increased with time (Figs. 2V-Y and Figs. 3A-D). Oogonium with oospore was found to be less in this study. *Pithophora cleveana* showed numerous akinetes and branching pattern with the time under 2, 4-D and kinetin exposure (Figs. 3E-L). At high kinetin level *Oedogonium rufescens f. minuta* showed variation in the formation of oogonium with large oospores (Figs. 3M-S). The vegetative cells of *Spirogyra orientalis* was increased with time in low kinetin (Figs. 3T-V), whereas high kinetin resulted the cell fragmentation and deformation of chloroplast from spiral to round shape (Figs. 4A-D). The terrestrial alga *Trentepohlia torulosa* formed globose sporangium within the cells after the influence of 2, 4-D and kinetin (Figs. 4E-P).

Table 1. Showing major vegetative and reproductive changes of different green algal taxa under the influence of 2, 4-D and kinetin

Algal taxa studied	**Major vegetative and reproductive changes**		
	Control	**1:2 (2, 4-D: kinetin)**	**2:1 (2, 4-D: kinetin)**
Pithophora mooreana	Large cells	Intercalary and terminal akinetes	Epiphytic growth of *Oedogonium hindustanense*
P. roettleri	Elongated cells with branching and akinetes	Epiphytic growth of *O. lemmermannii*	Large intercalary and terminal akinetes
P. cleveana	Cells cylindrical with branching	Orbicular and globose intercalary akinetes	Extensive branching, ovoid to globose intercalary akinetes
Cladophora glomerata	Cylindrical cells with branching	Branching with ovoid apical cells	Extensive branching, epiphytic growth of *O. mexicanum*
Spirogyra orientalis	Cells with spiral chloroplasts	Chloroplasts modified from spiral to spherical mass; fragmentation of filaments	Deformation of chloroplasts
Trentepohlia torulosa	Globose cells, sporangium	Oval to sub-globose sporangium	Branching, globose to orbicular sporangium
O. hindustanense	Young filaments with holdfast	Formation of oogonium	Long thread like filaments forming oogonium
O. mexicanum	Formation of young filaments	Cells become cylindrical forming oogonium	Extensive growth of filaments; cells with oogonium
O. lemmermannii	Newly formed filaments	Maturation of cells; formation of oogonium	Formation of oogonium
O. rufescens f. minuta	Immature filaments	Few celled filaments	Formation and maturation of globose oogonium

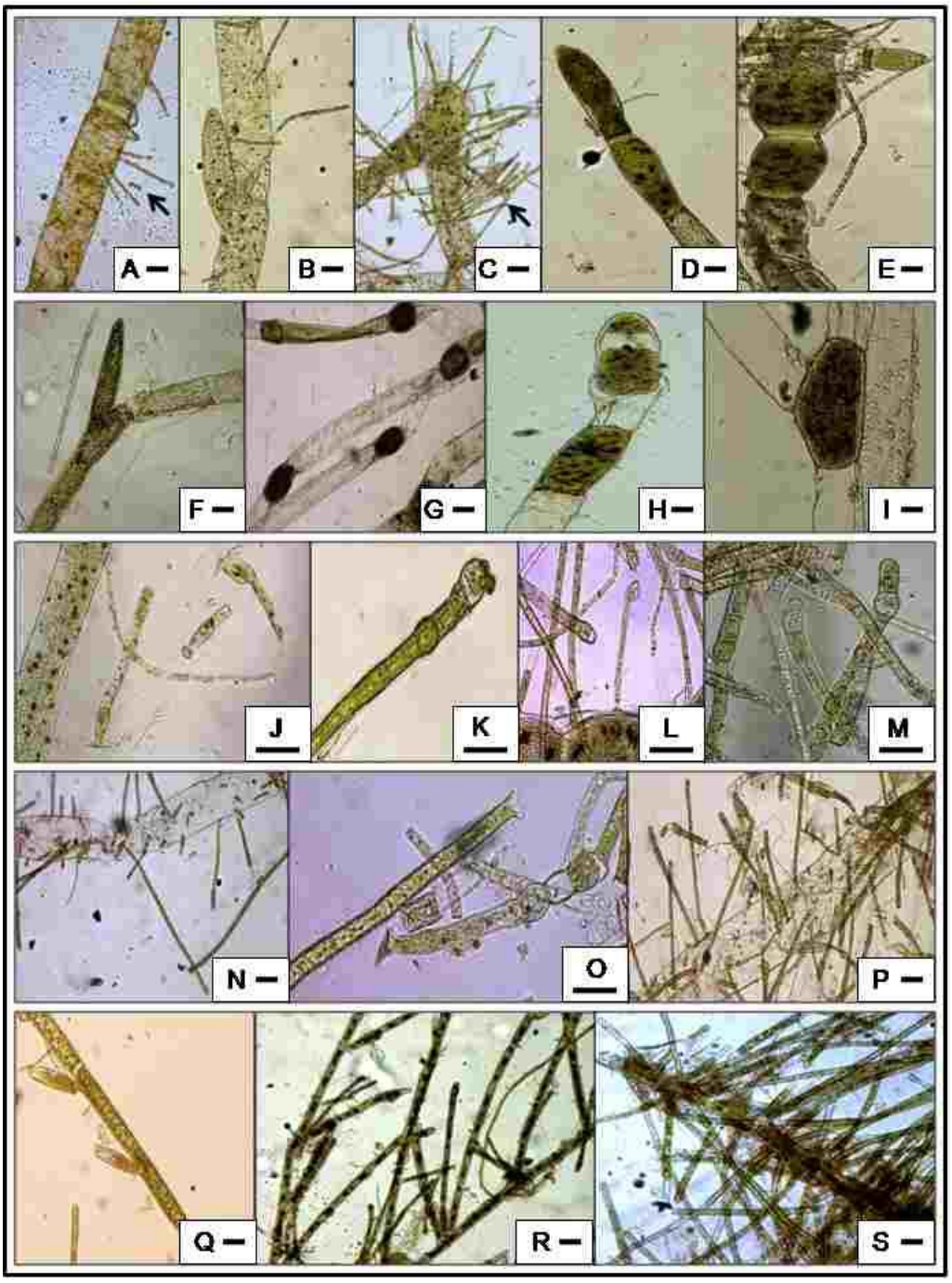

Fig. 1. Showing vegetative and reproductive changes of A-B. *Pithophora mooreana* in 2:1(2, 4-D: Kinetin) induced culture; C. Epiphytic *Oedogonium hindustanense* on *P. mooreana*; D-E. *P. mooreana* cells with large intercalary and terminal akinetes; F-I. *P. mooreana* in 1:2 (2, 4-D: Kinetin) induced culture, showing numerous akinetes; J-M. maturation of *O. hindustanense* cells and formation of oogonium in 2:1(2, 4-D: Kinetin) induced culture; N-P. Extensive growth of new filaments of *O. hindustanense* on *P. mooreana* in 1:2 (2, 4-D: Kinetin) induced culture; Q-S. Numerous branching of *Cladophora glomerata* and epiphytic growth of *Oedogonium mexicanum* in 2:1 (2, 4-D: Kinetin) induced culture after 21 days exposure. (Scale bar- 10µ).

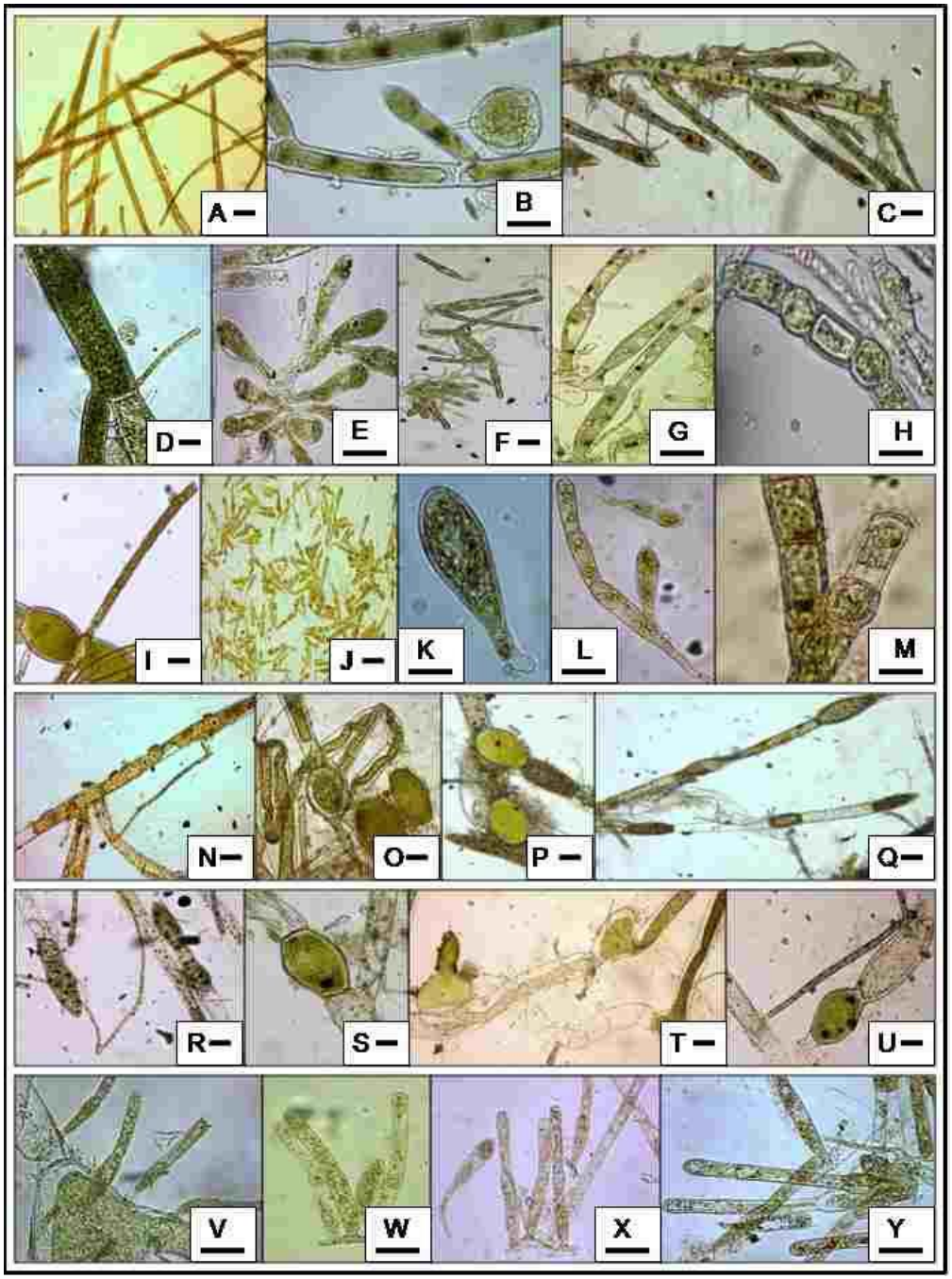

Fig. 2. Showing vegetative and reproductive changes of A-C. *Cladophora glomerata* in 1:2 (2, 4-D: Kinetin) induced culture showing numerous branches and bulbous tip cells after 21 days exposure; D-H. Maturation of *Oedogonium mexicanum* young filaments and formation of oogonium in 2:1 (2, 4-D: Kinetin) induced culture; I-M. Large club shaped filaments of *O. mexicanum* in1: 2 (2, 4-D: Kinetin) induced culture; N-Q. Large globose and orbicular akinetes of *Pithophora roettleri* in 2:1 (2, 4-D: Kinetin) induced culture; R-U. Cylindrical and orbicular akinetes of *P. roettleri* in 1:2 (2, 4-D: Kinetin) induced culture; V-Y. Matured intact filaments of *Oedogonium lemmermannii* in 2:1 (2, 4-D: Kinetin) induced culture. (Scale bar- 10µ).

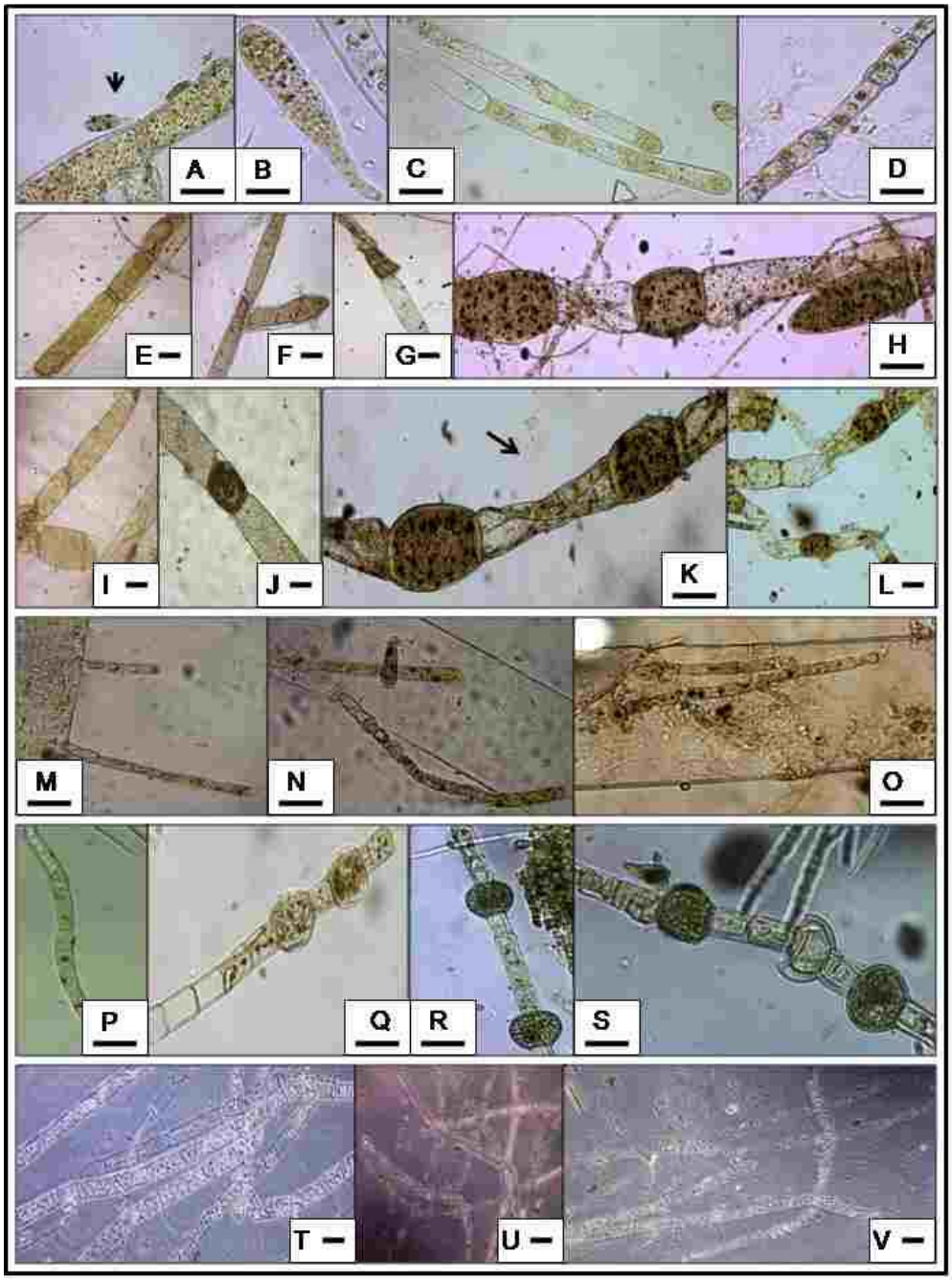

Fig. 3. Showing vegetative and reproductive changes of A-D. Germination and formation of oogonium on epiphytic *Oedogonium lemmermannii* in 1:2 (2, 4-D: Kinetin) induced culture; E-H. Initiation of branches and large akinetes in cells of *Pithophora cleveana* in 2:1 (2, 4-D: Kinetin) induced culture; I-L. Numerous orbicular akinetes of *P. cleveana* in 1:2 (2, 4-D: Kinetin) induced culture; M-O. Maturation of epiphytic *Oedogonium rufescens f. minuta* on *Pithophora mooreana* in 2:1 (2, 4-D: Kinetin) induced culture; P-S. Large oogonium with oopsores of *Oedogonium rufescens f. minuta* in 1:2 (2, 4-D: Kinetin) induced culture; T-V. *Spirogyra orientalis* in 2:1 (2, 4-D: Kinetin) induced culture showing disruption of chloroplasts and cell wall. (Scale bar- 10µ).

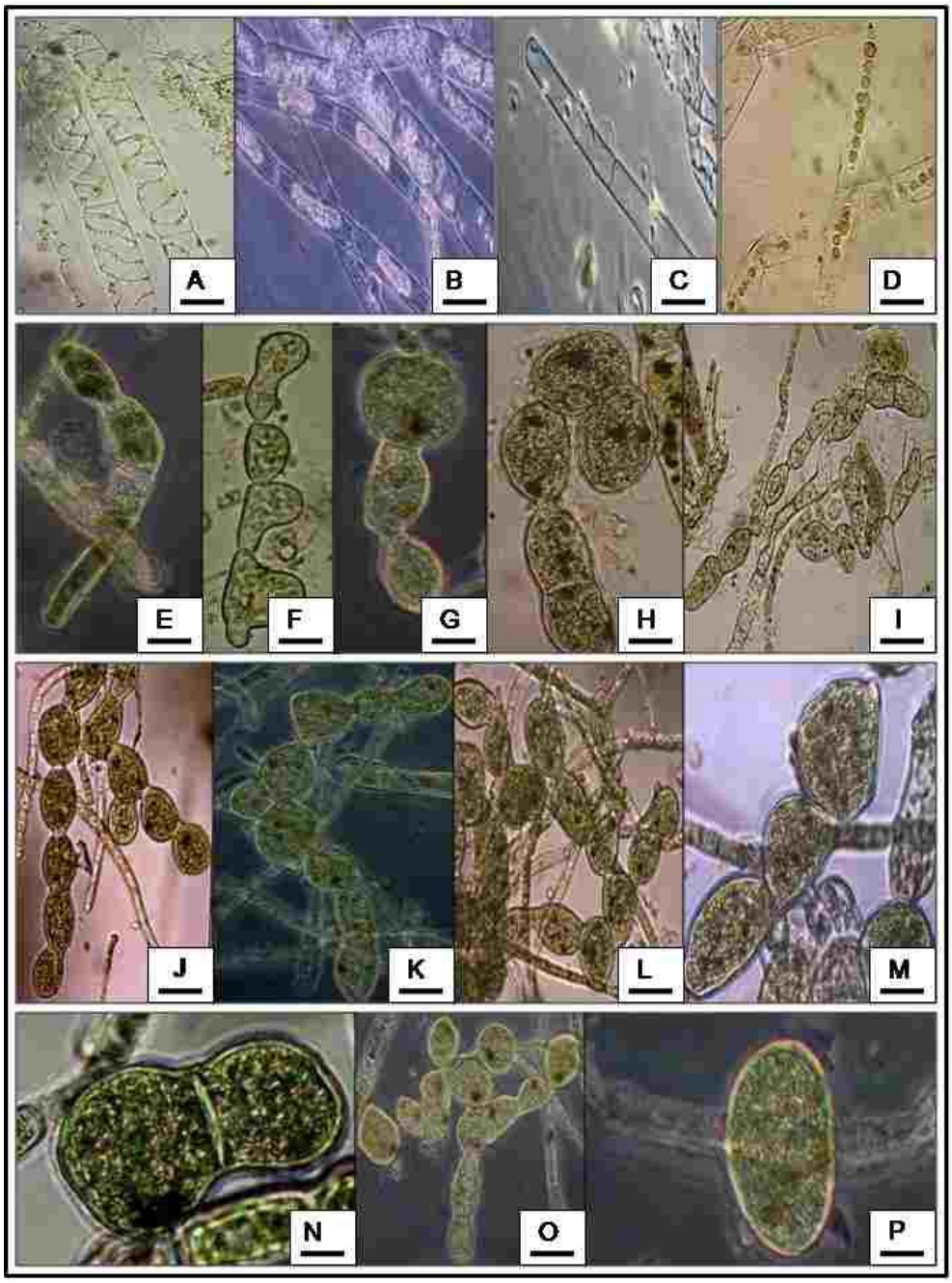

Fig. 4. Showing vegetative and reproductive changes of A-D. *Spirogyra orientalis* in 1:2 (2, 4-D: Kinetin) induced culture showing gradual defragmentation of chloroplasts and cells; E-I. *Trentepohlia torulosa* in 2:1 (2, 4-D: Kinetin) induced culture showing branching and development of sporangium; J-M & O. Germination and maturation of *T. torulosa* in 1:2 (2, 4-D: Kinetin) induced culture; N & P. Large bilobed and oval sporangia of *T. torulosa* in 1:2 (2, 4-D: Kinetin) induced culture. (Scale bar- 10µ).

Data analysis

A significant relationship ($p<0.05$) between cell length and cell breadth of *P. roettleri* and *O. lemmermannii* was found at different concentration of 2, 4-D and kinetin under different days interval (Table 2). This suggested that the induction of hormones on cultural behaviour of the green algae have positive role in increasing cell proliferation and development. Results obtained from Tukey's analysis showed (Table 3 and 4) significant relationship ($p<0.05$) of cell length and breadth of these two

algae under two distinct hormone concentrations at 95% confidence interval. Cell diameter of *P. mooreana* was significant ($p<0.0001$) under two variables (2:1 and 1:2). Other algal genera showed no significant relationship ($p>0.05$) of cell growth under two variables of hormone concentrations.

Table 2. ANOVA of cell length and cell breadth under two distinct variables 2:1 and 1:2 (2, 4-D: kinetin) at 95% confidence interval (* p<0.05).

Algal taxa	Cell length (µm)			Cell breadth (µm)		
	R^2	F	Pr>F	R^2	F	Pr>F
Pithophora mooreana	0.06	0.891	0.361	0.893	116.596	<0.0001*
P. roettleri	0.521	15.202	0.002*	0.579	19.231	0.001*
P. cleveana	0.231	3.01	0.113	0.144	1.68	0.224
Cladophora glomerata	0.026	0.376	0.549	0.002*	0.025	0.876
Spirogyra orientalis	0.091	0.802	0.397	0.37	4.696	0.062
Trentepohlia torulosa	0.06	0.508	0.496	0.055	0.469	0.513
Oedogonium hindustanense	0.066	0.982	0.339	0.059	0.878	0.365
O. mexicanum	0.186	3.203	0.095	0.087	1.328	0.268
O. lemmermannii	0.397	9.218	0.009	0.087	1.331	0.268
O. rufescens f. minuta	0.063	0.668	0.433	0.003	0.027	0.873

Table 3. Tukey's analysis of the difference of cell length at 2:1 and 1:2 (2, 4-D: kinetin) hormone ratio and 95% confidence interval.

Algal taxa	**Tukey's analysis of the difference of cell length at 2:1 and 1:2 (2, 4-D : kinetin) hormone ratio**				
	Difference	**Standardized difference**	**Critical value**	**Pr>diff.**	**Significance**
Pithophora mooreana	28.418	0.944	2.415	0.361	No
P. roettleri	424.666	3.899	2.145	0.002	Yes
P. cleveana	169.22	1.735	2.228	0.113	No
Cladophora glomerata	11.666	0.613	2.145	0.549	No
Spirogyra orientalis	19.8	0.896	2.306	0.397	No
Trentepohlia torulosa	6.6	0.712	2.306	0.496	No
Oedogonium hindustanense	3.713	0.991	2.145	0.339	No
O. mexicanum	23.331	1.79	2.145	0.095	No
O. lemmermannii	50.463	3.036	2.145	0.009	Yes
O. rufescens f. minuta	2.2	0.817	2.228	0.433	No

Table 4. Tukey's analysis of the difference of cell breadth at 2:1 and 1:2 (2, 4-D: kinetin) hormone ratio and 95% confidence interval.

Algal taxa	Tukey's analysis of the difference of cell breadth at 2:1 and 1:2 (2, 4-D : kinetin) hormone ratio				
	Difference	Standardized difference	Critical value	Pr>diff.	Significance
Pithophora mooreana	155.708	10.798	2.145	<0.0001	Yes
P. roettleri	41.418	4.385	2.145	0.001	Yes
P. cleveana	25.668	1.296	2.228	0.224	No
Cladophora glomerata	1.166	0.16	2.145	9.876	No
Spirogyra orientalis	3.96	2.167	2.306	0.062	No
Trentepohlia torulosa	7.04	0.685	2.306	0.513	No
Oedogonium hindustanense	1.1	0.937	2.145	0.365	No
O. mexicanum	5.25	1.152	2.145	0.268	No
O. lemmermannii	2.613	1.154	2.145	0.268	No
O. rufescens f. minuta	0.183	0.164	2.228	0.873	No

Discussion

Our study involved exposure of few green algae to two different combinations of 2, 4-D and Kinetin. Usually these two forms are applied in combination with each other at a definite ratio to induce required changes in plants. This was done in this experiment as well. We took two different combinations of 2, 4-D and Kinetin- 2:1 and 1:2 ratios respectively to determine the respective roles of each of these hormones. It is known that 2, 4-D is a synthetic auxin and promotes cell enlargement in plants (Schenck *et al*., 2010). A significant increase of growth of two green microalgae *Haematococcus pluvialis* and *Dunaliella salina* was studied under different concentration of 2, 4-D and kinetin (de Jesus Raposo and de Morais, 2013). Joseph and Chennubhotla (1999) studied role of 2, 4-D and gibberellic acid (GA) on growth of a green alga *Ulva fasciata* and two red algae *Gracilaria corticata* and *Hypnea valentiae* for a period of 30 days. When applied in combination with kinetin in the ratio of 2:1, 2, 4-D showed positive results for growth and cell enlargement.

A high 2, 4-D level induced cell enlargement resulting in larger cells, like, akinetes, oogonia, sporangia and elongated filaments respectively. It also induced branching in *Pithophora* and *Cladophora*. A high 2, 4-D: Kinetin ratio was also induced the dominance of the epiphytic algae on their respective host. This was a peculiar observation as most of the epiphytic genera grew extensively on the host at a high 2, 4-D level. However this observation could not be explained. When applied at a low concentration with 1:2 (2, 4-D: Kinetin) ratio, it induced quite different morphological changes.

A high kinetin level induced rapid cell division resulting in numerous short cells, thus promoting growth in a different way. A 1:2 ratio of 2, 4-D: kinetin also promoted earlier and increased growth and development of reproductive structures like akinetes in case of *Pithophora*, oogonia in *Oedogonium*, fragmentation in *Spirogyra* and sporangia in *Trentepohlia torulosa*. Unlike the previous scenario, a low 2, 4-D: Kinetin ratio did not show any abnormal growth of the epiphytic forms on the host. Instead certain other peculiar observations were made. In *Spirogyra orientalis*, it induced modification of the spiral chloroplast. The chloroplast morphology gradually decondensed from spiral to long beaded chloroplasts and ultimately developing into discoid spherical mass resembling the shape of spores. Thus these hormones can be used in mass cultivation of different algae for rapid increase in biomass and for the induction of reproductive structures. However they should be applied in the right combination and in low amounts as high levels of growth hormones can prove to be toxic.

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