STANDARDIZATION OF MICROPROPAGATION TECHNIQUES OF *Dendrobium* cv. Sonia Earsakul THROUGH SHOOT TIP CULTURE

Thesis

Submitted to

NAGALAND UNIVERSITY

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Doctor of Philosophy

in

Horticulture (Floriculture and Landscaping)

by

SABASTIAN KS

Admin. No. Ph-199/15Regn. No. 839/2019

Department of Horticulture
School of Agricultural Sciences and Rural Development,
Nagaland University, Medziphema Campus – 797 106
Nagaland

2021

Dedicated

to my

Beloved Family

DECLARATION

I, Sabastian KS, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis had not been submitted by me for any research degree in any other University/Institute.

This is being submitted to Nagaland University for the degree of Doctor of Philosophy in Horticulture.

Date:

Place: **(SABASTIAN KS)**

.....

Supervisor

NAGALAND UNIVERSITY
Medziphema Campus
School of Agricultural Sciences and Rural Development
Medziphema – 797 106, Nagaland

Dr. L. Hemanta
Assistant Professor
Department of Horticulture

CERTIFICATE – I

This is to certify that the thesis entitled **"Standardization of micropropagation techniques of *Dendrobium* cv. Sonia Earsakul through shoot tip culture"** submitted to Nagaland University in partial fulfilment of the requirements for the award of degree of Doctor of Philosophy in Horticulture is the record of research work carried out by Mr. Sabastian KS, Registration No. 839/2019 under my personal supervision and guidance.

The result of the investigation reported in the thesis has not been submitted for any other degree or diploma. The assistance of all kinds received by the student has been duly acknowledged.

Date :
Place :

.....
Dr. L. Hemanta
Supervisor

NAGALAND UNIVERSITY
Medziphema Campus
School of Agricultural Sciences and Rural Development
Medziphema – 797 106, Nagaland

CERTIFICATE – II

VIVA VOCE ON THESIS OF DOCTOR OF PHILOSOPHY IN HORTICULTURE

This is to certify that the thesis entitled **"Standardization of micropropagation techniques of *Dendrobium* cv. Sonia Earsakul through shoot tip culture"** submitted by Sabastian KS, Admission No. Ph - 199/15 Registration No. 839/2019 to the NAGALAND UNIVERSITY in partial fulfilment of the requirements for the award of degree of Doctor of Philosophy (Agriculture) in Horticulture has been examined by the Advisory Board on …….....

The performance of the student has been found **Satisfactory/Unsatisfactory.**

Member	Signature
1. Dr. L. Hemanta (Supervisor & Chairman)	…….
2. Dr. Anil K Singh (External Examiner)	……
3. Dr. Pro Vice Chancellor Nominee (Dean, SASRD, NU)	……
4. Dr. C.S. Maiti (Co-Supervisor)	……
5. Dr. Rokolhuü Keditsu	……
6. Dr. S.P. Kanaujia	……...
7. Dr. Sanjoy Das	…….....

Head
Department of Horticulture

Dean
School of Agricultural Science
& Rural Development

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Place :

Date :

(Sabastian KS)

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LIST OF ABBREVIATIONS

@	at the rate of
%	Percent
^{o}C	degree celsius
.	point
$^{-1}$	Per
±	plus or minus
½	Half
1N	1 normal
µM	Micrometre
BA	Benzyl adenine
BAP	Benzylaminopurine
$CaCl_2.4H_2O$	Calcium chloride tetrahydrate
CD	Critical difference
Cm	Centimetre
$CoCl_2.6H_2O$	Cobalt (II) chloride hexahydrate
CRD	Completely Randomized Design
$CuSO_4.5H_2O$	copper sulphate pentahydrate
cv.	Cultivar
et al.	Et alia (and others)
Fe.EDTA	Ferric ethylenediaminetetraacetic acid.
$FeSO_4.7H_2O$	Ferrous sulfateheptahydrate
Fig.	Figure
g	gram
HCl	Hydrochloric acid
H_3BO_3	Boric Acid

$HgCl_2$	Mercuric Chloride
IAA	Indole acetic acid
IBA	Indole 3 butyric acid
KC	Knudson C
KH_2PO_4	Mono potassium phosphate
KI	Potassium Iodide
KNO_3	Pottasium Nitrate
L^{-1}	Per litre
mg	Milligram
ml	Millilitre
MN	Minor
MR	Micro
$MnSO_4.4H_2O$	Manganese (II) sulphate tetrahydrate
$MgSO_4.7H_2O$	Magnesium sulphate heptahydrate
MS	Murashige and Skoog
N	NAA
NAA	Naphthaleneacetic acid
$Na_2.EDTA.2H_2O$	Ethylene diamine tetraacetic acid disodium salt dihydrate
$Na_2MoO_4.2H_2O$	Sodium molybdate dihydrate
NaOH	Sodium hydroxide
NH_4NO_3	Ammonium Nitrate
eg.	Example
NU	Nagaland University
PGRs	Plant growth regulators

PLB	Protocorm like body
pH	Potential of hydrogen
psi	Pound per square inch
RH	Relative humidity
SASRD	School of Agricultural Sciences and Rural development
SEm±	Standard Error Mean
Sp.	Species
UV	Ultraviolet
VW	*Vacin & Went*
$ZnSO_4.7H_2O$	Zinc sulphate heptahydrate

ABSTRACT

The investigation entitled "**Standardization of micropropagation techniques of *Dendrobium* cv. Sonia Earsakul through shoot tip culture**" was conducted during 2016-2019 at the Tissue Culture Laboratory, Department of Horticulture, NU:SASRD, Medziphema, Nagaland. Unavailability of the quality planting material, non endospermic seeds and slow mode of vegetative propagation is the main constraint of *Dendrobium* cultivation. The work aimed at standardization of protocol for producing large number of diseased free quality planting materials of *Dendrobium* cv. Sonia Earsakul by micropropagation techniques through shoot tip culture.

The first experiment aimed to study the *in vitro* shoot multiplication of *Dendrobium* cv. Sonia Earsakul. It consists of 2 factors in completely randomized block design with twenty (20) treatments; Benzylaminopurine @ 0, 0.5, 1, 1.5 and 2mgL^{-1} and Napthalene acetic acid @ 0, 0.5, 0.75 and 1mgL^{-1}. Among the various treatments, Benzylaminopurine @ 2 mgL^{-1} took the least days to callus formation (45.89 days), best percentage of response (51.62%), highest gain in weight of callus (13.05 g), least days to greening (63.98 days), earliest initiation of shoot (36.10 days), highest number of multiple shoot bud in first sub culturing (5.56) and second sub culturing (11.85), longest length of shoot (4.87 cm), highest number of leaves per shoot (4.86), heaviest fresh weight of shoot at 90 days of culturing (0.57 g) and during hardening stage (0.95 g). Napthalene acetic acid @ 0.5mgL^{-1} gave significant variation, it took least days to callus formation (47.60 days), highest percentage of response (51.19%), highest frequency of callus formation (11.97 g), least days to greening (65.42 days), earliest to initiate shooting (39.13 days), highest number of multiple shoot bud on first sub culturing (4.48) and second sub culturing (9.86), longest shoot bud (4.20 cm), highest number of leaves (4.21), heaviest fresh weight of shoot at 90 days of culturing (0.47 g) and at hardening stage (0.85 g). Results of the investigation revealed that BAP and NAA were least

efficient when used individually compared to when applied in combination. Among the different treatment combinations, BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1} resulted better than other treatment combinations and individual treatments. This treatment combination, took least days to callus formation (42.42 days), best percentage of response (55.84%), maximum gain in weight of callus (13.66 g), earliest to shoot initiation (34.77 days), maximum number of multiple shoot bud during first and second sub culturing (5.76 and 12.13), longest length of shoot bud (5.05 cm), maximum number of leaves per shoot (5.45) and heaviest fresh weight of shoot at 90 days and during hardening stage of shoot (0.61 g and 0.99 g).

The second experiment on *in vitro* root generation of *Dendrobium* cv. Sonia Earsakul was laid out in completely randomized block design with thirteen (13) treatments. Indole-3-butyric acid (@ 0.5 mg/l, 1 mg/l, 1.5 mg/l and 2 mg/l), Napthalene acetic acid (@ 0.5 mg/l, 1 mg/l, 1.5 mg/l and 2 mg/l) and Indole-3- acetic acid (@ 0.5 mg/l, 1 mg/l, 1.5 mg/l and 2 mg/l) were added to MS media. The study revealed that among the different treatments IBA @1 mg/l significantly took shortest days to root initiation (34.90 days), highest number of shoot per explant (2.13), highest number of root (8.39), highest number of functional root (6.39), longest root length (5.97 cm), longest shoot length (7.8 cm), highest survivability during primary hardening (34.30 %) and secondary hardening (94.29%).

Keyword: BAP, IBA, NAA, IAA, *in vitro, Dendrobium* cv. Sonia Earsakul

CHAPTER I

INTRODUCTION

INRODUCTION

Flowers symbolize beauty, purity, peace and love. Floriculture is a fast emerging, rapidly expanding industry in the present scenario both in domestic and international market. It is recognized as remunerative profession with much higher potential for return per unit area than field and other horticultural crops. Ornamental plants have great economic and cultural importance throughout the world. The major constraint in the growth of floriculture industry is the lack of correct information and non availability of large scale quality planting materials. Micropropagation is the most widely used biotechnological tool for large scale propagation of floricultural crops because of the fact that the marginal profit of *in vitro* propagation of floriculture crops are more when compared to cereals, grasses, fruit and forest tree (Datta and Chakrabaty, 2010). This economic proposition has led to intensive practice and research to evolve tissue culture techniques for mass propagation and production of virus free quality plant materials.

The term orchid had its origin from the greek words ‘Orchis’ meaning testicles, referring to the paired underground tubers of the terrestrial orchids. Orchids are highly evolved fascinating group of angiosperms known for their aesthetic beauty and great floral architecture. With amazing ornamentation, curious floral shape, brilliant colour combination and fragrance, they acquired highest admiration from botanical and flori-business point of view. In floriculture, the trend of international trade is shifted in favour of orchids which demand high price and occupies a place of trade among floriculture crops. Today horticulturists grow orchids worldwide not only because of its mysterious beauty, but mainly due to its high prices. Orchids are the most beautiful flowers in god’s creation and highly priced in the international florist trade due to their intricately designed spectacular flowers, brilliant colours,

delightful appearance, myriad sizes, shapes, forms and long lasting qualities. Among ornamentals, orchids are one of the most important global cut flower and pot plants and their sheer beauty has enchanted and fascinated people since early times (Silva, 2013). Orchidaceae family with its 2500 to 30,000 species in some 700 to 800 genera constitutes the largest family among the flowering plants. The greatest diversity of orchids occurs in the high humid tropical and subtropical regions. They have a variety of life form such as epiphytes, lithophytes, terrestrial or saprophytes with monopodial and sympodial branching system.

Dendrobium is a huge genus of orchids. It was established by Olof Swartz in 1799. The name is from the Greek word *'Dendron'* means tree and '*bios*' means 'life'; it means 'one who lives on tree', or essentially, 'epiphyte'. The true spectacular genus *Dendrobium* contains the largest diversity of horticulturally interesting specimens. *Dendrobium* the orchidaceae family, with about 1,100 species, of which at least 300 have been cultivated make *Dendrobium* the second largest orchid genus and most commonly encountered orchids in retail trade (Nanda *et al.*, 2014). This mesmerizing *Dendrobium* is well known in the orchidaceae family for their complex fabricated and long lasting colourful flowers and are highly valued in the flower industry as potted plant and cut flower for their marvelously long lasting flower (Khosravi *et al*., 2008, Talukder *et al.*, 2003). *Dendrobium* are sturdy orchids that can be added as a focal point to any room. *Dendrobium* is well known in the orchidaceae family for their complex, fabricated and long lasting colourful flowers. Different characteristics of *Dendrobium* such as rapid growth, easiness of plantlet regeneration, beauty of the flower and year round production in control flowering and long lasting of the flower stalk are very advantageous of this genus (Talukder *et al.,* 2003).

Dendrobium are originally from South East Asia. The geographic distribution is from India through China, Japan, Malaysia, Philippines and the

island of the South Pacific with the largest diversity in New Guinea. *Dendrobium* species are either epiphytic or occasionally lithophytic. They have adapted to a wide variety of habitat from the high altitude in the Himalayan mountains to low tropical forests and even to dry climate of the Australian desert. Several species of *Dendrobium* are originated from India. These species are well distributed in the Himalayan region of North-East India. About 37 species of *Dendrobium* are existing in the forest of Nagaland (Changkija *et al*., 1992). The *Dendrobium* orchid is a tropical flower that contains over 1600 species.

This genus of sympodial orchids develop pseudobulbs, which vary in length from under a centimeter (eg. *Dendrobium leucocyanum*) to several meter long (*Dendrobium discolor*) resembling cane. A few grow into long reed like stems. Leaf bases form sheaths that completely envelope the stem. *Dendrobiums* have cane like stems with racemose (clustered) flowers, sepal bright, consist of three strands of lancet shaped, tapered or rounded and sizes vary. Sepal, middle sepallum called the dorsal or eyelid back, while the two sides called sepal or petal sepal lateral side. Sepal base spurs united to form a triangle. Petal of three strands with third petal below is part of united to form a triangle. Petal of three strands with third petal below is part of a united to form the lips of flowers and petal shaped generally more rounded and bigger and smoother texture then the sepal. Lip (labellum) is the development of third petal. In some species the size of the lips can be enlarged and more brightly coloured.

Dendrobium Sonia (*Dendrobium caesar* × *Dendrobium tomie* Drake) bears purple and white coloured flower. Sepal is creamy white with purple markings and petals purple in colour. Lip is light purple with creamed colour regions, pseudobulbs are cup shaped and leaves are bright green in colour, broad and acute.

Generally, orchids are propagated both asexually and sexually. But the traditional asexual propagation is extremely slow which can give rise to 2-4 plants per year (Nasiruddin *et al.,* 2003). Micropropagation of orchids is the most frequently used convenient technique for their exploitation as a major trade in developed countries (Goh and Tan, 1982; Sagawa and Kunisaki, 1982). *In vitro* propagation of orchid offers an opportunity for the selection of various desirable traits and produces high quality and uniform plantlets through out the year under disease free conditions regardless of the seasons and weathers (Pola *et al.,* 2009). Orchids can be rapidly propagated through tissue culture techniques by using shoot tips (Saiprasad *et al.,* 2003).

Orchids have a low rate of multiplication under natural or greenhouse conditions. It thus requires a rapid and efficient micropropagation protocol for obtaining true-to-type regenerants without being detrimental to the survival of mother plant to meet the demand and saving its populations from getting rarer in nature. The development of protocols for extensive and clonal multiplication of highly priced varieties of *Dendrobium* serves as a foundation for commercial scale propagation.

Plant growth regulators are an essential part for *in vitro* regeneration of crop grown in artificial medium. Auxin and cytokinins are important plant growth substances for growth and differentiation of culture cell and tissue. A number of PGRs were reported to be least efficient when used individually compared to when applied in combination with other PGRs in order to produce the final effect by changing the amount and type of growth regulators in the medium, the cell can be stimulated to develop into shoots and roots or even may die (Alvard *et al*., 1993). The plant growth regulator, benzyl amino purine (BAP) is widely used and showed markedly enhanced cell division and regeneration of shoot (Chawla, 2009). Success in micropropagation largely depends upon the nutrients contained in the media required for culture. For successful regeneration of plant, concentration of plant growth regulators like

auxin and cytokinin are very important. Dunwell (1981) reported that each species requires a particular hormone concentration for optimum growth and differentiation. Auxin alone was conducive for protocorm multiplication whereas cytokinin alone caused necrosis, auxin and cytokinin combinations enhanced differentiation of protocorm (Mathew and Rao, 1985). Optimization of plant growth regulators is prerequisite for any *in vitro* micropropagtion work.

Thus, the present investigation entitled "Standardization of micropropagation techniques of *Dendrobium* cv. Sonia Earsakul through shoot tip culture" was carried out in the Tissue Culture Laboratory, Department of Horticulture, NU:SASRD with the following objectives.

1. To study the effect of growth regulators on the shoot induction from the shoot tip explants.
2. To study the effect of some auxins on root initiation and elongation of *in vitro* induced shoots.
3. To study the performance of rooted plantlets during hardening.

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Studies on *Dendrobium* have been carried out by a number of researchers in different aspects; some of the relevant literatures pertaining to the present study are highlighted in this chapter.

2.1 Shoot regeneration

Chookoh *et al*. (2019) in their experiment on *Tolumnia* orchid micropropagation reported that Murashige and Skoog (MS) medium supplemented with 2 mgL^{-1} BA and 0.5 mgL^{-1} NAA give the greatest rate of PLB induction (16.7%) for mass propagation in a short period of time.

Erawati *et al.* (2019) reported that emergence of vanilla shoot is not influenced by exogenous growth regulators and addition of BAP 3mgL^{-1} gave the most multiplication results of 3-4 shoots with shoot length of 2-2.5 cm at 28 days after inoculation.

Baby *et al*. (2019) develop a micropropagation protocol for *Vanda* hybrid ‘Dr. Anek, the results of their experiment showed that treating the explants shoot tip with 0.1 per cent carbendazim for 20 minutes, followed by 70% ethanol alcohol for 5 minutes and 0.1 per cent mercuric chloride for 5 minutes effectively reduced the microbial contamination of the culture with the highest percentage of explants survival.

Pareira *et al*. (2018) conducted an experiment to evaluate the efficiency of different BAP concentration on micropropagation of Farta Velhaco banana recorded that the highest number of shoot is recorded in 2.5 mgL^{-1} BAP concentration producing 3 shoots per explants in 4^{th} subculture. BAP @ 4mgL^{-1} reduced the number of shoots produced per explants.

Priyanka *et al.* (2018) reported that *in vitro* regeneration and multiplication of *Dendrobium* sp. among the different concentrations of NAA and BAP alone or combination of both hormones were used it was revealed that shoot regeneration from node was the best at 2.5 mg/l BAP supplemented

to MS medium. The highest number of shoots and leaves were found when 0.5 mgL^{-1} NAA with 1.0 mgL^{-1} BAP was supplemented into MS medium.

Beura *et al.* (2017) in their investigation to standardize the plant bio regulators for *in vitro* shoot proliferation of *Curcuma longa* L. cv. Roma using finger tip reported that MS media supplemented with BAP (3.0 mgL^{-1}) and NAA (0.2 mgL^{-1}) produces longer bud (0.7 cm).

Pandey (2017) noticed that on medicinal plants *Curcuma* MS media with 2.0 mgL^{-1} BAP+ 2.0 mgL^{-1} NAA was found good for shoot initiation and multiplication in all the cultivars he studied.

Rattana and Sangchanjiradet (2017) in their study revealed that in *Dendrobium signatum* Rchb.f. ½ MS media supplemented with 2 mg/l BA in combination with 0.5 mgL^{-1} NAA is most suitable medium for shoot proliferation.

Regmi *et al.* (2017) conducted an experiment on mass propagation of epiphytic orchid *Cymbidium aloifolium* (L.) Sw., through protocorm culture. It was found that MS media fortified with BAP (1 mgL^{-1}) and NAA (1 mgL^{-1}) resulted in maximum induction of rootless healthy shoots with an average value of 8-9 shoots per culture.

Thokchom and maitra (2017) studied micro propagation of *Anthurium andreanum* cv. Jewel and their result revealed that MS media supplemented with 0.5 mg L^{-1} BAP took minimum number of days (37.68) to initiate callus. However, maximum callus production was observed in the media containing 2 mgL^{-1} NAA (77.33%). MS media containing 3.0 mgL^{-1} BAP + 0.5 mgL^{-1} NAA took a minimum of 27.83 days to regenerate shoot from callus culture, while MS + 2 mgL^{-1} BAP + 0.5 mgL^{-1} NAA gave maximum regeneration percentage (98.89%) as well as shoot length of 2.23 cm. Maximum number of shoots per callus (5.83) and highest number of shoots following second sub culturing (22.60) were observed when MS basal medium was fortified with 2 mgL^{-1} BAP singly.

Gansau *et al.* (2016) observed that on endangered epiphytic orchid *Dendrobium lowii,* 2-4 μM NAA and 4-6 μM BAP in KC medium significantly enhanced growth and development. The supplementation of 6 μM NAA promote similar responses for growth index of 563.3. This treatment induced up to 86.7% and 83.7% of protocorms forming shoots and roots respectively.

Balilashaki and Ghehsareh (2016) reported that MS culture medium containing15 mg/l BAP + 3 mg/l NAA give the highest PLBs per explant (50.65) thereby considered as the best medium for *Phalaenopsis* micropropagation.

Meilasari and Iriawati (2016) showed that medium supplemented with 0.5 ppm NAA and 5 ppm BAP is the best medium for plant regeneration through PLBs.

Parveen *et al*. (2016) reported that hormonal combination of 3 mg/L BAP and 1 mgL^{-1} NAA showed excellent growth (100%) followed by 4 mgL^{-1} BAP and 1 mgL^{-1} NAA (75%). They revealed that MS medium containing 3 mgL^{-1} BAP and 1 mgL^{-1} NAA showed excellent growth and were most effective for germination of seeds in *Dendrobium macrostachyum.*

A study conducted on *Phalaenopsis* cv. Surabaya showed that MS media supplemented that 5 mgL^{-1} BAP and 2 mgL^{-1} NAA were most effective concentration for shoot regeneration (Balilashaki *et al*., 2015).

Goswani *et al*. (2015) investigated on *in vitro* regeneration of *Dendrobium* sp. of orchid using leaf tips as explants where the sub cultured PLBs were inoculated on MS medium supplemented with different combinations of NAA (0, 0.5, 2.5, 5 mgL^{-1}) and BAP (0, 0.5, 2.5, 5 mgL^{-1}) for shoot regeneration. The maximum number of shoot, the highest fresh weight and the highest shoot length were observed in 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP after 60 days of culture.

Islam *et al*. (2015) in their experiment to develop an efficient protocol for micropropagation in *Cymbidium finlaysonianum* Lindl. reported that the

best response for shoot multiplication and elongation was obtained in medium with 0.75 mg/L NAA and 1.5 mg/L BAP.

Kaur (2015) reported that for *in vitro* propagation of *Vanilla planifoli*a maximum of 6.0±0.81and 6.0±0.86 shoots were obtained in MS medium using BA (2 mgL^{-1}) and favored multiplication of shoot bud and early plantlet development within 11.65±0.41 weeks. NAA (1.2 mgL^{-1}) favored callusing at basal part of shoot bud and initiating multiple shoot buds.

Singh *et al*. (2015) conducted *in vitro* seed germination trials using three different basal media *viz*., half strength Murashige and Skoog (½ MS), and Knudson C (KC) and Vacin & Went (VW), of which ½ MS medium proved to be ideal for getting maximum percentage of seed germination, 83.97 ± 0.83% within 8 weeks. Multiple shoots were induced when the mature protocorms with shoot primordia were cultured on ½ MS medium supplemented with different combinations of NAA, BAP, and complex additives. Significant root induction in the multiplied shoots (10.34 ± 0.57) was observed in ½ MS medium fortified with 2 mgL^{-1} of NAA.

Anbazhagan *et al.* (2014) in their study on *in vitro* culture technique for the mass propagation of *Musa sp*. found that the best results were obtained from MS medium supplemented with BAP+NAA at the concentration of 3.0mgL^{-1} and 0.5mgL^{-1} respectively.

Bhattacharjee and Islam (2014a) developed an efficient protocol for regeneration of endangered medicinal orchid *Vanda tessellate* via multiple shoot using shoot segments as explants. It was recorded that among the different plant growth regulators (PGRs) combination tested for multiple shoot induction, the combination of 1.0 mgL^{-1} NAA and 1.0 mgL^{-1} BAP was proved to be the best medium formulation for multiple shoot development and elongation.

Bhattacharjee and Islam (2014b) developed an efficient protocol for *in vitro* seed germination and for growth and development of protocorm likes

bodies (PLBs) from three indigenous orchid species namely *Acampe premorsa (*Roxb.*), Agrostophylum khasianum* L. *and Phalaenopsis cornorerris*. The result revealed that MS medium with 1.0 mgL^{-1} BAP was the most efficient medium for seedling growth of *Acampe premorsa* and *Acampe khasianum*. But in the case of *Phalaenopsis cornorerris* the best seedling growth was observed using medium supplemented with 0.5 mgL^{-1} BAP and 0.1 mgL^{-1} NAA.

Islam *et al*. (2014) studied the effect of plant growth regulators on hybrid orchid (*Dendrobium alba* x *Ascanda dongtarm) in vitro* growth and development. It was recorded that among the different growth regulators used (IAA, NAA) maximum weight of PLBs (1.37g/explant) were noticed on MS medium supplemented with 1.5 mgL^{-1} NAA, and the highest number of plantlets (18.58/explant) were observed on MS medium containing 1.0 mgL^{-1} NAA.

Julkiflee *et al*. (2014) reported that the addition of BAP and NAA in combinations of BAP (4.44 or 8.88 μM) and NAA (8.88 μM) resulted in increased PLBs growth rate to 14 % compared to when added separately in their experiment on effective propagation of protocorm-like bodies (PLBs) of *Dendrobium* Sonia-28.

Nongdam and Tikendra (2014) studied the mass propagation of *Dendrobium chrysotoxum* through seed culture and found that BAP combined with low auxin resulted in pronounced shooting and leaf formation, cytokinin alone resulted in poor development of leaf indicating synergistic effect of auxin and cytokinin in leaf induction.

Panathula *et al*. (2014) studied regeneration of *Centella asiatica* (L.) from callus culture and observed that the maximum regeneration frequencies of shoots were recorded with BAP 2.5 mgL^{-1} + 0.5 NAA mgL^{-1}.

Devi *et al*. (2013) conducted a trial on plant regeneration system of *Aerides odorata* Lour. through foliar and shoot tip culture. It was recorded that the highest number of shoot was obtained in higher concentration of NAA

(2 mgL^{-1}) and BAP (4 mgL^{-1}) (4.80±0.18), showing combined effect of BAP and NAA, which may be due to the synergistic effect of cytokinin and auxin.

Jitsopakul *et al*. (2013) observed that on *Vanda coerulea* addition of 1 mgL^{-2} BAP to the modified VW medium induced the best shoots after 3 months of culture.

Kabir *et al*. (2013) in their study to optimize and develop efficient regeneration protocol for *in vitro* germination, micropropagation and root induction of *Dendrobium fimbriatum* revealed that MS medium fortified with 2.0 mgL^{-1} 6-benzylaminopurine (BAP) was the most effective for shoot elongation. MS medium supplemented with 1mg/l BAP was proven to be the best for multiple shoot formation and elongation. Furthermore, shortest duration for shoot induction was recorded on this medium.

Kumari *et al*. (2013) in their study on *in vitro* propagation of orchid *Dendrobium* Sonia recorded that ½ MS supplemented with 4 mgL^{-1} BA was observed to give early bud break and the shoot multiplication stage, treatment 0.1 mgL^{-1} NAA was found to give earliest shoot multiplication and maximum numbers of healthy shoots.

Mondal *et al*. (2013) in their work to develop an efficient protocol for *in vitro* propagation of *Doritis pulcherima* Lindl. through shoot tip culture. It was recorded that BAP and NAA show significant effect on the axillary shoot formation, protocorm like body induction and root formation. The highest frequency of axillary shoot formation was recorded in the medium containing 2 mgL^{-1} BAP and the PLB production was higher in the medium containing 2 mgL^{-1} NAA. A higher concentration of BAP showed inhibitory effects on the axillary shoot formation and PLB induction.

Maurya *et al*. (2013) investigated on *Rosa hybrid* L. cv. Benjamin Paul to develop an *in vitro* plant regeneration protocol. The results showed that the highest (100 %) shoot proliferation was obtained on modified MS medium containing 2.0 mgL^{-1} BAP and 0.1 mgL^{-1} NAA.

Paudel and Pant (2013) established a reliable protocol for micropropagation of *Esmeralda clarkei* Rchb.f., among the different nutrient media tried in their study, BAP (0.5-2.0 mgL^{-1}) considerably induced multiple shoot growth from single protocorm explants and multiple shoots from single shoot section explants after 120 days of culture.

Pradhan *et al*. (2013) studied *Dendrobium densiflorum* on regeneration from shoot tip explants obtained from *in vitro* grown seedlings by tissue culture technique. The maximum number of healthy shoot was observed on MS + BAP (2 mgL^{-1}) + NAA (0.5 mgL^{-1}) (4 shoots/ culture). The shoot multiplication started after three weeks of culture.

Zuraida (2013) conducted an experiment on *in vitro* propagation of *Curcuma caesia* and reported that explants cultured on MS medium supplemented with 3 mg/litre BAP showed highest proliferation rate (95%). *In vitro* microshoots that were subcultured on MS media supplemented with 3 mgL^{-1} BAP and 0.5 mgL^{-1} NAA performed better in terms of the number of shoots produced.

Asghar *et al.* (2011) reported in their experiment axillary buds of orchid *Dendrobium nobile* var. Emma white that maximum number of shoots (4.33), as well as fresh and dry weights (752.5 and 52.99 mg) were obtained at 2 mgL^{-1} BAP.

Manners *et al.* (2011) in their trial on *Vanda coerulea* through *in vitro* asymbiotic germination of seeds recorded that incorporation of either 5 µM 6-benzyl amino - purine (BAP) or 5 µM indole 3-acetic acid (IAA) separately in the MS medium attained seed germination to 94.4% and 92.6% respectively. A maximum shoot number and roots was obtained on MS medium supplemented with a combination of 5 µM BAP and 15 µM IAA.

Tao *et al*. (2011) reported that in *Cymbidium feberi* Rolfe MS media supplemented with 0.5 mgL^{-1} NAA was optimal medium for protocorm like

bodies turning green and its optimal concentration for growth and multiplication of PLB was BAP 2 mgL^{-1} and 1 NAA mgL^{-1}

Harb *et al*. (2010) in their trial on micropropagation of *Anthurium andraeanum* by shoot tip culture revealed that regenerated shoots were successfully rooted when cultured on half-strength MS medium fortified with 2.0 mgL^{-1} IBA.

Boudabous *et al* (2010) studied on apple *Malus domestica* L. cultivar Douce de Djerba through *in vitro* culture of axillary buds reported that MS medium supplemented with 1 mgL^{-1} and 0.1 mgL^{-1} gave the highest sprouting frequency (85.05%) and shoot differentiation. 1 and 2 mgL^{-1} BAP gave the highest multiple shoot formation. They reported that BAP had a positive effect on growth and multiplication of shoot but if the concentration exceeds beyond 4mgL^{-1} of BAP the growth decreases.

Janarthanam *et al.* (2009) reported that in *Stevia rebaudiana* MS supplemented with 4.44 μM BA and 1.34 μM NAA showed better growth response and produced 14.0 ± 1.0 shoots with an average length of 5.6 ± 0.1 cm after 28 days.

Rahman *et al*. (2009) in their study on *in vitro* micropropagation of orchid *Vanda tessellate* L. from shoot tip explants recorded that the combination of 1.5 mgL^{-1} NAA and 1.0 mgL^{-1} BAP was the best medium formulation for multiple shoot formation as well as maximum shoot elongation.

Sunitibala and Kishore (2009) in their experiment to develop an efficient protocol for micropropagation of *Dendrobium transparens* revealed that media containing 1 mgL^{-1} IAA showed better rooting parameters.

Pant and Thapa (2012) investigated to develop a protocol for rapid *in vitro* micropropagation of a critically endangered most important epiphytic orchid *Dendrobium primulinum* Lindl. through small shoot tip explants (0.3 to 0.5mm) derived from *in vitro* grown seedlings. It was recorded that the maximum number of rootless healthy shoots were observed on MS medium

fortified with BAP 1.5 mgL^{-1} with an average value of 4.5 shoots per culture where shoot multiplication was initiated after 5 weeks of culture of shoot tip. Among the different hormone concentrations, MS medium with BAP (1.5 mgL^{-1}) and NAA (0.5 mgL^{-1}) showed best result in shoot multiplication.

Khatun *et al*. 2010. studied the combination of BAP + NAA, BAP + IAA, BAP + IBA, and IAA + IBA at different concentrations and found that the highest shoot height (3.239 cm) and maximum number of rooted plantlets (4.473) was obtained from 1.0 mgL^{-1} each of BAP + NAA combination compared to other treatment combinations.

Parvin *et al*. (2009) investigated the effect of growth regulator NAA on *in vitro* shoot proliferation, rooting, and plantlet establishment. Among the different concentrations of NAA, the maximum increase in shoot weight and shoot number were observed from 0.1 mgL^{-1} NAA. The highest shoot length, number of leaves, number of roots, and root length were obtained with 0.2 mgL^{-1} NAA.

Rahman *et al.* (2009) reported that in *Vanda tessellate* L. the combination of 1.5 mgL^{-1} NAA and 1.0 mgL^{-1} BAP was proved to be the best medium formulation for multiple shoot formation as well as maximum shoot elongation.

Sunitibala and Kishore (2009) develop an efficient protocol for micropropagation of *Dendrobium transparens* cultured on medium with different concentration of BAP and NAA, it was revealed that for multiple shoot induction from axegenic nodal segment excised from 120 days old seedling, the media containing 1 mgL^{-1} NAA and 2 mgL^{-1} BAP gave the best response for shoot induction.

Talukder *et al.* (2003) reported for shoot proliferation of *Dendrobium* orchid with BAP and NAA that the use of different concentrations of BAP and NAA showed a significant effect. Among the concentrations the best shoot proliferation (1.90/explants), root formation (1.93/explants) leaf number

(4.25/plantlet) increment of shoot length (0.473 cm) and the least time requirement for regeneration (8.8 days) was obtained from 2.5 mgL^{-1} BAP + 0.5 mgL^{-1} NAA. At the same time, the lowest shoot proliferation (0.5/explants) was recorded in control and 0.1 mgL^{-1} NAA, leaf number (0.4/plantlet) from 5 mgl^{-1} BAP, increment of shoot length (0.252 cm) from control and highest days requirement (23.8 days) from 5 mgL^{-1} BAP+0.1 mgL^{-1} NAA was found.

Kosir *et al.* (2004) in their experiment on direct shoot regeneration from nodes of *Phalaenopsis* orchids with dormant buds from flower stalks obtained direct shoot regeneration without callus formation. Medium supplemented with 2 mgL^{-1} of 6-benzylaminopurine (BAP) and 0.5 mgL^{-1} of α–naphthalene aceticacid (NAA) was found to be the most appropriate of all the media used for rapid micropropagation of a large number of vegetative shoots without roots 160 days after inoculation. Medium containing BAP (2 mgL^{-1}) showed the highest multiplication rate for generative regenerants formation.

Park *et al.* (2001) studied the rapid propagation of *Phalaenopsis* and found that MS medium supplemented with N 6-benzyladenine (BA;88.8mM) and a-naphthalene acetic acid (NAA; 5.4 mM) produced an average of 10–13 protocorm-like bodies (PLBs) after12 weeks.

Mathews and Rao (1985) reported that auxin alone was conducive for protocorm multiplication, whereas cytokinin alone caused necrosis. Auxin and cytokinin combination enhanced differentiation of protocorm. Quantity of medium had a direct correlation with the rate of proliferation of isolated protocorms.

2.2 Root regeneration

Priyanka *et al.* (2018) in their experiment conducted on *in vitro* regeneration and multiplication of *Dendrobium sp*. recorded that application of 1.0 mgL^{-1} IBA was found to be the most effective. The well-rooted plantlets were successfully acclimatized under 70-80% humidity and planted in pots and

transferred to the shade house for establishment. Around 85% of plantlets survived in the field.

Rattana and Sangchanjiradet (2017) in their study revealed that in *Dendrobium signatum* Rchb.f. ½ MS media supplemented with 2 mgL^{-1} BA in combination with 0.5 mgL^{-1} NAA was most suitable medium for root induction.

Thokchom and maitra (2017) studied micro propagation of *Anthurium andreanum*cv. Jewel and their results revealed that ½ MS media supplemented with 2.0 mgL^{-1} NAA took the least days (33.55) to rooting. Maximum root induction per plant (5.83) and length (4.28cm) of root were recorded in ½ MS + 1.0 mgL^{-1} NAA.

Hrahsel and Thangjam (2015) in their study on asymbiotic *in vitro* seed germination and regeneration of *Vanda coerulea* observed that full strength MS basal medium showed higher rate of germination with subsequent formation of 'protocorm like bodies' (PLB) in comparison to half strength MS basal medium. Highest number of leaf emergence was observed in MS basal medium supplemented with 22.80 µMindole acetic acid (IAA).

Goswani *et al*. (2015) observed that on *in vitro* regeneration of *Dendrobium* sp. of orchid using leaf tips as explants recorded that the maximum number of root and the maximum root length were observed in 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP treatment combination after 60 days of culture.

Islam *et al*. (2015) develop an efficient protocol for micropropagation in *Cymbidium finlaysonianum* Lindl reported that MS + 1.0 mg/L IAA has proven the best for root induction comparing to IBA and NAA.

Anbazhagan *et al*. (2014) in their trial on *in vitro* culture technique for the mass propagation of *Musa* sp. found that the best root formation of *in vitro* developed shoots could be achieved on MS medium supplemented with IBA at concentration 1.0 mgL^{-1}.

Bhattacharjee and Islam (2014a and 2014b) developed an efficient protocol for regeneration of endangered medicinal orchid of *Vanda tessellate.* The developed shoot from multiple shoot bud were cultured in medium supplemented with NAA (0.5-2.0 mgL^{-1}), IAA (0.5-2.0 mgL^{-1}) and IBA (0.5-2.0 mgL^{-1}) for root induction. Maximum root induction and its growth was found in ½ MS medium supplemented with 1.0 mgL^{-1} IAA.

Panathula *et al*. (2014) studied regeneration of *Centella asiatica* (L.) from callus culture and it was observed that the well regenerated healthy micro shoots were cultured in rooting medium for rooting. MS medium augmented with IBA 1.0 mgL^{-1} showed maximum rooting frequency with high mean root number and root length.

Nongdam and Tikendra (2014) studied the mass propagation of *Dendrobium chrysotoxum* through seed culture and found that IBA was more effective in root induction as compared to NAA and IAA.

Devi *et al*. (2013) in their experiment on *Aerides odorata* Lour recorded that addition of NAA (0.5 mgL^{-1}) in ½ MS medium showed highest frequency of root induction.

Jitsopakul *et al*. (2013) in their experiment on *Vanda coerulea* observed that addition of 0.5 mgL^{-1} NAA gave the optimal number of roots per explant and plantlet height after 3 months of culture.

Kabir *et al*. (2013) in their study to optimize and develop efficient regeneration protocol for *in vitro* germination, micropropagation and root induction of *Dendrobium fimbriatum* revealed MS medium supplemented with 1.0 mgL^{-1} indole-3-acetic acid (IAA) was found suitable for effective induction and growth of adventitious roots on the micro-propagated orchid plantlets.

Kumari *et al*. (2013) investigated and reported that on *in vitro* propagation of orchid *Dendrobium* Sonia recorded that ½ MS supplemented with 0.5 mgL^{-1} NAA gave earliest rooting.

Maurya *et al*. (2013) conducted an experiment on *Rosa hybrid* L. cv. Benjamin Paul to develop an *in vitro* plant regeneration protocol. The results showed that MS medium containing 0.5 mg L^{-1} IBA was the most suitable medium for *in vitro* rooting.

Paudel and Pant (2013) established a reliable protocol for micropropagation of *Esmeralda clarkei* Rchb.f., among the different nutrient media tried in this study, it was recorded that more than 3 roots not less than 2 cm long were developed through *in vitro* rooting of shoots within 90 days of culture on MS medium containing NAA (0.5-1.0 mgL^{-1}).

Pradhan *et al*. (2013) conducted a study on *Dendrobium densiflorum* on regeneration from shoot tip explants. The induction of root was observed on all MS medium supplemented with different concentration and combination of plant growth regulators indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and NAA. Among all the treatments the most effective condition for *in vitro* rooting was observed on MS + IBA (1.5 mgL^{-1}).

Seyyedyousefi *et al.* (2013) reported that MS medium supplemented with 1.0 mgL^{-1} NAA resulted in the highest shoot length (6.30 cm), bud number (3.00) largest number of shoot (3.00) and rhizome (4.00) were obtained in MS medium containing 0.20 and 0.50 mgL^{-1} NAA in *Alstroemeria.*

Janarthanam *et al.* (2009) reported that in *Stevia rebaudiana* MS supplemented with 2.46 µM IBA produced profuse rooting within 25 and plantlets were transferred for hardening, with 90% of plantlets successfully established in the field.

Pant and Thapa (2012) studied to develop a protocol for rapid *in vitro* micropropagation of epiphytic orchid *Dendrobium primulinum* Lindl. Through shoot tip explants derived from *in vitro* grown seedlings. It was observed that MS medium supplemented with various concentrations of rooting hormones viz. NAA, IAA and IBA showed positive response in development of roots, except NAA 0.5 mgL^{-1}. The rooting was observed after 3 weeks of culture of

shoot tip. The various concentrations of IAA and IBA were found to be effective hormone for rooting of *D. primulinum* in comparison to NAA. The best rooting response was observed on MS medium with exogenous supply of IAA 0.5 mgL^{-1}.

Baker *et al.* (2011) reported that in *Orchis catasetum*a rare orchid, combination of 0.5 mgL^{-1} BA and 0.5 mgL^{-1} NAA was found to be suitable for maximum protocorm-like bodies (PLBs) regeneration (20.40 $plantlet^{-1}$). The largest number of root (7.16 $plantlet^{-1}$) and leaf (10.10 $plantlet^{-1}$), also the highest plant height (114.20 mm) and root length (193.40 mm) were obtained on MS medium supplemented with 0.5 mgL^{-1} BA along with 0.5 mgL^{-1} NAA.

Rahman *et al*. (2009) in their study on *in vitro* micropropagation of orchid V*anda tessellate* L. from shoot tip explants recorded that the maximum root induction was obtained in MS agarified medium having 0.5 mgL^{-1} NAA and 1.0 mgL^{-1} IBA.

Sunitibala and Kishore (2009) develop an efficient protocol for micropropagation of *Dendrobium transparens* revealed that media containing 1mgL^{-1} IAA show better rooting parameters. The plantlets were successfully hardened with more than 90% survivality in shade house.

Aktar *et al*. (2007) in their study revealed that the use of different concentrations of IBA on root formation of *Dendrobium* orchid showed significant results. The best results were obtained from 1.0 mg/l IBA treatment in which the number of root was 1.81 $plantlet^{-1}$, length of root 0.35 cm, fresh weight of root 0.16g at 30 DAI and the minimum days to root formation was 10.

Aktar *et al*. (2007) studied the effect of different concentrations of IBA (0, 0.5, 1.0, 1.5 and 2.0 mgL^{-1}) on root formation in *Dendrobium* orchid recorded that parameters such as number of roots per plantlet, length of root, fresh weight of root and days required for root formation were recorded at three days after innoculation (10, 20 and 30). The use of different concentrations of

IBA had significant effects on the parameters examined. The best results were obtained from 1.0 mgL^{-1} IBA treatment.

2.3 Hardening

Priyanka *et al.* (2018) carried out an experiment on *in vitro* regeneration and multiplication of *Dendrobium sp*. recorded that application of 1.0 mgL^{-1} IBA was found to be the most effective. The well-rooted plantlets were successfully acclimatized under 70-80% humidity and planted in pots and transferred to the shade house for establishment. Around 85% of plantlets survived in the field.

Anbazhagan *et al*. (2014) in their study reported that in *Musa* sp. MS media supplemented with IBA @ 1 mgL^{-1} resulted in best root formation for developed *in vitro* shoot and the rooted plant were grown in greenhouse for hardening and then planted in the open field condition. Around 85% of the planted sapling was successfully established in the open field condition.

Bhattacharjee and Islam (2014) developed an efficient protocol for regeneration of endangered medicinal orchid of *Vanda tessellate.* The developed shoot from multiple shoot bud were cultured medium supplemented with NAA (0.5-2.0 mgL^{-1}), IAA (0.5-2.0 mgL^{-1}) and IBA (0.5-2.0 mgL^{-1}) for root induction. Maximum root induction and its growth was found in medium supplemented with 1.0 mgL^{-1} IAA. The rooted plantlets were hardened successfully in the potting media containing coconut husk, charcoal, brick pieces in the ratio of 2:1:1.

Panathula *et al.* (2014) in their study reported that in *Centella asiatica* (L.) MS media augmented with IBA @ 1 mgL^{-1} resulted in better rooting parameters and the well rooted plant were hardened in field condition with surviving percentage of 80%.

Aslam *et al*. (2013) reported in their study on rapid multiplication of *Lilium orientalis* and *Lilium longiflorum* cv. White Fox, it was found that among different treatments used for culturing of the plant, the MS medium

supplemented with 6-benzylaminopurine (BAP) 3.0 mgL^{-1} was found to be the best for shoot initiation from scales of the bulb. After that plants were transferred to different media for multiple shooting. Out of different concentrations used the medium with 0.1 mgL^{-1} BAP + 0.1mgL^{-1} NAA increased frequency of shoot formation up to 100%. An average of about 10 ± 3.94 shoots/explants; well-developed roots and bulblet formation were obtained in this medium.

Pradhan *et al*. (2013) a study was conducted on *Dendrobium densiflorum* regeneration from shoot tip explants. For inducing root, MS media without and with auxins (IAA, IBA and NAA) were used. Among these, the most effective condition for *in vitro* rooting was observed on MS+ IBA (1.5 mgL^{-1}). The *in vitro* propagated plantlets were transferred in 2:1:1 ratio of cocopeat, litter and clay containing earthen pot for acclimatization. About 85% plantlets were successfully acclimatized in the greenhouse.

Asghar *et al.* (2011) reported in *Dendrobium nobile* var. Emma white that IBA at a level of 2 mgL^{-1} increased the rooting percentage (97.5%) number of roots (4.70) and root length (3.47 cm) more efficiently than NAA. Higher concentrations of IBA and NAA (3.0 mgL^{-1}) showed poor results of rooting.

Manners *et al.* (2011) in their trial on *Vanda coerulea in vitro* asymbiotic germination of seeds recorded that maximum shoot number and roots was obtained on MS medium supplemented with a combination of 5 μM BAP and 15 μM IAA. Well developed seedlings were hardened in a potting medium comprised of charcoal, brick pieces and decaying litter in a 1:1:1 ratio, with a top layer of moss, where in 91.2% survivability was obtained.

Rahman *et al.* (2009) reported that in *Vanda tessellate* L. the combination of 0.5 mgL^{-1} NAA and 1.0 mgL^{-1} IBA in MS medium resulted in maximum root induction

CHAPTER III

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation entitled "**Standardization of micropropagation techniques of *Dendrobium* cv. Sonia Earsakul through shoot tip culture"** was carried out at the Tissue Culture Laboratory, Department of Horticulture, School of Agricultural Sciences and Rural Development, Nagaland University, Medziphema campus during the year 2016-2019. The details of materials used and research methodology followed during the investigation for recording the various observations and analysis are presented in this chapter.

3.1 General information

3.1.1 Site of the experiment

The experimental work on the micropropagation was carried out at the Tissue Culture Laboratory, Department of Horticulture, NU:SASRD, Medziphema campus during the year 2016-2019.

3.1.2 Explants

A healthy and disease free shoot tip of *Dendrobium* cv. Sonia Earsakul is used as explants for the experimental work. The explants were collected from Orchidarium, Instructional Farm SASRD, Department of Horticulture, NU SASRD, Medziphema campus, Nagaland.

3.1.3 Plant growth regulators

The following plant growth regulators have been used in different experiments at different concentrations on shoot bud induction and root induction medium with MS basal medium

Cytokinins : 6-Benzylaminipurine (BAP)

Auxin : Napthalene acetic acid (NAA)

Indole-3-butyric acid (IBA)

Indole acetic acid (IAA)

3.1.4 Medium

Murashige and Skoog medium (1962) was used in the present study. MS media in full strength was used in all the experiments. Modification to the medium was done by adding growth regulators depending upon the investigation.

Table: 3.1 Composition of Murasige and Skoog (MS) medium

Ingredients	Amount (mgL^{-1})
1. Macro salts	
NH_4NO_3	1650
KNO_3	1900
$CaCl_2.4H_2O$	440
$MgSO_4.7H_2O$	370
KH_2PO_4	170
2.Micro salts	
KI	0.830
H_3BO_3	6.200
$MnSO_4.4H_2O$	22.300
$ZnSO_4.7H_2O$	8.600
$Na_2MoO_4.2H_2O$	0.250
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
3.Fe.EDTA	
$FeSO_4.7H_2O$	27.80
$Na_2.EDTA.2H_2O$	37.20

4. Organics	
Thiamine, HCl	0.10
Pyridoxine, HCl	0.50
Nicotinic Acid	0.50
Glycine	2.00
Myo-inositol	100.00
Sucrose	30,000.00

*pH = 5.7
*Agar = 0.8%
*Growth regulators are added as per the requirement of the experiments.

Table 3.2: Composition of stock solutions for the preparation of 1 litre modified MS medium for micropropagation of *Dendrobium* cv. Sonia Earsakul through shoot tip culture

Ingredients	**Amount (gL^{-1})**
1. Micro Stock-I	
$ZnSO_4.7H_2O$	1.72
H_3BO_3	1.24
$MnSO_4.4H_2O$	4.46
KI	0.17
2. Micro stock-II	
$NaMoO_4.2H_2O$	0.250
$CoCl_2.6H_2O$	0.025
$CuSO_4.5H_2O$	0.025

Table 3.3: Composition of Iron stock solutions used for the preparation of 1 litre modified MS medium for micropropagation of *Dendrobium* cv. Sonia Earsakul through shoot tip culture

Ingredients	**Amount (gL^{-1})**
$FeSO_4.7H_2O$	5.56
$Na_2.EDTA.2H_2O$	7.46

*Dissolved in boiling water through hot plate with constant stirring.

Table 3.4: Composition and amount of different vitamins and other organic stock solution used for the preparation of 1 litre modified MS medium for micropropagation of *Dendrobium* cv. Sonia Earsakul through shoot tip culture.

Ingredients	**Amount (g/100ml)**
Thiamine HCl	100
Nicotinic Acid	100
Pyridoxine HCl	100
Glycine	100

Table 3.5: Composition and amount of different growth regulators stock solutions used for the preparation of modified MS medium for micropropagation of *Dendrobium* cv. Sonia Earsakul through shoot tip culture

Stock	**Amounts (mg/100ml**
6-BAP	100
NAA	100
IBA	100
IAA	100

*All the growth hormones first dissolved in little (1-2 ml) amount of 1N NaOH Solution.

Table 3.6 Composition and amount of stock solutions used in the preparation of modified MS medium for micropropagation of *Dendrobium* cv. Sonia Earsakul through shoot tip culture.

Constituents	**Quantities**
A. Macro salts	
NH_4NO_3	$1.65gL^{-1}$
KNO_3	$1.90g\ L^{-1}$
$CaCl_2.4H_2O$	$0.44g\ L^{-1}$
$MgSO_4.7H_2O$	$0.37g\ L^{-1}$
KH_2PO_4	$0.09g\ L^{-1}$
B. MN-Minor stock	$5mlL^{-1}$
C. MR-Micro stock	$1mlL^{-1}$
D. MS-Iron stock	$5mlL^{-1}$

E. Vitamins, amino acids and other organics supplements	
Thiamine, HCl	0.1ml L^{-1}
Pyridoxine, HCl	0.5ml L^{-1}
Nicotinic Acid	0.5ml L^{-1}
Glycine	2ml L^{-1}
Myo-inositol	0.1g L^{-1}
F. Growth regulators	
6-BAP	(As per experiment)
NAA	(-do-)
IBA	(-do-)
IAA	(-do-)
G. Sugar	30g L^{-1}
H. Agar	8.7g L^{-1}
I. pH	5.6-5.7

3.2 Methodology

3.2.1 Preparation of nutrient media

MS media supplemented with growth regulators at different concentrations were used depending on the purpose of the individual experiment. Separate stock solutions were prepared for macro nutrients, micro nutrients (Table 3.2), iron stock (Table 3.3) vitamins (Table 3.4) and growth regulators (Table 3.5) separately.

3.2.2 Preparation of stocks

The stock solutions were prepared as given below with double distilled water, poured into well stopper conical flasks and were stored in refrigerator at 4°C.

Stock A: Micro nutrients -1000ml (10x) - Table: 3.2

Stock B : Macro Nutrient -1000ml (10x) - Table 3.3

Stock C: Vitamin -100ml (10x) Table 3.4

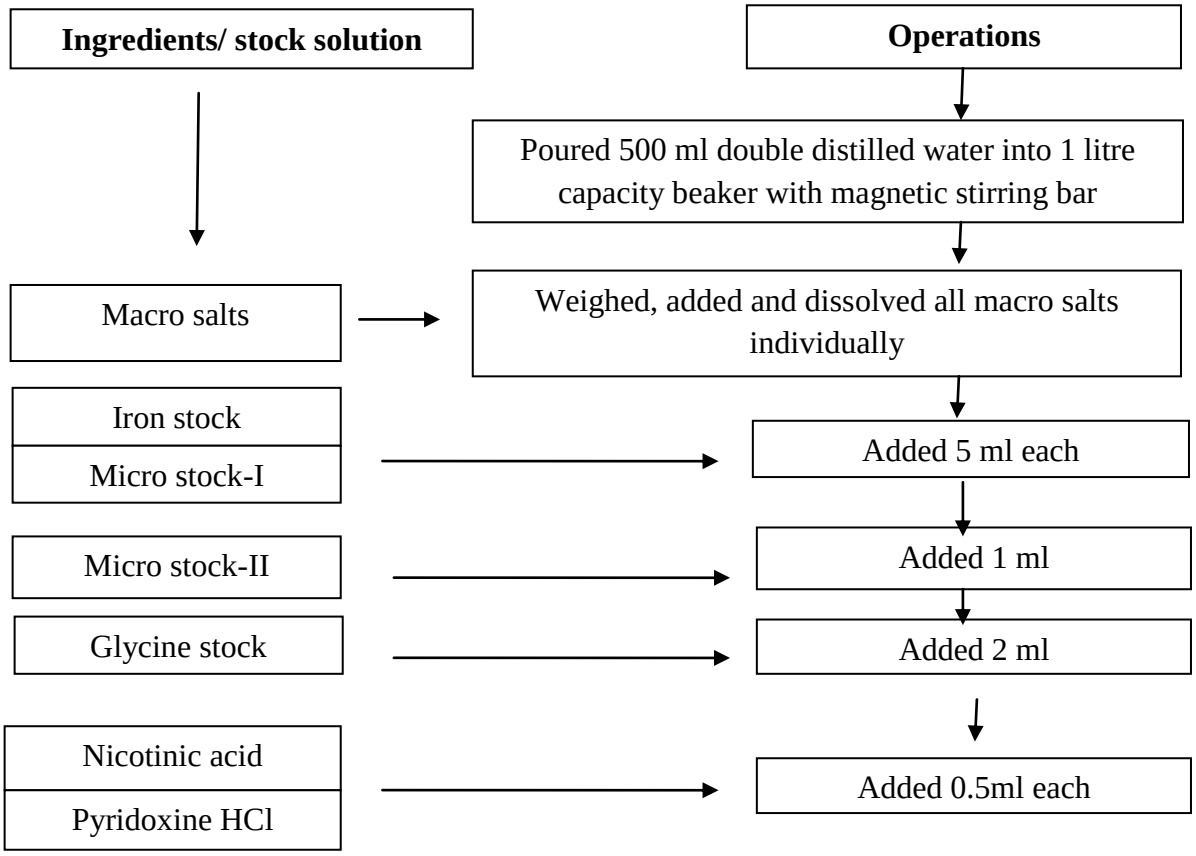
3.2.3 Preparation of growth regulator stocks

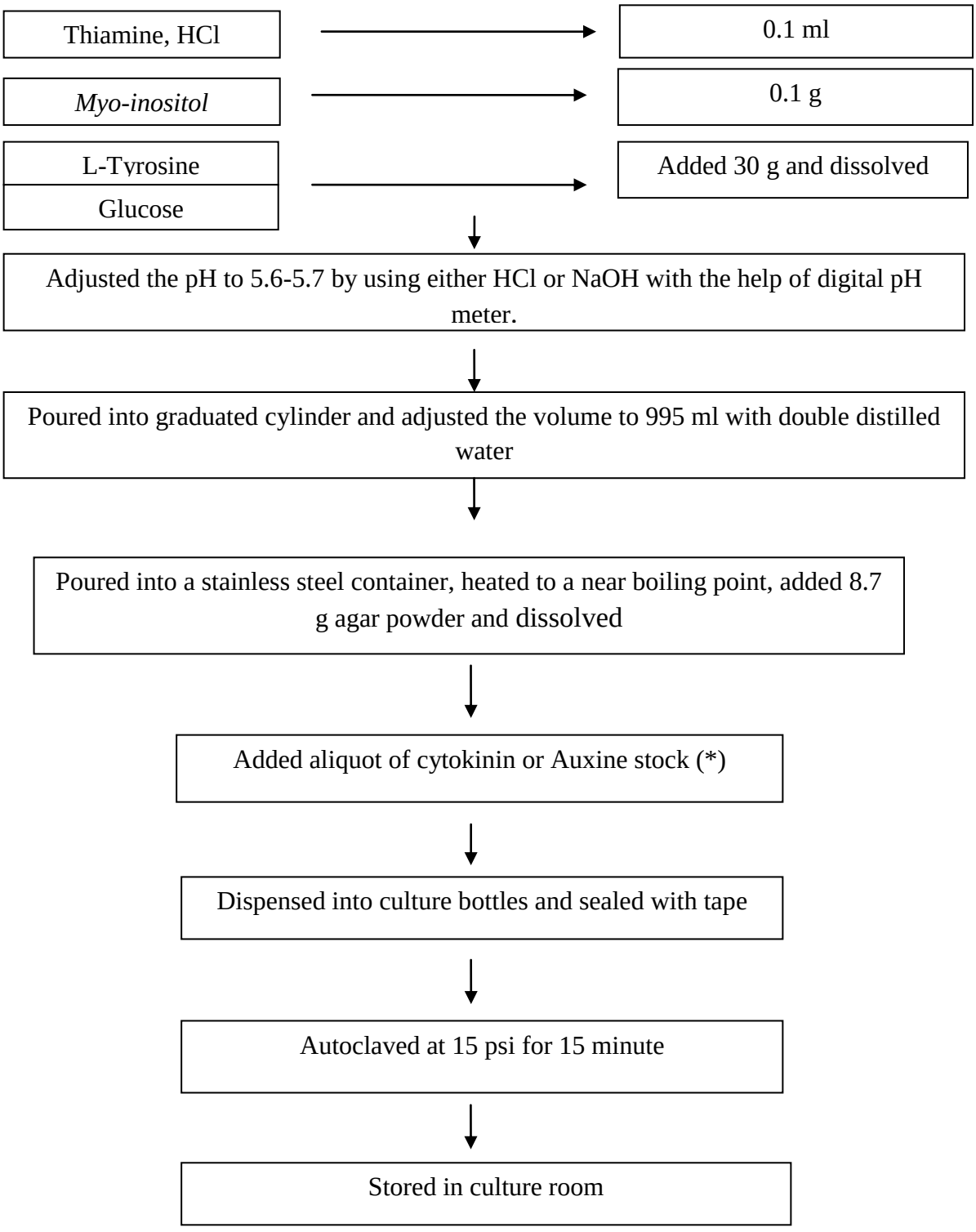
Stock solutions of 6-benzylaminopurine (BAP), NAA, IBA and IAA were prepared by dissolving them first in few drops of 1N NaOH and the

volume were made up to the required concentration with double distilled water Table: 3.5

3.2.4 Preparation and sterilization of media

The stock solutions were mixed in required proportion along growth regulators and sucrose. Sucrose 30 gL^{-1} and *myo-inositol* 100 mgL^{-1}were added fresh to the medium and the volume was finally made up by adding double distilled water. The pH was adjusted between 5.6-5.7 by using either HCl or NaOH with the help of digital pH meter. Agar @ 8.7 gL^{-1} was added and dissolved by gently heating up to 90°C, with frequent stirring to ensure uniform heating and to avoid boiling or frothing. About 60 ml of medium were dispensed into sterilized glass culture bottle (25cm x 60 cm) and plugged with plastic corks and sealed with tape to prevent any loss of moisture or contamination during handling and autoclaving. The media was autoclaved at 121°C at 16 *psi* for 15 minutes and then allowed to cool to room temperature and stored in culture room until further use.





- Only double distilled water was used for stock and media preparation.

- ❖ Only analytical grade chemicals and reagents were used.
- ❖ Stock solutions were checked properly for any contamination or precipitation before use.
- ❖ Glassware and equipments were properly sterilized at 150±5°C for 1 hour in hot oven prior to media preparation
- ❖ (*) indicates requirements as per the research experiment

Fig: 3.1 Schematic procedure for preparing 1 litre modified MS media

3.3 Experimental details

3.3.1 Experiment 1: Study on *in vitro* shoot multiplication of *Dendrobium* cv. Sonia Earsakul

3.3.2 Technical details

Shoot tips (0.5-1.5 cm) of *Dendrobium* cv. Sonia Earsakul were cultured on MS media supplemented with various concentrations of 6-benzylaminopurine (BAP) @ 0, 0.5, 1, 1.5 and 2 mgL^{-1} and naphthalene acetic acid (NAA) @ 0, 0.5, 0.75 and 1 mgL^{-1} alone and in combination were used for shoot regeneration.

Experimental Design	:	Completely Randomized Design (CRD)
Replication	:	3 (Three)
Number of factor	:	2 (Two)

Table 3.7 Composition of MS media with different hormone concentration tested for multiplication of *Dendrobium* cv. Sonia Earsakul

Treatment	Media Concentration (mgL^{-1})

	BAP (B)	**NAA (N)**
T_1:MS+$B_{1+}N_1$	0 mgL^{-1}	0 mgL^{-1}
T_2:MS+$B_{1+}N_2$	0 mgL^{-1}	0.5 mgL^{-1}
T_3:MS+$B_{1+}N_3$	0 mgL^{-1}	0.75 mgL^{-1}
T_4:MS+$B_{1+}N_4$	0 mgL^{-1}	1 mgL^{-1}
T_5:MS+$B_{2+}N_1$	0.5 mgL^{-1}	0 mgL^{-1}
T_6:MS+$B_{2+}N_2$	0.5mgL^{-1}	0.5 mgL^{-1}
T_7:MS+$B_{2+}N_3$	0.5mgL^{-1}	0.75 mgL^{-1}
T_8:MS+$B_{2+}N_4$	0.5mgL^{-1}	1 mgL^{-1}
T_9:MS+$B_{3+}N_1$	1 mgL^{-1}	0 mgL^{-1}
T_{10}:MS+$B_{3+}N_2$	1 mgL^{-1}	0.5 mgL^{-1}
T_{11}:MS+$B_{3+}N_3$	1 mgL^{-1}	0.75 mgL^{-1}
T_{12}:MS+$B_{3+}N_4$	1 mgL^{-1}	1 mgL^{-1}
T_{13}:MS+$B_{4+}N_1$	1.5 mgL^{-1}	0 mgL^{-1}
T_{14}:MS+$B_{4+}N_2$	1.5 mgL^{-1}	0.5 mgL^{-1}
T_{15}:MS+$B_{4+}N_3$	1.5 mgL^{-1}	0.75 mgL^{-1}
T_{16}:MS+$B_{4+}N_4$	1.5 mgL^{-1}	1 mgL^{-1}
T_{17}:MS+$B_{5+}N_1$	2 mgL^{-1}	0 mgL^{-1}
T_{18}:MS+$B_{5+}N_2$	2 mgL^{-1}	0.5 mgL^{-1}
T_{19}:MS+$B_{5+}N_3$	2 mgL^{-1}	0.75 mgL^{-1}
T_{20}:MS+$B_{5+}N_4$	2 mgL^{-1}	1 mgL^{-1}

Plate 1. General view of culture room.

3.3.4 Preparation of explants

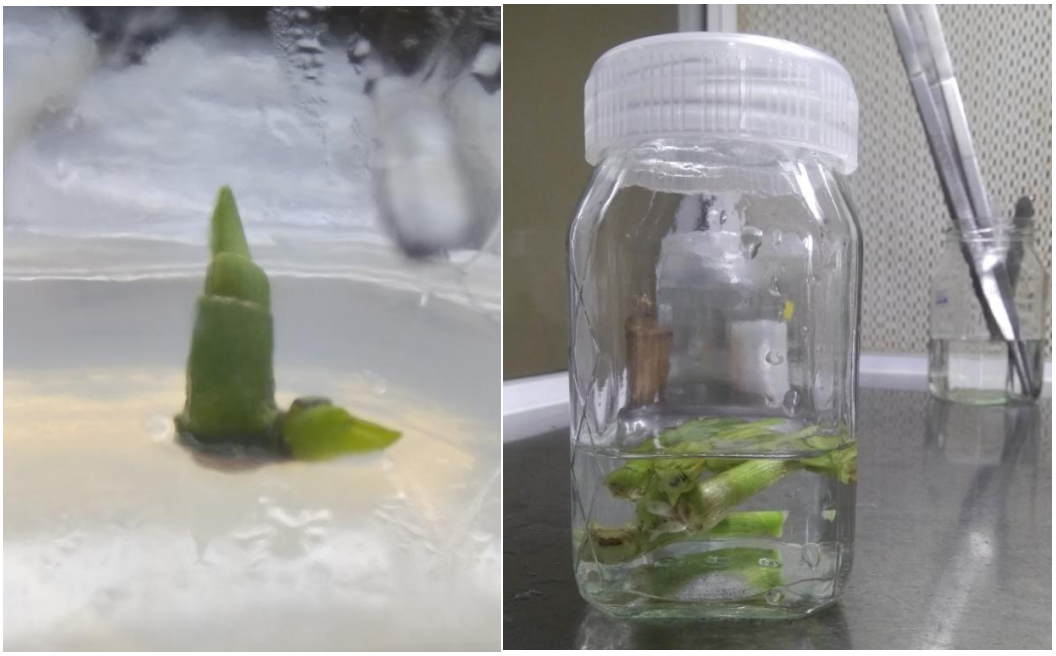
Disease and pest free healthy shoots (2-3 cm long) of *Dendrobium* cv. Sonia Earsakul were collected from the mother plants growing in green house of SASRD instructiona farm. The collected shoots were washed in running tap water and then washed with diluted Tween 20 solution, thereafter the traces of Tween 20 were removed by washing repeatedly for 3 to 4 times in tap water. The washed shoot tips were dipped in 1% carbendazim solution for 30 minutes with constant shaking in electric shaker and then washed with sterilized double distilled water for 5 minutes and taken to laminar airflow cabinet in inoculation room. The shoot tips were first sterilized with absolute alcohol (ethanol) with constant shaking for 1 minute, following washing with sterilized double distilled water 2-3 times. Thereafter, the shoot tips were again sterilized in freshly prepared 0.1% $HgCL_2$ for 5 minute with constant shaking. Finally the sterilized shoot tips were washed 3-4 times for 30 minutes with sterilized double distilled water and then dried in sterilized filter paper in the laminar air flow cabinet and ready for excision.

3.3.5 Excision and inoculation of explants

The floor and walls of the laminar air flow were wiped with 70% isopropyl alcohol, then the inoculation room and laminar air flow cabinet was exposed to UV light for 1 hour prior to excision and inoculation work. With the existing aseptic condition under the laminar air flow cabinet the sterilized shoot tips were placed on a sterile petridish. Holding steady with forceps, the superficial tissue, the unsheathing cone of leaf primordial were removed carefully with the help of scalpel through circular incision of each primordium, thereby loosening it from the basal portion. Holding the shoot with forceps the loosened outer leaves were removed.

The shoot tip with its typical conical morphology was reduced to about 0.5 to 1 cm height. The final excised shoot tips were transferred to cultures bottles containing various modified MS medium with the help of forceps and scalpel under laminar air flow. The excised shoot tips were transferred to culture bottles containing various modified MS medium with the help of forceps and scalpel under laminar air flow. The excised shoot tips were placed upright and inserted a little in the medium to ensure proper contact. The neck of the culture bottle and lid were flamed after the transfer of excised shoot tip. Thereafter, it WAS covered properly with lid and sealed tightly with plastic films, labelled and then transferred to culture room.

(A) **(B)**



(C) **(D)**

Plate 2. (A) Selected explants in mother plant. (B) Sterilized shoot tip ready for excision. (C) Excised shoot tip cultured in modified MS Media. (D) Sterilizing shoot tip.

3.3.6 Incubation and maintenance of cultures

The culture bottles were maintained at 25±2°C temperature and 50-70% RH, under alternate 12 hours light (intensity of 1000 lux) and dark cycle. Further light intensity and duration was enhanced up to 16 hours (3000 lux), during subsequent subculture. Cultures were observed daily. Days required for greening of explants, swelling of the base, and other parameters were recorded. After few days of inoculation the cut surface of the shoot tips turned black which hinder the uptake of the nutrient by the shoot tips, therefore the blackened tissues were cut and removed and the culture were transferred to new media.

3.4 Observations to be recorded

3.4.1 Days to callus initiation

The number of days taken to show initial callus formation from the days of inoculation of shoot tip was recorded and the mean number of days was taken for the analysis of data.

3.4.2 Percentage of response

The number of explants that initiate callus from the total number of explants used were recorded and converted to percent.

3.4.2 Growth rate of callus

The growth rate of callus were calculated by measuring the weight of callus after six (6) weeks of culturing the callus subtracting the initial weight of callus with the help of weighing balance and the value were expressed in gram (g).

3.4.3 Days to greening

The number of days taken for greening of inoculated explants from the days of inoculation of shoot tip was recorded and the mean numbers of days was taken for the analysis of data.

3.4.4 Days to initiation of shoot

The number of days taken to show initial differentiation of shoot from the date of inoculation of explants in different treatment was recorded and the mean days was taken for analysis of data.

3.4.5 Number of multiple shoots bud

Number of multiple shoot bud was counted and the mean number of shoots was taken for further analysis of data.

3.4.6 Number of multiple buds at different sub-culturing

The number of multiple shoot buds produced during first and second sub culturing were counted and the mean number of shoot were taken for analysis.

3.4.7 Length of shoot buds (cm)

The length of shoot bud was measured from the base to the tip of the plantlets with the help of measuring scale and the average length of the shoot was expressed in centimetre (cm).

3.4.8 Number of leaves per plantlet

The numbers of leaves per plantlets were counted and the mean numbers of leaves were taken.

3.4.9 Fresh weight of shoot (g)

Fresh weight of shoots were measured with the help of electronic weighing balance at 90 days of culture and during hardening stage and the fresh weight of shoot were expressed in gram (g).

3.5 Statistical analysis of data

The experiment design was factorial Completely Randomized Design (CRD) and the data collected on the effect of different treatments during the period of investigation were tested by analysis of variance; difference among the treatment means were tested by Duncan's Multiple Range Test (Duncan, 1955).

3.5 Experiment 2. Study on *in vitro* root regeneration of *Dendrobium* cv. Sonia Earsakul

3.5.1 Technical details:

The best treatments among the regeneration of shoot were used for *in vitro* root regeneration experiment. The propagules multiplied during various sub-culturing were very small and not yet capable of surviving in the external potting media. These shoots were therefore transferred to a media containing various root regeneration media. Different parameters like days to root initiation, number of root and shoot, number of functional root and length of root were recorded.

Experimental Design : Completely Randomized Design (CRD)

Replication : 3 (Three)

Number of treatment : 13 (Thirteen)

Table 3.8 Composition of MS media with different hormone concentration tested for *in vitro* root regeneration of *Dendrobium* cv. Sonia Earsakul**.**

Treatment	**Media Concentration (mgL^{-1})**
T_1	MS
T_2	MS + 0.5 mgL^{-1} IBA
T_3	MS + 1.0 mgL^{-1} IBA
T_4	MS + 1.5 mgL^{-1} IBA
T_5	MS + 2.0 mgL^{-1} IBA
T_6	MS + 0.5 mgL^{-1} NAA
T_7	MS + 1.0 mgL^{-1} NAA
T_8	MS + 1.5 mgL^{-1} NAA
T_9	MS + 2.0 mgL^{-1} NAA
T_{10}	MS + 0.5 mgL^{-1} IAA
T_{11}	MS + 1.0 mgL^{-1} IAA
T_{12}	MS + 1.5 mgL^{-1} IAA
T_{13}	MS + 2.0 mgL^{-1} IAA

3.5.3. Plantlets hardening and transfer of the *Dendrobium* cv. Sonia Earsakul to potting media

The well developed rooted shoots after 12 to 15 week of culturing in the rooting media were at first exposed to natural environment by opening the cultured bottle cap for 3 days prior to removal from the culture bottle in the culture room. The plantlet were carefully removed from the culture bottles and washed carefully in tap water to wash off the traces of media in the plantlet to prevent the contamination in the plantlet during hardening. Media for hardening are prepared by mixing soaked sterilized coco peat and agricultural perlite at the ratio of 4:1 and then filled it up in the disposable cup. The plantlets were then planted carefully in the prepared hardening media. The planted plantlets were kept in the room for 3 days and then transferred to green house for easy adapting to natural environment.

3.6 Observation to be recorded

3.6.1 Days to root initiation

The days taken to root initiation from the date of inoculation of developed shoot to rooting media was recorded.

3.6.2 Number of shoot and root per shoot

The number of developed shoots from a culture bottle and the number of root per shoot during the stage hardening stage of plantlets were recorded.

3.6.3 Number of functional roots

The numbers of functional roots were counted during the hardening stage of the plantlets when the plantlets were removed from the culture bottles for hardening.

3.6.4 Length of roots and shoots during the hardening stage of plantlets

The length of the shoots and root were measured with the help of linear scale, and the lengths are expressed in centimeter (cm).

3.6.5 Percentage of survivability during hardening

Percentage of survivability during hardening was calculated by dividing the number of surviving plantlets by total number of plantlets hardened multiply by 100.

$$\text{Percentage of survivability} = \frac{\text{Total Number of surviving plantlets}}{\text{Total number of plantlets hardened}} \times 100$$

3.7 Statistical analysis of data

The experiment design was completely Randomized Design (CRD) and the data collected on the effect of different treatment during the period of investigation were tested by Analysis of variance; difference among the treatment means were tested by Duncan's Multiple Range Test, (Duncan, 1955).

CHAPTER IV

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Dendrobium cv. Sonia Earsakul is an important flower crop having predominant position in both domestic and international market. Improvement in the production technology like quality planting material is an important consideration to make this industry a remunerative enterprise. Plant growth regulator has a vital role in *in-vitro* multiplication.

The findings thus obtained in the present investigation are discussed in this chapter with the support of available literature and reasoning.

4.1 Experiment I: Study on *in vitro* shoot multiplication of *Dendrobium* cv. Sonia Earsakul

4.1.1 Days to callus formation

Table 4.1 and Fig.3.1 and 3.2 depicted the results in the influence of graded level of BAP, NAA and their interaction on days to callus formation. During the year 2016-17, application of BAP in varied doses significantly reduced the days taken to callus formation. The minimum days (45.36) taken to callus formation was recorded in MS media supplement with BAP @ 2 mgL^{-1} BAP followed by 46.92 days in BAP @ 1.5 mgL^{-1} and the maximum (52.76 days) number of days was recorded in control followed by 50.05 days in BAP @ 0.5 mgL^{-1}. Similar results were obtained during the year 2017-18 with the least days (46.02) to callus formation which was recorded in media containing BAP @ 2 mgL^{-1} followed by (48.40 days) BAP @ 1.5 mgL^{-1} and the maximum number of days (52.11) was recorded in control. The pooled analysis of two year data showed that application of BAP significantly reduced the days to callus formation, the minimum days (45.69) taken to callus formation was recorded in treatment BAP @ 2 mgL^{-1} followed by treatment containing BAP @ 1.5 mgL^{-1} (47.66 days) and the longest period taken to callus formation (52.44 days) was recorded in control. In the present investigation, BAP @ 2

mgL^{-1} resulted in early callus formation which is similar with the findings of Chookoh *et al*., 2019. The balanced concentration of hormone helped in the early growth and development resulting in the earlier formation of callus as compared to other hormonal concentration.

MS media supplemented with NAA exhibited significant difference on the days taken to callus formation. In both the years, 2016-17 and 2017-18, the minimum days taken to callus formation was recorded in the MS media supplemented with NAA @ 0.5 mgL^{-1} (46.86 days and 47.81 days respectively) followed by 0.75 NAA @ mgL^{-1} (N_3) (48.31 days and 48.64 days respectively). The longest days (50.31 and 50.31 days) taken to callus formation was recorded in control in both the years. The pooled data revealed that the treatment containing NAA significantly reduced the days to callus formation, the least days (47.33 days) taken to callus formation were recorded in NAA @ 0.5 mgL^{-1}, followed by (48.48 days) NAA @ 0.75 mgL^{-1}, the longest days (50.31 days) taken to callus formation was recorded in control. NAA at lower concentration i.e. 0.5 mgL^{-1} in MS media resulted in the earlier days taken to callus formation, however with the increase in the concentration the days taken to callus formation increased. This might be due to the toxic effect of NAA at higher concentration which hinders the growth and development. Similar results were reported by Chookoh *et al*. (2019) in *Tolumnia* orchids and Tao *et al*. (2011) in *Cymbidium feberi* Rolfe.

The data presented in Table 4.2 revealed that interaction between various levels of BAP and NAA showed significant effect on the days to callus formation, the pooled data in the table showed that the least days (42.42) taken to callus formation was recorded in the treatment combination of BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1} which is statistically at par with treatment combination of BAP @ 2 mgL^{-1} + NAA @ 0.75 mgL^{-1} (44.52 days), the longest days (53.64) taken to callus formation was recorded in BAP @ 0 mgL^{-1} BAP +NAA @ 0 mgL^{-1}. BAP in combination with low concentration of NAA

resulted in earlier days required for callus formation. Similar results were reported by Chookoh *et al*. (2019), BAP or NAA alone resulted in longer days required for callus formation indicating the synergistic effect of BAP and NAA in days required for callus formation. Supplementation of cytokinin and auxin in optimum dose is basic requirement for growth and development.

Table 4.1: Effect of 6-BAP and NAA levels on days to callus formation in micropropagation of *Dendrobium* cv. Sonia Earsakul

Treatments	Days to callus formation		
	First year	*Second year*	*Pooled*
B_1 (0 mgL^{-1} BAP)	52.76^{a}	52.11^{a}	52.44^{a}
B_2 (0.5 mgL^{-1} BAP)	50.05^{b}	50.69^{b}	50.37^{b}
B_3 (1 mgL^{-1} BAP)	48.02^{c}	48.25^{c}	48.13^{c}
(B_4) (1.5 mgL^{-1} BAP)	46.92^{d}	48.40^{c}	47.66^{c}
B_5 (2 mgL^{-1} BAP)	45.36^{e}	46.02^{d}	45.69^{d}
Sem±	0.38	0.32	0.25
CD at 5%	1.08	0.92	0.70
N_1 (0 mgL^{-1} NAA)	50.31^{a}	50.31^{a}	50.31^{a}
N_2 (0.5 mgL^{-1} NAA)	46.86^{c}	47.81^{b}	47.33^{d}
N_3 (0.75 mgL^{-1} NAA)	48.31^{b}	48.64^{b}	48.48^{c}
N_4 (1 mgL^{-1} NAA)	49.01^{b}	49.62^{a}	49.31^{b}
SEm±	0.34	0.29	0.22
CD at 5%	0.97	0.82	0.63

Note: Mean values in a column having common letter subscribe are statistically identical and those having different letters are statistically different.

Table 4.2: Interaction effect of 6-BAP and NAA levels on days to callus formation in micropropagation of *Dendrobium* cv. Sonia Earsakul

Treatments	*First year*	*Second year*	*Pooled*
B_1N_1 (BAP 0 mgL^{-1} + NAA 0 mgL^{-1})	53.67^{a}	53.61^{a}	53.64^{a}
B_1N_2 (BAP 0 mgL^{-1} + NAA 0.5 mgL^{-1})	52.12^{ab}	50.83^{bcd}	51.48^{bc}
B_1N_3(BAP 0 mgL^{-1} + NAA 0.75 mgL^{-1})	52.26^{ab}	51.17^{bc}	51.72^{b}
B_1N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	52.99^{a}	52.83^{ab}	52.91^{ab}
B_2N_1(BAP 0.5 mgL^{-1} + NAA 0 mgL^{-1})	51.55^{abc}	51.30^{bc}	51.43^{bc}
B_2N_2(BAP 0.5 mgL^{-1} + NAA 0.5 mgL^{-1})	48.94^{def}	50.12^{cde}	49.53^{def}
B_2N_3(BAP 0.5 mgL^{-1} + NAA 0.75 mgL^{-1})	49.70^{bcde}	50.20^{cde}	49.95^{cde}
B_2N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	50.00^{bcd}	51.14^{c}	50.57^{bcd}
B_3N_1(BAP 1 mgL^{-1} + NAA 0 mgL^{-1})	49.26^{bcde}	49.60^{cdef}	49.43^{de}
B_3N_2(BAP 1 mgL^{-1} + NAA 0.5 mgL^{-1})	46.18^{fgh}	46.44^{gh}	46.31^{h}
B_3N_3(BAP 1 mgL^{-1} + NAA 0.75 mgL^{-1})	48.23^{def}	48.68^{def}	48.46^{efg}
B_3N_4(BAP 1 mgL^{-1} + NAA 1 mgL^{-1})	48.41^{def}	48.26^{efg}	48.34^{fg}
B_4N_1(BAP 1.5 mgL^{-1} + NAA 0 mgL^{-1})	48.98^{de}	48.85^{ef}	48.92^{efg}
B_4N_2(BAP 1.5 mgL^{-1} + NAA 0.5 mgL^{-1})	45.67^{gh}	48.21^{efg}	46.94^{gh}
B_4N_3(BAP 1.5 mgL^{-1} + NAA 0.75 mgL^{-1})	47.00^{efg}	48.48^{ef}	47.74^{gh}
B_4N_4(BAP 1.5 mgL^{-1} + NAA 1 mgL^{-1})	46.03^{fgh}	48.07^{ef}	47.05^{gh}
B_5N_1(BAP 2 mgL^{-1} + NAA 0 mgL^{-1})	48.08^{defg}	48.20^{ef}	48.14^{fg}
B_5N_2(BAP 2 mgL^{-1} + NAA 0.5 mgL^{-1})	41.40^{i}	43.43^{i}	42.42^{j}
B_5N_3(BAP 2 mgL^{-1} + NAA 0.75 mgL^{-1})	44.38^{h}	44.67^{h}	44.52^{i}
B_5N_4(BAP 2 mgL^{-1} + NAA 1mgL^{-1})	47.59^{de}	47.79^{fg}	47.69^{g}
SEm±	0.76	0.64	0.50
CD at 5%	2.17	1.84	1.40

Note: Mean values in a column having common letter subscribe are statistically identical and those having different letters are statistically different.

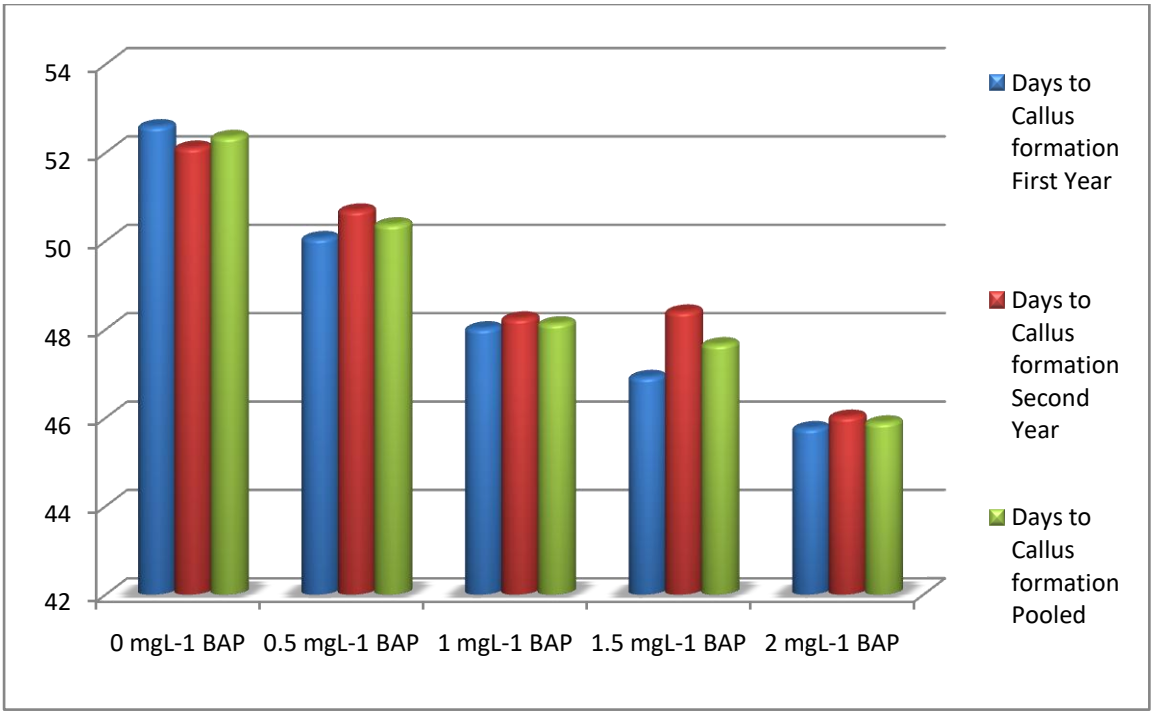


Fig. 4.1: Effect of 6-BAP levels on the number of days taken to callus formation

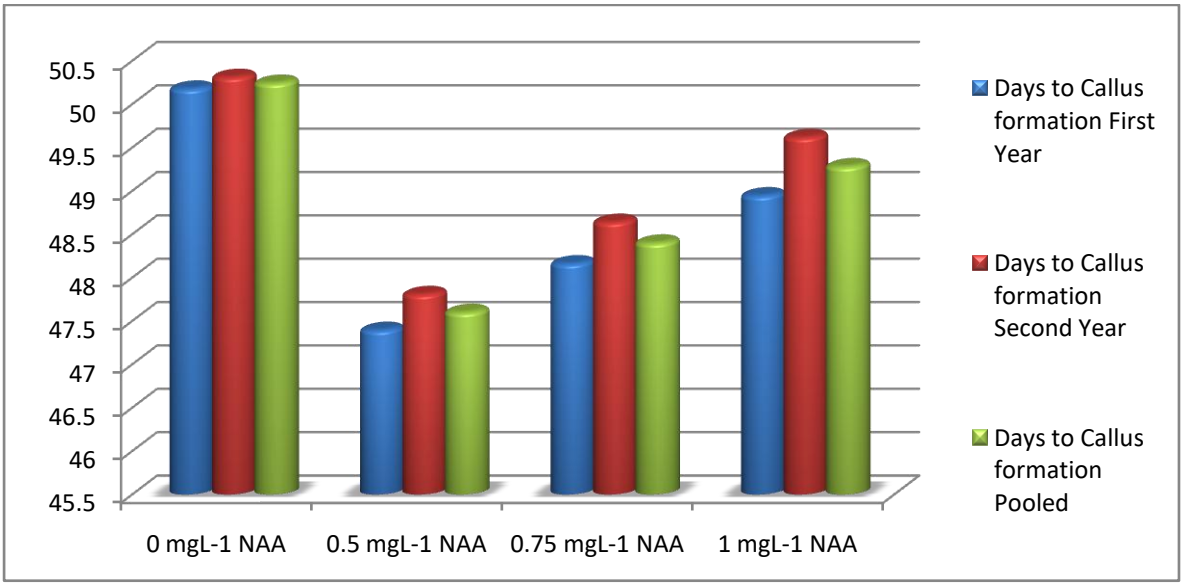


Fig. 4.2: Influence of NAA levels on the number of days taken to callus formation

(A) **(B)**

(C) **(D)**

Plate 3.(A) Initial weight of callus. (B) weight of callus after 6 weeks of culturing. (C) Culturing of Callus and PLBs for shoot induction. (D) Sub culturing of developed shoot.

4.1.2 Percentage of response

Data presented in table 4.3 revealed the influence of BAP and NAA in percentage of response. During the year 2016-17, application of BAP @ 2 mgL^{-1} resulted in the highest percentage of response (51.48%) followed by BAP @1.5 mgL^{-1} (50.12%) and the least percentage (41.95%) was recorded in BAP @ 0 mgL^{-1}. Even in the subsequent year 2017-18, BAP @ 2 mgL^{-1} resulted in highest percentage of response (51.62%) which is at par with BAP @ 1.5 mgL^{-1} (50.67%). The minimum percentage of response was recorded in BAP @ 0 mgL^{-1}. The pooled data depicted that supplementation of MS media with BAP @ 2 mgL^{-1} resulted in highest percentage of response (51.62%), which is followed by BAP @ 1.5 mgL^{-1} (50.67%) and the least percentage of response (42.14%) was recorded in BAP @ 0 mgL^{-1}. The current experimental findings are in conformity with the findings of Rattana and Sangchanjiradet (2017) in *Dendrobium signatum* Rchb.f., and Thokchom and Maitra (2017) on *Anthurium andreanum* cv. Jewel where highest regeneration percent (98%) was recorded and in line with Kosir *et al*. (2004) on *Phalaenopsis* orchids.

NAA application resulted in significant effect on the percentage of response, application of NAA @ 0.5 mgL^{-1} resulted in the highest percentage (50.48%) of response followed by NAA @ 0.75 mgL^{-1} (48.36%) and the least percentage of response was recorded in NAA @ 0 mgL^{-1} during the year 2016-17. During the year 2017-18, addition of NAA @ 0.5 mgL^{-1} resulted in the highest percentage (51.55%) of response which was followed by NAA @ 0.75 mgL^{-1} (48.58%) and the least percentage of response (44.22%) was recorded in NAA @ 0 mgL^{-1}. The Pooled data revealed that application of NAA @ 0.5 mgL^{-1} significantly resulted in highest percentage of response (51.19%) which is followed by NAA @ 0.75 mgL^{-1} (48.47%) and the least percentage of response was recorded in NAA @ 0 mgL^{-1}. NAA @ 0.5 mgL^{-1} resulted in highest percentage of response, above this concentration resulted in the lesser percentage which might be due to requirement of this hormone in very low

concentration. The current findings are in accordance with the report of Rattana and Sangchanjiradet (2017) in *Dendrobium signatum* Rchb.f, Thokchom and Maitra (2017) on *Anthurium andreanum* cv. Jewel and also in line with the finding of Kosir *et al*. (2004) on *Phalaenopsis* orchids.

The data presented Table 4.4 depicted the interaction effect of BAP and NAA on the percentage of response. During the year 2016-17, the highest percentage of response (55.50%) were recorded in the treatment combination of BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1}, which is followed by (53.67%) treatment combination of BAP @ 1.5 mgL^{-1} + NAA @ 0.5 mgL^{-1} and the least percentage of response (37.20%) was recorded in control. During the year 2017-18, the highest percentage of response (56.18%) was recorded in treatment combination of BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1} and the least percentage of response was recorded in control. The pooled data revealed that the treatment combination of BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1} resulted in the highest percentage (55.84%) of response and the least percentage of response (37.62%) was recorded in control. Optimum doses of cytokinin and auxin resulted in better growth and development of the cultured tissue. Similar results were reported by Rattana and Sangchanjiradet (2017) in *Dendrobium signatum* Rchb.f. where they recorded highest percentage of seed germination in media supplemented with BAP @ 2mgL^{-1} + NAA @ 0.5 mgL^{-1}, while Thokchom and Maitra (2017) studied on *Anthurium andreanum* cv. Jewel where regeneration percent (98%) was recorded in BAP @ 2mgL^{-1} + NAA @ 0.5 mgL^{-1} and in line with Kosir *et al*. (2004) on *Phalaenopsis* orchids.

Table 4.3: Influence of 6-BAP and NAA on percentage of response on *in vitro* multiplication*f Dendrobium* cv. Sonia Earsakul

Treatments	Percentage of response		
	First year	*Second year*	*Pooled*
B_1 (0 mgL^{-1} BAP)	41.95^{e}	42.33^{d}	42.14^{e}
B_2 (0.5 mgL^{-1} BAP)	45.07^{d}	45.64^{c}	45.35^{d}
B_3 (1 mgL^{-1} BAP)	47.45^{c}	48.33^{b}	47.89^{c}
(B_4) (1.5 mgL^{-1} BAP)	50.12^{b}	51.22^{a}	50.67^{b}
B_5 (2 mgL^{-1} BAP)	51.48^{a}	51.76^{a}	51.62^{a}
Sem±	0.24	0.22	0.16
CD at 5%	0.67	0.62	0.45
N_1 (0 mgL^{-1} NAA)	43.27^{d}	44.22^{d}	43.75^{d}
N_2 (0.5 mgL^{-1} NAA)	50.84^{a}	51.55^{a}	51.19^{a}
N_3 (0.75 mgL^{-1} NAA)	48.36^{b}	48.58^{b}	48.47^{b}
N_4 (1 mgL^{-1} NAA)	46.39^{c}	47.07^{c}	46.73^{c}
SEm±	0.21	0.20	0.14
CD at 5%	0.60	0.56	0.40

Note: Mean values in a column having common letter subscribe are statistically identical and those having different letters are statistically different

Table 4.4: Interaction effect of 6-BAP and NAA levels on percentage of response on i*n vitro* multiplication of *Dendrobium* cv. Sonia Earsakul

Treatments	*First year*	*Second year*	*Pooled*
B_1N_1 (BAP 0 mgL^{-1} + NAA 0 mgL^{-1})	37.20^{j}	38.03n	37.62^{l}
B_1N_2 (BAP 0 mgL^{-1} + NAA 0.5 mgL^{-1})	45.74^{g}	45.77^{jk}	45.75^{g}
B_1N_3(BAP 0 mgL^{-1} + NAA 0.75 mgL^{-1})	43.28^{h}	43.50^{l}	43.39^{i}
B_1N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	41.57^{i}	42.03^{m}	41.80^{j}
B_2N_1(BAP 0.5 mgL^{-1} + NAA 0 mgL^{-1})	40.51^{i}	41.20^{m}	40.85^{k}
B_2N_2(BAP 0.5 mgL^{-1} + NAA 0.5 mgL^{-1})	48.71^{f}	49.61^{efg}	49.16^{cd}
B_2N_3(BAP 0.5 mgL^{-1} + NAA 0.75 mgL^{-1})	46.70^{g}	46.67^{ij}	46.68^{f}
B_2N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	44.37^{h}	45.07^{k}	44.72^{h}
B_3N_1(BAP 1 mgL^{-1} + NAA 0 mgL^{-1})	43.47^{h}	43.63^{l}	43.55^{i}
B_3N_2(BAP 1 mgL^{-1} + NAA 0.5 mgL^{-1})	50.57^{de}	52.12^{cd}	51.34^{b}
B_3N_3(BAP 1 mgL^{-1} + NAA 0.75 mgL^{-1})	48.67^{f}	49.20^{f}	48.94^{d}
B_3N_4(BAP 1 mgL^{-1} + NAA 1 mgL^{-1})	47.10^{g}	48.35^{gh}	47.73^{e}
B_4N_1(BAP 1.5 mgL^{-1} + NAA 0 mgL^{-1})	46.04^{g}	47.72^{hi}	46.88^{ef}
B_4N_2(BAP 1.5 mgL^{-1} + NAA 0.5 mgL^{-1})	53.67^{b}	54.07^{b}	53.87^{b}
B_4N_3(BAP 1.5 mgL^{-1} + NAA 0.75 mgL^{-1})	51.00^{cd}	52.60^{c}	51.80^{b}
B_4N_4(BAP 1.5 mgL^{-1} + NAA 1 mgL^{-1})	49.77^{def}	50.51^{ef}	50.14^{c}
B_5N_1(BAP 2 mgL^{-1} + NAA 0 mgL^{-1})	49.13^{ef}	50.53^{ef}	49.83^{cd}
B_5N_2(BAP 2 mgL^{-1} + NAA 0.5 mgL^{-1})	55.50^{a}	56.18^{a}	55.84^{a}
B_5N_3(BAP 2 mgL^{-1} + NAA 0.75 mgL^{-1})	52.14^{c}	50.92^{de}	51.53^{c}
B_5N_4(BAP 2 mgL^{-1} + NAA 1mgL^{-1})	49.13^{ef}	49.41^{fg}	49.27^{cd}
SEm±	0.47	0.44	0.32
CD at 5%	1.35	1.25	0.90

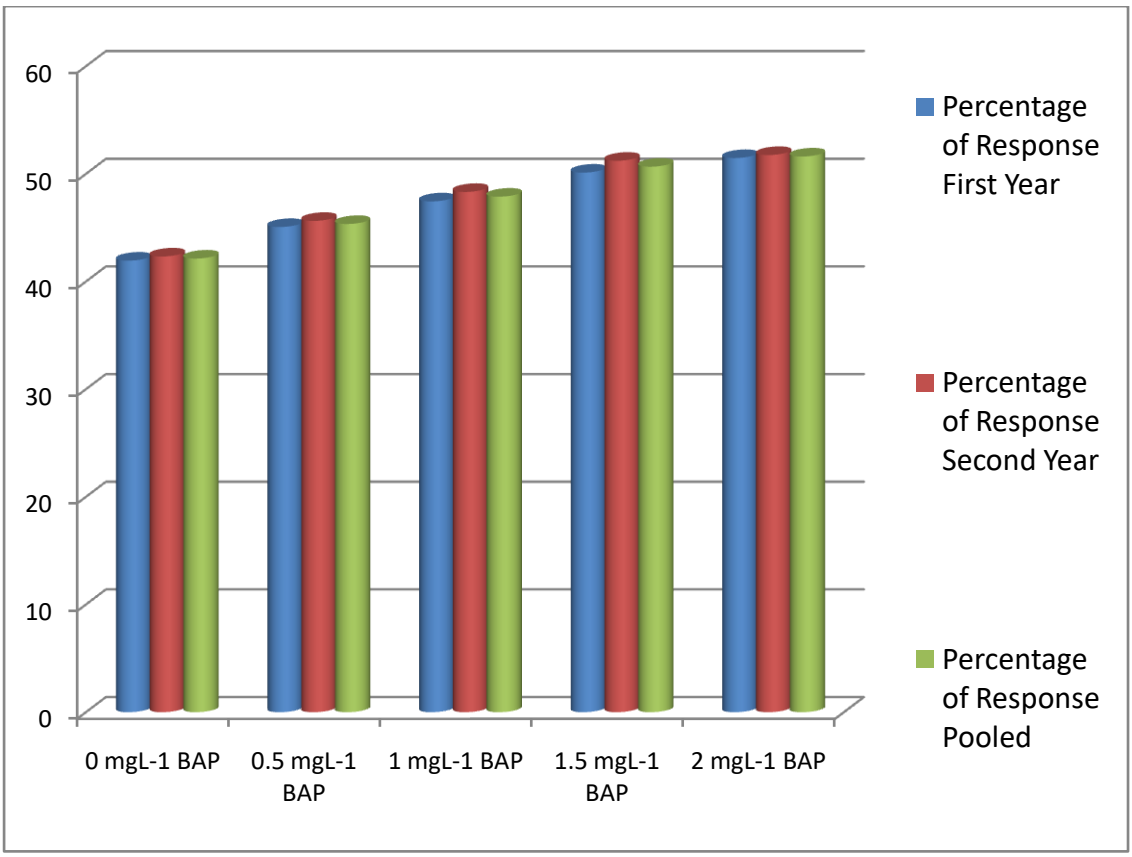


Fig. 4.3: Effect 6-BAP levels on the percentage of response

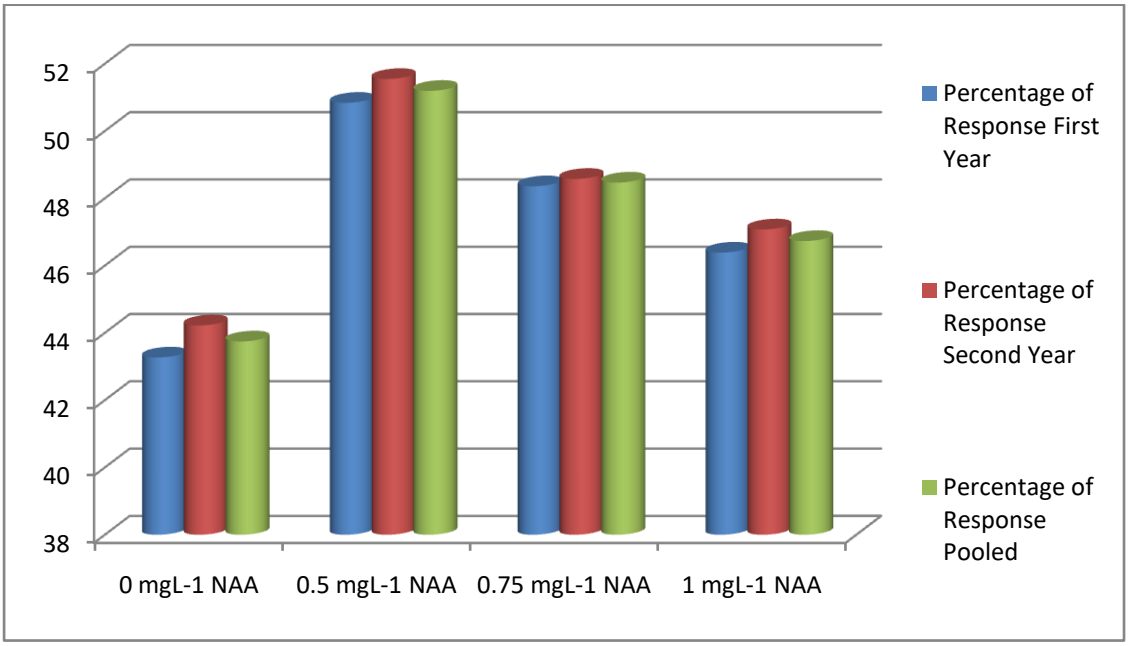


Fig. 4.4: Influence of NAA levels on the percentage of response

4.2.3 Gain in weight of callus

Table 4.5, Fig. 4.5 and Fig. 4.6 depict the results of the influence of various levels of BAP and NAA on gain in weight of callus. During the year 2016-17, augmentation of BAP in MS media resulted in significant difference in the growth rate of callus. The highest gain in weight of callus after 6 weeks of subculture (12.98 g) was recorded in treatment BAP @ 2 mgL^{-1} which is at par with BAP @ 1.5 mgL^{-1} (12.77 g) followed by BAP @ 1 mgL^{-1}(11.46 g) and the least gain in weight of callus was recorded in control (8.97 g). Similar results were obtained in the subsequent year 2017-18, the highest gain in weight of callus (13.13 g) was recorded in the treatment BAP @ 2 mgL^{-1} which is at par with BAP @ 1.5 mgL^{-1} (12.70 g) followed by BAP @ 1 mgL^{-1} (11.54 g) and the gain in weight of callus (9.07 g) after 6 weeks of sub culturing were recorded in control. Further analysis of pooled data revealed that the highest gain in weight of callus (13.05 g) were recorded in treatment BAP @ 2 mgL^{-1} which is at par with BAP @ 1.5 mgL^{-1} (12.74 g) followed by BAP @ 1 mgL^{-1} (11.54 g) and the least gain in weight of callus were recorded in control (9.07 g). It is evident from the present study that BAP in various level shave differential positive effect on gain in weight of callus on *in vitro* micropropagation. Among the various levels BAP @ 2 mgL^{-1} resulted in the better gain in weight of callus which might be due to availability of optimum hormone required for cell division. These experimental findings are in confirmation with the finding of Chookoh *et al*. (2019), Tao *et al*. (2011) *Cymbidium feberi* Rolfe and similar with the finding of Chookoh *et al*. (2019) in *Tolumnia* orchid.

NAA augmentation in MS media resulted in significance difference on gain in weight of callus. During the year 2016-17, the highest gain in weight of callus (11.90 g) was recorded in treatment NAA @ 0.5 mgL^{-1} followed by NAA @ 0.75 mgL^{-1} (11.46 g) which is at par with NAA @ 1 mgL^{-1} (11.13 g) and the least gain in weight of callus (10.63 g) was recorded in control. During

the year 2017-18, analysis of pooled data revealed that the best gain in weight of callus (11.97 g) were recorded in treatment NAA @ 0.5 mgL^{-1} followed by NAA @ 0.75 mgL^{-1} (11.47 g) which is at par with NAA @ 1 mgL^{-1} (11.38 g) and the least gain in weight of callus (10.75 g) were recorded in control. The current experimental finding revealed that the increase in the gain in weight of callus after six weeks of culturing was influenced by concentration of NAA in the media. Among the different concentration of NAA i.e 0.5 mgL^{-1} results in best growth of callus, with the increase in concentration there is adverse effect on the callus growth. Similar result was recorded by Chookoh *et al*. (2019) in *Tolumnia* orchid.

The data presented in Table 4.6 depicted the significant interaction effect of BAP and NAA interaction on the gain in weight of callus. The pooled data analysis revealed that the best gain in weight of callus (13.66 g) was recorded in treatment combination of BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1} followed by treatment combination of BAP @ 1.5 $mgL^{-1}$and NAA @ 0.5 mgL^{-1} (13.37 g) which was at par with BAP @ 1.5 mgL^{-1} +NAA @ 0.75 mgL^{-1} (13.13 g) and the least gain in weight of callus were recorded in control. A balance application of BAP and NAA result in better growth of callus comparing to singly application. In addition to acting directly, many hormones can interact with each other to control the development of these symbioses and these complex networks help in better uptake of nutrient from the media resulting in the better growth of callus. The current experimental findings are in line with the finding of Chookoh *et al*. (2019) in *Tolumnia* orchid.

Table 4.5: Influence of 6-BAP and NAA levels on gain in weight of callus in *in vitro* multiplication *Dendrobium* cv. Sonia Earsakul

Treatment	*First year*	*Second year*	*Pooled*
B_1 (0 mgL^{-1} BAP)	8.97^{d}	9.16^{d}	9.07^{d}
B_2 (0.5 mgL^{-1} BAP)	10.22^{c}	10.93^{c}	10.57^{c}
B_3 (1 mgL^{-1} BAP)	11.46^{b}	11.62^{b}	11.54^{b}
(B_4) (1.5 mgL^{-1} BAP)	12.77^{a}	12.70^{a}	12.74^{a}
B_5 (2 mgL^{-1} BAP)	12.98^{a}	13.13^{a}	13.05^{a}
Sem±	0.16	0.19	0.12
CD at 5%	0.45	0.54	0.34
N_1 (0 mgL^{-1} NAA)	10.63^{c}	10.87^{c}	10.75^{c}
N_2 (0.5 mgL^{-1} NAA)	11.90^{a}	12.05^{a}	11.97^{a}
N_3 (0.75 mgL^{-1} NAA)	11.46^{b}	11.49^{b}	11.47^{b}
N_4 (1 mgL^{-1} NAA)	11.13^{b}	11.63^{ab}	11.38^{b}
SEm±	0.14	0.17	0.11
CD at 5%	0.40	0.48	0.31

Table. 4.6: Interaction effect of 6-BAP and NAA levels on gain in weight of callus in *in vitro* multiplication of *Dendrobium* cv. Sonia Earsakul.

Treatments	*First year*	*Second year*	*Pooled*
B_1N_1 (BAP 0 mgL^{-1} + NAA 0 mgL^{-1})	8.43	9.00	8.72
B_1N_2 (BAP 0 mgL^{-1} + NAA 0.5 mgL^{-1})	9.49	9.41	9.45
B_1N_3(BAP 0 mgL^{-1} + NAA 0.75 mgL^{-1})	9.10	9.12	9.11
B_1N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	8.87	9.11	8.99
B_2N_1(BAP 0.5 mgL^{-1} + NAA 0 mgL^{-1})	9.49	9.85	9.67
B_2N_2(BAP 0.5 mgL^{-1} + NAA 0.5 mgL^{-1})	10.67	11.80	11.23
B_2N_3(BAP 0.5 mgL^{-1} + NAA 0.75 mgL^{-1})	10.40	10.93	10.66
B_2N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	10.32	11.13	10.72
B_3N_1(BAP 1 mgL^{-1} + NAA 0 mgL^{-1})	10.90	10.88	10.89
B_3N_2(BAP 1 mgL^{-1} + NAA 0.5 mgL^{-1})	12.11	12.20	12.16
B_3N_3(BAP 1 mgL^{-1} + NAA 0.75 mgL^{-1})	11.70	11.39	11.54
B_3N_4(BAP 1 mgL^{-1} + NAA 1 mgL^{-1})	11.11	12.01	11.56
B_4N_1(BAP 1.5 mgL^{-1} + NAA 0 mgL^{-1})	11.27	11.87	11.57
B_4N_2(BAP 1.5 mgL^{-1} + NAA 0.5 mgL^{-1})	13.46	13.27	13.37
B_4N_3(BAP 1.5 mgL^{-1} + NAA 0.75 mgL^{-1})	13.32	12.94	13.13
B_4N_4(BAP 1.5 mgL^{-1} + NAA 1 mgL^{-1})	13.03	12.73	12.88
B_5N_1(BAP 2 mgL^{-1} + NAA 0 mgL^{-1})	13.06	12.75	12.90
B_5N_2(BAP 2 mgL^{-1} + NAA 0.5 mgL^{-1})	13.77	13.55	13.66
B_5N_3(BAP 2 mgL^{-1} + NAA 0.75 mgL^{-1})	12.76	13.06	12.91
B_5N_4(BAP 2 mgL^{-1} + NAA 1mgL^{-1})	12.33	13.16	12.75
SEm±	8.43	9.00	8.72
CD at 5%	9.49	9.41	9.45

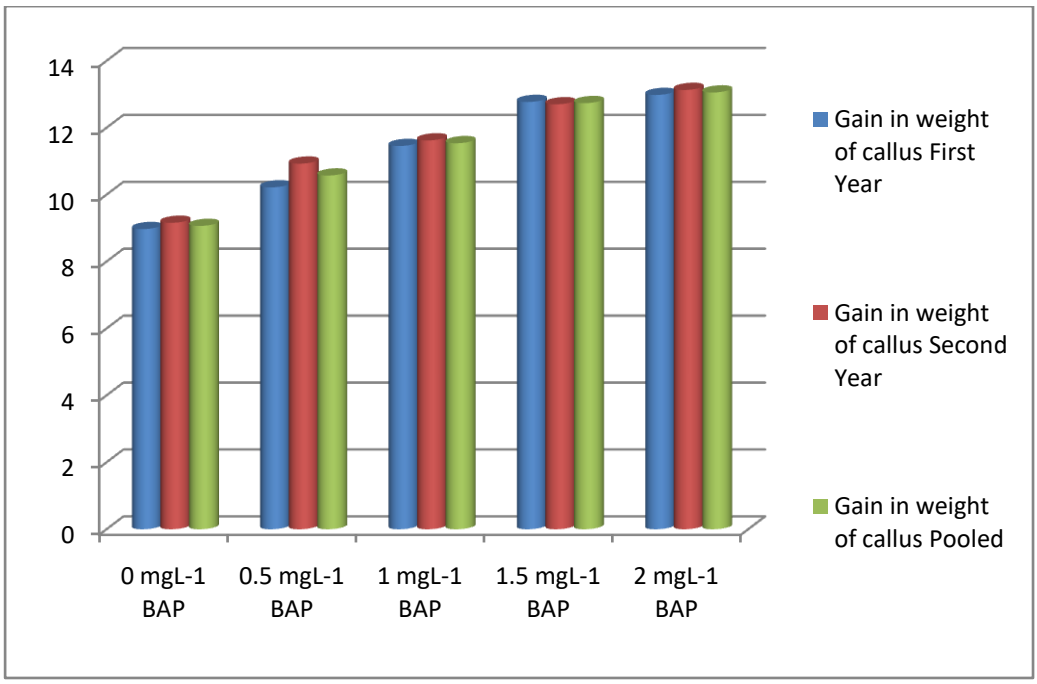


Fig. 4.5: Influence of BAP levels in gain in weight of callus

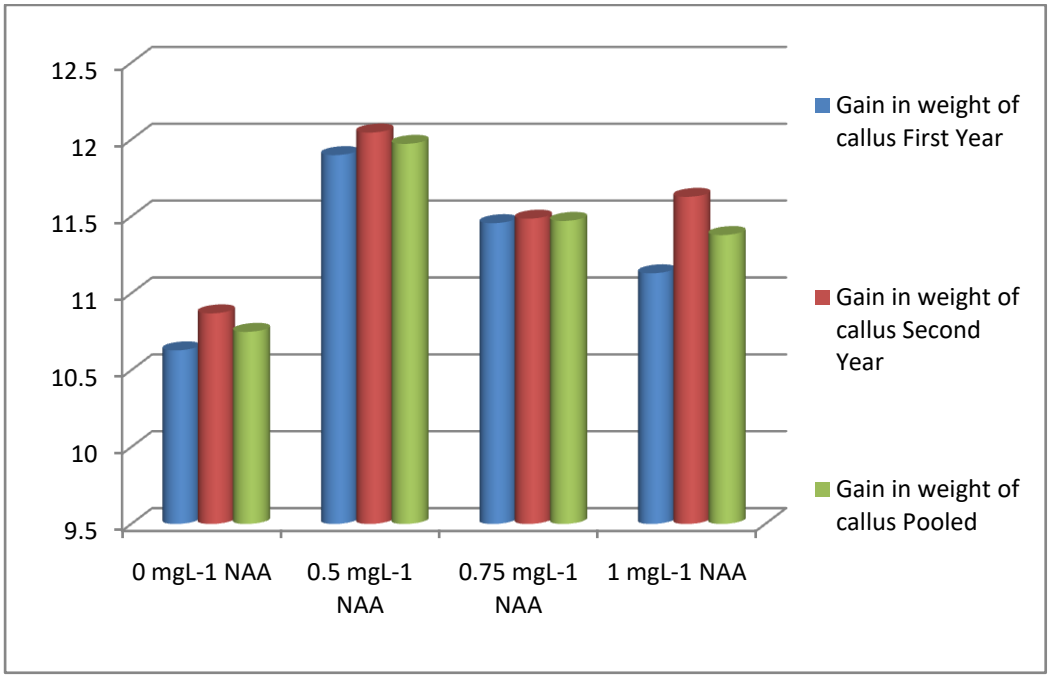


Fig. 4.6: Influence of NAA levels in gain in weight of callus

4.1.4 Days to greening

Table 4.7 and figure 4.7 depicted the perusal of the result pertaining to the effect of BAP on the days to greening. The treatments differed significantly on the days to greening, During the year 2016-17, the least days taken to greening (15.71 days) were recorded in the media supplement with BAP @ 2 mgL^{-1} followed by BAP @ 1.5 mgL^{-1} (17.28 days) and the longest days taken to greening were recorded in control (25.27 days). In the subsequent year 2017-18 similar result were recorded, the minimum days taken to greening (16.50 days) were recorded in the treatment BAP @ 2 mgL^{-1} followed by BAP @ 1.5 mgL^{-1} (18.10 days) and the maximum number of days taken to greening were recorded in control (25.79 days). Analysis of pooled data of both the year showed significant differences in the number of days taken to greening. The least number of days taken to greening (16.10 days) was recorded in MS media augmented with BAP @ 2 mgL^{-1} and the maximum number of days taken were recorded in control. Chookoh *et al.* 2019 orchids reported a similar result in Tolumnia, the above finding might be due to balanced hormonal effect comparing to other concentration on the explants that lead to early growth and development.

Table 4.7 and Fig. 4.8 showed that MS media supplement with different levels of NAA results in significant differences on the days taken to greening. During the year 2016-17, the least number of days taken to greening (18.09 days) were recorded in treatment NAA @ 0.5 mgL^{-1} followed by NAA @ 0.75 mgL^{-1} (20.09 days) and the maximum number of days taken were observed in control (22.31 days). In the second year study 2017-18 similar result were obtained, the least days taken to greening (19.50 days) were recorded in treatment NAA @ 0.5 mgL^{-1} and the maximum numbers of days taken (23.46 days) were recorded in control. Pooled data analysis of both the year data showed significant different on the days taken to greening, the least days (18.79 days) taken were recorded in treatment NAA @ 0.5 mgL^{-1} and the maximum

days take (22.89 days) were recorded in control. The results revealed that NAA in lower concentration promote growth and development, however in higher concentration above 0.5 mgL^{-1} results in negative effect. Similar result in earliness were reported by Tao *et al*. 2011 in *Cymbidium feberi* Rolfe and Chookoh *et al*. 2019 in *Tolumnia* orchids.

The data presented in Table 4.8 revealed that interaction between various level of BAP and NAA show significant effect on the days to greening. The pooled data result revealed that the least days taken to greening (14.77 days) of explants were recorded in treatment combination of BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1} followed by treatment combination of BAP @ 2 mgL^{-1} + NAA @ 0.75 mgL^{-1} (15.94 days) and the maximum number of days taken to greening (30.29 days) were recorded in control BAP @ 0 mgL^{-1} + NAA @ 0 mgL^{-1}. Application of BAP and NAA in combination results in the early greening as compared to when they are applied separately. Alvard *et al.* 1993 reported that numbers of PGRs were least efficient when they are applied individually as compared to when they are applied in combination. The current experimental findings are in line with the finding of Chookoh *et al*. 2019 in *Tolumnia* orchids.

Table 4.7: Influence of 6-BAP and NAA levels on days to greening in micropropagation of *Dendrobium* cv. Sonia Earsakul

Treatments	Days to callus formation		
	First year	*Second year*	*Pooled*
B_1 (0 mgL^{-1} BAP)	25.27^a	25.79^a	25.53^a
B_2 (0.5 mgL^{-1} BAP)	22.35^b	24.23^b	23.29^b
B_3 (1 mgL^{-1} BAP)	21.55^b	21.20^c	21.38^c
(B_4) (1.5 mgL^{-1} BAP)	17.28^c	18.10^d	17.69^d
B_5 (2 mgL^{-1} BAP)	15.71^d	16.50^e	16.10^e
Sem±	0.46	0.28	0.27
CD at 5%	1.31	0.80	0.75
N_1 (0 mgL^{-1} NAA)	22.31^a	23.46^a	22.89^a
N_2 (mgL^{-1} NAA)	18.09^d	19.50^d	18.79^d
N_3 (mgL^{-1} NAA)	20.09^c	20.40^c	20.24^c
N_4 (1 mgL^{-1} NAA)	21.24^b	21.29^b	21.27^b
SEm±	0.41	0.25	0.24
CD at 5%	1.17	0.72	0.68

Table 4.8: Interaction effect of 6-BAP and NAA levels on days to greening in micropropagation of *Dendrobium* cv. Sonia Earsakul

Treatments	*First year*	*Second year*	*Pooled*
B_1N_1 (BAP 0 mgL^{-1} + NAA 0 mgL^{-1})	31.00^{a}	29.59^{a}	30.29^{a}
B_1N_2 (BAP 0 mgL^{-1} + NAA 0.5 mgL^{-1})	19.61^{cde}	23.23^{ef}	21.42^{d}
B_1N_3(BAP 0 mgL^{-1} + NAA 0.75 mgL^{-1})	24.36^{c}	24.80^{cde}	24.58^{c}
B_1N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	26.13^{b}	25.54^{cd}	25.83^{b}
B_2N_1(BAP 0.5 mgL^{-1} + NAA 0 mgL^{-1})	23.20^{cd}	26.03^{bc}	24.61^{c}
B_2N_2(BAP 0.5 mgL^{-1} + NAA 0.5 mgL^{-1})	21.22^{e}	22.27^{fg}	21.75^{e}
B_2N_3(BAP 0.5 mgL^{-1} + NAA 0.75 mgL^{-1})	22.27^{de}	23.67^{ef}	22.97^{de}
B_2N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	22.71^{cde}	24.94^{cde}	23.83^{cd}
B_3N_1(BAP 1 mgL^{-1} + NAA 0 mgL^{-1})	22.43^{d}	23.95^{de}	23.19^{d}
B_3N_2(BAP 1 mgL^{-1} + NAA 0.5 mgL^{-1})	20.02^{f}	19.72^{g}	19.87^{f}
B_3N_3(BAP 1 mgL^{-1} + NAA 0.75 mgL^{-1})	21.31^{de}	20.04^{g}	20.67^{ef}
B_3N_4(BAP 1 mgL^{-1} + NAA 1 mgL^{-1})	22.44^{de}	21.10^{fg}	21.77^{e}
B_4N_1(BAP 1.5 mgL^{-1} + NAA 0 mgL^{-1})	18.62^{fg}	20.74^{fg}	19.68^{f}
B_4N_2(BAP 1.5 mgL^{-1} + NAA 0.5 mgL^{-1})	15.58^{ij}	16.76^{hi}	16.17^{g}
B_4N_3(BAP 1.5 mgL^{-1} + NAA 0.75 mgL^{-1})	17.17^{ghi}	16.95^{hi}	17.06^{gh}
B_4N_4(BAP 1.5 mgL^{-1} + NAA 1 mgL^{-1})	17.75^{gh}	17.97^{h}	17.86^{g}
B_5N_1(BAP 2 mgL^{-1} + NAA 0 mgL^{-1})	16.31^{hi}	17.00^{hi}	16.66^{gh}
B_5N_2(BAP 2 mgL^{-1} + NAA 0.5 mgL^{-1})	14.01^{j}	15.52^{i}	14.77^{i}
B_5N_3(BAP 2 mgL^{-1} + NAA 0.75 mgL^{-1})	15.34^{ij}	16.54^{hi}	15.94^{hi}
B_5N_4(BAP 2 mgL^{-1} + NAA 1mgL^{-1})	17.19^{ghi}	16.92^{hi}	17.06^{gh}
SEm(±)	0.91	0.56	0.54
CD at 5%	2.62	1.60	1.51

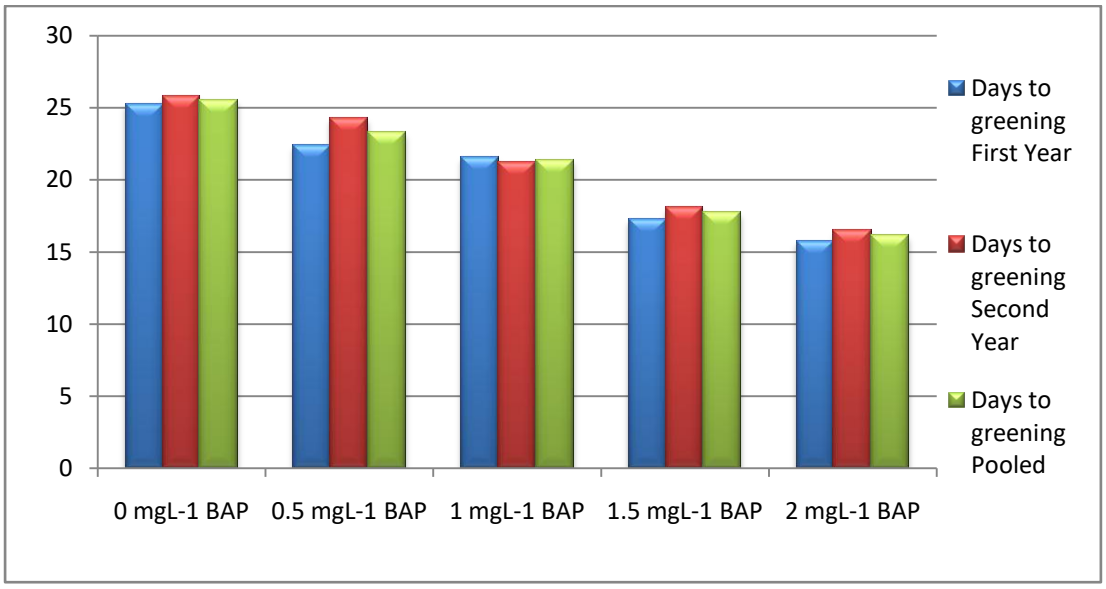


Fig.4.7: Effect of Various levels of 6-BAP in number of days taken to greening

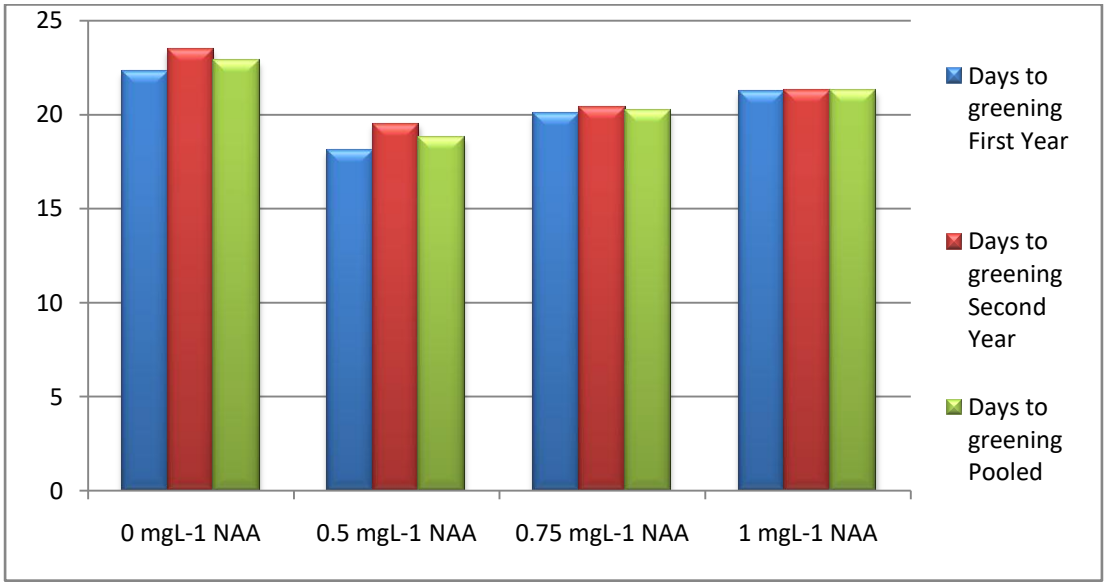


Fig. 4.8: Effect of Various levels of NAA in number of days taken to greening

4.1.5 Days to initiation of shoot

Perusals of the result pertaining in the influence of 6-BAP and NAA on the days taken to initiation of shoot are depicted in Table 4.9 Fig 4.9 and Fig. 10. Applications of 6-BAP showed significant effect on the days to initiation of shoot. During the year 2016-17, application of BAP in various level to MS media showed significant difference in the days to initiation of shoot in *in vitro* multiplication of *Dendrobium* cv. Sonia Earsakul. The least days (35.71 days) taken to initiation of shoot was observed in 2 mgL^{-1} BAP followed by (37.28 days) 1.5 mgL^{-1} BAP and the longest days (46.11 days) required for initiation of shoot was recorded in control. During the year 2017-18 BAP @ 2 mgL^{-1} resulted in minimum days (36.50 days) taken to shoot initiation which is followed by BAP @ 1.5 mgL^{-1} (38.10 days) and the maximum number of days taken was observed in BAP @ 0 mgL^{-1}. The pooled data revealed that BAP @ 2 mgL^{-1} resulted in minimum days taken to initiation of shoot (36.10 days) and the long days (45.95 days) taken to root initiation was observed in control 0mgL^{-1}. Among the different concentrations studied, BAP @ 2 mgL^{-1} recorded significantly lesser days to shoot initiation. This might be due to application of BAP which elicits plant growth and development by stimulating cell division. This result is in agreement with the findings of Chookoh *et al.* (2019) in *Tolumnia* orchid, Rattana and Sangchanjiradet (2017) in *Dendrobium signatum* Rchb.f, Kaur 2015 on *Vanilla planifolia* and Sunitibala and Kishore 2009 in *Dendrobium transparens.*

Further examination of the data revealed that application of NAA in various levels significantly influenced the duration to initiation of shoot. During the year 2016-17, incorporation of NAA @ 0.5 mgL^{-1} to MS media resulted in least days (38.76 days) to initiation of shoot followed by NAA @ 0.75 mgL^{-1} (40.09 days) and the maximum number of days taken was observed in control. In the subsequent year 2017-18, the least days taken to shoot initiation was also recorded in application of NAA @ 0.5 mgL^{-1} and the longest

duration taken to shoot initiation was observed in 0 mgL^{-1} of NAA. The perusal of pooled data of both the trials showed that NAA @ 0.5 mgL^{-1} in MS recorded the minimum days (39.13 days) taken to shoot initiation followed by NAA @ 0.75 mgL^{-1} (40.24 days) and the highest days taken was observed in 0mgL^{-1} NAA. NAA at higher concentration are toxic to plant resulting in the delay of shoot initiation. Similar results were reported by Chookoh *et al*. 2019 in *Tolumnia* orchid and Kosir *et al*. 2004 on *Phalaenopsis* orchids.

In table 4.10, the interaction effect of 6-BAP and NAA showed a significant effect on the days to initiation of shoot. The pooled data of both the years showed that the minimum days (34.77 days) taken to initiation of shoot were recorded in the treatment combination of 2 mgL^{-1} BAP + 0.5 mgL^{-1} NAA followed by (35.94 days) 2 mgL^{-1} BAP + 0.75 mgL^{-1} NAA. The maximum days (50.29 days) was recorded in control. When BAP and NAA are applied together in balanced dose, it results in early initiation of shoot as comparing to single application. This might be due to the working together of these two hormones to produce a combined effect. The current experimental findings are in line with the findings of Chookoh *et al*. 2019 in *Tolumnia* orchid and Kosir *et al*. 2004 on *Phalaenopsis* orchids.

Table 4.9 Influence of 6-BAP and NAA levels on days to initiation of shoot on *in vitro* multiplication of *Dendrobium* cv. Sonia Earsakul

Treatment	*First year*	*Second year*	*Pooled*
B_1 (0 mgL^{-1} BAP)	46.11[a]	45.79[a]	45.95[a]
B_2 (0.5 mgL^{-1} BAP)	42.35[b]	44.23[b]	43.29[b]
B_3 (1 mgL^{-1} BAP)	41.55[b]	41.20[c]	41.38[c]
(B_4) (1.5 mgL^{-1} BAP)	37.28[c]	38.10[d]	37.69[d]
B_5 (2 mgL^{-1} BAP)	35.71[d]	36.50[e]	36.10[e]
Sem±	0.30	0.28	0.20
CD at 5%	0.85	0.80	0.58
N_1 (0 mgL^{-1} NAA)	42.31[a]	43.46[a]	42.89[a]
N_2 (0.5 mgL^{-1} NAA)	38.76[d]	39.50[d]	39.13[d]
N_3 (0.75 mgL^{-1} NAA)	40.09[c]	40.40[c]	40.24[c]
N_4 (1 mgL^{-1} NAA)	41.24[b]	41.29[b]	41.27[b]
SEm±	0.27	0.25	0.18
CD at 5%	0.76	0.72	0.52

Table 4.10 Interaction effect of 6-BAP and NAA on days to initiation of shoot in *in vitro* multiplication of *Dendrobium* cv. Sonia Earsakul

Treatments	*First year*	*Second year*	*Pooled*
B_1N_1 (BAP 0 mgL^{-1} + NAA 0 mgL^{-1})	51.00^{a}	49.59^{a}	50.29^{a}
B_1N_2 (BAP 0 mgL^{-1} + NAA 0.5 mgL^{-1})	42.94^{cde}	43.23^{ef}	43.09^{d}
B_1N_3(BAP 0 mgL^{-1} + NAA 0.75 mgL^{-1})	44.36^{c}	44.80^{cde}	44.58^{c}
B_1N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	46.13^{b}	45.54^{cd}	45.83^{b}
B_2N_1(BAP 0.5 mgL^{-1} + NAA 0 mgL^{-1})	43.20^{cd}	46.03^{bc}	44.61^{c}
B_2N_2(BAP 0.5 mgL^{-1} + NAA 0.5 mgL^{-1})	41.22^{e}	42.27^{fg}	41.75^{e}
B_2N_3(BAP 0.5 mgL^{-1} + NAA 0.75 mgL^{-1})	42.27^{de}	43.67^{ef}	42.97^{de}
B_2N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	42.71^{cde}	44.94^{cde}	43.83^{cd}
B_3N_1(BAP 1 mgL^{-1} + NAA 0 mgL^{-1})	42.43^{d}	43.95^{de}	43.19^{d}
B_3N_2(BAP 1 mgL^{-1} + NAA 0.5 mgL^{-1})	40.02^{f}	39.72^{g}	39.87^{f}
B_3N_3(BAP 1 mgL^{-1} + NAA 0.75 mgL^{-1})	41.31^{de}	40.04^{g}	40.67^{ef}
B_3N_4(BAP 1 mgL^{-1} + NAA 1 mgL^{-1})	42.44^{de}	41.10^{fg}	41.77^{e}
B_4N_1(BAP 1.5 mgL^{-1} + NAA 0 mgL^{-1})	38.62^{fg}	40.74^{fg}	39.68^{f}
B_4N_2(BAP 1.5 mgL^{-1} + NAA 0.5 mgL^{-1})	35.58^{ij}	36.76^{hi}	36.17
B_4N_3(BAP 1.5 mgL^{-1} + NAA 0.75 mgL^{-1})	37.17^{ghi}	36.95^{hi}	37.06^{gh}
B_4N_4(BAP 1.5 mgL^{-1} + NAA 1 mgL^{-1})	37.75^{gh}	37.97^{h}	37.86^{g}
B_5N_1(BAP 2 mgL^{-1} + NAA 0 mgL^{-1})	36.31^{hi}	37.00^{hi}	36.66^{gh}
B_5N_2(BAP 2 mgL^{-1} + NAA 0.5 mgL^{-1})	34.01^{j}	35.52^{i}	34.77^{i}
B_5N_3(BAP 2 mgL^{-1} + NAA 0.75 mgL^{-1})	35.34^{ij}	36.54^{hi}	35.94^{hi}
B_5N_4(BAP 2 mgL^{-1} + NAA 1mgL^{-1})	37.19^{ghi}	36.92^{hi}	37.06^{gh}
SEm±	0.60	0.56	0.41
CD at 5%	1.71	1.60	1.15

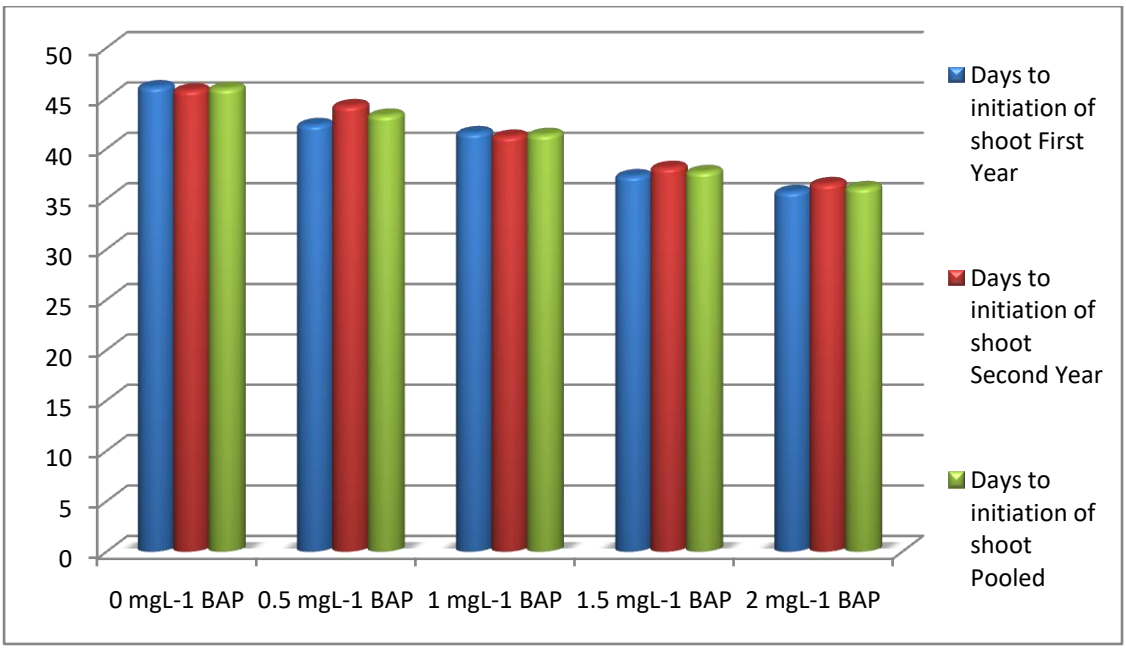


Fig. 4.9: Response of 6-BAP levels days to initiation of shoot

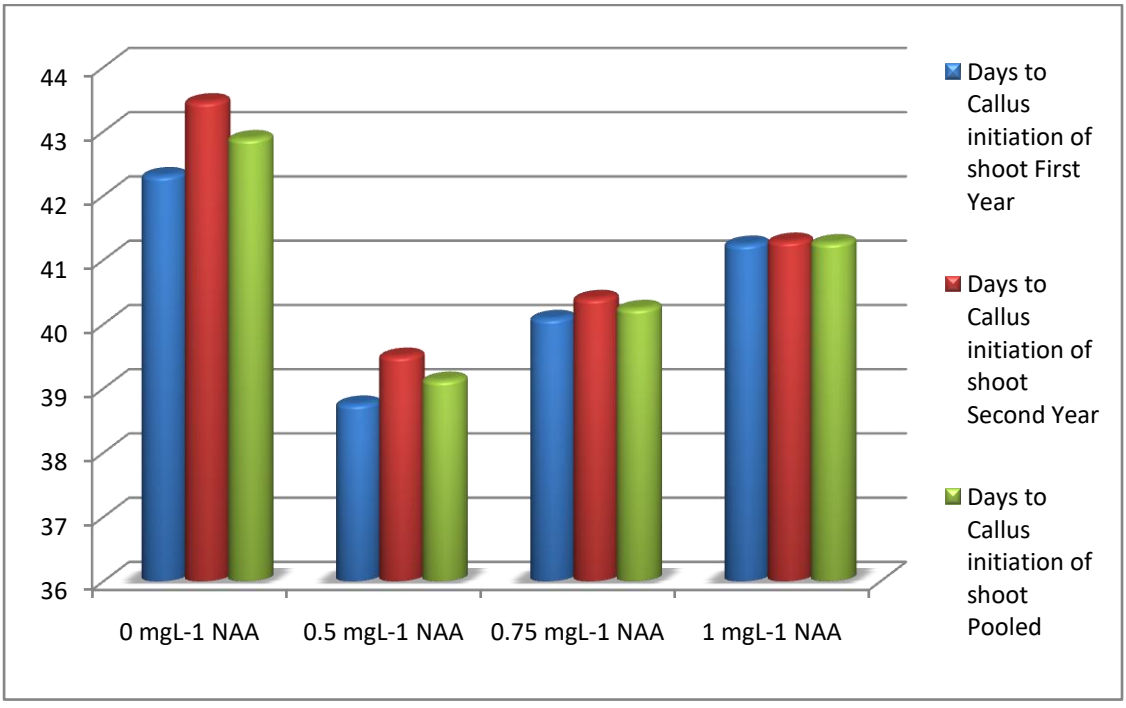
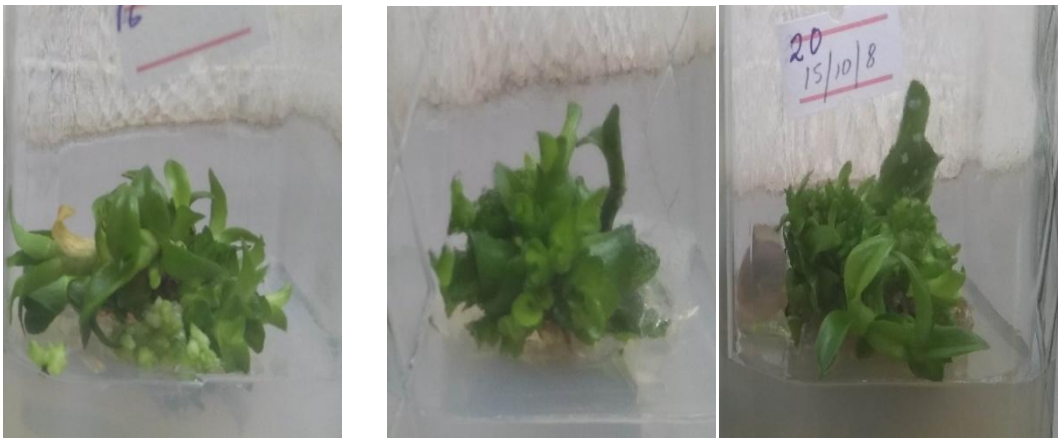


Fig. 4.10: Response of NAA levels days to initiation of shoot

A **B** **C**



D **E** **F**

Plate 4. Influence of 6-BAP and NAA on the number of shoot bud

(A) **Control**
(B) **BAP @ 1 mgL^{-1} + NAA @ 0.5 mgL^{-1}**
(C) **BAP @ 1 mgL^{-1} + NAA @ 0.75 mgL^{-1}**
(D) **BAP @ 1.5 mgL^{-1} + NAA @ 1 mgL^{-1}**
(E) **BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1}**
(F) **BAP @ 2 mgL^{-1} + NAA @ 1 mgL^{-1}**

4.1. Number of multiple shoot bud during first sub culturing

The data pertaining to the number of multiple shoot bud on first sub culturing as influenced by various levels of 6-benzylaminopurine and naphthalene acetic acid are presented in Table 4.11, Fig. 4.11 and Fig. 4.12. During the year 2016-17, application of BAP @ 2mgL^{-1} recorded the maximum number (5.61) of shoot bud which is at par with BAP @ 1.5 mgL^{-1} (5.37). The least number of shoot bud was recorded in control (2.84). During the year 2017-18, BAP in varied doses significantly increased the number of multiple shoot bud. Maximum number of shoot bud (5.50) were recorded in treatment containing BAP @ 2 mgL^{-1} followed by BAP @ 1.5 mgL^{-1} (4.66) and the least number (2.84). Analysis of two years pooled data showed that application BAP resulted in significant increase in number of shoot bud, the maximum number (5.56) of shoot bud was recorded in treatment containing BAP @ 2 mgL^{-1} followed by BAP @ 1.5 mgL^{-1} (5.02) and the least shoot bud (2.84) was recorded in control.

MS media incorporated with NAA exhibited significant effect on the number of multiple shoot bud on *Dendrobium* cv. Sonia Easrsakul *in vitro* multiplication. During the year 2016-17, maximum number (4.60) of shoot bud was recorded in treatment containing NAA @ 0.5 mgL^{-1} which is at par with NAA @ 1 mgL^{-1} and NAA @ 0.75 mgL^{-1} (4.53 and 4.52, respectively). The least number of shoot bud (3.96) was recorded in control. In the subsequent year trial 2017-18, the maximum number (4.60) of shoot bud was recorded in treatment NAA @ 0.5 mgL^{-1}which was at par with NAA @ 0.75 mgL^{-1} and NAA @ 1 mgL^{-1} (4.24 and 4.22, respectively). Pooled data of both the trial showed that MS media supplement with NAA @ 1.5 results in the highest number (4.48) of multiple shoot bud followed by (4.38) in the treatment containing NAA @ of 0.75 mgL^{-1} and NAA @ 1 mgL^{-1}. The least number of shoot bud (3.83) were recorded in control.

Table 4.12 depict the interaction effect of BAP and NAA on the number of multiple shoot buds during the first sub culturing. The pooled data of both the years study revealed that the highest number of multiple shoot buds (5.76) were recorded in the treatment combination of BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1} which is at par with BAP @ 2mgL^{-1} + NAA @0.75 and BAP @ 2 mgL^{-1} + NAA @ 1 mgL^{-1} (5.70 and 5.70 respectively).

4.1.7 Number of multiple shoot bud during second sub culturing

The analysis of the data given in Table 4.11, Fig. 4.13 and Fig. 4.14 revealed that the various levels of 6-Benzylaminopurine and naphthalene acetic acid had a significant effect on the number of multiple shoot during the second sub culturing. During the year 2016-17, application of BAP @ 2mgL^{-1} recorded the maximum number (11.61) of shoot bud followed by (10.50) BAP @ 1.5 mgL^{-1}. The least number of shoot buds (7.88) was recorded in control. During the year 2017-18, BAP in varied dose significantly increased the number of multiple shoot bud. Maximum number of shoot bud (12.8) were recorded in treatment containing BAP @ 2 mgL^{-1} followed by BAP @ 1.5 mgL^{-1} (10.87) and the least number (7.79) was recorded in control. Analysis of two years pooled data showed that application of BAP resulted in significant increase in number of shoot bud, the maximum number (11.85) of shoot bud were recorded in treatment containing BAP @ 2 mgL^{-1} followed by BAP @ 1.5 mgL^{-1} (10.69) and the least shoot bud (7.83) was recorded in control. The result of the study revealed that among all the treatments, BAP @ 2 mgL^{-1} resulted better in the number of shoot in both the sub culturing. This might be due to the optimum dose of BAP which stimulate cell division resulting in differentiation and outgrowth of axillary bud. The result of the study are in confirmation with the findings of researchers like Rattana and Sangchanjiradet 2017 in *Dendrobium signatum* Rchb.f., Thokchom and Maitra 2017 on *Anthurium andreanum* cv. Jewel, Maurya *et al.* 2013 on Rose (*Rosa hybrid* L.) cv. Benjamin Paul, Pradhan *et al*. 2013 on *Dendrobium densiflorum* Lindl.,

Boudabous *et al*. 2010 on *Malus domestica* L. cultivar Douce de Djerba, Kosir *et al*. (2004) on *Phalaenopsis* orchids and Sunitibala and Kishore 2009 on *Dendrobium transparens.*

MS media incorporated with NAA exhibited significant effect on the number of multiple shoot bud on *Dendrobium* cv. Sonia Easrsakul *in vitro* multiplication. During the year 2016-17, maximum number (9.81) of shoot bud were recorded in treatment containing NAA @ 0.5 mgL^{-1} followed by (9.68) NAA @ 1 mgL^{-1} and the least number of shoot bud (8.90) was recorded in control. In the subsequent year trial 2017-18, the maximum number (9.90) of shoot bud was recorded in treatment NAA @ 0.5 mgL^{-1} which was at par with NAA @ 0.75mgL^{-1} and NAA @ 1 mgL^{-1} (9.71 and 9.79 respectively). Pooled data of both the trials showed that MS media supplement with NAA @ 1.5 results in the highest number (4.48) of multiple shoot bud which is at par with NAA @ 1 mgL^{-1} of (9.74) and the least number of shoot bud (3.83) were recorded in control. As the level of NAA increased above 0.5 mgL^{-1} the number of shoots reduced as NAA in lower concentration helped in cell regeneration but in higher concentration NAA inhibited growth of lateral bud. The current experimental findings are in agreement with the findings of Priyanka *et al*. 2018 on *Dendrobium nobile,* Rattana and Sangchanjiradet 2017 in *Dendrobium signatum* Rchb.f., Goswani *et al*. 2015 in *Dendrobium* sp. Of orchid, Pradhan *et al*. 2013 on *Dendrobium densiflorum* Lindl. and Zuraida (2013) on *Curcuma caesia.*

The interaction effect of BAP and NAA on the number of multiple shoot bud during the second sub culturing of *Dendrobium* cv. Sonia Earsakul were given in the Table 4.13. MS media supplement with BAP and NAA in various levels give a significant result. From the given pooled data, the highest number (12.13) of multiple shoot bud during second sub culturing were recorded in the treatment combination of BA @ 2mgL^{-1} + NAA @ 0.5 mgL^{-1} which was at par with BAP @ 2 mgL^{-1} + NAA @ 0.75 mgL^{-1} and BAP @ 2 mgL^{-1} + NAA @ 1

mgL^{-1} (12.03 and 11.92 respectively). The least number of multiple shoot buds (7.65) were recorded in treatment containing BAP @ $0mgL^{-1}$ + NAA @ 0 mgL^{-1}. In both the first and second sub culturing, treatment combination of BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1} showed significantly higher multiple shoot bud. This right concentration of hormone might attribute synergy in regeneration of number of multiple shoot bud. The current experimental finding are in line with the findings of Rattana and Sangchanjiradet 2017 in *Dendrobium signatum* Rchb.f., Pradhan *et al*. 2013 in *Dendrobium densiflorum* Lindl. and Kosir *et al*. 2004 in *Phalaenopsis* orchids.

Fig. 4.11 Influence of 6-BAP and NAA levels on number of multiple shoot bud during first and second sub culturing in *in vitro* multiplication of *Dendrobium* cv. Sonia Earsakul

Treatments	Number of multiple shoot bud during first sub culturing			Number of multiple shoot bud during second sub culturing		
	First year	*Second year*	*Pooled*	*First year*	*Second year*	*Pooled*
B_1 (0 mgL^{-1} BAP)	2.84^{d}	2.84^{e}	2.84^{e}	7.88^{e}	7.79^{e}	7.83^{e}
B_2 (0.5mgL^{-1} BAP)	3.32^{c}	3.51^{d}	3.41^{d}	8.37^{d}	8.28^{d}	8.33^{d}
B_3 (1mgL^{-1} BAP)	4.87^{b}	4.13^{c}	4.50^{c}	9.04^{c}	8.92^{c}	8.98^{c}
(B_4)(1.5mgL^{-1}BAP)	5.37^{a}	4.66^{b}	5.02^{b}	10.50^{b}	10.87^{b}	10.69^{b}
B_5 (2 mgL^{-1} BAP)	5.61^{a}	5.50^{a}	5.56^{a}	11.61^{a}	12.08^{a}	11.85^{a}
Sem±	0.12	0.05	0.06	0.07	0.10	0.06
CD at 5%	0.34	0.15	0.18	0.21	0.28	0.17
N_1 (0 mgL^{-1} NAA)	3.96^{b}	3.69^{b}	3.83^{b}	8.90^{c}	8.95^{b}	8.92^{c}
N_2 (0.5mgL^{-1} NAA)	4.60^{a}	4.36^{a}	4.48^{a}	9.81^{a}	9.90^{a}	9.86^{a}
N_3(0.75mgL^{-1} NAA)	4.52^{a}	4.24^{a}	4.38^{b}	9.53^{b}	9.71^{a}	9.62^{b}
N_4 (1 mgL^{-1} NAA)	4.53^{a}	4.22^{a}	4.38^{b}	9.68^{ab}	9.79^{a}	9.74^{a}
SEm±	0.10	0.05	0.06	0.07	0.09	0.05
CD at 5%	0.30	0.13	0.16	0.19	0.25	0.15

Table 4.12 Interaction effect of 6-BAP and NAA levels on number of multiple shoot bud during first sub culturing in *in vitro* multiplication of *Dendrobium* cv. Sonia Earsakul

Treatments	*First year*	*Second year*	*Pooled*
B_1N_1 (BAP 0 mgL^{-1} + NAA 0 mgL^{-1})	2.80^{c}	2.84^{h}	2.82^{f}
B_1N_2 (BAP 0 mgL^{-1} + NAA 0.5 mgL^{-1})	2.88^{c}	2.87^{h}	2.87^{f}
B_1N_3(BAP 0 mgL^{-1} + NAA 0.75 mgL^{-1})	2.85^{c}	2.81^{h}	2.83^{f}
B_1N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	2.84^{c}	2.83^{h}	2.84^{f}
B_2N_1(BAP 0.5 mgL^{-1} + NAA 0 mgL^{-1})	2.87^{c}	3.17^{g}	3.02^{f}
B_2N_2(BAP 0.5 mgL^{-1} + NAA 0.5 mgL^{-1})	3.57^{bc}	3.73^{e}	3.65^{e}
B_2N_3(BAP 0.5 mgL^{-1} + NAA 0.75 mgL^{-1})	3.43^{bc}	3.57^{ef}	3.50^{e}
B_2N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	3.41^{bc}	3.55^{ef}	3.48^{e}
B_3N_1(BAP 1 mgL^{-1} + NAA 0 mgL^{-1})	3.80^{b}	3.40^{fg}	3.60^{e}
B_3N_2(BAP 1 mgL^{-1} + NAA 0.5 mgL^{-1})	5.20^{a}	4.50^{cd}	4.85^{cd}
B_3N_3(BAP 1 mgL^{-1} + NAA 0.75 mgL^{-1})	5.17^{a}	4.33^{d}	4.75^{cd}
B_3N_4(BAP 1 mgL^{-1} + NAA 1 mgL^{-1})	5.31^{a}	4.30^{d}	4.81^{cd}
B_4N_1(BAP 1.5 mgL^{-1} + NAA 0 mgL^{-1})	5.00^{a}	4.23^{d}	4.62^{d}
B_4N_2(BAP 1.5 mgL^{-1} + NAA 0.5 mgL^{-1})	5.58^{a}	4.93^{b}	5.26^{b}
B_4N_3(BAP 1.5 mgL^{-1} + NAA 0.75 mgL^{-1})	5.50^{a}	4.75^{bc}	5.12^{bc}
B_4N_4(BAP 1.5 mgL^{-1} + NAA 1 mgL^{-1})	5.40a	4.73^{bc}	5.07^{bc}
B_5N_1(BAP 2 mgL^{-1} + NAA 0 mgL^{-1})	5.33^{a}	4.80^{bc}	5.07^{bc}
B_5N_2(BAP 2 mgL^{-1} + NAA 0.5 mgL^{-1})	5.75^{a}	5.76^{a}	5.76^{a}
B_5N_3(BAP 2 mgL^{-1} + NAA 0.75 mgL^{-1})	5.67^{a}	5.73^{a}	5.70^{a}
B_5N_4(BAP 2 mgL^{-1} + NAA 1mgL^{-1})	5.70^{a}	5.70^{a}	5.70^{a}
SEm±	0.23	0.10	0.13
CD at 5%	0.67	0.29	0.36

Table 4.13 Interaction effect of 6-BAP and NAA levels on the number of multiple shoot bud during second sub culturing in *in vitro* multiplication of *Dendrobium* cv. Sonia Earsakul

Treatments	*First year*	*Second year*	*Pooled*
B_1N_1 (BAP 0 mgL^{-1} + NAA 0 mgL^{-1})	7.60^{j}	7.70^{h}	7.65^{f}
B_1N_2 (BAP 0 mgL^{-1} + NAA 0.5 mgL^{-1})	8.03^{ij}	7.90^{fg}	7.97^{f}
B_1N_3(BAP 0 mgL^{-1} + NAA 0.75 mgL^{-1})	7.97^{ij}	7.77^{gh}	7.87^{f}
B_1N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	7.90^{ij}	7.80^{gh}	7.85^{f}
B_2N_1(BAP 0.5 mgL^{-1} + NAA 0 mgL^{-1})	7.98^{ij}	7.90^{fg}	7.94^{f}
B_2N_2(BAP 0.5 mgL^{-1} + NAA 0.5 mgL^{-1})	8.63^{gh}	8.53^{de}	8.58^{e}
B_2N_3(BAP 0.5 mgL^{-1} + NAA 0.75 mgL^{-1})	8.53^{h}	8.27^{efg}	8.40^{e}
B_2N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	8.34^{hi}	8.43^{def}	8.39^{e}
B_3N_1(BAP 1 mgL^{-1} + NAA 0 mgL^{-1})	8.51^{h}	8.33^{defg}	8.42^{e}
B_3N_2(BAP 1 mgL^{-1} + NAA 0.5 mgL^{-1})	9.13^{ef}	9.23^{c}	9.18^{d}
B_3N_3(BAP 1 mgL^{-1} + NAA 0.75 mgL^{-1})	9.03^{fg}	8.91^{cd}	8.97^{d}
B_3N_4(BAP 1 mgL^{-1} + NAA 1 mgL^{-1})	9.50^{e}	9.18^{c}	9.34^{d}
B_4N_1(BAP 1.5 mgL^{-1} + NAA 0 mgL^{-1})	9.21^{ef}	9.40^{c}	9.30^{d}
B_4N_2(BAP 1.5 mgL^{-1} + NAA 0.5 mgL^{-1})	11.40^{bc}	11.46^{b}	11.43^{b}
B_4N_3(BAP 1.5 mgL^{-1} + NAA 0.75 mgL^{-1})	10.37^{d}	11.27^{b}	10.82^{c}
B_4N_4(BAP 1.5 mgL^{-1} + NAA 1 mgL^{-1})	11.03^{c}	11.36^{b}	11.20^{b}
B_5N_1(BAP 2 mgL^{-1} + NAA 0 mgL^{-1})	11.19^{c}	11.40^{b}	11.30^{b}
B_5N_2(BAP 2 mgL^{-1} + NAA 0.5 mgL^{-1})	11.87^{a}	12.40^{a}	12.13^{a}
B_5N_3(BAP 2 mgL^{-1} + NAA 0.75 mgL^{-1})	11.73^{ab}	12.32^{a}	12.03^{a}
B_5N_4(BAP 2 mgL^{-1} + NAA 1mgL^{-1})	11.65^{ab}	12.20^{a}	11.92^{a}
SEm±	0.15	0.19	0.12
CD at 5%	0.42	0.55	0.34

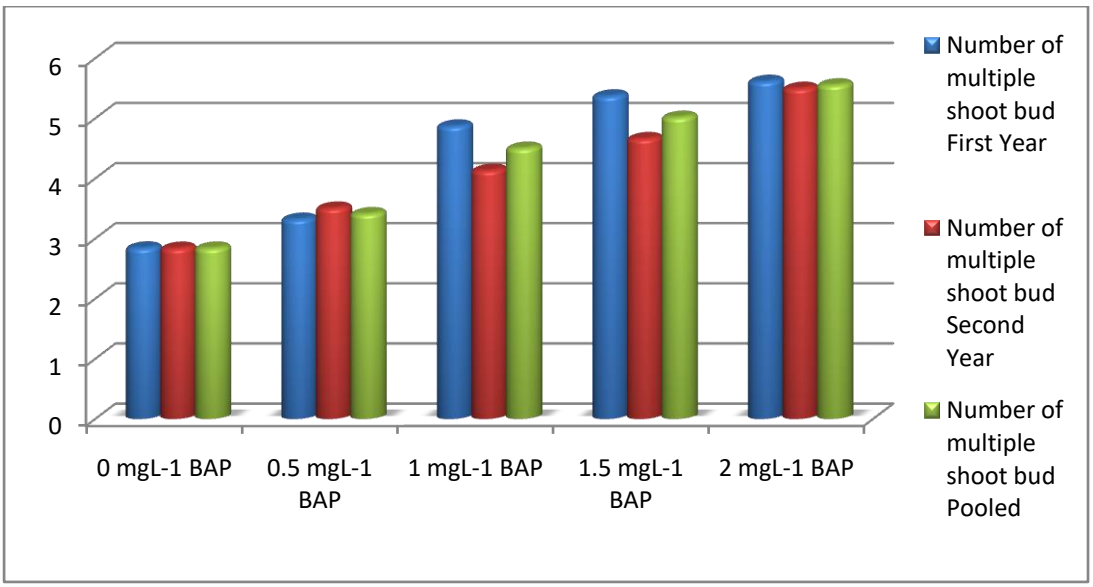


Fig. 4.11: Influence of 6-BAP on the number of multiple shoot bud during the first sub culturing

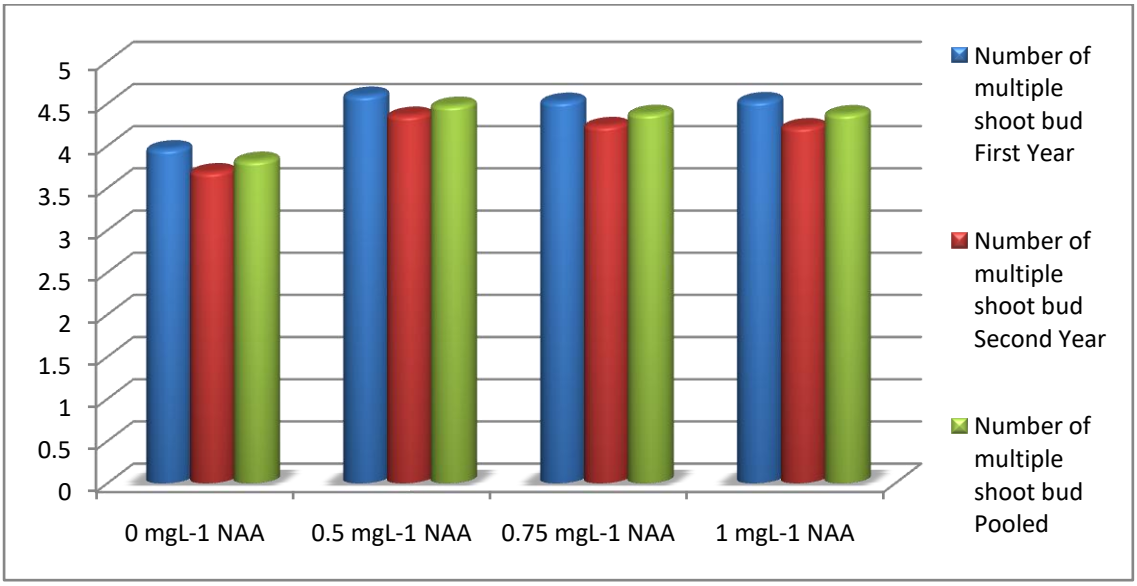


Fig. 4.12: Effect of NAA levels in number of multiple shoot bud during the first sub culturing

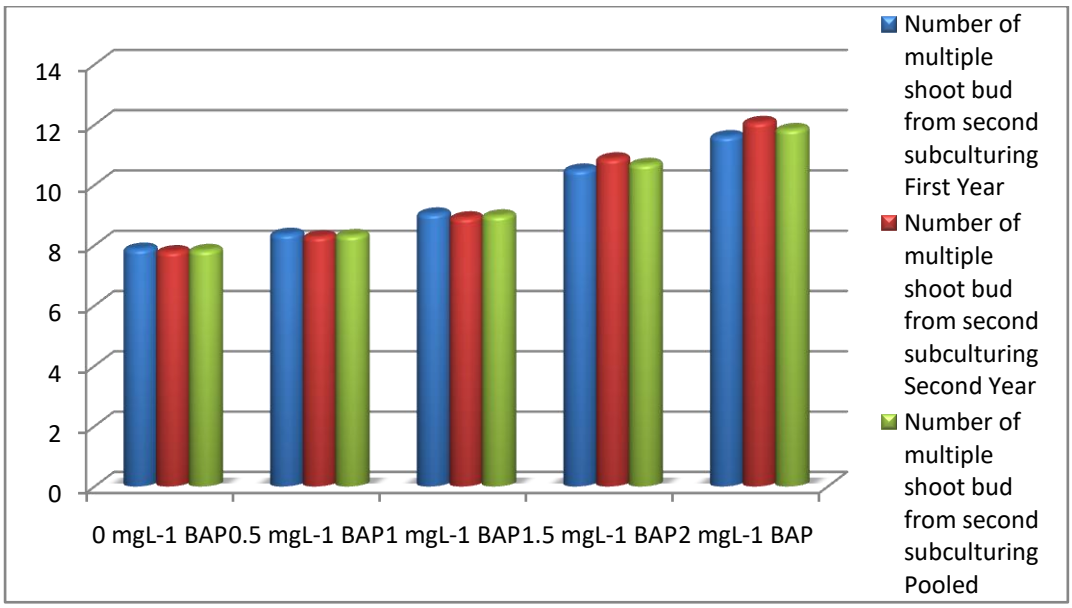


Fig. 4.13: Influence of BAP levels in number of multiple shoot bud during second sub culturing

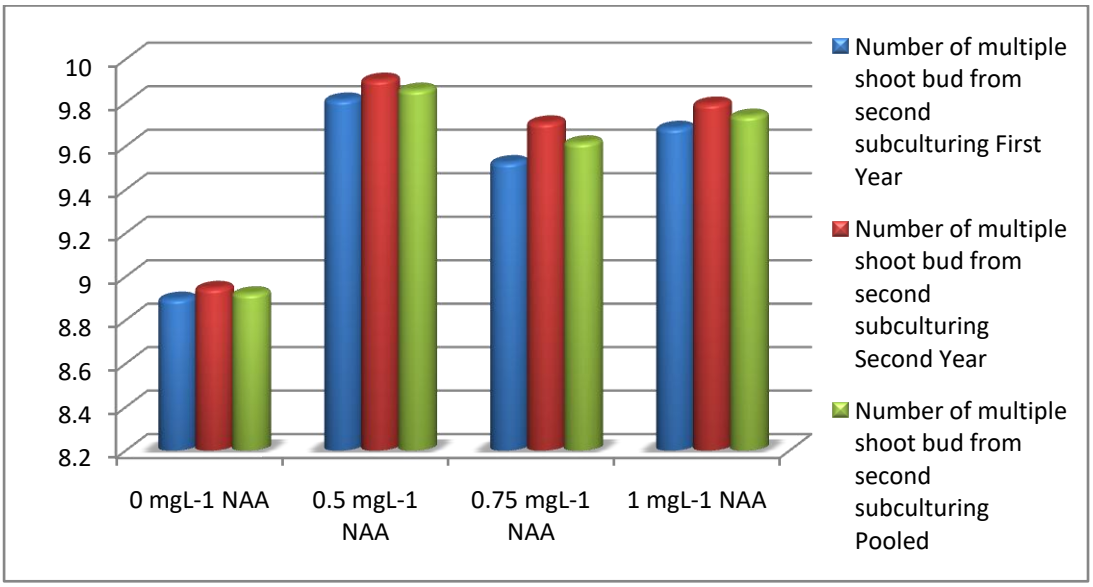


Fig. 4.14: Influence of NAA levels in number of multiple shoot bud during second sub culturing

4.1.8 Length of shoot bud

The data pertaining to length of shoot bud as influenced by various levels of BAP and NAA are shown in Table 4.14, Fig. 4.15 and Fig. 4.16. During the year 2016-17, application of BAP in varied doses significantly increased in the length of shoot bud. The longest length shoot bud (4.92 cm) were recorded in MS media supplement with BAP @ 2 mgL^{-1} followed by media containing BAP @ 1.5 mgL^{-1} (4.75 cm) and the shortest length of shoot bud was recorded in BAP @ 0 mgL^{-1} (2.95 cm). Similarly, during the subsequent year 2017-18 significant result were obtained with the application of BAP. The longest length of shoot bud (4.83 cm) was recorded in the media containing BAP @ 2 mgL^{-1} which is followed by MS media containing BAP @ 1.5 mgL^{-1} (4.58 cm) and the shortest length of shoot bud were recorded in control BAP @ 0 mgL^{-1} (2.92 cm). The pooled analysis of two year data showed that application of BAP showed a significant result in the length of shoot bud. The longest length of shoot bud (4.87 cm) were recorded in the MS media incorporated with BAP @ 2 mgL^{-1} which is followed by BAP @ 1.5 mgL^{-1} (4.66 cm) and the shortest length of shoot bud (2.94 cm) were recorded in control BAP @ 0 mgL^{-1}. Among the different treatments BAP @ 2 mgL^{-1} showed significantly result in elongated shoot which might be due to optimum concentration of hormone that promote RNA synthesis and stimulate protein and enzyme activities in the tissue resulting in the longer length of shoot bud. The current experimental finding is supported by researchers like Rattana and Sangchanjiradet 2017 in *Dendrobium signatum* Rchb.f., Thokchom and Maitra 2017 on *Anthurium andreanum* cv. Jewel, Kabir *et al*. 2013 in *Dendrobium fimbriatum,* Maurya *et al*. 2013 in Rose (*Rosa hybrid* L.) cv. Benjamin Paul and Kosir *et al*. 2004 on *Phalaenopsis* orchids.

MS media supplement with NAA in different level exhibit significant result on the length of *in vitro* shoot bud on both the year 2016-17 and 2017-18. During the year 2016-17, application of NAA @ 0.5 results in the longest

length of shoot bud (4.21 cm) which is followed by NAA @ 0.75 mgL^{-1} (4.11 cm) which is at par with NAA @1 mgL^{-1} (4.03 cm) and the shortest length of shoot bud (3.79 cm) was recorded in control NAA @ 0 mgL^{-1}. In the subsequent year 2017-18 similar result were obtained, the longest length of shoot bud (4.21 cm) were recorded in the treatment containing NAA @ 0.5 mgL^{-1} followed by NAA @ 0.75 mgL^{-1} (4.11 cm) which is at par with NAA @ 1 mgL^{-1} (4.03 cm) and the shortest length of shoot bud (3.79 cm) was recorded in NAA @ 0 mgL^{-1}. The pooled data revealed that NAA application in the MS media showed significant effect on the length of shoot bud. The tallest shoot bud (4.20 cm) were recorded in the treatment NAA @ 0.5 mgL^{-1} followed by NAA @ 0.75 mgL^{-1} (4.09 cm) which is at par with NAA @ 1 mgL^{-1} (4.03 cm) and the shortest length of shoot bud were recorded in NAA @ 0 mgL^{-1} (3.70 cm). The current experimental finding indicated that NAA at higher concentration have negative impact on the height of the plant which might be due to toxicity of hormone at higher concentration. NAA @ 0.5 mgL^{-1} result in most suitable concentration for the length of shoot bud which might be due maintaining of apical dominance promoted by NAA. Similar finding were advocated by Rattana and Sangchanjiradet 2017 in *Dendrobium signatum* Rchb.f., Thokchom and Maitra 2017 in Anthurium andreanum cv. Jewel, Goswani *et al*. 2015 in *Dendrobium* sp. of orchid and Kosir *et al*. 2004 in *Phalaenopsis* orchids.

The data presented in Table 4.15 depict the interaction between various level of BAP and NAA, the pooled data of both the year study revealed that BAP and NAA in their graded level show significant effect on the length of shoot bud. The longest length of shoot bud (5.10 cm) were recorded in treatment combination of BAP @ 2mgL^{-1} + NAA @ 0.5 mgL^{-1}, followed by BAP @ 2 mgL^{-1} + NAA @ 0.75 mgL^{-1} (4.95 cm) which is statistically at par with BAP @ 2mgL^{-1} + NAA @ 1 mgL^{-1} (4.80 cm) and BAP @ 1.5mgL^{-1} + NAA @ 0.5 mgL^{-1} (4.80 cm) and the shortest length of shoot bud (2.88 cm)

were recorded in treatment combination of BAP @ 0 mgL^{-1} + NAA @0 mgL^{-1}. It is evident from the current study result that when cytokinin and auxin are applied in combination they performed better than individual application. This might be due to the reason that they interact with each other to control the development of this symbioses, resulting in the better uptake of nutrient, vitamin and other substance from the media. Among the different treatment combination BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1} result in better performance with respect of shoot elongation. These finding was supported by the finding of Rattana and Sangchanjiradet 2017 in *Dendrobium signatum* Rchb.f., Thokchom and Maitra 2017 on *Anthurium andreanum* cv. Jewel and Kosir *et al*. 2004 on *Phalaenopsis* orchids.

Table 4.14: Effect of 6-BAP and NAA levels on length of shoot bud on the *in vitro* shoot regeneration of *Dendrobium* cv. Sonia Earsakul.

Treatment	*First year*	*Second year*	*Pooled*
B_1 (0 mgL^{-1} BAP)	2.95^{e}	2.92^{e}	2.94^{e}
B_2 (0.5 mgL^{-1} BAP)	3.32^{d}	3.42^{d}	3.37^{d}
B_3 (1 mgL^{-1} BAP)	4.23^{c}	4.12^{c}	4.18^{c}
(B_4) (1.5 mgL^{-1} BAP)	4.75^{b}	4.58^{b}	4.66^{b}
B_5 (2 mgL^{-1} BAP)	4.92^{a}	4.83^{a}	4.87^{a}
Sem±	0.03	0.04	0.03
CD at 5%	0.09	0.13	0.08
N_1 (0 mgL^{-1} NAA)	3.79^{c}	3.61^{c}	3.70^{c}
N_2 (0.5mgL^{-1} NAA)	4.21^{a}	4.19^{a}	4.20^{a}
N_3 (0.75 mgL^{-1} NAA)	4.11^{b}	4.08^{b}	4.09^{b}
N_4 (1 mgL^{-1} NAA)	4.03^{b}	4.02^{b}	4.03^{b}
SEm±	0.03	0.04	0.02
CD at 5%	0.08	0.11	0.07

Table 4.15: Interaction effect of 6-BAP and NAA levels on length of shoot bud in *in vitro* shoot regeneration of *Dendrobium* cv. Sonia Earsakul

Treatments	*First year*	*Second year*	*Pooled*
B_1N_1 (BAP 0 mgL^{-1} + NAA 0 mgL^{-1})	2.92^{hi}	2.84^{e}	2.88^{h}
B_1N_2 (BAP 0 mgL^{-1} + NAA 0.5 mgL^{-1})	3.08^{h}	3.07^{e}	3.08^{g}
B_1N_3(BAP 0 mgL^{-1} + NAA 0.75 mgL^{-1})	2.97^{hi}	2.94^{e}	2.95^{gh}
B_1N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	2.84^{i}	2.83e	2.84^{h}
B_2N_1(BAP 0.5 mgL^{-1} + NAA 0 mgL^{-1})	2.87^{i}	2.84^{e}	2.86^{h}
B_2N_2(BAP 0.5 mgL^{-1} + NAA 0.5 mgL^{-1})	3.57^{fg}	3.73^{d}	3.65^{e}
B_2N_3(BAP 0.5 mgL^{-1} + NAA 0.75 mgL^{-1})	3.43^{g}	3.57^{d}	3.50^{ef}
B_2N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	3.41^{g}	3.55^{d}	3.48^{f}
B_3N_1(BAP 1 mgL^{-1} + NAA 0 mgL^{-1})	3.70^{f}	3.47^{d}	3.59^{ef}
B_3N_2(BAP 1 mgL^{-1} + NAA 0.5 mgL^{-1})	4.48^{d}	4.39^{c}	4.44^{d}
B_3N_3(BAP 1 mgL^{-1} + NAA 0.75 mgL^{-1})	4.38^{d}	4.33^{c}	4.36^{d}
B_3N_4(BAP 1 mgL^{-1} + NAA 1 mgL^{-1})	4.36^{d}	4.30^{c}	4.33^{d}
B_4N_1(BAP 1.5 mgL^{-1} + NAA 0 mgL^{-1})	4.70^{c}	4.23^{c}	4.47^{d}
B_4N_2(BAP 1.5 mgL^{-1} + NAA 0.5 mgL^{-1})	4.84^{bc}	4.77^{ab}	4.80^{bc}
B_4N_3(BAP 1.5 mgL^{-1} + NAA 0.75 mgL^{-1})	4.76^{c}	4.65^{b}	4.70^{c}
B_4N_4(BAP 1.5 mgL^{-1} + NAA 1 mgL^{-1})	4.70^{c}	4.67^{b}	4.69^{c}
B_5N_1(BAP 2 mgL^{-1} + NAA 0 mgL^{-1})	4.74^{c}	4.67^{b}	4.70^{c}
B_5N_2(BAP 2 mgL^{-1} + NAA 0.5 mgL^{-1})	5.10^{a}	5.00^{a}	5.05^{a}
B_5N_3(BAP 2 mgL^{-1} + NAA 0.75 mgL^{-1})	5.00^{ab}	4.89^{ab}	4.95^{b}
B_5N_4(BAP 2 mgL^{-1} + NAA 1mgL^{-1})	4.83^{bc}	4.77^{ab}	4.80^{bc}
SEm±	0.06	0.09	0.05
CD at 5%	0.19	0.25	0.15

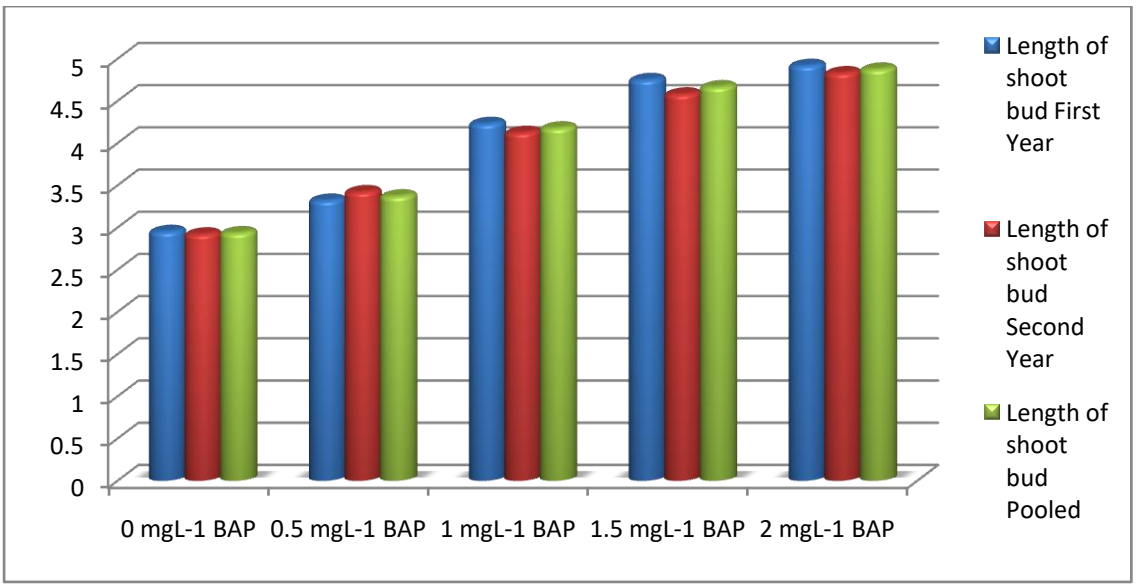


Fig. 4.15: Influence of BAP levels in length of shoot bud

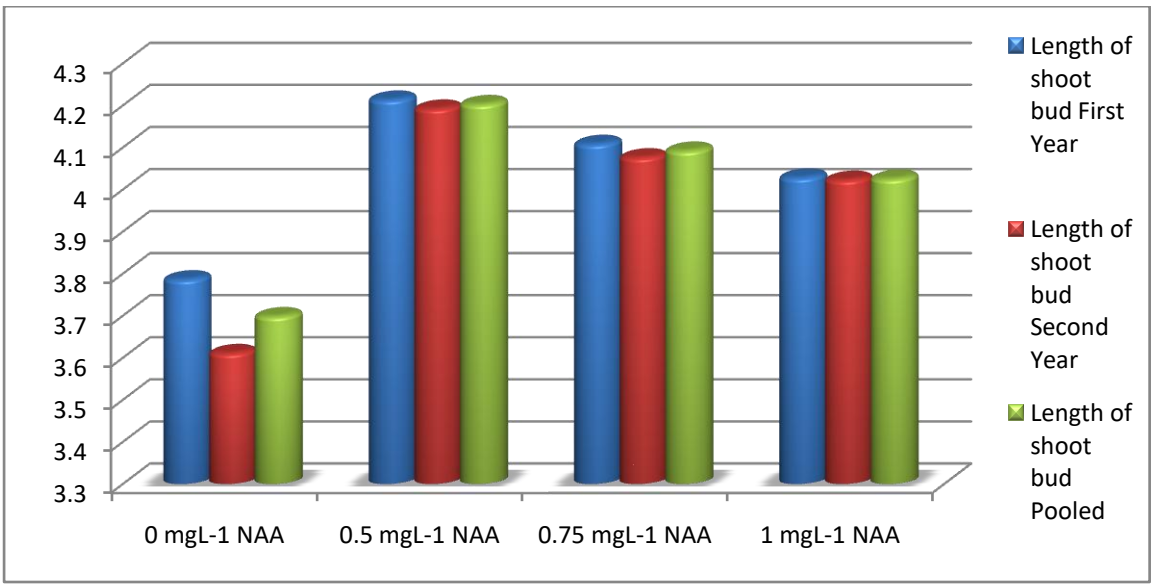


Fig. 4.16: Influence of NAA on the length of shoot

4.1.9 Number of leaves per shoot

Perusals of the results pertaining to the influence of BAP and NAA on the number of leaves on *in vitro* shoot regeneration of *Dendrobium* cv. Sonia Earsakul are presented in Table 4.16, Fig. 4.17 and Fig.4.18. During the year 2016-17, application of BAP in various levels significantly increases the number of leaves per shoot, the maximum number of leaves (4.90) was observed in BAP @ 2 mgL^{-1} followed by BAP @ 1.5 mgL^{-1} (4.71) whereas the least number of leaves (2.95) were recorded in BAP @ 0 mgL^{-1}. Similarly, in the subsequent year 2017-18 results in the significant effect, the maximum number of leaves (5.50) were observed in BAP @ 2 mgL^{-1} followed by BAP @ 1.5 mgL^{-1} (4.66) and the least number of leaves (2.84) were recorded in BAP @ 0 mgL^{-1}. The analysis of both the year showed that application of BAP showed significant results in the number of leaves, the maximum number of leaves (5.20) was observed in BAP @ 2 mgL^{-1} followed by (4.69) BAP @ 1.5 mgL^{-1} whereas the least number of leaves was observed in BAP @ 0 mgL^{-1}. The present experimental findings are in conformity with the findings of Rattana and Sangchanjiradet (2017) in *Dendrobium signatum* Rchb.f. and Kosir *et al*. (2004) on *Phalaenopsis* orchids.

Further observation of data revealed that MS media supplement with NAA exhibit significant difference on the number of leaves on in vitro shoot regeneration of *Dendrobium* cv. Sonia Earsakul. During the first year 2016-17, NAA @ 0.5 mgL^{-1} result in the maximum number of leaves (4.22) per shoot followed by NAA @0.75 mgL^{-1} (4.09) and the least number of leaves (3.74) was recorded in control NAA @0 mgL^{-1}. During the year 2017-18, NAA @ 0.5 mgL^{-1}gives the highest number of leaves (4.36) which is at par with NAA @of 0.75 mgL^{-1} (4.34) and NAA @ 1 mgL^{-1} (4.03) and the least number of leaves (3.69) was recorded in NAA @ 0mgL^{-1}. The pooled data revealed significant difference in the number of leaves per shoot as a result of NAA supplement in MS media. The highest number of leave (4.29) were recorded in treatment

NAA @ 0.5 mgL^{-1} which is followed by NAA @ 0.75 (4.16) which is at par with NAA @1 mgL^{-1} (4.12) and the least number of leave (3.71) were recorded in control. The result indicates that higher level of NAA above 0.5 mgL^{-1} has a negative impact on the numbers of leaves on the *in vitro* multiplication of *Dendrobium* cv. Sonia Earsakul. This might be due to the requirement of these plant growth regulators in low concentration, but promotes cell growth and elongationat optimum concentration. The current experimental findings are in accordance with the findings of Priyanka *et al*. (2018) on *Dendrobium nobile,* Rattana and Sangchanjiradet 2017 in *Dendrobium signatum* Rchb.f. and Kosir *et al*. 2004 on *Phalaenopsis* orchids.

The data presented in Table 4.17 revealed the interaction effect of BAA and NAA in varied doses. The pooled data of the both the study showed that the maximum number of leaves per shoot (5.45) were recorded in the treatment combination of 2 mgL^{-1} BAP + 0.5 mgL^{-1} NAA followed by 2 mgL^{-1} BAP + 1 mgL^{-1} NAA and the least number of leaves per shoot was recorded in treatment combination of 0 mgL^{-1} BAP + 0 mgL^{-1} NAA. A number of PGRs were reported to be least efficient when used individually compared to when applied in combination with other PGRs and the cell can be stimulated to develop into different parts of the plant or even may die Alvard *et al*., 1993. Better growth of the plant is proportional to the numbers of the leaves per shoot. The current experimental finding is in agreement with the findings of researchers like Rattana and Sangchanjiradet 2017 in *Dendrobium signatum* Rchb.f. and Kosir *et al*. 2004 on *Phalaenopsis* orchids.

Table 4.16 Influence of 6-BAP and NAA levels on number of leaves per shoot in *in vitro* multiplication of *Dendrobium* cv. Sonia Earsakul

Treatment	*First year*	*Second year*	*Pooled*
B_1 (0 mgL^{-1} BAP)	2.95^{e}	2.84^{e}	2.89^{e}
B_2 (0.5 mgL^{-1} BAP)	3.31^{d}	3.51^{d}	3.41^{d}
B_3 (1 mgL^{-1} BAP)	4.24^{c}	4.13^{c}	4.19^{c}
(B_4) (1.5 mgL^{-1} BAP)	4.71^{b}	4.66^{b}	4.69^{b}
B_5 (2 mgL^{-1} BAP)	4.90^{a}	5.50^{a}	5.20^{a}
Sem±	0.02	0.05	0.03
CD at 5%	0.06	0.15	0.08
N_1 (0 mgL^{-1} NAA)	3.74^{d}	3.69^{b}	3.71^{c}
N_2 (0.5 mgL^{-1} NAA)	4.22^{a}	4.36^{a}	4.29^{a}
N_3 (0.75 mgL^{-1} NAA)	4.09^{b}	4.24^{a}	4.16^{b}
N_4 (1 mgL^{-1} NAA)	4.03^{c}	4.22^{a}	4.12^{b}
SEm±	0.02	0.05	0.02
CD at 5%	0.06	0.13	0.07

Table 4.17 Interaction effect of 6-BAP and NAA levels in number of leaves per shoot in *in vitro* multiplication of *Dendrobium* cv. Sonia Earsakul

Treatments	*First year*	*Second year*	*Pooled*
B_1N_1 (BAP 0 mgL^{-1} + NAA 0 mgL^{-1})	2.95	2.84^{h}	2.89^{h}
B_1N_2 (BAP 0 mgL^{-1} + NAA 0.5 mgL^{-1})	3.07^{i}	2.87^{h}	2.97^{h}
B_1N_3(BAP 0 mgL^{-1} + NAA 0.75 mgL^{-1})	2.95^{ij}	2.81^{h}	2.88^{h}
B_1N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	2.82^{ij}	2.83^{h}	2.83^{h}
B_2N_1(BAP 0.5 mgL^{-1} + NAA 0 mgL^{-1})	2.75^{j}	3.17^{g}	2.96^{h}
B_2N_2(BAP 0.5 mgL^{-1} + NAA 0.5 mgL^{-1})	3.60^{g}	3.73^{ef}	3.67^{f}
B_2N_3(BAP 0.5 mgL^{-1} + NAA 0.75 mgL^{-1})	3.46^{h}	3.57^{ef}	3.52^{fg}
B_2N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	3.41^{h}	3.55^{ef}	3.48^{g}
B_3N_1(BAP 1 mgL^{-1} + NAA 0 mgL^{-1})	3.69^{g}	3.40^{fg}	3.55^{fg}
B_3N_2(BAP 1 mgL^{-1} + NAA 0.5 mgL^{-1})	4.54^{e}	4.50^{cd}	4.52^{d}
B_3N_3(BAP 1 mgL^{-1} + NAA 0.75 mgL^{-1})	4.35^{f}	4.33^{d}	4.34^{e}
B_3N_4(BAP 1 mgL^{-1} + NAA 1 mgL^{-1})	4.37^{f}	4.30^{d}	4.34^{e}
B_4N_1(BAP 1.5 mgL^{-1} + NAA 0 mgL^{-1})	4.65^{de}	4.23^{d}	4.44^{d}
B_4N_2(BAP 1.5 mgL^{-1} + NAA 0.5 mgL^{-1})	4.78^{cd}	4.93^{b}	4.86^{c}
B_4N_3(BAP 1.5 mgL^{-1} + NAA 0.75 mgL^{-1})	4.73^{cd}	4.75^{bc}	4.74^{c}
B_4N_4(BAP 1.5 mgL^{-1} + NAA 1 mgL^{-1})	4.68^{d}	4.73^{bc}	4.71^{c}
B_5N_1(BAP 2 mgL^{-1} + NAA 0 mgL^{-1})	4.64^{de}	4.80^{bc}	4.72^{c}
B_5N_2(BAP 2 mgL^{-1} + NAA 0.5 mgL^{-1})	5.13^{a}	5.76^{a}	5.45^{a}
B_5N_3(BAP 2 mgL^{-1} + NAA 0.75 mgL^{-1})	4.97^{b}	5.73^{a}	5.35^{ab}
B_5N_4(BAP 2 mgL^{-1} + NAA 1mgL^{-1})	4.85^{bc}	5.70^{a}	5.28^{b}
SEm±	0.05	0.10	0.06
CD at 5%	0.13	0.29	0.16

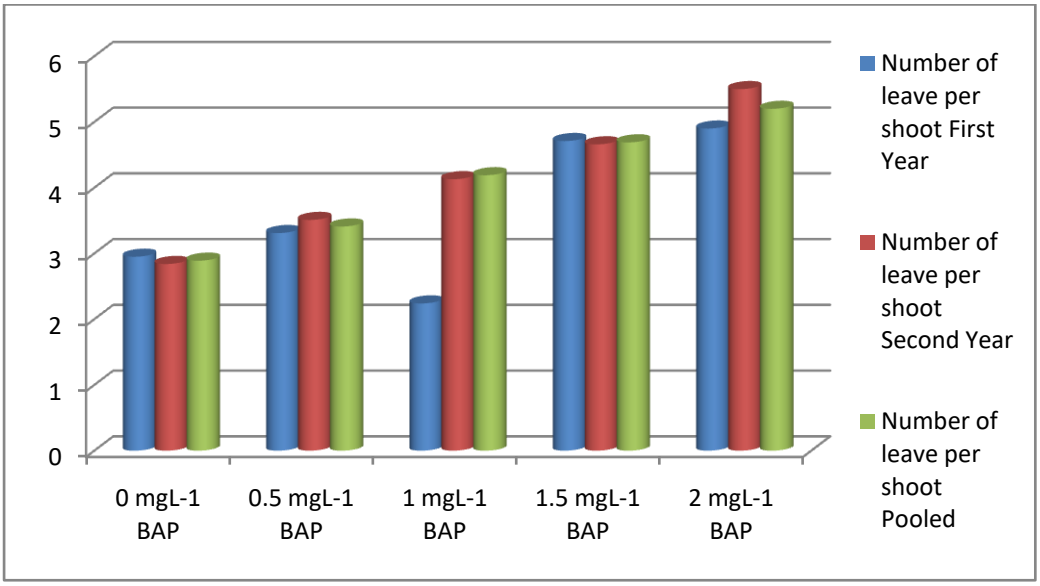


Fig. 4.17: Influence of BAP levels on the number of leave per shoot

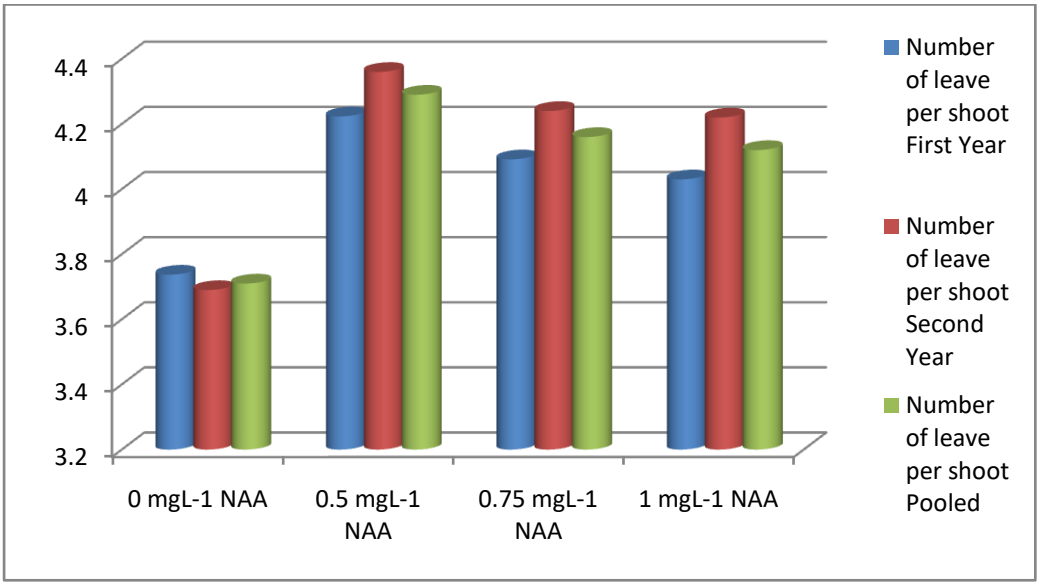


Fig. 4.18: Influence of NAA levels in number of leave per shoot

4.1.10 Fresh weight of shoot at 90 days

The data pertaining to the fresh weight of shoot at 90 days old shoot as influence by varied doses of BAP and NAA are shown in Table 4.18, Fig. 4.19 and Fig. 4.20. During the 2016-17, application of BAP in deferent level significantly increases in the fresh weight of shoot at 90 days. Application of BAP @ 2 mgL^{-1} result in the heaviest fresh weight of shoot (0.57g) followed by BAP @ 1.5 mgL^{-1} (0.55g) and the lightest weight of shoot(0.27g) were recorded in BAP @ 0 mgL^{-1}. Similar result were obtained during the subsequent experiment 2017-18, the heaviest weight fresh weight of shoot at 90 days (0.56 g) were recorded in BAP @ 2 mgL^{-1} followed by (0.51g) BAP @ 1.5 mgL^{-1} and the least weight were recorded in BAP @ 0mgL^{-1}. Pooled analysis of both the year data revealed that application of BAP significantly increased in the fresh weight of shoot at 90 days. BAP @ 2 mgL^{-1} record the heaviest fresh weight of shoot (0.57g) followed by BAP @ 1.5 mgL^{-1} (0.53g) and the least weight of shoot (0.27 g) were recorded in treatment BAP @ 0 mgL^{-1}.

MS media supplemented with varied levels of NAA resulted in significant difference on the fresh weight of shoot at 90 days. In both the year 2016-17 and 2017-18 and the pooled data analysis revealed that the heaviest fresh weight of shoot (0.48g, 0.47g, 0.47g respectively) were recorded in treatment NAA @ 0.5 mgL^{-1} which is followed by NAA @ 0.75 mgL^{-1} (0.44g, 0.44g, 0.44g, respectively). The least fresh weight of shoot (0.42g, 0.40g, 0.41g respectively) at 90 days was recorded in treatment NAA @ 0 mgL^{-1}.

The perusal of the data given in Table 4.19 revealed the significant difference due to interaction effect between various level of BAP and NAA. During the year 2016-17, maximum fresh weight of shoot (0.61g) at 90 days were recorded in the treatment combination of BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1}followed by BAP @ 2 mgL^{-1} + 0.75 mgL^{-1} (0.57g) and the least weight of shoot (0.23g) were recorded in treatment combination of BAP @ 0mgL^{-1} +

NAA @ 0 mgL^{-1}. In the subsequent year trial similar result were obtained, the heaviest fresh weight of shoot (0.60 g) were recorded in the treatment combination of BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1} followed by BAP @ 2 mgL^{-1} + 0.75 mgL^{-1} (0.57g) and the least fresh weight of shoot (0.22 g) were recorded in control. Pooled data analysis of both the year depict that the heaviest weight of shoot (0.61 g) were recorded in treatment combination of BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1} followed by BAP @ 2 mgL^{-1} + 0.75 mgL^{-1} (0.57g) which was at par with BAP @ 1.5 mgL^{-1} + 0.5 mgL^{-1} (0.57 g) and the least fresh weight of shoot at 90 days (0.22 g) were recorded in BAP @ 0 mgL^{-1} + 0 mgL^{-1}.

Table 4.18 Effect of 6-BAP and NAA levels in fresh weight of shoot in micropropagation of *Dendrobium* cv. Sonia Earsakul

Treatment	*First year*	*Second year*	*Pooled*
B_1 (0 mgL^{-1} BAP)	0.27^{e}	0.27^{e}	0.27^{e}
B_2 (0.5 mgL^{-1} BAP)	0.38^{d}	0.40^{d}	0.39^{d}
B_3 (1 mgL^{-1} BAP)	0.43^{c}	0.42^{c}	0.42^{c}
(B_4) (1.5 mgL^{-1} BAP)	0.55^{b}	0.51^{b}	0.53^{b}
B_5 (2 mgL^{-1} BAP)	0.57^{a}	0.56^{a}	0.57^{a}
Sem±	*0.00*	*0.00*	*0.00*
CD at 5%	*0.01*	*0.01*	*0.01*
N_1 (0 mgL^{-1} NAA)	0.42^{c}	0.40^{d}	0.41^{d}
N_2 (0.5 mgL^{-1} NAA)	0.48^{a}	0.47^{a}	0.47^{a}
N_3 (0.75 mgL^{-1} NAA)	0.44^{b}	0.44^{b}	0.44^{b}
N_4 (1 mgL^{-1} NAA)	0.42^{c}	0.42^{c}	0.42^{c}
SEm±	0.00	0.00	0.00
CD at 5%	0.01	0.01	0.01

Table 4.19: Interaction effect of 6-BAP and NAA levels in fresh weight of shoot in micropropagation of *Dendrobium* cv. Sonia Earsakul

Treatments	*First year*	*Second year*	*Pooled*
B_1N_1 (BAP 0 mgL^{-1} + NAA 0 mgL^{-1})	0.23^{j}	0.22^{k}	0.22^{m}
B_1N_2 (BAP 0 mgL^{-1} + NAA 0.5 mgL^{-1})	0.32^{h}	0.31^{i}	0.32^{k}
B_1N_3(BAP 0 mgL^{-1} + NAA 0.75 mgL^{-1})	0.27^{i}	0.29^{i}	0.28^{l}
B_1N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	0.27^{i}	0.27^{j}	0.27^{l}
B_2N_1(BAP 0.5 mgL^{-1} + NAA 0 mgL^{-1})	0.35^{g}	0.38^{h}	0.37^{j}
B_2N_2(BAP 0.5 mgL^{-1} + NAA 0.5 mgL^{-1})	0.41^{f}	0.42^{fg}	0.42^{hi}
B_2N_3(BAP 0.5 mgL^{-1} + NAA 0.75 mgL^{-1})	0.39^{f}	0.40^{gh}	0.40^{i}
B_2N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	0.36^{g}	0.38^{h}	0.37^{j}
B_3N_1(BAP 1 mgL^{-1} + NAA 0 mgL^{-1})	0.40^{f}	0.41^{fg}	0.40^{i}
B_3N_2(BAP 1 mgL^{-1} + NAA 0.5 mgL^{-1})	0.46^{d}	0.46^{e}	0.46^{g}
B_3N_3(BAP 1 mgL^{-1} + NAA 0.75 mgL^{-1})	0.44^{e}	0.43^{f}	0.44^{h}
B_3N_4(BAP 1 mgL^{-1} + NAA 1 mgL^{-1})	0.40^{f}	0.40^{gh}	0.40^{i}
B_4N_1(BAP 1.5 mgL^{-1} + NAA 0 mgL^{-1})	0.53^{c}	0.48^{e}	0.51^{f}
B_4N_2(BAP 1.5 mgL^{-1} + NAA 0.5 mgL^{-1})	0.58^{b}	0.55^{bc}	0.57^{bc}
B_4N_3(BAP 1.5 mgL^{-1} + NAA 0.75 mgL^{-1})	0.55^{c}	0.52^{cd}	0.53^{de}
B_4N_4(BAP 1.5 mgL^{-1} + NAA 1 mgL^{-1})	0.54^{c}	0.50^{d}	0.52^{e}
B_5N_1(BAP 2 mgL^{-1} + NAA 0 mgL^{-1})	0.57^{b}	0.53^{c}	0.55^{cd}
B_5N_2(BAP 2 mgL^{-1} + NAA 0.5 mgL^{-1})	0.61^{a}	0.60^{a}	0.61^{a}
B_5N_3(BAP 2 mgL^{-1} + NAA 0.75 mgL^{-1})	0.57^{b}	0.57^{b}	0.57^{bc}
B_5N_4(BAP 2 mgL^{-1} + NAA 1mgL^{-1})	0.54^{c}	0.55^{bc}	0.55
SEm±	0.01	0.01	0.01
CD at 5%	0.02	0.02	0.01

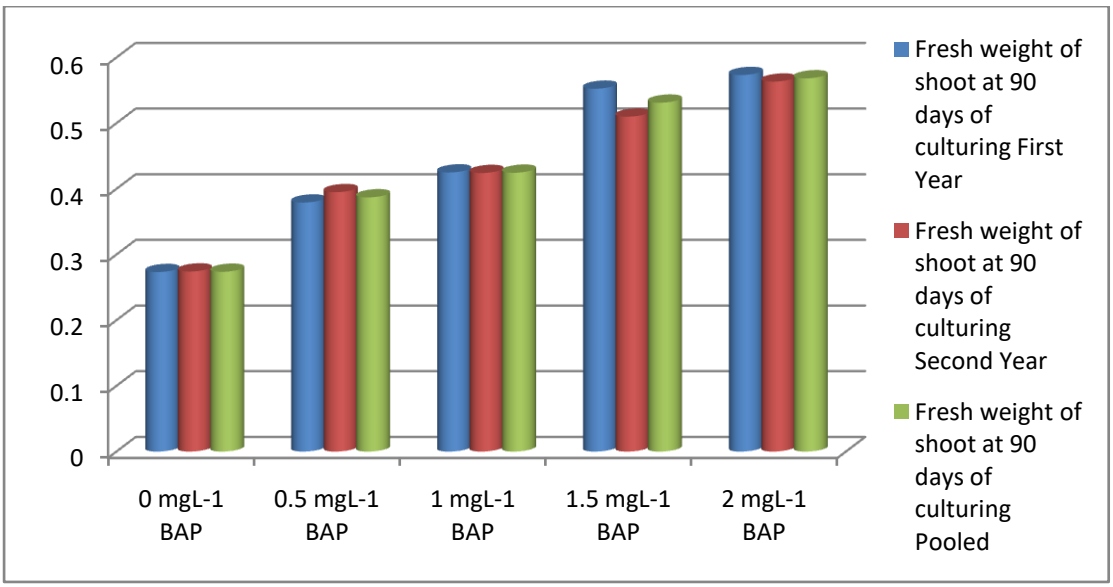


Fig. 4.19: Influence of BAP levels fresh weight of shoot at 90 days

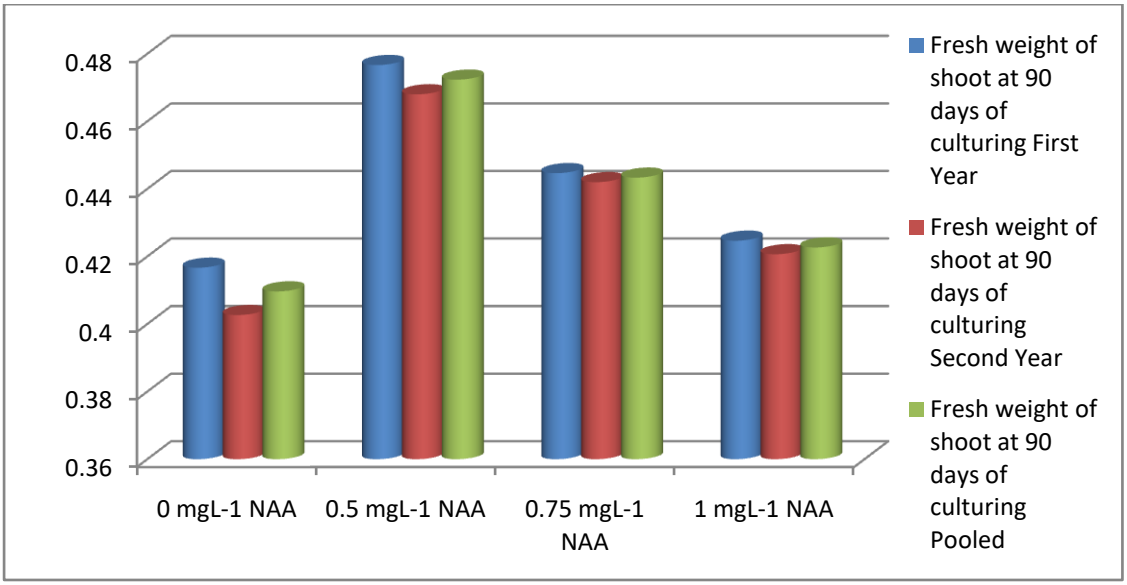


Fig. 4.20: Influence of NAA levels in fresh weight of shoot at 90 days

(A)

(B)

(C)

(D)

Plate 5. (A) Fresh weight of shoot during first sub culturing (B) Length of shoot during first sub culturing (C) Multiple shoot developed from callus culture (D) Mass of callus.

4.32 Fresh weight of shoot during hardening stage

Data on the fresh weight of shoot during hardening stage as influence by different level of BAP and NAA are presented in Table 4.20, Fig. 4.21 and Fig. 4.22. During the year 2016-17, application of BAP in varied doses result in significant effect on the fresh weight of shoot during hardening stage. BAP @ 2 mgL^{-1} result in the maximum fresh weight (0.92 g) of shoot during the hardening stage followed by BAP @ 1.5 mgL^{-1} (0.85 g) and the least fresh weight of shoot (0.70 g) during hardening stage were recorded in BAP @ 0 mgL^{-1}. Similar result were obtained during the subsequent year (2017-18) BAP in various doses showed significant difference, the highest fresh weight of shoot (0.98 g) were recorded in treatment BAP @ 2 mgL^{-1} followed by BAP @ 1.5 mgL^{-1} (0.91 g) and the lightest fresh weight (0.73 g) were observed in BAP @ 0 mgL^{-1}. The pooled data from both years revealed significant difference in the fresh weight of shoot with the application of BAP, the highest fresh weight (0.95 g) of shoot were recorded in treatment BAP @ 2 mgL^{-1} followed by BAP @ 1.5 mgL^{-1} (0.88 g) and the least fresh weight of shoot (0.73 g) was recorded in control. BAP is widely used in tissue culture work as it enhances cell division, facilitates numerous aspect of plant growth, induces chloroplast differentiation and regeneration of shoot (Chawla, 2009). The current experimental findings are in accordance with the findings of Kabir *et al*. (2013), Kosir *et al*. (2004) on *Phalaenopsis* orchids and Rattana and Sangchanjiradet (2017) in *Dendrobium signatum* Rchb.f.

Supplementation of MS media with NAA exhibited significant result on the fresh weight of shoot during hardening stage. During the year 2016-17, the heaviest fresh weight of shoot (0.82 g) was recorded in NAA @ 0.5 mgL^{-1} followed by NAA @ 0.75 mgL^{-1} (0.80 g) which was at par with NAA @ 1 mgL^{-1} (0.79 g) and the least fresh weight (0.76 g) was recorded in control. In the year 2017-18, similar result were observed, the heaviest fresh weight of shoot (0.88 g) was recorded in treatment NAA @ 0.5 mgL^{-1} followed by NAA

@ 1 mgL^{-1} (0.85 g) which is at par with NAA @ 0.75 mgL^{-1} (0.84 g) and the least fresh weight (0.80 g) were recorded in control. Pooled data analysis of both the year revealed significant difference, NAA @ 0.5 mgL^{-1} result in the heaviest fresh weight of shoot (0.58 g) during the hardening stage followed by NAA @ 0.75 mgL^{-1} and NAA @ 1 mgL^{-1} (82 g each) and the least fresh weight (0.78 g) were recorded in control. The present study results are in line with the findings of Goswani *et al*. (2015) in *Dendrobium* sp. of orchid Kosir *et al*. (2004) on *Phalaenopsis* orchids, Rattana and Sangchanjiradet (2017) in *Dendrobium signatum* Rchb.f.

The interaction effect of BAP and NAA on the fresh weight of shoot during hardening stage is presented in Table 4.21. The pooled data in the table depict that the highest fresh weight (0.99 g) of shoot during hardening stage were recorded in treatment combination of which is at par with BAP @ 2 mgL^{-1} + NAA @ 0.75 mgL^{-1} (0.95 g) and the least fresh weight of shoot during hardening stage were recorded in control, BAP @ 0 mgL^{-1} + NAA @ 0.75 mgL^{-1} and BAP @ 0 mgL^{-1} + NAA @ 1 mgL^{-1} (0.72 g each). It was observed that when BAP or NAA are added individually the fresh weight of the shoot is lesser comparing to combine effect of BAP and NAA. Mathew and Rao 1985, reported that auxin alone were conducive for protocorm multiplication whereas cytokinin alone cause necrosis, auxin and cytokinin combinations enhanced differentiation of protocorm. The current experimental finding are in line with the finding of Kosir *et al*. 2004 in *Phalaenopsis* orchids and Rattana and Sangchanjiradet 2017 in *Dendrobium signatum* Rchb.f.

Table 4.20: Effect of 6-BAP and NAA levels in fresh weight of shoot during hardening stage in *in vitro* shoot multiplication of *Dendrobium* cv. Sonia Earsakul

Treatment	*First year*	*Second year*	*Pooled*
B_1 (0 mgL^{-1} BAP)	0.70^{e}	0.75^{e}	0.73^{e}
B_2 (0.5 mgL^{-1} BAP)	0.72^{d}	0.77^{d}	0.75^{d}
B_3 (1 mgL^{-1} BAP)	0.77^{c}	0.81^{c}	0.79^{c}
(B_4) (1.5 mgL^{-1} BAP)	0.85^{b}	0.91^{b}	0.88^{b}
B_5 (2 mgL^{-1} BAP)	0.92^{a}	0.98^{a}	0.95^{a}
Sem±	0.01	0.01	0.00
CD at 5%	0.02	0.02	0.01
N_1 (0 mgL^{-1} NAA)	0.76^{c}	0.80^{c}	0.78^{c}
N_2 (0.5 mgL^{-1} NAA)	0.82^{a}	0.88^{a}	0.85^{a}
N_3 (0.75 mgL^{-1} NAA)	0.80^{b}	0.84^{b}	0.82^{b}
N_4 (1 mgL^{-1} NAA)	0.79^{b}	0.85^{b}	0.82^{b}
SEm±	0.01	0.01	0.00
CD at 5%	0.02	0.02	0.01

Table 4.21: Interaction effect of 6-BAP and NAA levels in fresh weight of shoot during hardening stage in *in vitro* shoot multiplication of *Dendrobium* cv. Sonia Earsakul.

Treatments	*First year*	*Second year*	*Pooled*
B_1N_1 (BAP 0 mgL^{-1} + NAA 0 mgL^{-1})	0.70^{h}	0.74^{e}	0.72^{f}
B_1N_2 (BAP 0 mgL^{-1} + NAA 0.5 mgL^{-1})	0.73^{gh}	0.76^{de}	0.74^{f}
B_1N_3(BAP 0 mgL^{-1} + NAA 0.75 mgL^{-1})	0.70^{h}	0.74^{e}	0.72^{f}
B_1N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	0.69^{h}	0.74e	0.72^{f}
B_2N_1(BAP 0.5 mgL^{-1} + NAA 0 mgL^{-1})	0.72^{gh}	0.74^{e}	0.73^{f}
B_2N_2(BAP 0.5 mgL^{-1} + NAA 0.5 mgL^{-1})	0.74^{g}	0.81^{cd}	0.77^{e}
B_2N_3(BAP 0.5 mgL^{-1} + NAA 0.75 mgL^{-1})	0.73^{gh}	0.76^{de}	0.74^{f}
B_2N_4(BAP 0.5 mgL^{-1} + NAA 1 mgL^{-1})	0.71^{gh}	0.76^{de}	0.74^{f}
B_3N_1(BAP 1 mgL^{-1} + NAA 0 mgL^{-1})	0.71^{gh}	0.76^{de}	0.74^{f}
B_3N_2(BAP 1 mgL^{-1} + NAA 0.5 mgL^{-1})	0.80^{f}	0.85^{c}	0.82^{d}
B_3N_3(BAP 1 mgL^{-1} + NAA 0.75 mgL^{-1})	0.79^{f}	0.81^{cd}	0.80^{de}
B_3N_4(BAP 1 mgL^{-1} + NAA 1 mgL^{-1})	0.78^{f}	0.83^{c}	0.81^{d}
B_4N_1(BAP 1.5 mgL^{-1} + NAA 0 mgL^{-1})	0.80^{f}	0.80^{cd}	0.80^{de}
B_4N_2(BAP 1.5 mgL^{-1} + NAA 0.5 mgL^{-1})	0.89^{cd}	0.97^{b}	0.93^{ab}
B_4N_3(BAP 1.5 mgL^{-1} + NAA 0.75 mgL^{-1})	0.86^{de}	0.93^{b}	0.90^{c}
B_4N_4(BAP 1.5 mgL^{-1} + NAA 1 mgL^{-1})	0.85^{e}	0.95^{b}	0.90^{c}
B_5N_1(BAP 2 mgL^{-1} + NAA 0 mgL^{-1})	0.88^{cd}	0.95^{b}	0.92^{bc}
B_5N_2(BAP 2 mgL^{-1} + NAA 0.5 mgL^{-1})	0.95^{a}	1.03^{a}	0.99^{a}
B_5N_3(BAP 2 mgL^{-1} + NAA 0.75 mgL^{-1})	0.93^{ab}	0.97^{b}	0.95^{a}
B_5N_4(BAP 2 mgL^{-1} + NAA 1mgL^{-1})	0.91^{bc}	0.96^{b}	0.93^{ab}
SEm±	0.01	0.02	0.01
CD at 5%	0.03	0.04	0.03

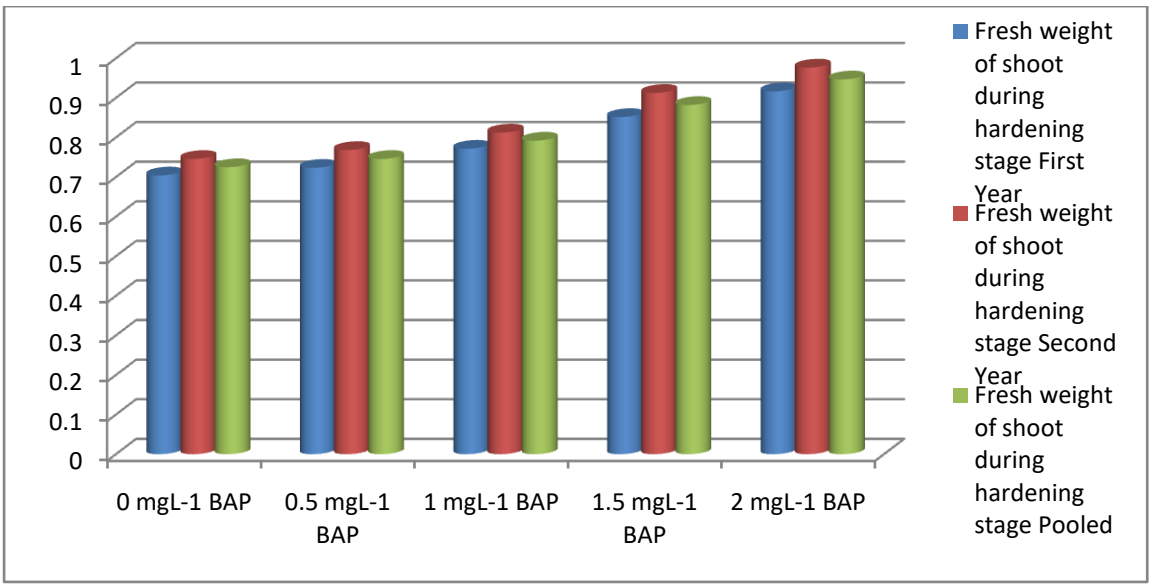


Fig. 4.21: Effect of BAP levels in fresh weight of shoot during hardening stage

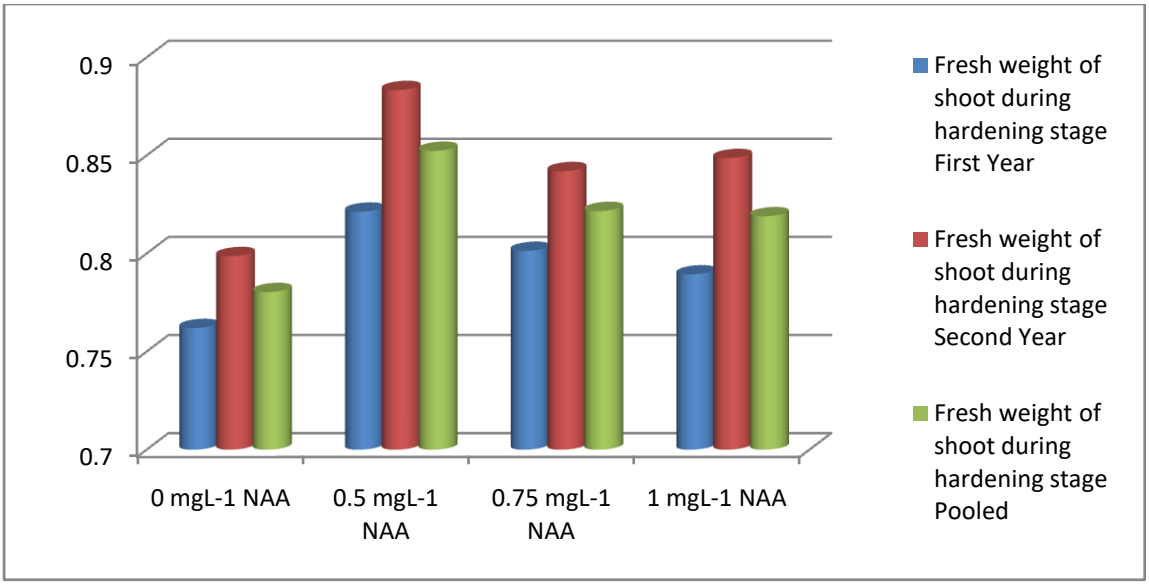


Fig. 4.22: Effect of NAA levels in fresh weight of shoot during hardening stage

4.2. Experiment 2: Study on *in vitro* root regeneration of *Dendrobium* cv. Sonia Earsakul

4.2.1 Days to root initiation

Perusals of the results pertaining to the influence of growth hormones on the days to initiation of shoot are depicted in Table 4.22 and Fig.4. The earliest days taken to root initiation (34.93 days) during the year 2017-18 were recorded in IBA @ 1 mgL^{-1} which is followed by IBA @ 1.5 mgL^{-1} (36.17 days) and maximum number of days required for initiation of root were recorded in control (46.68 days) which is statistically at par with IAA @ 2 mgL^{-1} (45.03 days) and NAA @ 2 mgL^{-1} (44.83 days). In the next year trial 2018-19, it was also observed that the earliest days (34.87 days) taken to root initiation were recorded in treatment IBA @ 1 mgL^{-1} followed by followed by IBA @ 1.5 mgL^{-1} (36.03 days) and IBA @ 2 mgL^{-1} (37.03 days) and the maximum number of days taken to root initiation were recorded in control (46.38 days) which is at par with NAA @ 2 mgL^{-1} (45.84 days) and IAA @ 2 mgL^{-1} (44.52 days). Further analysis of both the year pooled data revealed significant difference, the shortest days taken to root initiation (34.90 days) were recorded in IBA @ 1 mgL^{-1} which statistically at par with IBA @ 1.5 mgL^{-1} (36.10 days) which is followed by IBA @ 2 mgL^{-1} (37.18 days) and the maximum number of days (46.38 days) taken to root initiation were recorded in control (46.38 days) which is statistically at par with NAA @ 2 mgL^{-1} (45.34 days) and IAA @ 2 mgL^{-1} (44.78 days). Among the different type hormone use for regeneration of roots from developed shoot, IBA @ of 1 mgL^{-1} performed better NAA and IAA. Dunwell (1981) reported that each species require particular hormone concentration of optimum growth and differentiation. Nongdam and Tikendra 2014 reported IBA was more effective in root induction as compared to NAA and IAA, and the current experimental finding is in line Aktar *et al*. 2007 in *Dendrobium* orchid.

4.2.2 Number of shoots per explant

Analysis of data pertaining to the influence of growth hormones on the number of shoots per explant are given in Table 4.22 and Fig. 4.23**.** During the year 2017-18, results showed significant difference, the maximum number of

shoot per explants (2.10) was recorded in treatment IBA @ 1 mgL^{-1} which is at par with IBA @ 1.5 mgL^{-1} (2.0) followed by IBA @ 2 mgL^{-1} (1.83) and the least number of shoot per explants (1.47) was recorded in control which is at par with NAA @ 0.5 mgL^{-1} (1.57). In the subsequent year 2018-19, growth hormones application resulted in significant difference in the number of shoot per explants. The maximum number of shoot (2.15) was recorded in IBA @ 1 mgL^{-1} followed by IBA @ 1.5 mgL^{-1} (2.10) which is statistically at par with NAA @1 mgL^{-1} (1.93) and the least number of shoot was recorded in control which was statistically at par with NAA @ 0.5 mgL^{-1}. Pooled data analysis exhibited significant difference on the number of shoot, the maximum number of shoot (2.13) was recorded in IBA @ 1 mgL^{-1} followed by IBA @ 1.5 mgL^{-1} (2.05) which is statistically at par with NAA @ 1 mgL^{-1} (1.92) and the least number of shoot (1.48) was recorded in control. This might be due to the fact that better rooting resulted in better uptake of the nutrient from the MS media which resulted in production of more number of shoot. The findings are in line with the findings of Panathula *et al.* 2014 in *Centella asiatica* (L), Aktar *et al*. 2007 in *Dendrobium* orchid.

Table 4.22: Influence of growth hormone on days to root initiation and number of shoots per explants in *in vitro* root regeneration of *Dendrobium* cv. Sonia Earsakul

Treatment	*Days to root initiation*			*Number of shoot per explant*		
	2017-18	*2018-19*	*Pooled*	*2017-18*	*2018-19*	*Pooled*
T_1(MS)	46.68^{a}	46.07^{a}	46.38^{a}	1.47^{f}	1.50^{e}	1.48^{g}
T_2(MS + 0.5 mgL^{-1} IBA)	37.63^{cde}	37.54^{cde}	37.59^{ef}	1.73^{c}	1.77^{c}	1.75^{d}
T_3(MS + 1 mgL^{-1} IBA)	34.93^{e}	34.87^{f}	34.90^{g}	2.10^{a}	2.15^{a}	2.13^{a}
T_4(MS + 1.5 mgL^{-1} IBA)	36.17^{de}	36.03^{ef}	36.10^{fg}	2.00^{ab}	2.10^{ab}	2.05^{ab}
T_5(MS + 2 mgL^{-1} IBA)	37.33^{cde}	37.03^{def}	37.18^{ef}	1.83^{b}	1.85^{c}	1.84^{cde}
T_6(MS + 0.5 mgL^{-1} NAA)	38.97^{cd}	38.85^{cd}	38.91^{de}	1.57^{ef}	1.59^{d}	1.58^{f}
T_7(MS + 1 mgL^{-1} NAA)	36.87^{cd}	37.94^{cde}	37.41^{ef}	1.90^{bc}	1.93^{ab}	1.92^{bc}
T_8(MS + 1.5 mgL^{-1} NAA)	42.20^{b}	42.07^{b}	42.14^{c}	1.87^{bcd}	1.88^{bc}	1.88^{cd}
T_9(MS + 2 mgL^{-1} NAA)	44.83^{a}	45.84^{a}	45.34^{ab}	1.85^{bcd}	1.86^{c}	1.85^{cd}
T_{10}(MS + 0.5 mgL^{-1} 1AA)	39.37^{c}	39.53^{c}	39.45^{d}	1.67^{de}	1.72^{cde}	1.69^{ef}
T_{11}(MS + 1 mgL^{-1} 1AA)	38.60^{cd}	37.52^{cde}	38.06^{de}	1.83^{bcd}	1.85^{c}	1.84^{cde}
T_{12}(MS + 1.5 mgL^{-1} 1AA)	44.17^{ab}	43.88^{ab}	44.02^{b}	1.77^{cd}	1.78^{cd}	1.78^{cde}
T_{13}(MS + 2 mgL^{-1} 1AA)	45.03^{a}	44.52^{a}	44.78^{ab}	1.75^{cde}	1.77^{cd}	1.76^{de}
SEm±	0.89	0.76	0.59	0.06	0.07	0.05
CD at 5%	2.60	2.21	1.66	0.17	0.21	0.13

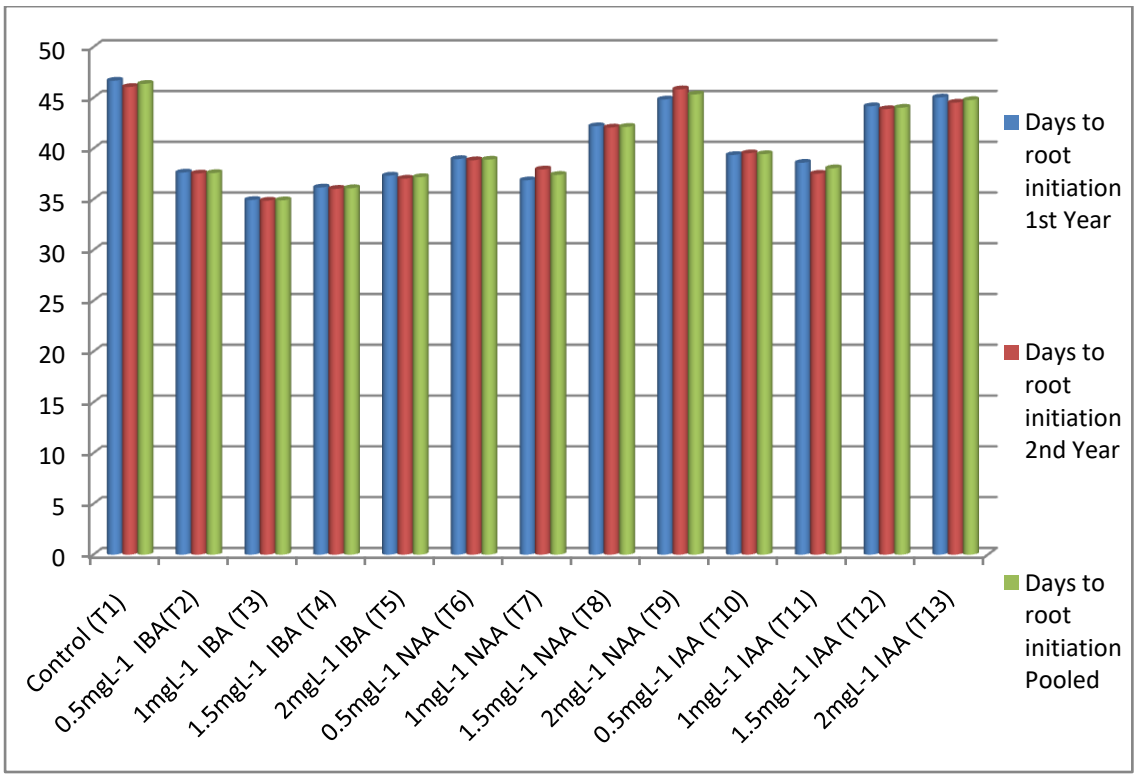


Fig. 4.23: Effect of growth hormones on the days to root initiation

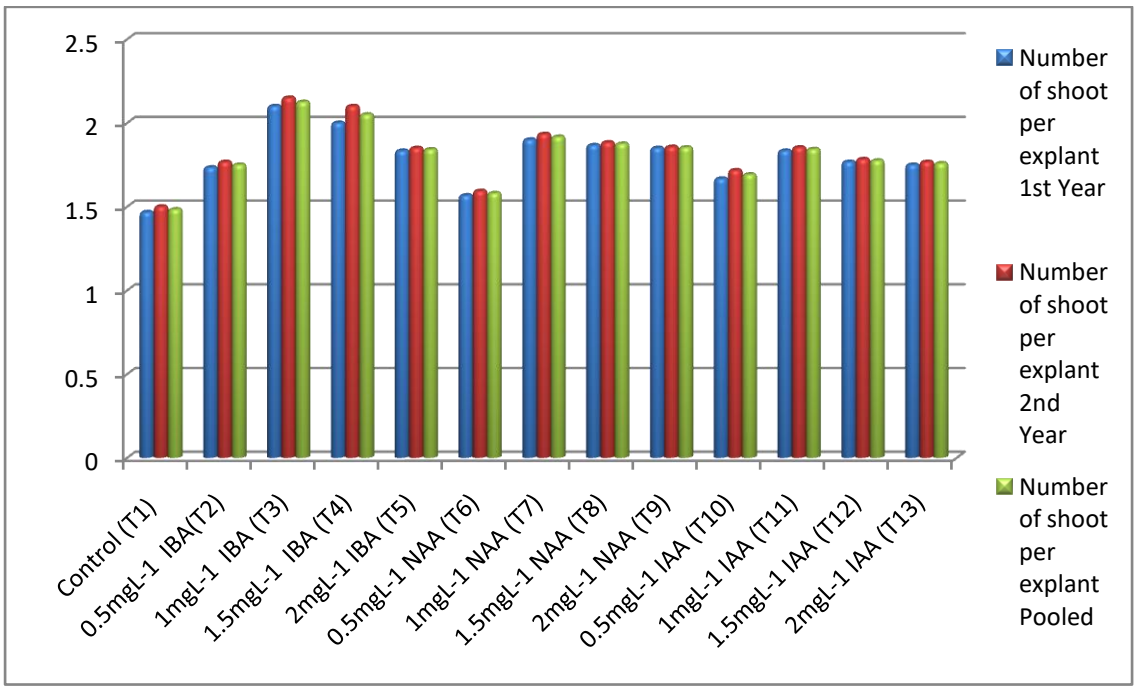


Fig. 4.24: Influence of growth hormones on the number of shoot per explants

(A) (B)

(C) (D)

Plate 6.

(A) Culturing of developed shoot in rooting media

(B) Root initiation in treatment IBA @ 1 mgL^{-1}

(C) Initial Hardening inside room to acclimatize in natural environment

(D) Opening the bottle cap prior to hardening

4.2 Number of root per shoot

The data pertaining to number of root per shoot as influence by different hormones in graded levels are presented in Table 4.23 and Fig. 4.24. Application of growth hormones IBA, NAA and IAA resulted in significant difference, the maximum number of root per shoot (8.46) were recorded in treatment IBA @ 1 mgL^{-1} followed by NAA @ 1 mgL^{-1} (8.20) and the least number of root per shoot (4.80) was recorded in control. In the subsequent year 2018-19, the highest number of root per shoot (8.32) was recorded in treatment IBA @ 1 mgL^{-1} which is at par with NAA @ 1 mgL^{-1} and the least number of roots per shoot (4.73) was recorded in control. Pooled data analysis revealed significant difference, the maximum number of root per shoot (8.39) was observed in treatment IBA @ 1mgL^{-1} followed by NAA @ 1mgL^{-1} (8.16) which is at par with IBA @ 1.5 mgL^{-1} (7.92) and the least number of roots per shoot (4.77) was recorded in control. Current experimental findings are in confirmation with the findings of Nongdam and Tikendra (2014) where IBA was more effective in root induction as compared to NAA and IAA. Similar findings on the number of roots were reported by Aktar *et al*. (2007) where highest number of root was recorded in treatment IBA @ 1 mgL^{-1}in *Dendrobium* orchid, Rahman *et al*. (2009) in *Vanda tessellate* L. where maximum root induction was recorded in treatment IBA @ 1 mgL^{-1}, Panuthula *et al*. (2014) reported maximum rooting frequency with high mean root number in *Centella asiatica* (L).

4.5 Number of functional root

Perusal of the results pertaining to the influence of plant growth regulators on number of functional root are depicted in Table 4.23 and Fig. 4.24. During the first year 2017-18, application of different plant growth regulators in different levels exhibited significant difference on the number of functional roots. The maximum number of functional roots (6.97) was obtained

in treatment IBA @ 1 mgL^{-1} followed by IBA @ 1.5 mgL^{-1} (6.82) which is at par with NAA @1 mgL^{-1} (6.80) and the least number of functional roots (3.93) was recorded in control. In the subsequent year 2018-19 study, the maximum number of functional root (6.90) was recorded in treatment IBA @ 1 mgL^{-1} which is at par with NAA @ 1 mgL^{-1} (6.86) followed by IBA @ 1.5 mgL^{-1} (6.82) which is at par with IBA @ 2 mgL^{-1} (6.78) and NAA @ 1.5 mgL^{-1} (6.72) and the least number of functional root was recorded in control (4.0). Pooled data analysis of both the years study revealed significant result, the maximum number of functional root (6.93) was recorded in IBA @ 1 mgL^{-1} followed by IBA @ 1.5 mgL^{-1} (6.82) which is at par with IBA @ 2 mgL^{-1} (6.78) and NAA @ 1 mgL^{-1} (6.83) and the least number of functional number of root were recorded in control (3.97). Among the different type of hormones used in graded doses, IBA @ 1 mgL^{-1} resulted in significant difference then the other treatments. Nongdam and Tikendra 2014 reported that IBA resulted better in root regeneration as compared to NAA and IAA. Results of the current study are in accordance with the findings of Aktar *et al*. 2007 in *Dendrobium* orchid, Rahman *et al*. 2009 in *Vanda tessellate*, Panuthula *et al*. 2014 in *Centella asiatica* (L).

Table 4.23: Effect of growth hormones on number of root per shoots and number of functional roots in *in vitro* root regeneration of *Dendrobium* cv. Sonia Earsakul

Treatment	*Number of root per shoot*			*Number of functional root*		
	2017-18	*2018-19*	*Pooled*	*2017-18*	*2018-19*	*Pooled*
T_1(MS)	4.80^{e}	4.73^{f}	4.77^{h}	3.93^{g}	4.00^{f}	3.97^{g}
T_2(MS + 0.5 mgL^{-1} IBA)	6.51^{cd}	6.48^{d}	6.49^{ef}	4.93^{e}	4.80^{e}	4.87^{f}
T_3(MS + 1 mgL^{-1} IBA)	8.46^{a}	8.32^{a}	8.39^{a}	6.97^{a}	6.90^{a}	6.93^{a}
T_4(MS + 1.5 mgL^{-1} IBA)	8.00^{ab}	7.84^{b}	7.92^{bc}	6.82^{ab}	6.82^{ab}	6.82^{ab}
T_5(MS + 2 mgL^{-1} IBA)	7.93^{ab}	7.75^{b}	7.84^{c}	6.77^{abc}	6.78^{ab}	6.78^{ab}
T_6(MS + 0.5 mgL^{-1} NAA)	6.14^{d}	5.92^{e}	6.03^{g}	4.87^{e}	4.93^{e}	4.90^{f}
T_7(MS + 1 mgL^{-1} NAA)	8.20^{ab}	8.12^{a}	8.16^{ab}	6.80^{ab}	6.86^{a}	6.83^{ab}
T_8(MS + 1.5 mgL^{-1} NAA)	7.87^{ab}	7.58^{b}	7.72^{c}	6.65^{bc}	6.72^{ab}	6.68^{bc}
T_9(MS + 2 mgL^{-1} NAA)	7.63^{b}	7.58^{b}	7.61^{c}	6.35^{d}	6.34^{d}	6.34^{e}
T_{10}(MS + 0.5 mgL^{-1} 1AA)	6.30^{d}	6.25^{d}	6.28^{fg}	4.58^{f}	4.77^{e}	4.68
T_{11}(MS + 1mgL^{-1} 1AA)	7.05^{c}	6.91^{c}	6.98^{d}	6.52^{bc}	6.64^{bc}	6.58^{cd}
T_{12}(MS + 1.5 mgL^{-1} 1AA)	7.77^{b}	7.68^{b}	7.73^{c}	6.48^{cd}	6.46^{cd}	6.47^{de}
T_{13}(MS + 2 mgL^{-1} 1AA)	7.67^{b}	7.60^{b}	7.63^{c}	6.47^{cd}	6.37^{d}	6.42^{de}
SEm±	0.19	0.08	0.10	0.09	0.06	0.05
CD at 5%	0.55	0.24	0.29	0.25	0.19	0.15

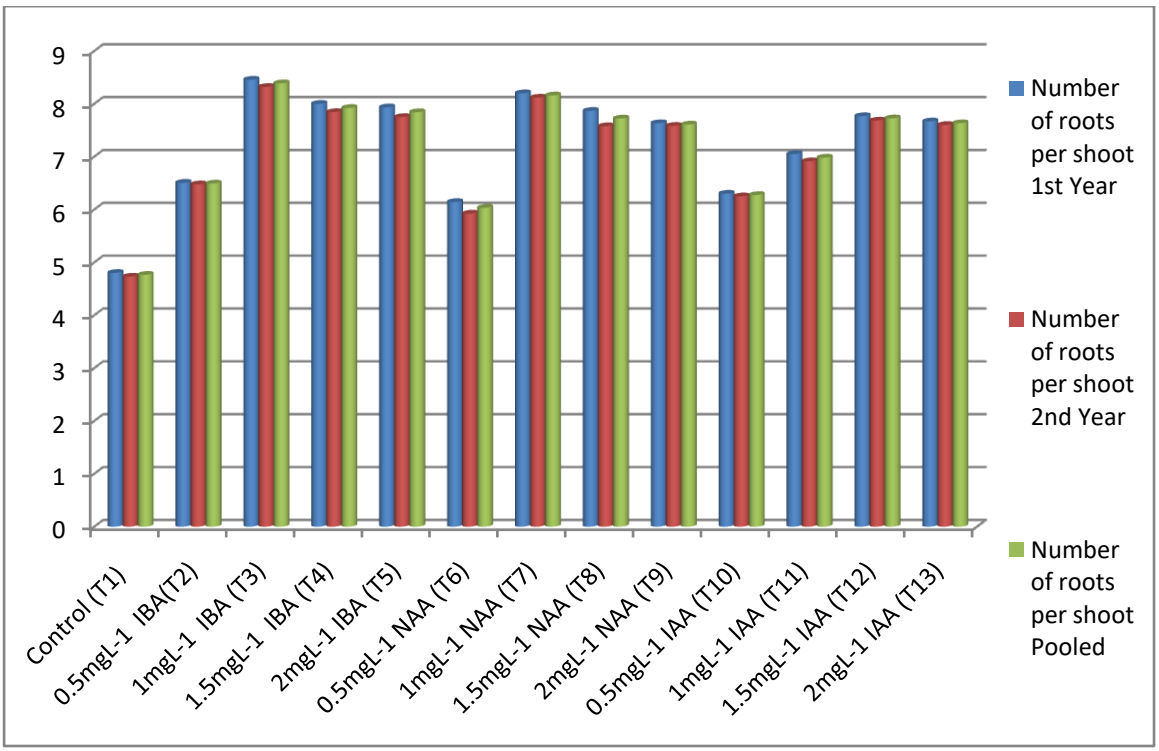


Fig. 4.25: Effect of plant growth regulators in number of roots per shoot

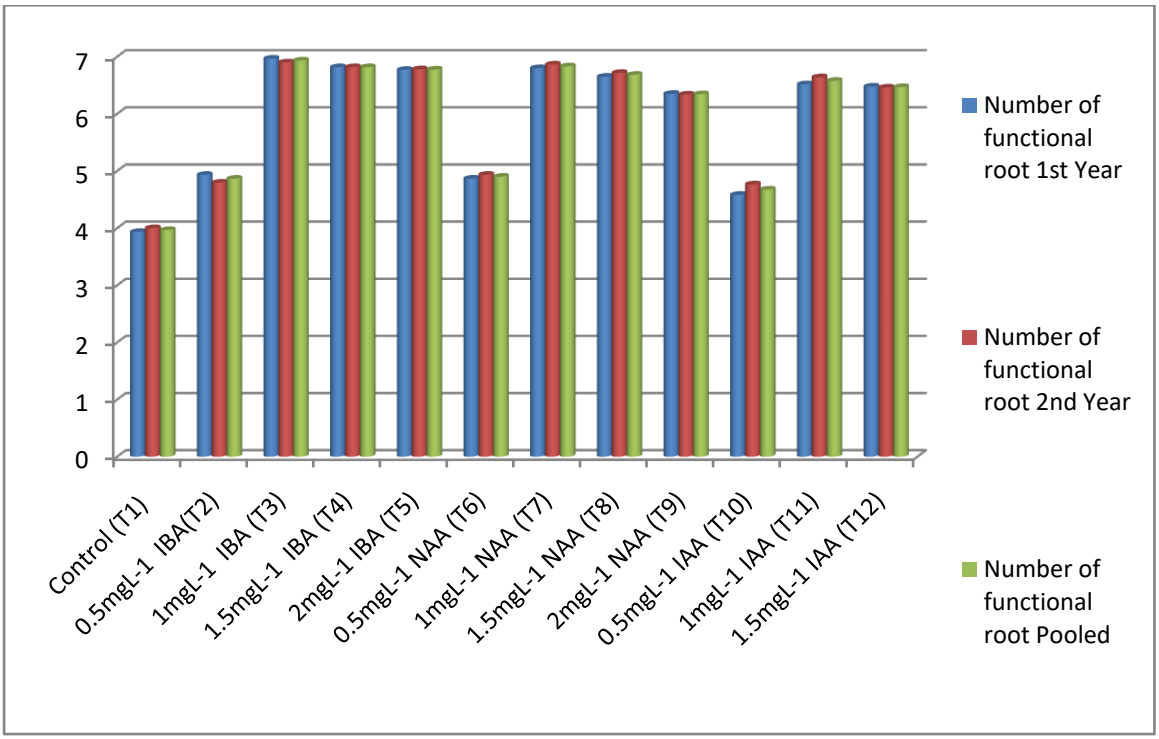


Fig. 4.26: Effect of plant growth regulators on the number of functional roots

Control | 0.5 mgL^{-1} IBA | 1.0 mgL^{-1} IBA

1.5 mgL^{-1} IBA | 2.0 mgL^{-1} IBA | 0.5 mgL^{-1} NAA

1.0 mgL^{-1} NAA | 1.5 mgL^{-1} NAA | 2.0 mgL^{-1} NAA

0.5 mgL^{-1} IAA | 1.0 mgL^{-1} IAA | 1.5 mgL^{-1} IAA

Plate 7. Rooted plantlets in different treatments

4.2.5 Length of root

Analysis of data given in Table 4.24 Fig. 4.26, revealed the effect of various plant growth regulators in different levels on the length of root of *Dendrobium* cv. Sonia Earsakul for *in vitro* root regeneration. During the year 2017-18, the longest length of root (6.05 cm) was recorded in IBA @ 1 mL^{-1} followed by IBA @ 0.5 mgL^{-1} (5.93 cm) and the shortest length of root (4.78 cm) was recorded in control. Similarly in the subsequent year 2018-19, the longest length of root (5.90 cm) was recorded in IBA @ 1 mgL^{-1} followed by IBA @ 1.5 mgL^{-1} (5.85 cm) which is at par with NAA @ 1 mgL^{-1} (5.83 cm) and the shortest length of root (4.63 cm) was recorded in control. Analysis of both the years revealed significant difference in the length of the root. The longest length of root (5.97 cm) was recorded in IBA @ 1 mgL^{-1} which is followed by IBA @ 0.5 mgL^{-1} (5.88 cm) which was at par with IBA @ 1.5 mgL^{-1} and NAA @ 1 mgL^{-1} (5.86 cm each). The shortest length of root (4.71 cm) was recorded in control. Each species prefer particular hormones concentrations reported by Dunwell (1981). The current findings are in conformity with the experimental finding of researchers Nongdam and Tikendra (2014) where they reported that IBA gave better result in root regeneration as compared to NAA and IAA. These results are in conformity with the findings of Aktar *et al*. (2007) in *Dendrobium* orchid, Panathula *et al.* (2014) in *Centella asiatica* (L.), Nongdam and Tikendra (2014) in *Dendrobium chrysotoxum* and Anbazhagan *et al*. (2014) in *Musa* sp. who reported that IBA @ 1 mgL^{-1} resulted in the longer root length compared to other treatments.

4.2.6 Length of shoot

The data on the length of shoot as influence by the plant growth regulators are presented in Table 4.24 and Fig. 4.27. The longest length of shoot (6.97 cm) during the year 2017-18 were recorded in IBA @ 1 mgL^{-1} followed by IBA @ 1.5 mgL^{-1} (6.82 cm) which was at par with NAA @ mgL^{-1} (6.80 cm) and the shortest length of shoot 3.93 were recorded in control.

Similar results were obtained in the subsequent year 2018-19, the longest length of shoot (6.90 cm) was recorded in IBA @ 1 mgL^{-1} followed by IBA @1.5 mgL^{-1} (6.82 cm) which is at par with NAA @ 1 mgL^{-1} (6.80 cm) and the shortest length of shoot (3.93 cm) was recorded in control. Pooled data analysis revealed that the longest length of shoot (6.93 cm) was recorded in IBA @ 1 mgL^{-1} followed by NAA @ 1 mgL^{-1} (6.83 m) which is at par with IBA @ 1.5 mgL^{-1} and IBA @ 2 mgL^{-1} (6.82 cm and 6.78 cm, respectively). *In vitro* multiplication of shoot was carried out at high concentration of cytokinin which inhibited the development of shoot, to induce root lower cytokinin/auxin ratio is favourable. The reason for getting such length of shoot might be due to the fact that better root formation results in better uptake of nutrient from the media which lead to better growth of the shoot. The current experimental findings are in line with the findings of Panathula *et al.* 2014 in *Centella asiatica* (L), Aktar *et al*. 2007 in *Dendrobium* orchid.

Table 4.24: Influence of growth hormones on length of root and shoot in *in vitro* regeneration *of Dendrobium* cv. Sonia Earsakul

Treatment	*Length of root*			*Length of shoot*		
	2017-18	*2018-19*	*Pooled*	*2017-18*	*2018-19*	*Pooled*
T_1(MS)	4.78^{d}	4.63^{e}	4.71^{g}	3.93^{h}	4.00^{f}	3.97^{h}
T_2(MS + 0.5 mgL^{-1} IBA)	5.93^{ab}	5.82^{ab}	5.88^{ab}	4.93^{f}	4.80^{e}	4.87^{f}
T_3(MS + 1 mgL^{-1} IBA)	6.05^{a}	5.90^{a}	5.97^{a}	6.97^{a}	6.90^{a}	6.93^{a}
T_4(MS + 1.5 mgL^{-1} IBA)	5.87^{abc}	5.85^{ab}	5.86^{ab}	6.82^{ab}	6.82^{ab}	6.82^{ab}
T_5(MS + 2 mgL^{-1} IBA)	5.83^{abc}	5.78^{ab}	5.81^{bc}	6.77^{abc}	6.78^{ab}	6.78^{ab}
T_6(MS + 0.5 mgL^{-1} NAA)	5.85^{abc}	5.78^{ab}	5.82^{abc}	4.87^{f}	4.93^{e}	4.90^{f}
T_7(MS + 1 mgL^{-1} NAA)	5.90^{abc}	5.83^{ab}	5.86^{ab}	6.80^{ab}	6.86^{a}	6.83^{ab}
T_8(MS + 1.5 mgL^{-1} NAA)	5.75^{bc}	5.68^{b}	5.72^{bc}	6.65^{bc}	6.72^{ab}	6.68^{bc}
T_9(MS + 2 mgL^{-1} NAA)	5.60^{c}	5.45^{c}	5.53^{de}	6.35^{e}	6.34^{d}	6.34^{e}
T_{10}(MS + 0.5 mgL^{-1} 1AA)	5.62^{c}	5.70^{b}	5.66^{cd}	4.58^{g}	4.77^{e}	4.68^{g}
T_{11}(MS + 1mgL^{-1} 1AA)	5.80^{abc}	5.75^{ab}	5.78^{bc}	6.52^{cd}	6.64^{bc}	6.58^{cd}
T_{12}(MS + 1.5 mgL^{-1} 1AA)	5.78^{abc}	5.17^{d}	5.48^{ef}	6.48^{de}	6.46^{cd}	6.47^{de}
T_{13}(MS + 2 mgL^{-1} 1AA)	5.63^{bc}	5.06^{d}	5.35^{f}	6.47^{de}	6.37^{d}	6.42^{de}
SEm±	0.08	0.05	0.05	0.09	0.06	0.05
CD at 5%	0.24	0.16	0.14	0.25	0.19	0.15

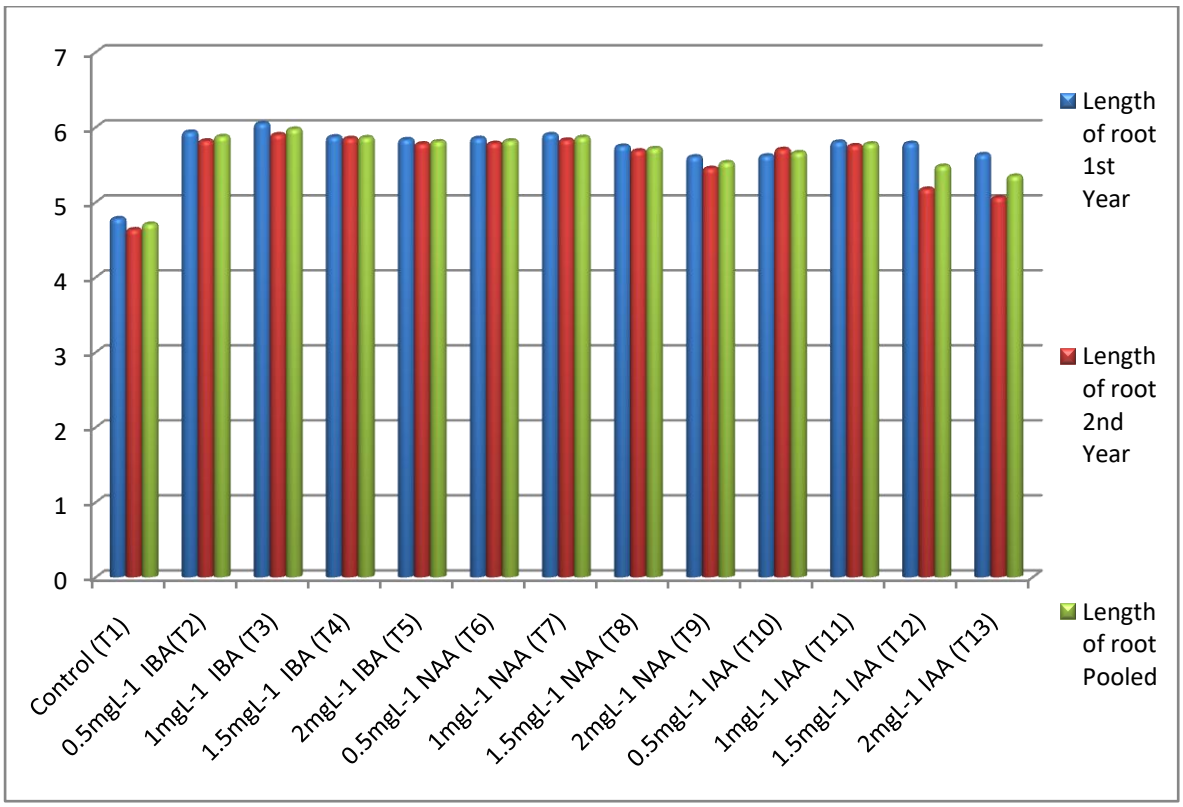


Fig. 4.27: Influence of different growth hormones in length of root

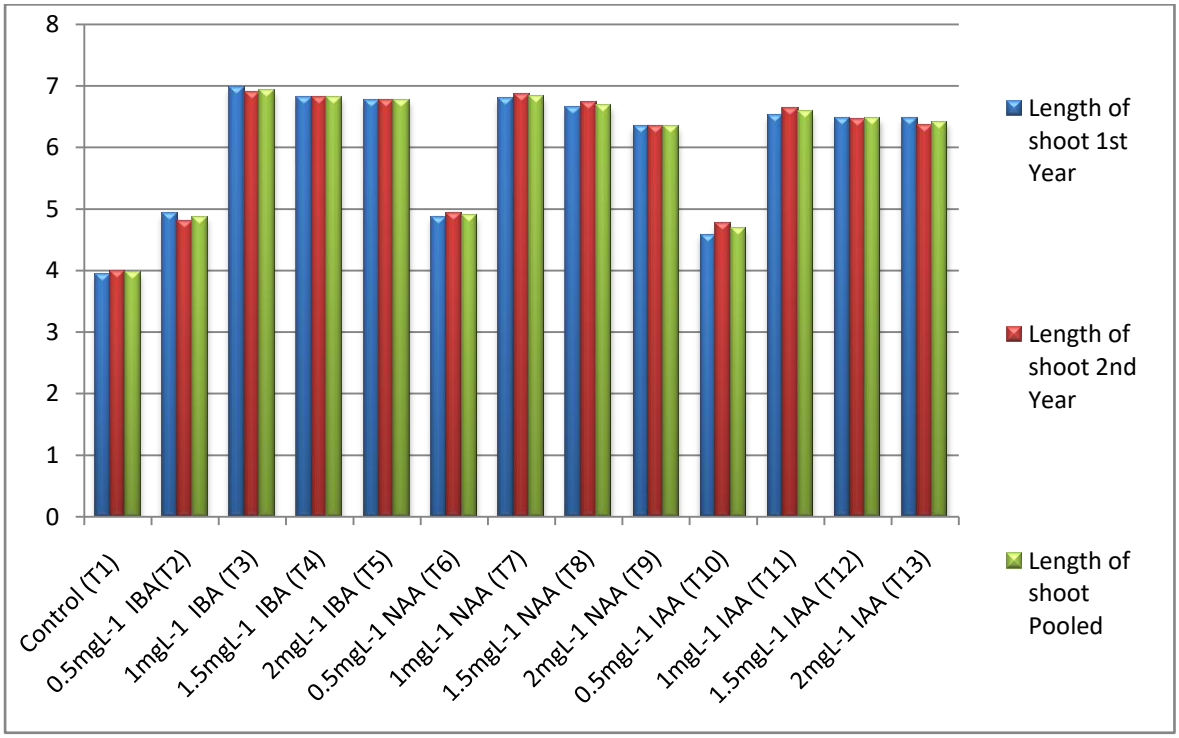


Fig. 4.28: Influence of different growth hormones in length of shoot

4.2.7 Percentage of survival during primary hardening

The data pertaining to percentage of survival during the primary hardening as influenced by different rooting hormones in various levels are presented in Table 4.25 and Fig. 4.28. During the first year study 2017-18, results revealed significant difference, application of IBA @ 1 mgL^{-1} resulted in the highest percentage of survival (34.69 %) followed by IBA @ 1.5 mgL^{-1} (33.57%) and the least percentage of survival (27.33 %) was recorded in control. In the subsequent year study 2018-19, IBA @ 1 mgL^{-1} resulted in the highest percentage of surviving (33.91%) followed by IAA @ 1 mgL^{-1} (32.42%) and the least percentage of survival (26.87%) was recorded in control. Further analysis of pooled data resulted in significant difference in the percentage of survival during primary hardening. MS media supplemented with IBA @ 1 mgL^{-1} resulted in highest percentage of survival (34.30%) followed by NAA @ 1 mgL^{-1} (33.05%) which is at par with IBA @ 1.5 mgL^{-1} (32.92%), IAA @ 1 mgL^{-1} (32.59%), NAA @ 1.5 mgL^{-1} (32.42%) and IBA @ 2 mgL^{-1} (32.24%) whereas the least percentage of survival (27.10%) was recorded in control.

4.2 Percentage of survival during secondary hardening

Table 4.25 and Fig. 4.29 depicted the results of the influence of plant growth regulator on the percentage of surviving on the hardening of *Dendrobium* cv. Sonia Earsakul. During the first year studied 2017-18, the highest percentage of surviving (94.29%) were recorded treatment IBA @ 1 mgL^{-1} which is followed by IBA @ 1.5 mgL^{-1} (92.54%) which is at par with IBA @ 2 mgL^{-1} (91.92%) and the least percentage of surviving (82.20%) were recorded in control. In the second year study 2018-19, the highest percentage of surviving (96.37%) were recorded in treatment IBA @ 1 mgL^{-1} which is at par with IBA @ 1.5 mgL^{-1} (95.75%) followed by IBA @ 2 mgL^{-1} (93.26%) and the least percentage of surviving were recorded in control (83.77). Pooled data analysis showed that the highest percentage of surviving (95.33%) were

recorded in treatment IBA @ 1 mgL^{-1} which is at par with IBA @ 1.5 mgL^{-1} (94.15%) which was followed by IBA @ 2 mgL^{-1} (92.59%) and the least percentage surviving were recorded in control (82.99%). During the primary hardening, the overall percentage of survival was less but during the secondary hardening the rate of survival increased. During the primary hardening the tender seedlings from *in vitro* culture bottle were acclimatized and hardened in natural environment. Survival percentage in primary hardening was less which might be due to the sudden shock, tenderness of plants, adverse natural environment, susceptibility to pests, diseases and other factors. However, the percentage of survival was quite satisfactory in the secondary hardening, as the plant were getting sturdy and adapted to the natural environment. In both the primary and secondary hardenings the best percentage of survival was recorded in treatment containing IBA @ 1 mgL^{-1}, which might be due to proper root development in that treatment where *in vitro* plant were growing in culture bottle comparing to other treatments. Plantlets with proper root development will establish easily in the natural environment during hardening and thus resulting in proper growth and development. These findings are in accordance with the findings of Priyanka *et al*. (2018) in *Dendrobium* sp. where 85% of plantlets developed from media augmented with IBA @ IBA 1 mgL^{-1} survived in shade house, Panathula *et al*. 2014 also reported in *Centella asiatica* (L.). that IBA @ 1 mgL^{-1} resulted in 85% survival during hardening and the finding was in line with the finding of Anbazhagan *et al*. 2014 in *Musa* sp. and Pananthula *et al*. 2009 in *Centella asiatica* (L.).

Table 4.25: Influence of growth hormones on survival percentage of rooted plantlets during of *Dendrobium* cv. Sonia Earsakul

Treatment	*Primary hardening*			*Secondary hardening*		
	2017-18	*2018-19*	*Pooled*	*2017-18*	*2018-19*	*Pooled*
T_1 (MS)	27.33^{g}	26.87^{g}	27.10^{f}	82.20^{f}	83.77^{f}	82.99^{g}
T_2(MS + 0.5 mgL^{-1} IBA)	31.00^{de}	30.34^{de}	30.67^{c}	89.77^{de}	92.16^{bc}	90.97^{c}
T_3(MS + 1 mgL^{-1} IBA)	34.69^{a}	33.91^{a}	34.30^{a}	94.29^{a}	96.37^{a}	95.33^{a}
T_4(MS + 1.5 mgL^{-1} IBA)	33.57^{ab}	32.27^{bc}	32.92^{b}	92.54^{ab}	95.75^{a}	94.15^{a}
T_5(MS + 2 mgL^{-1} IBA)	32.45^{bc}	32.03^{bc}	32.24^{b}	91.92^{bc}	93.26^{b}	92.59^{b}
T_6(MS + 0.5 mgL^{-1} NAA)	29.67^{ef}	29.37^{ef}	29.52^{de}	88.12^{e}	89.62^{d}	88.87^{de}
T_7(MS + 1 mgL^{-1} NAA)	33.18^{b}	32.92^{ab}	33.05^{b}	89.60^{d}	92.12^{bc}	90.86^{c}
T_8(MS + 1.5 mgL^{-1} NAA)	32.83^{bc}	32.00^{bc}	32.42^{b}	91.37^{bcd}	91.14^{bcd}	91.26^{bc}
T_9(MS + 2 mgL^{-1} NAA)	31.00^{de}	31.00^{cd}	31.00^{c}	88.84^{e}	91.04^{cd}	89.94^{cde}
T_{10}(MS + 0.5 mgL^{-1} 1AA)	28.50^{fg}	28.70^{f}	28.60^{e}	84.29^{f}	86.28^{e}	85.29^{f}
T_{11}(MS + 1mgL^{-1} 1AA)	32.77^{bc}	32.42^{ab}	32.59^{b}	88.40^{e}	89.11^{d}	88.76^{de}
T_{12}(MS + 1.5 mgL^{-1} 1AA)	31.60^{cd}	30.27^{de}	30.93^{c}	89.41^{d}	91.12^{bcd}	90.27^{cd}
T_{13}(MS + 2 mgL^{-1} 1AA)	30.97^{de}	29.63^{de}	30.30^{cd}	88.08^{e}	88.91^{d}	88.49^{e}
SEm±	0.46	0.50	0.34	0.72	0.67	0.49
CD at 5%	1.33	1.45	0.96	2.09	1.96	1.40

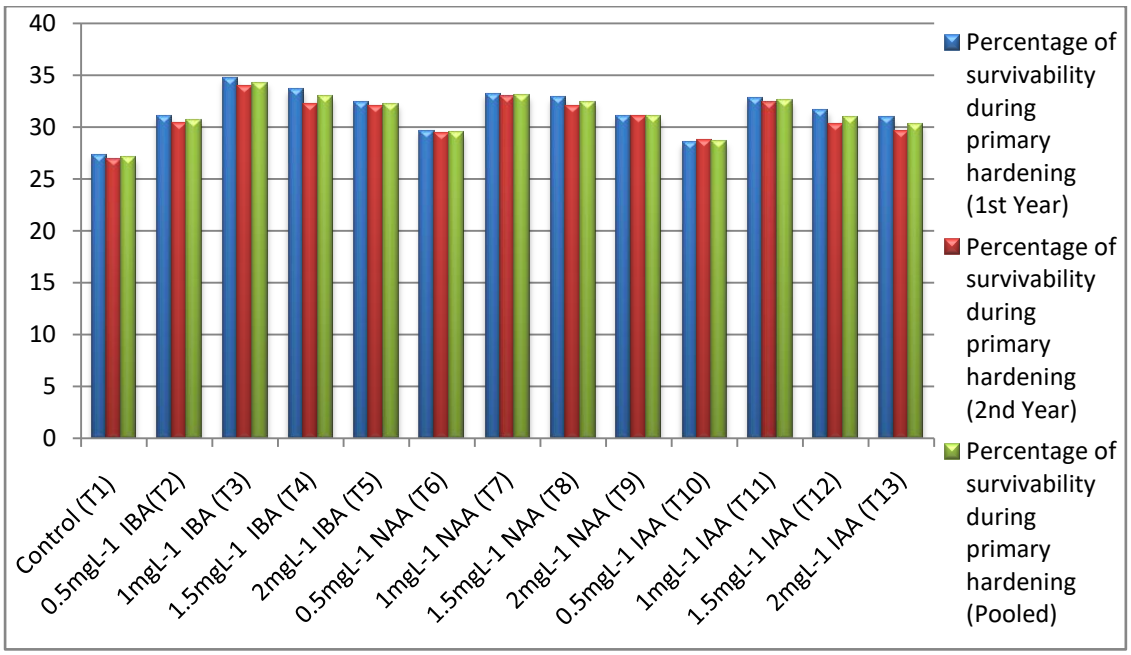


Fig. 4.29: Influence of growth hormones in Percentage of survival during Primary hardening

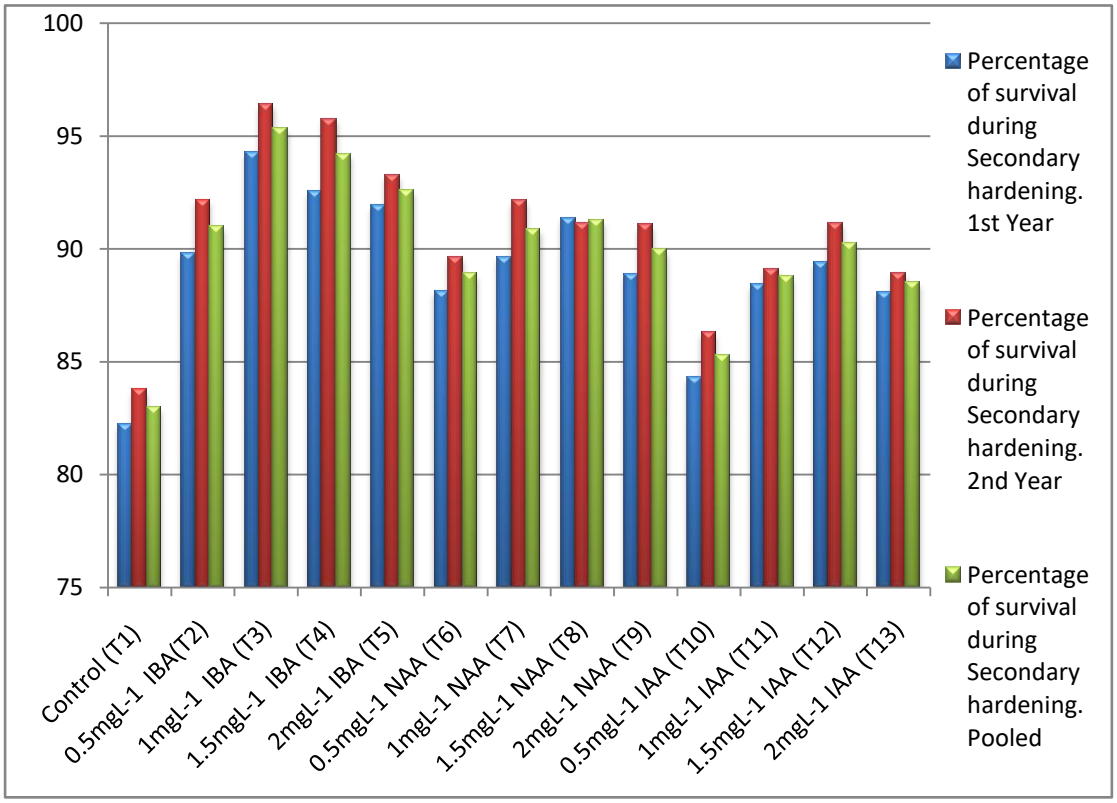


Fig. 4.30: Influence of growth hormones in Percentage of survival during secondary hardening

Plate 8. General view of hardening in polyhouse

CHAPTER V

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The salient findings of the investigation entitled "Standardization of micropropagation techniques of *Dendrobium* cv. Sonia Earsakul through shoot tip culture" carried out in Tissue Culture laboratory, Department of Horticulture, School of Agricultural Sciences and Rural Development, Nagaland University, Medziphema campus during the year 2016-2019 are summarized below.

6.1 *In vitro* shoot multiplication

- BAP @ 2 mgL^{-1}, NAA @ 0.5 mgL^{-1} and treatment combination of BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1} showed earliest days taken to callus formation **(**45.69 day, 47.33 and 42.42 days, respectively).
- BAP @ 2 mgL^{-1}, NAA @ 0.5 mgL^{-1} and treatment combination of BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1} resulted in the highest percentage of response (51.62%, 51.19% and 55.84%, respectively).
- Highest growth rate of callus (13.05 g) was recorded in treatment BAP @ 2 mgL^{-1} and NAA @ 0.5 mgL^{-1} (11.97g). Treatment combination of BAP @ 2 mgL^{-1} and NAA @ 0.5 mgL^{-1} yield better result in the growth rate of callus (13.66 g) as compared to other treatment combinations and single application.
- BAP @ 2 mgL^{-1}, NAA @ 0.5 mgL^{-1} and treatment combination of BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1} resulted in earlier days taken to initiation of shoot bud (36.10 days, 39.13 days and 34.77 days respectively).
- In both the first and second sub culturing, BAP @ 2 mgL^{-1} resulted in highest number of multiple shoot bud (5.56 and 11.85 respectively). Similarly, NAA @ 0.5 mgL^{-1} yield higher number of shoot bud in both the sub culturing (4.48 and 9.86, respectively). Treatment combination

of BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1} resulted in more number of shoot bud in both the sub culturing (5.76 and 12.13, respectively).

- The longest length of shoot bud (4.87 cm, 4.20 cm and 5.10 cm respectively) was recorded in treatment BAP @ 2 mgL^{-1}, NAA @ 0.5 mgL^{-1} and treatment combination of BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1}.
- The maximum number of leaves (5.20 and 4.29, respectively) was observed in BAP @ 2 mgL^{-1} and NAA @ 0.5 mgL^{-1} and treatment combination of BAP @ 2 mgL^{-1} + NAA @ 0.5 $mgL^{-1,}$ resulted in maximum number of leaves (5.45).
- The maximum fresh weight of shoot at 90 days of culturing and during hardening stage (0.57g and 0.95 g) were recorded in treatment BAP @ 2 mgL^{-1}. Similarly, NAA @ 0.5 mgL^{-1} resulted in the maximum fresh weight of shoot at 90 days of culturing and during hardening stage (0.47g and 0.58 g, respectively). Treatment combination of BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1} yield better result in fresh weight of shoot (0.61 g and 0.99 g respectively) at 90 days of culturing and during hardening stage comparing to single application and other treatment combinations.

6.2 *In vitro* root regeneration of *Dendrobium* cv Sonia Earsakul.

- The shortest days taken to root initiation (34.90 days) was recorded in IBA @ 1 mgL^{-1}.
- The maximum numbers of shoot (2.13) was recorded in IBA @1 mgL^{-1}.
- The maximum number of root per shoot (8.39) was observed in treatment IBA @ 1mgL^{-1}.
- The maximum number of functional root (6.93) was recorded in IBA @ 1 mgL^{-1}.
- The maximum length of root (5.97 cm) was recorded in IBA @ 1mgL^{-1}.
- Maximum length of shoot (6.93 cm) was recorded in IBA @ 1 mgL^{-1}.

6.3 Hardening of *in vitro* regenerated rooted shoot

- MS media supplemented with IBA @ 1 mgL^{-1} resulted in highest percentage of survival (34.30%) comparing to IAA and NAA during the primary hardening.
- The highest percentage of survival (95.33%) was recorded in treatment IBA @ 1 mgL^{-1} and the least percentage of survival were recorded in control (82.99%) during the secondary hardening.

Conclusion

1. From the above summary, it could be easily concluded that BAP @ 2 mgL^{-1} and NAA @ 0.5mgL^{-1} proved to be the best concentrations for *in vitro* shoot multiplication of *Dendrobium* cv. Sonia Earsakul. Similarly, interaction of BAP @ 2 mgL^{-1} + NAA @ 0.5 mgL^{-1} yield superior result as compared to single application for *in vitro* shoot multiplication.
2. Likewise, among the different levels of IBA, NAA and IAA studied, IBA @ 1mgL^{-1} showed better performance in the rooting parameters for *in vitro* root regeneration of *Dendrobium* cv. Sonia Earsakul.
3. In both the primary and secondary hardening, plantlets regenerated from MS media augmented with IBA @ 1 mgL^{-1} showed better percentage of survivability during hardening.

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APPENDICES

ANNOVA – 1: Days to callus formation

ANOVA TABLE Pooled						
Source of Variance	***Degree of Freedom***	***Sum of Square***	***Mean Sum of Square***	***F Cal***	***F Tab at 5%***	***S/NS***
Years	1	6.66	6.66	2.10	3.96	***NS***
Factor A	8	663.99	83.00	55.99	2.06	***Significant***
Factor B	6	147.08	24.51	16.54	2.21	***Significant***
A x B interaction	24	76.01	3.17	2.14	1.65	***Significant***
Error	80	118.59	1.48			
Total	119	1012.32				

ANNOVA – 2: Percentage of response

ANOVA TABLE Pooled						
Source of Variance	***Degree of Freedom***	***Sum of Square***	***Mean Sum of Square***	***F Cal***	***F Tab at 5%***	***S/NS***
Years	1	12.39	12.39	4.20	3.96	***Significant***
Factor A	8	1454.63	181.83	293.22	2.06	***Significant***
Factor B	6	879.88	146.65	236.49	2.21	***Significant***
A x B interaction	24	70.83	2.95	4.76	1.65	***Significant***
Error	80	49.61	0.62			
Total	119	2467.34				

ANNOVA – 3: Gain in weight of callus

ANOVA TABLE Pooled						
Source of Variance	***Degree of Freedom***	***Sum of Square***	***Mean Sum of Square***	***F Cal***	***F Tab at 5%***	***S/NS***
Years	1	1.57	1.57	3.95	3.96	***NS***
Factor A	8	258.23	32.28	89.94	2.06	***Significant***
Factor B	6	23.58	3.93	10.95	2.21	***Significant***
A x B interaction	24	9.53	0.40	1.11	1.65	***NS***
Error	80	28.71	0.36			
Total	119	321.61				

ANNOVA – 4: Days to initiation of shoot

ANOVA TABLE Pooled						
Source of Variance	***Degree of Freedom***	***Sum of Square***	***Mean Sum of Square***	***F Cal***	***F Tab at 5%***	***S/NS***
Years	1	9.53	9.53	2.74	3.96	***NS***
Factor A	8	1573.45	196.68	195.19	2.06	***Significant***
Factor B	6	234.79	39.13	38.83	2.21	***Significant***
A x B interaction	24	83.37	3.47	3.45	1.65	***Significant***
Error	80	80.61	1.01			
Total	119	1981.75				

ANNOVA – 5: Number of multiple shoot bud during first sub culturing

ANOVA TABLE Pooled						
Source of Variance	***Degree of Freedom***	***Sum of Square***	***Mean Sum of Square***	***F Cal***	***F Tab at 5%***	***S/NS***
Years	1	1.57	1.57	6.08	3.96	***Significant***
Factor A	8	134.76	16.85	171.50	2.06	***Significant***
Factor B	6	7.67	1.28	13.02	2.21	***Significant***
A x B interaction	24	6.21	0.26	2.63	1.65	***Significant***
Error	80	7.86	0.10			
Total	119	158.07				

ANNOVA – 6: Number of multiple shoot bud during second sub culturing

ANOVA TABLE Pooled						
Source of Variance	***Degree of Freedom***	***Sum of Square***	***Mean Sum of Square***	***F Cal***	***F Tab at 5%***	***S/NS***
Years	1	0.35	0.35	0.98	3.96	***NS***
Factor A	8	273.79	34.22	390.23	2.06	***Significant***
Factor B	6	15.94	2.66	30.29	2.21	***Significant***
A x B interaction	24	8.68	0.36	4.12	1.65	***Significant***
Error	80	7.02	0.09			
Total	119	305.78				

ANNOVA – 7: Length of shoot bud

ANOVA TABLE Pooled						
Source of Variance	***Degree of Freedom***	***Sum of Square***	***Mean Sum of Square***	***F Cal***	***F Tab at 5%***	***S/NS***
Years	1	0.10	0.10	1.28	3.96	***NS***
Factor A	8	66.61	8.33	465.27	2.06	***Significant***
Factor B	6	4.37	0.73	40.72	2.21	***Significant***
A x B interaction	24	1.93	0.08	4.49	1.65	***Significant***
Error	80	1.43	0.02			
Total	119	74.44				

ANNOVA – 8: Number of leave per shoot

ANOVA TABLE Pooled						
Source of Variance	***Degree of Freedom***	***Sum of Square***	***Mean Sum of Square***	***F Cal***	***F Tab at 5%***	***S/NS***
Years	1	0.35	0.35	3.16	3.96	***NS***
Factor A	8	86.05	10.76	577.29	2.06	***Significant***
Factor B	6	5.90	0.98	52.74	2.21	***Significant***
A x B interaction	24	2.63	0.11	5.89	1.65	***Significant***
Error	80	1.49	0.02			
Total	119	96.42				

ANNOVA – 9: Fresh weight of shoot 90 days

ANOVA TABLE Pooled						
Source of Variance	***Degree of Freedom***	***Sum of Square***	***Mean Sum of Square***	***F Cal***	***F Tab at 5%***	***S/NS***
Years	1	0.00	0.00	3.58	3.96	***NS***
Factor A	8	1.34	0.17	1076.16	2.06	***Significant***
Factor B	6	0.07	0.01	72.54	2.21	***Significant***
A x B interaction	24	0.01	0.00	2.89	1.65	***Significant***
Error	80	0.01	0.00			
Total	119	1.43				

ANNOVA – 10: Fresh weight of shoot during hardening

ANOVA TABLE Pooled						
Source of Variance	***Degree of Freedom***	***Sum of Square***	***Mean Sum of Square***	***F Cal***	***F Tab at 5%***	***S/NS***
Years	1	0.07	0.07	43.32	3.96	***Significant***
Factor A	8	0.85	0.11	187.10	2.06	***Significant***
Factor B	6	0.08	0.01	24.17	2.21	***Significant***
A x B interaction	24	0.04	0.00	3.02	1.65	***Significant***
Error	80	0.05	0.00			
Total	119	1.09				

ANNOVA – 11: Days to root initiation

ANOVA Table of Pooled Final						
Source of Variance	***Degree of Freedom***	***Sum of Square***	***Mean Sum of Square***	***F Cal***	***F Tab at 5%***	***S/NS***
Years	1	0.14	0.14	0.07	4.03	***NS***
Treatment	12	1075.60	89.63	43.41	1.94	***Significant***
Years x Treatment	12	6.18	0.51	0.25	1.94	***NS***
Error	52	107.38	2.07			
Total	77	1189.30				

ANNOVA – 12: Number of shoot per ex plant

ANOVA Table of Pooled Final						
Source of Variance	***Degree of Freedom***	***Sum of Square***	***Mean Sum of Square***	***F Cal***	***F Tab at 5%***	***S/NS***
Years	1	0.02	0.02	1.54	4.03	***NS***
Treatment	12	2.15	0.18	13.49	1.94	***Significant***
Years x Treatment	12	0.01	0.00	0.07	1.94	***NS***
Error	52	0.69	0.01			
Total	77	2.86				

ANNOVA – 13: Number of root per shoot

ANOVA Table of Pooled Final						
Source of Variance	***Degree of Freedom***	***Sum of Square***	***Mean Sum of Square***	***F Cal***	***F Tab at 5%***	***S/NS***
Years	1	0.28	0.28	4.41	4.03	***Significant***
Treatment	12	77.16	6.43	101.51	1.94	***Significant***
Years x Treatment	12	0.11	0.01	0.14	1.94	***NS***
Error	52	3.29	0.06			
Total	77	80.84				

ANNOVA – 14: Number of functional roots

ANOVA Table of Pooled Final						
Source of Variance	***Degree of Freedom***	***Sum of Square***	***Mean Sum of Square***	***F Cal***	***F Tab at 5%***	***S/NS***
Years	1	0.01	0.01	0.40	4.03	***NS***
Treatment	12	75.18	6.26	359.59	1.94	***Significant***
Years x Treatment	12	0.14	0.01	0.69	1.94	***NS***
Error	52	0.91	0.02			
Total	77	76.23				

ANNOVA – 15: Length of roots

ANOVA Table of Pooled Final						
Source of Variance	***Degree of Freedom***	***Sum of Square***	***Mean Sum of Square***	***F Cal***	***F Tab at 5%***	***S/NS***
Years	1	0.46	0.46	30.53	4.03	***Significant***
Treatment	12	8.06	0.67	45.02	1.94	***Significant***
Years x Treatment	12	0.76	0.06	4.23	1.94	***Significant***
Error	52	0.78	0.01			
Total	77	10.05				

ANNOVA – 16: Length of shoot

ANOVA Table of Pooled Final						
Source of Variance	***Degree of Freedom***	***Sum of Square***	***Mean Sum of Square***	***F Cal***	***F Tab at 5%***	***S/NS***
Years	1	0.01	0.01	0.40	4.03	***NS***
Treatment	12	75.18	6.26	359.59	1.94	***Significant***
Years x Treatment	12	0.14	0.01	0.69	1.94	***NS***
Error	52	0.91	0.02			
Total	77	76.23				

ANNOVA – 17: Survival percentage during Primary hardening

ANOVA Table of Pooled Final						
Source of Variance	***Degree of Freedom***	***Sum of Square***	***Mean Sum of Square***	***F Cal***	***F Tab at 5%***	***S/NS***
Years	1	7.07	7.07	10.29	4.03	***Significant***
Treatment	12	288.58	24.05	34.98	1.94	***Significant***
Years x Treatment	12	4.46	0.37	0.54	1.94	***NS***
Error	52	35.75	0.69			
Total	77	335.87				

ANNOVA – 18: survival percentage during Secondary hardening

ANOVA Table of Pooled Final						
Source of Variance	***Degree of Freedom***	***Sum of Square***	***Mean Sum of Square***	***F Cal***	***F Tab at 5%***	***S/NS***
Years	1	54.97	54.97	37.75	4.03	***Significant***
Treatment	12	792.60	66.05	45.36	1.94	***Significant***
Years x Treatment	12	14.32	1.19	0.82	1.94	***NS***
Error	52	75.73	1.46			
Total	77	937.61				