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**IN VITRO MICRO PROPAGATION OF
CYMBIDIUM**

By:

Eva Ahuja(071554)
Shalki Shabnam(071574)
Supervisor-Dr Hemant Sood

**A THESIS SUBMITTED IN FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF**

**BACHELOR OF TECHNOLOGY
IN
BIOTECHNOLOGY**



**JAYPEE UNIVERSITY OF INFORMATION
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Eva Ahuja
Eva Ahuja

Shalki Shabnam

CERTIFICATE

This is to certify that the thesis entitled "**In vitro procedures for micro propagation of orchids**" submitted by **Ms. Eva Ahuja and Ms. Shalki Shabnam** to the Jaypee University of Information Technology, Waknaghat in partial fulfillment of the requirement for the award of the degree of **Bachelor of Technology in Biotechnology** is a record of bona fide research work carried out by them under my guidance and supervision and no part of this work has been submitted to any other university or institute for this or any other degree or diploma.

die Kood
[Dr. (Mrs.) Hemant Sood]
22/5/17

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We express our heartfelt gratitude to all those who have contributed directly or indirectly towards obtaining our baccalaureate degree and at the same time, we cherish the years spent in the department of Bioinformatics and Biotechnology. We are highly indebted to our esteemed supervisor, Dr. (Mrs.) Hemant Sood, who has guided us through thick and thin. This project would not have been possible without her guidance and active support. Her positive attitude towards research and zest for high quality research work has prompted us for its timely completion. We deem it a privilege to be working under Dr.(Mrs.) Hemant Sood, who has endeared herself to her students and scholars.

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Needless to say, errors and omissions are ours.

Eva Ahuja
Eva Ahuja

Shalki
Shalki Shabnam

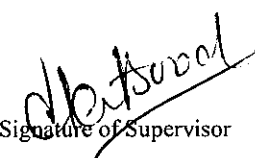
Summary

Cymbidium or **boat orchids**, is a genus of 52 evergreen species in the orchid family Orchidaceae. The name is derived from the Greek word *kumbos*, meaning 'hole, cavity'. It refers to the form of the base of the lip. The genus is abbreviated **Cym** in horticultural trade. As the plant is commercially very important (Rs500-600), having long shelf life and exploited very little for carrying out successful and more significant *in vitro* studies. This study was carried out with the objective of developing some rapid procedures for its micro propagation so that their production should be enhanced. As the plant is very tough to culture and gave very slow responses in the nutrient media and also prone to many viral disease, therefore its exploration is always a challenging research. As the plant is very sensitive to its temperature ranges therefore we choose the tropical to temperate variety of cymbidium which we procured from *Adarsh Pushpa Kendra* Chail. Surface sterilization was done with the help of Bavistin and Mercuric Chloride. Culturing was done by using different explants such as petals, tubers, bulbs etc. Various combinations of media were tried for achieving good response and growth. Out of all the combinations the best results were obtained for shoot formation from *in vitro* derived calli from bulbs cultured on different media supplemented with 2, 4-D and NAA. Different media were tried for increasing the number of protocorm. Shoot proliferation was obtained best on MS+Gibberelic acid. So the outcome of this study would definitely play an important role for carrying out large scale multiplication and propagation of this commercially important flower and its tough species, which would be great contribution for strengthening the status of this very flower in floriculture industry.

Signature of Students

Name: Eva Ahuja, Shalki Shabnam

Date:


Signature of Supervisor

Name: Dr. Hemant Sood

Date: 23/5/11

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
°C	Degree Celsius
μM	Micro molar
BAP	Benzyl Amino Purine
cv.	Cultivars
Gm.	Gram
HCl	Hydrochloric Acid
IBA	Indole -3Butyric Acid
Kg	Kilogram
KN	Kinetin
l / lt.	Litre
Mg	Milligram
MS media	Murashige and Skoog Media
NaOH	Sodium Hydroxide
GA ₃	Gibberilic Acid
2-4 D	2, 4 dichlorophenoxyacetic acid

CHAPTER 1

Introduction

Cymbidium or **boat orchids**, is a genus of 52 evergreen species in the orchid family Orchidaceae. The name is derived from the Greek word *kumbos*, meaning 'hole, cavity'. It refers to the form of the base of the lip. The genus is abbreviated **Cym** in horticultural trade. It is one of the most popular and desirable orchids in the world because of the beautiful flowers. These plants make great houseplants, and are also popular in floral arrangements and corsages. They have been cultivated for thousands of years, especially in China. *Cymbidiums* became popular in Europe during the Victorian era. One feature that makes the plant so popular is the fact that it can survive during cold temperatures (as low as 7° C or 45° F). Orchid hobbyists in temperate climates appreciate the fact that they can bloom in winter, when few other orchids are blooming. This genus is distributed in tropical and subtropical Asia (such as northern India, China, Japan, Malaysia, the Philippines, and Borneo) and northern Australia. The larger flowered species from which the large flowered hybrids are derived grow at high altitudes. The species *Cymbidium hookerianum* is considered a delicacy in Bhutan where it is traditionally cooked in a spicy curry or stews and called "olatshe" or "olachoto". *Cymbidium* plants are sympodial and grow to a height of 60 cm and the racemes as high as 90 cm. The raceme grows from the base of the most recent pseudo bulb. Each flower can have a diameter of 5 to 10 cm, according to the species. They bloom during the winter, and each plant can have up to fifteen or more flowers. The fantastic range of colors for this genus include white, green, yellowish-green, cream, yellow, brown, pink, and red [and orange] (and there may be markings of other color shades at the same time), but not blue and black. The flowers last about ten weeks. They have a waxy texture. The rounded sepals and

petals have about the same dimensions. They show very diverse color patterns, different for every species.

Facts about Cymbidium

- *Cymbidiums* have been cultivated for thousands of years, especially in ancient China.
- In his writings, Confucius referred to the Asian Cymbidium as the **King of Fragrance**
- *Cymbidiums* usually bloom once a year during winter and spring seasons and the flowers last for more than four weeks.
- *Cymbidiums* may be divided into two main groups- Standard Cymbidiums (large-flowered type) and Miniature Cymbidiums (small-flowered type).
- A cymbidium plant has three types of bulbs-
 1. **Old back bulbs without leaves**, which reserve food supply for emergencies.
 2. **Old bulbs with leaves**, Supporting the new growth and may produce flowers.
 3. **New leads or bulbs**, from where flowers grow.

ECONOMIC IMPORTANCE

The orchid family is probably one of the most important of plant families from a horticultural point of view. Other than the horticultural uses to which orchids are put, the family is notably lacking in species from which products are derived. The only commercially important product derived from orchids is vanilla. Most vanilla is produced from one species, *Vanilla planifolia*, although two additional species are also cultivated commercially (*V. pompona* and *V. tahitensis*). The principal vanilla-growing areas are Madagascar, Mexico, French Polynesia, Réunion, Dominica, Indonesia, the West Indies, Seychelles, and Puerto Rico. Vanilla.

CURRENT STATUS

Cymbidium has retained its status as a celebrity orchid for thousands of years since the time of ancient China. People love them so much because Cymbidium orchid hybrids produce up to 30 flamboyant and sometimes fragrant blooms on a single spike, and the flowers last couple of months. Even if we cut those spikes and put them in a vase, the flowers will last just as long. No wonder Cymbidium is one of the most popular orchids for corsages. And even though there are only 44 species, thousands of hybrids have been developed to satisfy people's demand for variety.

Standard Cymbidium Orchid

"Standard" Cymbidium refers to the large-flowered species from the Himalayas and China. These well-loved plants produce large, attractive flowers that come in many different colors. The roundness of these flowers is a very desirable trait and commercial hybridizers work hard to develop plants that produce the fullest looking flowers. Even though they are tolerant of extreme temperatures, to thrive and to produce flowers, they require frost-free cool nights (below 53°F/12°C) and warm days. Cymbidium demands a little more fertilizer than most other orchids, so be sure to apply fertilizer twice a month at half the strength that is prescribed on the instruction label. We can also use the slow-release fertilizer once a year and forget about it for the rest of the year.

CHAPTER 2

Review of Literature

Review is discussed in the light of available literature relevant to the research problem both on orchid as well as on other plants.

2.1 GENERAL MICROPROPAGATION

Tissue culture is the most widely and successfully used technology by the commercial industry for the mass production of horticultural crops. Tissue culture has been applied effectively in the clonal propagation of many plant species including ornamental and other crop plants. The use of meristem and shoot tip cultures for the recovery and establishment of pathogen free plants has also become common practice in the production of virus free stocks of vegetatively propagated plants in many commercial nurseries in the developed world.

2.2 CULTURE CONDITIONS

2.2.1 MEDIUM

Murashige and Skoog (MS) medium (1962) supplemented with agar-agar, sucrose and hormones at desired levels are most commonly used for micro propagation of *Cymbidium*.

2.2.2 Modified media

According to **Li Cheng-xiu** the best induced rooting in the culture medium including 1/4MS, 2.0mg/L NAA, 0.1mg/L 6-BA and 10 percent banana mud, and were the best induced root stock in the culture medium including MS, 2.0mg/L NAA, 1.0mg/L 6-BA and 10 percent coconut milk, and the seedlings transplanted out of aseptic bottles can survive 93.8 percent micro propagation.

Susmita et al. (2000) reported TCS (thin cross sections) were cultured on Murashige and Skoog's medium supplemented with any of the three cytokinins

such as zeatin riboside (ZR), N⁶-benzyladenine (BA) or kinetin (KN). For induction of PLBs, ZR at 14.0 µM was most effective for *C. aloifolium* while BA at 11.0 µM was optimum for *D. nobile*. Average number of PLBs per TCS explant was high in both the cases (28.2 in *C. aloifolium*, and 34.0 in *D. nobile*) though inhibitory effect was noticed with cytokinins above the optimal level.

WU Kai-yun et al. (2000) suggested different concentrations of 2,4-D, 6-BA, and NAA on the PLB formation of *C. hybridum* were investigated in different level of concentration. The results showed that the effects of 2,4-D and 6-BA on the frequency of PLB formation of *C. hybridum* differed extremely significantly. 2,4-D on lower level was an important stimulative to induce PLB, but the inducing of PLB would be restrained when the concentration of 2,4-D was above 0.60 mg·L⁻¹. The frequency of PLB formation increased basically with the increase of concentrations of 6-BA. The frequency of PLB formation was also related to the genotype, NAA was an important stimulative factor for some special genotype of *C. hybridum*.

CHEN Xiao-qiang et al saw the effects of different basic culture media (1/3 MS, 1/2 MS, MS), different organic compounds (coconut milk, banana and apple) and plant hormones (6-BA, NAA) on the propagation of PLB of *Cymbidium hybridum* were studied by *Cymbidium hybridum* PLB. The results indicate that 1/2 MS was the optimum basic medium for the propagation of PLB. Organic compounds have influence on the multiplication of PLB, the promoting effects of coconut milk added into the basic media were better than banana mud and apple juice. 1.5 g·L⁻¹ activated carbon could absorb phenols effectively, which inhibited the PLB browning. The concentration of 6-BA and NAA could also affect the multiplication of PLB. The lower level of NAA (0.2 mg·L⁻¹) with the higher level of 6-BA (1.0 mg·L⁻¹) could have significant roles on the promotion of the multiplication of PLB.

TAN Tian et al reported the effects of different mediumson inducement and multiplication of *C.hybridium* protocorms,using shoot tips and stem sections as explants. The results showed that inducing percentage of the shoot tips was higher than that of stem sections, the optimal medium for inducing *C.hybridium* protocorm was 1/2MS+ 2.0 mg/L 6-BA + 0.2 mg/L NAA + 1.0% aloe juice.The optimal medium for the multiplication of protocorms was 1/2MS + 2.0 mg/L 6-BA + 0.2 mg/L NAA +150 g/L banana mud + 1.0% aloe juice. This research will provide scientific references for factory-scale nursery system of C.hybridium.

2.2.3 Light

The effects of light intensity were studied, culture method and cytokines on plant formation from callus-derived rhizomes of *Cymbidium ensifolium* var. *misericors*. The results demonstrated that one piece of rhizome produced seven shoot buds in 45 days when cultured in 1/2 MS basal liquid medium supplemented with 0.17 μM N⁶-(2-isopentenyl) adenine, 0.17 μM thidiazuron, 33 μM 6-aminopurine adenine and 1.5 μM naphthalene acetic acid under 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ artificial light and agitated at 60 rpm on a rotary shaker. These shoot-cm plantlets in 5 mo. when transferred to the same Gelrite-gel basal medium supplemented with 50 g l⁻¹ banana pulp. Plantlets were acclimated and grew well when potted in the greenhouse.

2.3 Sterilization of explants

T. Yanagawa, et al(2003) used simple sterile cultures for micro propagation of orchids using direct application of chlorine disinfectants by preparing sterile medium without autoclaving and inoculating explants without the laminar air-flow cabinet. The sterile medium could be prepared without autoclaving by immediately incorporating chlorine disinfectants into the medium. In these cases, all chlorine disinfectants tested were effective for sterile medium preparation. The media could be used for sterile cultures in various micro propagation processes. Spraying the surface of a medium and the whole explants with chlorine

disinfectants after inoculating was effective for inoculating explants sterilely and for subsequent sterile cultures under non-sterilized conditions. These techniques could be applied to the following cultures, shoot tips of chrysanthemum and *Cymbidium*, stem section explants of chrysanthemum and carnation, PLB explants of *Cymbidium*, *Cymbidium* plantlets and *Phalaenopsis* plantlets. The treated levels of incorporated and sprayed chlorine disinfectants suppressed in vitro contamination and did not appear to be toxic to shoots, PLBs and plantlets of orchids tested. Propagated plantlets which were cultured on the disinfectant incorporated medium and handled with spraying treatments under non-sterile conditions could survive without harming tissues and were raised without in vitro contamination.

2.4 In vitro Shoot Regeneration

Nihar Ranjan Nayak et al(2000)induced high frequency direct shoot proliferation in the shoot segments of three epiphytic orchids, *Cymbidium aloifolium* (L.) Sw., *Dendrobium aphyllum* (Roxb.) Fisch. and *Dendrobium moschatum* (Buch-Ham) Sw. on Murashige and Skoog's medium (MS) containing N⁶-benzyladenine (BA) or thidiazuron (TDZ) with the latter being more effective at 2.2–4.5 µM.

2.5Compost

While preparing a compost mix it should be borne in mind that the compost provides adequate aeration around the root system by being porous, sufficient nutrition, adequate water supply and firmness to hold the plants in the container. It must also be seen that the compost is free from fungal spores and insects that damage the plants. Accordingly, a mixture consisting of humus soil + river sand + charcoal (1/2 to 1 cm size)is prepared in 1:1: 1 proportion and heated up over a tin frying pan to remove undesired fungal spores and insect larvae. This mixture is also supplemented with half-burnt dry leaf chopping and charred wood-chips (1 cm size). The compost can be supplemented with N:P: K (30:10:10) fertilizers at an interval of 45-60 days during growth season (summer) and with a fortnightly spray of micro-nutrients and hormone. Slow release fertilizers like Osmocote/soil-

rite greatly help in healthy growth. Cymbidium can not tolerate high levels of fertilizers or salts and hence should be avoided.

2.6 Potting or planting:

The compost thus prepared is filled into the earthen or plastic pots up to 2/3rd of the container or nursery beds (0.40 x 1 x 6m size) which is already layered at the bottom with crocks consisting of brick bats or stones and pebbles to allow excess water to drain down avoiding stagnation of water. Then the plant with roots or back bulb is placed at the center of the container and filled up with the same compost. In the nursery beds however, 6 seedlings can be planted up per metre. After planting the compost is also supplemented with soil-rite in the top layer to give additional nutrition to the plants. In case of young mericlones viz. seedlings just removed from or pricked out of the culture flasks, absolutely sterile medium consisting of perlite + soil-rite or charcoal+ brickbats + tree-fern fiber should be used in trays/poly pots or clay-pots. Seedlings also must be treated with fungicides to prevent infection. This medium should be sprayed with nutrient solution after planting up the seedlings and placed in the clean environment in the hardening house (polythene or fibre-glass house) maintaining humidity temperature around 24° C and ventilation. The seedlings with the new growth of leaves and roots can be transferred to larger containers after hardening for 45-60 days. They should however be sprayed with nutrients, fungicides and pesticides periodically to avoid infections that damage the plant.

2.7 Propagation :

Cymbidiums can be propagated vegetatively by division of back bulbs or by adopting aseptic culture technique of seeds and meristematic tissues.

a. Vegetative propagation : By 3-4th year, Cymbidiums form a cluster which can be separated from the base by dividing it with the help of a sharp garden knife. It must be ensured that the back bulbs (pseudobulbs formed in the previous year) always have a new shoot emerging from the base along with roots .Such divisions can be planted up with fresh compost as described earlier.

b. Aseptic culture: Aseptic culture technique involves propagation under controlled conditions of the laboratory in an artificial nutrient medium in a clean and disease free environment. Seeds or meristematic tissues are inoculated into the flasks and allowed to propagate and differentiate into seedlings. This is an elaborate technique involving technicians for culturing and sub-culturing and when the seedlings attain 1-3" in size, they are transplanted in composts and hardened in controlled green-house/glass-house. After about 45-60 days they are transferred to larger pots or ground beds.

CHAPTER 3

Materials and Methods

Selection of plant material and establishment of cultures

The orchids were procured from *Adarsh Pushpa Kendra*, Chail, H.P., India and planted in the green house in Deptt. of Biotechnology and Bioinformatics, Jaypee University of Information Technology. Bulbs were isolated from the pot grown plants and were surface sterilized in 0.5% Bavistin and 0.1% Mercuric Chloride followed by 4-5 washings in sterile water. The sterile root tubers were cultured on MS medium supplemented with different concentrations and combinations of auxins and cytokinins.

Preparation of media and incubation of cultures

Modifications of MS media supplemented with different concentrations and combinations of IBA, KN, GA₃, NAA and BAP were prepared (Murashige and Skoog 1962). The pH of the media was adjusted to between 5.5 - 5.7 using 0.1N HCl and 0.1N NaOH and finally agar-agar 0.8% (w/v) was added as a gelling agent. The media were autoclaved at 121°C and 15 lb/in² pressure for 15-20 minutes. The autoclaved media was kept under Ultraviolet radiation 20 minutes prior to culturing. After 20 minutes explant was cultured on the respective media. The cultures were subcultured after every 4 weeks on shoot proliferation media for 2 months so as to obtain good growth, followed by subculturing on the rooting media prior to transfer to the potting mixtures for the acclimatization.

Establishment of callus cultures

Different explants were tried for callus induction petals, bulbs and tuber sections in 10 different concentrations and combination of auxins such as NAA(0-2)mg/l

2,4 D (0-3)mg/l and IBA(0-2)mg/l. The data were collected for percentage of callus ind and days to callus initiation.

Protocorm formation from bulbs

Different auxins and cytokinins were used for protocorm formation where 5 different concentrations of KN(0-2)mg/l and IBA(0-3)mg/l were tried and data were collected for protocorm formation and number of protocorm formed.

***In vitro* shoot formation from protocorm**

Protocorm bodies were cultured on 6 different media containing KN (0-2)mg/l, IBA(0-4)mg/l, Gibberelic acid(0-2)mg/l for shoot formation. Data were collected for shoot formation as number of shoots formed and days to shoot formation.

CHAPTER 4

Results and Discussions

Establishment of surface sterilization

Sterilization of explants:

Sterilization is a term referring to any process that eliminates (removes) or kills all forms of life, including transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) present on a surface, contained in a fluid, in medication, or in a compound such as biological culture media. Living plant materials from the environment are naturally contaminated on their surfaces (and sometimes interiors) with microorganisms, so surface sterilization of starting materials (explants) in chemical solutions (usually alcohol or bleach) is required. Mercuric chloride is seldom used as a plant sterilant today, as it is dangerous to use, and is difficult to dispose of. Explants are then usually placed on the surface of a solid culture medium, but are sometimes placed directly into a liquid medium, particularly when cell suspension cultures are desired.

The explants were subjected to bacterioside and fungicide agents for different time durations. Bavistin and HgCl_2 are the most commonly used agents.

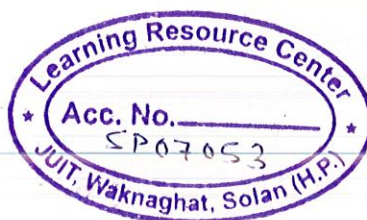


Table 1 : Different concentrations and time of exposure of explants of *Cymbidium* to sterilizing agents.

Sterilizing agents	Concentration	Time of exposure (in mins)
HgCl ₂	0.05%	1 , 1.5 , 2
	0.1%	1 , 1.5 , 2
	0.15%	1 , 1.5 , 2
Bavistin	0.45%	2 , 3 , 5
	0.5%	2 , 3 , 5
	0.55%	2 , 3 , 5

Callus induction from different explants

As callusing is quite tough in orchids as plant is monocot in nature. It took maximum of 50-55 days to form good calli. 54% of callus is formed from bulbs on media containing MS+ 2,4D (2 mg/l), NAA (2 mg/l).

Table 2 :Different concentration and combinations of auxins tried for callus induction in *Cymbidium*

MS+growth hormone			Days to callus initiation	Percentage of callus initiation	
2-4-D	NAA	IBA		Bulbs	Leaves
0	0	0			
1	0	1	55-57	22	1
1	1	0	55-57	28	2
1	0	2	45-55	28	3
1	2	0	40-45	35	5
2	0	1	50-55	32	4
2	1	0	45-55	48	7
2	2	0	45-50	54	10
3	0	2	45-50	40	5
3	2	0	45-55	42	5
4	3	0	45-55	52	8

Protocorm formation

Initially the protocorm like bodies were developed from the calli. 5 different tested media containing MS+KN and IBA and protocorm were developed with in 30-40 days of their culturing . The maximum number of protocorms (3-4) were observed in MS+ KN(2mg/l) and IBA(3 mg/l.and their number was increased in same medium after sub culturing.

Table3: Effect of different concentrations of growth hormones on protocorm formation.

MS +growth hormones KN	IBA	Days to protocorm formation	Number of protocorms
0	0		
0	1	40-45	0
1	1	30-35	1
1	2	35-40	1-2
2	3	25-30	3-4

In vitro shoot formation

From the developed protocorm the shoot initiation was best and observed in medium containing MS+ IBA(2 mg/l), Kinetin(2 mg/l), GA3(4 mg/l) The media formed maximum of 3-4 shoots with in 30-35 days of their culturing. The shoots are still in the process of sub culturing as plant responded very slowly to their sub culturing.

Table 4 : Effect of different concentrations and combinations of growth hormones on shoot formation in *Cymbidium*

KN	<u>MS+growth hormones</u>		Days to multiple shoot formation from protocorm	Number of shoots per explants
	GA ₃	IBA		
0	0	0		
1	0	0	30	0
1	1	1	30	0
1	1	2	40-45	1-2
2	2	2	35-40	2-3
2	2	4	30-35	3-4



Fig. 1 Callus induction from bulbs.

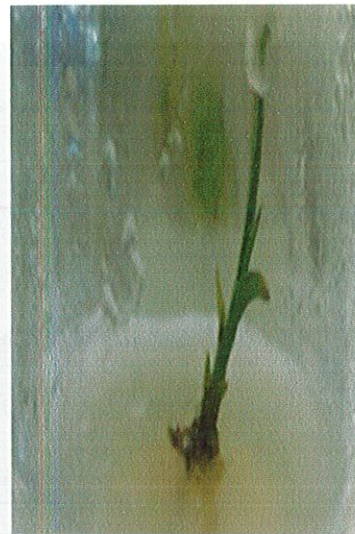
a. Tuber inoculated in auxin containing medium

b. Callus initiated and start growing in media after 45-50 days of incubation

a.



b



c.

Fig. 2

***In vitro* shoot formation from protocorm**

a. Protocorm in shooting medium

b. Shoot formed after 30-35 days

c. Shoot grown after 40- 45 days



Fig 3. Protocorm formation and increase in their number after change of concentration of media

CHAPTER 5

CONCLUSION

The identification of MS media supplemented with KN(2mg/l) + Gibberelic acid(1mg/l) + IBA(3mg/l) for better growth of orchids. Orchids are commercially very important flowers. This genus is distributed in tropical and subtropical Asia (such as northern India, China, Japan, Malaysia, the Philippines, and Borneo) and northern Australia. The larger flowered species from which the large flowered hybrids are derived grow at high altitudes. It is one of the most popular and desirable orchids in the world because of the beautiful flowers. As the plant is commercially very important (Rs500-600), having long shelf life and exploited very little for carrying out successful and more significant in vitro studies. This study was carried out with the objective of developing some rapid procedures for its micro propagation so that their production should be enhanced. As the plant is very tough to culture and give very slow responses in the nutrient media and also prone to many viral disease, therefore its exploration is always a challenging research. Culturing was done by using different explants such as petals, tubers, bulbs etc. Out of all the combinations the best results were obtained for shoot formation from in vitro derived calli from bulbs cultured on different media supplemented with 2,4-D and NAA. Different media were tried for increasing the number of protocorms. Various combinations of media were used for shoot formation and the best combination comprised of Kinetin, IBA, Gibberelic acid which not only increases but also accelerate the growth of shoot formation. So these tissue culture methodologies would definitely be an important catalyst for accelerating the growth and production of cymbidiums. As flowers are commercially very important and difficult to grow but these procedures will

provide another ground for the production of disease free and commercially important flower of floriculture industry.

STOCK D [100X]

Na ₂ B ₄ O ₇	1.75g
FeSO ₄ ·7H ₂ O	1.75g

STOCK E [100X]

KI	1.75g
----	-------

STOCK F [100X]

H ₂ BO ₃	1.75g
CoCl ₂ ·6H ₂ O	1.75g
ZnSO ₄ ·7H ₂ O	1.75g
CuSO ₄ ·5H ₂ O	1.75g
MnSO ₄ ·4H ₂ O	1.75g
Na ₂ MoO ₄ ·2H ₂ O	1.75g

APPENDIX – I

Media components (Inorganic)		
Components		Amount (gm/lt.)
STOCK A [10X]		
	KNO ₃	19.00
	MgSO ₄ .7H ₂ O	3.70
	KH ₂ PO ₄	1.70
STOCK B [20X]		
	NH ₄ NO ₃	33.00
STOCK C [100X]		
	CaCl ₂ .2H ₂ O	44.00
STOCK D [100X]		
	Na ₂ EDTA	3.726
	FeSO ₄ .7H ₂ O	3.785
STOCK E [100X]		
	KI	0.083
STOCK F [100X]		
	H ₃ BO ₃	0.62
	CoCl ₂ .6H ₂ O	0.0025
	ZnSO ₄ .7H ₂ O	0.86
	CuSO ₄ .5H ₂ O	0.0025
	MnSO ₄ .4H ₂ O	2.23
	Na ₂ MoO ₄ .2H ₂ O	0.025

APPENDIX – II

Media components (Organic)		
Components		Amount (gm/lt.)
STOCK G [100X]		
	m – Inositol	10.00
	Glycine	0.20
STOCK H [100X]		
	Pyridoxin HCl	0.05
	Nicotinic Acid	0.05
	Thiamine HCl	0.01

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SHALKI SHABNAM

Department of Biotechnology & Bioinformatics,

Jaypee University of Information Technology,

Waknaghat, Solan (H.P).

Cell: +91-9418450100

E-mail: shalki.shabnam@gmail.com

OBJECTIVE

To work with devotion, dedication and sincerity to achieve my professional and personal goals.

ACADEMIC QUALIFICATIONS

Standard	College/School	Year	CGPA/Percentage
B.Tech (Biotechnology)	JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY, SOLAN.	2011 (Still pursuing)	(Up till 7 th sem) 6.6 72%
12 th (C.B.S.E.)	ARMY PUBLIC SCHOOL NAHAN	2007	74.4%
10 th (I.C.S.E.)	CARMEL CONVENT SCHOOL NAHAN	2005	67.8%

TECHNICAL SKILLS

- **Programming languages:** C.
- **Bioinformatics Tools:** Blast, FASTA, Primer3.
- **Core subjects:** Microbiology, Genetics, Food and agricultural biotechnology, Genetic Engineering

AREAS OF INTEREST

- Bioterrorism
- Stem Cells and Healthcare
- Contemporary Marketing Practices
- Microbiology

PROJECTS

- **Currently involved in a project for the micro propagation of orchids.**
- Team Size: 2
- **Prepared cornstarch in my biotechnology lab.**
- Team Size:4

INDUSTRIAL TRAINING

- Successfully completed six week **training in Animal Biotechnology Center in National Dairy Research Institute Karnal, Haryana.**

PERSONAL ACHIEVEMENTS

- Member of **Hospitality Committee** FOR *LE-FIESTUS'08, 09, 10.*
- Member of **Literary Club** FOR *LE-FIESTUS'09.*
- An **active volunteer** of world's largest N.G.O "**The art of living**", having organized three YES! + Courses with a strength of 100+ participants.
- Have undergone **YES! + Course thrice** in JUIT.
- Delivered a seminar on the topic "**BIOTERRORISM**" and it was graded **excellent** in **NDRI.**
- Organized various events in Literary Club such as **Theme collage, Pentathlon etc.**
- Have done **stage compering** of various events in school and college.
- Blue House **captain** at school in 2005-2006.
- Participated in **Inter School Debate Contest Of ICSE Schools CHANDIGARH Region** held in **St. Xaveir's Public School Chandigarh.**
- Have undergone a workshop on "**INDIAN THEATRE AND PERSONALITY DEVELOPMENT**".
- Active Participant in **Dramatics.**
- Participated in various **Debate competitions** in school.

HOBBIES

- Reading books (preferably Indian writers)
- Listening music
- Cooking

LANGUAGES

- Hindi
- English
- Punjabi
- Learning French

PERSONAL DETAILS

Date of Birth : 31ST OCTOBER, 1989.

Father's Name : Dr.Pradeep Shabnam

Mother's Name : Dr. Neeru Shabnam

Residential Address: The Mall

Above Uco Bank, First floor

Nahan, Himachal Pradesh

Pin-1732001

Phone No. : +91-9418450100.


(Shalki Shabnam)

EVA AHUJA

Department of Biotechnology & Bioinformatics,
Jaypee University of Information Technology,
Waknaghat, Solan (H.P).
Cell: +91-8894886629
E-mail: evaa8652@gmail.com

OBJECTIVE

To make the best use of my skills to add value to the organization and imbibe maximum knowledge .

ACADEMIC QUALIFICATIONS

Standard	College/School	Year	CGPA/Percentage
B.Tech (BIOTECH)	JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY, SOLAN.	2011 (STILL PURSUING)	(Up till 7 th sem) (CGPA=7.2) 77%
12 th (C.B.S.E.)	DAV SEN. SEC SCHOOL,BATHINDA	2006	66%
10 th (C.B.S.E.)	ST JOSEPH CONVENT SECONDARY SCHOOL,BATHINDA	2004	87%

TECHNICAL SKILLS

- **Programming languages:** Basics of C.
- **Bioinformatics Tools:** BLAST, FASTA,PRIMER 3
- **Core subjects:** Fermentation and Downstream Processing, Microbiology, Genetics.

AREAS OF INTEREST

- Comparative and Functional Genomics.
- Fermentation technology
- Environmental biotechnology

PROJECTS

- **Currently involved in a project for the micro propagation of orchids.**
- Team Size: 2
- **Prepared cornstarch in my biotechnology lab.**
- Team Size: 4

INDUSTRIAL TRAINING

- Successfully completed the **training in Animal Biotechnology Center in National Dairy Research Institute Karnal, Haryana.**

PERSONAL ACHIEVEMENTS

- Delivered a seminar on the topic **PROBIOTICS** and was awarded an **excellent** grade in NDRI.
- Member of **Synapse (BIO CLUB).**
- Member of **Cultural Club** for **LE-FIESTUS'07**
- Have successfully contributed to **The Yeast Molecular Biology And Biochemical Analysis** workshop held in JUIT.
- Participated in **JUIT IDOL.**
- Have successfully organized a **blood donation camp** in JUIT.
- Have organized various events such as **sandwich making, water games and cultural night.**
- Have undergone two **YES+** workshops in JUIT.
- Secured **first position** in the skit competition in school.
- Participated in **group dance competition** and secured the **first position**
- Was a **lead singer** in many group singing competitions.
- Have done **compering** of various events in school.

HOBBIES

- Singing
- Listening music
- Reading (preferably Indian authors)

- Mimicry

LANGUAGES KNOWN

- English
- Hindi
- Punjabi

PERSONAL DETAILS

Date of Birth : 16th January, 1989
Father's Name : Mr. SUNEET AHUJA
Mother's Name : Mrs. MANJU AHUJA
Residential Address: 373 MODEL TOWN, PHASE-1, BATHINDA (PUNJAB)
Phone No. : +91-8894886629

(Eva Ahuja)