

COMPARATIVE EFFECT OF DIFFERENT LIQUID CULTURE MEDIA ON REGENERATION FROM DIFFERENT EXPLANTS OF *NOTOTHYLAS KHASIANA* UDAR ET SINGH

B.I. Chaudhary¹ and Vandana Vijaivargiya^{2*}

^{1,2}Bryology laboratory, Department of Botany, College of Science, Mohan Lal Sukhadia University, Udaipur

E-mail: vandanavijay80@yahoo.co.in

ABSTRACT

Effect of different culture media (half knop's full knop's and beneck's) was studied on the percentage of regeneration of different explants of *Notothylas khasiana* Udar et Singh. It was found that regeneration percentage was maximum in HKM liquid medium followed by FKM and Beneck's liquid media respectively in all the three explants i.e. apical, middle and basal. A positive influence of time period was noticed on percentage of regeneration.

Key words: Half knop's medium, Full knop's medium, Beneck's medium, *Notothylas khasiana*, Regeneration.

INTRODUCTION

Regeneration illustrates the process by which living organism replaces its lost or artificially isolated part by new growth and differentiation and produces in this way a complete or a repaired individual (Goebel, 1908). Studies on regeneration play an important role in understanding the problems of morphogenesis. The use of bryophytes for such investigations was due to the fact that these plants readily regenerate under ordinary conditions and secondly because regenerants can be obtained from haploid as well as diploid generations. Due to these two features, experiments could be designed to understand the various processes involved in normal plant growth and development.

In a study of effect of various culture media on regeneration of *Asterella angusta* Singhvi (1979) observed that half knop's is the best followed by

full knop's and Beneck's. Mertia (1992) studied the effect of various culture media on regeneration from various explants of *Funaria nutans*. He noted that Half Knop's liquid culture medium gave the maximum percentage of regeneration in comparison to Full Knop's and Beneck's media.

Chaudhary and Bapna (1995), made regeneration studies on *Asterella angusta* Steph. Regeneration percentage was highest in apical followed by basal and middle explants. Regeneration was higher in liquid culture media, namely Half Knop's, Full Knop's and Beneck's medium in comparison to solid ones. In Half Knop's (liquid as well as solid) medium maximum regeneration percentage was observed.

While studying the effect of various culture media on regeneration, Chouhan (2002) concluded that Half Knop's liquid culture medium was the most suitable culture medium

for regeneration of various explants of *Hydrogonium consaguineum*.

MATERIALS AND METHODS

Living materials of *Notothylas khasiana* was collected from Pawagadh, 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 the washed thoroughly with distilled water several times. All the three standard liquid culture media Half knop's, Full knop's and Beneck's were prepared.

The composition of these culture media is as under:

1. Half Knop's liquid medium:

NaNO ₃	0.375 gm
CaCl ₂ .6H ₂ O	0.125 gm
KH ₂ PO ₄	0.125 gm
MgSO ₄	0.125 gm
KCL	0.06 gm
FeCl ₃ .6H ₂ O	4.00 mg
Modified Nitsche's element	Trace 1ml/lit
Double Distilled water	1000 ml

2. Modified Nitsche's Trace element:

H ₂ SO ₄ Sp. Gr.-1.83	0.5 ml
MnSO ₄ .4H ₂ O	3000.00 mg
ZnSO ₄ .7H ₂ O	500.00 mg
H ₃ Bo ₃	500.00 mg
CuSO ₄ .5H ₂ O	25.00 mg
Na ₂ MoO ₄ .5H ₂ O	25.00 mg
CoCl ₂	25.00 mg
NiCl ₂	25.00 mg
Double distilled water	1000 ml

3. Full Knop's liquid medium:

Ca(NO ₃) ₂ .4H ₂ O	1.00 gm
MgSO ₄ .7H ₂ O	0.25 gm
KH ₂ PO ₄	0.25 gm
KCl	0.12 gm
FeCl ₃ .6 H ₂ O	0.004 gm
Modified Nitsche's element	Trace 1 ml/lit
Double distilled water	1000 ml

4. Beneck's liquid medium:

(NH ₄) ₂ CO ₃	0.2 gm
MgSO ₄ .7 H ₂ O	0.1 gm
KH ₂ PO ₄	0.1 gm
CaCl ₂	0.1 gm
FeCl ₃	4.0 mg
Modified Nitsche's element	Trace 1 ml/l
Double Distilled Water	1000ml

These culture media were autoclaved at 15 lb pressure.

Five explants of apical, middle and basal parts were laid in each petri dish upon what man's filter paper number 1 under aseptic conditions. 5ml of each culture media was poured in each petri dish. All these operations were done in a glass chamber thoroughly sterilized by spraying ethyl alcohol or in a 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 was maintained at 22 ±2°C. Observations were recorded at 10, 20th and 30th day. Each experiment was comprised a minimum of three replicates and repeated thrice.

RESULTS AND DISCUSSION

10th day: For apical explants maximum percentage of regeneration was observed in HKM (46.67) followed by FKM (33.33) and Beneck's (20.00) liquid media respectively. For middle explants the percentage of regeneration was highest in HKM (26.67) followed by FKM (20.00) and Beneck's (13.33). For basal explants HKM resulted in the highest percentage of regeneration (33.33) followed by FKM (26.67) and Beneck's (13.33) liquid media respectively.

Time period positively affected the process of regeneration i.e. as the time passed, regeneration percentage also increased (Table 2 and 3).

Further on 20th and 30th day also regeneration percentage was maximum in HKM followed by FKM and Beneck's respectively for all the three explants as shown in Table 1, 2 and 3.

Table 1: Showing effect of Half Knop's (HKM), Full Knop's (FKM) and Beneck's liquid culture media on regeneration from apical explants of thallus of *Notothylas khasiana* Udar et Singh.

Culture medium	10 th day	20 th day	30 th day
HKM	46.67	60.00	60.00
FKM	33.33	46.67	46.67
Beneck's	20.00	26.67	33.33
<i>Mean</i>	33.333	44.444	46.667

CRD ANOVA for 10th day

SN.	SOURCE	DF	MS	F	SE
1	Treatment	2	533.33333	1.500	10.9

CRD ANOVA for 20th day

SN.	SOURCE	DF	MS	F	SE
1	Treatment	2	844.44444	9.500*	5.44

CRD ANOVA for 30th day

SN.	SOURCE	DF	MS	F	SE
1	Treatment	2	533.33333	6.000*	5.44

* = Significant at 5% level of significance

Table 2: Showing effect of Half Knop's (HKM), Full Knop's (FKM) and Beneck's liquid culture media on regeneration from middle explants of thallus of *Notothylas khasiana* Udar et Singh.

Culture medium	10 th day	20 th day	30 th day
HKM	26.67	40.00	40.00
FKM	20.00	26.67	33.33
Benecks	13.33	13.33	20.00
<i>Mean</i>	20.000	26.667	31.111

CRD ANOVA for 10th day

SN.	SOURCE	DF	MS	F	SE
1	Treatment	2	133.33333	1.500	5.44

CRD ANOVA for 20th day

SN.	SOURCE	DF	MS	F	SE
1	Treatment	2	533.33333	6.000*	5.44

CRD ANOVA for 30th day

SN.	SOURCE	DF	MS	F	SE
1	Treatment	2	311.11111	7.000*	3.85

* = Significant at 5% level of significance

Table 3: Showing effect of Half Knop's (HKM), Full Knop's (FKM) and Beneck's liquid culture media on regeneration from basal explants of thallus of *Notothylas khasiana* Udar et Singh.

Culture medium	10 th day	20 th day	30 th day
HKM	33.33	46.67	53.33
FKM	26.67	40.00	40.00
Benecks	13.33	26.67	26.67
Mean	24.444	37.778	40.000

CRD ANOVA for 10th day

SN.	SOURCE	DF	MS	F	SE
1	Treatment	2	311.11111	2.333	6.67

CRD ANOVA for 20th day

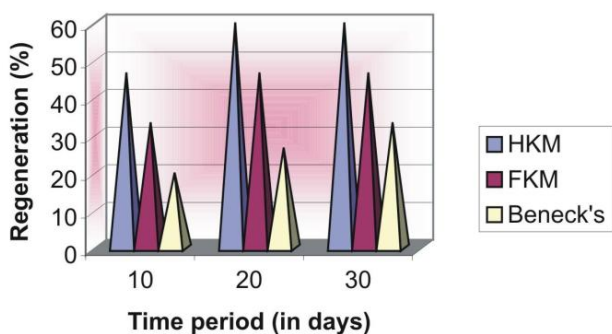
SN.	SOURCE	DF	MS	F	SE
1	Treatment	2	311.11111	1.400	8.61

CRD ANOVA for 30th day

SN.	SOURCE	DF	MS	F	SE
1	Treatment	2	533.33333	2.400	8.61

These results reveal that atmosphere is most suitable for metabolism of regeneration principle in HKM liquid medium so it is more active in this.

Figure-1. Effect of Half Knop's (HKM) and Beneck's liquid culture media on regeneration from apical explants of thallus of *Notothylas khasiana* Udar et Singh.



Results of the present work are in conformity with Singhvi (1979), Mertia (1992) and Chouhan (2002) who noticed the effect of HKM, FKM and Beneck's liquid media on regeneration of *Asterella angusta*, *Funaria nutans* and

Figure-2 & 3. Effect of Half Knop's (HKM) and Beneck's liquid culture media on regeneration from middle and basal (Fig-3) explants of thallus of *Notothylas khasiana* Udar et Singh.

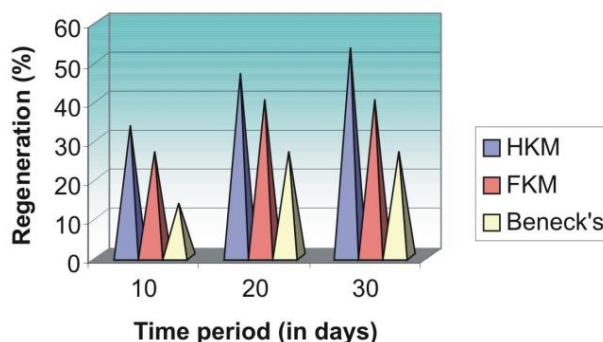
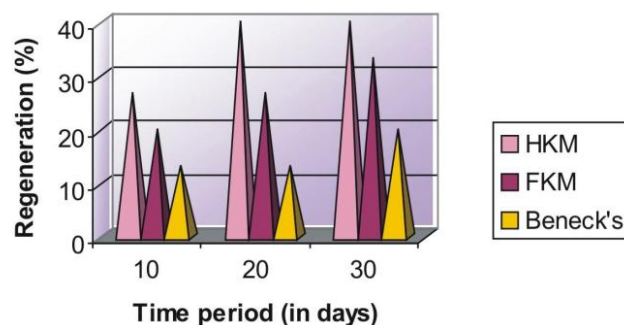
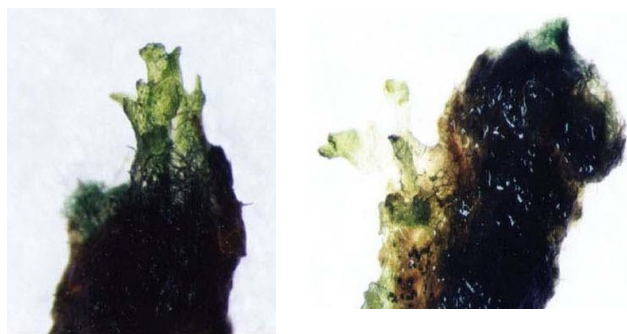
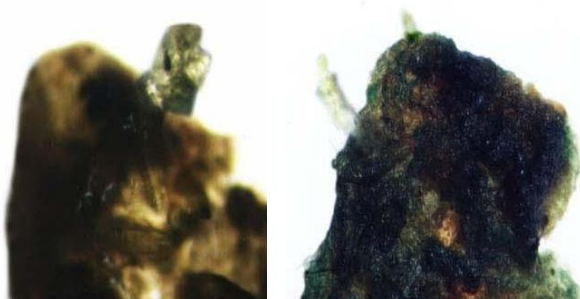


Figure-4: Effect of various liquid culture media on regeneration from different explants of *Notothylas khasiana* Udar et Singh



HKM (Middle part) FKM (Apical part)



FKM (Basal part) Beneck's (Apical part)

Hydrogonium consanguineum respectively and found that regeneration was maximum in HKM followed by FKM and Beneck's liquid media respectively

Acknowledgement

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

REFERENCES

1. **Chaudhary and Bapna** (1995). Regeneration studies on *Asterella angusta*. Recent studies on Indian Bryophytes, 131-139.
2. **Chouhan, K.** (2002). Morphogenetic and ecophysiological studies on *Hydrogonium*

consanguineum. Ph.D. Thesis, Mohan LaL Sukhadia University, Udaipur.

3. **Goebel, K.** (1908). Einleitung in die experimental Morphologie der pflanzen. Teubner, Leipzig.
4. **Mertia** (1992) Morphogenetic and ecophysiological studies on *Funaria nutans*. Ph. D. Thesis, Mohan Lal Sukhadia University, Udaipur.
5. **Singhvi, U.** (1979). Studies on reproductive biology of certain species of *Asterella*. Ph. D. Thesis submitted to the University of Udaipur, Udaipur.

DOI:

<https://dx.doi.org/10.5281/zenodo.7193176>

Received: 11 October 2013;

Accepted; 29 November 2013;

Available online : 6 December 2013

