ejpmr, 2015,2(4), 238-249

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

IN VITRO MICRO PROPAGATION AND TUBERIZATION OF EULOPHIA NUDA LIND AN ENDANGERED TERRESTRIAL ORCHID

[1]Ruchi Kurapa Shroti and [2]Dr. Ravi Upadhyay

Research Scholar Govt. P. G College Hoshangabad (M.P).

Professor Govt. P. G College Pipariya (M.P).

Article Received on 19/04/2015 **Article Revised on 10/05/2015** **Article Accepted on 01/06/2015**

***Correspondence for Author**

Ruchi Kurapa Shroti

Research Scholar Govt. P. G College Hoshangabad (M.P).

ABSTRACT

Eulophia nuda lind commonly known as amarkand, malakand,belongs to family Orchidaceae is an important medicinal orchid so it posses position in Astraverga the known class of medicine in ayurvedic system of medicine due to its medicinal uses and beautiful flowers it is being Over exploited by local tribes, herbal health care, destruction of habitats by reclamation, loss of pollinators ,fragmentation of population is the fore most reason of it endangered stage (Brooks *et al.*, 2002) (Appendix II of CITES), (Annex-2 PC-19 DOC.11.3-P-7) Orchids whose parts and derivatives traded mainly for Traditional Asian Medicine(TAM) or SALEP. It propagates at very low rate because seeds are very minute, non-endospermic and requires mycorrizhal association >0.1 % seeds germinates in nature the seeds so called 'dust seeds ('Fleischer 1929,Ziegenspech 1936.), therefore ,it is the high time to save of this potential source of medicine for sustainable utilization in mankind, so here we propose standardized protocol for invitro micro propagation of *Eulophia nuda lind* So here we described an efficient and reproducible protocol standardized for in vitro micro propagation of *Eulophia nuda lind* through axillary bud culture initiation induced in MS+3.0 (mg/l Bap),after 15 days of inoculation , shoot elongation in MS+3.0 mg/l BAP+1MG/L IAA, Spontaneous rooting obtained in MS +3MG/L BAP+1 MG/L IBA.

KEYWORDS: Endangered orchid, medicinal, micropropaation.

INTRODUCTION

Eulophia, includes 210 species of orchids. It was first described by John Lindley in 1821. The name "Eulophia" was derived from the Greek words "eu" (well) and "lophos" (plume),

referring to the crested ridges of the labellum (lip) in most species. This genus is abbreviated Eupha in horticultural trade.

Distribution

Eulophia nuda lind is distributed in Eastern Himalaya Tropical Himalayas from Nepal eastward to Assam Deccan from Konkan southwards, M.P, Nepal, Srilanka, China, Cambotia, Laos, Vietnam, Myanmar, Thailand, Malayasiya, Java, Borneo, etc its grows in open grassland and swamps, and hot to warm growing orchid with sub terranean.

Description

The plant is terrrestial with almost round pseudo bulbs enveloped by a few ,sheath carrying 3 to 4 lanceolate ,plicate,acuminate,long petiolated leaf that wraps and enfold a long grooved stalk which has several leaf like bracts. The plants blooms in spring with tall thick fleshy few to several[2 to 20]flowered inflororencecs.

Medicinal importance

Many researchers and scientist have proven its medicinal values and arrived a conclusion that Eulophia nuda is a medicinally Important terrestrial Orchid used for tumours and various health problems, The local healers use its juice for the treatment of snake bite.

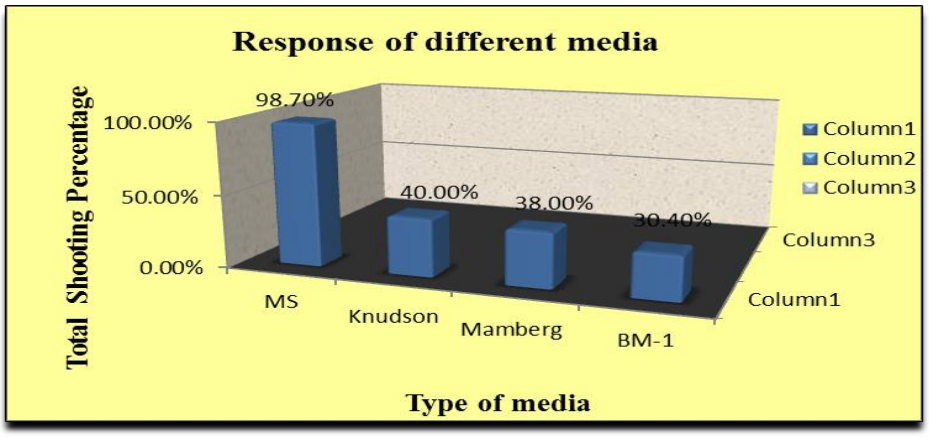
1. Its unique ethno medicinal perception of tribal communities of chitrakoot M.P have been studied by R.L.S Sikarwall IN Indian journal of traditional knowledge vol 7(4) October 2008.
2. Its properties have also been studied by Amrit Paal singh and sanjeev Duggal in Ethnomedicinal leaf leats -2009, over view in journal Ethnobotanical leaflets.
Published in-2009
3. Eulophia nuda contains active ingredients.
4. The tubers of the plant are useful for tumors ,Scrofulous affection of the glands of the neck and in disease of the blood,the plant is also useful as an antihelmintic ,and cases of broncritis, its clamed to useful in tuberculosis (Ref-Chopra R.N Glosary of Indian medicinal plant 1956, page no.112 and review of work on Indian Medicinal plants(1955 p-98)No.3 March 1962 ,Indian Academy of Science**.**

MATERIAL AND METHODS

Collection of explants: The explants were collected from Forest of Kesla M.P.

Explant were collected and and sterilization procedure were standardized using different concentration of Sodium hypochloride and ethanol. Explants were thoroughly washed under running tap water for 10 min. Then after removing leaves explants were washed with distilled water containing few drops of laboline for 5 min and then washed three times with distilled water. After that they were surface sterilized with 0.1% bavistin (w/v in lukewarm distilled water) solution for 15 min and thoroughly washed with distilled water. Then the explants were surface sterilized in the LAF with 70% methanol for 2 min and washed three times with DDW ,after that they were surface sterilized with 0.1% mercuric chloride for 2 ,5 and 10 min and again washed with DDW for three times. Salts of MS medium and vitamins supplemented with various combination and concentration of different growth regulators namely I-NAA,2-4D,BAP,Kinetin,6(Y,Y dimethylallylamino-purine,2-ip,gibberelic acid ,and sucrose were used for regeneration studies .The PH was adjusted to 5.8 before autoclaving at 15 lbs.for 15 mins. All the cultures were maintained at 24-26Ċ with 24 hrs light ,and 32%relative humidity.

Observation and data analysis



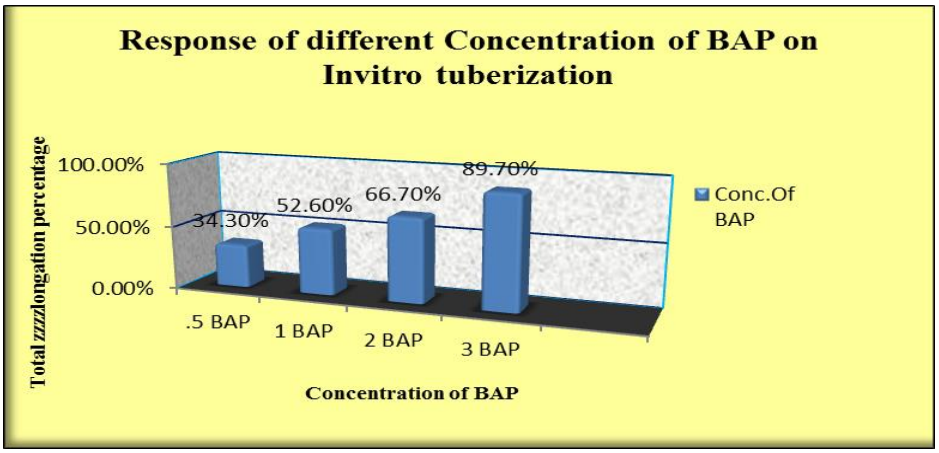


Table 1: Standardization of Media for shoot initiation

Sr. No.	Media	% of shooting	Shoot No. Mean±SD	Shoot length Mean±SD (cm)
1.	**Knudson**	10%	0.27±0.02	0.21±0.07
2.	Knudson+0.5 mg/l BAP	21%	0.9 ±0.29	0.39±0.22
3.	Knudson +1.0 mg/lBAP	35%	0.39±0.22	0.63±0.17
4.	Knudson+2.0 mg/l BAP	37%	1.32±0.82	1.02±0.22
5.	Knudson+3.0 mg/l BAP	40%	1.63±0.12	1.20±0.71
1.	MS	20%	0.40± 0.02	0.40± 0.01
2.	MS+.5mg/l BAP	34.30	1.15±0.13	0.91±0.21
3.	MS+1.0 mg/l BAP	52.60	1.05±0.21	1.08±0.26
4.	MS+2.0 mg/l BAP	66.70%	1.42±0.04	1.21±0.10
5.	**MS+3.0 mg/l BAP**	**98.70%**	**2.82±0.11**	**1.72±0.23**
1.	BM-1	15%	0.35±0.27	0.28±0.12
2.	BM-1+0.5 mg/l BAP	40%	1.09±0.34	0.85±0.14
3.	BM+1.0 mg/l BAP	47%	1.13±0.15	0.89±0.66
4.	BM+2.0 mg/l BAP	58%	1.45±0.67	1.05±0.43
5.	**BM+3.0 mg/l BAP**	**62%**	**1.56±0.09**	**1.16±0.22**

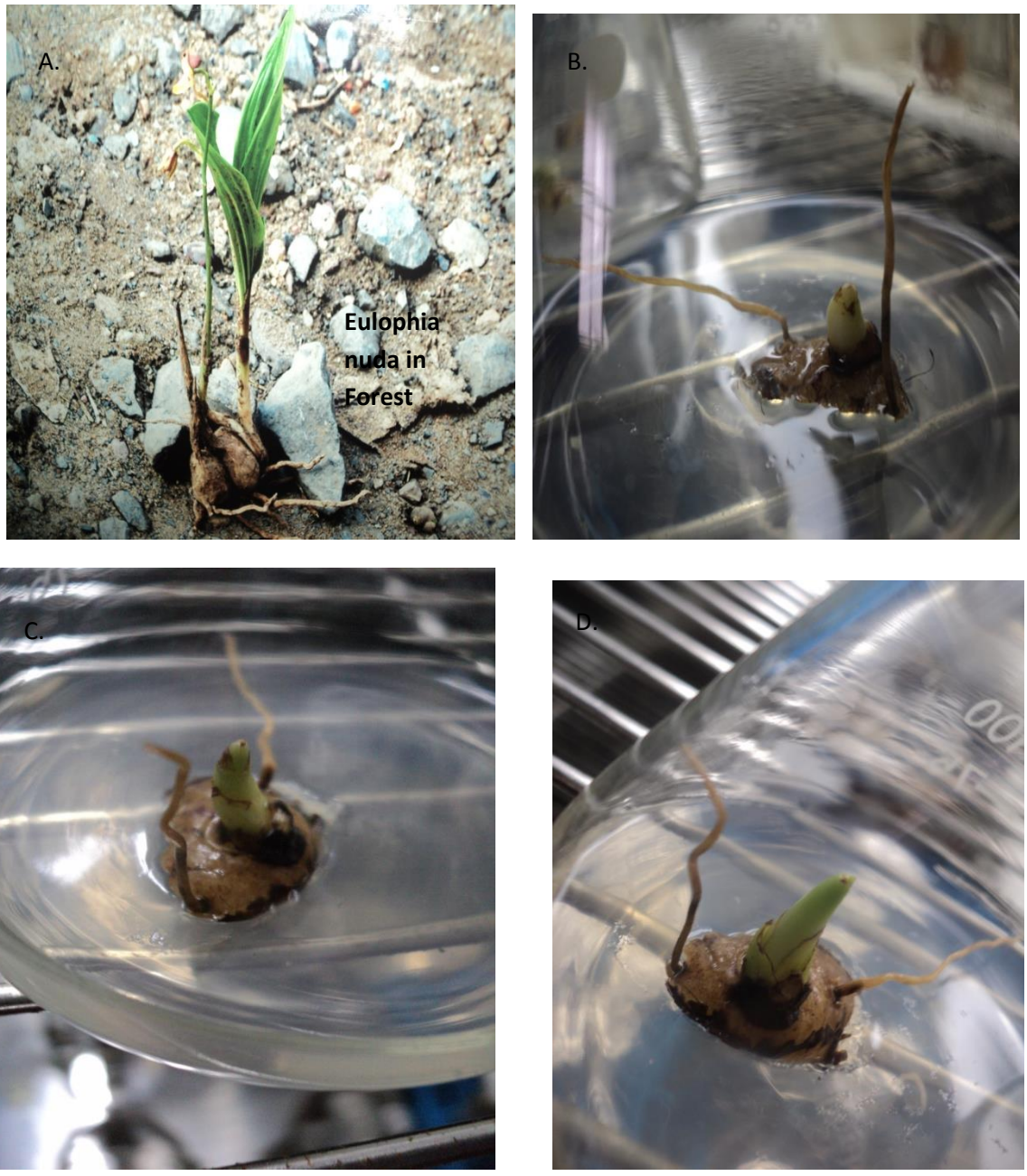
Table 2: Standardization of Media for shoot elongation

1.	MS	20%	0.40± 0.02
2.	MS+0.5 mg/l BAP+.5 mg/l IAA	44%	0.86±0.37
3.	MS+0.25 mg/l BAP+1 mg/L IAA	54%	0.96±0.33
4.	MS+1.0 mg/l BAP + 0.5 mg/l NAA	34%	0.61±0.02
5.	MS+.5mg/L BAP +.5 IBA	60%	**1.52±0.07**
6.	MS+1mg/L BAP+.8 mg/L IBA	62%	**1.56±0.09**
7.	MS+2mg/L BAP+1 mg/L IBA	66.70%	1.42±0.04
8.	**MS+3mg/L BAP+1 mg/L IAA**	**90%**	**1.84±0.13**

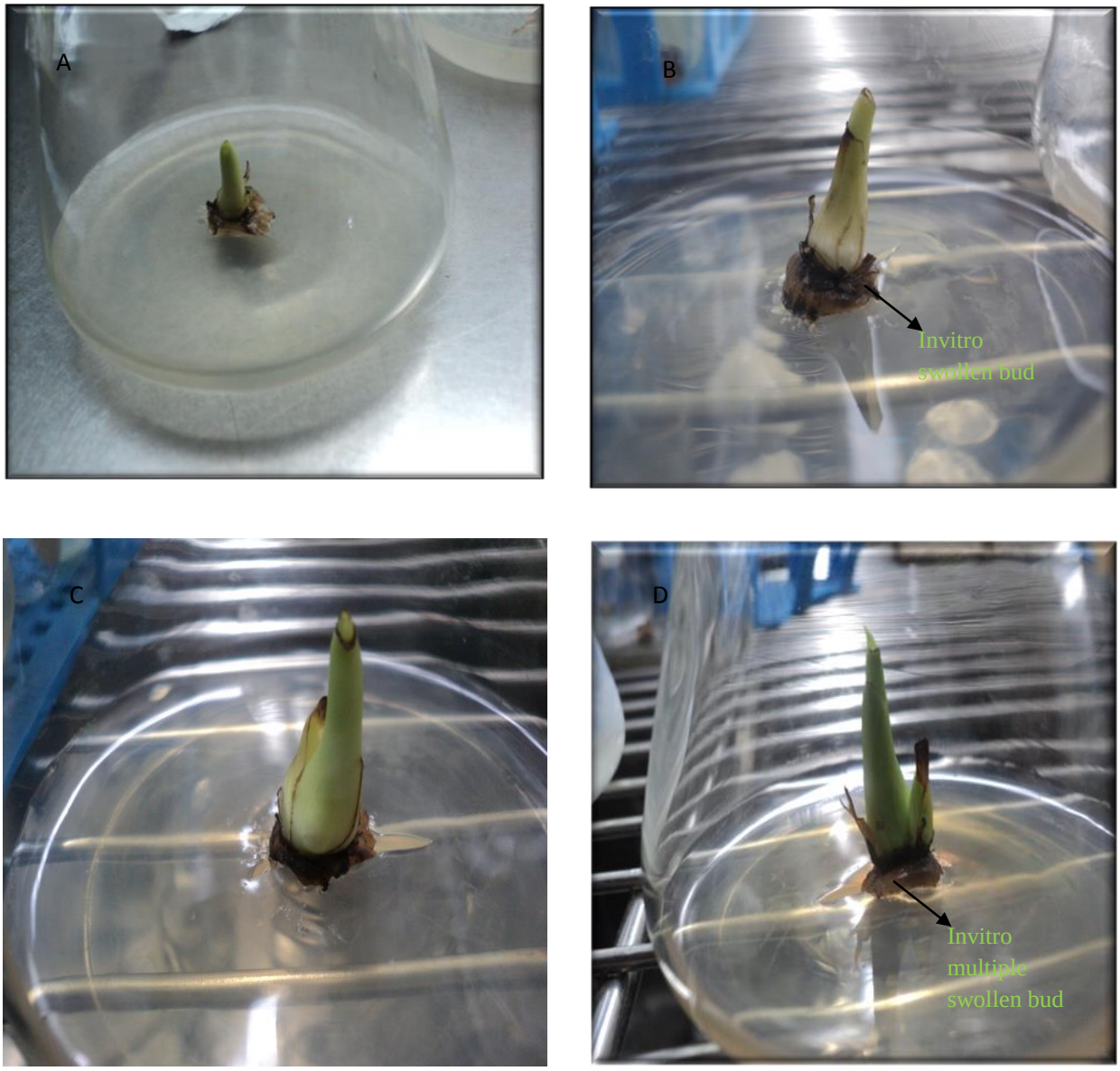
Table 3: Standardization of media for In vitro rooting and tuberization

Sr. No.	Treatments	Leaf number Mean± SD	Shoot length (in c.m.) Mean± SD
1	MS+.5mg/L BAP+0.5 mg/LNAA	1.6±0.54	1.6± 0.89
2	MS+1 .0mg/L BAP+1 mg/L NAA	2.6±0 .89	1.9 ±1.64
3	MS+2.0 mg/L BAP+0.5 mg/L NAA	2.2 ±.083	1.84 ±0.20
4	MS+2.0 mg/L BAP+1 mg/L NAA	2 .0±1.0	1.78 ±0.13
5	MS+2.0 mg/L BAP+0.5 mg/L IBA	4.6 ± 1.34	2.24±0.38
6	**MS+2.0mg/L BAP+1 mg/L IBA**	**5 .0± 1.58**	**2. 44±0.38**

PHOTOGRAPS & DESCRIPTION

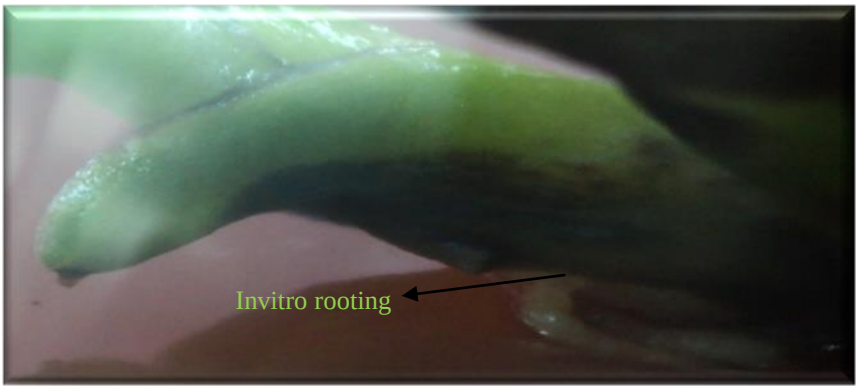


A.Eulophia nuda lind in natural condition(b)Inoculation of explants (axillary bud)3-4 mm (c)swelling of explant after 8 days of inoculation (d)Initiation of shooting after 21 days of culture MS media fortified with 3mg/L BAP.



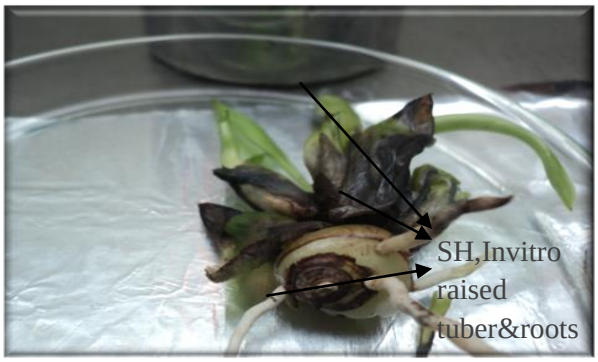
A.2nd Subculturing of invitro bud in MS media fortified with 3mg/L BAP+1mg/L IAA.(B,C,D)Development of shoots from axillary bud of Euolphia nuda lind on MS

medium supplemented with 3mg/L BAP+1mg/L IAA +3% sucrose(Bar 1.5 cm),E.Initiation of Invitro tuber formation.


Invitro rooting


Sprouted in
vitro tubers


Invitro raised sw
bud&tuber


SH,Invitro
raised
tuber&roots


PLAYER BOY

A.In vitro raised plants in 1%Bavestin+0.1%cuso4 for 10 seconds &washed with water B.Invitro raised plants in coconut husk+sand+sterilized soil+dead leafs and mosses.C.Invitro raised plants in green house established in pot after 45 days of transfer(8 CM)with 72%relative humidity .

Observation and Data Analysis

The observation were monitored at the interval of every three days for above objective like contamination, shoot initiation, shoot elongation, callus initiation, caulogenesis etc. The growth hormone combination and response were also recorded.

For data analysis ANOVA was used. In statistics, analysis of variance (ANOVA) is a collection of statistical models, and their associated procedures, in which the observed variance in a particular variable is partitioned into components attributable to various sources

of variation. In its simplest form, ANOVA provides a statistical test of whether or not the means of several groups are all equal. ANOVA are useful in comparing two, three, or more means. ANOVA: Two factor without Replication and Descriptive statistics tools in Microsoft excel were used to analyze the present data. Shoot length and leaf number were represented by using two statistical values that is mean and standard deviation.

RESULT AND DISCUSSION

RESULTS

Preparation of Media

MS , Knudson, Mamberg, BM-1, medium with various hormone combination were used for the study. Medium contains all the nutrients which are required for the growth of plant *in vitro*. This medium contains essential nutrients, vitamin, amino acid, carbon source. Agar was used as gelling agent. Stock solution was prepared for an easy handling because chemical used in preparation of stocks were required in very small quantity. Medium pH was adjusted to 5.7 with the help of 1N HCl and 1N NaOH.

Optimization of Surface Sterilizing Agent for *Eulophia nuda lind.*

Surface sterilization of explant was done. Treatment of explant with 70% alcohol for 2 min and 0.1 % $HgCl_2$ for 5 min was the best for *Eulophia nuda lind* in this study.

Standardization of Media for Shoot Initiation

In the present study micropropagation of *Eulophia nuda lind* was initiated. Axillary bud 3-4 mm gave better response as compared to tuber section. MS medium containing 3 mg/L BAP was the best medium for initiation. (Refer Table No. 1)

Standardization of Media for Shoot Elongation

In the present study, initiated tissue was used for shoot elongation. Higher concentration of BAP is required for shoot induction and shoots multiplication of *Eulophia nuda lind*. For shoot elongation and multiplication MS+3mg/L BAP with various concentrations of IAA,NAA, and IBA were used. The best result was obtained in MS +3mg/L BAP+1 mg/L IAA. (Refer Table No. 2)

Standardization of media for in vitro rooting

Elongated shoots sub cultured on ms media supplemented with MS+2.0mg/L BAP+1 mg/L IBA, 5 .0$\pm$ 1.58 Leaf number(Mean$\pm$ SD), 2. 44$\pm$0.38 Shoot length(in c.m.) (Refer Table No. 3)

DISCUSSION

The present study was under taken to study the effect of plant growth regulator on growth invitro micropropagation and tuberization of *Eulophia nuda lind*. First of all explants were collected and surface dust was removed by washing under the running tap water then explants were surface sterilized with 0.1 % (w/v) Bavistin for 15 min and washed with DW, then firstly with 70% alcohol and then with 0.1 % (w/v) $HgCl_2$ for 5 min.

After surface sterilization explants were inoculated in MS media with various hormonal combinations. Among the various hormone combinations shoot induction was obtained in the following concentration: ½ MS, full strength MS, MS+0.2 mg/L BAP ,MS+0.5 mg/L BAP,MS+1 mg/L BAP, MS+2.0 mg/L BAP, MS+3.0 mg/L BAP . The best result for shoot initiation (100%) was obtained in the concentration 3mg/L BAP. The best result for shoot elongation 1.84±0.13); in the concentration MS+3mg/L BAP+1 mg/L IAA.

ACKNOWLEDGEMENT

Mrs. Sharad trivedi Upadhyay for support and guidance, Dr.K.W Shah for providing lab facilities,Mr.Sharad kurapa & Dr. Abhishek Shroti for proper support, Mr. Rishabh kurapa for support, Miss. Saurav Tiwari, Mr. Mukesh Dwivedi for help and support.

REFERENCES

1. Agarwal A, Khokhar D, Vishwnath Conservation through *in vitro* propagation of a critically endangered medicinal plant, *Dactylorhiza hatagirea* (D. Don) Soo. In: Reddy MV (ed). Wildlife Biodiversity Conservation. Daya Publishing House, 2008; pp. 294-299.
2. Arditti J Aspects of physiology of orchids. Adv. Bot. Res, 1979; 7: 421-655.
3. Barroso J, Fevereiro P, Oliveira MM, Pais MSS In vitro seed germination, differentiation and production of minitubers from Ophrys lutea Cav., Ophrys fusca Link and Ophrys speculum Link. Sci Hortic (Amsterdam), 1990; 42: 329–337.
4. Correa MN Orchidaceae. In: Zuloaga F, Morrone O (eds) Cata´logo de plantas vasculares de la Repu´blica Argentina I, Monogr Syst Bot Missouri Bot Gard, vol 60. Missouri Botanical Garden, Missouri, USA, 1996; pp 242–271.
5. Debeljak N, Regvar M, Dixon K, Sivasithamparam K Induction of tuberization in vitro with jasmonic acid and sucrose in an Australian terrestrial orchid, Pterostylis sanguinea. Plant Growth Regul, 2002; 36: 253–260. doi:10.1023/A:1016570319387.
6. Ernst R Studies on asymbiotic culture of orchids. Am. Orch. Soc. Bull, 1975; 44: 12-18.

7. Fernie AR, Willmitzer L Update on tuber formation, dormancy and sprouting: molecular and biochemical triggers of potato tuber development. Plant Physiol, 2001; 127: 1459–1465. doi10.1104/pp.010764.
8. Ghosh S, Ghosh B, Jha S In vitro tuberization of Gloriosa superba L. on basal medium. Sci Hortic (Amsterdam), 2007; 114: 220–223.
9. Hussey G, Stacey NJ Factors affecting the formation of in vitro tubers of potato (Solanum tuberosum L.). Ann Bot (Lond), 1984; 53: 565–578.
10. IUCN. IUCN guidelines for the prevention of biodiversity loss due to biological invasion. Species, 1999; 31–32: 28–42.
11. McAlister, B.G. and J. Van Staden. *In vitro* culture of *Eulophia* species. South Afr. J. Bot, 1998; 64**:** 264-266.
12. Mirta Mabel Faloci Æ Luis Amado Mroginski In vitro tuberization and plant regeneration from multinodal segment culture of Habenaria bractescens Lindl., an Argentinean wetland orchid Plant Cell Tiss Organ Cult, 2009; 97: 91–101.
13. Ndoumou DO, Tsala GN, Kanmegne G, Balange´ AP In vitro induction of multiple shoots, plant regeneration and tuberization from shoot tips of cocoyam. C R Acad Sci Parı´s, Sciences de la vie, 1995; 318: 773–778.
14. Omokolo ND, Boudjeko T, Tsafack Takadong JJ In vitro tuberization of Xanthosoma sagittifolium L. Schott: effects of phytohormones, sucrose, nitrogen and photoperiod. Sci Hortic(Amsterdam), 2003; 98: 337–345. doi:10.1016/S0304-4238(03)00066-9.
15. Oyono PO, Kevers EC, Dommes EJ Axillary proliferation and tuberisation of Dioscorea cayenensis—D. rotundata complex, 2007.
16. Plant Cell Tissue Organ Cult 91: 107–114. doi:10.1007/s11240- 007-9238.
17. Roy J, Banerjee N Rhizome and shoot development during in vitro propagation of Geodorum densiflorum (Lam.) Schltr. Sci Hortic (Amsterdam), 2002; 94: 181–192. doi:10.1016/S0304-4238(01) 00373-9.
18. Sengupta J, Mitra GC, Sharma AK Organogenesis and tuberization in cultures of Dioscorea floribunda. Plant Cell Tissue Organ Cult, 1984; 3: 325–331. doi:10.1007/BF00043084.
19. Vij, S.P., A. Sood, and P. Pathak. On the utility of rhizome segments in micropropagating *Eulophia hormusj*i Duth. J. Orchid Soc. India, 1989; 1**,**3(2): 41-45.
20. Walter KS, Gillet H. The IUCN Red List of threatened plants. Gland and Cambridge: World Conservation Union; 1998.

21. Wu, J.S. Pollination mechanism of *Eulophia graminea*. National Science Council Undergraduate project NSC 92-2581-C-006-086-B 27P (Chinese), 2004.
22. Zhou SP, He YK, Li SJ Induction and characterization of in vitro corms of diploid-taro. Plant Cell Tissue Organ Cult, 1999; 57: 173–178. doi:10.1023/A:1006335813056.