In vitro Propagation of *Dendrobium* Orchid var. Earsakul

Thesis

Submitted to the
Central Agricultural University, Imphal
in partial fulfilment of the requirements for the award of the degree of

Master of Science (Horticulture)

In

Floriculture and Landscape Architecture

By

Khaling Lallemmoi, B.Sc. Horticulture

U-19-AR-01-005-M-H-004

DEPARTMENT OF FLORICULTURE AND LANDSCAPE ARCHITECTURE

COLLEGE OF HORTICULTURE AND FORESTRY
Pasighat, Pin: 791102, Arunachal Pradesh, India

December, 2021

In vitro Propagation of *Dendrobium* Orchid var. Earsakul

Thesis

Submitted to the
Central Agricultural University, Imphal
in partial fulfilment of the requirements for the award of the degree of

Master of Science (Horticulture)

In

Floriculture and Landscape Architecture

By

Khaling Lallemmoi, B.Sc. Horticulture

U-19-AR-01-005-M-H-004

DEPARTMENT OF FLORICULTURE AND LANDSCAPE ARCHITECTURE

COLLEGE OF HORTICULTURE AND FORESTRY

Pasighat, Pin: 791102, Arunachal Pradesh, India

December, 2021

Affectionately

Dedicated

To

My beloved Family

COLLEGE OF HORTICULTURE AND FORESTRY

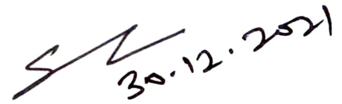
CENTRAL AGRICULTURAL UNIVERSITY

Pasighat, Pin-791102, Arunachal Pradesh, India

CERTIFICATE - I

Certified that **Ms. Khaling Lallemmoi [Registration No. U-19-AR-01-005-M-H-004]** has satisfactorily prosecuted her course of research for a period of not less than two semesters and that the title of the thesis entitled **"*In vitro* Propagation of *Dendrobium* Orchid var. Earsakul"** submitted by her to the Central Agricultural University, Imphal -795004 (Manipur) in partial fulfilment of the requirements for the award of the degree of **Master of Science (Horticulture)** in the subject of **Floriculture and Landscape Architecture** is the result of original research work conducted by her under my supervision and is sufficiently of high standard to warrant its presentation to the examination.

I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any University.



Date: 30.12.2021

(Sunil Kumar)

Name and Signature of the chairperson

Student's Advisory Committee

Tel : 0368-2224887. Te Fax: 0368-2225066

Email: pa_deanchf@hotmail.com/ripondam@gmail.com

COLLEGE OF HORTICULTURE AND FORESTRY

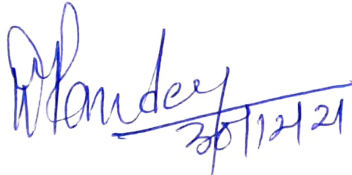
CENTRAL AGRICULTURAL UNIVERSITY

Pasighat, Pin-791102, Arunachal Pradesh, India

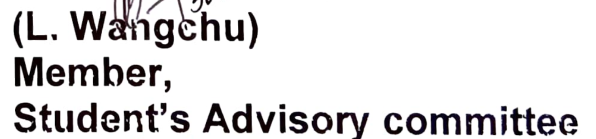
CERTIFICATE – II

This is to certify that the thesis entitled **"*In vitro* Propagation of *Dendrobium* Orchid var. Earsakul"** submitted by **Ms. Khaling Lallemmoi [Registration No. U-19-AR-01-005-M-H-004]** submitted to the Central Agricultural University, Imphal-795004 (Manipur) in partial fulfillment of the requirements for the award of the degree of **Master of Science (Horticulture)** in the subject of **Floriculture and Landscape Architecture** has been approved by the Student's Advisory Committee after oral examination jointly with an External Examiner.

(Sunil Kumar)
Chairperson,
Student Advisory Committee

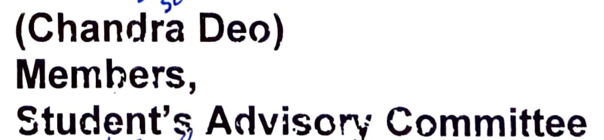


(D. K. Pandey)
Dean's Nominee

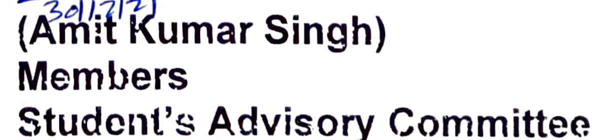


(L. Wangchu)
Member,
Student's Advisory committee

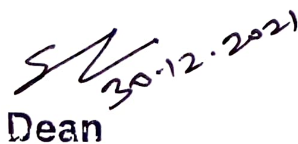
Head of the Department



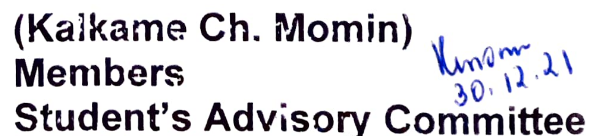
(Chandra Deo)
Members,
Student's Advisory Committee



(Amit Kumar Singh)
Members
Student's Advisory Committee



Dean



(Kalkame Ch. Momin)
Members
Student's Advisory Committee

DECLARATION

I hereby declare that the thesis entitled **"*In vitro* Propagation of *Dendrobium* Orchids var. Earsakul"** is an authentic record of work done by me and that no part thereof has been presented for the award of any other degree, diploma, associateship, fellowship or any other similar title.

(Khaling Lallemmoi)

Date 30-12-2021

Place: Pasighat

ACKNOWLEDGEMENTS

I thank God for the bountiful blessings and the perfect plan he has for me.

I am immensely thankful to the chairman of my advisory committee Dr. Sunil Kumar, Professor and Head, Department of Floriculture and Landscape Architecture, College of Horticulture and Forestry, Central Agricultural University, for his constant guidance, motivation, constructive suggestion and criticism without which I would not be able to complete my thesis and shall remain grateful eternally.

I wish to convey my sincerest gratitude to the members of my advisory committee Dr. L. Wangchu, Associate Professor and Head, Department of Fruit Science, Dr. Chandra Deo, Associate Professor and Head, Department of Vegetable Science, Dr. Amit Kumar Singh, Assistant Professor, Department of Basic Sciences and Humanities and Dr. Kalkame Ch. Momin, Assistant Professor Department of Floriculture and Landscape Architecture for their constant support, valuable suggestion and insightful remarks.

I convey my gratitude to Dr B.N. Hazarika, Dean, College of Horticulture and Forestry, Pasighat for his advice and all the necessary facilities for my research.

I would like to extend my unfeigned gratitude towards my teachers, Dr. A.K. Sahu, Associate Professor and Head, Basic Sciences and Humanities, Dr. Siddhartha Singh, Assistant Professor Basic Sciences and Humanities, Dr. Khwairakpam Lily Devi, Assistant Professor, Department of Floriculture and Landscape Architecture, Dr. Bharghav Veluru, Assistant Professor, MTTC and VTC and Dr. Arunkumar Phurailakpam, Associate Professor, Department of Floriculture and Landscape Architecture for their professional guidance.

My deepest gratitude to Dr. N. Surmina Devi, Assistant Professor, Department of Plant Protection, Dr. Shivani Dobhal, Assistant Professor, Department Forest Biology and Tree Improvement, Ms. Tasso Yatung, Assistant Professor, MTTC and VTC and Dr. Asieleavio John, Assistant Professor Deparment of Forest Product and Utilization for their unceasing support.

I extend my gratitude toward the non-teaching staffs of Floriculture and Landscape Architecture, Ms. Sudipta, Mr. Kamal, Ms. Jopi, Ms. Nang and others for always readily extending their helping hand.

My cordial appreciation toward Mr. Chandrakumar and Mr Santhosh, non-teaching staffs of Basic Sciences and Humanities, for always readily helping me during my research and Mr. Manoj, non-teaching staff, Department of Natural Resource Management for always readily extending help when approached.

I express my sincere gratitude to my seniors, Che Alice, Che Rina, Che Leima, Che Bidya (Chaobi), Sis. Oying, Sis. Jome, Sis. Monya, Sis. Megha, Sis. Mounika, Brother Theja and Brother Vivek for their guidance during my research.

My earnest and heartfelt gratitude toward all my friends Gunnu, Omem, William, Anush, Kripa, Unshani, Mashine, Bidya, Indira, Sunayna, Geying, Olivia, Parmoi, Tetei,Koku, Aruna and Shweta who supported me through thick and thin.

My beloved family to whom I owe everything, I am beyond thankful for all the love and support I receive constantly.

Many more are not mentioned but none are forgotten.

Date : 30-12-2021

Place: Pasighat

(Khaling Lallemmoi)

CONTENTS

LIST OF TABLES		viii
LIST OF FIGURES		ix
LIST OF SYMBOLS/ABBREVIATIONS		xi
ABSTRACT		xiv
1	**INTRODUCTION**	**1**
2	**REVIEW OF LITERATURE**	**5**
2.1	Influence of different plant growth regulators (PGRs) for callus induction	5
2.2	Effect of light and dark in callus induction and formation	8
2.3	Effect of plant growth hormones on shoot and root proliferation	9
3	**MATERIALS AND METHODS**	**13**
3.1	Experimental site	13
3.2	Climate and weather	13
3.3	Experimental materials	13
3.4	Experimental methods	15
3.5	Observations recorded	16
3.6	Statistical analysis	19
4	**RESULTS**	**22**
4.1	Performance of plant growth regulators combination for callus initiation and formation under 16 hr normal light.	22
4.2	Performance of plant growth regulators combination for callus initiation and formation under complete dark	32

4.3	Performance of plant growth regulators for shoot proliferation	40
4.4	Performance of plant growth regulators for root proliferation	53
5	**DISCUSSION**	**64**
5.1	Performance of plant growth regulators combination for callus initiation and formation under 16 hr normal light.	64
5.2	Performance of plant growth regulators combination for callus initiation and formation under complete dark	65
5.3	Performance of plant growth regulators for shoot proliferation	65
5.4	Performance of plant growth regulator for root proliferation.	66
6	**SUMMARY AND CONCLUSION**	**68**
6.1	Performance of plant growth regulators combination for callus initiation and formation under 16 hr normal light	68
6.2	Performance of plant growth regulators combination for callus initiation and formation under complete dark	68
6.3	Performance of plant growth regulators for shoot proliferation,	68
6.4	Performance of plant growth regulators for root proliferation	69
6.5	Conclusion	69
6.6	Future scope of research.	69
	BIBLIOGRAPHY	70

LIST OF TABLES

Table	Title	Page
Table 4.1.1	Effect of PGRs on number of days taken for callus initiation under 16 hr normal light. (square root value)	24
Table 4.1.2	Effect of PGRs on eight of callus (mg) under 16 hr normal light.	25
Table 4.1.3	Effect of PGRs on percentage of callus Formation under 16 hr normal light (arcsine value).	26
Table 4.2.1	Effect of PGRs on number of days taken for callus initiation under complete dark. (square root value)	34
Table 4.2.2	Effect of PGRs on weight of the callus under complete dark (mg)	35
Table 4.2.3	Effect of PGRs on percentage of callus formation under complete dark (arcsine value)	36
Table 4.3.1	Effect of PGRs on days taken for shoot initiation (square root value)	43
Table 4.3.2	Effect of PGRs on number of shoot. (square root value)	44
Table 4.3.3	Effect of PGRs shoot length (cm)	45
Table 4.3.4	Effect of PGRs on number of leaves (square root value)	46
Table 4.3.5	Effect of PGRs on Leaf length (cm)	47
Table 4.3.6	Effect of PGRs on shoot weight (mg)	48
Table 4.4.1	Effect of PGRs on days taken for initiation of root (square root value)	55
Table 4.4.2	Effect of PGRs on number of roots (square root transformation)	56
Table 4.4.3	Effect of PGRs on Root length (cm)	57
Table 4.4.4	Effect of PGRs on Root weight (mg)	58

LIST OF FIGURES

Fig 3.1	Sterilization, Inoculation and Tissue culture laboratory	21
Fig. 4.1.1	Effect of PGRs on number of days taken for callus initiation under 16 hr normal light. (square root value)	24
Fig. 4.1.2	Effect of PGRs on weight of callus (mg) under 16 hr normal light.	25
Fig. 4.1.3	Effect of PGRs on percentage of Callus Formation under 16 hr normal light (arcsine value).	26
Fig. 4.1.4	Callus under 16 hr normal light on 15th day after inoculation	28
Fig.4.1.5	Callus under 16 hr normal light on 30th day after inoculation	29
Fig 4.1.6	Callus under 16 hr normal light on 45th day after inoculation	30
Fig 4.1.7	Callus under 16 hr normal light on 60th day after inoculation	31
Fig. 4.2.1	Effect of PGRs on number of days taken for callus initiation under complete dark. (square root value)	34
Fig. 4.2.2	Effect of PGRs on weight of the callus under complete dark (mg)	35
Fig. 4.2.3	Effect of PGRs on percentage of callus formation under complete dark (arcsine value)	36
Fig 4.2.4	Callus under complete dark on 30th day after inoculation	37
Fig.4.2.5	Callus under complete dark on 45th day after inoculation	38
Fig 4.2.6	Callus under complete dark on 60th day after inoculation	39

Fig. 4.3.1	Effect of PGRs on days taken for shoot initiation (square root value)	43
Fig. 4.3.2	Effect of PGRs number of shoot. (square root value)	44
Fig. 4.3.3	Effect of PGRs shoot length (in cm)	45
Fig. 4.3.4	Effect of PGRs on number of leaves (square root value)	46
Fig. 4.3.5	Effect of PGRs on Leaf length (cm)	47
Fig. 4.3.6	Effect of PGRs on shoot weight (mg)	48
Fig 4.3.7	Shoot proliferation on 15th day	49
Fig 4.3.8	Shoot proliferation on 30th day	50
Fig 4.3.9	Shoot proliferation on 45th day	51
Fig 4.3.9	Shoot proliferation on 60th day	52
Fig. 4.4.1	Effect of PGRs on days taken for initiation of root (square root value)	55
Fig. 4.4.2	Effect of PGRs on number of roots (square root transformation)	56
Fig. 4.4.3	Effect of PGRs on Root length (cm)	57
Fig. 4.4.4	Effect of PGRs on Root weight (mg)	58
Fig.4.4.5	Root proliferation after 15th days	59
Fig.4.4.6	Root proliferation after 30th days	60
Fig.4.4.7	Root proliferation after 45th days	61
Fig.4.4.8 (a)	Root proliferation after 60th days	63
Fig.4.4.8 (b)	Root proliferation after 60th days	63

LIST OF SYMBOLS/ABBREVIATIONS

%	Percentage
.	Points
@	at the rate
$^{-1}$	per
1/2	Half
µl	microliter
2,4,D	2,4- Dichlorophenoxyacetic Acid
BA	Benyladenine
BAP	Benzyl aminopurine
℃	Degree Celsius
$CaCl_2.4H_2O$	Calcium chloride tetra tetrahydrate
CAU	Central Agricultural University
CD	Critical difference
cm	centimeter
$CoCl_2.6H_2O$	Cobalt (II)chloride hexahydrate
CRD	Completely Randomized Design
$CuSO_4.5H_2O$	Copper sulphatepentahydrate
cv.	Cultivar
eg.	Example
et al.	Et alia (and others)
Fe.EDTA	Ferric ethylenediaminetetraacetic acid

$FeSO_4.7H_2O$	Ferrous Sulfateheptahydrate
Fig.	Figure
g	gram
H_3BO_3	Boric Acid
HCl	Hydrochloric acid
$HgCl_2$	Mercuric Chloride
i.e.	that is
IAA	Indole acetic acid
IBA	Indole 3 butyric acid
KC	Knudson C
KH_2PO_4	Monopotassiumphosphate
KI	Potassium Iodide
KIN	Kinetin
KNO_3	Potassium Iodide
L^{-1}	per litre
mg	milligram
$MgSO_4.7H_2O$	Magnesium sulphate Heptahydrate
ml	millilitre
$MnSO_4.4H_2O$	Manganese (II) sulphate tetrahydrate
MS	Murashige and Skoog
N	Normal

$Na_2.EDTA.2H_2O$	Ethylenediaminetetraacetic acid disodium salt dihydrate
$Na_2MoO_4.2H_2O$	Sodium Molybdate dihydrate
NAA	Naphthaleneacetic acid
NaOH	Sodium hydroxide
NH_4NO_3	Ammonium Nitrate
PGR	Plant Growth Regulator
pH	Potential of hydrogen
PLB	Protocrom like body
Psi	pound per square inch
RH	Relative humidity
UV	Ultraviolet
var.	variety
viz	namely
$ZnSO_4.7H_2O$	Zincsulphate heptahydrate

Name: Khaling Lallemmoi
Chairperson: Dr. Sunil Kumar

Registration No.: U-19-AR-01-005-M-H-004
Degree: M.Sc. (Horticulture)
Major: Floriculture and Landscape Architecture

Thesis title: *In vitro* propagation of *Dendrobium* Orchid var. Earsakul

ABSTRACT

The present experiment was conducted at the tissue culture laboratory of the Department of Basic Sciences and Humanities, College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh during the year 2020-2021. Murashige and Skoog media with pH 5.6 were used as growing media for *in vitro* culture. *Dendrobium* orchid var. Earsakul explant of 5 to 6 months old was collected from the Orchidarium of College of Horticulture and Forestry, Pasighat for *in vitro* culture. The present study was conducted with four experiments.

Significant responses of plant growth regulator (PGR) combinations for callus initiation and formation under 16 hr normal light were observed during first experimentation. Earliness in callus initiation (3.24 days) was recorded in treatment combinations 0.25 mg/l KIN + 5 mg/l NAA followed by 0.5 mg/l KIN + 0.5 mg/l 2,4D (3.50 days). However, maximum callus weight was associated with 0.5 mg/l KIN + 0.5 mg/l NAA (308.10mg) followed by 0.25 mg/l KIN + 5 mg/l NAA (261.10 mg) and was at par with 0.25 mg/l BAP + 0.05 mg/l IAA (255 mg). Maximum percentage callus formation (66.66 percent) recorded in 0.25 mg/l BAP + 0.05 mg/l IAA which was at par with 0.25 mg/l KIN + 5mg/l NAA (65.86 percent) and 0.5 mg/l KIN + 7.5mg/l NAA (65.31 percent).

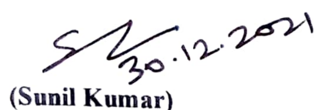
Significant effect of plant growth regulator (PGR) combinations for callus initiation and formation under complete dark were noticed during second experimentation. Minimum days taken for callus initiation (4.28 days) was recorded in 0.25 mg/l BAP + 0.05 mg/l IAA followed by 1 mg/l KIN + 1 mg/l 2,4-D (4.46 days). Highest percentage callus formation (43.55 percent) were obtained with treatment combinations 0.5 mg/l KIN + 7.5 mg/l NAA) on the 60th days and was at par with 0.25 mg/l KIN + 5mg/l NAA (42.69 percent). Whereas, increased callus weight was noticed in 0.5 mg/l KIN + 7.5mg/l NAA (247.45 mg) followed by 1mg/l KIN +10mg/l NAA (234.30 mg)

Significant result of plant growth regulator (PGR) combinations for shoot proliferation were revealed during third experimentation. Shortest days for shoot initiation (3.41 days) had been noticed with treatment combinations 3 mg/l BAP + 0.6 mg/l NAA which was at par with 4 mg/l BAP + 0.8 mg/l NAA (3.50 days) and 2.5 mg/l BAP + 0.5 mg/l NAA (3.56 days). However, the highest number of the shoots (1.73) was found in treatment combination 2.5 mg/l BAP + 0.5 mg/l NAA and was at par with 3.5mg/l BAP +0.7 mg/l NAA(1.72). Maximum shoot length (1.93 cm) was recorded in 1.5mg/l BAP + 0.125mg/l NAA which was at par with 2mg/l BAP +0.25 NAA (1.82 cm). Moreover, increased leaf length in 3.5mg/l BAP+0.7 mg/l NAA (1.91 cm) was recorded, however, maximum number of leaves was found in 3 mg/l BAP + 0.6 mg/l NAA (2.04) and was at par with 3.5mg/l BAP + 0.7 mg/l NAA (2.01). Enhanced shoot weight with the treatment combinations 2 mg/l BAP + 0.25 mg/l NAA (619.03 mg) was noticed and at par with 0.5 mg/l BAP + 0.06 mg/l NAA (603.13 mg).

Significant consequence of plant growth regulator (PGR) combinations for root proliferation were ascertained during fourth experimentation. Minimum days taken for root proliferation (2.96 days) was obtained from the treatment combinations 0.5 mg/l BAP + 1 mg/l IBA followed by 1 mg/l BAP + 2 mg/l IBA (3.24 days) and 0.25 mg/l BAP + 0.5 mg/l IBA (3.74 days). Highest number of roots (3.39) was found in 0.5 mg/l BAP + 1 mg/l IBA which was at par with 0.25 mg/l BAP + 0.5 mg/l IBA (3.36) and 1 mg/l BAP + 2 mg/l IBA(3.31). However, increased root weight (11.08 mg) in 0.25 mg/l BAP + 0.5 mg/l IBA followed by 0.5 mg/l BAP + 1 mg/l IBA (10.77 mg) was noted. Moreover, highest root length (1.38 cm) in 0.25 mg/l BAP + 0.5 mg/l IBA followed by 0.5 mg/l BAP + 1 mg/l IBA (1.25cm) were showed.

Thus, the present experimentation reveals that addition of Kinetin and NAA for callus induction and formation under 16 hr normal light as well as BAP and NAA for shoot proliferation and BAP and IBA for root proliferation showed positive responses. It is apparent through entire investigation that 0.5 mg/l KIN + 7.5mg/l NAA application was significantly associated with callus induction and formation for *in vitro* propagation of *Dendrobium* Orchid var. Earsakul when explant placed in the 16 hr normal light. However, the treatment combinations 2.5 mg/l BAP+0.5 mg/l NAA became more pertinent for shoot proliferation and 0.5 mg/l BAP +1 mg/l IBA for root proliferation.

Keyword: *Dendrobium, in vitro*, PGR, callus, shoot, root



(Sunil Kumar)

Chairperson

(Khaling Lallemmoi)

Author

Chapter-1
Introduction

Orchids belonging to the family Orchidaceae, are diverse flowering plants both in ecology and morphology. It is the second-largest flowering plant in the world comprising about 779 genera and 22,500-35,000 species (Monalisha *et al*., 2017). The number of species under the family Orchidaceae constitutes roughly 10% of all flowering plants in the plant kingdom. Orchids are found to grow in all the continents except Antarctica. It adapts to broad environmental conditions from thick cloudy forests, lowlands, tundra and desert. Large numbers of Orchid species are found in tropical forests around the equatorial area. In addition to the species, many hybrids and cultivated forms continue to increase at a steady rate year after year. Orchids are popular in global trade as it accounts for about 10% of the total international fresh flower trade. India share in international Orchid trade is only 0.02% in 2007 (De and Medhi, 2015) even though Orchids alone made 9% of the Indian flora, about 1,350 species found in India which are distributed in different regions of India particularly North-western Himalayas, North-eastern and Western Ghat (Kataki *et al.*, 1984). Approximately 900 species (nearly 69 %) are from North East India (NE) alone and around 150 species are endemic to this region (De and Singh, 2015).

The genus *Dendrobium* is the third-largest in the family Orchidaceae (Leitch *et al.,* 2009). It is a diverse genus of Orchids that are commonly used as a cut flower because of their sturdy stems and distinctive colours (Singh, 2006). The genus *Dendrobium* has more than 70 species making it the largest epiphytic genus (Ninawe and Swapna, 2017) the name *Dendrobium* is derived from the Greek word *Dendron* which means tree and *bios* meaning life or simply one who lives on a tree or simply epiphyte. It grows best at night temperatures between 15-18°C and day temperatures 23-29°C. The light intensity required for better growth and flowering is 25 to 30 Kilo Lux and relative humidity 50-75%. They can be propagated by separating the keikis, division, stem cutting and micropropagation.

Dendrobium Earsakul is a mutant of *Dendrobium* Sonia BOM, *Dendrobium* Sonia (*Dendrobium ceasar* X *Dendrobium tomei* Drake). The number of inflorescence per plant is more than 5, Inflorescence is erect, medium-length (20 -40 cm), flower number per inflorescence is few (<7), does not have fragrance, flower longevity on the plant is long (>30 days), sepal dominant colour is purple, sepal colour

pattern inside mixed, petal predominant colour is pink, lip predominant colour is purple, have double lip colour purple and green-yellow.

This concept of *In vitro* propagation or micropropagation was first proposed by Haberlandt in the year 1902, a German Botanist, who made the first attempt to use the *In vitro* method to grow plant tissues. Moore and Noel Bernard also attempted *in vitro* symbiotic germination (Yam *et al.*, 2002). Asymbiotic germination was possible only after the formulation of Knudson B and C medium. Sexual propagation is less desirable for commercial cultivars in Orchids due to the long juvenile period and Orchids are out breeder so, sexual propagation leads to the production of the heterozygous plant. In the year 1949, Rotor at Cornell University made a demonstration that plantlet could be induced by aseptic culturing of the dormant buds on the basal nodes of *Phalaenopsis* inflorescence. Morel (1960) cultured shoot tips for obtaining virus-free *Cymbidium* clones. It is possible to obtain more than four million plants in a year from a single bud by repeatedly sectioning and sub-culturing the protocorm like bodies (PLBs) through *In vitro* propagation, this have also created interest among the Orchid growers and has revolutionized the Orchid industry (Chugh *et al.*, 2009).

Micropropagation is the most efficient means for mass production of a selected species or hybrid. Plants are totipotent in nature this property has been exploited to multiply the plant. In this method any part of the plant is taken as explant, sterilized, cut into minute pieces and inoculated into a flask containing artificial culture media. It has many applications such as rapid propagation, disease eliminations, embryo rescue, germplasm conservation, induced somaclonal variation, somatic hybridization, genetic engineering, production of secondary metabolites, etc.

The content in the culture media greatly affects the growth and development of the explant into plantlet. Growth regulators and plant hormones are known as intrinsic factors which are also important for plant survival. There are synthetic growth regulators that can be added extrinsically to the plants to regulate their growth and development. Cytokinins and auxins are one of the main constituents in a culture media that plays an important role in the micropropagation of Orchids and they are important for organogenesis in *in vitro* culture. Auxin mainly helps in ovule/ovary maturation, formation of vegetative structure, root induction and others. Cytokinin is mainly responsible for the induction of axillary shoots without intervening callus (Sarmah *et al.,* 2017).

Intensive studies have been made in the field of tissue culture for the last 50 years but literature is scarce concerning the production behaviour of Orchids especially *Dendrobium* in different climatic conditions (Reddy *et al.,* 2021).

Dendrobium propagules are limited so, purchasing the plantlet from a different state or country become expensive for the grower, a working protocol for rapid multiplication using micropropagation is necessary to meet the demand locally and export to earn foreign currency. Hence, keeping in view the above points the objectives for the experiment was as follows:

1. To standardize plant growth hormones for callus induction and formation.
2. To study the effect of light and dark in callus induction and formation.
3. To standardize plant growth hormones for shoot and root proliferation.

Chapter-2
Review of Literature

Dendrobium Orchid var. Earsakul is a popular commercial variety used as cut flower in both domestic and international markets. *In vitro* propagation is popular for the production of quality planting material thus various studies have been conducted by many researchers hence, a brief review of literature embracing the relevant references related to the different aspects of the present investigation has been presented below.

2.1 Influence of different plant growth regulators (PGRs) for callus induction
2.2 Effect of light and dark in callus formation
2.3 Effect of plant growth hormones on shoot and root proliferation

2.1 Influence of different plant growth regulators (PGRs) for callus induction

Mohammadi and Kaviani (2019) studied micropropagation of *Phalaenopsis amabilis* Blume var. Grandiflora by culturing protocrom like body (PLBs) in Murashige and Skoog (MS) medium containing different concentrations of Kinetin (KIN; 0.00, 0.50, 1.00, 2.00 and 3.00 mg /l) and Indole 3 butyric acid (IBA; 0.00, 0.10, 0.20, 0.50 and 1.00 mg /l), either individually or in combination and activated charcoal (AC; 0.00, 0.50 and 1.00 g /l). Cultured medium containing 0.20 mg /l IBA and 0.50 mg/l KIN induced largest number of callus (9.10±0.611).

Thokchom and Maitra (2017) studied micropropagation of *Anthurium andreanum* cv. Jewel and reported that MS media supplemented with 0.5 mg/l BAP took minimum days for callus initiation but maximum callus was observed in 2mg/l NAA (77.33%), minimum days (27.83) for shoot regeneration was observed in 3.0 BAP + 0.5 mg/l NAA, 2 mg/l BAP + 0.5 mg/l NAA gives highest regeneration percentage (98.89%) and maximum shoot length (2.23 cm).

Refish *et al.* (2016) experimented to establish and optimise embryonic callus induction in *Dendrobium candidium* through shoot explant using 2, 4-D and NAA or Kinetin and observed significant differences between callus and PLB's (protocrom like body) using the MS medium containing 0.5 mg/l 2, 4-D, 0.25 mg/l NAA or 0.5 mg/l Kinetin after 6-9 months.

Romeida *et al.* (2016) conducted an experiment to study the embryonic callus induction in pencil orchid (*Papilionanthe hookeriana Rchb.f.*) through *in vitro* culture and found that the best treatment was MS medium added with 1.00 mgL^{-1} 2, 4-D which produced the highest number of embryogenic calli, and the biggest callus diameter (3.5 cm), characterized by transparent green colour and friable callus structure.

Hardjo *et al.* (2016) carried out an experiment to study induction of Somatic Embryo from Basal Leaf Segments of *Vanda tricolor* Lindl. var. Pallida. The best medium for embryogenic callus formation and proliferation was 0.05 mgL^{-1} NAA in combination with 0.01 mgL^{-1} BAP. The callus after 30 days were cultured in half strength MS with PGR to form the shoot.

In an experiment conducted by Tarrahi and Rezanejad (2013), to study Callogenesis and production of anthocyanin and chlorophyll in callus cultures of vegetative and floral explants in *Rosa gallica* and *Rosa hybrida* (Rosaceae). The investigation was carried out using different combinations of 2,4-dichlorophenoxyacetic acid, 6-benzylaminopurine, and gibberellic acid on modified Murashige and Skoog medium. The highest callogenesis was obtained on Murashige and Skoog medium containing ratios of 2 and 3 mg L^{-1} of 2,4-dichlorophenoxyacetic acid to 1 mg L^{-1} of 6-benzylamino purine. Stem explants in *R. gallica* initiated callus after 4 days.

Mei *et al*. (2012) conducted an experiment to develop a protocol of callus induction and protocrom like body regeneration of *Dendrobium*' Sonia-28. The PLBs segments were cultured on half-strength MS semi-solid medium supplemented with different concentrations of 1-naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) alone and in combination and reported that 1.0 mg/l NAA and 0.1 mg/l 2,4-D was optimal for callus induction.

Ng and Saleh (2011) studied *in vitro* propagation of *Paphiopedilum* orchid through the formation of protocorme-like bodies (PLB). The highest number of secondary PLBs formed was obtained on half-strength MS medium supplemented with 4.0 μM Kinetin, with an average of 4.1 PLBs per explant after 8 weeks of culture.

Kumar *et al.* (2010) studied *in vitro* callogenesis and differential callus growth rate from hypocotyl, cotyledon and leaf explants of three cultivars of *Capsicum annum* L. X-235, PC-1 and Pusa Jwala and found that the best medium for a maximum frequency of callus induction from hypocotyl explants of these 3 genotypes

was MS medium supplemented with 2,4-D (1.0 mg/l) and BAP (2.0 mg/l). The hypocotyl explants manifested maximum callus fresh weight (1.16 g) in comparison to cotyledon and leaf explants.

Maridass *et al.* (2010) conducted an experiment on *in vitro* propagation of *Dendrobium nanum* using rhizome bud as an explant. The maximum percentage of callus induction was obtained from the rhizome bud explants cultured on MS basal medium at 2.0 $\mu M/l^{-1}$ NAA and 1.2 $\mu M/l^{-1}$ Kinetin. The obtained micropropagated orchid was successfully reintroduced into their natural habitat (85% of survival after 3 months).

Yu *et al.* (2009) studied plant regeneration by callus-mediated protocrom like body induction of *Anthurium andraeanum* Hort. optimum callus proliferation was manifested on a 1/2 strength MS medium containing 0.90 µmol L^{-1} 2,4-dichlorophenoxyacetic acids (2,4-D) and 8.88 µmol L^{-1} N6-benzyladenine (BA) which was furthered subculturing on 1/2 MS medium containing 0.90 µmol L^{-1} 2,4-D and 4.44 µmol L^{-1} BA.

Khosravi *et al.* (2008) establish an *in vitro* propagation protocol for *Dendrobium* cv. Serdang Beauty. The propagation protocol utilized calli tissues that were successfully initiated from protocrom-like bodies (PLBs) explants. Calli was successfully regenerated on media supplemented with either Kinetin or BAP (cytokinins) and combined treatments of Kinetin and IAA (4 mg/l) or NAA (1.5 mg/l).

Roy *et al.* (2007) studied direct and callus mediated protocrom like body induction from shoot tips of *Dendrobium chrysotoxum* Lindl. The frequency of callusing was best in the presence of 2 mM thidiazuron (TDZ) or N6-benzylaminopurine (BAP). BAP was evident to advance callus growth and PLB differentiation.

Roy and Banerjee (2003) investigated induction of callus and to regenerate *Dendrobium fimbriatum* from shoot tip explant. Different concentrations of N6 benzylaminopurine (BAP) and α Naphthaleneacetic acid (NAA) showed callus development in 2 weeks. The media with 0.5 mgL^{-1} NAA and 1 mgL^{-1} BAP showed optimum results.

Tokuhara and Masahiro (2001) examined in their studies to induced embryogenic callus and cell suspension culture from shoot tips explant of *Phalaenopsis* orchid where 73 % of callus development was recorded from culture media

supplemented with 0.5 µM α-naphthaleneacetic acid, 4.4µM 6-benzylaminopurine and 29.2 mM sucrose.

Mandal and Datta (2002) established organogenic callus culture was established from immature flower buds of gerbera on a modified MS medium supplemented with IAA and BAP.

2.2 Effect of light and dark in callus induction and formation.

Chen *et al.* (2019) performed an experiment on the effect of light intensity and plant growth regulators on callus proliferation and shoot generation in *Haworthia*. The results showed that the highest callus proliferation (93.15%) was obtained with 1.0 mg L^{-1} BA+0.1 mg L^{-1} NAA under a light intensity of 10 µmol m^{-2} s^{-1}.

The effect of light and dark on callus induction and regeneration in Tobacco (*Nicotiana tabacum L.)* was investigated and early callus initiated on the 5th day was resulted from nodal segment explant and the highest amount of callus (97.20%) and highest plant regeneration (95.56%) was recorded in light conditions which were around five times higher than in dark. (Siddique and Islam, 2015)

Kumari *et al.* (2013) standardized *in vitro* propagation protocol in *Dendrobium Sonia* 'Earsakul' using stem nodal explant on ½ MS medium supplemented with 4mg/L BAP and observed early bud break.

Afshari *et al.* (2011) described the effects of light, different concentrations of PGR and micro and macro elements on callogenesis in rapeseed (*Brassica napus* L.) and observed that auxins had an inhibitory effect on chlorophyll formation, whereas cytokinin promotes it. However callogenesis was enhanced when 2,4,D and BAP where supplemented in combination and the explants under light conditions is more beneficial for callogenesis when compared to dark.

Ram *et al.* (2011) induced anthocyanin pigments in callus cultures of *Rosa hybrida* L.cv. 'Pusa Ajay' in response to sucrose and ammonical nitrogen level for explants petal and leaf discs under light and dark, Profuse and early callus induction on solid Murashige and Skoog (MS) medium supplemented with 4.0 mgL^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) under dark condition was observed.

Gow *et al.* (2009) inspected the effect of light on the embryogenesis of *Phalaenopsis* orchids and reported direct embryogenesis was retarded when explants

were placed in light and the larger embryos that are whitish to pale green in colour was observed.

Chung *et al.* (2005) examined the effects of light and dark on direct embryo induction of *Dendrobium* cv. Chiengmai Pink was cultured on 1/2 Murashige and Skoog (MS) medium supplemented with different auxins and cytokinins and observed that explants cultured in the light had a higher embryogenic response compared with those cultured in darkness.

Pelkonen *et al.* (1999) investigated the effects of light and different auxins on regeneration in lily (*Lilium regale* Wil.) callus at a morphologic and anatomic level. Their results showed a high regeneration capacity for lily callus and a rate of somatic embryogenesis and differentiation was promoted when the callus was kept in the dark during propagation and transferred into light only before regeneration.

Khui and Sink (1982) investigated callus induction of *Rosa Manetti* Hort. and *R. hybrida* L. 'Tropicana'where the explants were cultured either in dark or in light (2 Klux 16 h/day) at 26±2 ℃ which resulted in formation of Friable, fast-growing callus after 3 weeks on MS supplemented with 2.0 mg/l 2,4-D, 0.25 mg/l Kinetin and 2.0 g/l casein hydrolysate.Callus initiation occurred faster in dark than in light, but deteriorated when continuously sub-cultured in the dark regardless of media.

2.3 Effect of plant growth hormones on shoot and root proliferation

Micropropagation on *Dendrobium palpebrae* Lindl. using *in vitro* developed pseudobulb culture as explant in MS medium supplemented with auxins (IAA, IBA, NAA, Picloram) and cytokinins (BAP, Kinetin) reported average highest number of multiple shoot buds (6.43 ± 0.40-8.21 ± 0.44) on MS medium with 1.0 mg/l NAA + 2.0 mg/l BAP (Bhowmik and Raman 2020).

Erawati *et al.* (2020) reported that vanilla shoot was not influenced by exogenous growth regulator and BAP 3 mg/l was most suitable for shoot multiplication and development.

Chookoh *et al.* (2019) experimented on *Tolumnia* orchid to induce protocrom like body through micropropagation and found MS medium supplemented with 2mg/l BA and 0.5 mg/l NAA to give the highest rate of PLB induction (16.7%) for mass propagation in a short period.

Priyanka *et al.* (2018) conducted an experiment on *in vitro* regeneration and multiplication of *Dendrobium sp*. on different concentrations of NAA and BAP alone and combination and found that MS medium with BAP at 2.5mg showed the best result for shoot regeneration, the highest number of shoot and leaves were recorded for MS medium supplemented with 0.5 mg/l NAA with 0.1 mg/l BAP.

Beura *et al.* (2017) carried out an investigation to standardize the plant bioregulator for *in vitro* shoot proliferation of *Curcuma longa* L.Cv. Roma using fingertip as explant, the best performance in shoot proliferation was recorded in MS media supplemented with BAP (3.0mg/l) and NAA (0.2mg/l).

Regmi *et al.* (2017) develop a protocol for *in vitro* propagation of *Cymbidium aloifolium,* through protocrom culture and observed that MS medium supplemented with various concentration of plant growth regulators, 6-Benzylaminopurine (BAP, 0.5; 1; 1.5; 2 mg/l) or α-Naphthalene Acetic Acid (NAA, 0.5; 1 mg/l) or their combination. BAP (1 mg/l) and NAA (1 mg/l) resulted in maximum induction of rootless healthy shoots.

Riva *et al.* (2016) conducted an experiment on *in vitro* regeneration and multiplication of *Dendrobium bensoniae*, media supplemented with 0.5 mg/l BA with 1.0 mg/l IBA showed the most effective rooting.

Goswami *et al.* (2015) reported that *in vitro* regeneration of *Dendrobium* orchid using lip tip explant by supplementing with PGRs (2,4-D, NAA and BAP) added to Murashige and Skoog (MS). The maximum PLB was found to be developed in a medium containing 10 mgL^{-1} 2, 4-D, and optimum shoot and root were developed in medium supplemented with 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP in 60 days.

Pradhan *et al*. (2013) studied the plant regeneration from the shoot tip of *Dendrobium densiflorum* and observed shoot multiplication after three weeks of culture as well as the maximum number of healthy shoots using the treatment combination BAP (2 mg/l) with NAA (0.5 mg/l).

Abubacker *et al.* (2013) worked to develop an efficient protocol for micropropagation of orchid *Dendrobium barbatum* Lindl. where Murashige Skoog (MS) was used as basal medium supplemented with 1.0 mg/l of Naphthalene Acetic Acid (NAA) + 2.0mg/l of Benzyl Amino Purine (BAP) for multiple shoot induction and the shoot display the best rooting in MS medium with 1 mg/l IBA.

Kadu (2013) developed a protocol for rapid clonal micropropagation of gerbera by axillary bud and obtained a high efficient micropropagation protocol for commercial multiplication of gerbera where, MS medium supplemented with BAP with NAA was found good.

Asghare et al (2011) performed an investigation on *in vitro* propagation of *Dendrobium nobile* var. Emma white and recorded maximum number of shoots (4.33), as well as fresh and dry weights (752.5 and 52.99 mg), were obtained at 2 mg/l BAP and root induction percentage was found to be best in IBA at a level of 2 mg/l.

Khatun *et al.* (2010**)** examined the combined effect of different plant growth regulators for root formation and plantlet development from protocrom like bodies (PLBS) of orchids. The maximum number of roots from 1.0 mg/l each of IAA and IBA combination and root length from 2.0 mg/l BAP and 1.0 mg/l IBA with charcoal supplementation was recorded.

Rehena *et al.* (2009) studied the influence of plant growth regulators on shoot proliferation from the shoot tip of banana on 4 banana cultivars using different concentrations of BAP the highest percentage (60 %) of a single shoot at 4.0 mg/l BAP within 10-15 days. The best root formation in multiplied shoots of 'Amritsagar' was found on MS medium containing 2.0 mg/l IBA after 15 days of culture.

An experiment was conducted to develop a plant regeneration protocol of *Ocimum sactum* Linn. using different concentrations of BAP, Kinetin, 2, 4-D, IAA and IBA for shoot multiplication using shoot tip as explant and noticed an increase in shoot multiplication in culture media supplemented with 0.2 mg/l BAP (Banu and Bari, 2007).

Zhao *et al.* (2007) worked on *in vitro* propagation protocol for *Dendrobium candidum* Wall ex Lindl. The explant used was a transverse thin cell layer and It was observed that medium with half-strength macronutrients and 2% sucrose, supplemented with 1.2 mg/l naphthaleneacetic acid (NAA) and 1.2 mg/l 6-benzyladenine (6-BA), was optimal for shoot regeneration.

Aktar *et al.* (2007) carried out an experiment to form *in vitro* root in *Dendrobium* orchid plantlets with IBA and found the best results from 1.0 mg/l IBA treatment in which the number of the root was 1.81 per plantlet, length of root 0.35 cm, fresh weight of root 0.16g at 30 DAI and the minimum days to root formation was 10.8.

Talukder *et al.* (2003) studied the effect of IBA and BAP for *in vitro* shooting in *Dendrobium* orchid and found the best shoot proliferation in the least time required for regeneration (8.8 days) with treatment combination 2.5 mg/l BAP + 0.5 mg/l NAA.

Nayak *et al*. (2002) established a procedure for rapid micropropagation of *Cymbidium aloifolium* (L). Sw. and *Dendrobium nobile* Lindl. from thin cross-sections of protocorm-like bodies. PBA at 11.0 μM was optimum for PLB production.

Chapter -3
Materials and Methods

The details of materials and method adopted during the course of conducting the present experiment are enumerated as follows.

3.1 Experimental site:

The present experimentation was carried out in the Tissue culture laboratory, Department of Basic Sciences and Humanities, College of Horticulture and Forestry, Central Agricultural University, Pasighat. East Siang District, Arunachal Pradesh, during the year 2020-2021. College of Horticulture and Forestry is geographically located 28° 04' 43" N latitude and 95° 19'26" E at an altitude of 153 m above mean sea level.

3.2 Climate and weather:

The experiment was conducted under control atmosphere with temperature at 27°C, humidity at 60% and 16 hour normal light.

3.3 Experimental materials:

3.3.1 Description of the crops:

3.3.1.1 Crop: *Dendrobium* Orchid var. Earsakul

3.3.1.2 Age of explant: 5-6 months old

3.3.1.3 Duration of the experiment: 2020-21

3.3.1.4 Factor of experiment: 1 - plant growth regulators and 2 - light.

3.3.2 Plant growth regulators used:

The plant growth regulators used were mainly the combination of different cytokinin and auxin at different concentration. The cytokinin used were 6-Benzylaminopurine (BAP) and Kinetin (KIN) and auxin used were Indole-3-butyric acid (IBA), Indole-3-Acetic Acid (IAA), 1-Naphthalene Acetic Acid (NAA) and 2,4-Dichlorophenoxyacetic acid (2,4-D) which were supplemented at different concentration on the MS media. For the callus induction 9 combinations of the PGRs with 1 control (basal MS media) was used for both dark and light condition for callus initiation, 7 treatment combinations of PGRs with 1 control was used for shoot proliferation and 5 treatment combinations of PGRs with 1 control was used for root proliferation.The treatment details are given below at 3.3.3

3.3.3 Treatments

3.3.3.1 Performance of plant growth regulators combination for callus initiation and formation under 16 hour normal light.

T_0 = MS basal media (control)

T_1 = 0.0125 mg/l BAP + 0.025 mg/l IAA

T_2 = 0.25 mg/l BAP + 0.05 mg/l IAA

T_3 = 0.5 mg/l BAP + 0.10 mg/l IAA

T_4 = 0.25 mg/l KIN + 0.25 mg/l 2, 4-D

T_5 = 0.5 mg/l KIN + 0.5 mg/l 2, 4-D

T_6 = 1 mg/l KIN + 1mg/l 2, 4-D

T_7 = 0.25 mg/l KIN + 5mg/l NAA

T_8 = 0.5 mg/l KIN + 7.5mg/l NAA

T_9 = 1 mg/l KIN + 10 mg/l NAA

3.3.3.2. Performance of plant growth regulators combination for callus initiation and formation under complete dark.

T^0_0 = MS basal media (control)

T^0_1 = 0.0125 mg/l BAP + 0.025 mg/l IAA

T^0_2 = 0.25 mg/l BAP + 0.05 mg/l IAA

T^0_3 = 0.5 mg/l BAP + 0.10 mg/lAA

T^0_4 = 0.25 mg/l KIN + 0.25 mg/l 2, 4-D

T^0_5 = 0.5 mg/l KIN + 0.5 mg/l 2, 4-D

T^0_6 = 1 mg/l KIN + 1mg/l 2, 4-D

T^0_7 = 0.25 mg/l KIN + 5mg/l NAA

T^0_8 = 0.5 mg/l KIN + 7.5mg/l NAA

T^0_9 = 1 mg/l KIN + 10 mg/l NAA

3.3.3.3. Performance of plant growth regulators for shoot proliferation.

S_0 = MS basal media (control)

S_1 = 0.5 mg/l BAP + 0.06 mg/l NAA

S_2 = 1.5 mg/l BAP + 0.125 mg/l NAA

S_3 = 2 mg/l BAP + 0.25 mg/l NAA

S_4 = 2.5 mg/l BAP + 0.5 mg/l NAA

S_5 = 3 mg/l BAP + 0.6 mg/l NAA

S_6 = 3.5mg/l BAP + 0.7 mg/l NAA

S_7 = 4 mg/l BAP + 0.8 mg/l NAA

3.3.3.4. Performance of plant growth regulators for root proliferation.

R_0 = MS basal media (control)

R_1 = 0.06 mg/l BAP + 0.125 mg/l IBA

R_2 = 0.125 mg/l BAP + 0.25 mg/l IBA

R_3 = 0.25 mg/l BAP + 0.5 mg/l IBA

R_4 = 0.5 mg/l BAP + 1 mg/l IBA

R_5 = 1 mg/l BAP + 2 mg/l IBA

3.4 Experimental methods

3.4.1 Experimental design

The experiment was laid out in completely randomized design (CRD) 10 treatment combination for callus initiation and formation under 16 hr normal light , 10 treatment combination for callus initiation and formation in dark, 8 treatment combination for shoot proliferation and 6 treatment combination for root proliferation respectively were undertaken with 4 replication for all the experiment.

3.4.2 Media preparation:

3.4.2.1 Major stock (10X) 250 ml.

KNO_3	4.75 g
NH_4NO_3	4.125 g
$MgSO_4.7H_20$	0.925 g
$CaCl_2.2H_20$	1.1 g
KH_2PO_4	0.43 g

3.4.2.2 Minor stock (100X) 50ml

H_3BO_3	310 mg
$MnSO_4.4H_2O$	1115 mg
$ZnSO_4.7H_2O$	430 mg
$Na_2MoO_4.2H_2O$	12.5 mg
$CuSO_4.5H_2O$	1.25 mg
$CoCl_2.6H_2O$	1.25 mg

3.4.2.3 Vitamins (100X) 50 ml

Nicotinic acid	25mg
Thiamine HCI	25mg
Pyridoxine HCI	5 mg
Myo inositol	5000 mg

3.4.2.4 Iron EDTA solutions (10X) in 100 ml

$FeSO_4.7H_2O$	0.557 g in 35 ml (heat was applied)
Na_2 EDTA	0.745 g in 35 ml (heat was applied)

Both the solution was mixed and were made upto 100 ml

3.4.2.5 Preparation of basic MS media 1L

Doubled distilled water	400 ml
Major salts stock	100 ml
Minor salt stock	10 ml
Vitamin stock	10 ml
Iron EDTA stock	5 ml
Sucrose	30 g
Clerigel	7.0 g
PH	5.6

3.4.3 Sterilization of explant:

Five-six months old young shoots of *Dendrobium* were collected from orchidarium of College of Horticulture and Forestry which were sterilized using chemicals and detergents. First, the explants were washed under running water for 5 min to remove adhering dirt. The further sterilizing steps were carried out inside the laminar flow, where the explants were submerged in 0.1% $HgCl_2$ solution for 5 min, followed by treatment with 70% ethyl alcohol for 30 second to ensure sterilisation. The explants were kept for 15 min inside a conical flask containing 0.5% Carbendazim solution and treated with 70% ethyl alcohol for 30 sec. followed by antibiotic treatment using Gentamycin (400 ppm) for 40 min. The final treatment was 12 min of shaking in a solution containing 4% sodium hypochlorite with 5 drops of Tween 20. Every chemical treatment was followed by thorough rinsing using sterilized double distilled water for three to four times.

3.5 Observations recorded

3.5.1 Performance of plant growth regulators combination for callus initiation and formation under 16 hr normal light.

3.5.1.1 Number of days taken for callus initiation

The day on which callus development was first observed were recorded.

3.5.1.2 Weight of the callus at 15th, 30th, 45th and 60th days (mg)

The weight of callus was measured using electronic balance. The weight of the culture bottle were measured first than the calli were inoculated to new culture bottles. The culture bottles after removal of calli were measured again. Thus, the weight of callus was obtained by subtracting the weight of the bottle containing media with callus and without callus.

3.5.1.3 Percentage of callus formation at 15th, 30th, 45th and 60th days.

The percentage of callus formed was obtained by the following formula:

$$\text{Percentage of callus} = \frac{\text{Number of explant that formed callus}}{\text{Number of all the explant inoculated}} \times 100$$

3.5.2 Performance of plant growth regulators combination for callus initiation and formation under complete dark.

3.5.2.1 Number of days taken for callus initiation

The day on which callus development 1st observed was recorded for every treatment.

3.5.2.2 Weight of the callus at 15th, 30th, 45th and 60thdays (mg).

The weight of callus was measured using electronic balance. The weight of the culture bottle were measured first than the calli were inoculated to new culture bottles. The culture bottles after removal of calli were measured again. Thus, the weight of callus was obtained by subtracting the weight of the bottle containing media with callus and without callus.

3.5.2.3 Percentage of callus formation at 15th, 30th, 45th and 60thdays.

The percentage of callus formed was obtained by the following formula:

$$\text{Percentage of callus} = \frac{\text{Number of explant that formed callus}}{\text{Number of all the explant inoculated}} \times 100$$

3.5.3 Performance of plant growth regulators for shoot proliferation.

3.5.3.1 Days taken for shoot initiation

The day on first shoot initiation observed was recorded.

3.5.3.2 Number of shoot at 15th, 30th, 45th and 60thdays.

The number of shoots developed for every treatment was recorded.

3.5.3.3 Shoot length at 15th, 30th, 45th and 60th days (cm).

The shoot length was measured using measuring scales.

3.5.3.4 Number of leaves at 15th, 30th, 45th and 60th days.

The number of leaves on every shoot were counted and recorded.

3.5.3.5 Leaf length at 15th, 30th, 45th and 60th days (cm).

Leaf length was measured using the measuring scale.

3.5.3.6 Shoot weight at 15th, 30th, 45th and 60th days (mg).

Shoot weight was measured using electronic balance. the weight of the culture bottles with plantlet were measured first than the plantlets were inoculated to the new culture bottles, the culture bottles after removal of the plantlets were measured again. Thus, the weight of the callus was obtained by subtracting the weight of the bottle containing media with or without plantlet.

3.5.4 Performance of plant growth regulators for root proliferation.

3.5.4.1 Days taken for root initiation after inoculating

The plant that have developed shoot in the rooting media (the days on which shoot developed where recorded)

3.5.4.2 Number of root at 15th, 30th, 45th and 60th days

The numbers of roots were recorded for every treatment and replication

3.5.4.3 Root length at 15th, 30th, 45th and 60th days (cm)

The root lengths were measured using measuring scale for every treatment and replication at 15 days interval till 60th days.

3.5.4.4 Root weight at 15th, 30th, 45th and 60th days (mg)

The root weight was measured for every replication after every 15 days for every treatment and replication.

3.6 Statistical analysis:

Observations recorded during experiment and data obtained from laboratory analysis was subjected to the statistical analysis of variance by Completely Randomized Design (CRD). Significance and non-significance of the variance due to different treatments was determined by calculating the respective 'F' values as the method described by Gomez and Gomez (2010).

3.6.1 ANOVA for Completely randomized design (CRD)

Source of Variation	d.f.	SS	MS	Expectations	'F' Test
Treatment	(t-1)	Trss	Trss/t-1 (TrMs)	$\sigma^2 e + r\,\sigma^2 t$	TrMS/EMS
Error	t (r-1)	ESS	ESS/t (r-1) (EMS)	$\sigma^2 e$	
Total	(tr-1)				

Where,

t= number of treatments

r= number of replications

TrMS= Mean sum of square due to treatment

EMS= Mean sum of square due to error

The critical difference (CD) has calculated after list of significance among treatments as follows

CD at 0.05%= SEdx 't' value at error d.f. at 0.05% P level [where SEd=$\sqrt{2}$ EMS/r]

3.6.2 Estimation of mean and standard error

Mean values of each characters was worked out by dividing the totals by corresponding number of observation (x = xi/ n). Standard error of difference of two treatment means was calculated as follows:

$$SEd = \pm\sqrt{\frac{2Mse}{r}}$$

Where,

r = Number of replication

Mse = Error mean square

SEd = Standard error of the difference between two treatment

The formula for coefficient of variation is given below:

$$CV = \frac{SEd \times 100\%}{M}$$

Fig. 2.1: Sterilisation, Inoculation and Tissue Culture laboratory

Chapter - 4
Results

The present investigation entitled "*in vitro* propagation of *Dendrobium* Orchid var. Earsakul" was carried out in the year 2020-2021. The result obtained from different experiments has been presented under the following heads.

4.1 Performance of plant growth regulators combination for callus initiation and formation under 16 hr normal light.

4.2 Performance of plant growth regulators combination for callus initiation and formation under complete dark

4.3 Performance of plant growth regulators for shoot proliferation.

4.4 Effect of plant growth regulators for root proliferation

4.1 Performance of plant growth regulators combination for callus initiation and formation under 16 hr normal light.

4.1.1 Number of days taken for callus initiation:

Positive responses on callus initiation had been ascertained with different treatment combinations of auxin and cytokinin supplemented in the MS media and is presented in Table 4.1.1 and Fig. 4.1.1. Earliness in callus initiation (3.24 days) was observed in treatment combinations T_7, 0.25 mg/l KIN + 5mg/l NAA followed by T_5, 0.5 mg/l KIN + 0.5 mg/l 2, 4-D (3.50 days) and T_2, 0.25 mg/l BAP + 0.05 mg/l IAA (3.62 days). However, delay in callus initiation was noticed in T_0, control (5.24 days).

4.1.2 Weight of the callus:

Enhanced callus weight was recorded when supplemented with plant growth regulators in different treatment combinations and the detailed results are presented in Table 4.1.2 and Fig. 4.1.2. Weight of the callus after 15 days of inoculation was significantly higher (234.20 mg) in the treatment T_8, 0.5 mg/l + 7.5 mg/l NAA followed by T_7, 0.25 mg/l KIN +5 mg/ NAA (190.00 mg). However, callus induction was not observed in T_0 (control), T_1 (0.0125 mg/l BAP + 0.025 mg/l IAA) and T_3 (0.5 mg/l BAP + IAA 0.10 mg/l).

While, on the 30th day the callus initiation was perceived in all the treatments including control. The callus weight was significantly highest (259.40 mg) for T_8, 0.5 mg/l + 7.5 mg/l NAA, followed by T_2, 0.25 mg/l BAP +0.05 mg/l IAA (240.50 mg) and T_7, 0.25 mg/l KIN +5 mg/ NAA (214.80 mg). The lowest (65.20 mg) callus weight was noted in T_0, control.

Furthermore, the highest callus weight was exhibited on 45th day after inoculation in the treatment T_8, 0.5 mg/l KIN + 7.5 mg/l NAA (293.70 mg) followed by T_2, 0.25 mg/l BAP + 0.05 mg/l IAA (249.64 mg).

Significantly, the best treatment combination for callus growth and development on the 60th day after inoculation was recorded in T_8, 0.5 mg/l KIN + 7.5mg/l NAA (308.10 mg) followed by T_7, 0.25 mg/l KIN +5 mg/ NAA (261.10 mg) and T_2, 0.25 mg/l BAP + 0.05 mg/l IAA (255.00 mg). The lowest callus weight was found in T_0, control (98.3 mg),

4.1.3 Percentage of callus formation:

The percentage of callus formation was estimated using the formula: -

$$\text{Percentage of callus } = \frac{\text{Number of explant that formed callus}}{\text{Number of all the explant inoculated}} \times 100$$

First observation was recorded on 15th day, the highest callus percentage was recorded in T_8, 0.5 mg/l KIN + 7.5 mg/l NAA (52.82%) followed by T_7, 0.25 mg/l KIN +5 mg/l NAA (50.03 %) (Table 4.1.3 and Fig. 4.1.3).

Subsequently on the 30th day, the highest percentage (58.72%) of callus was noted same in T_7, 0.25 mg/l KIN +5 mg/ NAA and T_8, 0.5 mg/l KIN+ 7.5mg/l NAA which was statistically at par with T_2, 0.25 mg/l BAP + 0.05 mg/l IAA (55.86%). The lowest callus formation was recorded in the treatment T_0, control (20.45%).

In the next observation registered on 45th day, the greater callus percentage was in treatment T_7, 0.25 mg/l KIN +5 mg/ NAA (62.40%) followed by T_2, 0.25 mg/l BAP + 0.05 mg/l IAA (62.20%) and T_8, 0.5 mg/l KIN+ 7.5mg/l NAA (61.08%)which was at par with each other. Whereas, the lowest percentage of callus formation was noticed in T_0, control (27.93%).

However, on 60th day (final observation), the highest percentage of callus formation was found in T_2, 0.25 mg/l BAP + 0.05 mg/l IAA (66.66%) followed by T_8, 0.5 mg/l KIN+ 7.5mg/l NAA (65.86%) and T_7, 0.25 mg/l KIN +5 mg/ NAA (65.31%) which were at par with each other. On contrary, the lowest percentage of callus formation was noted in T_0, control (34.57%).

Table 4.1.1 Effect of PGRs on number of days taken for callus initiation under 16 hr normal light. (Square root value)

Treatment	Days of Callus Initiation
T_0 (MS basal media control)	5.24
T_1 (0.0125 mg/l BAP + 0.025 mg/l IAA)	5.02
T_2 (0.25 mg/l BAP + 0.05 mg/l IAA)	3.62
T_3 (0.5 mg/l BAP + 0.10 mg/l IAA)	4.18
T_4 (0.25 mg/l KIN + 0.25 mg/l 2, 4-D)	3.72
T_5 (0.5 mg/l KIN + 0.5 mg/l 2, 4-D)	3.50
T_6 (1 mg/l KIN + 1 mg/l 2, 4-D)	3.94
T_7 (0.25 mg/l KIN + 5 mg/l NAA)	3.24
T_8 (0.5 mg/l KIN + 7.5 mg/l NAA)	3.69
T_9 (1 mg/l KIN + 10 mg/l NAA)	3.74
SEM	0.05
CD (p=0.05)	0.16
CV%	2.36

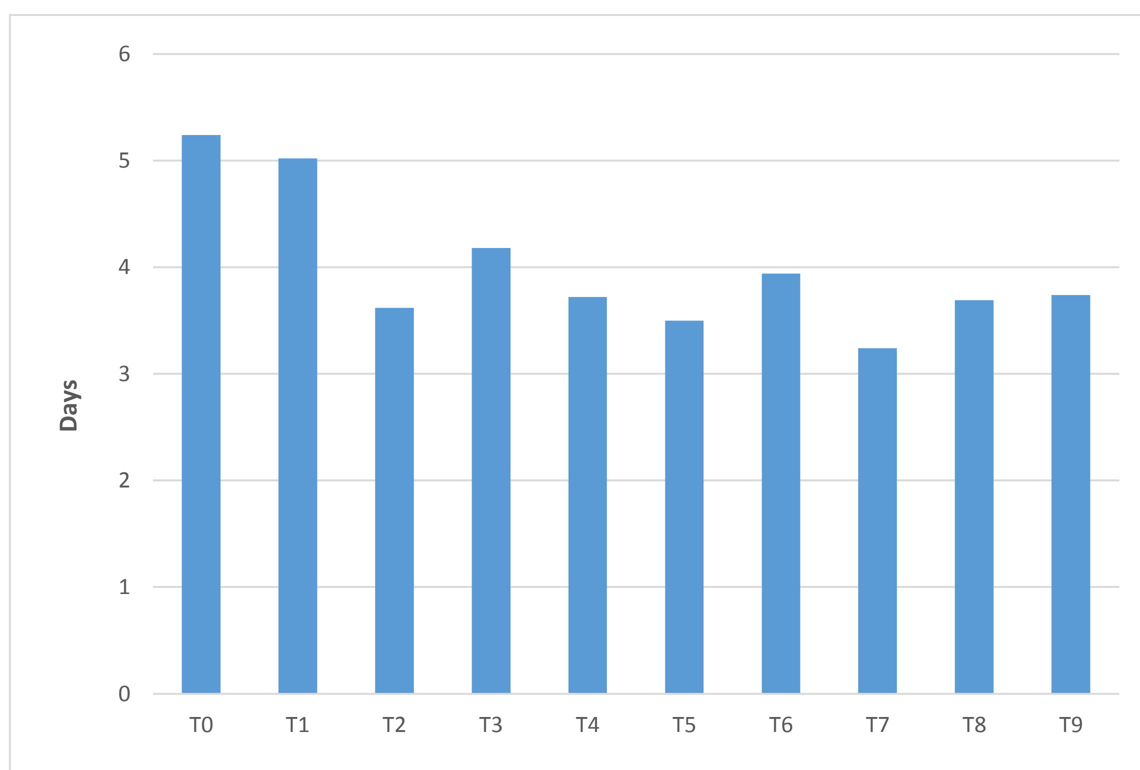


Fig 4.1.1 Effect of PGRs on number of days taken for callus initiation under 16 hr normal light. (Square root value)

Table 4.1.2 Effect of PGRs on weight of callus (mg) under 16 hr normal light.

Treatment	15 days	30 days	45 days	60 days
T_0 (MS basal media control)	0.00	65.20	84.30	98.3
T_1 (0.0125 mg/l BAP + 0.025 mg/l IAA)	0.00	106.50	113.80	119.4
T_2 (0.25 mg/l BAP + 0.05 mg/l IAA)	172.00	240.50	249.64	255.00
T_3 (0.5 mg/l BAP + 0.10 mg/l IAA)	0.00	96.90	113.50	123.7
T_4 (0.25 mg/l KIN + 0.25 mg/l 2,4-D)	86.10	183.20	222.50	222.7
T_5 (0.5 mg/l KIN + 0.5 mg/l 2, 4-D)	97.10	98.60	102.70	112.4
T_6 (1 mg/l KIN + 1 mg/l 2, 4-D)	80.40	93.70	93.95	103.30
T_7 (0.25 mg/l KIN + 5 mg/l NAA)	190.00	214.80	224.00	261.1
T_8 (0.5 mg/l KIN + 7.5 mg/l NAA)	234.20	259.40	293.70	308.1
T_9 (1 mg/l KIN + 10 mg/l NAA)	158.50	182.50	216.10	225.10
SEM	2.15	2.25	2.600	2.34
CD (p=0.05)	7.18	7.50	8.68	7.81
CV%	4.22	2.92	3.03	2.56

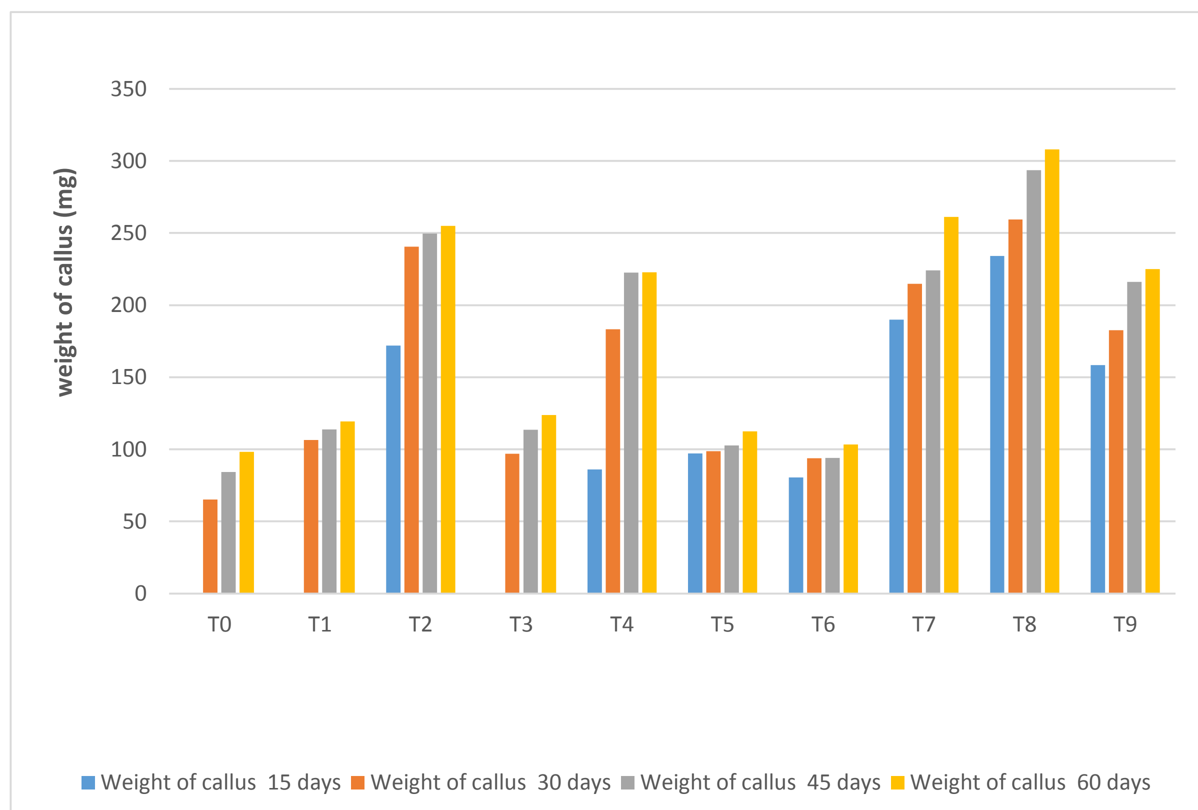


Fig. 4.1.2 Effect of PGRs on weight of callus (mg) under 16 hr normal light.

Table 4.1.3 Effect of PGRs on percentage of callus formation under 16 hr normal light (arcsine value).

Treatment	15 days	30 days	45 days	60 days
T_0 (MS basal media control)	0.91	20.45	27.93	34.57
T_1 (0.0125 mg/l BAP + 0.025 mg/l IAA)	0.91	42.55	52.83	57.29
T_2 (0.25 mg/l BAP + 0.05 mg/l IAA)	46.71	55.86	62.20	66.66
T_3 (0.5 mg/l BAP + 0.10 mg/l IAA)	0.91	40.23	49.45	51.51
T_4 (0.25 mg/l KIN + 0.25 mg/l 2,4-D)	35.33	35.50	43.84	51.94
T_5 (0.5 mg/l KIN + 0.5 mg/l2,4-D)	29.47	33.19	39.07	43.55
T_6 (1 mg/l KIN + 1mg/l 2,4-D	29.10	35.35	35.79	40.52
T_7 (0.25 mg/l KIN + 5mg/l NAA)	50.03	58.72	62.40	65.86
T_8 (0.5 mg/l KIN + 7.5 mg/l NAA)	52.82	58.72	61.08	65.31
T_9 (1 mg/l KIN + 10 mg/l NAA)	27.95	29.47	33.96	37.59
SEM	0.66	0.99	0.93	1.06
CD (p=0.05)	2.20	3.29	3.11	3.53
CV%	4.82	4.81	3.97	4.11

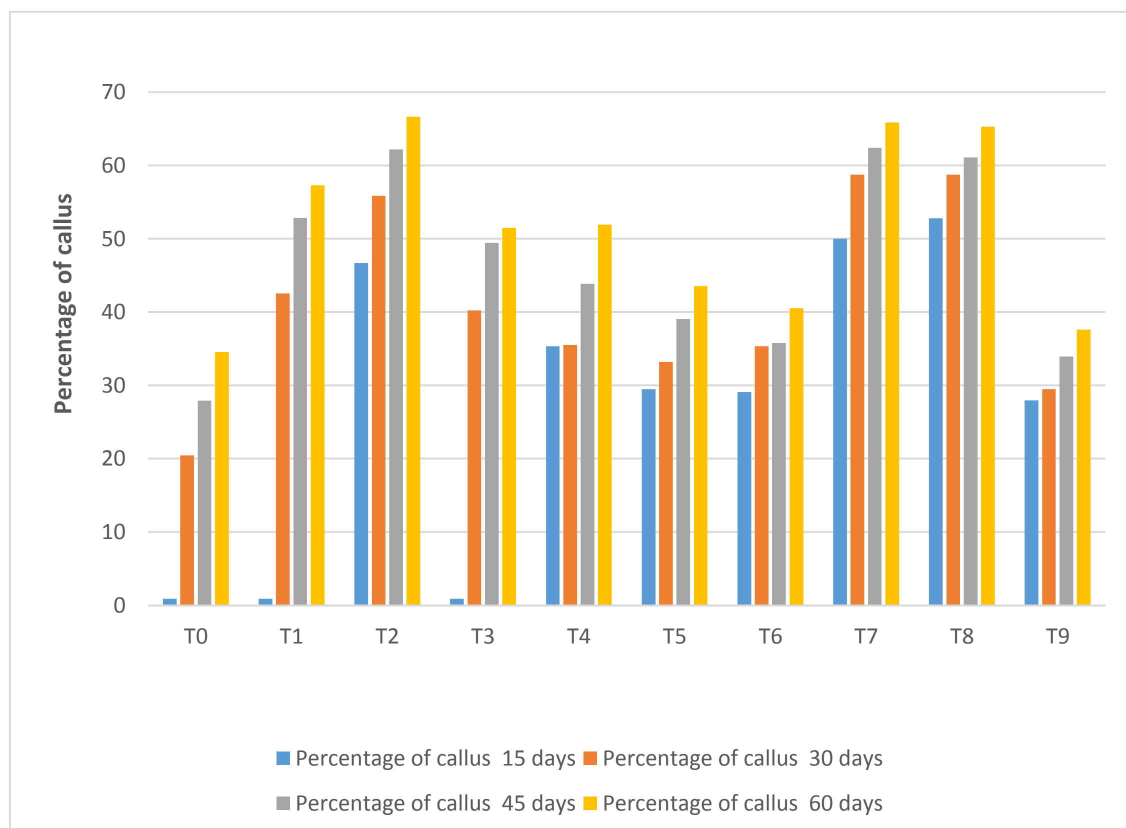
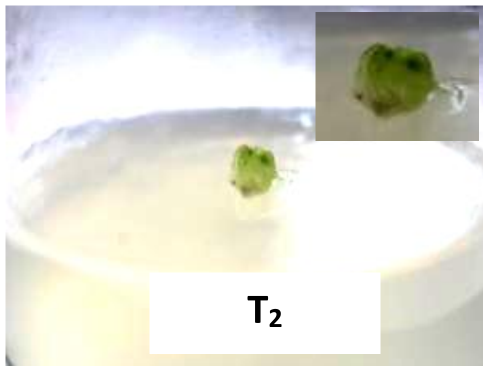
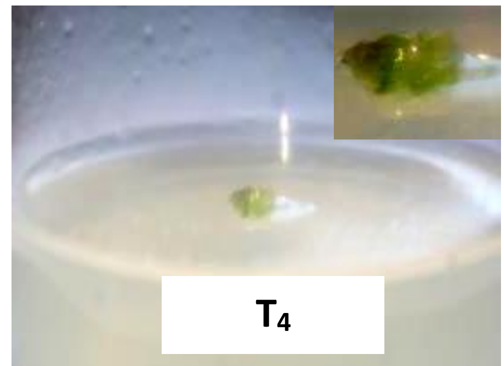
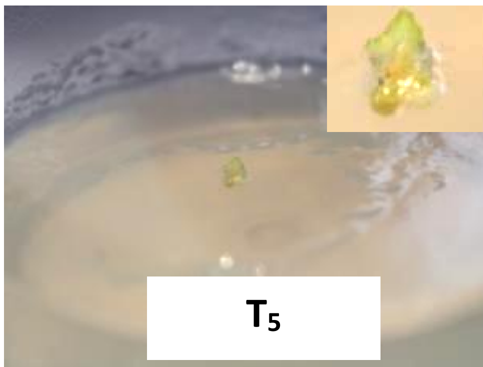
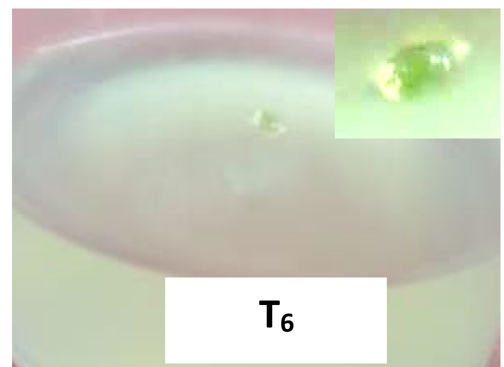


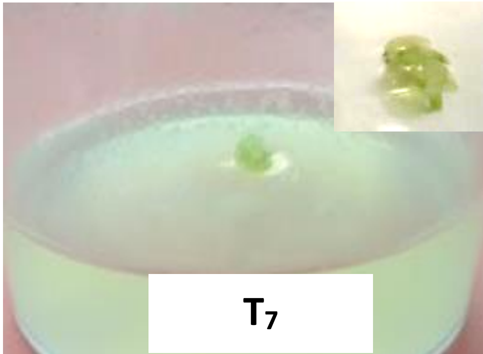
Fig 4.1.3 Effect of PGRs on percentage of callus formation under 16 hr normal light (arcsine value)











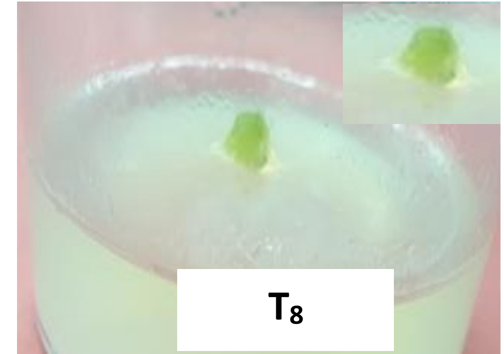
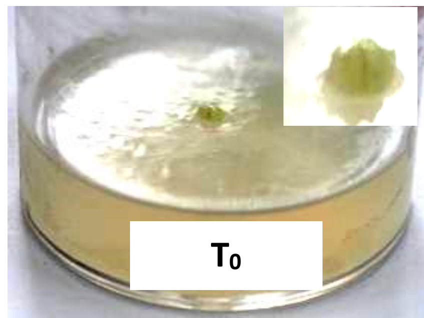
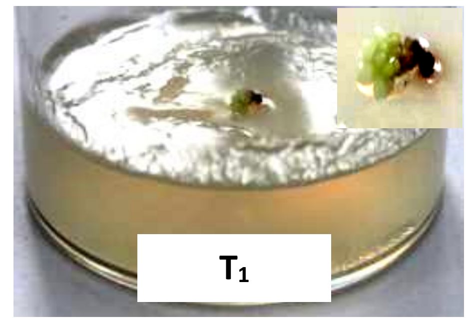
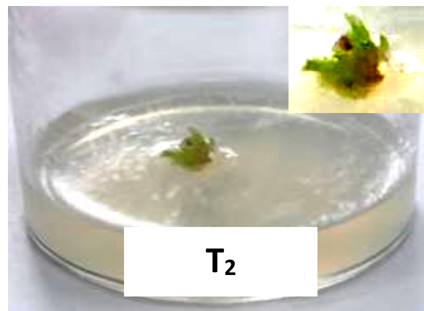
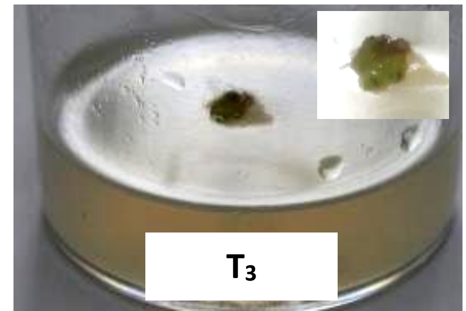


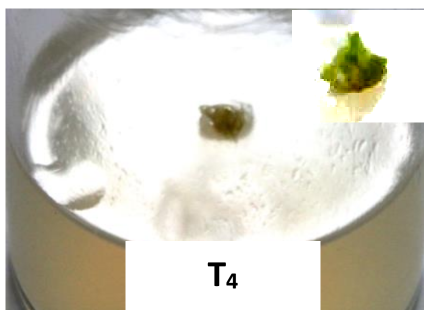
Fig.4.1.4: Callus under 16 hr normal light on 15th day after inoculation

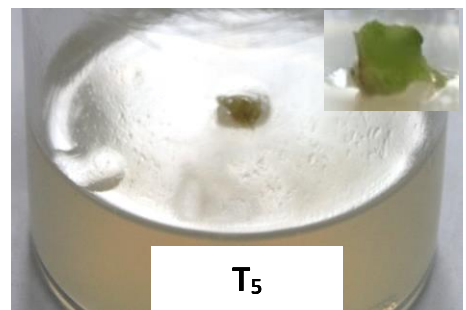


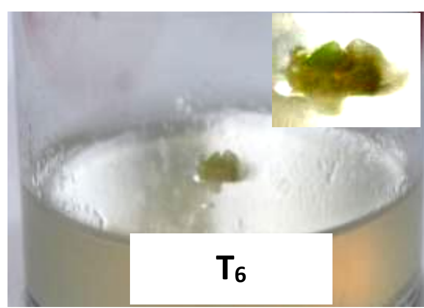


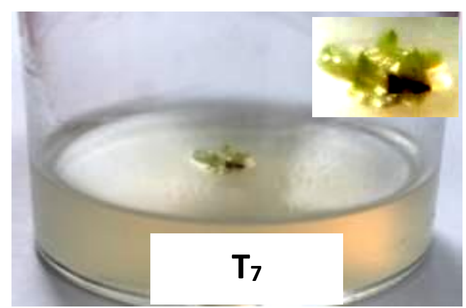


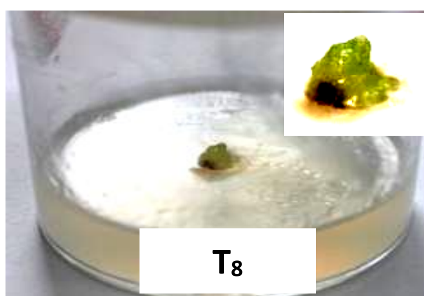












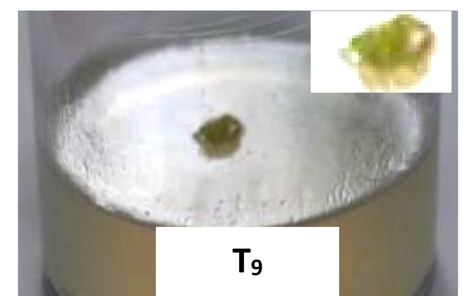
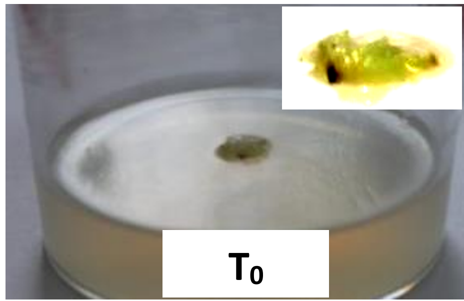
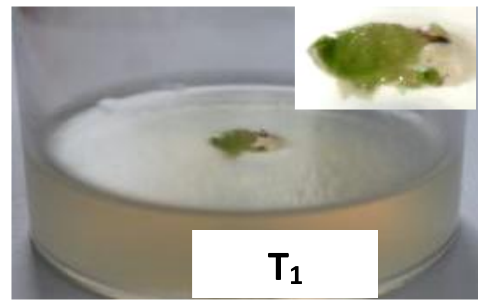
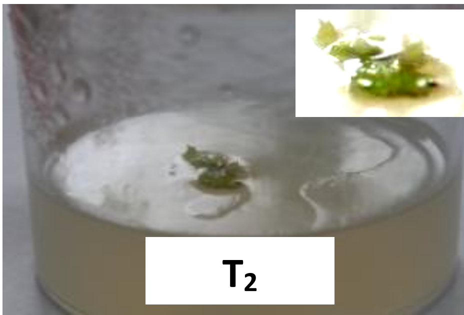
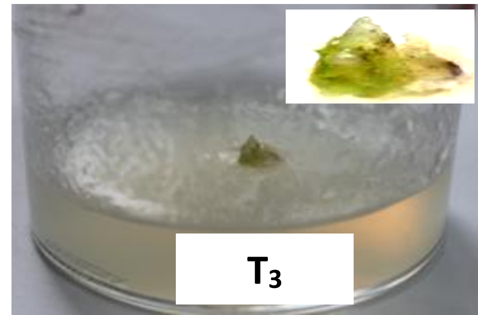


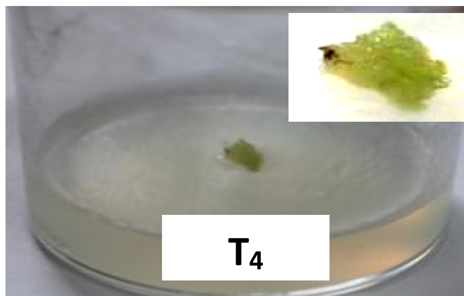
Fig.4.1.5: Callus under 16 hr normal light on 30th day after inoculation

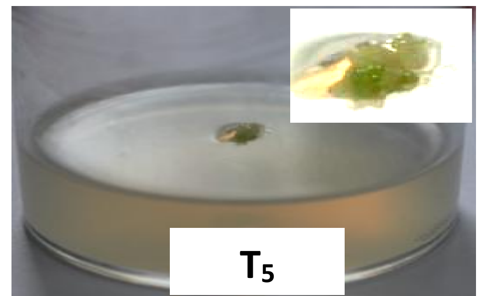


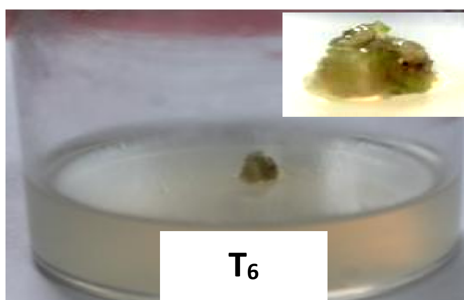


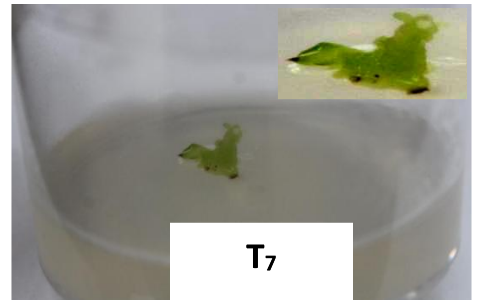


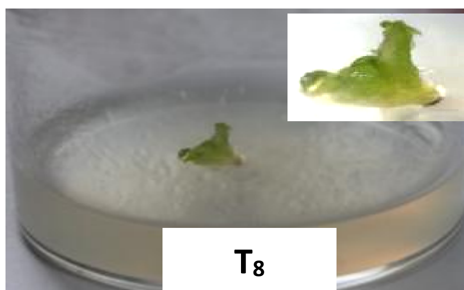












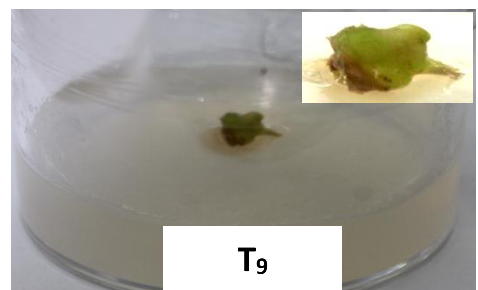


Fig.4.1.6: Callus under 16 hr normal light on 45th day after inoculation

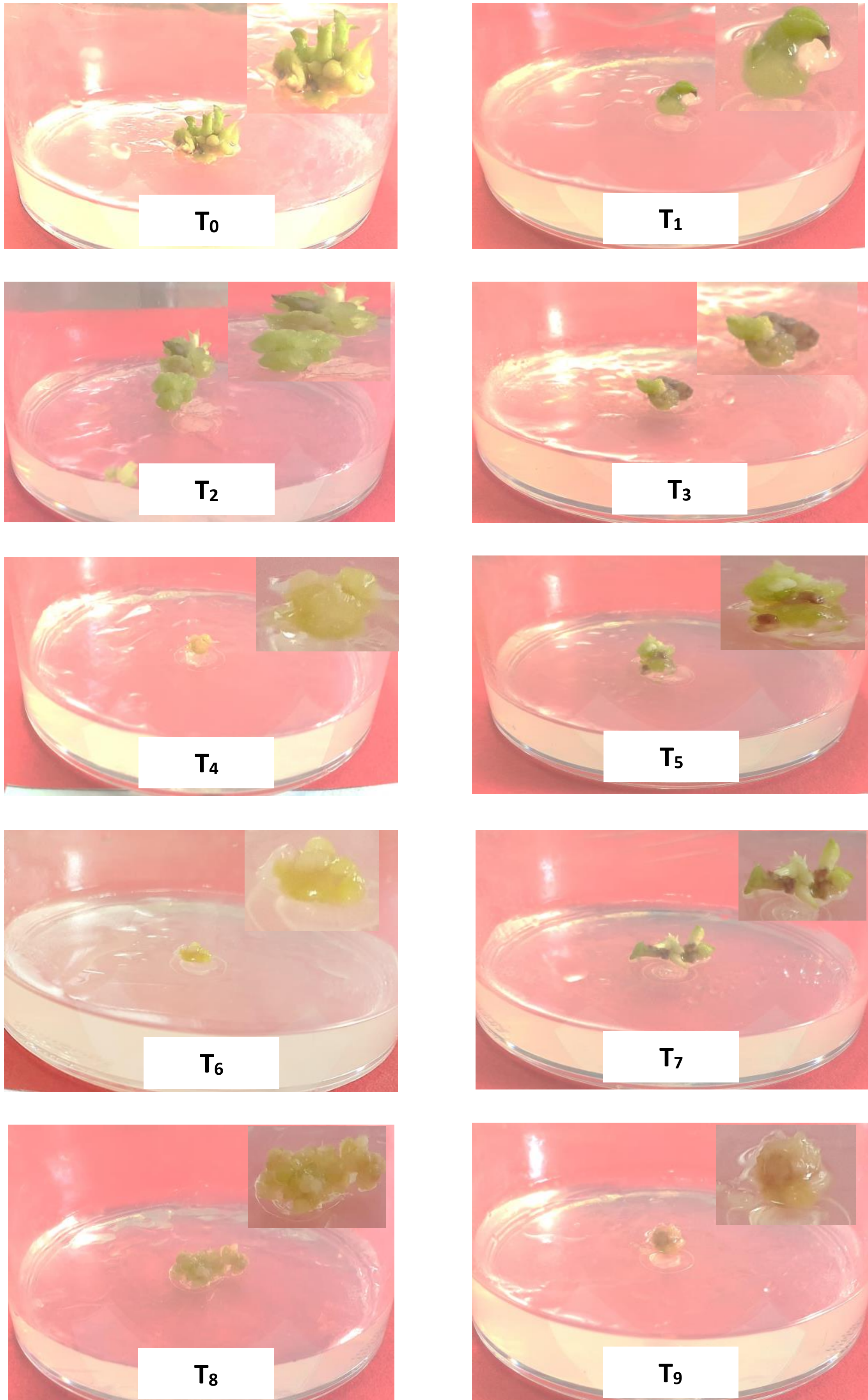


Fig.4.1.7: Callus under 16 hr normal light on 60th day after inoculation

4.2 Performance of plant growth regulator combination for callus initiation and formation under complete dark

4.2.1 Number of days taken for callus initiation:

The earliest (4.28 days) callus emergence under complete dark conditions was evident in treatment T^0_2, 0.25 mg/l BAP +0.05 mg/l NAA followed by T^0_6, 1 mg/l KIN + 1mg/l 2, 4-D (4.46 days) and T^0_4, 0.25 mg/l KIN + 0.25 mg/l 2,4-D (4.55 days) which were at par with each other. Whereas the longest time (6.00 days) taken for callus induction was captured from the treatment T^0_0, control. Further results are presented in Table 4.2.1 and Fig. 4.2.1

4.2.2 Weight of the callus:

The weight of calli were taken at 15 days interval, counted from the day of inoculation. Whereas, the callus formation was completely inhibited under the dark condition in all the treatment combinations and illustrated in Table 4.2.2 and Fig 4.2.2.

On the 30th day after inoculation, notable callus induction was observed under dark condition on all the treatment combinations except T^0_0, control. While, loftier callus weight was observed in T^0_9,1 mg/l KIN + 10 mg/l NAA (147.05 mg) followed by T^0_7, 0.25 mg/l KIN + 5mg/l NAA (132.90 mg) and at par with T^0_8, 0.5 mg/l KIN + 7.5mg/l NAA (141.15 mg)

In further observation recorded on 45th day, the maximum weight of callus was recorded in T^0_8, 0.5 mg/l KIN + 7.5mg/l NAA (232.15 mg) followed by $T^0_{9,}$ 1 mg/l KIN + 10 mg/l NAA (204.70 mg). However, T^0_0, control recorded minimum callus weight (63.11 mg).

While, on the 60th day of observation, maximum weight of callus (247.45 mg) under the treatment combination T^0_8, 0.5 mg/l KIN + 7.5mg/l NAA followed by T^0_9, 1 mg/l KIN + 10 mg/l NAA (234.30 mg) was observed. However, the minimum callus weight was noted in $T^0_{0,}$ control (83.03 mg).

4.2.3 Percentage of callus formation:

Significant callus formation was not evident in any of the treatment combinations under the dark condition till 15th day after inoculation and the detail observation is presented in Table 4.2.3 and Fig 4.2.3.

However, on the 30th day considerable callus initiation was noted in all the treatments except for T^0_0, control. Among all treatments, significant percentage of callus initiation was recorded in $T^0_{8,}$ 0.5 mg/l KIN + 7.5mg/l NAA (38.63%) followed by T^0_7, 0.25 mg/l KIN + 5mg/l NAA (36.26%)

Further observation on 45th day revealed 41.97% callus initiation in T^0_8, 0.5 mg/l KIN + 7.5mg/l NAA which was at par with T^0_7, 0.25 mg/l KIN + 5mg/l NAA (40.23%).

In the final observation which was recorded on the 60th day, the best treatment combination with maximum percentage of callus formation (43.55%) was recorded in the treatment T^0_8, 0.5 mg/l KIN + 7.5mg/l NAA and was at par with T^0_7, 0.25 mg/l KIN + 5mg/l NAA (42.69%). The lowest percentage of callus formation (19.53%) remained in T^0_0, control.

Table 4.2.1 Effect of PGRs on number of days taken for callus initiation under complete dark. (Square root value)

Treatments	Days taken for callus initiation
T^0_0 (MS basal media (control)	6.00
T^0_1 (0.0125 mg/l BAP + 0.025 mg/l IAA)	5.05
T^0_2 (0.25 mg/l BAP + 0.05 mg/l IAA)	4.28
T^0_3 (0.5 mg/l BAP + 0.10 mg/l IAA)	4.93
T^0_4 (0.25 mg/l KIN + 0.25 mg/l 2, 4-D)	4.55
T^0_5 (0.5 mg/l KIN + 0.5 mg/l 2, 4-D)	5.29
T^0_6 (1 mg/l KIN + 1mg/l 2, 4-D)	4.46
T^0_7 (0.25 mg/l KIN + 5mg/l NAA)	4.66
T^0_8 (0.5 mg/l KIN + 7.5mg/l NAA)	4.80
T^0_9 (1 mg/l KIN + 10 mg/l NAA)	5.01
SEM	0.04
CD (p=0.05)	0.15
CV%	1.81

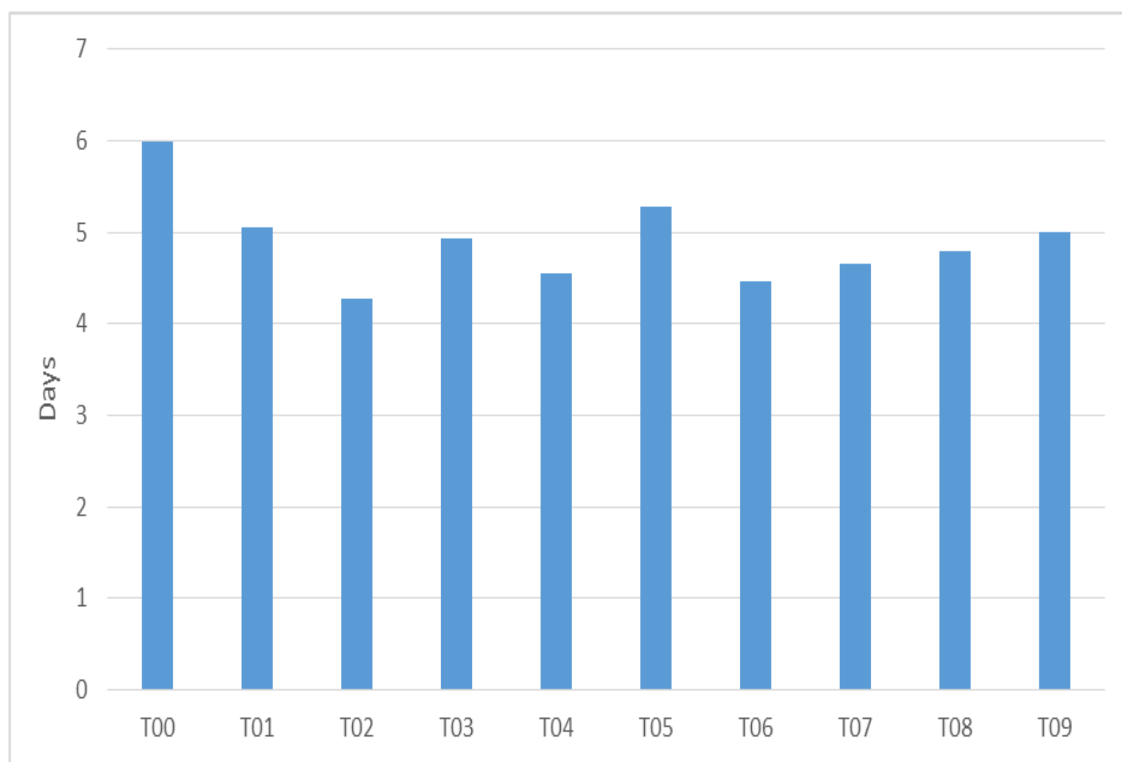


Fig 4.2.1 Effect of PGRs on number of days taken for callus initiation under complete dark. (Square root value)

Table 4.2.2 Effect of PGRs on weight of the callus under complete dark (mg)

Treatments	15 days	30 days	45 days	60 days
T^0_0 (MS basal media (control)	0.00	0.00	63.11	83.03
T^0_1 (0.0125 mg/l BAP + 0.025 mg/l IAA)	0.00	90.00	121.30	153.22
T^0_2 (0.25 mg/l BAP + 0.05 mg/l IAA)	0.00	100.10	101.25	109.10
T^0_3 (0.5 mg/l BAP + 0.10 mg/l IAA)	0.00	95.10	99.78	100.88
T^0_4 (0.25 mg/l KIN + 0.25 mg/l 2, 4-D)	0.00	92.35	97.40	101.76
T^0_5 (0.5 mg/l KIN + 0.5 mg/l 2, 4-D)	0.00	78.60	86.06	88.10
T^0_6 (1 mg/l KIN + 1mg/l 2, 4-D)	0.00	82.15	89.90	90.80
T^0_7 (0.25 mg/l KIN + 5mg/l NAA)	0.00	132.90	143.20	155.43
T^0_8 (0.5 mg/l KIN + 7.5mg/l NAA)	0.00	141.15	232.15	247.45
T^0_9 (1 mg/l KIN + 10 mg/l NAA)	0.00	147.05	204.70	234.30
SEM	0.00	2.34	1.35	1.78
CD (p=0.05)	0.00	7.81	4.52	5.95
CV%	0.00	4.88	2.19	2.61

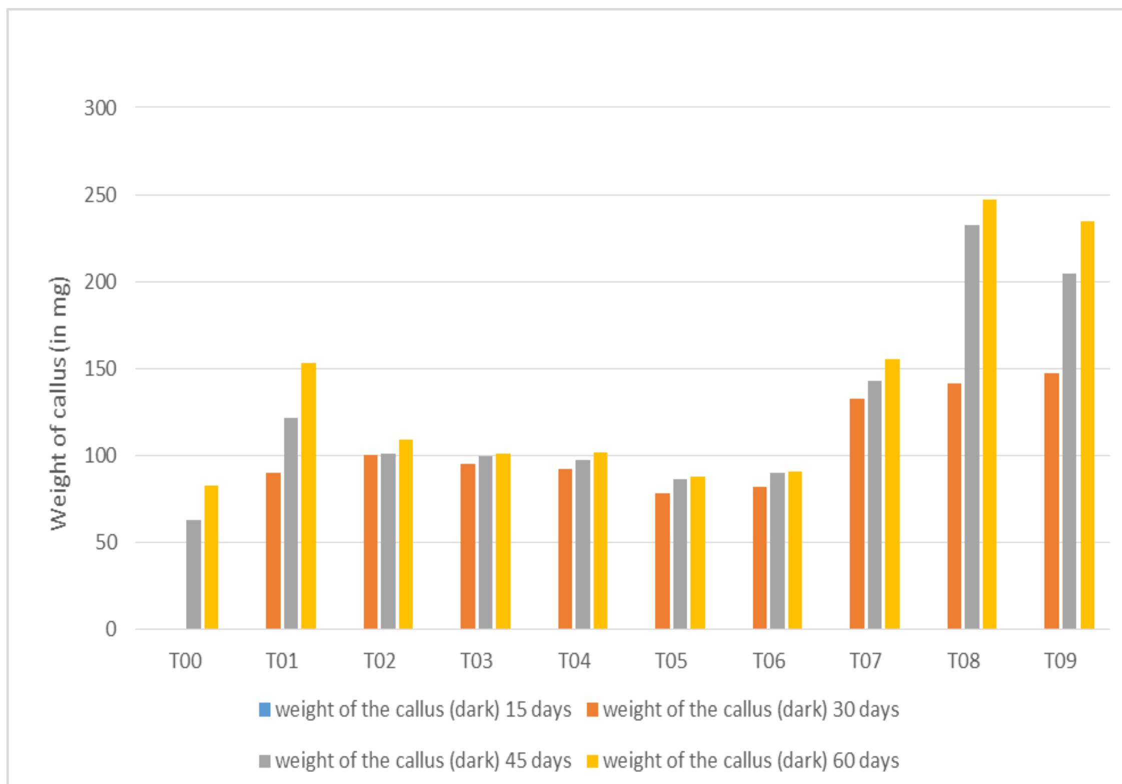


Fig 4.2.2 Effect of PGRs on weight of the callus under complete dark (mg)

Table 4.2.3 Effect of PGRs on percentage of callus formation under complete dark (arcsine value)

Treatment	15 days	30 days	45 days	60 days
T^0_0 (MS basal media (control)	0	0.91	17.65	19.53
T^0_1 (0.0125 mg/l BAP + 0.025 mg/l IAA)	0	21.11	23.34	24.31
T^0_2 (0.25 mg/l BAP + 0.05 mg/l IAA)	0	33.20	37.44	38.33
T^0_3 (0.5 mg/l BAP + 0.10 mg/l IAA)	0	26.55	28.80	29.81
T^0_4 (0.25 mg/l KIN + 0.25 mg/l 2, 4-D)	0	22.77	25.81	28.97
T^0_5 (0.5 mg/l KIN + 0.5 mg/l 2, 4-D)	0	28.64	30.79	32.41
T^0_6 (1 mg/l KIN + 1mg/l 2, 4-D)	0	21.11	24.12	26.16
T^0_7 (0.25 mg/l KIN + 5mg/l NAA)	0	36.26	40.23	42.69
T^0_8 (0.5 mg/l KIN + 7.5mg/l NAA)	0	38.63	41.97	43.55
T^0_9 (1 mg/l KIN + 10 mg/l NAA)	0	29.32	30.80	31.93
SEM	0	0.65	0.63	0.60
CD (p=0.05)	0	2.18	2.09	2.01
CV%	0	4.83	4.15	3.80

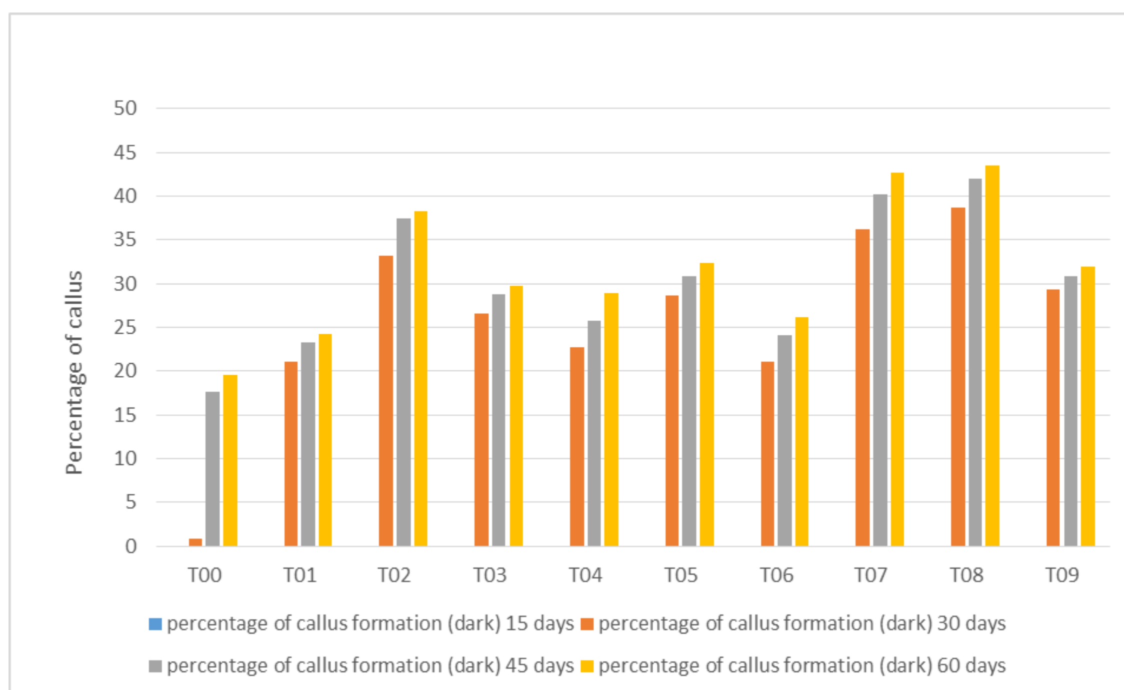


Fig 4.2.3 Effect of PGRs on percentage of callus formation under complete dark (arcsine value)

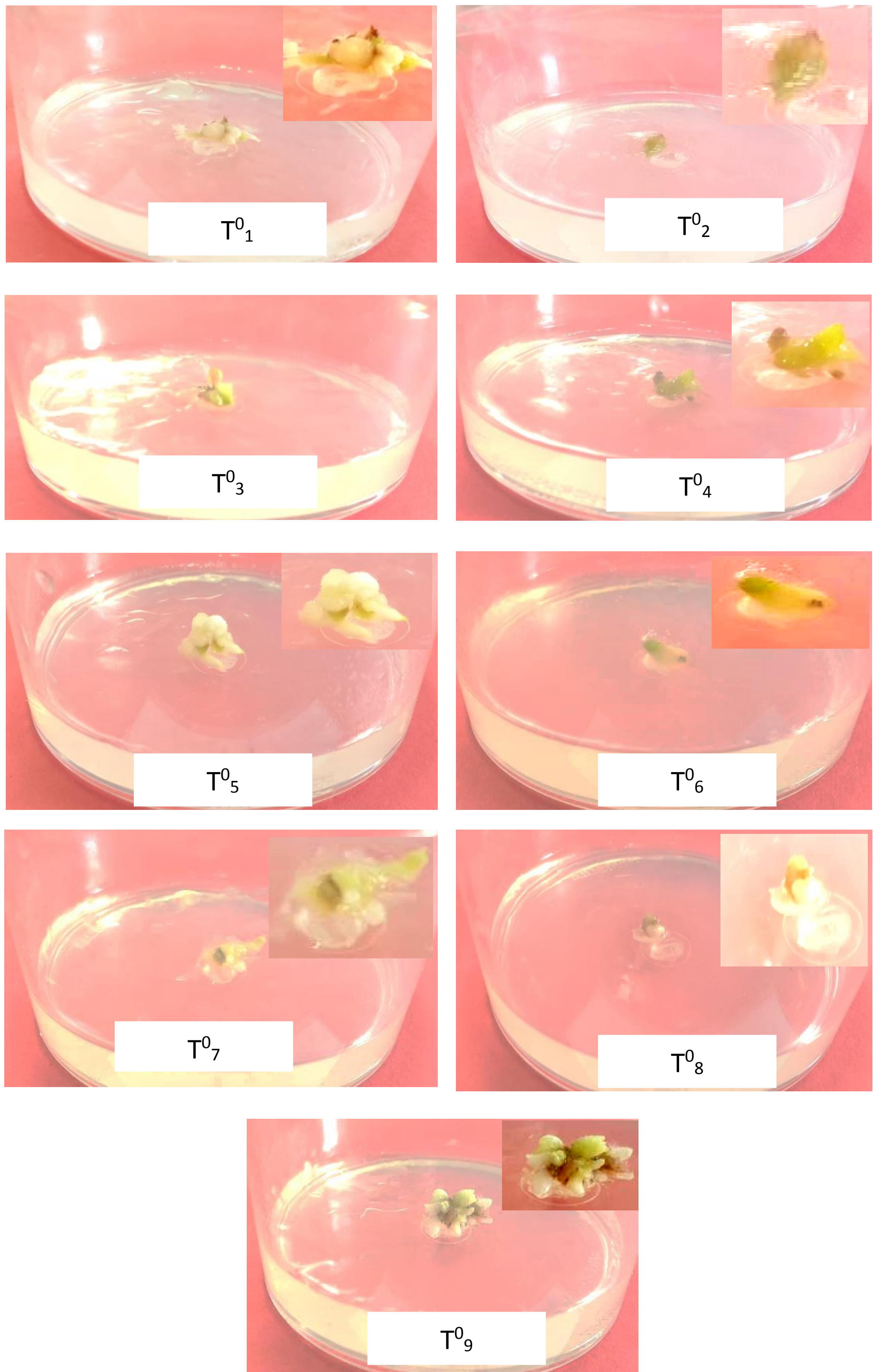


Fig.4.2.4: Callus under complete dark on 30th day after inoculation

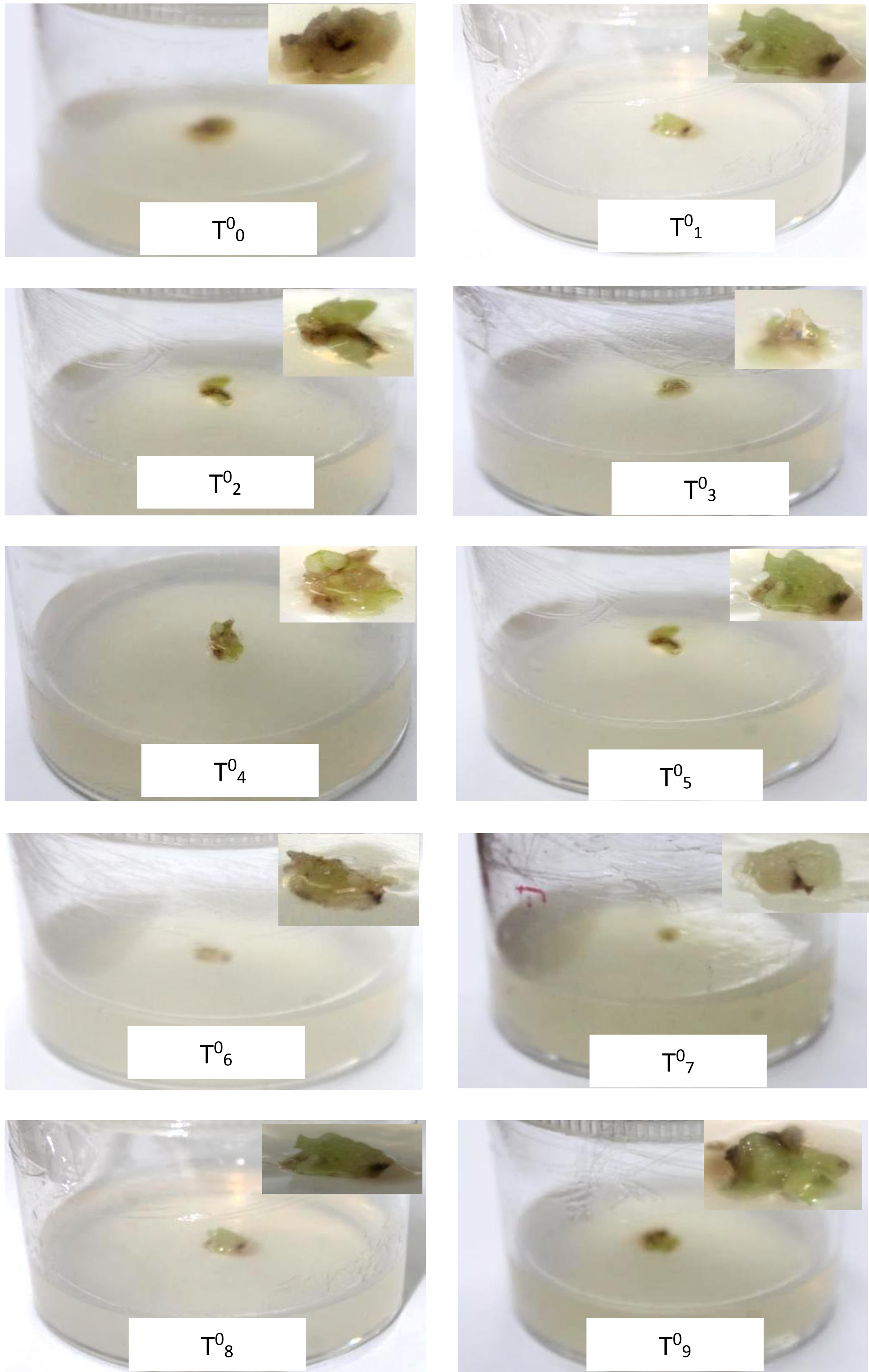


Fig.4.2.5: Callus under complete dark on 45th day after inoculation

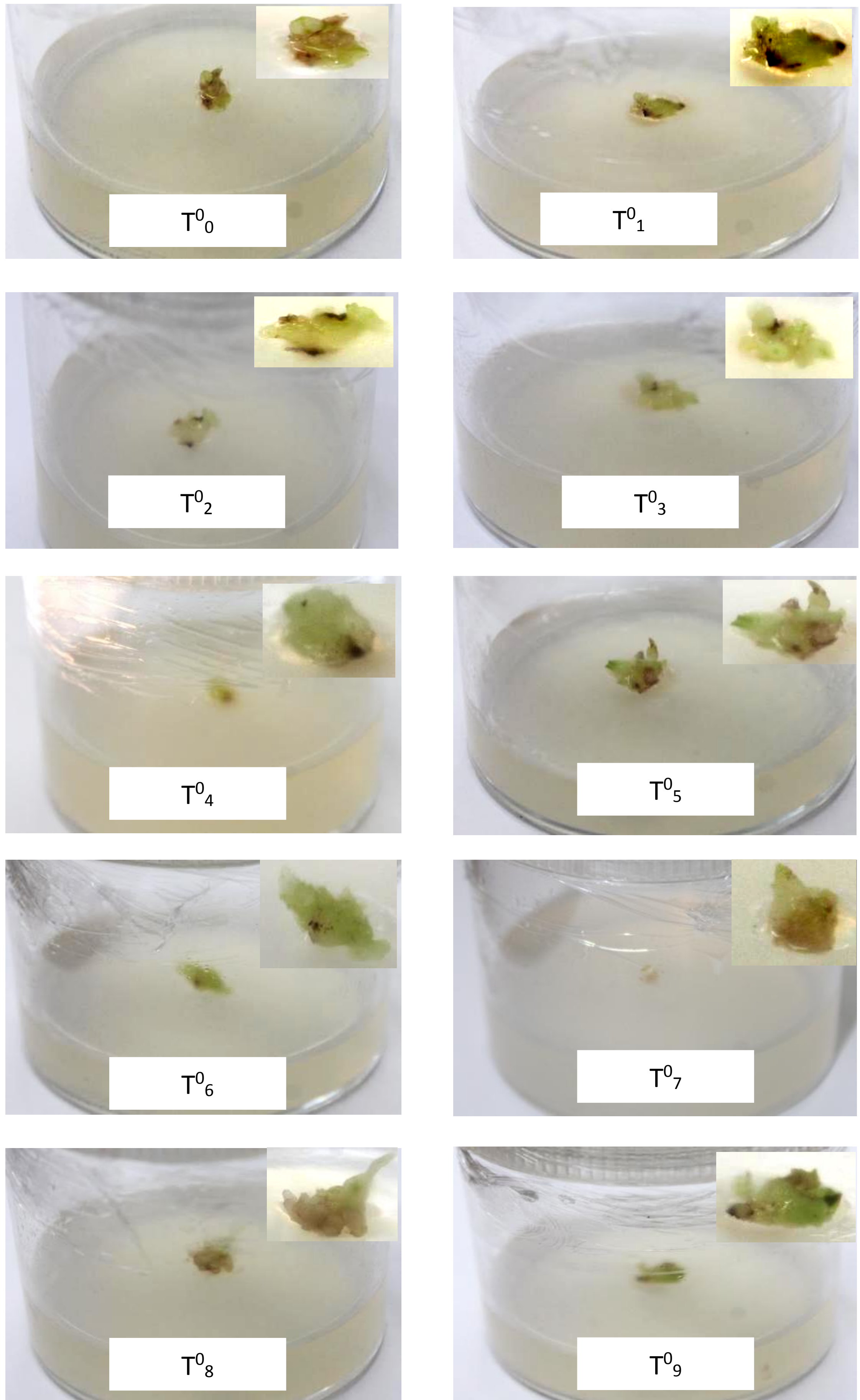


Fig.4.2.6: Callus under complete dark on 60th day after inoculation

4.3 Performance of plant growth regulators for shoot proliferation.

4.3.1 Days taken for shoot initiation:

The plant growth regulators for shoot proliferation were administered in 7 different concentration combinations of NAA and BAP and a control. It was observed that earliest shoot initiation (3.41 days) was recorded in S_5, 3 mg/l BAP + 0.6 mg/l NAA and the values were tabulated in Table 4.3.1 and Fig 4.3.1. Treatment S_5, 3 mg/l BAP + 0.6 mg/l NAA was statistically at par with S_7, 4 mg/l BAP + 0.8 mg/l NAA (3.50 days) and S_4, 2.5 mg/l BAP + 0.5 mg/l NAA (3.56 days). Treatment S_0, control, took the longest time (4.88 days) for shoot initiation.

4.3.2 Number of shoots:

The maximum number of shoots counted on the 15th day was in S_4, 2.5 mg/l BAP + 0.5 mg/l NAA (1.41) followed by S_3, 2 mg/l BAP + 0.25 mg/l NAA (1.24) and was at par with all other treatment combinations *viz.* S_2, 1.5 mg/l BAP + 0.125 mg/l NAA (1.22), S_7, 4 mg/l BAP + 0.8 mg/l NAA (1.23), S_1, 0.5 mg/l BAP + 0.06 mg/l NAA (1.22), S_5, 3 mg/l BAP + 0.6 mg/l NAA (1.22), S_6, 3.5mg/l BAP + 0.7 mg/l NAA (1.22) except S_0, control (0.71) (Table 4.3.2 and Fig. 4.3.2).

However, on the 30th day of observation the maximum number of shoots was significantly higher in the treatments S_4, 2.5 mg/l BAP + 0.5 mg/l NAA and S_6, 3.5mg/l BAP + 0.7 mg/l NAA (1.58).

Similarly, on the 45th day the maximum number of shoots were in S_6, 3.5mg/l BAP + 0.7 mg/l NAA (1.66) which was statistically at par with S_4, 2.5 mg/l BAP + 0.5 mg/l NAA (1.58) and the lowest number was recorded in S_0, control (1.23).

The 60th day observation gave the highest value of number of shoots in S_4, 2.5 mg/l BAP + 0.5 mg/l NAA (1.73) which is at par with S_6, 3.5mg/l BAP + 0.7 mg/l NAA (1.72) followed by S_2, 1.5 mg/l BAP + 0.125 mg/l NAA (1.58), S_5, 3 mg/l BAP + 0.6 mg/l NAA (1.47).

4.3.3 Shoot length

The maximum shoot length on the 15th day of observation was in S_3, 2 mg/l BAP + 0.25 mg/l NAA (0.98 cm, followed by S_2, 1.5 mg/l BAP + 0.125 mg/l NAA (0.63). However, it was observed that S_0, control, which contains only MS basal media failed in shoot initiation.

On the 30th day, shoot initiation was evident in all the treatment and maximum shoot length (1.50 cm) was registered in S_2, 1.5 mg/l BAP + 0.125 mg/l NAA followed by S_3, 2 mg/l BAP + 0.25 mg/l NAA (1.20cm).

In the subsequent observation on 45th day the maximum shoot length(1.45cm) was observed in S_7, 4 mg/l BAP + 0.8 mg/l NAA which was statistically at par with S_2, 1.5 mg/l BAP + 0.125 mg/l NAA (1.44 cm) and followed by S_3, 2 mg/l BAP + 0.25 mg/l NAA (1.33cm).

The maximum shoot length among all treatment combinations was recorded on the 60th day in the treatment S_2, 1.5 mg/l BAP + 0.125 mg/l NAA (1.93 cm) followed by S_7, 4 mg/l BAP + 0.8 mg/l NAA (1.65 cm) and at par with S_3, 2 mg/l BAP + 0.25 mg/l NAA (1.82 cm). While, the minimum shoot length in S_0, control (1.08 cm) was recorded.

4.3.4 Number (No.) of leaves:

In first observation (15th day) the number of leaves were highest (1.73) in S_6, 3.5mg/l BAP + 0.7 mg/l NAA (Table 4.3.4 and Fig. 4.3.4) followed by S_5, 3 mg/l BAP + 0.6 mg/l NAA (1.60) and at par with S_1, 0.5 mg/l BAP + 0.06 mg/l NAA (1.67).

Similarly, the number of leaves was maximum in S_6, 3.5mg/l BAP + 0.7 mg/l NAA (1.89) on the 30th day, followed by S_7, 4 mg/l BAP + 0.8 mg/l NAA (1.75) and S_3, 2 mg/l BAP + 0.25 mg/l NAA (1.75) which was at par with each other, S_5, 3 mg/l BAP + 0.6 mg/l NAA (1.74), S_1, 0.5 mg/l BAP + 0.06 mg/l NAA (1.73) and S_2, 1.5 mg/l BAP + 0.125 mg/l NAA (1.73). The lowest number of leaves was recorded in S_0, control (1.23).

Subsequently, on the 45th day the highest number of leaves recorded was in S_6, 3.5mg/l BAP + 0.7 mg/l NAA (1.98) was at par with S_3, 2 mg/l BAP + 0.25 mg/l NAA (1.89), S_7, 4 mg/l BAP + 0.8 mg/l NAA (1.89) and S_5, 3 mg/l BAP + 0.6 mg/l NAA (1.88)

Interestingly, the maximum number of leaves recorded on the 60th day was in S_5, 3 mg/l BAP + 0.6 mg/l NAA (2.04), which was statistically at par with S_6, 3.5mg/l BAP + 0.7 mg/l NAA (2.01), S_7, 4 mg/l BAP + 0.8 mg/l NAA (1.93) and S_3, 2 mg/l BAP + 0.25 mg/l NAA (1.91). The lowest number of leaves was recorded in S_0, control (1.47).

4.3.5 Leaf length

On 15th day observation (Table 4.3.5 and Fig.4.3.5) the maximum leaf length was noted in S_4, 2.5 mg/l BAP + 0.5 mg/l NAA (1.01 cm) followed by S_5, 3 mg/l BAP + 0.6 mg/l NAA (0.79 cm). Whereas, the lowest leaf length was recorded in S_2, 1.5 mg/l BAP + 0.125 mg/l NAA (0.39 cm).

On the 30th day, it was recorded that the leaf length in S_5, , 3 mg/l BAP + 0.6 mg/l NAA (1.33 cm) was significantly higher than the other treatment followed by S_4, 2.5 mg/l BAP + 0.5 mg/l NAA (1.19 cm).

Similarly, on the 45th day the leaf length was highest in S_5, 3 mg/l BAP + 0.6 mg/l NAA (1.50 cm) followed by S_6, 3.5mg/l BAP + 0.7 mg/l NAA (1.35 cm) and S_4, 2.5 mg/l BAP + 0.5 mg/l NAA (1.20 cm).

Nevertheless, the 60th day data revealed that the maximum leaf length was recorded in treatment S_6, 3.5mg/l BAP + 0.7 mg/l NAA (1.91 cm) followed by S_5, 3 mg/l BAP + 0.6 mg/l NAA (1.73 cm) and the lowest leaf length was recorded in S_2, 1.5 mg/l BAP + 0.125 mg/l NAA (1.23 cm) followed by S_0, control (1.25 cm) and S_3, 2 mg/l BAP + 0.25 mg/l NAA (1.25 cm).

4.3.6 Shoot weight

The shoot weight on 15th day, was maximum in the treatment S_3, 2 mg/l BAP + 0.25 mg/l NAA (498.75 mg) followed by S_7, 4 mg/l BAP + 0.8 mg/l NAA (338.48 mg) and S_2, 1.5 mg/l BAP + 0.125 mg/l NAA (321.20 mg). However, there was no shoot formation in treatment S_0, control on the same day of observation (Table 4.3.6 and Fig 4.3.6).

Shoot weight on the 30th day was recorded highest (554.03 mg) in S_3, 2 mg/l BAP + 0.25 mg/l NAA followed by S_7, 4 mg/l BAP + 0.8 mg/l NAA (495.28 mg) and S_1, 0.5 mg/l BAP + 0.06 mg/l NAA (480.63 mg) which was at par witheach other.

Further, the observation on the 45th day showed that the highest shoot weight was recorded in S_3, 2 mg/l BAP + 0.25 mg/l NAA (594.13 mg) followed by S_1, 0.5 mg/l BAP + 0.06 mg/l NAA (553.35 mg) and S_7, 4 mg/l BAP + 0.8 mg/l NAA (531.15 mg).

Results obtained on the 60th day of observation were in consonance with that of earlier results. The highest shoot weight recorded was in S_3, 2 mg/l BAP + 0.25 mg/l NAA (619.03 mg) followed by S_1, 0.5 mg/l BAP + 0.06 mg/l NAA (603.13 mg) and the lowest shoot weight observed was in S_0, control (226.90 mg).

Table 4.3.1 Effect of PGRs on days taken for shoot initiation (square root value)

Treatment	Day of shoot initiation
S_0 (MS basal media control)	4.88
S_1 (0.5 mg/l BAP + 0.06 mg/l NAA)	3.70
S_2 (1.5 mg/l BAP + 0.125 mg/l NAA)	3.96
S_3 (2 mg/l BAP + 0.25 mg/l NAA)	3.70
S_4 (2.5 mg/l BAP + 0.5 mg/l NAA)	3.56
S_5 (3 mg/l BAP + 0.6 mg/l NAA)	3.41
S_6 (3.5mg/l BAP + 0.7 mg/l NAA)	3.77
S_7 (4 mg/l BAP + 0.8 mg/l NAA)	3.50
SEM	0.05
CD (p=0.05)	0.18
CV%	2.81

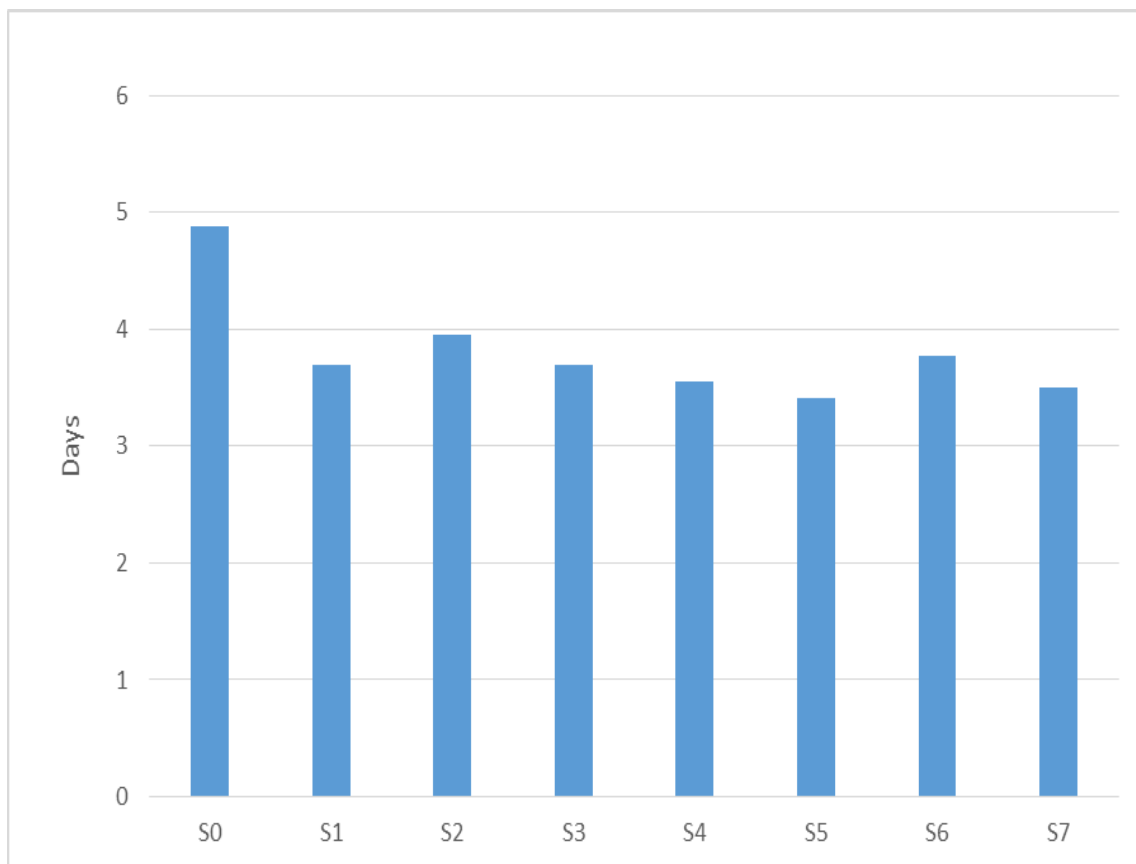


Fig 4.3.1 Effect of PGRs on days taken for shoot initiation

Table 4.3.2 Effect of PGRs on number of shoots. (square root value)

Treatment	15 days	30 days	45 days	60 days
S_0 (MS basal media control)	0.71	1.22	1.23	1.41
S_1 (0.5 mg/l BAP + 0.06 mg/l NAA)	1.22	1.29	1.33	1.45
S_2 (1.5 mg/l BAP + 0.125 mg/l NAA)	1.24	1.41	1.44	1.58
S_3 (2 mg/l BAP + 0.25 mg/l NAA)	1.22	1.29	1.41	1.45
S_4 (2.5 mg/l BAP + 0.5 mg/l NAA)	1.41	1.58	1.58	1.73
S_5 (3 mg/l BAP + 0.6 mg/l NAA)	1.22	1.28	1.45	1.47
S_6 (3.5mg/l BAP + 0.7 mg/l NAA)	1.22	1.58	1.66	1.72
S_7 (4 mg/l BAP + 0.8 mg/l NAA)	1.23	1.41	1.43	1.47
SEM	0.03	0.03	0.03	0.03
CD (p=0.05)	0.09	0.11	0.09	0.09
CV%	4.35	4.91	3.76	3.29

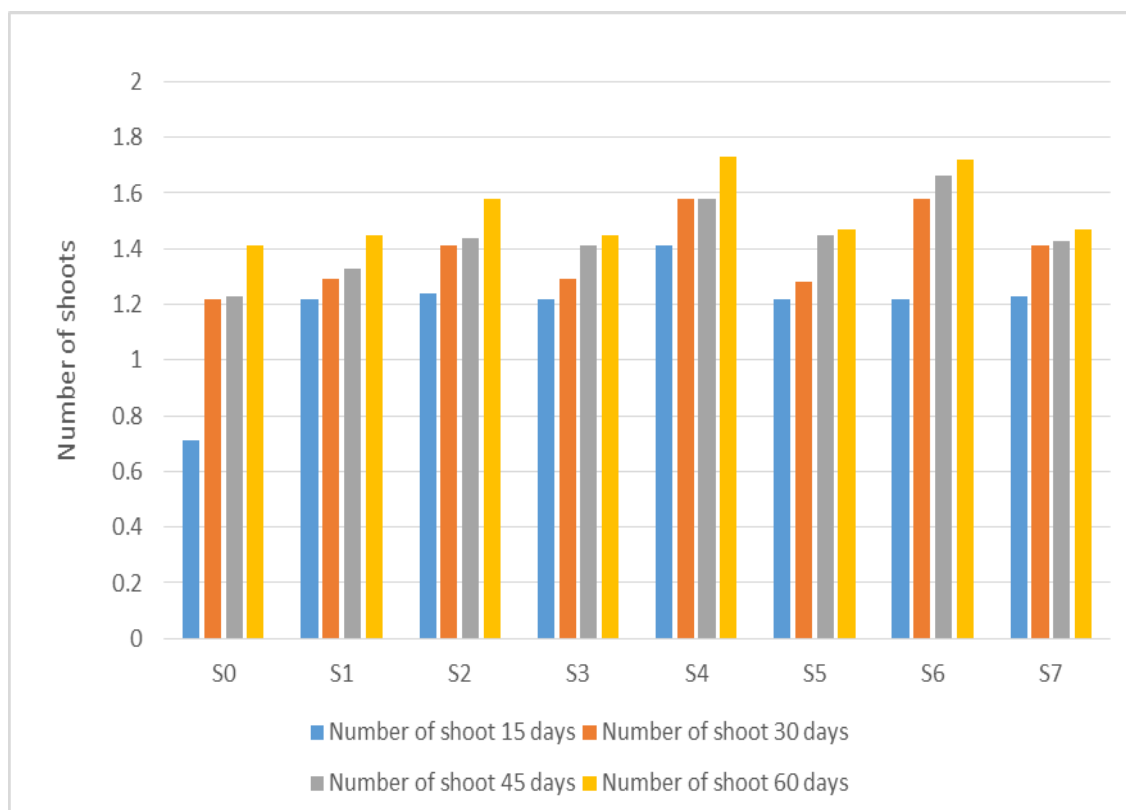


Fig 4.3.2 Effect of PGRs on number of shoots. (Square root value)

Table: 4.3.3 Effect of PGRs on shoot length (cm)

Treatment	15 days	30 days	45 days	60 days
S_0 (MS basal media control)	0.00	0.57	0.72	1.08
S_1 (0.5 mg/l BAP + 0.06 mg/l NAA)	0.54	0.94	1.20	1.48
S_2 (1.5 mg/l BAP + 0.125 mg/l NAA)	0.63	1.50	1.44	1.93
S_3 (2 mg/l BAP + 0.25 mg/l NAA)	0.98	1.20	1.33	1.82
S_4 (2.5 mg/l BAP + 0.5 mg/l NAA)	0.46	0.68	0.89	1.38
S_5 (3 mg/l BAP + 0.6 mg/l NAA)	0.50	0.70	1.01	1.25
S_6 (3.5mg/l BAP + 0.7 mg/l NAA)	0.43	0.54	1.01	1.30
S_7 (4 mg/l BAP + 0.8 mg/l NAA)	0.56	0.86	1.45	1.65
SEM	0.01	0.02	0.02	0.03
CD (p=0.05)	0.04	0.06	0.08	0.12
CV%	4.91	4.06	4.26	4.65

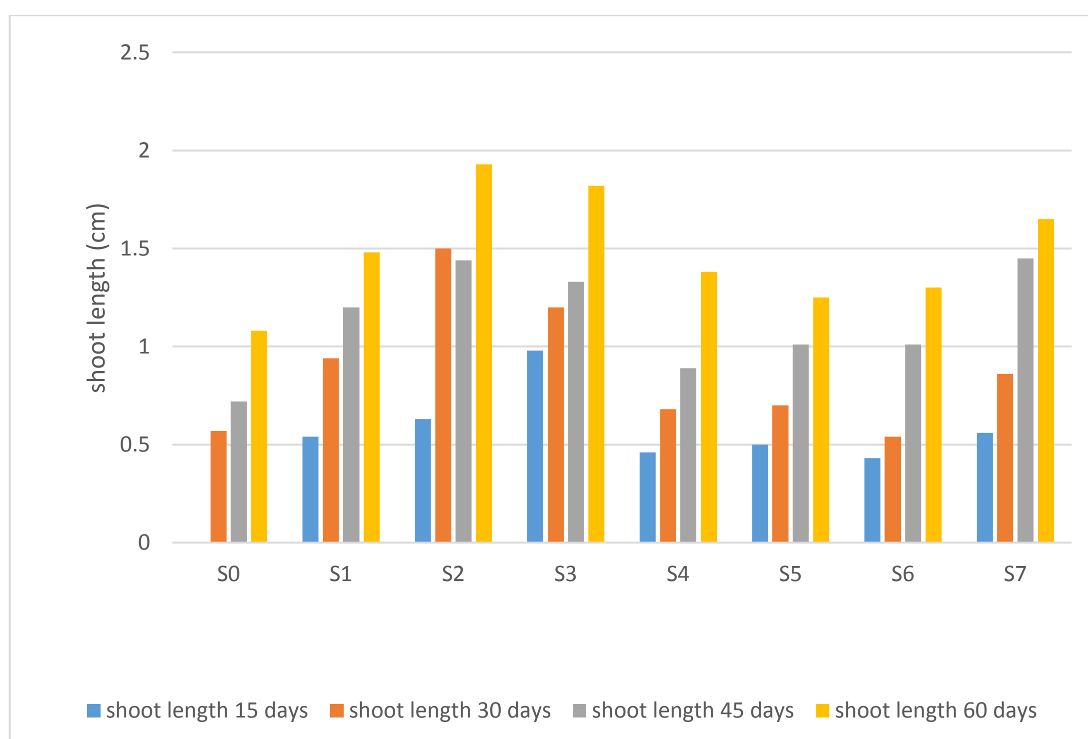


Fig 4.3.3 Effect of PGRs on shoot length (cm)

Table 4.3.4 Effect of PGRs on number of leaves (square root value).

Treatment	15 days	30 days	45 days	60 days
S_0 (MS basal media control)	0.71	1.23	1.27	1.42
S_1 (0.5 mg/l BAP + 0.06 mg/l NAA)	1.67	1.73	1.77	1.87
S_2 (1.5 mg/l BAP + 0.125 mg/l NAA)	1.57	1.73	1.75	1.77
S_3 (2 mg/l BAP + 0.25 mg/l NAA)	1.58	1.75	1.89	1.91
S_4 (2.5 mg/l BAP + 0.5 mg/l NAA)	1.41	1.47	1.58	1.78
S_5 (3 mg/l BAP + 0.6 mg/l NAA)	1.60	1.74	1.88	2.04
S_6 (3.5mg/l BAP + 0.7 mg/l NAA)	1.73	1.89	1.98	2.01
S_7 (4 mg/l BAP + 0.8 mg/l NAA)	1.41	1.75	1.89	1.93
SEM	0.03	0.03	0.04	0.03
CD (p=0.05)	0.10	0.11	0.13	0.10
CV%	4.12	3.79	4.46	3.29

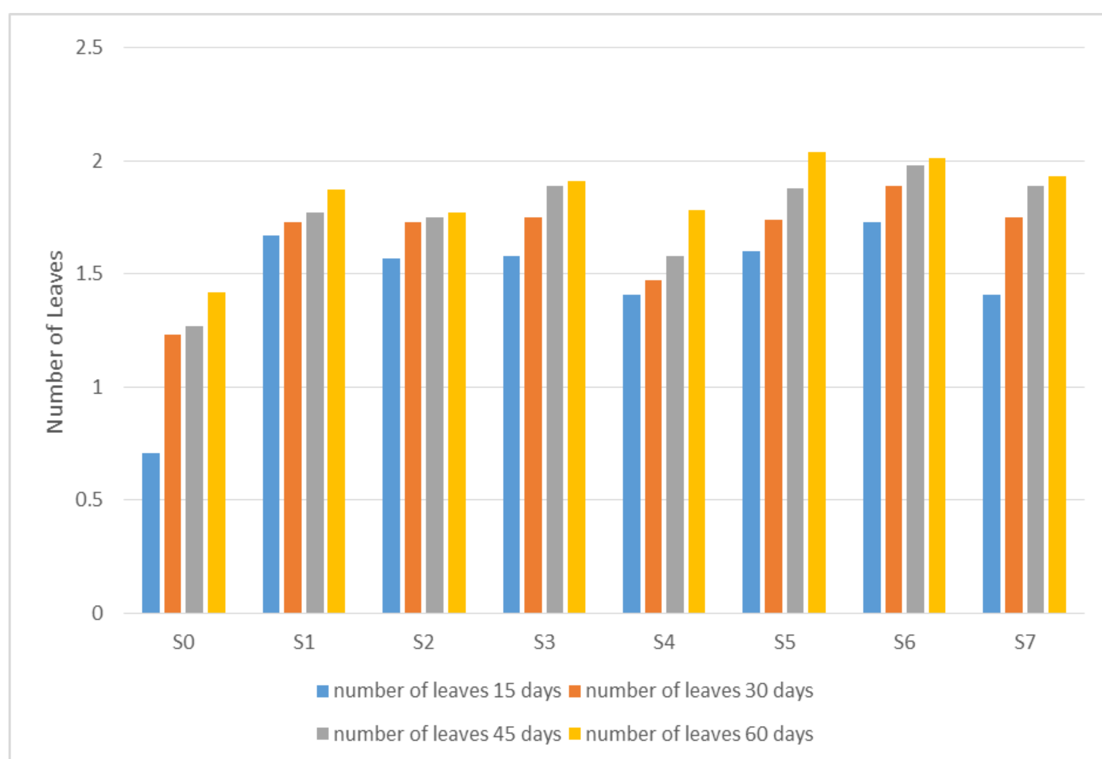


Fig 4.3.4 Effect of PGRs on number of leaves (square root value).

Table 4.3.5 Effect of PGRs on Leaf length (cm).

Treatment	15 days	30 days	45 days	60 days
S_0 (MS basal media control)	0.00	0.58	0.80	1.25
S_1 (0.5 mg/l BAP + 0.06 mg/l NAA)	0.48	0.84	1.23	1.37
S_2 (1.5 mg/l BAP + 0.125 mg/l NAA)	0.39	0.59	1.10	1.23
S_3 (2 mg/l BAP + 0.25 mg/l NAA)	0.44	0.57	0.83	1.25
S_4 (2.5 mg/l BAP + 0.5 mg/l NAA)	1.01	1.19	1.20	1.33
S_5 (3 mg/l BAP + 0.6 mg/l NAA)	0.79	1.33	1.50	1.73
S_6 (3.5mg/l BAP + 0.7 mg/l NAA)	0.41	0.85	1.35	1.91
S_7 (4 mg/l BAP + 0.8 mg/l NAA)	0.68	0.85	1.00	1.27
SEM	0.01	0.02	0.02	0.03
CD (p=0.05)	0.04	0.06	0.08	0.10
CV%	4.70	4.28	4.21	4.26

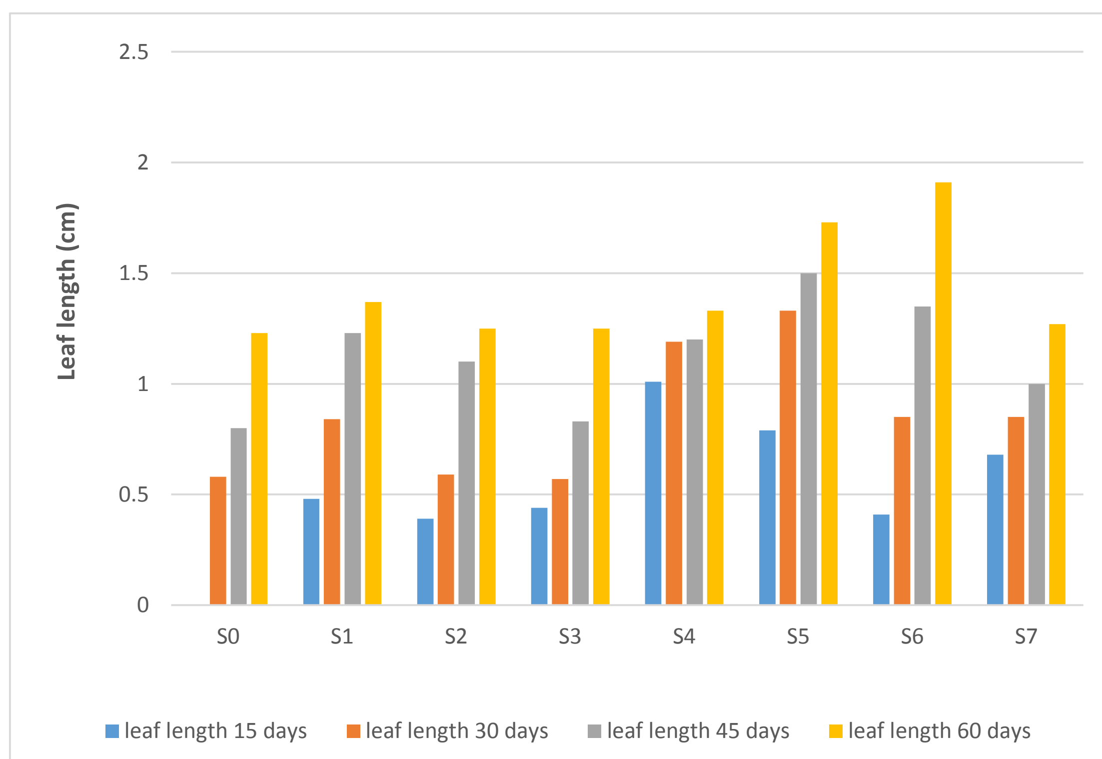


Fig 4.3.5 Effect of PGRs on Leaf length (cm).

Table 4.3.6 Effect of PGRs on shoot weight (mg).

Treatment	15 days	30 days	45 days	60 days
S_0 (MS basal media control)	0.00	147.60	193.28	226.90
S_1 (0.5 mg/l BAP + 0.06 mg/l NAA)	242.53	480.63	553.35	603.13
S_2 (1.5 mg/l BAP + 0.125 mg/l NAA)	321.20	390.30	422.05	432.18
S_3 (2 mg/l BAP + 0.25 mg/l NAA)	498.75	554.03	594.13	619.03
S_4 (2.5 mg/l BAP + 0.5 mg/l NAA)	249.90	354.10	384.43	405.63
S_5 (3 mg/l BAP + 0.6 mg/l NAA)	213.53	371.68	457.35	493.38
S_6 (3.5mg/l BAP + 0.7 mg/l NAA)	239.20	356.13	392.65	414.03
S_7 (4 mg/l BAP + 0.8 mg/l NAA)	338.48	495.28	531.15	574.88
SEM	3.68	4.35	5.64	5.33
CD (p=0.05)	12.43	14.71	19.09	18.04
CV%	2.80	2.21	2.56	2.26

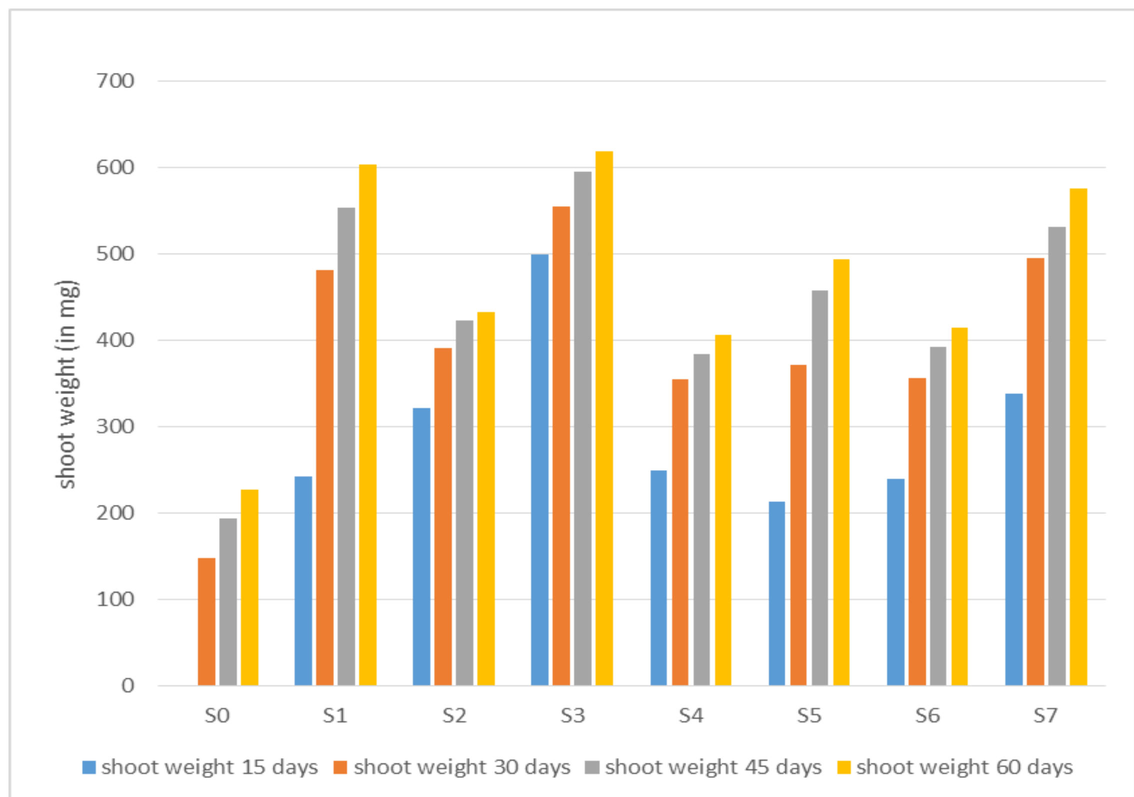
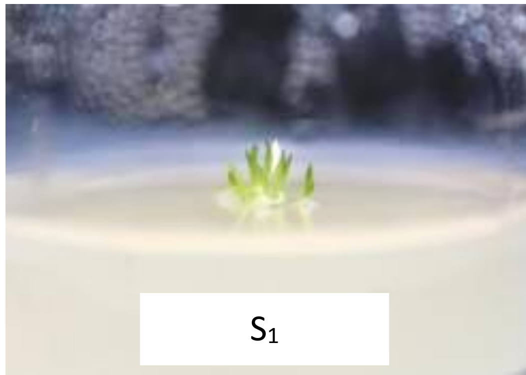
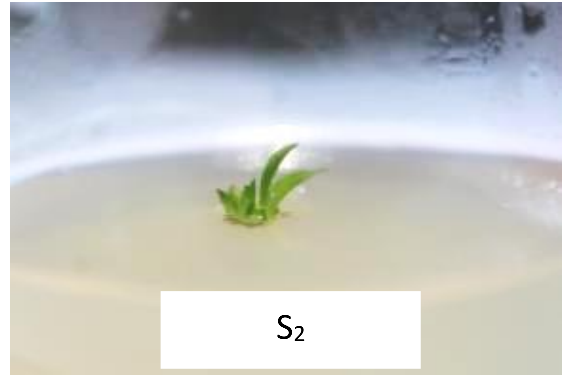
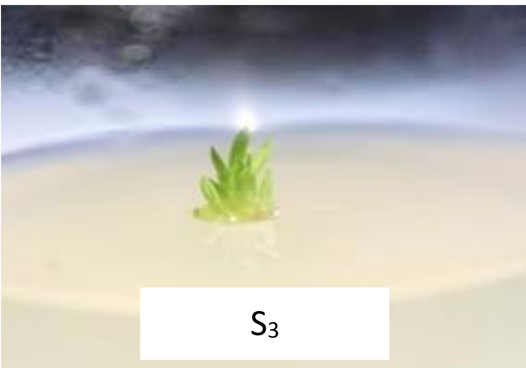
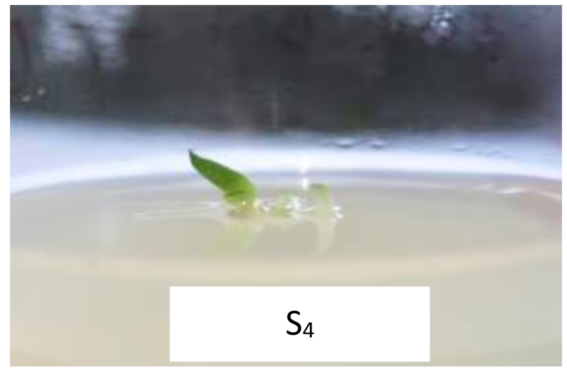


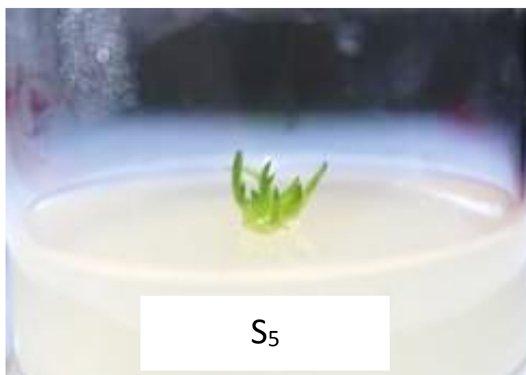
Fig 4.3.6 Effect of PGRs on shoot weight (mg)

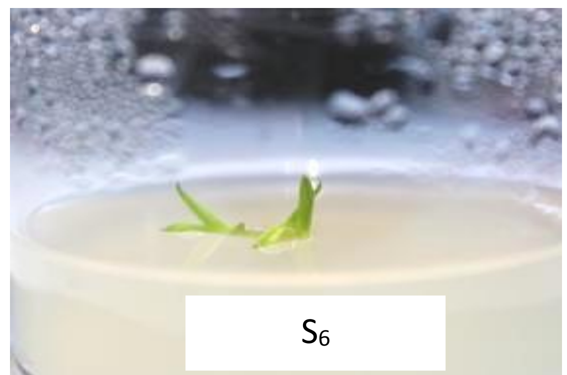












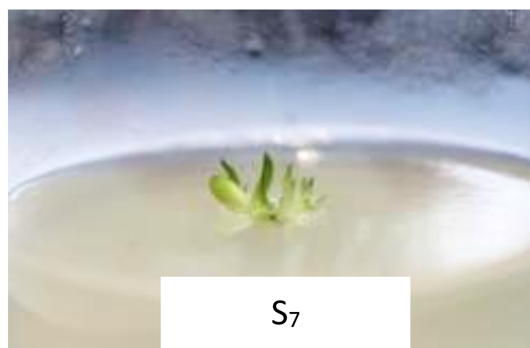
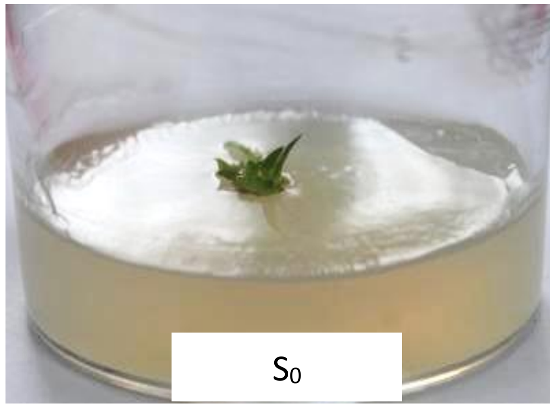
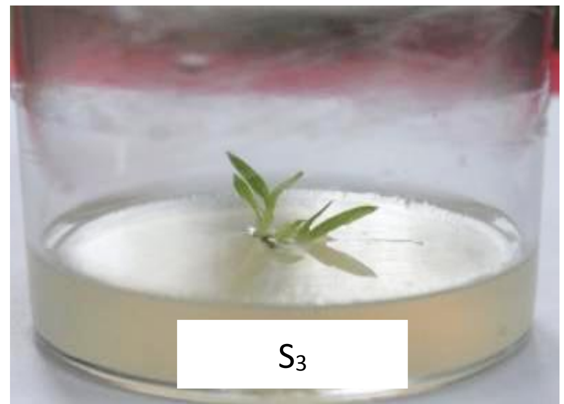
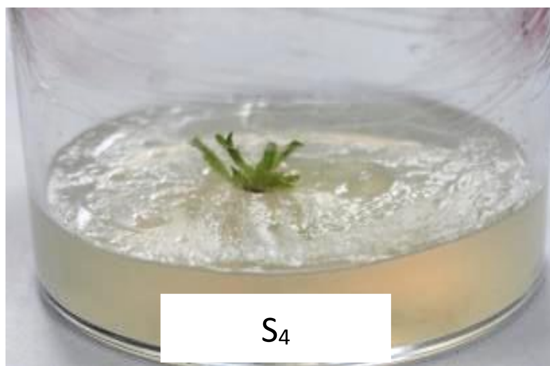


Fig. 4.3.7: Shoot proliferation after 15 days







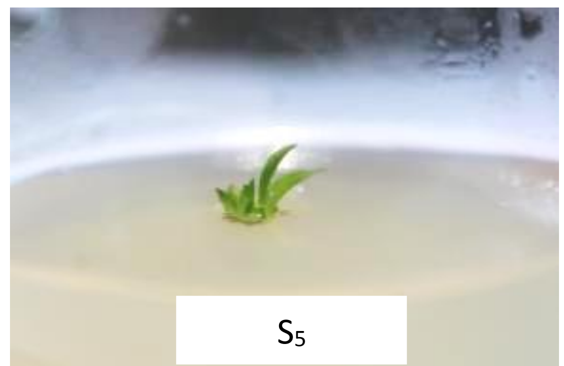
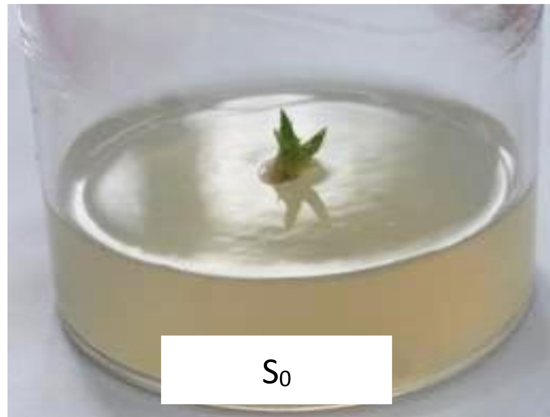
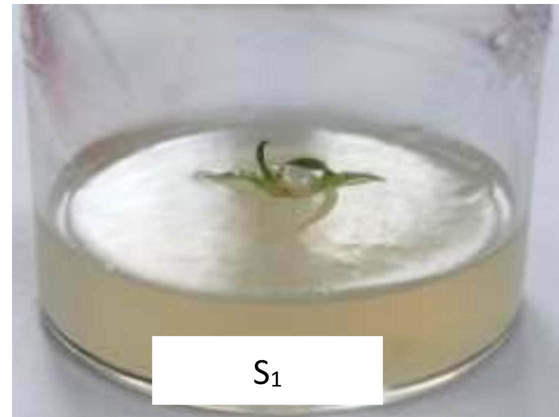


Fig. 4.3.8: Shoot proliferation after 30 days





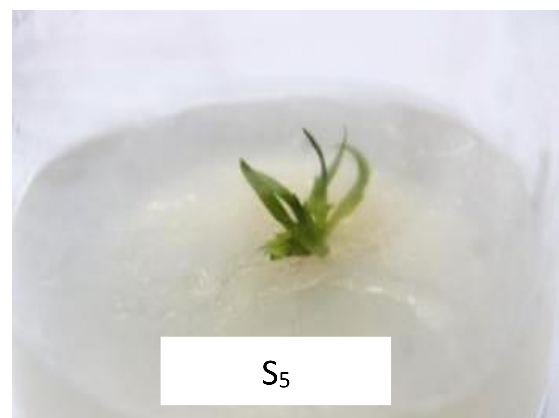
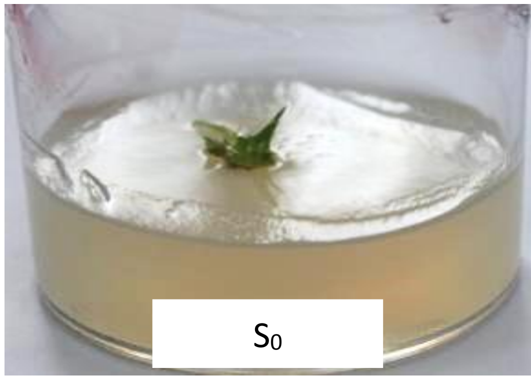
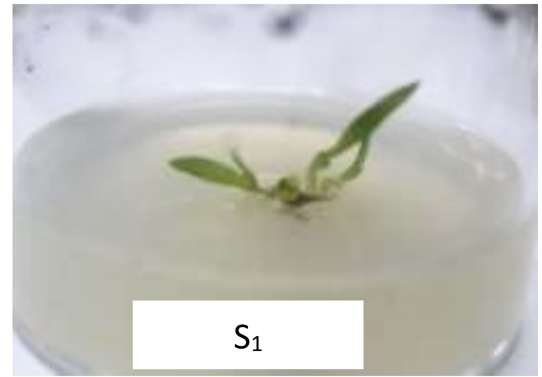
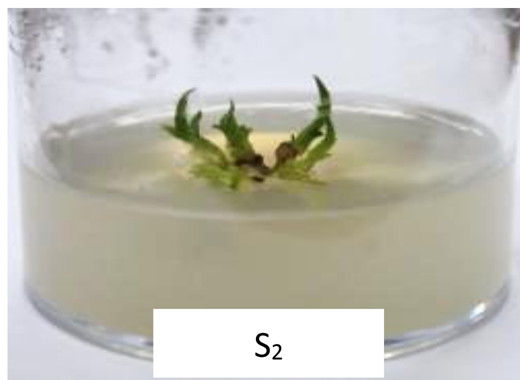
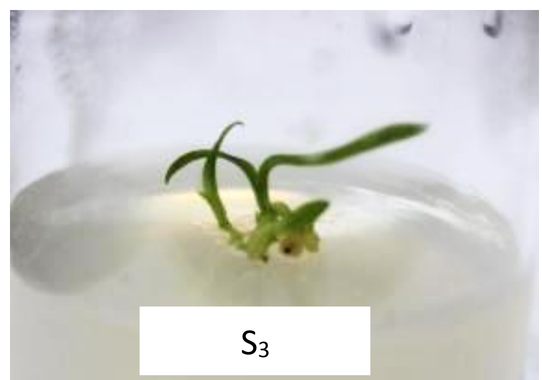


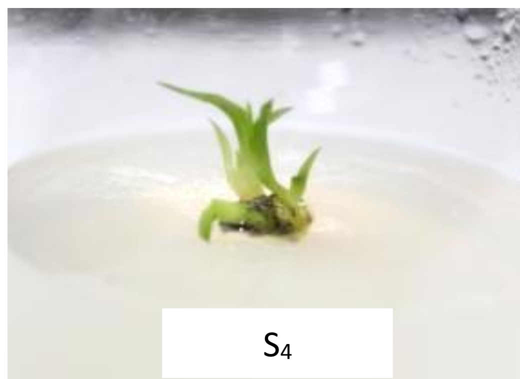
Fig. 4.3.9: Shoot proliferation after 45 days











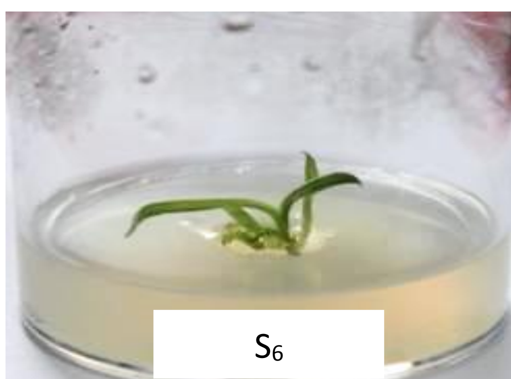


Fig. 4.3.10: Shoot proliferation after 60 days

4.4 Effect of plant growth regulators for root proliferation

4.4.1 Days taken for root initiation.

Earliness in root initiation was recorded with supplement of plant growth regulator on the MS media and are presented in Table 4.4.1 and Fig. 4.4.1. The minimum number of days recorded for significant root initiation was recorded in R_4, 0.5 mg/l BAP + 1 mg/l IBA (2.96) followed by R_5, 1 mg/l BAP + 2 mg/l IBA (3.24 days) and R_3, 0.25 mg/l BAP + 0.5 mg/l IBA (3.74 days). While, the maximum days took for root initiation was observed in R_0, control (5.12 days).

4.4.2 Root length:

Positive effect with the supplement of plant growth regulator was noted on root proliferation and presented in Table 4.4.2 and Fig 4.4.2. The longest root length (0.70 cm) was recorded in R_4, 0.5 mg/l BAP + 1 mg/l IBA followed by R_5, 1 mg/l BAP + 2 mg/l IBA (0.58 cm) and R_3, 0.25 mg/l BAP + 0.5 mg/l IBA (0.40 cm). it was noticed that R_0, control, R_1, 0.06 mg/l BAP + 0.125 mg/l IBA and R_2, 0.125 mg/l BAP + 0.25 mg/l IBA fail to show root initiation on the 15th day after inoculation.

On the subsequent observation the 30th day it was observed that root initiation was recorded in all the treatment. Wherein, the longest root length (1.10 cm) was observed in R_4, 0.5 mg/l BAP + 1 mg/l IBA followed by R_5, 1 mg/l BAP + 2 mg/l IBA (0.90cm) than R_3, 0.25 mg/l BAP + 0.5 mg/l IBA (0.80 cm) and the lowest root length was recorded for R_0, control (0.27 cm).

However, on the 45th days the longest root length (1.28 cm) was recorded for R_3, 0.25 mg/l BAP + 0.5 mg/l IBA followed by R_4, 0.5 mg/l BAP + 1 mg/l IBA (1.13 cm) than R_5, 1 mg/l BAP + 2 mg/l IBA (0.95 cm).

Similarly, the longest root on the 60th day was recorded for R_3, 0.25 mg/l BAP + 0.5 mg/l IBA (1.38 cm) followed by R_4, 0.5 mg/l BAP + 1 mg/l IBA (1.25 cm) than R_5, 1 mg/l BAP + 2 mg/l IBA (1.10 cm). and the shortest root was noticed in R_0 (0.35 cm).

4.4.3 Number of root:

Significant number of root (2.17) as observed on the 15th days in R_5, 1 mg/l BAP and 2 mg/l IBA) followed by R_4, 0.5 mg/l BAP + 1 mg/l IBA (2.00) and R_3, 0.25 mg/l BAP + 0.5 mg/l IBA (1.87) respectively,(Table 4.4.3 and Fig 4.4.3).

However, the number of root on the 30th day was recorded to be higher (2.53) in R_4, 0.5 mg/l BAP + 1 mg/l IBA was at par with R_5, 1 mg/l BAP + 2 mg/l IBA (2.46) followed by R_3 (2.06).

Nonetheless, on the 45th day the highest number (3.04) of root was observed in R_4, 0.5 mg/l BAP + 1 mg/l IBA followed by R_5, 1 mg/l BAP and 2 mg/l IBA (2.90). while, the lowest number of root was recorded in R_0, control (1.63).

Similarly, on the 60th day the number of root was maximum (3.39) in R_4, 0.5 mg/l BAP + 1 mg/l IBA which is at par R_3, 0.25 mg/l BAP + 0.5 mg/l IBA (3.36) and R_5, 1 mg/l BAP and 2 mg/l IBA (3.31) R_0, control recorded minimum number of roots (1.67) .

4.4.4 Root weight:

Enhanced root weight with addition of plant growth regulator was noted and presented on Table 4.4.4 and Fig. 4.4.4. On the 15th day highest root weight (6.63 mg) was observed in R_3, 0.25 mg/l BAP + 0.5 mg/l followed by R_5, 1 mg/l BAP + 2 mg/l IBA (4.31 mg) and R_4, 0.5 mg/l BAP + 1 mg/l IBA (4.22 mg) respectively.

Similarly, on the 30th day the highest root weight (8.18 mg) was obtained in R_3, 0.25 mg/l BAP + 0.5 mg/l which was followed by R_4, 0.5 mg/l BAP + 1 mg/l IBA (6.66mg) and the lowest root weight recorded in R_0, control (1.75 mg).

Correspondingly, on the 45th day maximum root weight (11.04 mg) was recorded in R_3, 0.25 mg/l BAP + 0.5 mg/l IBA followed by R_5, 1 mg/l BAP + 2 mg/l IBA (8.84 mg) and R_4, 0.5 mg/l BAP + 1 mg/l IBA (8.19 mg) respectively.

On the 60th day maximum root weight (11.08 mg) was recorded in R_3, 0.25 mg/l BAP + 0.5 mg/l followed by R_5, 1 mg/l BAP + 2 mg/l IBA (10.46 mg) and at par with R_4, 0.5 mg/l BAP + 1 mg/l IBA (10.77 mg). The lowest root weight (2.23) was noted in R_0, control.

Table 4.4.1 Effect of PGRs on days taken for initiation of root (square root value)

Treatment	Days taken for root initiation
R_0 (MS basal media control)	5.12
R_1 (0.06 mg/l BAP + 0.125 mg/l IBA)	4.53
R_2 (0.125 mg/l BAP + 0.25 mg/l IBA)	4.15
R_3 (0.25 mg/l BAP + 0.5 mg/l IBA)	3.74
R_4 (0.5 mg/l BAP + 1 mg/l IBA)	2.96
R_5 (1 mg/l BAP + 2 mg/l IBA)	3.24
SEM	0.04
CD (p=0.05)	0.14
CV%	2.11

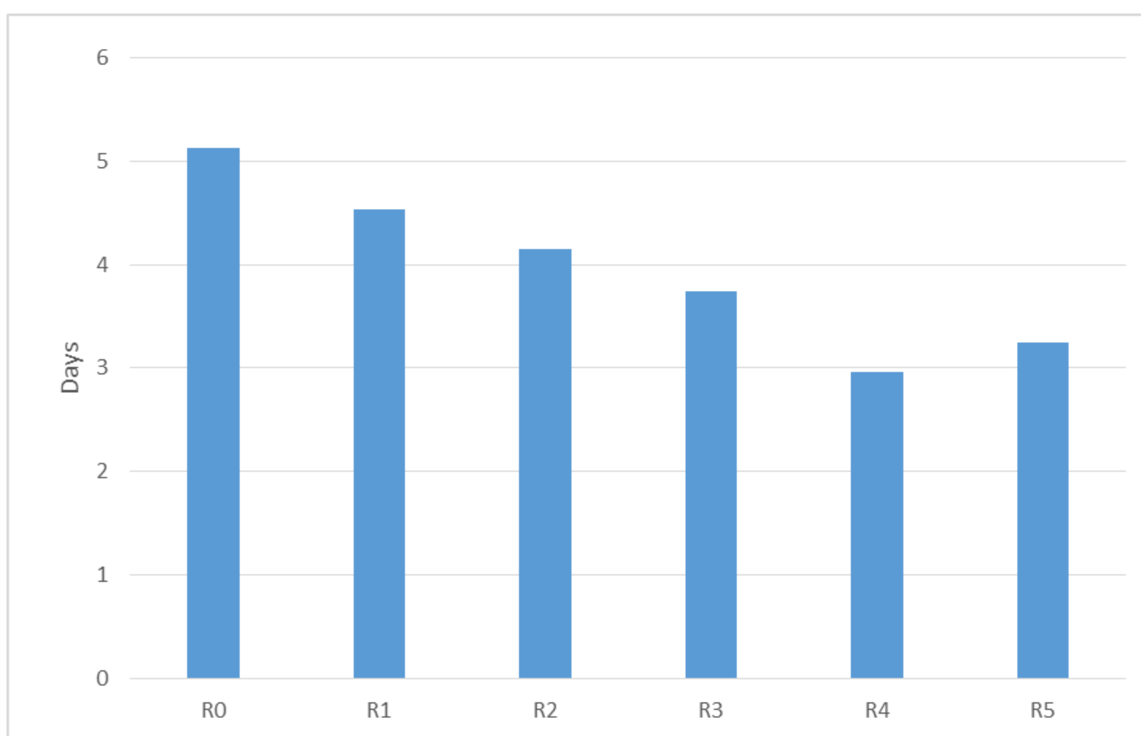


Fig. 4.4.1 Effect of PGRs on days taken for initiation of root (square root value)

Table 4.4.2 Effect of PGRs on number of roots (square root transformation)

Treatment	15 days	30 days	45 days	60 days
R_0 (MS basal media control)	0.71	1.40	1.63	1.67
R_1 (0.06 mg/l BAP + 0.125 mg/l IBA)	0.71	1.66	1.81	2.09
R_2 (0.125 mg/l BAP + 0.25 mg/l IBA)	0.71	1.58	1.99	2.34
R_3 (0.25 mg/l BAP + 0.5 mg/l IBA)	1.87	2.06	2.50	3.36
R_4 (0.5 mg/l BAP + 1 mg/l IBA)	2.00	2.53	3.04	3.39
R_5 (1 mg/l BAP + 2 mg/l IBA)	2.17	2.46	2.90	3.31
SEM	0.02	0.02	0.04	0.03
CD (p=0.05)	0.06	0.08	0.12	0.12
CV%	2.76	2.41	3.12	2.59

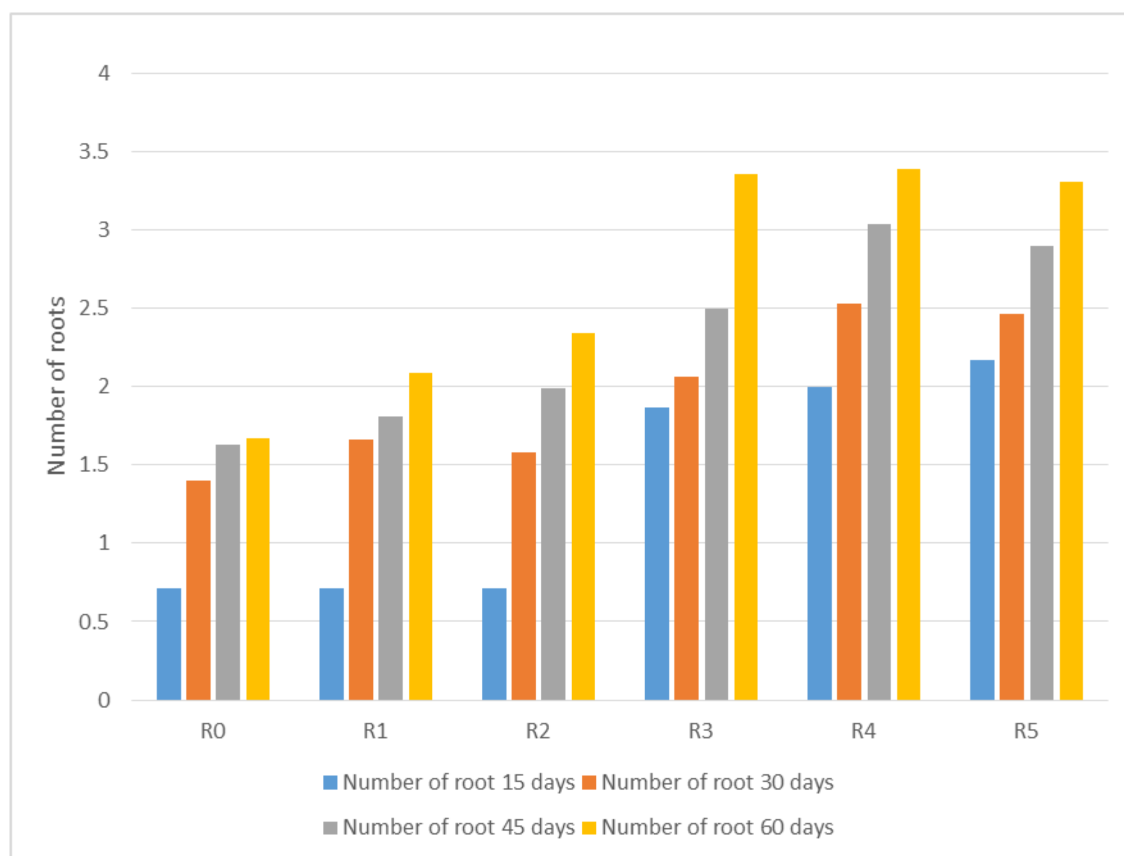


Fig 4.4.2 Effect of PGRs on number of roots (square root transformation)

Table 4.4.3 Effect of PGRs on Root length (cm)

Treatment	15 days	30 days	45 days	60 days
R_0 (MS basal media control)	0.00	0.27	0.30	0.35
R_1 (0.06 mg/l BAP + 0.125 mg/l IBA)	0.00	0.30	0.43	0.50
R_2 (0.125 mg/l BAP + 0.25 mg/l IBA)	0.00	0.37	0.40	0.65
R_3 (0.25 mg/l BAP + 0.5 mg/l IBA)	0.40	0.80	1.28	1.38
R_4 (0.5 mg/l BAP + 1 mg/l IBA)	0.70	1.10	1.13	1.25
R_5 (1 mg/l BAP + 2 mg/l IBA)	0.58	0.90	0.95	1.10
SEM	0.01	0.01	0.01	0.01
CD (p=0.05)	0.02	0.03	0.03	0.03
CV%	4.71	3.04	2.36	2.17

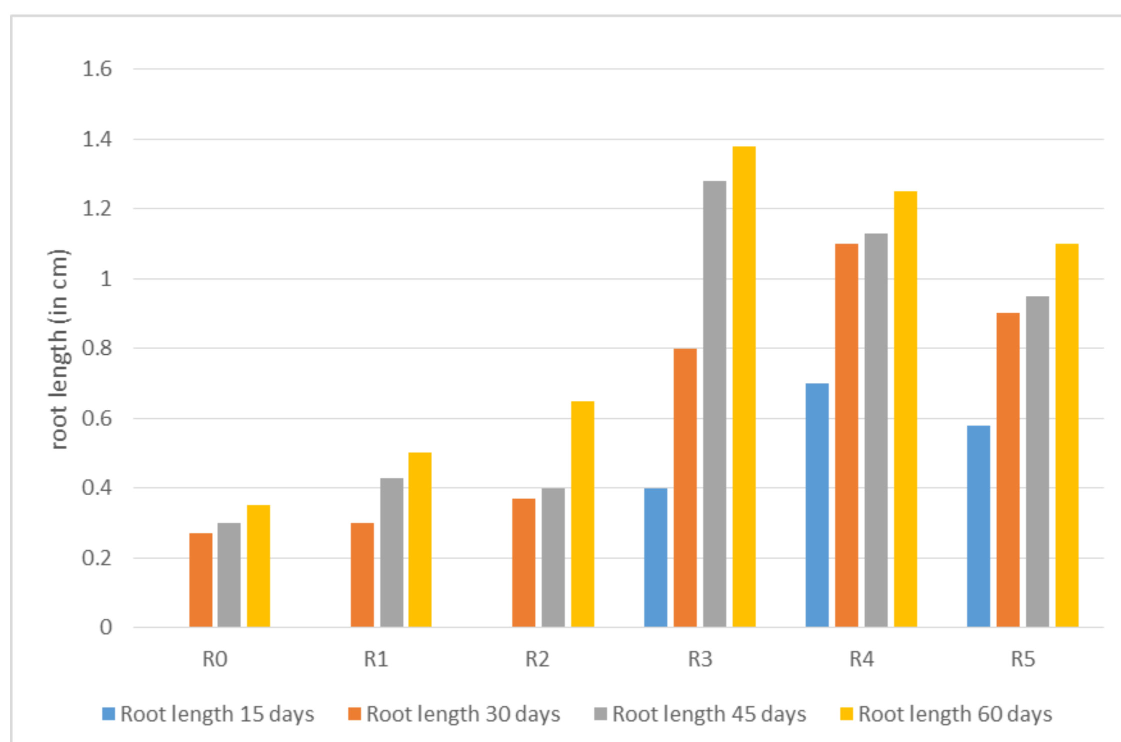


Fig. 4.4.3 Effect of PGRs on Root length (cm)

Table 4.4.4 Effect of PGRs on Root weight (mg)

Treatment	15 days	30 days	45 days	60 days
R_0 (MS basal media control)	0.00	1.75	2.03	2.32
R_1 (0.06 mg/l BAP + 0.125 mg/l IBA)	0.00	2.77	3.05	3.78
R_2 (0.125 mg/l BAP + 0.25 mg/l IBA)	0.00	2.88	3.1325	4.90
R_3 (0.25 mg/l BAP + 0.5 mg/l IBA)	6.63	8.18	11.04	11.08
R_4 (0.5 mg/l BAP + 1 mg/l IBA)	4.22	6.66	8.19	10.77
R_5 (1 mg/l BAP + 2 mg/l IBA)	4.31	4.91	8.84	10.46
SEM	0.04	0.059	0.09	0.12
CD (p=0.05)	0.13	0.21	0.30	0.41
CV%	2.98	2.63	2.89	3.27

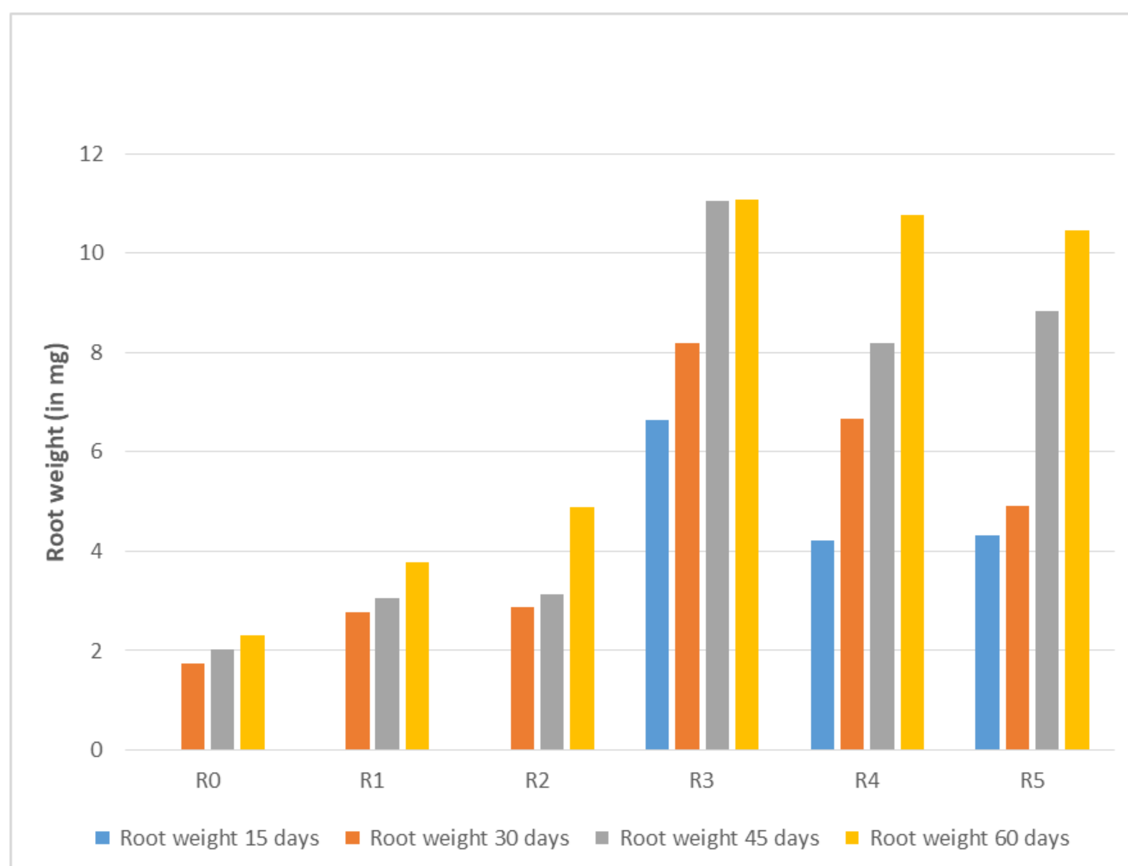
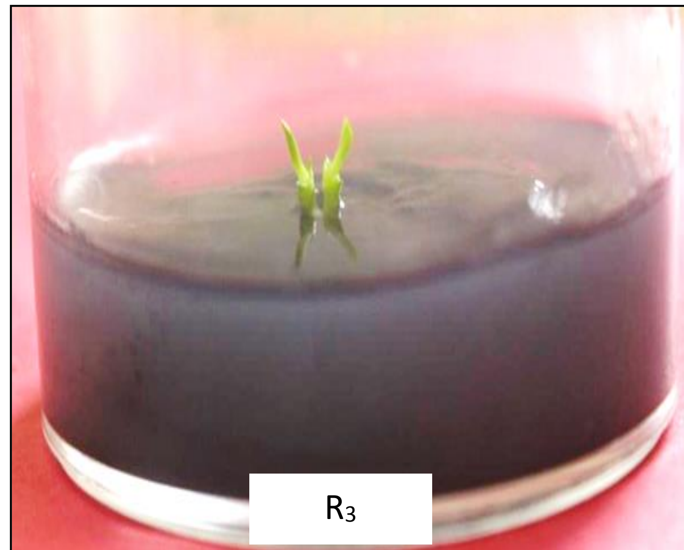
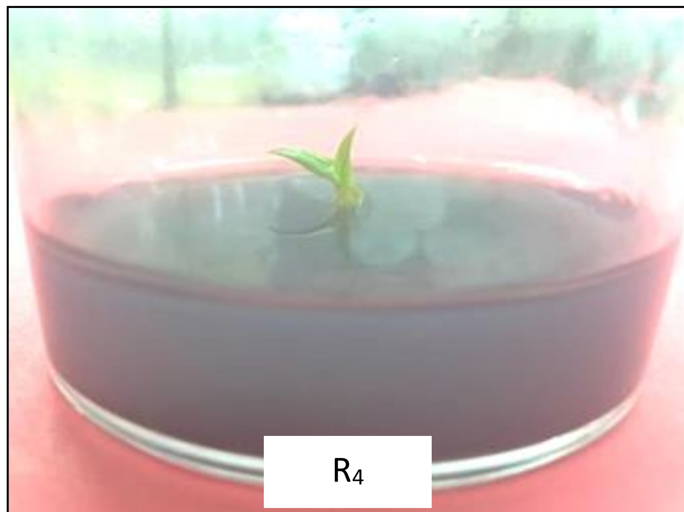


Fig. 4.4.4 Effect of PGRs on Root weight (mg)





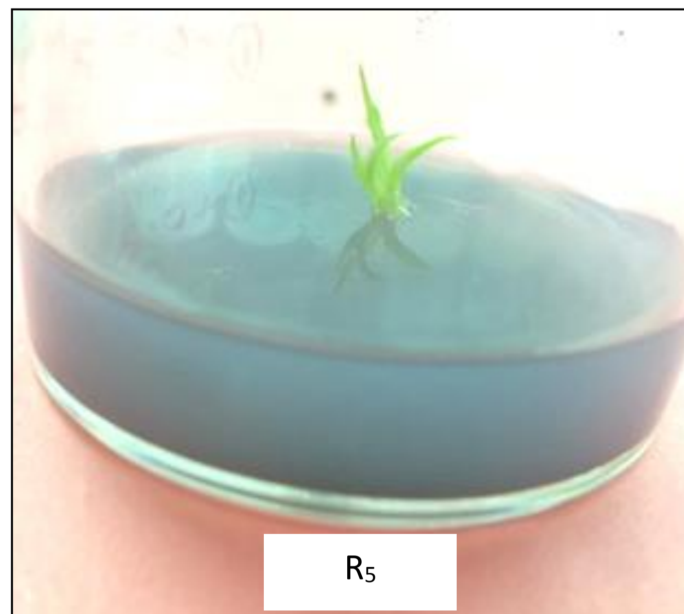
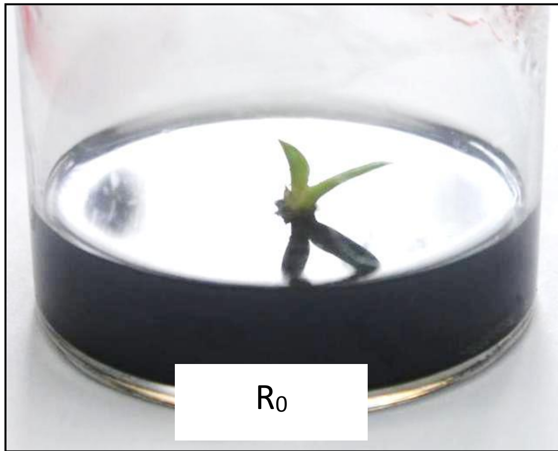
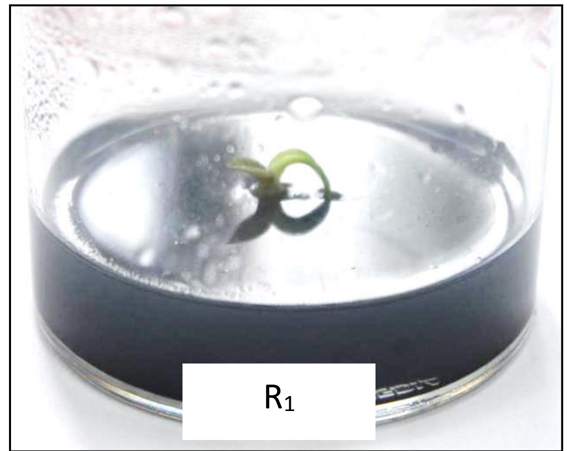
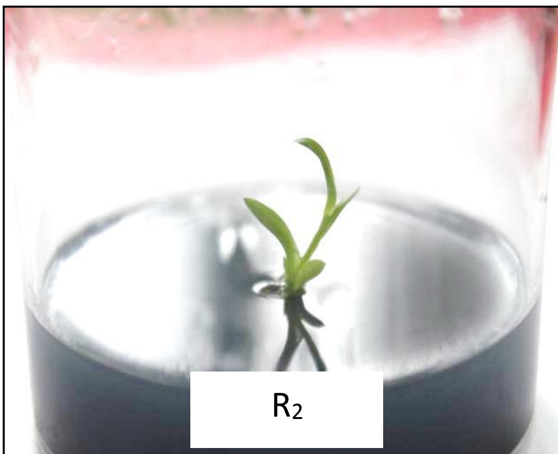
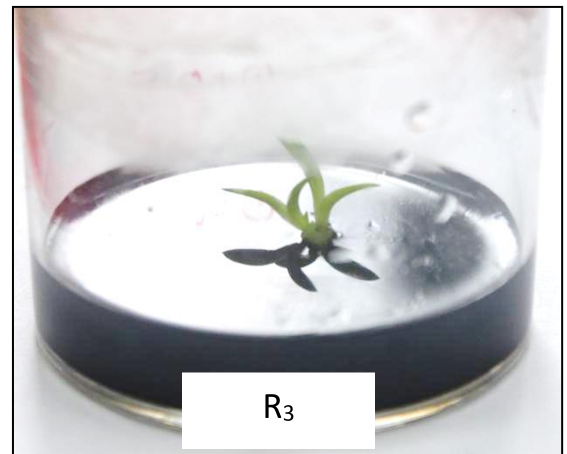


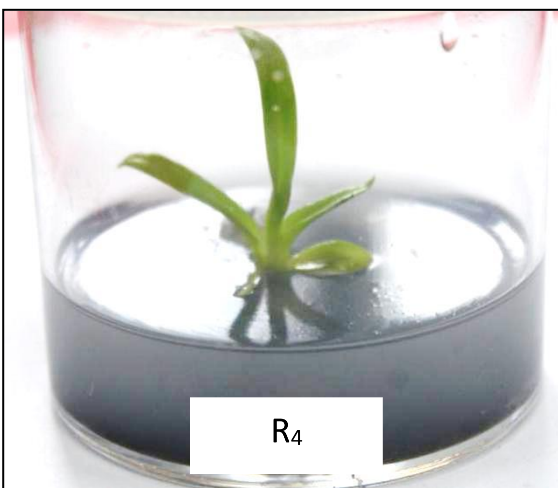
Fig.4.4.5: Root proliferation after 15 days











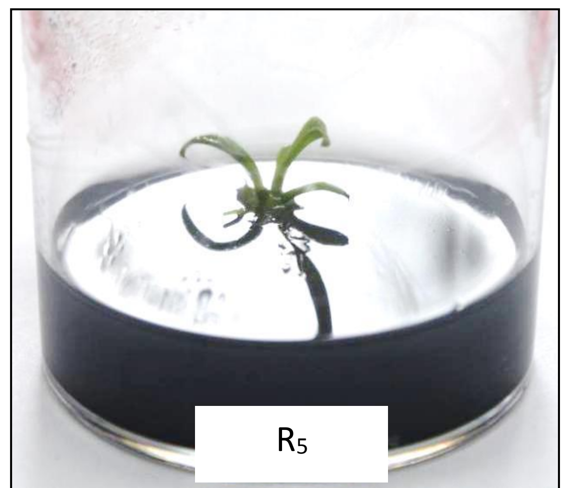
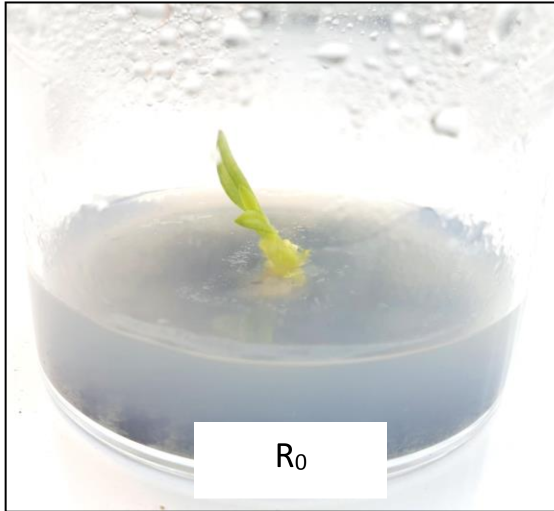
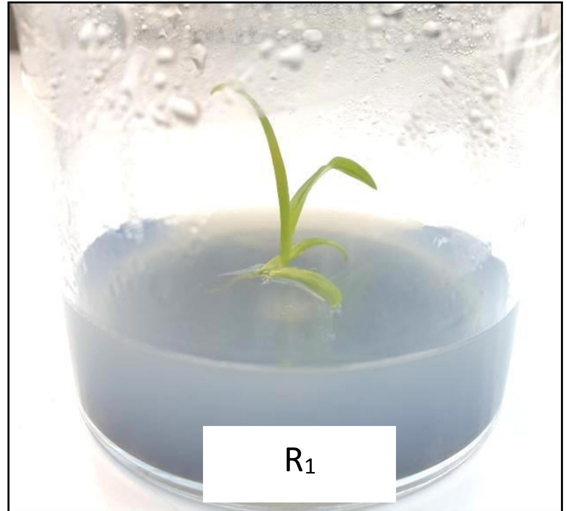
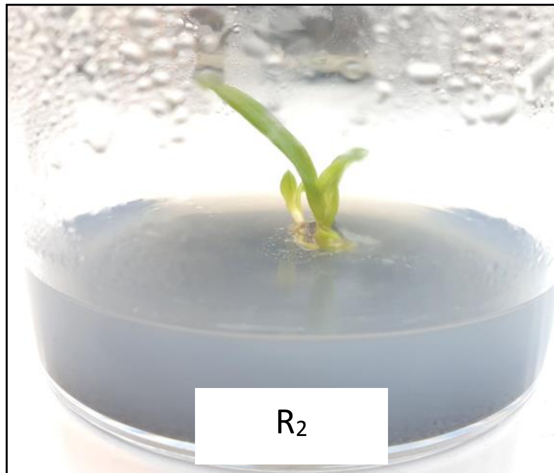
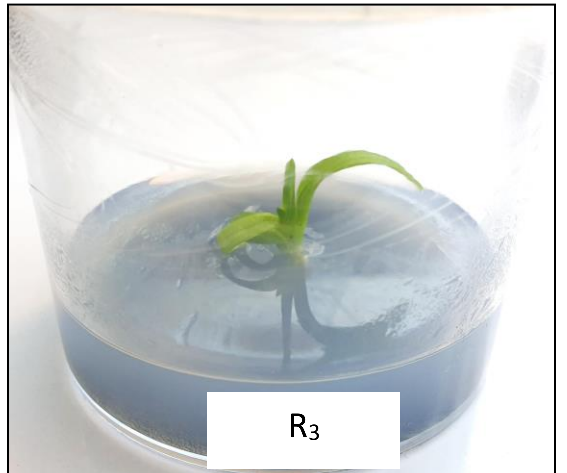


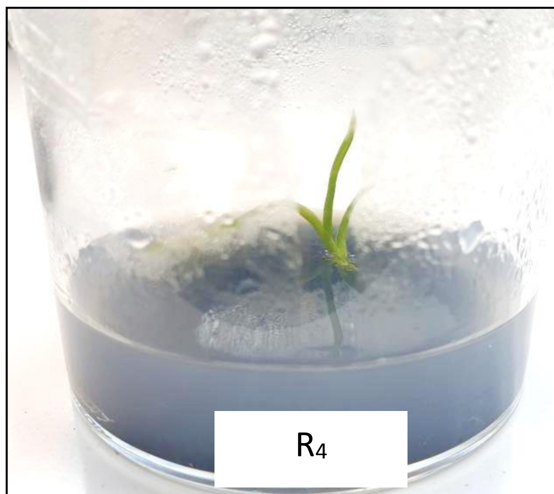
Fig.4.4.6: Root proliferation after 30 days











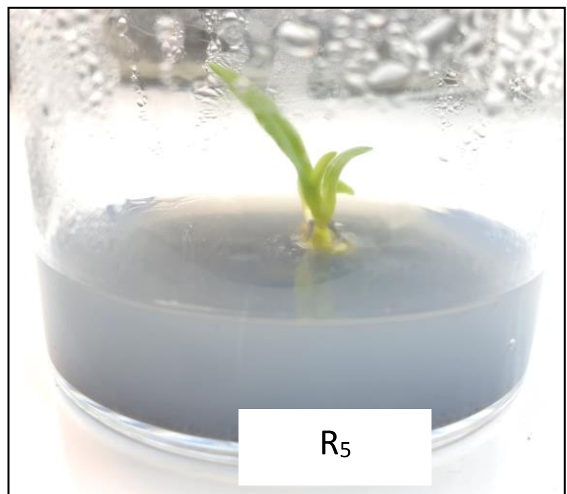
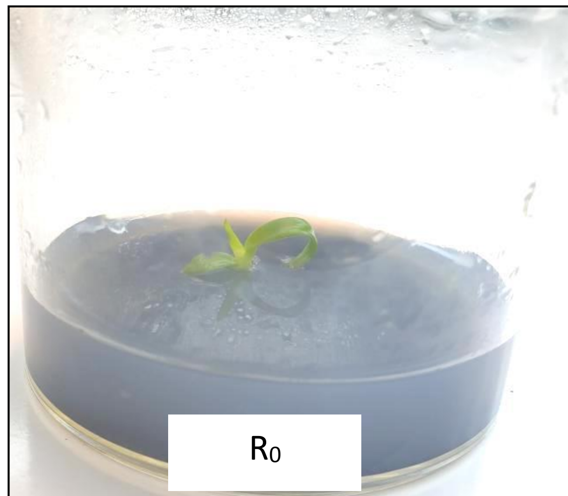
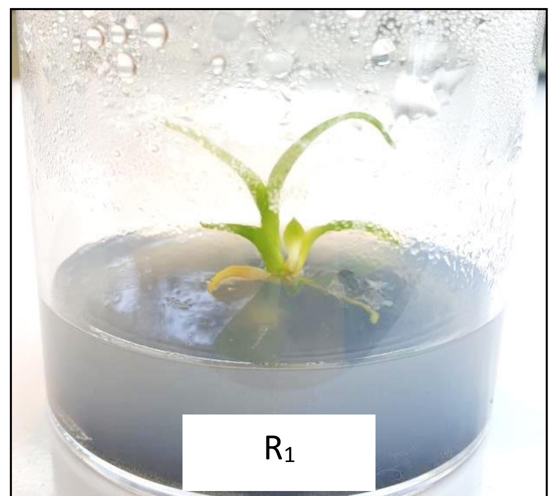
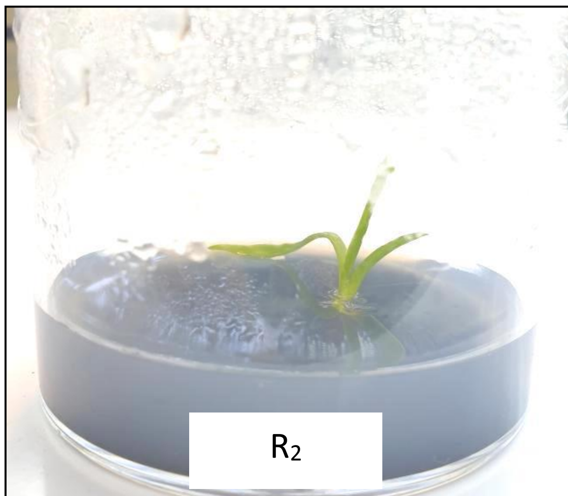
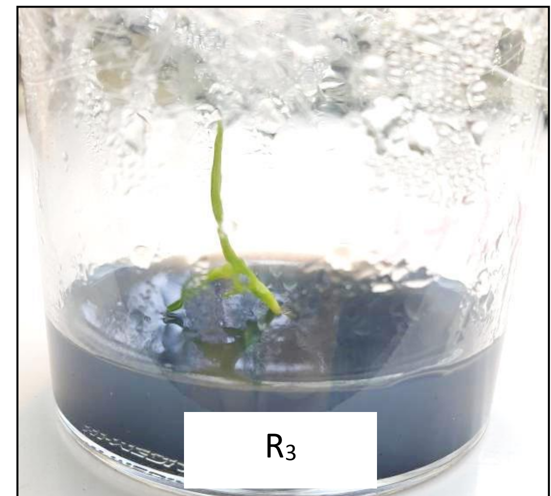


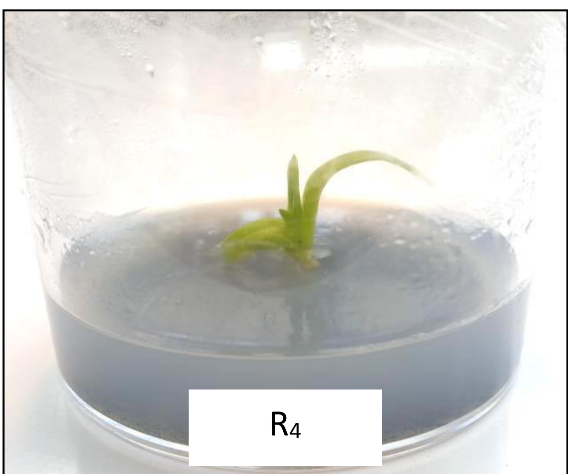
Fig.4.4.7: Root proliferation after 45 days











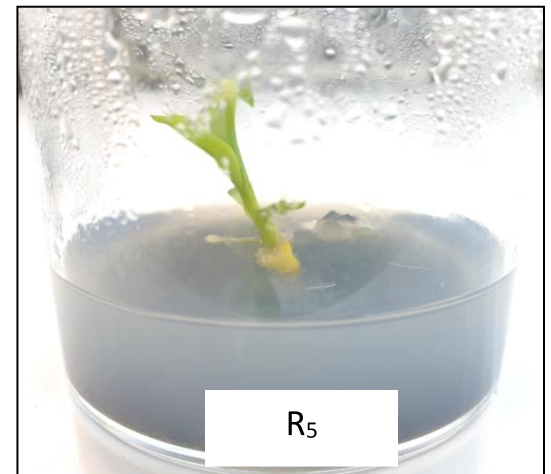


Fig.4.4.8(a): Root proliferation after 60 days

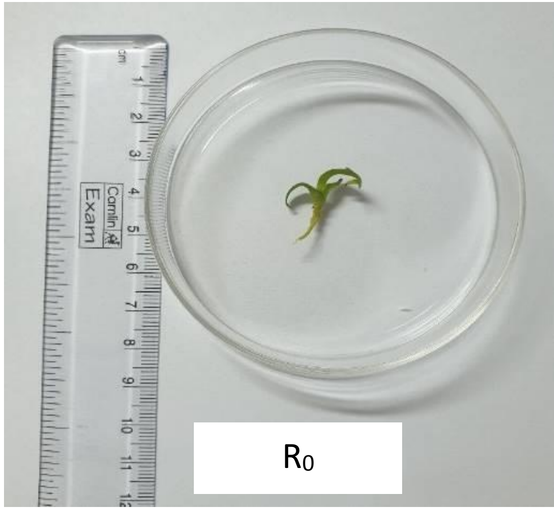


Fig.4.4.8(b): Root proliferation after 60 days

Chapter 5
Discussion

Dendrobium Orchids var. Earsakul is a popular commercial cut flower which has higher demand and sold at a better price in the market when compared to its ancestral clone *Dendrobium* sonia 'BOM'. *In vitro* propagation of *Dendrobium* is rapid, ensured clonal stability (Li *et al.*, 2013) and the use of PGRs to induce the growth and development is vital in micropropagation.

The findings obtained from the present investigation are discussed briefly in this chapter by presenting the literature and reasoning to support the findings.

5.1 Performance of plant growth regulators combination for callus initiation and formation under 16 hr normal light

The callus initiation and development are found to be significantly affected by the addition of PGRs in the MS medium, the earliest callus initiation (3.24 days) was recorded in T_8 (0.5 mg/l KIN + 7.5 mg/l NAA). Similarly, maximum callus weight (308.10 mg) was obtained on T_8, 0.5 mg/l KIN + 7.5 mg/l NAA. It was also noticed MS basal media alone without PGR took the longest time for callus initiation. The results of this investigation corroborate with the findings of Maridass *et al.*, 2010, where, NAA and KIN were reported to be effective in callus initiation of *Dendrobium nanum.* Cytokinin and auxin are important hormones in cell division which might have magnified the rate of cell division and allowed early initiation of callus. Callus initiation is influenced by many factors among which PGRs and the explant chosen are very important (Refish *et al.*, 2016).

The percentage of the callus formation was recorded to be highest (66%) in T_3 (0.5 mg/l BAP + 0.10 mg/l IAA) which was at par with T_7 MS media supplemented with 0.25 mg/l KIN + 5 mg/l NAA. Higher concentration of auxin and lower concentration cytokinin favour the callus development and similar result are reported by Hardjo *et al.* (2016) in *Vanda tricolor* Lindl. var. Pallida; Tarrahi and Rezanejad (2013) in Rose, while, it is also contradicted by Chookoh *et al* (2019) in *Tolumnia* orchids.

5.2 Performance of plant growth regulators combination for callus initiation and formation under complete dark

Minimum days taken for callus initiation (4.28 days) was recorded in T^0_2 which contain 0.25 mg/l BAP + 0.05 mg/l IAA, whereas,maximum weight of callus (247.45 mg) and percentage of callus formation (43.55%) was pertained in T^0_8. (0.5 mg/l KIN + 7.5 mg/l NAA)

All the observation recorded for callus formation and proliferation is optimum under the 16 hr light in all the treatment with respect to days taken for callus initiation, callus weight, and percentage of callus induction. The poor performance of the culture placed under dark might be due to lack of uptake of nutrients by the explant in total the dark condition. Similar results have been reported in *Dendrobium chiengmai* pink (Chung *et al.*, 2005) and *Brasicca napus* L (Afshari *et al.*, 2011) culture placed under light gave better embryogenic response, callus induction and growth in comparision with the medium in dark. Early initiation and a higher percentage of callus development were also reported in Tobacco (Siddique and Islam, 2015). The better performance under light may be attributed to the autotrophic nature of calli due to the development of photosynthetic pigment, which lead to the synthesis of carbohydrates and other necessary metabolites required for growth (Kami *et al.*, 2010).

5.3 Performance of plant growth regulators for shoot proliferation.

A shooting media need to have a balance between auxin and cytokinin present in the medium, a higher concentration of cytokinin with respect to auxin is critical for shoot development. The shoot initiation was tested with different concentrations of PGR *viz* NAA and BAP in 7 combinations out of which the 2 to 4 mg/l BAP + 0.5 to 0.8 mg/l NAA showed statistically similar results for days taken (3.41-3.77 days) maximum number of shoot (1.73) was observed in S_4 (2.5 mg/l BAP + 0.5 mg/l NAA) produced more shoot, a comparable number of shoots (1.72) was also recorded in S_6 (3.5 mg/l BAP + 0.7 mg/l NAA). However, the shoot length is maximum (1.93) in S_2 (1.5 mg/l BAP + 0.125 mg/l NAA) and at par with S_3 (2 mg/l BAP + 0.25 mg/l) where shoot length 1.82 cm was recorded. A slightly lower concentration of BAP and NAA seems to favour shoot growth. Similarly, maximum shoot weight (619.03 mg) was also noticed in $S_{3,}$ higher shoot growth favour increased in shoot weight. The best PGRs concentration for shooting was BAP at 2 mg/l + 0.25 mg/l NAA which showed superior result in shoot proliferation and development. However, It was noticed that a maximum and a comparable number of leaves was obtained in the medium that was supplemented with S_5, 3mg/l BAP + 0.6 mg/l NAA (2.04) and S_6,3.5 mg /l BAP + 0.7

mg/l NAA(2.01). Similarly, the leaf length was maximum (1.91cm) in PGR at S_6, 3.5 mg /l BAP + 0.7 mg/l NAA which was followed by S_5, 3mg/l BAP + 0.6 mg/l NAA (1.73 cm). Higher concentrations of BAP and NAA are noticed be ideal for development of leaves.

PGR plays an important role to regenerate the cell differentiation the combined treatment of BAP and NAA are found to give a positive effect on shoot growth and multiplication as reported in *Dendrobium* orchid (Taluker *et al.,*2003*), Dendrobium chiengmai* pink (Chung *et al., 2005) and Dendrobium nanum (*Maridass *et al.,* 2010). The physiological process of interaction between both the hormones might have a synergistic effect on the shoot initiation and growth, cytokinin was found to proliferate embryogenesis but NAA inhibits the process (Regmi *et al.* 2005 in *Cymbidium aliofolium*; Maridass *et al.,* 2010 in *Dendrobium nanum*). Whereas, a decline in the number of shoot formations was observed in MS media supplemented with BAP concentration at 4 mg/l with 0.8 mg/l NAA which might have been caused by toxicity due to higher concentration.

5.4 Performance of plant growth regulators for root proliferation.

The interaction between BAP and IBA showed significant results in root development. The root initiation was earliest (2.93 days) in the MS media supplemented with treatment combination of R_4, 0.5 mg/l BAP + 1 mg/l IBA similarly, maximum root weight (11.08 mg) was also recorded for the same treatment. However, maximum elongation in root length (1.38 cm) was observed in MS supplemented with a combination of 0.25 mg/l BAP +0.5 mg/l IBA, (R_3) followed by R_4, 0.5 mg/l BAP + 1 mg/l IBA in combination. A high concentration of cytokinin induces the growth of shoot buds, while a high concentration of auxin induces root formation. The interaction of BAP and IBA with the addition of activated charcoal gave a synergistic effect to form roots. The findings were in close agreement with Refish *et al.,2016 in Dendrobium candidum; N*ayak *et al.,*1997 in *Acampe praemorsa ;* However, Maridass *et al.*(2010) findings disagree to the result, as per their findings IBA supplemented in media did not display root initiation in *Dendrobium nanum*.

Chapter 6
Summary and Conclusions

The conspicuous features of the investigation entitled "*In vitro* propagation of *Dendrobium* orchid var. Earsakul carried out in tissue culture laboratory of Basic science and Humanities under College of Horticulture and Forestry, Central Agricultural University, Pasighat during the year 2020-2021. The pivotal consequences of the research are recapitulated below:

6.1 Performance of plant growth regulators (PGR) combination for callus initiation and formation under 16 hr normal light

Minimum day (3.24 days) for callus initiation was recorded when 0.25 mg/l KIN + 5 mg/l NAA was supplemented to MS media.

The maximum weight of callus (308.1 mg) was obtained at treatment supplemented with 0.5 mg/l KIN + 7.5 mg/l NAA.

Maximum percentage of callus formation was recorded at 0.25 mg/l BAP + 0.05 mg/l IAA (66.66 %).

6.2 Performance of plant growth regulators (PGR) combination for callus initiation and formation under complete dark

The shortest time (4.28 days) was taken for callus initiation at 0.25 mg/l BAP + 0.05 mg/l IAA.

The maximum weight of callus (247.45 mg) was noted at 0.5 mg/l KIN + 2.5 mg/l BAP + 0.5 mg/l NAA (3.56 days).

Highest number of shoot was exhibited by 2.5 mg/l BAP + 0.5 mg/l NAA (1.73) which was at par with 3.5 mg/l BAP + 0.7 mg/l NAA.(1.72) .

Shoot length was maximum (1.93 cm) at 1.5 mg/l BAP + 0.125 mg/l NAA which was at par with 2 mg/l BAP + 0.25 mg/l NAA (1.82 cm).

Maximum leaf length (1.91 cm) was obtained at 3.5mg/l BAP + 0.7 mg/l NAA

The shoot weight was maximum (619.03 mg) at 2 mg/l BAP + 0.25 mg/l NAA which was at par with 0.5 mg/l BAP + 0.06 mg/l NAA (603.13 mg)

6.4 Performance of plant growth regulators for root proliferation

It was determined that 0.5 mg/l BAP + 1 mg/l IBA significantly took shortest day (2.96 days) for root initiation

The maximum number of roots (3.39) was found in 0.5 mg/l BAP + 1 mg/l IBA which was at par with 0.25 mg/l BAP + 0.5 mg/l IBA (3.36) and 1 mg/l BAP + 2 mg/l IBA (3.31).

Root weight was recorded to be maximum (11.08 mg) in 0.25 mg/l BAP + 0.5 mg/l IBA(10.77 mg).

The root length was highest in R_4 initially till 30th days but later R_3 (1.38 cm) exhibited higher root length followed by R_4 (1.25 cm).

6.5 Conclusion

Thus, the present experimentation reveals that addition of Kinetin and NAA for callus induction and formation under 16 hr normal light as well as BAP and NAA for shoot proliferation and BAP and IBA for root proliferation showed positive responses. It is apparent through entire investigation that 0.5 mg/l KIN + 7.5mg/l NAA application was significantly associated with callus induction and formation for *in vitro* propagation of *Dendrobium* Orchid var. Earsakul when explant placed in the 16 hr normal light. However, the treatment combinations 2.5 mg/l BAP + 0.5 mg/l NAA became more pertinent for shoot proliferation and 0.5 mg/l BAP +1 mg/l IBA for root proliferation.

6.6 Future scope of research.

- Mass production of disease free quality planting material.
- Development of protocol for acclimatization.

Bibliography

Abubacker, M.N., Durgadevi, J., Sathya, C. and Rose, K L. (2013). Micropropagation of orchid *Dendrobium barbatum* Lindl.(Orchidaceae) from fleshy stem segments. *Biosci. Biotechnol. Res. Asia.*, *10* (1): 411-414.

Afshari, R.T., Angoshtari, R. and Kalantari, S. (2011). Effects of light and different plant growth regulators on induction of callus growth in rapeseed (*Brassica napus* L.) genotypes. *Plant Omics J.*, 4(2): 60-67.

Aktar, S., Nasiruddin, K.M. and Huq, H. (2007). *In vitro* root formation in Dendrobium orchid plantlets with IBA. *J.Agric.Rural Dev,* pp. 48-51.

Asghar, S., Ahmed T., Hafiz, I.A. and Yaseen, M. (2011). *In vitro* propagation of orchid (*Dendrobium nobile*) var. Emma white. *Afr J Biotechnol*., 10(16): 3097-3103

Banu, L.A. and Bari, M.A. (2007). Protocol establishment for multiplication and regeneration of *Ocimum sanctum* Linn. An important medicinal plant with high religious value in Bangladesh. *Plant Sci*., 2(5): 530-537.

Beura, S., Sahu, A., Rout, S., Beura, R. and Jagadev, P.N. (2017). Standardization of Plant Bio-Regulators for *In Vitro* Shoot Proliferation of *Curcuma longa* L. cv. Roma. *Int. J. Curr. Microbiol. App. Sci*., 6(5): 386-394.

Bhowmik, T.K. and Rahman, M.M. (2020). Micropropagation of Commercially Important Orchid *Dendrobium palpebrae* Lindl. through *In Vitro* Developed Pseudobulb Culture. *J. Adv. Biotechnol. Exp. Ther.,* 3(3): 225-232.

Chen, Y.M., Huang, J.Z., Hou, T.W. and Pan, I.C. (2019). Effects of light intensity and plant growth regulators on callus proliferation and shoot regeneration in the ornamental succulent *Haworthia*. *Bot. Stud*., 60(1): 1-8.

Chookoh, N., Chiu, Y.T., Chang, C., Hu, W.H. and Dai, T.E. (2019). Micropropagation of *Tolumnia* orchids through induction of protocorm-like bodies from leaf segments. *Hort. Sci*., 54(7): 1230-1236.

Chugh, S., Guha, S. and Rao, I.U. (2009). Micropropagation of orchids: a review on the potential of different explants. *Sci. Hort.,* 122(4): 507-520.

Chung, H.H., Chen, J.T. and Chang, W.C. (2005). Cytokinins induce direct somatic embryogenesis of *Dendrobium chiengmai* pink and subsequent plant regeneration. *In Vitro Cell Dev. Biol. Plant,* 41(6): 765-769.

De, L.C. and Medhi, R.P. (2015). Orchid–a diversified fcomponent of farming systems for profitability and livelihood security of small and marginal farmers. *J. Glob. Biosci.*, 4(2): 1393-1406.

De, L.C. and Singh. D.R. (2015). Biodiversity, conservation and bio-piracy in orchids- An overview. *J. Glob. Biosci*., 4(4): 2030-2043.

Erawati, D.N., Wardati, I., Humaida, S. and Fisdiana, U. (2020). Micropropagation of Vanilla (Vanilla planifolia Andrews) with Modification of Cytokinins. *Environ. Earth Sci.,* 411(1): 146-153

Gomez, A.K. and Gomez, A.A. (2010). Statistical procedures for agricultural research. 2nd edn. Wiley India Private Limited, New Delhi, pp. 134-138.

Goswami, K., Yasmin, S., Nasiruddin, K. M., Khatun, F. and Akte, J. (2015). *In vitro* regeneration of *Dendrobium* sp. of orchid using leaf tip as explant. *J. Environ. Sci. Nat. Resour.*, *8*(2): 75-78.

Gow, W.P., Chen, J.T., and Chang, W.C. (2009). Effects of genotype, light regime, explant position and orientation on direct somatic embryogenesis from leaf explants of *Phalaenopsis* orchids. *Acta. Physiologiae Plantarum*., *31* (2), 363-369.

Hardjo, P.H, Binarto C.W.S, and Savitri.W.D. (2016) Induksi Protokorm-Like Bodies (PLBs) *Vanda tricolor* Lindl. var. Pallida Proceeding of Seminar Nasional Biodiversitas, pp.172-179

Kadu, A.R. (2013). *In vitro* micropropagation of gerbera using auxillary bud. *Asian J. Bio. Sci.*, *8*(1): 15-18.

Kami, C., Lorrain, S., Hornitschek, P. and Fankhauser, C. (2010). Light-regulated plant growth and development. *Curr. Topics Dev. Biol.,* 91: 29-66.

Kataki, S.K., Jain, S.K. and Sastry, A.R.K. (1984). Distribution of Orchids of Sikkim and North East India. Plant Conservation Bull 5, *Bot. Surv. Ind*., 5: 1-38

Khatun, M.M., Khatun, H., Khanam, D. and Al-Amin, M.D. (2010). *In vitro* root formation and plantlet development in *Dendrobium* orchid. *Bangladesh J. Agric. Res.*, *35*(2): 257-265.

Khosravi, A.R., Kadir, M.A., Kazemin, S.B., Zaman, F.Q. and De Silva, A.E. (2008). Establishment of a plant regeneration system from callus of *Dendrobium* cv. Serdang Beauty. *African J. Biotech., 7* (22): 4093-4099,

Khui, K.M. and Sink, K.C. (1982). Callus induction and culture of *Rosa*. *Sci. Hort.*, *17*(4), 361-370.

Kumar, A.O., Tata. S.S. and Rupavati, T. (2010). *In vitro* induction of callusogenesis in chili peppers (*Capsicum annuum* L.). *Int. J. Curr. Res.*, 3: 42-45.

Kumari, I.P., George, T.S. and Rajmohan, K. (2013). Influence of plant growth regulators on *in vitro* clonal propagation of *Dendrobium* Sonia 'Earsakul'. *J. Bio Innovation*., 2(2): 51-58.

Leitch, J., Suda, J., Hanson, L., Ingrouille, M.J., Chase, M.W. and Fay, M.F. (2009). Genome size diversity in orchids: Consequences and evolution. *Ann. Bot.,* 104: 469-481.

Li, X., He, T. and Chun, Z. (2013). Detecting genetic diversity of *Dendrobium* tissue culture seedlings with ISSR method. *Chin. J. App. Environ. Biol.*, 19:249–254

Mandal, A.K.A. and Datta, S.K. (2002). Introduction of gerbera cultivation in Lucknow agro-climate through tissue culture of young flower head. *Ind. J. Biotechnol.,* 01(2): 212-214.

Maridass, M., Mahesh, R., Raju, G., Benniamin, A. and Muthuchelian, K. (2010). *In vitro* propagation of *Dendrobium nanum* through rhizome bud culture. *Int. J. Biol. Tech.*, 1: 50-54

Mei, T.A., Danial, M., Mahmood, M. and Subramaniam, S. (2012). Exquisite protocol of callus induction and protocorm-like bodies (PLBs) regeneration of *'Dendrobium'* sonia-28. *Australian. J. Crop Sci.*, 6(5): 793-800.

Mohammadi, M., Kaviani, B. and Sedaghathoor, S. (2019). Micropropagation of two near threatened orchid. Part 2: *Phalaenopsis amabilis* Blume var. Grandiflora. *Adv. Hort. Sci.,* 33(4): 485-493.

Monalisha, B., Palai, S.K., Swarnaprabha, C., Priyanka, S. and Basabdatta, B. (2017). Evaluation of ten commercial cultivars of *Dendrobium* under Odisha condition. *Biosci. Trends.,* 10 (6): 1400-1403.

Morel, G.M. (1960). Producing virus free Cymbidiums. *Amer Orchid. Soc. Bull.*, 29: 495-497

Nayak, N. R., Patnaik, S., and Rath, S.P. (1997). Direct shoot regeneration from foliar explants of an epiphytic orchid, *Acampe praemorsa* (Roxb.) Blatter and Mc Cann. *Plant Cell Rep.*, *16*(8): 583-586.

Nayak, N.R., Sahoo, S., Patnaik, S. and Rath, S.P. (2002). Establishment of thin cross section (TCS) culture method for rapid micropropagation of *Cymbidium aloifolium* (L.) Sw. and *Dendrobium nobile* Lindl. (Orchidaceae). *Sci. Hort.*, 94(1-2): 107-116.

Ng, C.Y. and Saleh, N.M. (2011). *In vitro* propagation of *Paphiopedilum* orchid through formation of protocorm-like bodies. *Plant Cell, Tissue Organ Cult.,* 105 (2): 193-202.

Ninawe, A.S. and Swapna, T.S. (2017). Orchid diversity of northeast India–Traditional Knowledge and strategic plan for conservation. *J. Orchid Soc. Ind.*, 31: 41-56.

Pelkonen, V. P., and Kauppi, A. (1999). The effect of light and auxins on the regeneration of lily (*Lilium regale* Wil.) cells by somatic embryogenesis and organogenesis. *Int. J. Plant Sci.*, *160*(3): 483-490.

Pradhan, S., Paudel, Y.P. and Pant, B. (2013). Efficient regeneration of plants from shoot tip explants of *Dendrobium densiflorum* Lindl., a medicinal orchid. *African J. Biotechnol.*, 12(12): 1378-1383

Priyanka, S., Verma, L.S., Satyanarayana, E. and Subhankar (2018). *In vitro* regeneration and rapid multiplication of *Dendrobium nobile*. *Int. J. Chem. Stud.,* 6(6): 1286-1288

Ram, M., Prasad, K.V., Kaur, C., Singh, S.K., Arora, A. and Kumar, S. (2011). Induction of anthocyanin pigments in callus cultures of *Rosa hybrida* L. in response to sucrose and ammonical nitrogen levels. *Plant Cell, Tissue Organ Cult.,* 104(2): 171-179.

Reddy, J. Niveshika, Shaju, A., Jose, A., Betty, A, Yarmichon, H. (2021)Plant Growth Regulators Used For *In Vitro* Micropropagation Of Orchids. *Int. J. Biol. Res.* 8(1): 37-42

Refish, N.M.R., Wang, L., Fu, C., Xu, X., Jin, W., Li, M. and Yu, L. (2016). Establishment and optimization of high efficiency embryogenic callus induction system in *Dendrobium candidum*. *African J. Plant Sci.,* 10(4): 77-83.

Regmi, T., Pradhan, S. and Pant, B. (2017). *In vitro* mass propagation of an epiphytic orchid, *Cymbidium aloifolium* (L.) Sw., through protocorm culture. *Biotechnol. J. Int.,* 19(1): 1-6.

Rehana, S., Alam, M.S., Islam, K.S. and Samad, M.A. (2009). Influence of growth regulators on shoot proliferation and plantlet production from shoot tips of banana. *Progress. Agric.,* 20(1-2): 9-16.

Riva, S.S., Islam, A. and Hoque, M.E. (2016). *In vitro* regeneration and rapid multiplication of *Dendrobium bensoniae*, an indigenous ornamental orchid. *Agriculturist.*, 14(2): 24-31.

Romeida, A. Ganefianti, D.W. and Rustikawati, R. (2016). Embryogenic Callus Induction of Pencil Orchid (*Papilionanthe hookeriana* Rchb. f.) Through *in Vitro* Culture. *Int. J. Adv. Sci. Eng. Inf. Technol.,* 6(2): 196-200.

Roy J, and Banerjee, N. (2003) Induction of callus and plant regeneration from shoot-tip explant of *Dendrobium fimbriatum* Lindl. var. oculatum Hk. f. *Sci. Hort.,* 97: 333–340

Roy, J., Naha, S., Majumdar, M. and Banerjee, N. (2007). Direct and callus-mediated protocorm-like body induction from shoot-tips of *Dendrobium chrysotoxum* Lindl.(Orchidaceae). *Plant Cell, Tissue Organ Cult.*, 90(1): 31-39.

Sarmah, D., Kolukunde, S., Sutradhar, M., Singh, B. K., Mandal, T. and Mandal, N. (2017). A review on: *in vitro* cloning of orchids. *Int. J. Curr. Microbiol. App. Sci.*, *6*(9): 1909-1927.

Siddique, A.B. and Islam, S.S. (2015). Effect of light and dark on callus induction and regeneration in tobacco (*Nicotiana tabacum* L.). *Bangladesh J. Bot.*, 44(4): 643-651.

Singh, A.K. (2006). Orchids: In Flower Crops: Cultivation and Management, New India Publishing, pp. 269-296.

Talukder, S.K., Nasiruddin, K.M., Yasmin, S., Hassan, L. and Begum, R. (2003). Shoot proliferation of *Dendrobium* orchid with BAP and NAA. *J. Biol. Sci.*, 3(11): 1058-1062.

Tarrahi, R. and Rezanejad, F. (2013). Callogenesis and production of anthocyanin and chlorophyll in callus cultures of vegetative and floral explants in *Rosa gallica and Rosa hybrida* (Rosaceae). *Turk. J. Bot.*, 37(6): 1145-1154.

Thokchom, R. and Maitra, S. (2017). Micropropagation of *Anthurium andreanum* cv. Jewel from leaf explants. *J. Crop Weed,* 13(1): 23-27.

Tokuhara, K. and Masahiro, M. (2001). Induction of embryogenic callus and cell suspension culture from shoot tips excised from flower stalk buds of *Phalaenopsis* (Orchidaceae). *In Vitro Cell. Dev. Biol. Plant.*, 37(4): 457-461.

Yam, T.W., Yeung, E.C., Ye, X.L., Zee, S.Y., Arditti, J., Kull,T. and Arditt, J. (2002). Orchid embryos. Orchid biology: reviews and persectives, pp. 287-385.

Yu, Y.X., Ling, L.I.U., Liu, J.X. and Jing, W.A.N.G. (2009). Plant Regeneration by Callus-Mediated Protocorm-Like Body Induction of *Anthurium andraeanum Hort. Agric. Sci. China*, 8(5): 572-577.

Zhao, P., Wang, W., Feng, F.S., Wu, F., Yang, Z. Q. and Wang, W.J. (2007). High-frequency shoot regeneration through transverse thin cell layer culture in *Dendrobium Candidum* Wall Ex Lindl. *Plant Cell, Tissue Organ Cult.*, 90(2): 131-139.