



EFFECT OF DIFFERENT GROWTH HORMONES ON REGENERATION OF *NOTOTHYLAS KHASIANA* UDAR ET SINGH

B. L. Chaudhary and Vandana Vijaivargiya*

Bryology Laboratory, Deptt. of Botany, College of Science, Mohan Lal Sukhadia University, Udaipur (Rajasthan), India.

Abstract

Present paper deals with the effect of various growth hormones (BAP, IAA and GA) on regeneration capacity of hornwort *Notothylas khasiana*. Regeneration was maximum in BAP followed by IAA and GA. Further regeneration percentage was maximum in apical explants followed by basal and middle explants. Time period positively affected the process of regeneration. Higher concentration of growth hormones shared inhibitory effect due to unfavourable chemical changes in the treated explants of thallus.

Key words : BAP, IAA, GA, *Notothylas khasiana*, regeneration.

Introduction

Bryophytes have been powerful experimental tools for the elucidation of complex biological processes. Because of their short life cycle, simplicity of organization, small size, relatively small genome, plasticity in their differential capacity, sensitivity to small variations in the environment, their definite responses to plant hormones and a conspicuous well defined haploid phase in life cycle, they hold many attractions as model organisms for research in plant sciences.

In the present investigation, experiments were set to study the effects of different growth hormones (BAP, GA and IAA) on regeneration of different explants of *Notothylas khasiana*.

Chouhan (2002) observed that in *Hydrogonium consangium* the percentage of regeneration was higher in various concentrations of Kinetin followed by GA₃ and IAA in leaf, stem and rhizoid explants in comparison to their respective controls.

Chaturvedi and Vashistha (2002) observed the effect of some auxins (2, 4 di chloro phenoxyacetic acid, indole 3 acetic acid, β naphtoxy acetic acid) and three cytokinins (6-benzyl amino purine, 6-y Methallyl amino purine, 6-Furfuryl amino purine) on protonemal growth and differentiation in the moss *Bryum capillarae* L. ex. Hedw.

According to Sztein *et al.* (1999) the plant hormone auxin (Indole-3-acetic acid, IAA) is involved in the control of many phenomena during plant development.

Materials and Methods

Living material of *Notothylas khasiana* was collected from Pavagadh, Gujarat. Thallus of plants were cut into apical, middle and basal parts. These parts were surface sterilized with 2% of calcium hypochlorite for 1-3 minutes and then washed thoroughly with distilled water several times.

Different concentrations of growth hormones (BAP, IAA and GA) like 1, 5, 10, 50 ppm were prepared by serial dilution with Half Knop's liquid culture medium. Pure Half Knop's medium was treated as control. 5ml of each ppm solution was poured in each petri dish upon Whatmans filter paper No. 1 under asptic conditions. All these operations were done in a glass chamber thoroughly sterilized by spraying ethyl alcohol in laminar air flow bench. All experimental petri dishes were laid in a growth chamber with florescent tube light with an intensity of 3500 to 4000 lux and temperature maintained at 25±2°C. Observations were recorded at 10th, 20th and 30th day. Each experiment was comprised a minimum of three replicates and repeated thrice.

*Author for correspondence: E-mail: vandnavijay80@yahoo.co.in

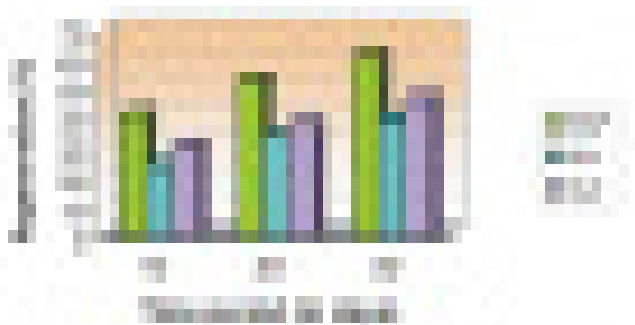


Fig. 1 : Effect of growth hormones on regeneration from apical explants of thallus of *Notothylas khasiana* Udar et Singh in Half knop's liquid medium.

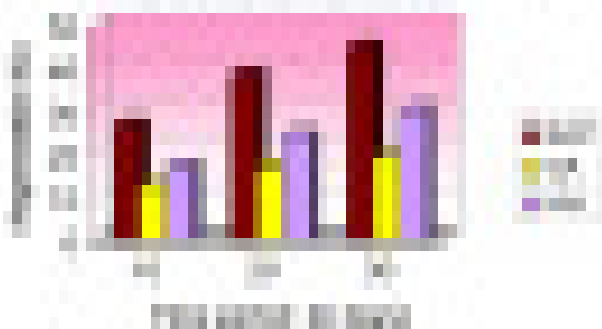


Fig. 2 : Effect of growth hormones on regeneration from middle explants of thallus of *Notothylas khasiana* Udar et Singh in Half knop's liquid medium.

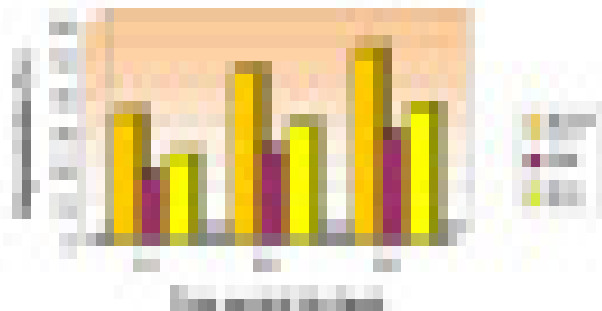
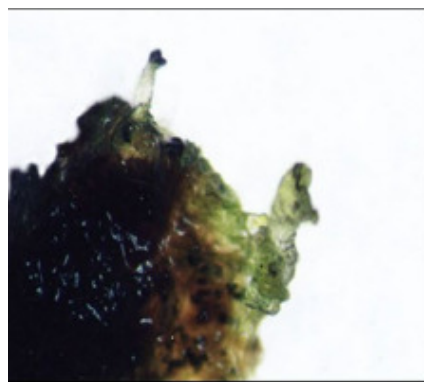


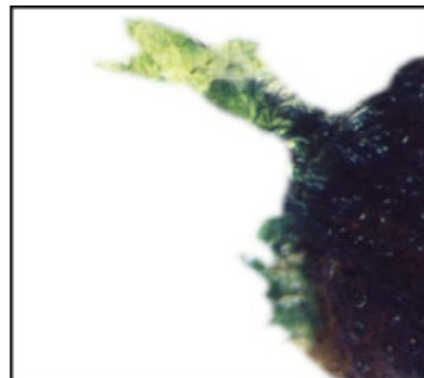
Fig. 3 : Effect of growth hormones on regeneration from basal explants of thallus of *Notothylas khasiana* Udar et Singh in Half knop's liquid medium.

Results and Discussion

Experiments on regeneration from various explants of *Notothylas khasiana* regarding influence of growth regulants show that BAP was most effective followed by IAA and GA, respectively. On 10th day 33.33% of regeneration was observed in control for apical explants. The maximum percentage of regeneration was observed in BAP (80.00) at 10 ppm concentration followed by IAA (60.00) and GA (46.66) at 5 ppm concentrations (table

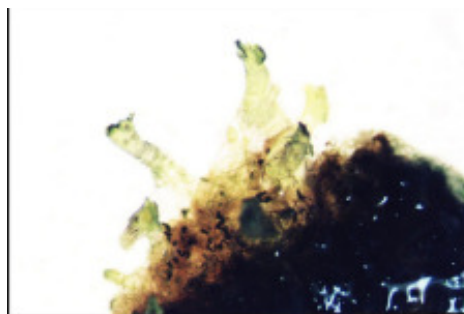


5 ppm IAA (Apical part)

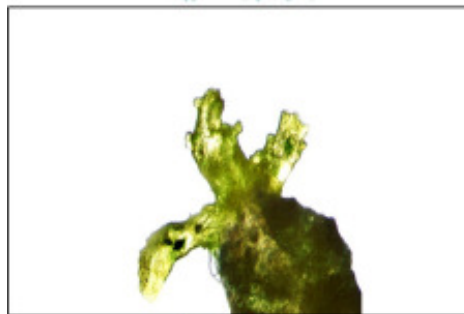


10 ppm GA (Middle part)

Plate 1 : Effect of growth hormones on regeneration from different explants of *Notothylas khasiana* in Half knop's liquid medium.



10 ppm BAP (Apical part)



1 ppm GA (Basal part)

Plate 2 : Effect of growth hormones on regeneration from different explants of *Notothylas khasiana* Udar et Singh in Half knop's liquid medium.

Table 1 : Showing effect of growth hormones on regeneration from apical explants of thallus of *Notothylas khasiana* Udar et Singh in Half Knop's liquid medium.

Concentration (ppm)	10 th day						20 th day						30 th day					
	BAP	GA	IAA	Mean	BAP	GA	IAA	Mean	BAP	GA	IAA	Mean	BAP	GA	IAA	Mean		
	Control	33.333	33.333	33.333	33.333	46.667	46.667	46.667	46.667	53.333	53.333	53.333	53.333	53.333	53.333	53.333	53.333	
1 ppm	60.000	40.000	53.333	51.111	66.667	66.667	60.000	60.000	66.667	66.667	60.000	60.000	86.667	66.667	73.333	75.556		
5 ppm	66.667	46.667	60.000	57.778	73.333	73.333	60.000	66.667	73.333	60.000	66.667	66.667	86.667	73.333	80.000	80.000		
10 ppm	80.000	40.000	46.667	55.556	93.333	93.333	53.333	66.667	53.333	53.333	53.333	66.667	93.333	60.000	66.667	73.333		
20 ppm	46.667	13.333	26.667	28.889	66.667	66.667	40.000	44.444	80.000	33.333	40.000	44.444	80.000	33.333	46.667	53.333		
30 ppm	26.667	13.333	20.000	20.000	40.000	40.000	20.000	28.889	40.000	20.000	26.667	28.889	40.000	20.000	33.333	31.111		
40 ppm	13.333	6.667	6.667	8.889	26.667	26.667	13.333	17.778	33.333	13.333	13.333	17.778	33.333	13.333	20.000	22.222		
50 ppm	6.667	0.000	0.000	2.222	13.333	13.333	0.000	4.444	20.000	0.000	0.000	4.444	20.000	0.000	0.000	6.667		
Mean	41.667	24.167	30.833	32.222	53.333	53.333	34.167	41.944	61.667	40.000	38.333	41.944	61.667	40.000	46.667	49.444		

CRD ANOVA for 10th day

S.no.	Source	DF	MS	F	SE(m)	CD(5%)	CD(1%)
1	A	2	1872.22	9.108**	2.93	8.322	11.11
2	B	7	4063.49	19.76**	4.78	13.59	18.14
3	AXB	14	170.635	0.830	8.28	23.54	31.41

CRD ANOVA for 20th day

S.no.	Source	DF	MS	F	SE(m)	CD(5%)	CD(1%)
1	A	2	2438.89	15.138**	2.59	7.367	9.832
2	B	7	4805.56	29.828**	4.23	12.03	16.06
3	AXB	14	191.27	1.187	7.33	20.84	27.81

CRD ANOVA 30th day

S.no.	Source	DF	MS	F	SE(m)	CD(5%)	CD(1%)
1	A	2	2955.56	16.121**	2.76	7.859	10.49
2	B	7	6587.3	35.931**	4.51	12.83	17.13
3	AXB	14	168.254	0.918	7.82	22.23	29.66

A = Growth hormones,
BAP = Benzyl amino purine

B = Concentrations,
GA = Gibberellic acid

** = Significant at 1% level of significance
IAA = 3 - Indoleacetic acid

Table 2 : Showing effect of growth hormones on regeneration from middle explants of thallus of *Notothydas khasiana* Udar et Singh in Half Knop's liquid medium.

Concentration (ppm)	10 th day				20 th day				30 th day			
	BAP	GA	IAA	Mean	BAP	GA	IAA	Mean	BAP	GA	IAA	Mean
	Control	20.000	20.000	20.000	20.000	26.667	26.667	26.667	26.667	26.667	26.667	26.667
1 ppm	40.000	26.667	33.333	33.333	53.333	33.333	46.667	44.444	66.667	40.000	60.000	55.556
5 ppm	46.667	33.333	40.000	40.000	66.667	46.667	60.000	57.778	80.000	53.333	73.333	68.889
10 ppm	60.000	13.333	33.333	35.556	80.000	20.000	40.000	46.667	86.667	20.000	46.667	51.111
20 ppm	26.667	6.667	6.667	13.333	40.000	6.667	20.000	22.222	53.333	13.333	20.000	28.889
30 ppm	13.333	0.000	0.000	4.444	20.000	0.000	0.000	6.667	20.000	0.000	13.333	11.111
40 ppm	6.667	0.000	0.000	2.222	13.333	0.000	0.000	4.444	20.000	0.000	0.000	6.667
50 ppm	0.000	0.000	0.000	0.000	6.667	0.000	0.000	2.222	6.667	0.000	0.000	2.222
Mean	26.667	12.500	16.667	18.611	38.333	16.667	24.167	26.389	45.000	19.167	30.000	31.389

CRD ANOVA for 10th day

S.no.	Source	DF	MS	F	SE(m)	CD(5%)	CD(1%)
1	A	2	1272.22	7.897*	2.59	7.367	9.832
2	B	7	2323.02	14.419**	4.23	12.03	16.06
3	AXB	14	180.159	1.118	7.33	20.84	27.81

CRD ANOVA for 20th day

S.no.	Source	DF	MS	F	SE(m)	CD(5%)	CD(1%)
1	A	2	2905.56	14.943**	2.85	8.094	10.8
2	B	7	4107.14	21.122**	4.65	13.22	17.64
3	AXB	14	283.333	1.457	8.05	22.89	30.55

CRD ANOVA for 30th day

S.no.	Source	DF	MS	F	SE(m)	CD(5%)	CD(1%)
1	A	2	4038.89	24.233**	2.64	7.493	10
2	B	7	5503.97	33.024**	4.3	12.24	16.33
3	AXB	14	375.397	2.252	7.45	21.19	28.28

A = Growth hormones, B = Concentrations
 BAP = Benzyl amino purine, GA = Gibberellic acid
 ** = Significant at 5% and 1% level of significance respectively
 IAA = 3 - Indoleacetic acid.

Table 3 : Showing effect of growth hormones on regeneration from basal explants of thallus of *Notothylas khasiana* Udar et Singh in Half Knop's liquid medium.

Concentration (ppm)	10 th day						20 th day						30 th day					
	BAP	GA	IAA	Mean	BAP	GA	IAA	Mean	BAP	GA	IAA	Mean	BAP	GA	IAA	Mean		
	Control	20.000	20.000	20.000	20.000	40.000	40.000	40.000	40.000	40.000	40.000	40.000	40.000	40.000	40.000	40.000	40.000	
1 ppm	53.333	33.333	46.667	44.444	66.667	46.667	53.333	55.556	73.333	53.333	66.667	55.556	73.333	53.333	66.667	64.444		
5 ppm	66.667	46.667	53.333	55.556	66.667	53.333	60.000	60.000	80.000	60.000	80.000	60.000	73.333	60.000	73.333	71.111		
10 ppm	73.333	13.333	40.000	42.222	86.667	26.667	53.333	55.556	93.333	33.333	53.333	55.556	60.000	20.000	33.333	60.000		
20 ppm	33.333	13.333	13.333	20.000	53.333	13.333	20.000	28.889	60.000	20.000	20.000	28.889	60.000	20.000	33.333	37.778		
30 ppm	13.333	6.667	6.667	8.889	26.667	6.667	20.000	17.778	33.333	13.333	20.000	17.778	33.333	13.333	20.000	22.222		
40 ppm	13.333	0.000	0.000	4.444	20.000	0.000	0.000	6.667	20.000	0.000	0.000	6.667	20.000	0.000	0.000	6.667		
50 ppm	0.000	0.000	0.000	0.000	6.667	0.000	0.000	2.222	13.333	0.000	0.000	2.222	13.333	0.000	0.000	4.444		
Mean	34.167	16.667	22.500	24.444	45.833	23.333	30.833	33.333	51.667	27.500	35.833	38.333	51.667	27.500	35.833	38.333		

CRD ANOVA for 10th day

S.no.	Source	DF	MS	F	SE(m)	CD(5%)	CD(1%)
1	A	2	1905.56	16.333**	2.2	6.269	8.367
2	B	7	3809.52	32.653**	3.6	10.24	13.66
3	AXB	14	292.857	2.510	6.24	17.73	23.66

CRD ANOVA for 20th day

S.no.	Source	DF	MS	F	SE(m)	CD(5%)	CD(1%)
1	A	2	3150	13.186**	3.15	8.971	11.97
2	B	7	4736.51	19.827**	5.15	14.65	19.55
3	AXB	14	305.556	1.279	8.92	25.37	33.86

CRD ANOVA for 30th day

S.no.	Source	DF	MS	F	SE(m)	CD(5%)	CD(1%)
1	A	2	3616.67	17.132**	2.97	8.433	11.25
2	B	7	5965.08	28.256**	4.84	13.77	18.38
3	AXB	14	276.984	1.312	8.39	23.85	31.83

A = Growth hormones,
BAP = Benzyl amino purine

B = Concentrations
GA = Gibberellic acid

** = Significant at 1% level of significance
IAA = 3 - Indoleacetic acid.

1). For middle explants control resulted in 20.00% of ultimate regeneration. The highest percentage of regeneration was observed in BAP (60.00) at 10 ppm concentration followed by IAA (40.00) and GA (33.33) at 5 ppm concentrations (table 2). For basal explants 20.00% of regeneration was recorded in control. The maximum percentage of regeneration was observed in BAP (73.33) at 10 ppm concentrations followed by IAA (53.33) and GA (46.66) at 5 ppm concentrations (table 3). With the passage of time percentage of regeneration also increased *i.e.* on 20th and 30th day regeneration percentage was comparatively higher.

Cytokinins are chemicals which promote cytokinesis in cells of various plant origins (Skoog, Strong and Miller, 1965; vide Fox 1969). The function of gibberellins is the stimulation of cell enlargement in plant organs while the most characteristic action of auxin is to promote cell enlargement, which may be accompanied by cell division.

Results of the present work are in conformity with regeneration studies of Khuntaila (1991) on *Semibarbula orientalis*, Agarwal (2003) on *Riccia billardieri* and slightly in contrast with regeneration studies of Khuntaila (1991) on *Physcomitrium japonicum* and Chouhan (2002) on *Hydrogonium consanguineum* who found that effectiveness of growth regulants was in decreasing order of Kinetin, GA and IAA.

Among apical, middle and basal explants of thalli apical explant showed the highest percentage of regeneration followed by basal and middle explants, which proves the strict polar localization of regeneration principle. Vochting (1885) stated that transverse pieces taken from young thalli, regeneration was always polar.

In the present investigation, it was observed that growth regulants in lower concentrations promote growth and development of regenerant thalli, but higher concentrations were inhibitory.

These results are in conformity with Kaul *et al.* (1962) in *Marchantia nepalensis*, Vyas (1984) in *Plagiochasma appendiculatum*, Shukla and Kaul (1977,

1978) in *Marchantia nepalensis*, who also observed that the stimulation of growth responses occurs at lower concentrations of growth regulants while higher concentrations appear to have an inhibitory effect.

A positive correlation of regeneration percentage and time period, noted in present investigation, may be the result of increase in activity of growth regulants with increasing time period.

Acknowledgement

We are thankful to Head, Department of Botany, College of Science, M.L.S. University, Udaipur (Rajasthan), India for providing laboratory facilities.

References

- Chouhan, K. (2002). Morphogenetic and ecophysiological studies on *Hydrogonium consanguineum*. *Ph.D. Thesis*, Mohan Lal Sukhadia University, Udaipur.
- Chaturvedi, P. and B. D. Vashistha (2002). Effect of auxins and cytokinins on protonemal differentiation in *Bryum capillare* L. ex. Hedw. Abstract in World conference of Bryology, **62** held at Lucknow.
- Kaul, K. N., G. C. Mitra and B. K. Tripathi (1962). Responses of *Marchantia* in aseptic culture to well known auxins and anti auxins. *Ann. bot. (N.S.)*, **26** : 447-466.
- Noguchi, A. and M. Furuta (1958). Regeneration in *Brothera leana*. *Bryologist*, **61** : 361-366.
- Shukla, R. M. and A. Kaul (1977). Effect of gibberellic acid on regeneration of *Marchantia nepalensis* Lehm. *et al.* Lindenbg. *Rev. Bryol. Lichenol.*, **43** : 347-352.
- Sztein, A. E., J. D. Cohen, Ines Garcia de la Fuente and T. J. Cooke (1999). *Auxin metabolism in mosses and liverworts*.
- Vochting, H. (1885). Uber die Regeneration der Marchantieen. *Jb. Wiss. Bot.*, **16** : 367-414.
- Vyas, T. (1984). Ecophysiological and morphogenetic studies on *Plagiochasma appendiculatum*. *Ph. D. thesis*, Mohan Lal Sukhadia University, Udaipur.