

# HIGH FREQUENCY MULTIPLICATION OF ORCHIDS THROUGH *IN VITRO* CULTURE

**A**

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**By**

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*Lord Shiva*

*Artista*



*You are both special in every way,  
Encouraging me more & more each passing day.*

*You both are the reason why I'm so strong,  
With you two at the helm not a thing could go  
wrong.*

*You've both helped me through many trials &  
tribulations,  
You've made things better in every situation.*

*Thank you both for always being there,  
And showing me that you truly care.*

*Words could never explain how I feel about  
you,  
But I hope you know that I truly love you two!*


*My Parents*

# CERTIFICATE-II

This is to certify that the thesis entitled “**HIGH FREQUENCY MULTIPLICATION OF ORCHIDS THROUGH INVITRO CULTURE**”, submitted by **Subhalaxmi Sahoo** to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for award of the degree of **MASTER OF SCIENCE IN AGRICULTURE (FLORICULTURE & LANDSCAPING)** has been approved by the Students Advisory Committee after an oral examination on the same in collaboration with an external examiner.

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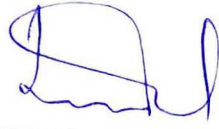
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**Bhubaneswar**

**Dated 31-07 2012**

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# ABSTRACT

Title of the thesis : **HIGH FREQUENCY MULTIPLICATION OF ORCHIDS THROUGH *IN VITRO* CULTURE**

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The thesis entitled “High frequency multiplication of Orchids through *in vitro* culture” was carried out in the Laboratory of Biotechnology-cum-Tissue Culture Centre, Department of Floriculture & Landscaping, Orissa University of Agriculture and Technology, Bhubaneswar during the year 2011-12 with the objectives for standardization of media supplements for shoot proliferation and root formation of Orchids (*Vanda* and *Dendrobium*) and for hardening of the *in vitro* plantlets. The experiment was carried out in the completely randomized design with various treatments.

The result indicated that MS medium supplemented with 3.0 mg/l BAP and 100 mg/l AdS was found to be the best for shoot in *Vanda spp* as far as number of leaves/clump (2.60), leaf length (0.41) and shoot initiation was concerned.

MS medium fortified with 2.0 mg/l BAP increased the shoot height (2.45 cm). Maximum leaf length (1.75 cm) was observed in MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA. The number of leaves (12.39) was best observed in MS medium fortified with BAP 2.5 mg/l, 0.5 mg/l NAA and AdS 100 mg/l. MS medium fortified with 3.0 mg/l BAP and 100 mg/l AdS presented the highest number of suckers (3.91) in *Dendrobium* cv. Sonia- 17 after 60 days of culture.

MS medium enriched with kinetin 5mg/l increased the shoot height (1.66 cm) and number of leaves/ clump (9.6). The best leaf length was noticed in MS medium fortified with 6 mg/l kinetin and the maximum number of suckers (3.82) was observed in MS medium fortified with 4 mg/l in *Dendrobium* cv. Sonia- 17 after 45 days of culture.

Half MS medium supplemented with 0.5 mg/l IBA alone or in combination with 0.5 mg/l NAA was found to be most suitable for root initiation and formation after 30 days of culture in *Vanda spp*. However, other characters related to rooting were found to be statistically non significant.

As regard to root initiation and development, half MS medium fortified with 1.0 mg/l IBA was found to be best for obtaining higher number of roots/ bottle (15.0), number of roots/plantlet(3.75) and root length(5.44 cm) in *Dendrobium* cv. Sonia-17 after 90 days of culture.

The media combinations such as Brick pieces: Charcoal: Cocopeat: Spahgnum moss: Decayed wood (1:1:1:1) was found to be the most suitable for hardening of *Dendrobium* cv. Sonia-17 as the combination had profound effect on enhancing significantly the plant height (12.63 cm), leaf number (8.33), leaf length (9.73 cm) and leaf width (0.80 cm) after 60 days of planting.

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# ABBREVIATIONS

AdS	:	Adenine Sulphate
BAP	:	Benzyl Amino Purine
Ca	:	Callus
CD (5%)	:	Critical difference with significance level of 5%
Cft	:	Cubic feet
cv.	:	Cultivar
CW	:	Coconut water
DAI	:	Days After Inoculation
DAT	:	Days After Transplantation
DAE	:	Days After Establishment
Dm	:	Decimeter
Fig.	:	Figure
GA3	:	Gibberellic acid
HCl	:	Hydrochloric acid
IAA	:	Indole 3-acetic acid
IBA	:	Indole 3-butyric acid
KC	:	Knudson C medium
KN	:	Kinetin
l	:	litre
M	:	Mitra media
Min	:	Minute
ml	:	milliliter
mg	:	milligram
MM	:	Modified Malmgren
MS	:	Murahige and Skoog medium
MVW	:	Modified Vacin and Went medium
N	:	Normal
NAA	:	Napthalene Acetic acid
NaOH	:	Sodium hydroxide
PBRs	:	Plant Bio Regulators
PLBs	:	Protocorm Like Bodies
PM	:	Phytamax media
SE (m)	:	Standard Error Mean
Sl no.	:	Serial number
TAB	:	Table
TDZ	:	Thiadiazuron
YE	:	Yeast extract
Zea	:	Zeatin
2ip	:	Isopentyl adenine
2,4-D	:	2,4-Dichlorophenoxy acetic acid



## CHAPTER I

# *Introduction*

**An Odyssey starts with  
Single step....**

# INTRODUCTION

Orchids have their own identity because of their exclusive beauty. The long life span coupled with exquisite colours and shapes contribute to their popularity and demand in international market. Orchids belong to the family Orchidaceae consisting of about 800 genera and 35,000 species and over 74,400 natural and manmade hybrids. They are of immense horticultural importance and also play a very useful role to balance the forest ecosystems (Kaushik, 1983).

India is the centre of origin of many Orchid species and they form 9% of our flora. Nearly 1300 species and 140 genera dwell in the country with Himalayas as their habitat and others scattered in eastern and Western Ghats (Jain, 1980). In general, terrestrial Orchids are more common in North Western India and the epiphytic ones in North Eastern India.

Orchid growing has become a great hobby all over the world in addition to its commercial value for cut flower industry. In the past few years, the Orchid trade has increased in volume and value throughout the world. Orchids rank first in the global trade of tropical cut flowers. Thailand (1080 ha), Australia (1000 ha), Japan (425 ha), Singapore (100 ha), Philippine, Korea and USA (1000 modern Orchid growers) are the major producers of Orchids and Hawaii, Mauritius and Caribbean countries are other important Orchid producing countries which together produce about 50 million stems from about 260 ha in the world (Prakash *et al.*, 2004).

Orchids are marketed both as cut flowers as well as potted plants. Taiwan is the largest producer of Orchids for pot plants. Under green houses the Orchids are grown in an area of about 196 ha. Over 125 million Orchids are exported from the country. Japan, Australia, Europe, Switzerland and USA are important buyers of the Orchid cut flowers.

In India the commercial cultivation of Orchids is gradually picking up. In the states of Kerala and Karnataka Orchids are grown on commercial scale under protected condition. Besides, these are also grown in some parts of Tamil Nadu and in north-eastern parts include Sikkim, Kalimpong, Darjeeling and Arunachal Pradesh.

The important genera which have given maximum number of manmade hybrids are *Cattleya*, *Cymbidium*, *Paphiopedilum*, *Dendrobium*, *Vanda* etc. Of many Orchid varieties the *Phalaenopsis*, *Cymbidium* and *Dendrobium* Orchids command high price for their cut flowers and have contributed immensely to the international trade in floriculture.

*Dendrobium* alone accounts for 85 percent in tropical Orchid cut flowers. It is the second largest genus in the family Orchidaceae comprising 1600 species distributed in India, Burma, Malaysia, Australia, New Zealand, China and Japan (Bose and Bhattacharjee, 1980). It has becoming increasingly popular due its floriferous flower sprays, wide range of colour, size and shape, year round availability and long flowering life of several weeks to months.

The small proportion (< 5%) of the total international cut flower trade occupied by Orchid highlights their potential to take a great share of the market. The aforesaid analysis clearly highlights the commercial significance of Orchids both in national and international floricultural trade. In the

commercial cultivation of Orchids, availability of quality planting materials appears to be a major constraint. However with the phenomenal development in the biotechnological science, *in vitro* culture is considered as an excellent avenue for the multiplication of quality planting materials of many horticultural plants. Quite a large number of Orchid species are commercially multiplied through *in vitro* culture. In the present investigation an attempt has been made to fine tune the protocol of *in vitro* culture for *Dendrobium* on which some basic work has been done and to develop a standard protocol for *Papilionanthe teres* of Chandaka forest range, Odisha on which practically no work has been done.

There are three methods of multiplying Orchids such as:

- i) Through division of rhizomes, cuttings, air layering, separation of offshoots and keikis.
- ii) By meristem or mericlone culture i.e. by growing and dividing growing tissues in culture media.
- iii) By seeds.

Propagation of Orchids through seeds is quite interesting although the rate of multiplication is slow in nature. However, culture of seeds under aseptic condition is commercially employed by nursery men and breeders for fast and large scale multiplication of several Orchids. Orchid seeds are unique in many ways. The seeds are extremely small and are often referred to “dust seeds” as these are tiny and powder like substance (80-130 micro m wide and 470-510 micro m long). Each seed contains an undifferentiated embryo composed of 80-100 cells with no functional endosperms. Although the seeds are produced in a large number i.e. two to three million per capsule, lack of metabolic machinery

and functional endosperm, only 0.2-0.3 percent seeds germinate in nature. Orchid seeds require a symbiotic relationship with a fungus called the Orchid mycorrhiza in order to germinate under natural condition which supplies sugar and nutrients to germinating seeds.

However, propagation and cultivation of Orchids was revolutionized in 1921-1922 by the discovery that Orchid seeds could be germinated *in vitro* on a simple sugar medium asymbiotically without association of mycorrhizal fungus (Arditti, 1979). In *in vitro* seed culture, the seeds are germinated in glass or plastic jars or dishes on an agar based medium which contains all the sugar, minerals and plant bio regulators that seeds need to germinate.

*In vitro* culture of Orchid seeds is beneficial in several ways. This technique is used in most commercial laboratories for rapid mass production of Orchids within short time. Several Orchid species and varieties including *Dendrobium* are self-compatible. Hence seed propagation would produce true to the type plants in these species/ varieties. Hence *in vitro* culture of seeds is a greater significance in this respect. Since many native Orchids are rare, threatened or endangered, *in vitro* seed culture has been applied to species conservation.

Generally, it takes about 4-17 months for a capsule to mature and ripened for collection of seeds in Orchids depending upon species. However, a major advancement in increasing the germination of Orchid seeds and reducing flowering time is the development of the green pod culture process which has now replaced the dry seed culture process on commercial basis.

*In vitro* seed culture techniques have been standardized and protocols have been developed for several Orchid species and varieties. However, the

techniques in terms of use of nutrient media, carbon source, growth regulators and other additives are specific for specific Orchids. Hence there is need of further studies to standardize the technique for other species and varieties for their rapid multiplication which can be employed for commercial production of plants as well as to save the native endangered species which are in the process of extinction.

Therefore, keeping the above facts in view, the present study on “High frequency multiplication of Orchids through *in vitro* culture” has been planned and executed with the following objectives:

1. Standardization of media supplements for shoot proliferation of Orchids (*Papilionanthe* and *Dendrobium*).
2. Standardization of media supplements for root initiation and development.
3. Standardization of media mixture for hardening of the *in vitro* plantlets.





## CHAPTER II

# *Review of Literature*

**We are because of  
our past.....**

## REVIEW OF LITERATURE

Creation of hybrids and *in vitro* culture of seeds for raising seedlings in flasks are being practiced by the commercial Orchid growers throughout the world since this is a very useful method for rapid mass production of plants within a short time. Besides, this technique is also useful in species conservation since many native Orchids are rare, threatened or endangered which can be gainfully utilized on hybridization programme for creation of new hybrids.

Between the two methods of seed culture *viz.*, symbiotic and asymbiotic association of mycorrhizal fungi is essential for symbiotic germination of seeds whereas asymbiotic seed culture involves germinating seeds and growing seedlings under aseptic conditions in an agar medium supplemented with nutrients required by the plant. Technique of asymbiotic seed culture is widely used for mass propagation of various tropical Orchid species and hybrids.

An attempt has been made in this chapter to review the available literature pertaining to media and media supplements standardized by a number of scientists for successful germination of Orchid seeds and subsequent growth of seedlings.

As early as in 1921, while studying the influence of carbohydrates on green plants, Knudson observed that a symbiotic fungus produced sugars for the initial development of an Orchid embryo. Based on this, he successfully germinated *Cattleya*, *Laelia* and *Epidendrum* on agar medium containing sugar

and concluded that fungus was not necessary for Orchid seed germination (Knudson, 1925). He also grew *Laelia*, *Cattleya* hybrid asymbiotically from seed to flower. This was followed by aseptic culture of *Dendrobium* by Tanaka (1957) and *Oncidium* by Livingston (1962).

The validity of asymbiotic seed germination was firmly established and the original medium developed by Knudson was improved to germinate the other species of Orchids. Inorganic salts, carbohydrates, organic substances like vitamins, amino acids, growth hormones, water and proper pH along with optimum light and temperature were necessary for success in germination. The procedure of asymbiotic seed culture with respect to media, media supplements and other related factors influencing the germination of seeds and seedling growth are reviewed below.

## **2:1: CULTURE MEDIA**

Several standardized media have been used for the germination of Orchid seeds. The commonly used nutrient media for Orchid seed culture are those proposed by Knudson (1946), Vacin and Went (1949), Murashige and Skoog (1962) and Nitsch (1967). Media such as Knudson C, Vacin and Went are broad spectrum used for seed germination in many Orchid species.

Vacin and Went (1949) reported higher percentage and faster seed germination in liquid culture of Orchid seeds as compared to that cultured in semi-solid VW medium.

Irawati *et al.* (1977) reported that the best growth and survival rates were obtained in *Dendrobium* when cultured in Knudson C medium.

Effect of media on seed germination has also reported by Singh and Prakash (1985) who found that Knudson C medium or Vacin and Went medium supplemented with coconut water (150 ml) were most ideal for germination of tropical Orchid seeds.

Devi *et al.* (1990) pointed out the preferred medium for *Dendrobium* seed germination which varied with the species. *Dendrobium farmari* and *Dendrobium primulinum* gave 50-60 percent higher seed germination on Vacin and Went medium.

Reddy (1992) reported that MS medium was rich in vitamin and micronutrients which gave better result than other media especially for tropical Orchids.

Zhang *et al.* (1992) reported that since MS medium contained high ionic concentration of nutrients, half strength media could adequately support rapid protocorm production in Orchids.

The morphogenesis potential of shoot tip explants procured from axenic seedlings was investigated by Devi (1997) in *Dendrobium moschatum* and *Cymbidium alatifolium* for production of virus free Orchids. Out of the five different media tested, Nitsch medium was found to be the best for formation and proliferation of protocorm like bodies. However, in a similar study Abhijit *et al.* (1998) reported that Knudson C medium was effective for inducing seed germination in *Dendrobium aphyllum*.

Pathania *et al.* (1998) reported that stem explants procured from shoots emerging from *Dendrobium* cv. Sonia pseudobulbs grown in Vacin and Went and Knudson C media favoured formation of protocorm like bodies (PLBs) and

subsequent development of plantlets. KC medium supplemented with BAP (1.5 mg/l) and Paclobutrazol (1.0 mg/l) was the best for further multiplication of PLBs.

Response of *Dendrobium* cv. Sonia to MS medium was studied by Prasad *et al.* (2001) who reported that the number of days required for meristem differentiation and first leaf initiation were the lowest for MS medium. Besides, MS medium also gave the highest mean shoot and root length, mean number of leaves, number of multiple shoots and roots formed, leaf area, fresh weight and dry weight. The highest number of multiple shoots formed was observed with BA (0.1 mg/l) + NAA (0.1 mg/l). On the other hand, the highest number of roots formed was obtained with NAA alone at 1.0mg/l.

Influence of different media on *in vitro* seed propagation of *Dendrobium* was also studied by Alam *et al.* (2002). Among the four different media namely Hyponex, Murashige and Skoog (MS), OKFI and Knudson C (KC) used, MS medium was found to be the best followed by Hyponex medium.

Raju *et al.* (2003) observed that MS medium was the best for initial culture establishment of *Dendrobium osterholt*, 60 days after inoculation with a higher rate of multiple shoot production and higher average shoot length. The maximum rooting was recorded as a result of application of IBA 1mg/l + NAA 1mg/l with ½ strength MS medium.

## **2:2: CARBON SOURCE FOR IN VITRO SEED GERMINATION**

Most of the Orchid seeds utilize disaccharides such as sucrose, which is the most commonly used carbon source (Singh, 1994). It supports initial germination and subsequent growth, whether autoclaved or filtered sterilized,

its effect depends upon concentration. It has also been reported that organogenesis is promoted at sub optimal concentrations while protocorm proliferation is enhanced by supra optimal concentrations (Arditti, 1979). Some species of *Cymbidium* however, prefer glucose to sucrose while *Phalaenopsis* prefers fructose to glucose. However, after a certain stage of development the Orchid seedlings require no exogenous supply of sugar and hence this can be drastically reduced.

### **2:3: pH OF MEDIA**

pH of media plays a major role on the germination of Orchid seeds. Orchid seeds prefer slightly acidic medium and pH between 4.5- 6.5 is ideal.

Knudson (1951) stated that pH in the medium greatly influenced plantlet growth. He observed the inability of *Cattleya* seeds to germinate when the initial pH of the medium was below 4.5. *Denrobium nobile* germinated better within a pH range of 4.0 to 5.0 where as many other Orchids responded favourably to media at pH between 5.0 & 6.0.

Kotamari and Murashige (1965) observed that the pH at 5.2-5.5 was favourable for successful seed germination in *Cymbidium mastersii*.

According to Segawa and Sehgal (1967) *Vanda* explants grew best on white medium with pH 5.5 whereas according to Churchii *et al.* (1970) *Epidendrum* leaf tips were best grown at pH 5.5 on modified MS medium. Churchii in 1972 also reported that *Epidendrum* root tips grew on modified Ojima and Fujiwara medium with a pH of 5.0.

Prasad & Mitra (1975) and Rosa & Laheri (1977) reported that pH of 5.2 for *Cattleya* & *Phalaenopsis* and 6.0 for *Cymbidium* and *Paphiopedilum* were satisfactory for seed germination.

Ichihasi and Kako (1997) observed that *Cattleya* shoot tips when cultured on a solid medium turned brown and died eventually. The activity was greatest at pH of 6.5 and was inhibited at lower pH.

Arditti (1979) reported that Orchid seeds germinated well within a pH of 4.8-5.2 with germination commencing at pH 3.6 and tapering off at 7.6.

Maximum germination and optimal growth of protocorm was observed at pH 5.0 in *Dendrobium chrysanthemum*, *Sarcanthus pallidus* (Raghuwanshiet al., 1986).

*Paphiopedilum ciliolare* Pfitz. seeds were sown asymbiotically on a modified Thomale medium by Pierik et al. (1988) who observed the optimal germination of *Paphiopedilum ciliolare* at a pH of 6.0.

George et al. (1997) reported that the optimum growth of protocorm in *Dendrobium osterholt* resulted when the pH was adjusted to 5.8.

## **2:4: GROWTH REGULATORS AND THEIR EFFECTS ON**

### **2:4:1: Seed Germination**

Orchid seeds germinate rather slowly. Various growth regulators have been added to culture media to enhance seed germination and seedling growth. Among these are auxin, cytokinin and gibberellins. The beneficial effect of IAA or NAA on Orchid seedlings has been known for a long time.

Auxins and Cytokinins are most frequently used in nutrient media to increase the percentage of germination of seeds. Although Orchids pollinia contain large amount of auxin, only traces of this hormone have been detected in Orchid seeds. Auxins were the first hormone added to the Orchids in cultures in majority of cases. IAA, IBA, NAA enhance seed germination and seedling growth.

There are only a few cases where auxin has been reported an inhibitory, these are usually when high auxin concentrations have been used. Generally auxin stimulates root formation and cytokinins enhance shoot development and cell division.

Use of 2,4-D is not recommended as it tends to cause abnormal growth in Orchid protocorms and shoots although it has been successfully used with *Paphiopedilum* and with many other genera when leaves or roots provide the explants (Sagawa and Kunisaki, 1982).

Cytokinins have different effect on different species. Benzyl Amino Purine (BAP) has known to retard development and differentiation of *Cymbidium* protocorms. As reported by Mitra (1989), cytokinins as such or in combination with auxin-indolacetic acid (IAA) stimulate germination.

Effect of growth regulators on seed germination of *Spathoglottis plicata* was reported by Sangama & Singh (1994). They observed that germination was faster in the KC medium (19.5 days) compared to MS medium (24 days) and V & W medium (50 days). A lower concentration of NAA, GA3 and BA (0.1 mg/l) enhanced seed germination (82%, 92.6% & 81.6%) and gibberellins was the most effective in accelerating seed germination respectively. Also they found that although a lower level of NAA, GA3 and BA was required for

higher germination of seeds, subsequent seedling growth was better with the combination of a higher level of IBA, GA3 and BA each at 5.0 mg/l in the KC medium.

A study of two Orchid species, viz., *Cymbidium elegant* Lindl. and *Dendrobium densiflorum* Lindl. ex Wall., was carried out on Murashige and Skoog's (MS) medium, supplemented with different concentrations and combination of 6-benzylaminopurine (BAP) and  $\alpha$ -Naphthalene acetic acid (NAA). The hormone-free MS medium and MS medium supplemented with various growth hormones were found effective for *in vitro* seed germination of both species. Hormone-free MS basal medium was found most effective for seed germination of *D. densiflorum*; whereas, basal medium supplemented with BAP (1mg/l) was effective for *C. elegans* (Pradhan *et al.*, 2009).

#### **2:4:2: Callus Induction**

Champagnat *et al.* (1970) reported that when seedling leaves of *Cattleya* hybrid were used to induce further growth and development of axillary meristem at leaf base these gave rise to callus with the supplement of IAA, kinetins, CH casein hydrolysate liver extract and vitamins induced variable growth.

In another study Mitra *et al.* (1976) reported that excellent callusing of some but not all embryos of *Dendrobium fimbriatum* was observed only in the basal medium without auxin or cytokinin.

Begum *et al.* (1994) initiated the embryogenic callus (EC) from inner tissues of protocorm like bodies of *Cymbidium* Orchid when cultured on MS medium supplemented with NAA or 2, 4-D. EC was obtained within 15 days

on a MS medium containing 2 mg/l NAA or 0.5 mg/l 2, 4-D which was the most effective among the concentrations tested.

Callus induction and plant regeneration through somatic embryogenesis in *Phalaenopsis* Richard Shaffer 'Santa Cruz' were examined by Ishii *et al.* (1998). Protocorm-like body (PLB) segments formed calli in Vacin and Went medium with sucrose. Medium containing 200 ml/l coconut water together with 40g/l sucrose was effective for callus induction.

Madera *et al.* (2003) conducted a study on somatic embryogenesis and plant regeneration with the use of plant growth regulators by using young leaves and nodes of *in vitro* cultured plantlets of *Grammatophyllum scriptum*. The explants were cultured in Modified Vacin and Went (MVW) medium supplemented with 2, 4-D (0 to 10 mM). Callus formation was observed to occur in all auxin concentrations except in MVW with no auxin supplementation.

Roy *et al.* (2003) cultured shoot-tip explants of *Dendrobium fimbriatum* Lindl. var. *oculatum* Hk.f. on modified nutrient solution of Knudson's C, supplemented with 10% (v/v) coconut water, 0.5 mg/l niacin, 0.5 mg/l pyridoxine HCl, 0.1 mg/l thiamine HCl and different concentrations of N6-benzylaminopurine (BAP) and naphthaleneacetic acid (NAA) in factorial combinations. Optimum callusing was recorded in the presence of 0.5mg/l NAA and 1 mg/l BAP (66.7%).

Nasiruddin *et al.* (2003) conducted a study to investigate the effects of different concentrations of 2, 4-D on callusing. The fastest callusing (9.32 days) with greatest amount (0.73g/ explant) was observed with 1 mg/l 2, 4-D at 20 DAE.

Huan *et al.* (2004) induced embryogenic calli from longitudinally bisected segments of protocorm like bodies (PLBs) of *Cymbidium* Twilight Moon 'Day Light', on modified Vacin and Went medium supplemented with 1-naphthalenecetic acid (NAA) or 2,4 dichlorophenoxyacetic acid (2,4-D) alone or in combination with N-phenyl-N'-1,2,3-thiadiazol-5yl urea (TDZ) within one month. The medium containing the combination of 0.1mg/l NAA and 0.01 mg/l TDZ was optimal for callus formation. Calli proliferated very well when being sub-cultured every 4 weeks on the same medium.

Embryonic calli were produced from segments of young internodes of flower stalks of *Vanda sanderiana* cultured in vitro on 1.2 times of Vacin and Went's basal medium supplemented with 1 mg 6-benzylaminopurine (benzyladenine)/l, 0.1 mg kinetin/l and 20 g sucrose/l. cut internodes, 3mm thick each, from 3-4 cm long flower stalks were the most suitable for the production of embryonic calli. (Linc, 2005).

### **2:4:3: Protocorm Formation**

Different culture media have been used for efficient plant regeneration in Orchid tissue culture. Among, them MS medium (Murashige and Skoog, 1962) was found to be efficient for PLBs formation.

Morel (1974) successfully regenerated protocorms from explants of *Ophrys* when both NAA and Kinetin were incorporated in the culture medium and for *Paphiopedilum* when 2, 4-D and BA were used.

Helena and Rao in 1980 established a successful tissue culture procedures for rapid multiplication of three interspecific precious hybrids of *Vanda*. Among the different growth adjuvants investigated, yeast extract (YE),

peptone, urea and naphthaleneacetic acid (NAA) markedly enhanced the growth and differentiation of protocorms.

Apical flower buds of *Cymbidium georingii* Reichenbachfil. (ca 2mm long) excised from inflorescence (ca 5mm long) were explanted on modified Murashige and Skoog medium (MS medium) supplemented with N<sup>6</sup>-benzyladenine (BA) and  $\alpha$ -naphthaleneacetic acid (NAA). Within 107 days of culture, swelling growth, chlorophyll synthesis, and subsequent rhizome differentiation were observed. (Kazuhiko *et al.*, 1991).

According to De Pauw *et al.* (1995), the importance of auxin and cytokinin germination is limited but increases during the development of protocorms. Rasmussen (1995) reported a positive effect of adding cytokinins i.e., BA or Kinetin on protocorm development.

Seeds of *Dendrobium transparens* were inoculated on B5 medium supplemented with 6-furfuryl amino purine (KN) and Naphthalene acetic acid by Hazarika and Sarma (1995). They showed signs of swelling and the embryos which emerged out developed into globular, yellowish- green protocorms within 25 days of culture.

Pathania (1998) reported that stem explants procured from shoots emerging from *Dendrobium* cv. Sonia pseudobulb grown in different culture media like Vacin and Went and Knudson C media supplemented with BAP 1.5 mg/l, NAA 0.4mg/l and paclobutrazol 1mg/l was best for further multiplication of protocorm like bodies.

Immature seeds from unripe capsules of *Calanthe sieboldii*, were sown on one of the three sterilized media: MS; modified MSH [i.e., the inorganic salts of

MS plus the organic elements of Schenk and Hildebrandt]; and Hyponex. Germination and protocorm development occurred on the MS and MSH media within eight weeks, but percent germination was low (Park *et al.*, 2000).

Efficiency of protocorm like bodies (PLBs) production as a function of various growth regulators was assessed in three Orchid genera viz. *Dendrobium* 'Sonia', *Oncidium* 'Gower Ramsay' and *Cattleya leopoldii* by Saiprasad *et al.* (2002). Growth regulators viz., BAP, kinetin, NAA, IAA, 2,4-D and GA3 were supplemented to MS medium at concentrations of 0.25, 0.5 and 1.0 mg/l. Maximum number of PLBs were produced in BAP 1.0 mg/l. The relative ranking among the plant growth regulators (highest to lowest) for obtaining maximum number of PLBs was BAP > Kinetin > NAA > 2,4-D > GA3.

Talukdar *et al.* (2002) conducted a study on the embryo culture of two intergeneric hybrids of Orchid. The embryos of *Spathoglottis plicata* x *Dendrobium densiflorum* and *Dendrobium muschatum* x *Arundina bambusifolia* germinated with a very high frequency to form protocorms in Nitsch medium.

Yung *et al.* (2003) induced the totipotent callus of *Cypripedium formosanum* from seed derived protocorm segments on a quarter-strength MS medium containing 4.52  $\mu$ M 2, 4-D and 4.54  $\mu$ M thidiazuron. The callus proliferated well and was maintained by sub-culturing on the same medium. On an average, 13 protocorm-like bodies could be obtained from a piece of 4 mm callus after being transferred to the medium with 4.44  $\mu$ M benzyladenine after 8 weeks of culture.

Nagaraju (2003) reported that MS medium was the best for better growth and rapid proliferation of protocorm which was regulated in vitro from

meristem explants of *Cymbidium*. Supplement of BA in the media increased total number of PLBs. BA with 0.5mg/l was optimum for the multiplication of PLBs.

Nasiruddin *et al.* (2003) conducted a study to investigate the effects of different concentrations of BAP (0, 2, 4 and 6 mg/l) and NAA (0, 0.5 and 1 mg/l) on formation of PLBs and plantlet regeneration from *Dendrobium formosum* leaf callus. The number of PLBs was the highest (10.52/explant) at 60 DAE. Time required for PLB formation was the longest (32.72 days) with 0.50 mg/l 2, 4-D.

Kitsaki *et al.* (2004) investigated the effect of culture medium on the in vitro germination and development of protocorms and plantlets from seeds of 13 different *Ophrys* species (*O.apifera*, *O. attica*, *O. cornuta*, *O. delfinensis*, *O. ferrum-equinum*, *O. lutea*, *O.mammosa*, *O.speculum*, *O. spruneri*, *O. umbilicata*, *O. argolica*, *O.irricolor* and *O. tenthredinifera*). Mature seeds and immature seeds were cultured in a coconut milk-enriched or a pineapple enriched medium (CEM or PEM, respectively). The highest percentage of protocorm formation (52%) was observed in mature seeds of *O. spruneri* in the CEM. Protocorm formation was significantly lower in immature seeds than in mature seeds in both culture media.

Puchoa (2004) studied the potential of different media, supplements and conditions for reliable *Dendrobium* Orchid plantlet regeneration from in vitro grown leaf explants. In all three media under investigation, regeneration was via the production of protocorm-like bodies (PLBs). Maximum number of PLBs was obtained in Murashige and Skoog (MS) liquid medium agitated at 80 rpm and supplemented with 0.1 mg/l of BA, 1.0 mg/l of NAA and 15% (v/v) coconut water.

Seeds of *Cymbidium dayanum* Reichb. were sown in vitro and germinated to produce protocorms one month later. Protocorms elongated, and the unicellular cell absorbing hairs were initiated at the base. After two months, the protocorms grew, with the structure similar to that of rhizomes with nodes and lateral buds. (Chang and Chen *et al.*, 2005).

In *Rhynchostylis retusa*, the Knudson C medium supplemented with peptone (1g/l), NAA (1.5 mg/l) gave best subsequent growth and development of protocorms and in *Spathoglottis plicata* on KC medium supplemented with Kin 0.1mg/l and NAA 0.1 mg/l resulted in healthier seedling growth (Madhusmita *et al.*, 2005).

Effects of six asymbiotic media (Modified Lucke, Murashige & Skoog, Lindemann, Vacin and Went, Malmgren Modified, Knudson C), four exogenous cytokinins (BA, Zea, Kin, 2-iP), and three photoperiods (0/24, 16/8, 24/0 h L/D) were examined on seed germination and early protocorm development of *Habenaria macroceratitis*. Protocorm development was enhanced on MM medium after both seven and 16 weeks. Final percent of protocorm development was increased in the absence of light (0/24 h L/D) (Scott *et al.*, 2006)

Shylaraj *et al.* (2007) conducted a study to standardize a protocol for rapid mericlone of tropical Orchid *Dendrobium* cv. Sonia from the shoot tip explants. The shoot tip explants were cultured in agitated (80-100 rpm) liquid Vacin and Went medium supplemented with 3% sucrose, 2-2.5 mg/l BA and 0.5-1.0 mg/l NAA could induce a large number of protocorms within a period of two weeks.

Ping *et al.* (2008) developed an efficient micro propagation protocol for *Dendrobium densiflorum* Lindl. ex Wall., through protocorm-like bodies

(PLBs) from nodal stem segments using 6-benzylamino-purine (BAP) and the *Lanthanoid neodymium*. The highest percentage of explants producing PLBs (72%), with an average of 15 PLBs per explants, was induced by culturing stem segments on Murashige and Skoog (MS) medium supplemented with 5.0 mg/l BAP.

#### **2:4:4: Shoot Proliferation**

Paek and Edward (1991) cultured the rhizomes of *Cymbidium forrestii* on Murashige and Skoog medium supplemented with various growth substances. Leaf formation and development was retarded in the presence of auxin but benzyladenine (BA) could induce shoot formation *in vitro*. A 5% (w/v) sucrose concentration was most effective in shoot induction when combined with 5 mg/l BA.

Effect of growth regulators on *in vitro* seeding growth of *Spathoglottis plicata* was reported by Sangama and Singh (1994). They found that although a lower level of NAA, GA3 and BA was required for higher germination of seeds, subsequent seedling growth was better with the combination of a higher level of IBA, GA3 and BA each at 5mg/l in the KC medium.

High frequency direct shoot proliferation was induced in the shoot segments of three epiphytic Orchids, *Cymbidium aloifolium* (L.) Sw., *Dendrobium aphyllum* (Roxb.) Fisch. A and *Dendrobium moschatum* (Buchham) Sw. on Murashige and Skoog's medium (MS) containing N6-benzyl adenine (BA) or thiadiazuron (TDZ) with the later being more effective at 2.2-4.5 $\mu$ M as observed by Nayak *et al.*(1997). In both *Dendrobium* species the number of shoot bud formation was greatly influenced by explants orientation.

An *in vitro* method was developed by Talukdar (2001) to regenerate multiple shoots of *Dendrobium aphyllum* Roxb. Upto a maximum of seven shoots were regenerated in Knudson medium supplemented with kinetin (10 mg/l), coconut water (15% v/v) and banana extract (6% v/v) within 13 weeks of culture.

Nagaraju and Das (2002) tested the shoots of *in vitro* grown cultures of *Ascocentrum ampullaceum*, *Dendrobium nobile* and *Cattleya maxima* for their response to Nitsch media with Triaquontinal (0, 1, 2 and 3 mg/l). *C. maxima* showed better response with maximum number of shoots (9.2). *A. ampullaceum* showed better shoot growth (15.6 mm). Among the four concentrations, 2mg/l was optimum for number of shoots and leaves/shoots.

Pyati *et al.* (2002) cultured the nodal explants of *Dendrobium macrostachyum* on MS basal medium and MS medium supplemented with N6-Benzyladenine (BA-2.22, 4.44 and 8.88  $\mu$ M), Kinetin (KN 2.32, 4.65 and 9.29  $\mu$ M) and coconut water (CW- 5, 10 and 15) individually or in combination with 2.69  $\mu$ M alpha-naphthalene acetic acid (NAA). Axillary shoots were induced directly from nodal explants in medium containing BA, KN or CW.

A study conducted at KAU by Sobhana and Rajeevan (2002) for refinement of culture media for growing *Dendrobium* New Pink  $\times$  Emma white seedlings revealed that MS medium at 1/4<sup>th</sup> strength, Vacin and Went and Knudson-C media supplemented with Kinetin, BAP, NAA and IBA had pronounced influence on growth characters like height, leaf length and leaf number of seedlings.

Raju *et al.* (2003) observed that MS medium was the best for initial culture establishment of *Dendrobium osterholt*, 60 days after inoculation with a higher rate of multiple shoot production and higher average shoot length.

Talukder *et al.* (2003) investigated the effect of BAP (0, 1, 2.5 and 5 mg/l) and NAA (0, 0.1, 0.5 and 1 mg/l) on Orchid shoot regeneration with MS media. The best shoot proliferation, root formation, leaf number, increment of shoot length was obtained from 2.5 mg/l+ 0.5 mg/l NAA.

Ket *et al.* (2004) induced the multiple shoot proliferation of an elite cultivar of Jewel Orchid in shoot tip explants on Hyponex media supplemented with 1 mg/cubic dm benzyladenine or 1-2 mg/cubic thiadiazuron (TDZ). Addition of activated charcoal (1 g/cubic dm) to the TDZ containing medium protocorm multiple shoot formation (11.1 shoots per explant).

Leaf explants from flower stalk node derived shoots of *Dendrobium* hybrids Sonia 17 and 28 cultured on half-strength Murashige and Skoog (MS) medium supplemented with 44.4  $\mu$ M N6-benzyladenine (BA) developed more than seven shoots per explants. Half-strength MS medium containing 6.97  $\mu$ M kinetin (KN) facilitated conversion of more than 90% PLBs to shoots (Martin *et al.*, 2006).

Kishor *et al.*, (2007) inoculated the pseudobulb segments of *Dendrobium* hybrid (*D. nobile* x *D. chrysotoxum* L.) on full strength and half strength MS medium with different concentrations and combinations NAA, BAP and Kinetin. An average of two shoot buds per explants were regenerated on  $\frac{1}{2}$  MS+2.0 mg/l KN and  $\frac{1}{2}$  MS+ 0.1 mg/l NAA+ 1.0 mg/l BAP.

Kurup *et al.* (2007) conducted a study on Plant regeneration from mesophyll protoplast of *Dendrobium* var. Sonia. The protoplast from the

*Dendrobium* var. Sonia was successfully isolated and regeneration of shoots and roots were obtained in MS medium containing BA 5 mg/l+ NAA 1mg/l.

Parvin *et al.* (2009) conducted a study to investigate the effect of growth regulator NAA on *in vitro* shoot proliferation of *Dendrobium* Orchid. Among the different concentrations of NAA, the maximum increase in shoot weight (0.25 g) and shoot number (8.83) were observed from 0.1mg/l NAA. The highest shoot length (2.60 cm) and number of leaves (4.83) were obtained with 0.2 mg/l NAA at 60 DAT.

### **2:4:5: Root Formation**

Pathania *et al.* (1998) reported that VW and KC media favoured rooting in *Dendrobium* cv. Sonia when supplemented with IBA (1 mg/l) or NAA (1.8 mg/l) and Paclobutrazol (0.5 mg/l).

Alam *et al.* (2002) tested four different media viz., Hyponex, Murashige and Skoog (MS), OKFI and Knudson C (KC) for multiplication of *Dendrobium transparens* Wall. via seeds. More number of roots per plantlet with highest root length was observed in MS medium supplemented with NAA 1mg/l which showed significantly better result for *in vitro* seed propagation.

Pyati *et al.* (2002) cultured the nodal explants of *Dendrobium macrostachyum* on MS basal medium and MS medium supplemented with N6-Benzyladenine (BA-2.22, 4.44 and 8.88  $\mu$ M), Kinetin (KN- 2.32, 4.65 and 9.29  $\mu$ M) and coconut water (CW – 5,10 and 15) individually or in combination with 2.69 $\mu$ M alpha –naphthalene acetic acid (NAA). Well-developed shoots rooted at an average 5 roots per shoot in half strength MS medium devoid of any growth regulators.

Sobhana and Rajeevan (2002) conducted a study on refinement of embryo culture media for growing *Dendrobium* New pink × Emma white hybrid seedlings. It was found that MS medium at ¼ strength, Vacin and Went and Knudson C supplemented with Kinetin BAP, NAA and IBA had profound influence on seedling growth as well as root length and number.

Indhumati *et al.* (2003) reported that the root number and root length of micro shoots of *Dendrobium* hybrid Sonia-17 were the highest in KC medium supplemented with IBA 0.5 mg/l.

Nasiruddin *et al.* (2003) investigate the effect of different concentrations of BAP (0, 2, 4 and 6 mg/l) and NAA (0, 0.5 and 1 mg/l) on *Dendrobium formosum* leaf callus. Observations recorded on root formation, root length and number of roots per plantlet revealed that BAP at 2.5 mg/l showed the best results in combination with 1 and 2 mg/l NAA.

Raju *et al.* (2003) obtained maximum rooting of in vitro cultured *Dendrobium osterholt* in half strength MS media with IBA 1mg/l +NAA 1mg/l.

Le Van *et al.* (2004) conducted a trial on *Cymbidium* Twilight Moon 'Day Light' on modified Vacin and Went medium supplemented 1-naphthaleneacetic acid (NAA) or 2,4- dichlorophenoxyacetic acid (2,4-D) alone or in combination with N-phenyl-N'-1,2,3- thiadiazol-5yl urea (TDZ) within one month. Shoots with well-developed roots were observed on the medium without plant growth regulators.

Ket *et al.* (2004) induced the shoot proliferation of an elite cultivar of jewel Orchid on Hyponex media supplemented with 1 mg/cubic dm benzyl

adenine or 1-2 mg/cubic dm thiadiazuron (TDZ). Rooting was induced in 100 percent of the regenerated shoots in the same media.

Martin *et al.* (2006) found that half strength MS medium with 2 g/l activated charcoal was the best for *in vitro* rooting of *Dendrobium* hybrids Sonia17 and 28.

Kalimuthu *et al.* (2007) achieved *in vitro* regeneration, multiplication and rooting of plantlets from immature seeds of *Oncidium* on MS medium supplemented with BAP (2mg/l) whereas Kurup *et al.* (2007) obtained regeneration of roots of *Dendrobium* var. Sonia in MS medium containing BA 5mg/l and NAA 1 mg/l.

Hossain (2008) induced strong and stout root system of *Epidendrum ibaguense* Kunth. *In vitro* seedlings on half strength PM (Phytamax) and M (Mitra) media fortified with 0.5- 1.0mg/l IAA. Well rooted seedlings were transferred to green house with 90 percent survival.

Ping *et al.* (2008) developed a protocol for *Dendrobium densiflorum* Lindl.ex Wall, through PLBs from the nodal stem segments using 6-BAP and lanthanoid neodymium. Shoots produced an average of 22 roots per plantlet when cultured on MS medium supplemented with 2.0 mg/l neodymium nitrate. The result suggested that thalanthanoids could be used effectively to initiate rooting in *D. densiflorum*.

Parvin *et al.* (2009) conducted a study to investigated the effect of growth regulator NAA on *in vitro* rooting of *Dendrobium* Orchid. The highest number of roots (5.15) with maximum root length (2.67 cm) were obtained with 0.2 mg/l NAA at 60 DAT.

Vyas *et al.* (2011) reported that morphologically uniform plantlets with elongated leaves and well developed roots of *Dendrobium lituiflorum* were formed on Knudson C (KC) medium supplemented with 20% (v/v) banana extract (BE).

## 2:5: HARDENING

Pine-bark, cork-bark, tree fern pieces, sphagnum moss, gravel, charcoal pieces and coconut fibres are commonly used potting materials for Orchids (Suresh Kumar, 1992).

Sharma and Chauhan (1995), the potting mixture comprising brick, charcoal, tree fern, bark pieces, leaf mould and dry sphagnum in 1:1:1:1:1:2 ratios with green sphagnum on top supported the maximum survival of *Dendrobium chrysanthum* seedlings.

According to Pathania *et al.* (1998) hardening medium containing bark pieces, brick pieces, moss, charcoal pieces (1:1:1:1) proved to be suitable for the establishment of *in vitro* rooted plantlets of Sonia-17.

The establishment of plantlets of *Dendrobium* Hybrid Sonia-17 in terms of plant height, leaf size, new growth and root length was good in the medium consisting of charcoal+brick+cocopeat (1:1:1), root number was highest in pure charcoal medium. Spraying with half KC nutrient solution improved the growth and establishment of the *in vitro* derived plantlets (Indhumati *et al.*, 2003)

Arumugam and Jawaharlal (2004) reported that the vase life of *Dendrobium* cultivar Sonia-17 was found highest in coconut fibre medium

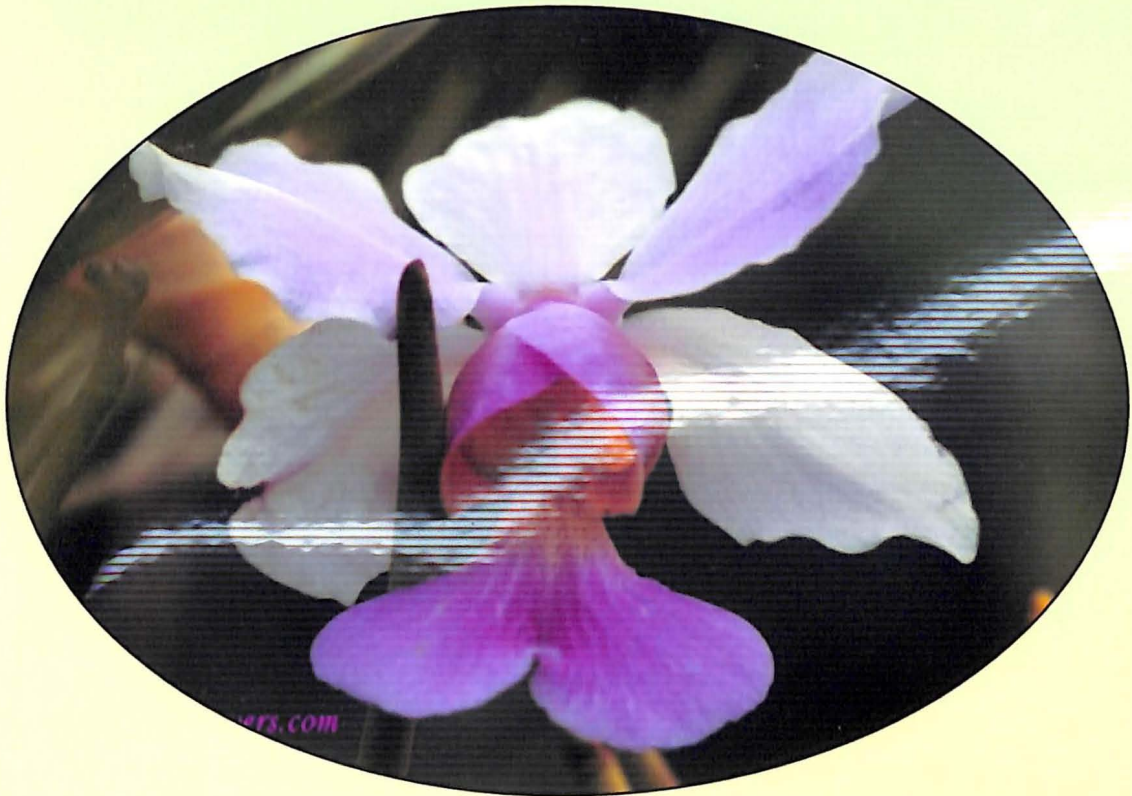
(21.20 days). Early flowering (177.00 days) was recorded in coconut fibre medium while late flowering (198.66 days) was observed in brick pieces.

Mani and Nagaraju (2004) reported that cocopith alone and in combination with sand best suitable for the acclimatization of *in vitro* raised seedlings of *Cymbidium* hybrids.

Sugapriya *et al.* (2011) suggested among the different varieties of *Dendrobium* evaluated, Sonia-17 recorded maximum plant height, length of internode and number of pseudobulbs per plant under partially environment controlled Greenhouse.

Vyas *et al.* (2011) stated that the tissue culture plantlets of *Dendrobium lituiflorum* are best hardened in *on agar-agar gelled medium, Luffa sponge medium and cocopeat: perlite (9:1)* as support matrices, each containing one-half strength KC major salts.





## CHAPTER III

# *Materials and methods*

**Zeal for work is a  
key to success....**

# MATERIALS AND METHODS

The present investigation was undertaken during the year 2011-12. The materials used and the techniques adopted during the course of investigation are described in this chapter.

## 3:1: EXPERIMENTAL SITE

The experiment on “High frequency multiplication of orchids through *in vitro* culture” was carried out in the Laboratory of Biotechnology-cum-Tissue Culture Centre, Department of Floriculture and Landscaping, Orissa University of Agriculture and Technology, Bhubaneswar.

## 3:2: VARIETAL DESCRIPTION

The *Papilionanthe teres* and *Dendrobium* cv. Sonia-17 were selected for this experiment. The important characteristics of the said species are presented as follows.

The *Papilionanthe teres* is an epiphytic, monopodial orchid with ornamental flowers. Aerial roots and inflorescence developed from the leaf axil. It prefers open sun light for growth and development.

The *Dendrobium* cv. Sonia-17 is an epiphytic, sympodial orchid with white and purple coloured flowers. Sepals creamy white with purple markings and petals purple in colour. It prefers partial shade, high humidity and performs well under tropical conditions. It is one of the important commercial varieties of *Dendrobium* having very good demand in the domestic as well as in the international flower market.

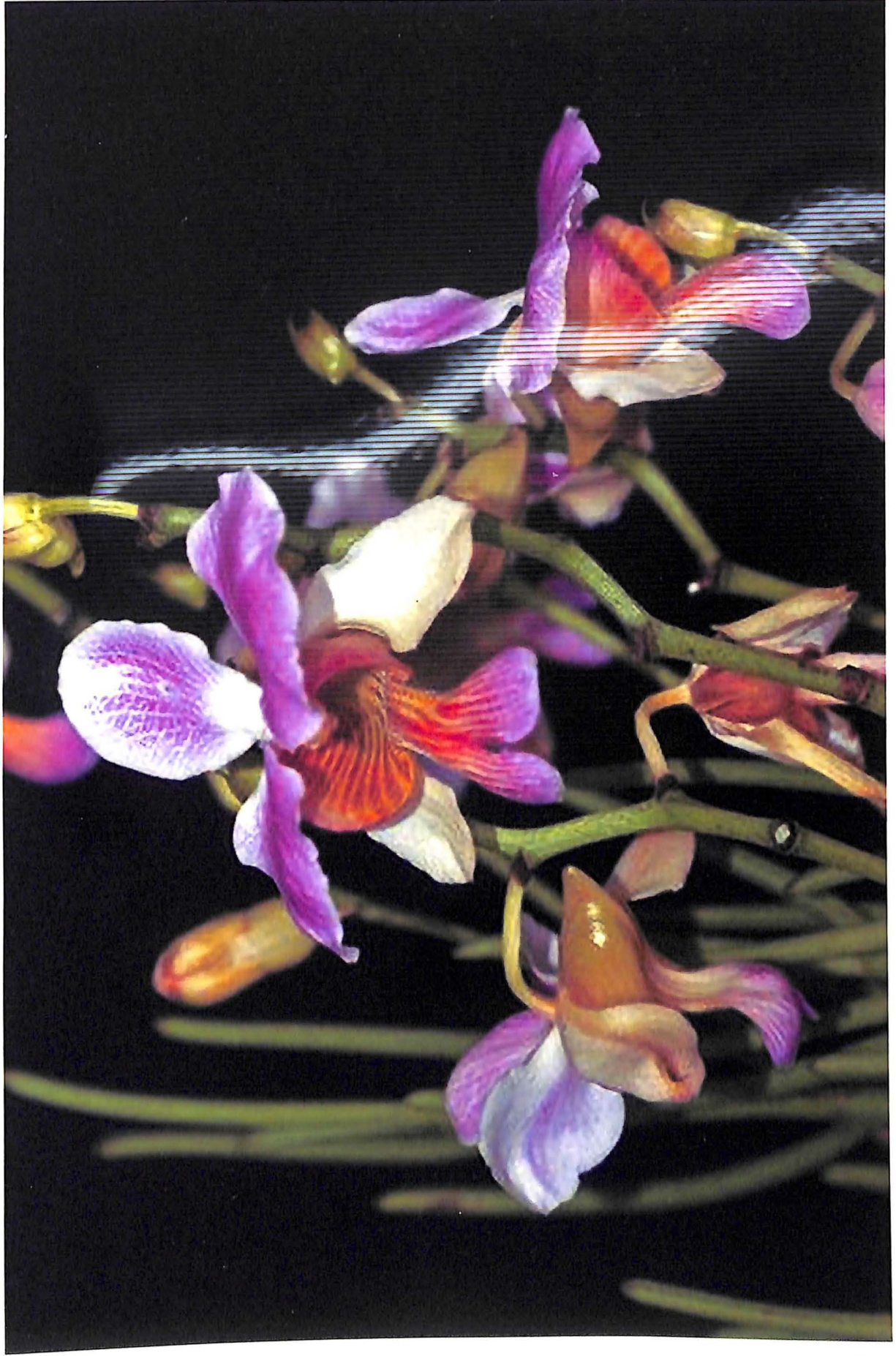


Plate 1. Full blooming of *Papilionanthe teres* (*Vanda teres*)

### **3:3: COLLECTION OF EXPERIMENTAL MATERIALS**

The pods of *Papilionanthe teres* were collected from Derace Chandaka forest range which is very adjacent to Bhubaneswar city. The *Dendrobium* cv. Sonia-17 was collected from the Regional Plant Resource Centre (RPRC), Bhubaneswar.

### **3:4: EXPLANTS**

The existing protocorms of *Papilionanthe teres* and shoot initials maintained in the culture of the Tissue culture laboratory of *Dendrobium* cv. Sonia-17 were used as the explants for further study.

### **3:5: EXPERIMENTAL DETAILS**

The following steps were undertaken for conducting the whole investigations:

1. Standardization of media supplements for shoot proliferation of orchids (*Papilionanthe* and *Dendrobium*).
2. Standardization of media supplements for root initiation and development.
3. Standardization of media mixture for hardening of the *in vitro* plantlets.

### **3:6: SOURCE OF CHEMICALS**

The chemicals used for the study were analytical reagents of excel and grade of Titan Biotech Ltd., Ranbaxy Laboratory Ltd, Merck (India), Qualigen Fine Chemicals and Himedia Laboratories Ltd. (India). Auxins, Cytokinins, Myo-inositol and Fe-EDTA were supplied by Sigma (USA) and Agar from Ranbaxy Laboratory Ltd.

### 3:7: GLASSWARE AND EQUIPMENTS

**Table:1** Detail of glassware, equipments with name of their supplier company

Glassware and Equipment	Supplier Company
Culture tubes, beakers, bottles, pipettes, measuring cylinders	BOROSIL, India
pH meter	Elico
Microoven	LG Instruments, India
Micropipettes	BOROSIL, India
Digital balance	Sartorius Basic
Laminar air flow system	Klenzoids, India
Autoclave	Satyam
Surgical Tools	Forceps, Scissors, Scalpel, Blade etc.

### 3:8: FORMULATION OF MEDIA

The formulation of MS medium (Murashige and Skoog, 1962) was used as a basal medium for the entire study and the composition of the same is given as follows:

**Table:2** Inorganic and Organic constituents of MS medium (Murashige and Skoog, 1962)

Stock solution constituents	Concentration of stock solution (mg/l)	Volume of stock solution taken (ml/l)	Concentration in culture medium (mg/l)
<b>Macronutrients (10 times concentrated)</b>			
NH <sub>4</sub> NO <sub>3</sub>	16500	100	1650
KNO <sub>3</sub>	19000		1900
CaCl <sub>2</sub> .2H <sub>2</sub> O	4400		440
MgSO <sub>4</sub> .7H <sub>2</sub> O	3700		370
KH <sub>2</sub> PO <sub>4</sub>	1700		170

Stock solution constituents	Concentration of stock solution (mg/l)	Volume of stock solution taken (ml/l)	Concentration in culture medium (mg/l)
<b>Micronutrients (100 times concentrated)</b>			
KI	83	10	0.83
H <sub>3</sub> BO <sub>3</sub>	620		6.2
MnSO <sub>4</sub> .4H <sub>2</sub> O	2230		22.3
ZnSO <sub>4</sub> .7H <sub>2</sub> O	860		8.6
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	25		0.25
CuSO <sub>4</sub> .5H <sub>2</sub> O	2.5		0.025
CoCl <sub>2</sub> .6H <sub>2</sub> O	2.5		0.025
<b>Iron compound (100 times concentrated)</b>			
FeSO <sub>4</sub> .7H <sub>2</sub> O	2780	10	27.8
Na <sub>2</sub> EDTA.2H <sub>2</sub> O	3730		37.3
<b>Vitamins (100 times concentrated)</b>			
Myo-Inositol	10000	Added fresh	100
Glycine	200	10	2.0
Nicotinic acid	50		0.5
Pyridoxine-HCL	50		0.5
Thiamine-HCL	10		0.1
<b>Other substances</b>			
Sucrose			30000
Agar			7000
pH			5.7 ± 0.1

### 3:9: PREPARATION OF STOCK SOLUTION

The stock solution for macro and micro nutrients were prepared separately by dissolving the salt as mentioned in the above table in sterile double distilled water with due care. For preparing Fe-EDTA stock solution, Na<sub>2</sub>EDTA and FeSO<sub>4</sub>.7H<sub>2</sub>O were dissolved in luke warm distilled water separately and then these were combined and the final volume of the solution was made up by adding distilled water. Instead of Na<sub>2</sub>EDTA and FeSO<sub>4</sub>.7H<sub>2</sub>O,

sodium ferric salt of EDTA was added fresh to the medium @ 40 mg/l. Myoinositol and sucrose were also added to it. Fresh stock solutions of vitamins like Pyridoxin-HCl (50mg), thiamine-HCl (10mg), nicotinic acid (50mg) and glycine (200mg) were separately dissolved in 100 ml of double distilled water and during media preparation 1 ml from each were added to the media. The stock solutions were stored in low temperature varying from 4<sup>0</sup>C to 20<sup>0</sup>C in refrigerator.

### **3:10: PREPARATION OF STOCK SOLUTION FOR PLANT BIO REGULATORS (PBRS)**

The stock solutions of plant bio regulators were prepared as follows.

#### **3:10:1: Auxin**

##### **a. IBA (Indole-3 Butyric Acid)**

Exactly 100 mg of IBA was weighed in an electric balance and first dissolved in small quantity of 0.1 N NaOH and then sterile double distilled water was added slowly and the volume was made up to 100 ml.

##### **b. NAA (Naphthalene Acetic Acid)**

Exactly 100 mg of NAA was weighed in an electric balance and first dissolved in small quantity of 0.1 N NaOH and then sterile double distilled water was added slowly and the volume was made up to 100 ml.

#### **3:10:2: Cytokinin**

##### **i. BAP (Benzyl Amino Purine)**

Exactly 100 mg of BAP was weighed and few drops of 0.1N HCl were added to dissolve it initially. Sterile double distilled sterile double distilled water was added slowly and the volume was made up to 100 ml.

## **ii. Kinetin**

Exactly 100 mg of kinetin was dissolved in 0.1 N HCL and then the volume was made up to 100 ml with sterile distilled water.

### **3:10:3: Adenine sulphate**

It was added fresh to the medium at 100 mg/l.

### **3:11: PREPARATION OF CULTURE MEDIUM**

For preparation of culture medium required quantities of macronutrients, micronutrients, Fe-EDTA, vitamins and growth regulators were taken from the stock solution and mixed in distilled water. Required quantities of sucrose dissolved in water was also added fresh to the solution prepared. The pH of solution was adjusted to  $5.7 \pm 0.1$  using 0.1 N NaOH or 0.1 N HCl. Then the final volume was made up to one litre with distilled water. After the volume was made up, the required quantity of agar was added and boiled in a beaker till it got dissolved and looked transparent. The hot medium was distributed to sterilized culture bottles.

#### **3:11:1: Sterilization of culture medium**

The bottles with culture medium were autoclaved for 25 minutes at  $121^{\circ}\text{C}$  and 15 psi pressure. The autoclaved medium was kept in a laminar air flow bench for cooling.

#### **3:11:2: Sterilization of glassware and instruments**

All the glassware were dipped in detergent solution overnight and washed under running tap water. The glassware were again rinsed with distilled water and dried in an oven for two hours at  $150^{\circ}\text{C}$ . Petridishes, scalpel and forceps were cleaned properly with alcohol, wrapped with a paper and aluminium foil and were kept in a clean sterilized base followed by sterilization in autoclave at 15 psi and  $121^{\circ}\text{C}$  for 30 minutes.

### **3:11:3: Sterilization of laminar air flow cabinet**

The laminar air flow cabinet was cleaned by wiping with absolute ethyl alcohol. Filtered air (80-100 cft/min) was blown for five minutes to remove the dirt particles from the working area. All the instruments that were to be used were kept inside the chamber and exposed to UV light for 25 minutes. While executing the inoculation work, filtered air was continuously passed through the laminar air flow cabinet.

### **3:12: CULTURE ROOM**

The temperature of the culture room was maintained at  $25\pm 2^{\circ}\text{C}$  and relative humidity of 80% by providing air conditioner in the room and 16 hour photo period (3000-3200 lux) supplied by fluorescent tubes exclusively for maintaining the light requirements.

### **3:13: STANDARDIZATION OF MEDIA SUPPLEMENTS FOR SHOOT PROLIFERATION OF ORCHIDS (*PAPILIONANTHE* AND *DENDROBIUM*)**

The existing protocorm like bodies in *Papilionanthe teres* and shoot initiation of *Dendrobium* cv. Sonia-17 maintained at the tissue culture laboratory were cut into pieces of more or less uniform size with the help of a sterilized sharp blade and pieces were inoculated at the rate of five pieces per bottle in MS media containing different concentrations of auxin (NAA) and cytokinins (BAP, Kinetin) and the adjuvant adenine sulphate (AdS) for better spread and development of callus, shoot height, leaf length and sucker formation. In total there are ten treatments with three replications per treatment for *Papilionanthe teres* and the PLBs were cultured for 30 days (Table-3). For *Dendrobium* cv. Sonia-17, there are thirteen and nine treatments with three replications per treatment and cultured for 30, 45 and 60 days (Table-4 and Table-5).

**Table:3 Impact of Plant bioregulators and adjuvant on shoot initiation in *Papilionanthe teres***

**Basal medium: MS**

Treatments	Plant Bioregulators		Adjuvants
	BAP (mg/l)	NAA (mg/l)	AdS(mg/l)
T <sub>1</sub>	-	-	-
T <sub>2</sub>	2.0	-	-
T <sub>3</sub>	2.5	-	-
T <sub>4</sub>	3.0	-	-
T <sub>5</sub>	2.0	0.5	-
T <sub>6</sub>	2.5	0.5	-
T <sub>7</sub>	3.0	0.5	-
T <sub>8</sub>	2.0	-	100
T <sub>9</sub>	2.5	-	100
T <sub>10</sub>	3.0	-	100

The following observations were recorded after 30 days of inoculation in *Vanda spp.*

- i. **Number of leaves/ clump** : It was obtained by dividing the total number of leaves with number of clumps used in the culture bottle.
- ii. **Length of leaf**: The average length of the leaves in the culture bottles was measured with the help of a scale from outside the bottle and recorded in cm.
- iii. **Calli /Protocorm/ Shoot formation**: A visual estimate was made by observing the calli mass and protocorms.
- iv. **Colour of calli/protocorm/shoot mass**: Visually observed colour of calli/protocorm/shoot mass under various treatments in the whole phase was noted.

**Table:4 Impact of plant bioregulators and adjuvant on shoot proliferation of *Dendrobium* cv Sonia-17**

**Basal medium: MS**

Treatments	Plant Bioregulators		Adjuvants
	BAP (mg/l)	NAA (mg/l)	AdS(mg/l)
T <sub>1</sub>	-	-	-
T <sub>2</sub>	2.0	-	-
T <sub>3</sub>	2.5	-	-
T <sub>4</sub>	3.0	-	-
T <sub>5</sub>	2.0	0.5	-
T <sub>6</sub>	2.5	0.5	-
T <sub>7</sub>	3.0	0.5	-
T <sub>8</sub>	2.0	-	100
T <sub>9</sub>	2.5	-	100
T <sub>10</sub>	3.0	-	100
T <sub>11</sub>	2.0	0.5	100
T <sub>12</sub>	2.5	0.5	100
T <sub>13</sub>	3.0	0.5	100

**Table:5 Impact of plant bioregulators on shoot proliferation of *Dendrobium* cv Sonia-17**

**Basal medium: MS**

Treatment	Kinetin (mg/l)	NAA (mg/l)
T <sub>1</sub>	-	-
T <sub>2</sub>	3.0	-
T <sub>3</sub>	4.0	-
T <sub>4</sub>	5.0	-
T <sub>5</sub>	6.0	-
T <sub>6</sub>	3.0	0.5
T <sub>7</sub>	4.0	0.5
T <sub>8</sub>	5.0	0.5
T <sub>9</sub>	6.0	0.5

The following observations were recorded after 30 days, 45 days, 60 days of inoculation in *Dendrobium* cv Sonia-17.

- i. **Plantlet height:** The average height of the plantlets present in culture bottles.
- ii. **Number of leaves:** The average number of leaves formed inside the culture bottles.
- iii. **Leaf length:** The average length of the leaves in the culture bottle under different treatments was measured with the help of a scale from outside the bottle and recorded in cm.
- iv. **Number of suckers:** The average number of suckers generated per explant per bottle was recorded.

### 3.14 STANDARDIZATION OF MEDIA SUPPLEMENTS FOR ROOT INITIATION AND DEVELOPMENT

The shoots developed in culture bottles were transferred into the half MS media supplemented with different concentrations of IBA and NAA alone or in combination, for initiation and better development of roots. There were nine treatments (Table-7) which were replicated thrice in this experiment.

**Table: 6 Impact of plant bioregulators on root initiation and development in *Papilionanthe teres* and *Dendrobium* cv Sonia-17**

Treatments	Basal medium: MS	
	IBA (mg/l)	NAA (mg/l)
T <sub>1</sub>	-	-
T <sub>2</sub>	0.5	-
T <sub>3</sub>	1.0	-
T <sub>4</sub>	-	0.5
T <sub>5</sub>	-	1.0
T <sub>6</sub>	0.5	0.5
T <sub>7</sub>	0.5	1.0
T <sub>8</sub>	1.0	0.5
T <sub>9</sub>	1.0	1.0

Observations were recorded thrice, once at 30 days after inoculation, 60 days after inoculation and 90 days after inoculation. The parameters which were noted are as follows:

- i. **Number of roots:** Total number of roots formed in the entire rooting phase were counted and noted.
- ii. **Number of roots/plantlet:** It was computed by dividing the total number of roots with the number of plantlets developed in the tube.
- iii. **Root length:** The roots with maximum length were measured with a scale and recorded in cm.

### **3:15: STANDARDIZATION OF MEDIA MIXTURE FOR HARDENING OF THE *IN VITRO* RAISED PLANTLETS OF *DENDROBIUM CV. SONIA-17***

The well developed seedlings of 4-5 cm height were taken and thoroughly washed with tap water to remove adhering medium completely without causing any damage to the roots. The media mixtures are prepared with brick pieces, charcoal, cocopeat, sphagnum moss, decayed wood and with their different combinations in equal proportions and filled in the pots. The components of the media like cocopeat were fumigated with formalin (40%) and sphagnum moss was treated with fungicide (bavistin 2%) before used in combinations. The seedlings were planted carefully in the respective mediums so that the roots of seedlings passed through the media and kept under the green house. The half MS liquid medium was fertigated to the seedlings at an interval of two days up to two weeks and then one fourth MS liquid medium was applied at an interval of two days the next two weeks. Then irrigation was given with the drippers present in the green house. In total for conducting up to experiment there were nineteen treatments replicated thrice with 5 plants per replication and the observations were recorded 30 and 60 days after the planting.

**Table: 7** Impact of media mixture for hardening of the *in vitro* plantlets of *Dendrobium cv. Sonia-17*

Treatment	Composition
T <sub>1</sub>	Brick pieces
T <sub>2</sub>	Charcoal
T <sub>3</sub>	Cocopeat
T <sub>4</sub>	Brick pieces + Charcoal
T <sub>5</sub>	Brick pieces + Cocopeat
T <sub>6</sub>	Charcoal +Cocopeat
T <sub>7</sub>	Brick pieces +Charcoal +Cocopeat
T <sub>8</sub>	Brick pieces + Sphagnum moss
T <sub>9</sub>	Brick pieces +Decayed wood
T <sub>10</sub>	Brick pieces + Sphagnum moss+Decayed wood
T <sub>11</sub>	Charcoal + Sphagnum moss
T <sub>12</sub>	Charcoal+ Decayed wood
T <sub>13</sub>	Charcoal + Sphagnum moss+ Decayed wood
T <sub>14</sub>	Cocopeat + Sphagnum moss
T <sub>15</sub>	Cocopeat+ Decayed wood
T <sub>16</sub>	Cocopeat + Sphagnum moss+ Decayed wood
T <sub>17</sub>	Brick pieces + Charcoal + Cocopeat+ Sphagnum moss
T <sub>18</sub>	Brick pieces + Charcoal + Cocopeat+ Decayed wood
T <sub>19</sub>	Brick pieces + Charcoal + Cocopeat+ Sphagnum moss+ Decayed wood

### 3:16: STATISTICAL ANALYSIS

The data concerned to CRD with three replications, each consisting of three culture bottles. Each bottle containing five cultures for shoot proliferation and rooting were used. For hardening total nineteen number of treatments replicated thrice with five plantlets per pot were used for study. Various parameters were analyzed statistically wherever necessary using the methods suggested by Gomez and Gomez (1984) in Completely Randomized Design (CRD). The analysis of variance (ANOVA) table was prepared. The treatment

effects were tested by 'F' test at 5% level of significance. The critical difference at 5% level was calculated for comparing the treatment means.

$$CD = SE (m) \times t, \text{ where } SE (m) \pm$$

= Standard Error Mean

$$= \sqrt{\frac{2 \times \text{Error Mean Square}}{\text{Number of replication}}}$$

T = Two sides table value at error degree of freedom at 5 % level of significance.



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$$CD = SE (m) \times t, \text{ where } SE (m) \pm$$

= Standard Error Mean

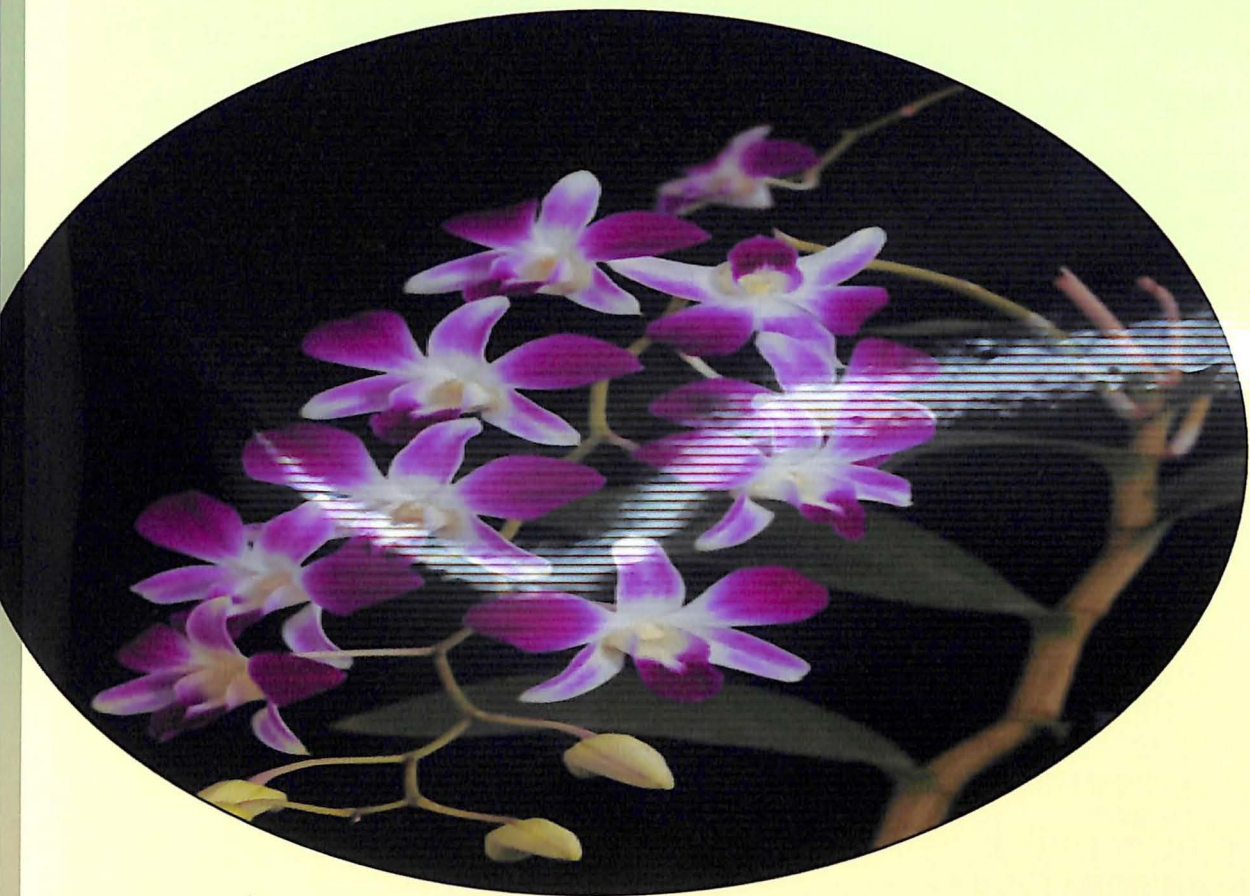
$$= \sqrt{\frac{2 \times \text{Error Mean Square}}{\text{Number of replication}}}$$

T = Two sides table value at error degree of freedom at 5 % level of significance.





Plate 2. *Dendrobium* Orchid cv. Sonia-17



## CHAPTER IV

# *Experimental Findings*

**Arise, awake and stop not till  
reach your goal.....**

## EXPERIMENTAL FINDINGS

In this chapter, the results of investigations are presented under the appropriate heads of the study.

1. Standardization of media supplements for shoot proliferation of orchids (*Papilionanthe* and *Dendrobium*).
2. Standardization of media supplements for root initiation and development.
3. Standardization of media mixture for hardening of the *in vitro* plantlets.

### **4:1: STANDARDIZATION OF MEDIA SUPPLEMENTS FOR SHOOT PROLIFERATION OF ORCHIDS (*PAPILIONANTHE* AND *DENDROBIUM*)**

#### **4:1:1: Standardization of media supplements for shoot initiation in *Papilionanthe teres***

It was evident from the data presented in the Table-8 that MS medium fortified with 2.0 mg/l BAP and 100 mg/l AdS significantly enhanced the number of leaves per explant (2.60), which stood at par with 2.0 mg/l or 2.5 mg/l BAP alone or in combination with 0.5 mg/l NAA, 2.5 or 3.0 mg/l BAP with 100 mg/l AdS and MS medium alone. Increase in concentration of BAP from 2.0 mg/l to 2.5 mg/l increased the number of leaves per explant. As far as leaf length is concerned MS medium supplemented with 3.0 mg/l BAP along with 100 mg/l AdS significantly increased the length of the leaf (0.41 cm), which stood at par with all the treatments except BAP 3.0 mg/l alone or in combination with 0.5 mg/l NAA and BAP 2 mg/l or 2.5 mg/l along with AdS 100 mg/l. All the treatments produced greenish yellow colour calli like mass

along with PLBs and shoots. The treatment T<sub>10</sub> i.e. MS medium supplied with 3.0 mg/l BAP along with 100 mg/l AdS initiated shoots with prominent leaves along with calli mass and PLBs.

Considering the above mentioned characters for shoot initiation studies in genus *Papilionanthe*, it has been concluded that T<sub>10</sub> (MS medium supplemented with 3 mg/l BAP and 100 mg/l AdS) was found to be the best treatment for shoot initiation (Fig. 1 and Plate 3, 4 and 5).

**Table:8 Efficacy of Plant Bioregulators and Adjuvant on shoot initiation of *Papilionanthe* spp**

**Basal Medium: MS**

**Duration:30 days**

Treatment	Plant Bioregulators		Adjuvant	No. of leaves/clump	Leaf length (cm)	Calli mass along with PLBs and shoots	Colour of the calli, PLBs and shoots
	BAP (mg/l)	NAA (mg/l)	AdS (mg/l)				
T <sub>1</sub>	-	-	-	2.33	0.23	More calli with less PLBs and shoots	Greenish Yellow
T <sub>2</sub>	2.0	-	-	2.00	0.21	More PLBs and shoots	Greenish Yellow
T <sub>3</sub>	2.5	-	-	2.07	0.23	Shoots with rudimentary leaves and calli	Greenish Yellow
T <sub>4</sub>	3.0	-	-	1.53	0.17	More calli	Greenish Yellow
T <sub>5</sub>	2.0	0.5	-	2.00	0.21	Shoots with rudimentary leaves with calli	Greenish Yellow
T <sub>6</sub>	2.5	0.5	-	2.40	0.24	Shoots with more leaves with calli mass	Greenish Yellow
T <sub>7</sub>	3.0	0.5	-	1.40	0.10	Shoots with rudimentary leaves with calli	Greenish Yellow
T <sub>8</sub>	2.0	-	100	<b>2.60</b>	0.15	Shoots with rudimentary leaves with more calli	Greenish Yellow
T <sub>9</sub>	2.5	-	100	2.30	0.18	Less calli	Greenish Yellow
T <sub>10</sub>	3.0	-	100	2.30	<b>0.41</b>	More calli with PLBs and shoots with prominent leaves	Greenish Yellow
SE(m)±				0.21	0.04		
CD (5%)				0.61	0.11		

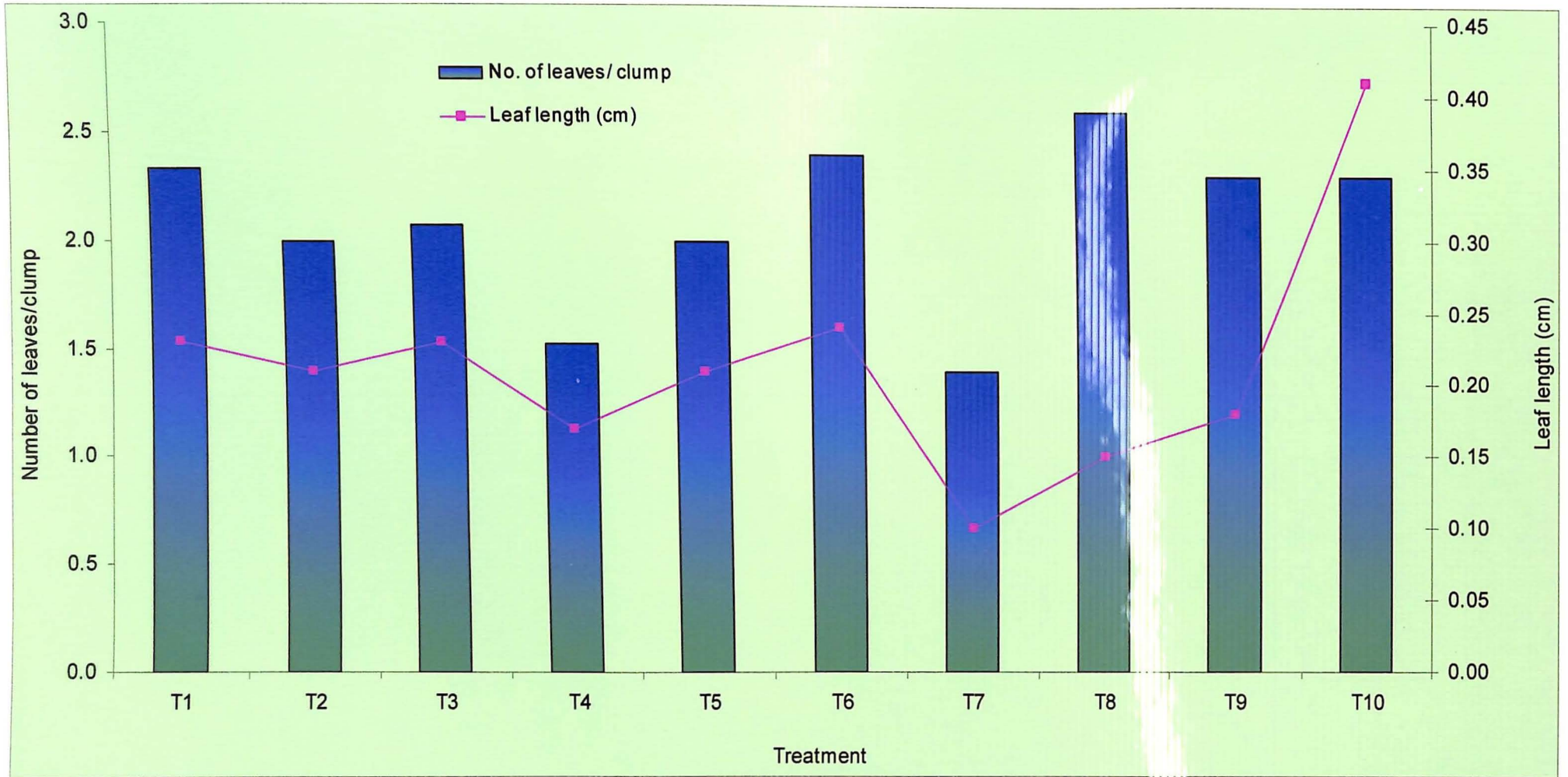


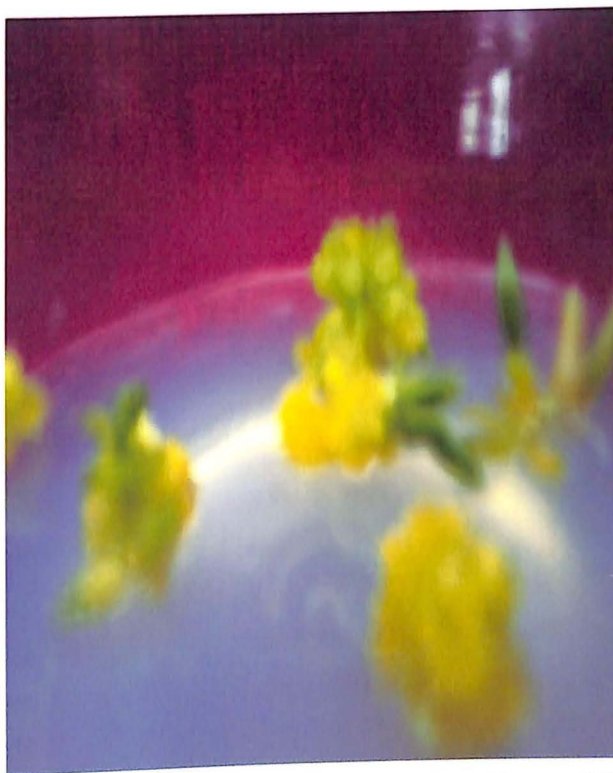
Fig. 1. Effect of plant bioregulators and adjuvant on shoot initiation (number of leaves/clump) and leaf length of *Papilionanthes teres*



**Plate 3.** PLBS taken as explant for *in vitro* study in *Papilionanthe teres*



**Plate 4.** Greenish yellow calli mass with protocorms in MS + 2.5 mg/l BAP after 30 days of culture



**Plate 5.** Greenish yellow calli with protocorms and prominent leaves in MS + 3.0 mg/l BAP + 100mg AdS

#### **4:1:2 Standardization of media supplements for shoot proliferation *Dendrobium cv. Sonia-17***

The perusal of the data in the Table-9 revealed that MS medium fortified with 2.0 mg/l BAP significantly enhanced the height of the shoot (2.17 cm) in *Dendrobium cv. Sonia-17* after 30 days of culture, followed by the treatment T<sub>6</sub> i.e. MS medium supplemented with 2.5 mg/l BAP and 0.5 mg/l NAA. Significantly maximum number of leaves (7.11) was recorded under the treatment T<sub>12</sub> where MS medium was fortified with 2.5 mg/l BAP, 0.5 mg/l NAA and 100 mg/l AdS followed by the treatment T<sub>11</sub> (2.0 mg/l BAP+ 0.5mg/l NAA+ 100 mg/l AdS). Length of the leaf was significantly longer (1.15cm) in the treatment T<sub>13</sub> (3.0mg/l BAP, 0.5 mg/l NAA and 100 mg/l AdS). However significantly maximum number of suckers (3.53) was recorded in the treatment T<sub>11</sub> (2.0 mg/l BAP, 0.5 mg/l NAA and 100 mg/l AdS).

Considering all the above mentioned characters, it has been concluded that after 30 days of culture MS medium supplemented with 2.0 mg/l BAP increased the shoot height (2.17 cm). A critical analysis of the data relating to shoot proliferation study presented in Table-9 clearly indicated that MS medium supplemented with 0.5 mg/l NAA, 100 mg/l AdS along with three different concentrations of BAP separately has given very interesting results, when BAP @ 2.5 mg/l was supplemented significantly increased the number of leaves (7.11) ;when it was supplemented @ 3.0 mg/l significantly increased the leaf length (1.15 cm) and it was when supplemented @ 2.0 mg/l significantly enhanced the number of suckers (3.53) per clump after 30 days of culture (Fig.2, 3 and Plate 6).

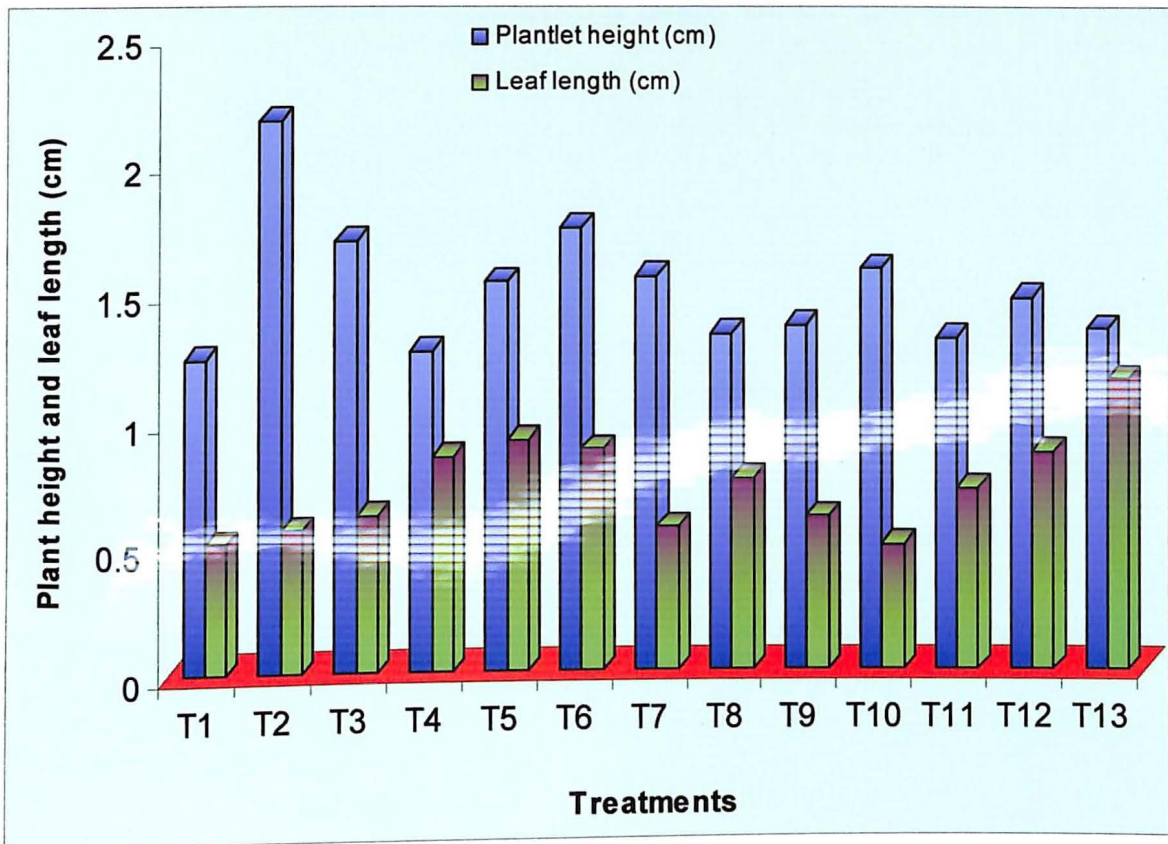


Fig. 2. Effect of plant bioregulators and adjuvant on shoot proliferation (plant height and leaf length) of *Dendrobium* cv. Sonia-17 after 30 days

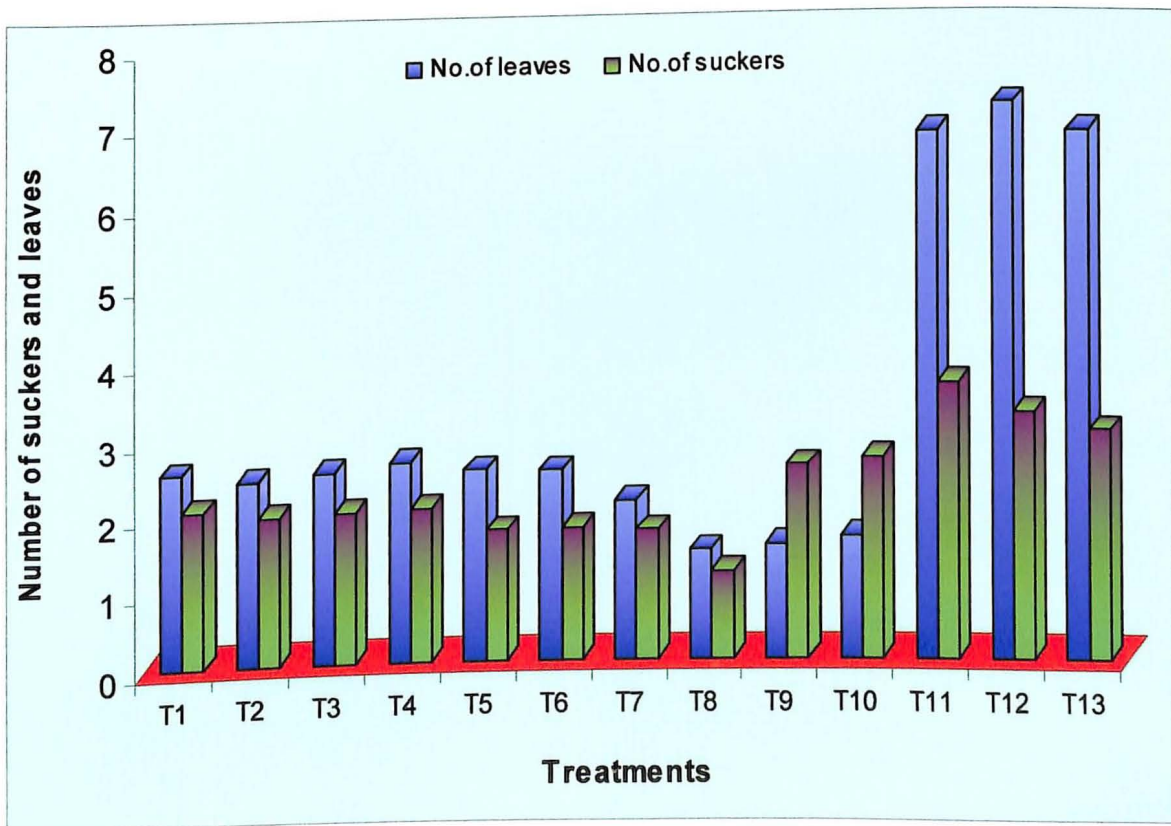


Fig. 3. Efficacy of plant bioregulators and adjuvant on shoot proliferation (number of suckers and number of leaves) of *Dendrobium* cv. Sonia-17 after 30 days

**Table: 9 Impact of Plant Bioregulators and Adjuvant on shoot proliferation of *Dendrobium* cv. Sonia-17**

**Basal Medium: MS**

**Duration:30 days**

Treatment	Plant Bioregulators		Adjuvant	Plantlet height (cm)	Number of leaves	Leaf length (cm)	Number of suckers
	BAP (mg/l)	NAA (mg/l)	AdS(mg/l)				
T <sub>1</sub>	-	-	-	1.24	2.53	0.51	2.03
T <sub>2</sub>	2.0	-	-	<b>2.17</b>	2.40	0.56	1.93
T <sub>3</sub>	2.5	-	-	1.69	2.49	0.61	1.95
T <sub>4</sub>	3.0	-	-	1.26	2.60	0.84	1.97
T <sub>5</sub>	2.0	0.5	-	1.53	2.47	0.91	1.67
T <sub>6</sub>	2.5	0.5	-	1.74	2.45	0.87	1.68
T <sub>7</sub>	3.0	0.5	-	1.55	2.03	0.56	1.64
T <sub>8</sub>	2.0	-	100	1.33	1.37	0.75	1.09
T <sub>9</sub>	2.5	-	100	1.36	1.44	0.60	2.47
T <sub>10</sub>	3.0	-	100	1.58	1.55	0.49	2.57
T <sub>11</sub>	2.0	0.5	100	1.31	6.75	0.71	<b>3.53</b>
T <sub>12</sub>	2.5	0.5	100	1.47	<b>7.11</b>	0.86	3.16
T <sub>13</sub>	3.0	0.5	100	1.35	6.74	<b>1.15</b>	2.93
SE(m)±				0.05	0.11	0.03	0.09
CD (5%)				0.15	0.33	0.10	0.27

The data presented in Table-10 revealed that MS medium fortified with 2.0 mg/l BAP significantly enhanced height of the shoot (2.27cm) of *Dendrobium* cv. Sonia-17 after 45 days of culture, followed by the treatment T<sub>6</sub> i.e. MS supplemented with 2.5 mg/l BAP and 0.5 mg/l NAA. Significantly maximum number of leaves (9.43) was recorded in treatment T<sub>12</sub> where MS medium was fortified with BAP 2.5 mg/l, 0.5 mg/l NAA and 100mg/l AdS followed by the treatment T<sub>11</sub> (2.0 mg/l BAP + 0.5 mg/l NAA + 100 mg/l AdS). Length of the leaf was significantly higher (1.37 cm) in treatment T<sub>8</sub> (2.0 mg/l BAP +100 mg/l AdS). However significantly maximum number of suckers (3.73) was recorded under Treatment T<sub>11</sub> ( 2.0 mg/l BAP, 0.5 mg/l NAA and 100 mg/l AdS) which was found statistically at par with treatment T<sub>12</sub> (2.0 mg/l BAP, 0.5 mg/l NAA and 100 mg/l AdS).

A critical analysis of the data, relating to shoot proliferation presented in Table-10 clearly indicated that after 45 days of culture MS medium supplemented with 2.0 mg/l BAP increased the shoot height (2.27 cm). The leaf length(1.37 cm) was best observed in MS medium fortified with BAP 2.0 mg/l, AdS 100 mg/l. MS medium fortified with 0.5 mg/l NAA, 100 mg/l AdS with two different concentrations of BAP has given spectacular results, i.e. when BAP @ 2.5 mg/l was supplemented significantly increase the number of leaves (9.43) and when supplied @ 2 mg/l significantly increased the number of suckers (3.73) per clump after 45 days of culture (Fig. 4 and plate 7,8).

**Table:10 Efficacy of Plant Bioregulators and Adjuvant on shoot proliferation of *Dendrobium* cv. Sonia-17**

Basal Medium: MS				Duration:45 days			
Treatment	Plant Bioregulators		Adjuvant	Plantlet height (cm)	Number of leaves	Leaf length (cm)	Number of suckers
	BAP (mg/l)	NAA (mg/l)	AdS (mg/l)				
T <sub>1</sub>	-	-	-	1.38	3.03	0.79	2.53
T <sub>2</sub>	2.0	-	-	<b>2.27</b>	2.73	0.76	2.20
T <sub>3</sub>	2.5	-	-	1.82	2.81	0.77	2.43
T <sub>4</sub>	3.0	-	-	1.67	3.17	0.92	2.73
T <sub>5</sub>	2.0	0.5	-	1.73	2.82	0.93	2.33
T <sub>6</sub>	2.5	0.5	-	1.96	2.69	0.91	2.34
T <sub>7</sub>	3.0	0.5	-	1.74	2.57	0.73	1.88
T <sub>8</sub>	2.0	-	100	1.51	2.23	<b>1.37</b>	1.58
T <sub>9</sub>	2.5	-	100	1.57	2.34	0.83	2.82
T <sub>10</sub>	3.0	-	100	1.73	2.54	0.66	3.17
T <sub>11</sub>	2.0	0.5	100	1.58	8.60	0.83	<b>3.73</b>
T <sub>12</sub>	2.5	0.5	100	1.63	<b>9.43</b>	1.12	3.69
T <sub>13</sub>	3.0	0.5	100	1.42	7.9	1.32	3.20
SE(m)±				1.10	0.08	0.03	0.07
CD (5%)				0.30	0.22	0.09	0.22

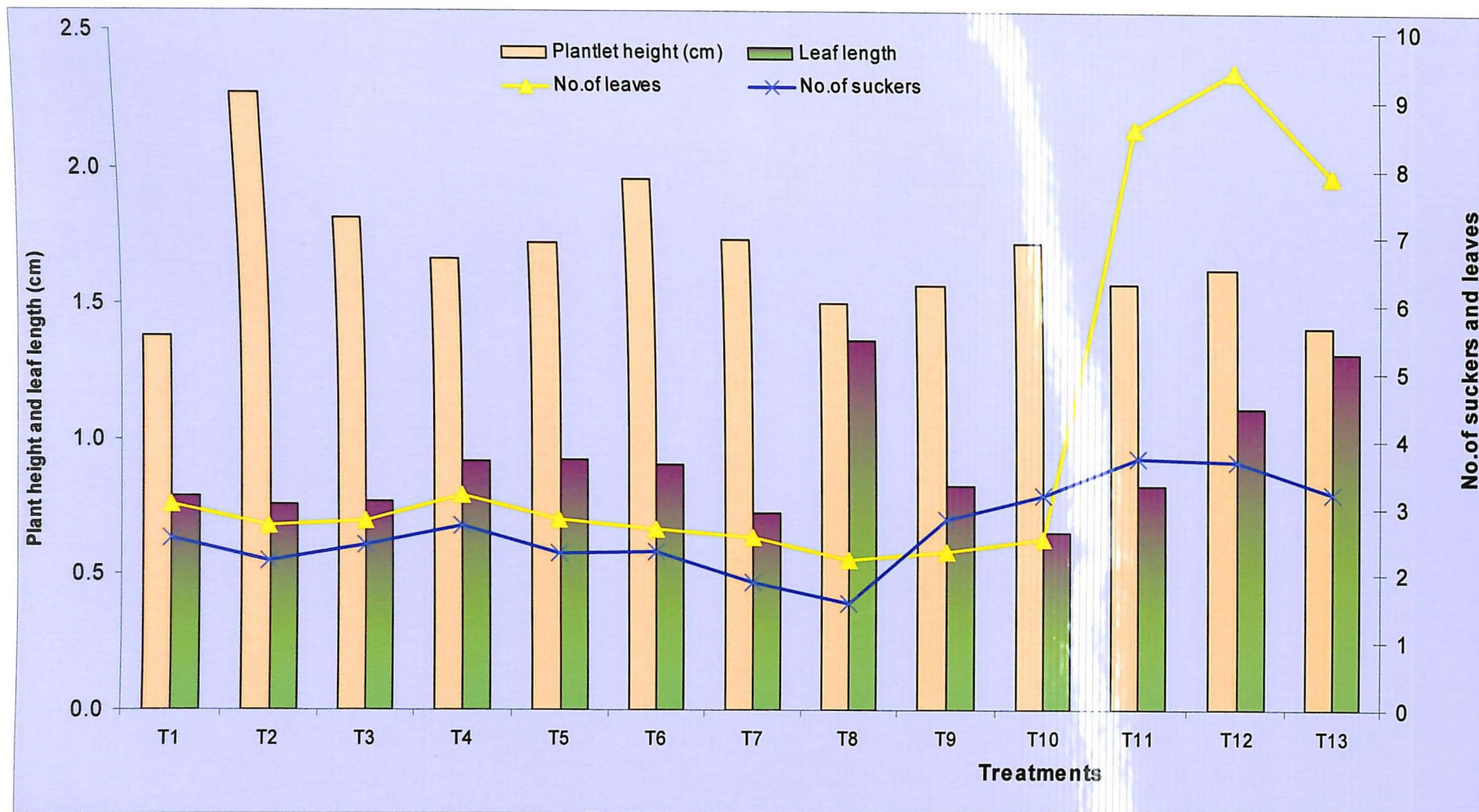


Fig. 4. Effect of plant bioregulators and adjuvant on shoot proliferation of *Dendrobium* cv. Sonia-17 after 45 days

The data pertaining to shoot proliferation study presented in Table-11 revealed that MS medium fortified with 2.0 mg/l BAP significantly enhanced the height of the shoots (2.45 cm) of *Dendrobium* cv. Sonia-17 after 60 days of culture, statistically at par with the treatment T<sub>3</sub> i.e. MS medium supplemented with 2.5 mg/l BAP with a shoot height (2.34 cm). Significantly maximum number of leaves (12.39) was recorded in the treatment T<sub>12</sub> where MS medium was fortified with BAP 2.5 mg/l, 0.5 mg/l NAA, 100mg/l AdS followed by treatment T<sub>11</sub> (2.0 mg/l BAP + 0.5 mg/l NAA + 100 mg/l AdS). Length of the leaf was significantly higher (1.75 cm) in treatment T<sub>5</sub> (2.0 mg/l BAP + 0.5 mg/l NAA) followed by treatment T<sub>6</sub> (2.5 mg/l BAP + 0.5 mg/l NAA). However significantly maximum number of suckers (3.91) was recorded in Treatment T<sub>10</sub> (3.0 mg/l BAP and 100 mg/l AdS) followed by treatment T<sub>11</sub> (2.0 mg/l BAP and 0.5 mg/l NAA) and 100 mg/l AdS.

Considering the above observations, a critical analysis of the data relating to shoot proliferation after 60 days of culture clearly indicated that MS medium supplemented with 2.0 mg/l BAP increased the shoot height (2.45cm). The number of leaves (12.39) was best observed in MS medium fortified with BAP 2.5 mg/l, 0.5 mg/l NAA and AdS 100 mg/l. Significantly longer leaf (1.75 cm) was observed in MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA. MS medium fortified with 3.0 mg/l BAP, 100 mg/l AdS gave the highest number of suckers (3.91) after 60 days of culture ( Plate 9).

**Table:11 Impact of Plant Bioregulators and Adjuvant on Shoot proliferation of *Dendrobium* cv. Sonia-17**

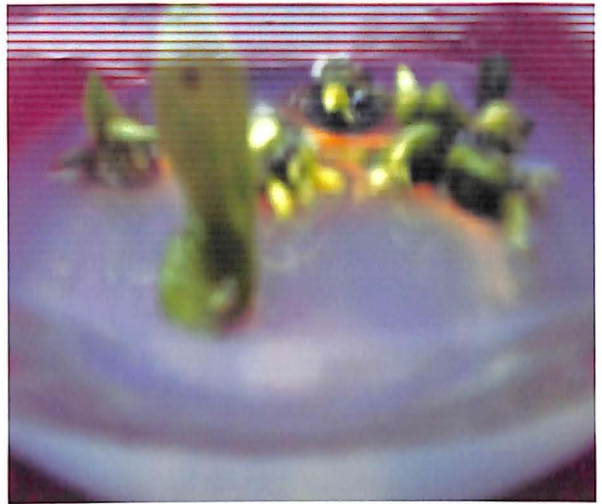
**Basal Medium: MS**

**Duration: 60 days**

Treatment	Plant Bioregulators		Adjuvants	Plantlet height (cm)	Number of leaves	Leaf length (cm)	Number of suckers
	BAP (mg/l)	NAA (mg/l)	AdS (mg/l)				
T <sub>1</sub>	-	-	-	1.41	3.82	1.24	2.61
T <sub>2</sub>	2.0	-	-	<b>2.45</b>	3.04	0.91	2.36
T <sub>3</sub>	2.5	-	-	2.34	3.07	0.92	2.55
T <sub>4</sub>	3.0	-	-	2.26	3.80	1.08	3.01
T <sub>5</sub>	2.0	0.5	-	1.93	3.33	<b>1.75</b>	2.71
T <sub>6</sub>	2.5	0.5	-	2.07	3.17	1.52	2.73
T <sub>7</sub>	3.0	0.5	-	1.95	2.77	1.40	2.28
T <sub>8</sub>	2.0	-	100	1.83	2.87	1.53	1.77
T <sub>9</sub>	2.5	-	100	1.93	3.16	1.15	2.95
T <sub>10</sub>	3.0	-	100	2.08	3.40	0.93	<b>3.91</b>
T <sub>11</sub>	2.0	0.5	100	1.91	10.10	1.34	3.33
T <sub>12</sub>	2.5	0.5	100	1.99	<b>12.39</b>	1.35	2.13
T <sub>13</sub>	3.0	0.5	100	1.81	9.14	1.43	2.06
SE(m)±				0.04	0.05	0.04	0.08
CD (5%)				0.11	0.15	0.11	0.23



**Plate 6.** Shoot proliferation in *Dendrobium* cv. Sonia-17 after 30 days of culture in MS + BAP 2.5 mg/l + NAA 0.5 mg/l



**Plate 7.** Multiple shoot generation with leaves in *Dendrobium* cv. Sonia-17 after 45 days of culture in MS + BAP 2.5 mg/l + NAA 0.5 mg/l



**Plate 8.** Shoot proliferation and development of plantlet after 45 days in *Dendrobium* cv. Sonia-17 in MS + 2.0 mg/l BAP + 0.5 mg/l NAA



**Plate 9.** Shoot proliferation and development of plantlet after 60 days in *Dendrobium* cv. Sonia-17 in MS + 2.0 mg/l BAP + 0.5 mg/l NAA

The data analysed in Table-12 revealed that MS medium fortified with 5 mg/l kinetin significantly enhanced the height of the shoot (1.35 cm) of *Dendrobium cv. Sonia-17* after 30 days of culture, followed by treatment T<sub>3</sub> i.e. MS medium fortified with 4.0 mg/l kinetin. Significantly maximum number of leaves (7.40) was recorded in MS medium fortified with 5.0 mg/l kinetin and stood statistically at par with treatment T<sub>3</sub> i.e. MS medium fortified with 4 mg/l kinetin followed by treatment T<sub>5</sub> i.e. MS medium fortified with 6.0 mg/l kinetin. Significantly maximum length of leaves (0.74 cm) was obtained in treatment T<sub>4</sub> i.e. MS medium fortified with 6.0 mg/l kinetin which is significantly at par with Treatment T<sub>4</sub> i.e. MS medium fortified with 5.0 mg/l kinetin possessing leaf length of 0.73 cm and Treatment T<sub>3</sub> i.e. MS medium fortified with 4.0 mg/l kinetin with leaf length 0.71cm. However significantly maximum number of suckers (3.33) was recorded in Treatment T<sub>3</sub> i.e. MS medium fortified with 4.0 mg/l kinetin and stood statistically at par with basal MS medium showing result of (3.17) and by treatment T<sub>2</sub> i.e. MS medium fortified with 4.0 mg/l kinetin (3.11).

A critical analysis of the data, relating to shoot proliferation presented in Table-12 clearly indicated that after 30 days of culture MS medium supplemented with kinetin 5.0 mg/l increased the shoot height (1.35 cm) and number of leaves (7.40). The best leaf length (0.74 cm) was observed in MS medium fortified with 6.0 mg/l kinetin. The highest number of suckers (3.33) observed in MS medium fortified with 4.0 mg/l kinetin (Fig. 5 to 8 and Plate 10).

**Table:12 Efficacy of Plant Bioregulators on Shoot proliferation of *Dendrobium* cv. Sonia-17**

**Basal Medium: MS**

**Duration:30 days**

Treatment	Kinetin (mg/l)	NAA (mg/l)	Plantlet height (cm)	Number of leaves	Leaf length (cm)	Number of suckers
T <sub>1</sub>	-	-	0.95	5.80	0.66	3.17
T <sub>2</sub>	3.0	-	0.95	5.37	0.67	3.11
T <sub>3</sub>	4.0	-	1.22	7.33	0.71	<b>3.33</b>
T <sub>4</sub>	5.0	-	<b>1.35</b>	<b>7.40</b>	0.73	2.77
T <sub>5</sub>	6.0	-	0.96	6.37	<b>0.74</b>	2.75
T <sub>6</sub>	3.0	0.5	0.87	4.10	0.64	0.83
T <sub>7</sub>	4.0	0.5	1.14	4.17	0.63	0.85
T <sub>8</sub>	5.0	0.5	0.85	4.43	0.62	1.55
T <sub>9</sub>	6.0	0.5	0.83	4.57	0.53	1.59
SE(m)±			0.02	0.07	0.01	0.05
CD (5%)			0.04	0.21	0.04	0.14

The data presented in Table-13 revealed that MS medium fortified with 5.0 mg/l kinetin significantly enhanced the height of the shoot (1.66 cm) of *Dendrobium* cv. Sonia-17 after 45 days of culture, followed by Treatment T<sub>3</sub> i.e. MS medium fortified with 4.0 mg/l kinetin. Significantly maximum number of leaves (9.60) was also recorded in MS medium fortified with 5.0 mg/l kinetin which is statistically at par with basal MS medium possessing number of leaves. Significantly longer leaf was recorded (0.87 cm) in treatment T<sub>5</sub> i.e. MS medium fortified with 6.0 mg/l kinetin and stood statistically at par with treatment T<sub>6</sub> i.e. 3.0 mg/l kinetin and 0.5 mg/l NAA possessing leaf length of (0.83 cm). However maximum number of suckers (3.82) was recorded in

treatment T<sub>3</sub> i.e. MS medium fortified with 4.0 mg/l kinetin which found statistically at par with treatment T<sub>2</sub> i.e. MS medium fortified with 3 mg/l kinetin (3.77).

Considering the above observations, a critical analysis of the data relating to shoot proliferation after 45 days of culture clearly indicated that MS medium supplemented with kinetin 5.0 mg/l increased the shoot height (1.66 cm) and number of leaves (9.60). The best leaf length (0.87 cm) was observed in MS medium fortified with 6.0 mg/l kinetin. The highest number of suckers (3.82) observed in MS medium fortified with 4.0 mg/l kinetin (Fig. 5 to 8 and Plate 11,12).

**Table:13 Efficacy of Plant Bioregulators on Shoot proliferation of *Dendrobium cv. Sonia-17***

Basal Medium: MS			Duration:45 days			
Treatment	Kinetin (mg/l)	NAA (mg/l)	Plantlet height (cm)	Number of leaves	Leaf length (cm)	Number of suckers
T <sub>1</sub>	-	-	1.03	9.40	0.67	3.45
T <sub>2</sub>	3.0	-	1.18	7.40	0.73	3.77
T <sub>3</sub>	4.0	-	1.53	8.67	0.75	<b>3.82</b>
T <sub>4</sub>	5.0	-	<b>1.66</b>	<b>9.60</b>	0.78	2.83
T <sub>5</sub>	6.0	-	1.13	7.72	<b>0.87</b>	2.82
T <sub>6</sub>	3.0	0.5	1.19	4.67	0.83	1.27
T <sub>7</sub>	4.0	0.5	1.25	5.53	0.73	1.73
T <sub>8</sub>	5.0	0.5	1.15	5.60	0.65	2.14
T <sub>9</sub>	6.0	0.5	0.96	6.47	0.53	2.33
SE(m)±			0.02	0.10	0.01	0.06
CD (5%)			0.06	0.30	0.04	0.18

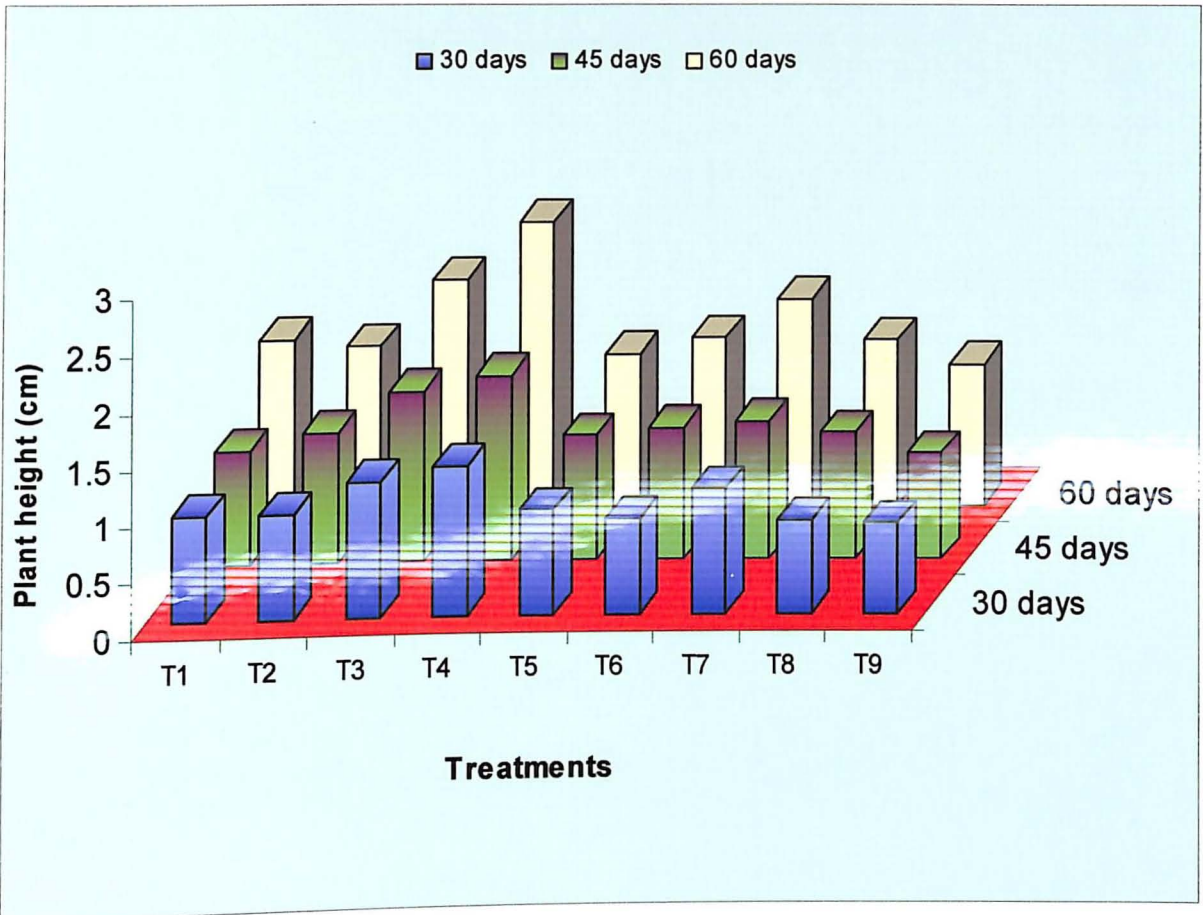


Fig. 5. Impact of plant bioregulators on shoot proliferation (plant height) of *Dendrobium* cv. Sonia-17 after 30, 45 and 60 days of culture

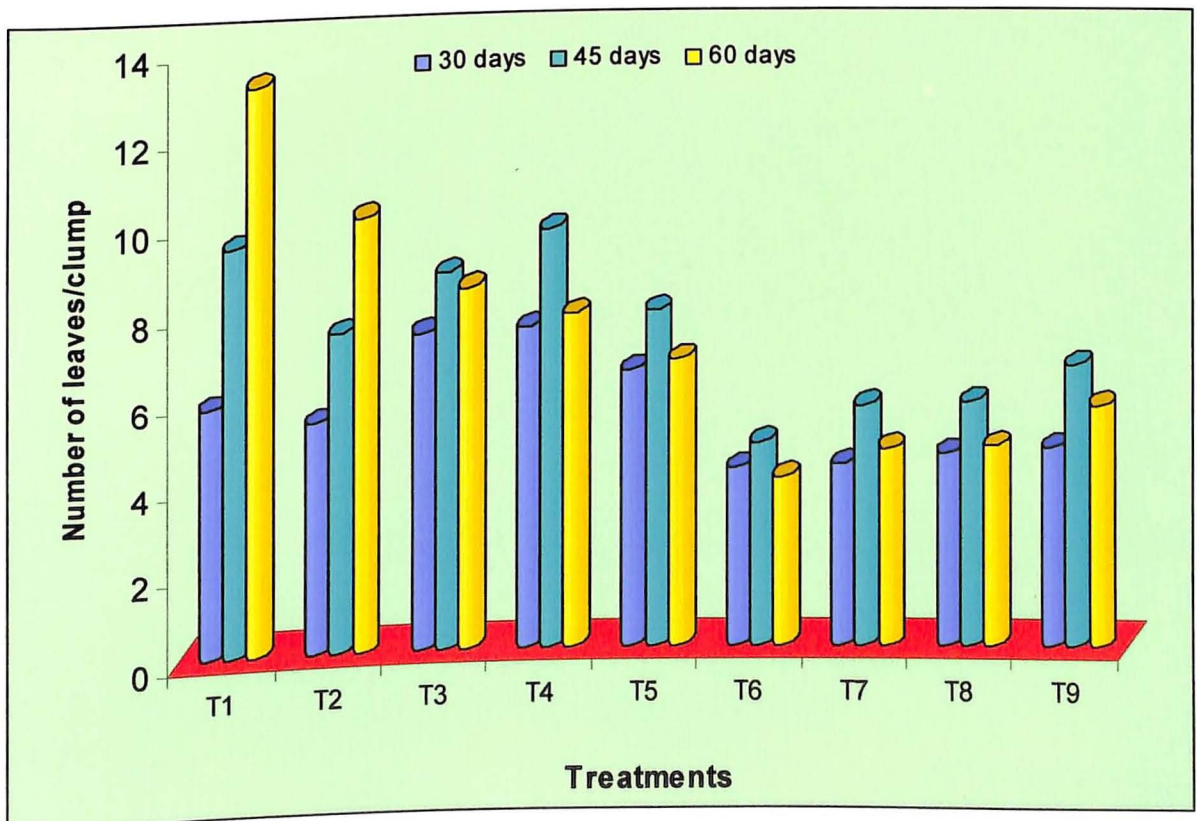


Fig. 6. Effect of plant bioregulators on shoot proliferation (number of leaves/clump) of *Dendrobium* cv. Sonia-17 after 30, 45 and 60 days of culture

The data analysed in Table-14 presented that MS medium fortified with 5.0 mg/l kinetin significantly enhanced the height of the shoot (2.54 cm) of *Dendrobium* cv. Sonia-17 after 60 days of culture, followed by treatment T<sub>3</sub> i.e. MS medium fortified with 4.0 mg/l kinetin (2.02 cm). Significantly maximum number of leaves (13.07) was recorded in basal MS medium followed by treatment T<sub>2</sub> i.e. MS medium fortified with 3.0 mg/l kinetin (9.99). Significantly higher (1.15 cm) in treatment T<sub>5</sub> i.e. MS medium fortified with 6 mg/l kinetin and stood statistically at par with treatment T<sub>6</sub> i.e. 3.0 mg/l kinetin and 0.5 mg/l NAA possessing leaf length (1.14 cm). ). However maximum number of suckers (3.45) was recorded in treatment T<sub>1</sub> i.e. basal MS medium followed by treatment T<sub>2</sub> i.e. MS medium fortified with 3.0 mg/l kinetin (3.17).

A critical analysis of the data, relating to shoot proliferation presented in Table-14 clearly indicated that after 60 days of culture MS medium supplemented with kinetin 5.0 mg/l increased the shoot height (2.54 cm). The maximum number of leaves (13.07) and highest number of suckers (3.45) were observed in basal MS medium after 60 days of culture. The best leaf length (1.15 cm) was observed in MS medium fortified with 6.0 mg/l kinetin. Very interesting result was obtained and as far as number of suckers was concerned Number of suckers per clump remained same in the treatment T<sub>1</sub> after 45 as well as 60 days of culture and it was decreased in other treatments due to prolonged culture up to 60 days. As the number of suckers per clump was significantly higher in the treatment T<sub>3</sub> (MS medium + 4.0 mg/l kinetin) after 45 days of culture, the shoot proliferation study can be continued for 45 days and beyond that the result was showing detrimental effect (decaying of the suckers) as far as number of suckers was concerned (Fig. 5 to 8 and Plate 13).

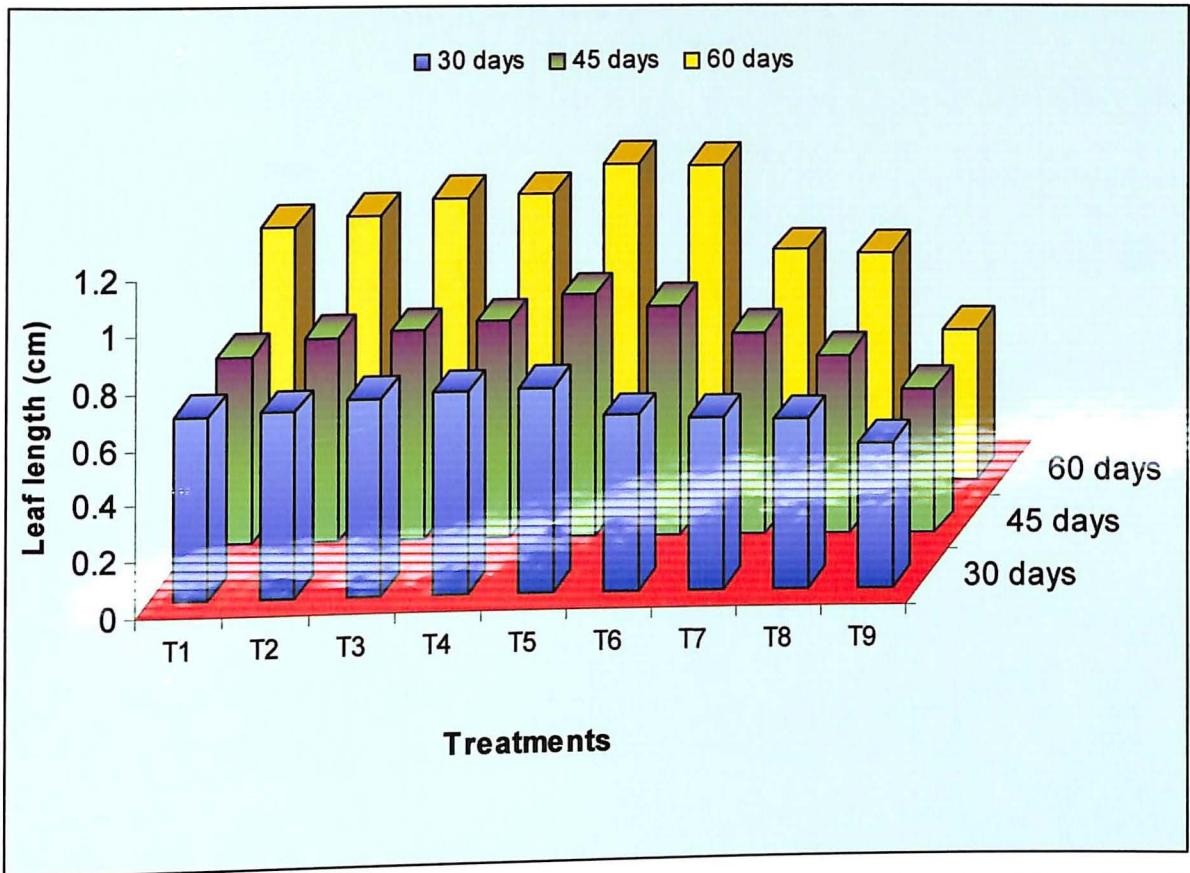


Fig. 7. Efficacy of plant bioregulators on shoot proliferation (leaf length) of *Dendrobium* cv. Sonia-17 after 30, 45 and 60 days of culture

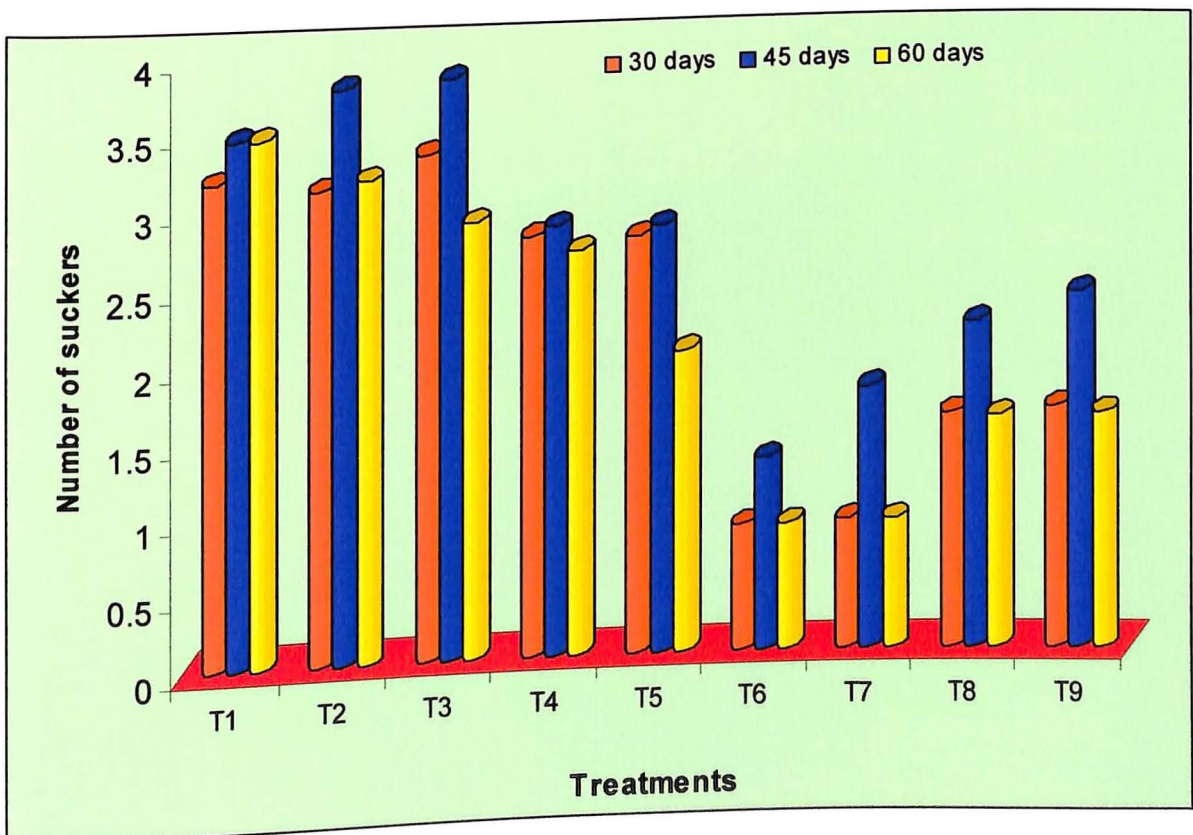


Fig. 8. Impact of plant bioregulators on shoot proliferation (number of suckers) of *Dendrobium* cv. Sonia-17 after 30, 45 and 60 days of culture

**Table:14 Impact of Plant Bio regulators on Shoot proliferation of *Dendrobium* cv. Sonia-17**

**Basal Medium: MS**

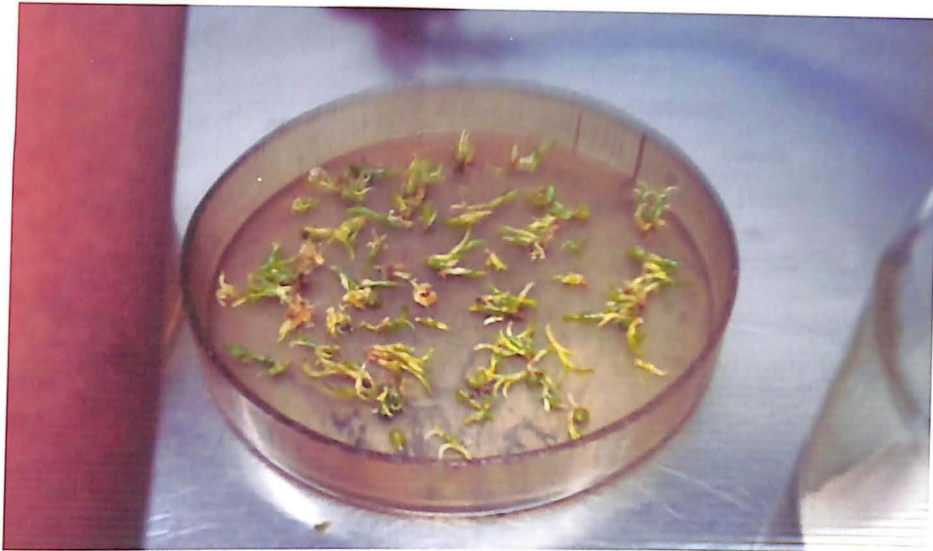
**Duration:60 days**

Treatment	Kinetin (mg/l)	NAA (mg/l)	Plantlet height (cm)	Number of leaves	Leaf length (cm)	Number of suckers
T <sub>1</sub>	-	-	1.50	13.07	0.93	3.45
T <sub>2</sub>	3.0	-	1.43	9.99	0.97	3.17
T <sub>3</sub>	4.0	-	2.02	8.33	1.03	2.87
T <sub>4</sub>	5.0	-	2.54	7.67	1.04	2.67
T <sub>5</sub>	6.0	-	1.35	6.60	1.15	2.00
T <sub>6</sub>	3.0	0.5	1.49	3.87	1.14	0.83
T <sub>7</sub>	4.0	0.5	1.83	4.53	0.83	0.85
T <sub>8</sub>	5.0	0.5	1.47	4.60	0.82	1.53
T <sub>9</sub>	6.0	0.5	1.26	5.53	0.54	1.55
SE(m)±			0.03	0.12	0.02	0.09
CD (5%)			0.07	0.36	0.07	0.26

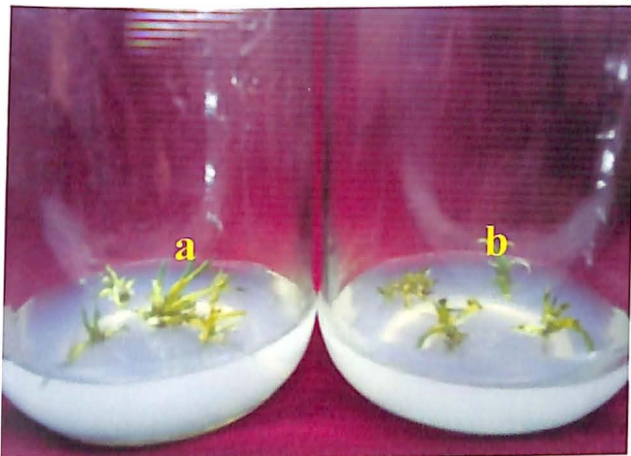
#### **4:2: STANDARDIZATION OF MEDIA SUPPLEMENTS FOR ROOT INITIATION AND DEVELOPMENT**

##### **4:2:1: Standardization of media supplements for root initiation and development of *Papilionanthe* spp**

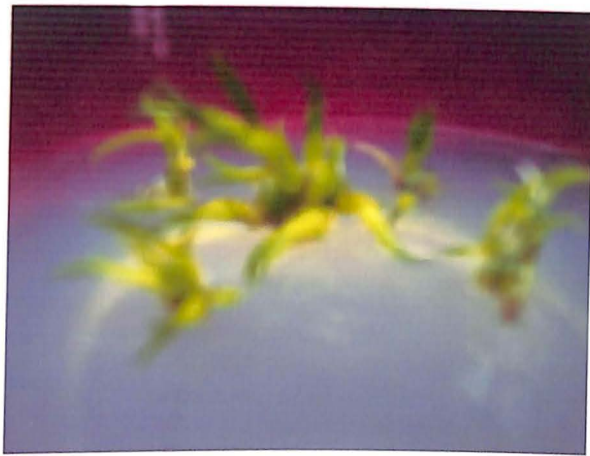
The data pertaining to root formation in *Papilionanthe* presented in the Table-15 after 30 days of inoculation revealed that half MS medium supplemented with 0.5 mg/l IBA along with 0.5 mg/l NAA significantly increased the number of roots per culture bottle (1.33) and the data remained at par with all the treatments except the treatment T<sub>4</sub> (NAA 0.5 mg/l) and T<sub>8</sub> (1.0 mg/l IBA + 0.5 mg/l NAA). Number of roots per plantlet was highest (0.33) and the root length was maximum (1.43 cm) in treatment T<sub>6</sub> (0.5 mg/l IBA and 0.5 mg/l NAA).



**Plate 10. Protocorms of *Dendrobium* cv. Sonia-17 used as the explant for shoot proliferation**



**Plate 12. Comparison study in the shoot proliferation in *Dendrobium* cv. Sonia-17 (a) MS + kinetin 5.0 mg/l, (b) MS + kinetin 3.0 mg/l + 0.5 mg/l NAA**



**Plate 11. Shoot proliferation in *Dendrobium* cv. Sonia-17 after 45 days of culture in MS + 5.0 mg/l Kinetin**



**Plate 13. After 60 days of culture the shoots showing the degeneration of suckers in *Dendrobium* Sonia-17**

It was concluded that half MS medium supplemented with 0.5 mg/l IBA along with 0.5 mg/l or 1.0 mg/l NAA has given best results in number of roots per culture bottle. The number of roots per plantlet was highest (0.33) and the root length was maximum (1.43 cm) in half MS medium supplemented with 0.5 mg/l IBA and 0.5 mg/l NAA in *Papilionanthe teres* after 30 days of inoculation. However the data shown non-significant results for character such as number of roots/ plantlet and root length (Fig. 9).

**Table: 15 Impact of Plant Bioregulators on root initiation and development of *Papilionanthe teres***

**Basal Medium: half MS**

**Duration: 30 days**

Treatment	IBA (mg/l)	NAA (mg/l)	No. of roots/ bottle	No. of roots/ plantlet	Root length (in cm)
T <sub>1</sub>	-	-	0.67	0.17	0.70
T <sub>2</sub>	0.5	-	1.00	0.25	0.42
T <sub>3</sub>	1.0	-	1.00	0.25	1.10
T <sub>4</sub>	-	0.5	0.00	0.00	0.00
T <sub>5</sub>	-	1.0	1.00	0.25	0.24
T <sub>6</sub>	0.5	0.5	<b>1.33</b>	<b>0.33</b>	<b>1.43</b>
T <sub>7</sub>	0.5	1.0	1.33	0.33	1.02
T <sub>8</sub>	1.0	0.5	0.00	0.00	0.00
T <sub>9</sub>	1.0	1.0	0.33	0.08	0.73
SE(m)±			0.33	NS	NS
CD (5%)			0.99		

The data pertaining to the root formation studies in *Papilionanthe teres* after 60 days presented in the Table-16 showed non-significant results for all the characters under study. However half MS medium enriched with 0.5.mg/l IBA alone or in combination 0.5 mg/l NAA increased the number of roots per culture bottle (2.0). IBA @ 0.5 mg/l alone or in combination with NAA 1.0

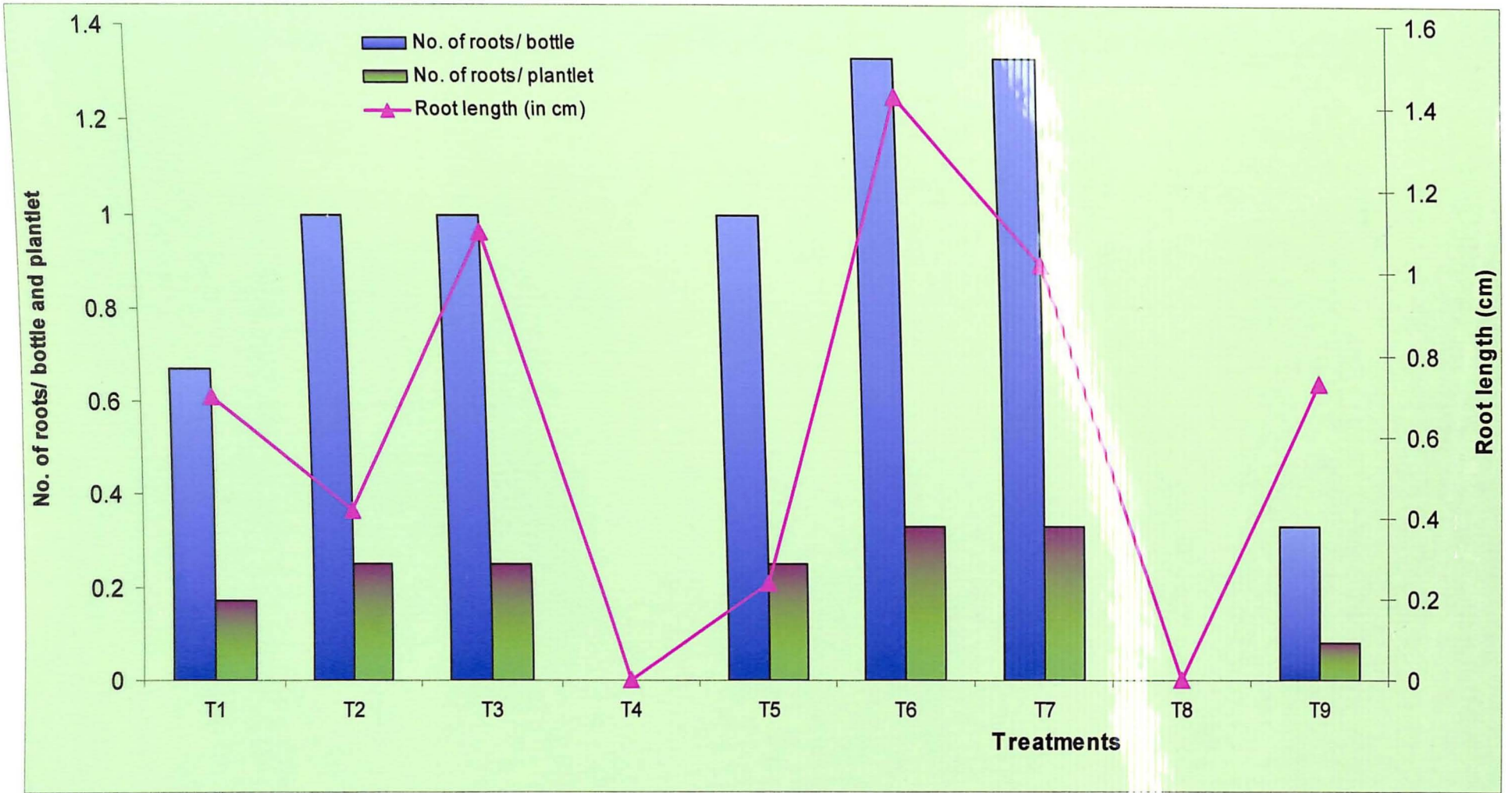


Fig. 9. Impact of plant bioregulators root initiation and development of *Papilionanthes teres* after 30 days of culture

mg/l increased the number of roots per plantlet (0.5). Root length was found to be maximum (1.60 cm) in the treatment T<sub>6</sub> (0.5 mg/l IBA + 0.5 mg/l NAA).

Considering the above mentioned characters it was concluded that half MS medium enriched with 0.5.mg/l IBA alone or in combination 0.5 mg/l NAA increased the number of roots per culture bottle (2.0). IBA @ 0.5 mg/l alone or in combination with NAA 1.0 mg/l increased the number of roots per plantlet (0.5). Root length was found to be maximum (1.60 cm) in half MS medium supplemented with 0.5 mg/l IBA, 0.5 mg/l NAA in *Papilionanthe* after 60 days of culture . However all the parameters gave the non significant results (Plate 14 and 15).

**Table 16: Efficacy of Plant Bioregulators on root initiation and development of *Papilionanthe spp***

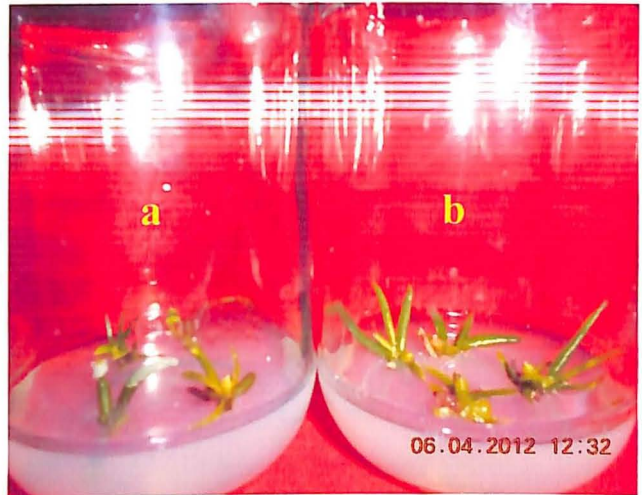
Basal Medium: half MS			Duration:60 days		
Treatment	IBA (mg/l)	NAA (mg/l)	No. of roots/ bottle	No. of roots/ plantlet	Root length (in cm)
T <sub>1</sub>	-	-	0.67	0.42	0.75
T <sub>2</sub>	0.5	-	<b>2.00</b>	<b>0.50</b>	0.61
T <sub>3</sub>	1.0	-	1.67	0.33	1.35
T <sub>4</sub>	-	0.5	0.00	0.00	0.00
T <sub>5</sub>	-	1.0	1.00	0.25	0.24
T <sub>6</sub>	0.5	0.5	<b>2.00</b>	<b>0.50</b>	<b>1.60</b>
T <sub>7</sub>	0.5	1.0	1.67	0.35	1.53
T <sub>8</sub>	1.0	0.5	0.67	0.17	0.37
T <sub>9</sub>	1.0	1.0	0.67	0.17	1.33
SE(m)±			NS	NS	NS
CD (5%)					

The data presented in Table-17 for *invitro* root formation in *Papilionanthe* after 90 days of inoculation recorded non-significant results for all the characters such as number of roots/ bottle, number of roots/ plantlet, and root length. However half MS medium fortified with IBA (0.5 mg/l) increased the number of roots/bottle (3.67); IBA @ 1.0 mg/l increased the number of roots per plantlet (0.75) and IBA (1.0 mg/l) with NAA 1.0 mg/l increased the root length (1.32 cm).

From the above mentioned observations it has been concluded that half MS medium supplemented with IBA 0.5 mg/l resulted higher number of roots per bottle (3.67) and with 1.0 mg/l resulted maximum number of roots per plantlet (0.75). MS medium fortified with IBA (1.0 mg/l) along with NAA (1.0 mg/l) resulted maximum root length (1.32 cm) after 90 days of culture. It has been noticed from the table-17 after 90 days of observation that in the treatment T<sub>3</sub> (IBA @ 1.0 mg/l) , T<sub>6</sub> (IBA @ 0.5 mg/l and NAA 0.5 mg/l), T<sub>7</sub> (IBA @ 0.5 mg/l and NAA @ 1.0 mg/l) and T<sub>9</sub> (IBA @ 1.0 mg/l and NAA @ 1.0 mg/l) the length of root has been reduced than that of 60 days after inoculation, as the tips of roots have degenerated and became brown. Hence the Orchids in rooting culture should be maintained for 60 days only (Plate 16 and 17).



**Plate 14. Explants used for root initiation and formation in *Papilionanthe teres***



**Plate 15. Root formation studies after 60 days of culture in *Papilionanthe teres* (a) Half MS + 1.0 mg/l IBA, (b) Half MS + 0.5 mg/l NAA**



**Plate 16. Root formation in *Papilionanthe teres* after 90 days of culture showing whitish roots**



**Plate 17. The culture room showing the *Papilionanthe teres* kept in rooting medium**

**Table:17 Efficacy of Plant Bioregulators on root initiation and development of *Papilionanthe sps***

**Basal Medium: half MS**

**Duration:90 days**

Treatment	IBA (mg/l)	NAA (mg/l)	No. of roots/bottle	No. of roots/plantlet	Root length (in cm)
T <sub>1</sub>	-	-	1.00	0.42	0.90
T <sub>2</sub>	0.5	-	<b>3.67</b>	0.58	0.61
T <sub>3</sub>	1.0	-	3.00	<b>0.75</b>	0.96
T <sub>4</sub>	-	0.5	1.53	0.53	0.50
T <sub>5</sub>	-	1.0	1.00	0.25	0.24
T <sub>6</sub>	0.5	0.5	2.53	0.58	0.78
T <sub>7</sub>	0.5	1.0	1.67	0.52	1.07
T <sub>8</sub>	1.0	0.5	0.67	0.17	0.55
T <sub>9</sub>	1.0	1.0	1.0	0.25	<b>1.32</b>
SE(m)±			NS	NS	NS
CD (5%)					

**4:2:2: Standardization of media supplements for root initiation and development of *Dendrobium cv. Sonia-17***

The perusal of the data presented in the Table- 18 revealed that half MS medium supplemented with 1.0 mg/l IBA significantly increased number of roots per bottle (3.33) over other treatments in of *Dendrobium cv. Sonia-17* after 30 days of culture. The same treatment significantly enhanced the number of roots per plantlet (0.83) followed by the treatment T<sub>8</sub> (1.0 mg/l IBA and 0.5 mg/l NAA). The root length was significantly higher (1.72 cm) in treatment T<sub>1</sub> (half MS only) followed by the treatment T<sub>2</sub> (0.5 mg/l IBA).

It has been concluded that half MS medium enriched with 1.0 mg/l IBA was most suitable treatment for initiation of roots in the *Dendrobium cv. Sonia-17* after 30 days of culture. However longer roots are produced in the treatment T<sub>1</sub> i.e. half MS alone (1.72 cm).

**Table 18: Impact of Plant Bioregulators on root initiation and development of *Dendrobium* cv. Sonia-17**

**Basal Medium: half MS**

**Duration:30 days**

Treatment	IBA (mg/l)	NAA (mg/l)	No. of roots/ bottle	No. of roots/ plantlet	Root length (in cm)
T <sub>1</sub>	-	-	1.67	0.42	1.72
T <sub>2</sub>	0.5	-	1.00	0.25	1.33
T <sub>3</sub>	1.0	-	<b>3.33</b>	<b>0.83</b>	0.57
T <sub>4</sub>	-	0.5	1.33	0.33	1.23
T <sub>5</sub>	-	1.0	1.67	0.42	0.24
T <sub>6</sub>	0.5	0.5	0.00	0.00	0.00
T <sub>7</sub>	0.5	1.0	1.67	0.42	0.45
T <sub>8</sub>	1.0	0.5	1.33	0.53	0.55
T <sub>9</sub>	1.0	1.0	0.00	0.00	0.00
SE(m)±			0.36	0.09	0.06
CD (5%)			1.14*	0.29*	0.18*

The data pertaining to Table-19 revealed that the treatment T<sub>3</sub> (1.0 mg/l IBA) significantly produced maximum number of roots per bottle (12.33) after 60 days of inoculation in *Dendrobium* cv. Sonia-17. It is quite interesting to observe that, the same treatment was significantly enhanced the number of roots per plantlet (3.08) and root length (3.33 cm). Root formation was observed in all the treatments except the treatment T<sub>6</sub> i.e. when half MS was fortified with 0.5 mg/l IBA and 0.5 mg/l NAA.

It was concluded that the treatment T<sub>3</sub> (half MS medium fortified with 1.0 mg/l IBA) was found to be best treatment for recording highest number of roots/ bottle (12.33), number of roots/plantlet (3.08) and root length (3.33 cm) (Plate 18 and 19).

**Table 19: Efficacy of Plant Bioregulators on root initiation and development of *Dendrobium* cv. Sonia-17**

Basal Medium: half MS			Duration:60 days		
Treatment	IBA (mg/l)	NAA (mg/l)	No. of roots/bottle	No. of roots/plantlet	Root length (in cm)
T <sub>1</sub>	-	-	3.53	0.83	2.06
T <sub>2</sub>	0.5	-	1.33	0.33	2.80
T <sub>3</sub>	1.0	-	<b>12.33</b>	<b>3.08</b>	<b>3.33</b>
T <sub>4</sub>	-	0.5	2.67	0.67	1.35
T <sub>5</sub>	-	1.0	3.00	0.75	0.31
T <sub>6</sub>	0.5	0.5	0.00	0.00	0.00
T <sub>7</sub>	0.5	1.0	2.00	0.50	0.79
T <sub>8</sub>	1.0	0.5	2.67	0.57	0.89
T <sub>9</sub>	1.0	1.0	2.17	0.67	0.67
SE(m)±			0.54	0.14	0.05
CD (5%)			1.62	0.40	0.14

The data presented in Table-20 clearly indicated that the treatment T<sub>3</sub> i.e. half MS medium fortified with 1.0 mg/l IBA significantly enhanced the number of roots per bottle (15.00), number of roots per plantlet (3.75) and root length (5.44 cm) over other treatments after 90 days of inoculation in *Dendrobium* cv. Sonia-17. However the treatment T<sub>6</sub> (0.5 mg/l NAA and 0.5 mg/l IBA) was failed to initiate roots even after 90 days of inoculation (Fig. 10 and Plate 20, 21).

Considering the above mentioned characters it has been found that half MS medium enriched with 1.0 mg/l IBA was most suitable for root primordia initiation for further growth and development in *Dendrobium* cv. Sonia-17.

**Table 20: Efficacy of Plant Bioregulators on root initiation and development of *Dendrobium* cv. Sonia-17**

**Basal Medium: half MS**

**Duration:90 days**

Treatment	IBA (mg/l)	NAA (mg/l)	No. of roots/ bottle	No. of roots/ plantlet	Root length (in cm)
T <sub>1</sub>	-	-	4.0	1.00	2.33
T <sub>2</sub>	0.5	-	3.33	0.83	3.09
T <sub>3</sub>	1.0	-	<b>15.00</b>	<b>3.75</b>	<b>5.44</b>
T <sub>4</sub>	-	0.5	4.33	1.08	1.75
T <sub>5</sub>	-	1.0	10.33	2.58	0.34
T <sub>6</sub>	0.5	0.5	0.00	0.00	0.00
T <sub>7</sub>	0.5	1.0	2.67	0.67	0.96
T <sub>8</sub>	1.0	0.5	4.33	1.08	0.89
T <sub>9</sub>	1.0	1.0	6.00	1.48	1.15
SE(m)±			0.83	0.21	0.082
CD (5%)			2.47	0.62	0.24

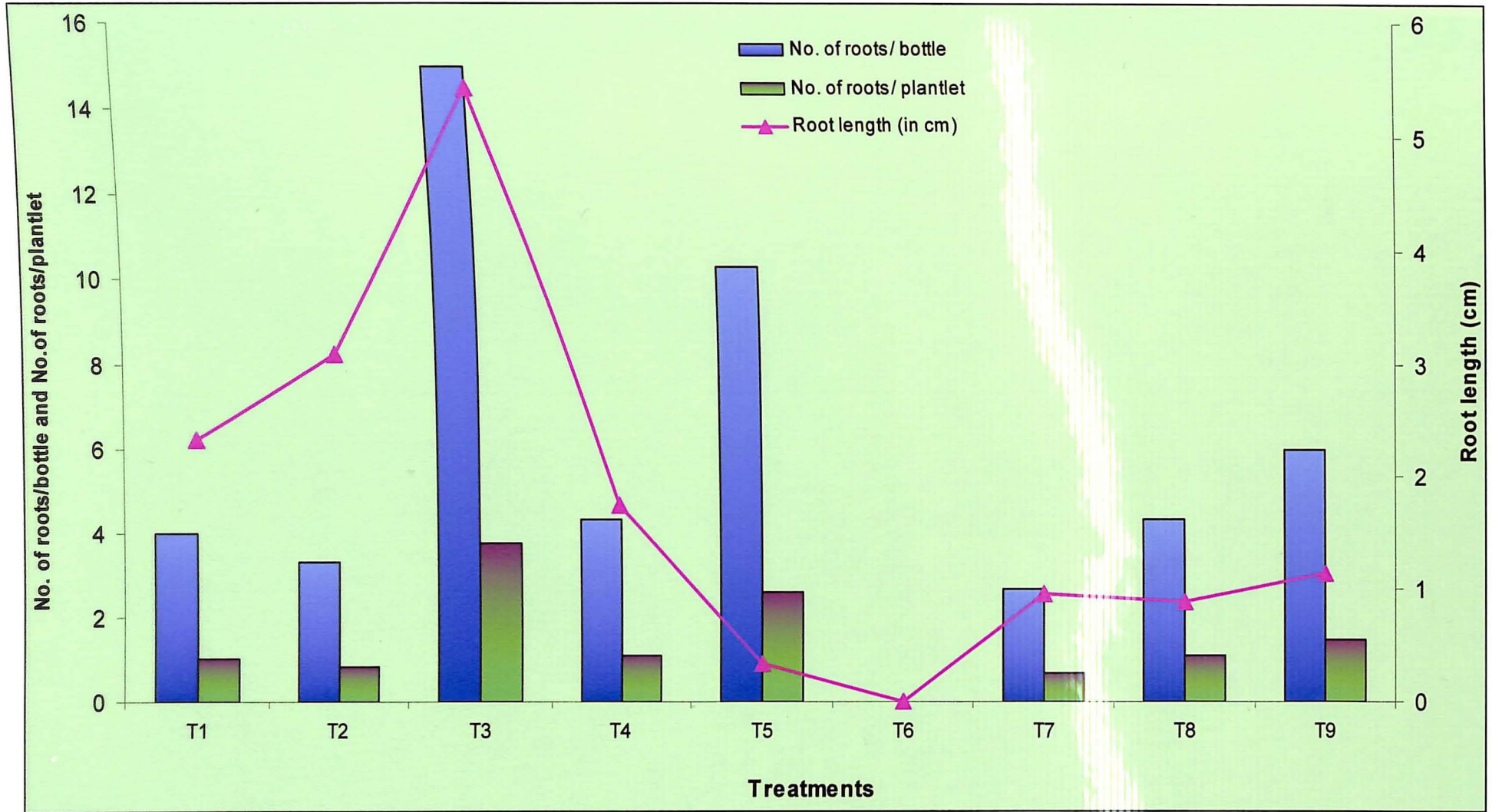


Fig. 10. Efficacy of plant bioregulators on root initiation and development of *Dendrobium* cv. Sonia-17 after 90 days of culture



**Plate 18. Root formation and development in *Dendrobium* cv. Sonia-17 after 60 days of culture (a) Half MS medium, (b) Half MS + 1.0 mg/l IBA + 1.0 mg/l NAA**



**Plate 19. Different treatments showing root initiation and development in *Dendrobium* Sonia-17**



**Plate 20. Development of prominent roots after 90 days of culture in *Dendrobium* Sonia-17 in half MS + 1.0 mg/l IBA**



**Plate 21. Root initiations and development observed after 90 days in *Dendrobium* Sonia 17 in (a) Half MS + 0.5 mg/l IBA + 1.0 mg/l NAA, (b) Half MS + 1.0 mg/l IBA**



Plate 22. Observations of *Dendrobium* cv. Sonia-17 taken inside the culture room of the laboratory

#### 4:3: STANDARDIZATION OF MEDIA MIXTURE FOR HARDENING OF THE *IN VITRO* PLANTLETS

The data presented in the Table-21 revealed that the media combination i.e. Brick Pieces : Charcoal : Cocopeat : Spahgnum moss : Decayed wood in 1:1:1:1:1 proportion significantly enhanced the plant height (10.2 cm) after 30 days of hardening and data stood at par with treatment T<sub>18</sub> i.e. Brick Pieces : Charcoal : Cocopeat : Decayed wood in 1:1:1:1 proportion. Minimum plant height (4.37 cm) was recorded when Brick Pieces were used as medium for hardening. As far as the leaf number was concerned again the same treatment T<sub>19</sub> i.e. Brick Pieces: Charcoal : Cocopeat : Spahgnum moss : Decayed wood in 1:1:1:1:1 proportion significantly increased the number of leaves per plant (8.0) followed by the treatment T<sub>8</sub> i.e. Brick Pieces : Charcoal : Cocopeat(1:1:1) and treatment T<sub>15</sub> i.e. Cocopeat : Decayed wood (1:1). It has been interesting to note that the same treatment T<sub>19</sub> i.e. Brick Pieces: Charcoal: Cocopeat: Spahgnum moss: Decayed wood (1:1:1:1:1) significantly enhanced the leaf length (9.43 cm) followed by treatment T<sub>18</sub> i.e. Brick Piece : Charcoal : Cocopeat: Decayed wood (1:1:1:1). Significantly maximum leaf width (0.70 cm) was recorded in both the treatments such as T<sub>19</sub> i.e. Brick Pieces: Charcoal: Cocopeat: Spahgnum moss: Decayed wood (1:1:1:1:1) and T<sub>6</sub> i.e. Charcoal: Cocopeat (1:1) which stood at par with the treatment T<sub>16</sub> i.e. Cocopeat : Spahgnum moss: Decayed wood (1:1:1).

Considering the above mentioned characters it has been concluded that the media combinations such as Brick Pieces: Charcoal: Cocopeat: Spahgnum moss: Decayed wood (1:1:1:1:1) was found to be the most suitable for hardening of *Dendrobium* cv. Sonia-17 after 30 days of planting. (Plate. 23-27)



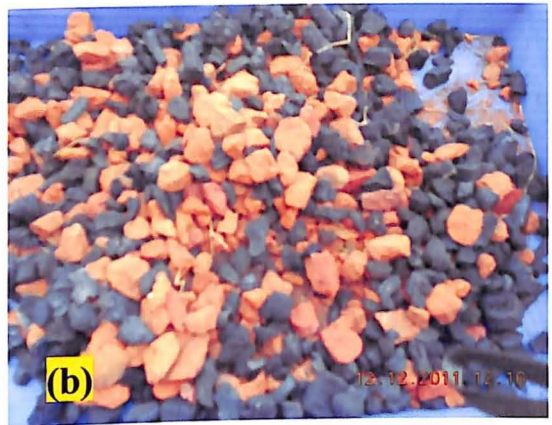
**Plate 23.** *Dendrobium* cv. Sonia-17 plantlets ready for hardening

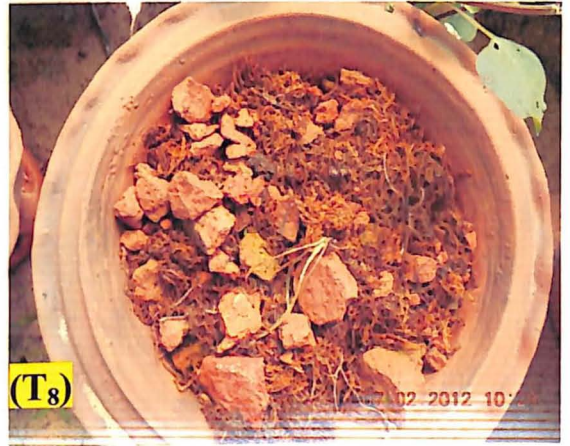


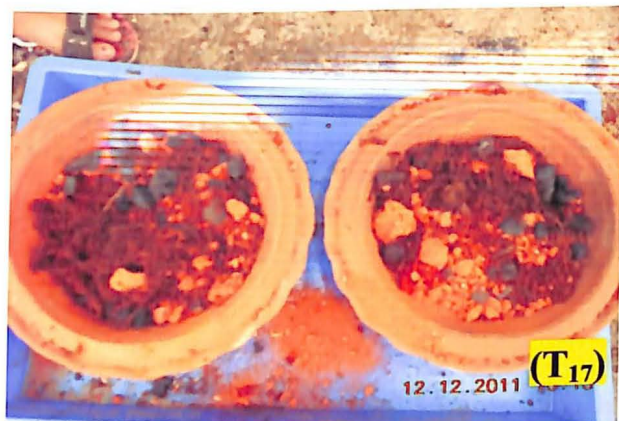
**Plate 24.** *Dendrobium* cv. Sonia-17 plantlets after washing in tap water



**Plate 25.** *Dendrobium* cv. Sonia-17 plantlets before transplanting in the pots







**Plate 26. Preparation of media mixture for hardening of *in vitro* plantlets of *Dendrobium cv. Sonia-17***

(T<sub>1</sub>) Brick pieces, (T<sub>2</sub>) Charcoal, (a) Brick pieces + cocopeat mixture, (b) Charcoal and brick pieces mixture (T<sub>3</sub>) Cocopeat, (T<sub>4</sub>) Brick pieces + Charcoal, (T<sub>5</sub>) Brick pieces + cocopeat, (T<sub>6</sub>) Charcoal + cocopeat (T<sub>7</sub>) Brick pieces +Charcoal +Cocopeat, (T<sub>8</sub>) Brick pieces + Sphagnum moss, (T<sub>9</sub>) Brick pieces +Decayed wood, (T<sub>10</sub>) Brick pieces + Sphagnum moss+Decayed wood, (T<sub>11</sub>) Charcoal + Sphagnum moss, (T<sub>12</sub>) Charcoal+ Decayed wood, (T<sub>13</sub>) Charcoal + Sphagnum moss+ Decayed wood, (T<sub>14</sub>) Cocopeat + Sphagnum moss, (T<sub>15</sub>) Cocopeat+ Decayed wood, (T<sub>16</sub>) Cocopeat + Sphagnum moss+ Decayed wood, (T<sub>17</sub>) Brick pieces + Charcoal + Cocopeat+ Sphagnum moss, (T<sub>18</sub>) Brick pieces + Charcoal + Cocopeat+ Decayed wood, (T<sub>19</sub>) Brick pieces + Charcoal + Cocopeat+ Sphagnum moss+ Decayed wood

**Table 21: Efficacy of media mixture for hardening of the in vitro plantlets of *Dendrobium* cv. Sonia-17 after 30 days of planting**

Treatment	Constituents	Plant height (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)
T <sub>1</sub>	Brick Pieces	4.37	3.67	3.60	0.23
T <sub>2</sub>	Charcoal	4.50	3.67	3.73	0.33
T <sub>3</sub>	Cocopeat	5.27	4.33	4.63	0.50
T <sub>4</sub>	Brick Pieces+ Charcoal	4.57	3.67	5.30	0.33
T <sub>5</sub>	Brick Pieces + Cocopeat	5.63	5.67	3.73	0.37
T <sub>6</sub>	Charcoal +Cocopeat	7.70	5.33	4.93	0.70
T <sub>7</sub>	Brick Pieces +Charcoal +Cocopeat	4.10	5.33	5.63	0.33
T <sub>8</sub>	Brick Pieces + Sphagnum moss	4.70	3.67	6.20	0.27
T <sub>9</sub>	Brick Pieces +Decayed wood	7.97	5.33	7.50	0.30
T <sub>10</sub>	Brick Pieces + Sphagnum moss+Decayed wood	9.63	5.67	5.63	0.37
T <sub>11</sub>	Charcoal + Sphagnum moss	8.40	5.33	4.63	0.27
T <sub>12</sub>	Charcoal+ Decayed wood	5.73	4.33	4.57	0.40
T <sub>13</sub>	Charcoal + Sphagnum moss+ Decayed wood	6.73	5.33	5.73	0.40
T <sub>14</sub>	Cocopeat + Sphagnum moss	8.37	5.00	6.00	0.43
T <sub>15</sub>	Cocopeat+ Decayed wood	6.90	6.00	3.93	0.43
T <sub>16</sub>	Cocopeat + Sphagnum moss+ Decayed wood	8.23	5.00	5.37	0.57
T <sub>17</sub>	Brick Pieces + Charcoal + Cocopeat+ Sphagnum moss	8.73	4.33	5.83	0.47
T <sub>18</sub>	Brick Pieces + Charcoal + Cocopeat+ Decayed wood	9.77	6.67	7.90	0.53
T <sub>19</sub>	Brick Pieces + Charcoal + Cocopeat+ Sphagnum moss+ Decayed wood	<b>10.20</b>	<b>8.00</b>	<b>9.43</b>	<b>0.70</b>
SEm(±)		0.32	0.44	0.31	0.05
CD (5%)		0.92	1.26	0.90	0.15



Plate 27. *In vitro* hardening of *Dendrobium* cv. Sonia-17 in pots in different media combination after 30 days of transplanting

The data presented in Table-22 that the treatment T<sub>19</sub> i.e. Brick Pieces: Charcoal: Cocopeat: Spahgnum moss: Decayed wood (1:1:1:1:1) significantly enhanced the plant height (12.63 cm) followed by the treatment T<sub>18</sub> i.e. Brick Pieces: Charcoal: Cocopeat:Decayed wood (1:1:1:1) and T<sub>13</sub> i.e. Charcoal :Sphagnum moss: Decayed wood (1:1:1) and lowest in treatment T<sub>1</sub> (Brick Pieces pieces only). As far as leaf number and length are concernred again the treatment T<sub>19</sub> i.e. Brick Pieces: Charcoal: Cocopeat: Spahgnum moss: Decayed wood (1:1:1:1:1) significantly enhanced the leaf number per plant (8.33) and leaf length (9.73 cm) followed by the treatment T<sub>18</sub> i.e. Brick Pieces : Charcoal : Cocopeat: Decayed wood (1:1:1:1). It is quite interesting to note that again the same treatment T<sub>19</sub> i.e. Brick Pieces: Charcoal: Cocopeat: Spahgnum moss: Decayed wood (1:1:1:1:1) and T<sub>6</sub> i.e. Charcoal :Cocopeat (1:1) significantly enhanced the leaf width (0.80 cm) which remained at par with the treatment T<sub>16</sub> i.e. Cocopeat :Sphagnum moss: Decayed wood (1:1:1). In all the cases Brick Pieces alone was failed for hardening of the *Dendrobium* cv. Sonia-17 showing minimum values for all the characters recorded after 60 days of hardening.

It has been concluded that the treatment T<sub>19</sub> i.e. Brick Pieces: Charcoal: Cocopeat: Spahgnum moss: Decayed wood (1:1:1:1:1) was most spectacular treatment for hardening of the tissue culture plantlets of *Dendrobium* cv. Sonia-17 after 60 days of planting (Fig. 11 and Plate.28,29) .

**Table 22: Efficacy of media mixture for hardening of the in vitro plantlets of *Dendrobium* cv. Sonia-17 after 60 days of planting**

Treatment	Constituents	Plant height (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)
T <sub>1</sub>	Brick Pieces	6.36	4.00	4.27	0.27
T <sub>2</sub>	Charcoal	6.59	5.33	7.83	0.40
T <sub>3</sub>	Cocopeat	6.97	4.33	6.50	0.50
T <sub>4</sub>	Brick Pieces+ Charcoal	6.47	4.33	6.33	0.37
T <sub>5</sub>	Brick Pieces + Cocopeat	7.20	5.67	4.53	0.43
T <sub>6</sub>	Charcoal +Cocopeat	8.93	5.67	5.87	0.80
T <sub>7</sub>	Brick Pieces +Charcoal +Cocopeat	6.30	6.00	6.43	0.53
T <sub>8</sub>	Brick Pieces+ Sphagnum moss	8.17	4.67	6.90	0.33
T <sub>9</sub>	Brick Pieces+Decayed wood	8.40	5.00	7.70	0.37
T <sub>10</sub>	Brick Pieces+ Sphagnum moss+Decayed wood	9.63	5.67	6.07	0.40
T <sub>11</sub>	Charcoal + Sphagnum moss	9.73	6.00	5.47	0.37
T <sub>12</sub>	Charcoal+ Decayed wood	9.33	5.00	5.60	0.40
T <sub>13</sub>	Charcoal + Sphagnum moss+ Decayed wood	10.07	5.33	6.37	0.52
T <sub>14</sub>	Cocopeat + Sphagnum moss	9.57	5.33	6.57	0.53
T <sub>15</sub>	Cocopeat+ Decayed wood	8.10	6.00	4.53	0.55
T <sub>16</sub>	Cocopeat + Sphagnum moss+ Decayed wood	8.50	6.33	6.43	0.77
T <sub>17</sub>	Brick Pieces+ Charcoal + Cocopeat+ Sphagnum moss	9.50	4.67	6.77	0.53
T <sub>18</sub>	Brick Pieces+ Charcoal + Cocopeat+ Decayed wood	10.37	7.00	8.77	0.60
T <sub>19</sub>	Brick Pieces+ Charcoal + Cocopeat+ Sphagnum moss+ Decayed wood	<b>12.63</b>	<b>8.33</b>	<b>9.73</b>	<b>0.80</b>
SE(m)±		0.23	0.42	0.22	0.04
CD (5%)		0.67	1.20	0.63	0.11

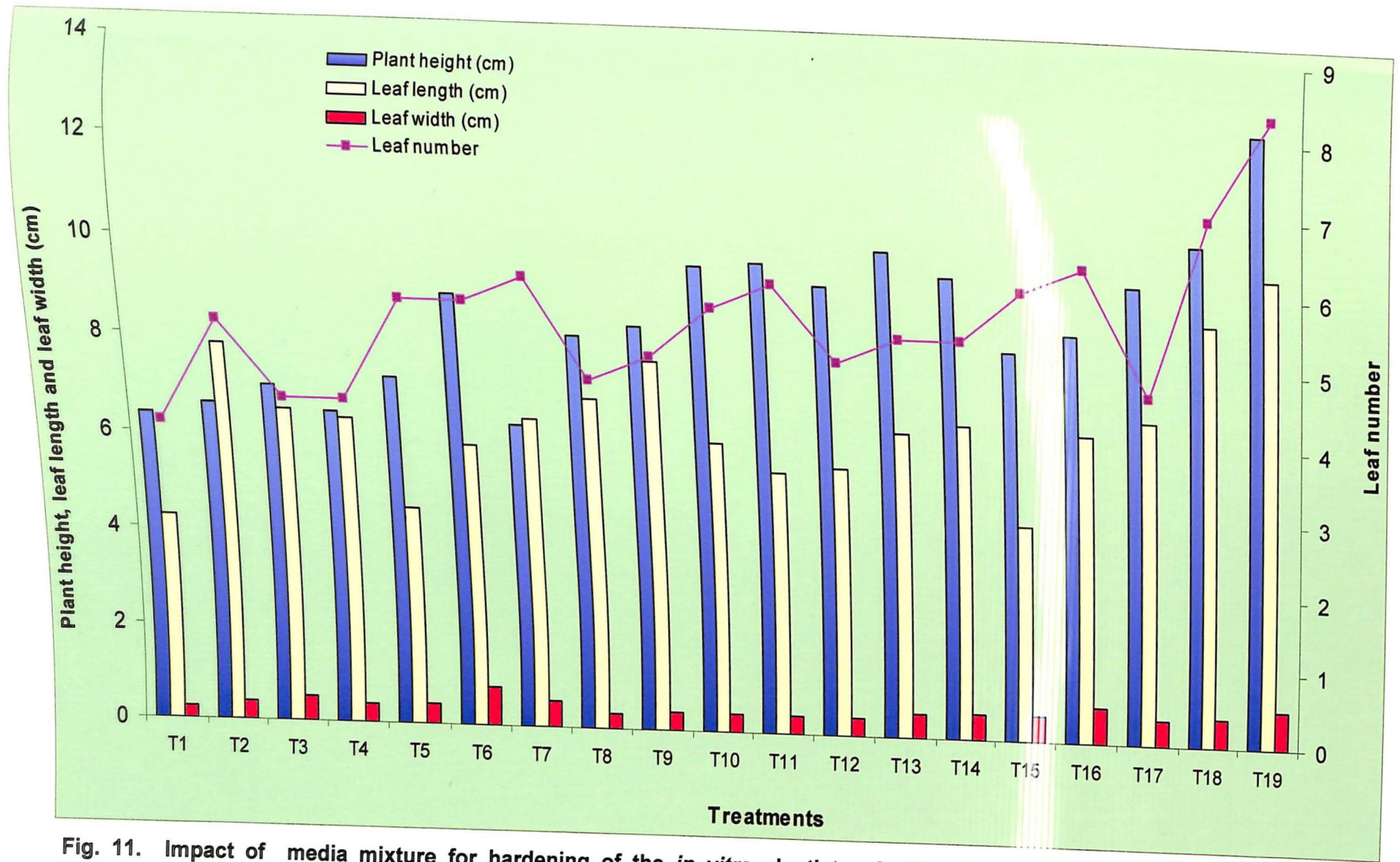


Fig. 11. Impact of media mixture for hardening of the *in vitro* plantlets of *Dendrobium* cv. Sonia-17 after 60 days of transplanting



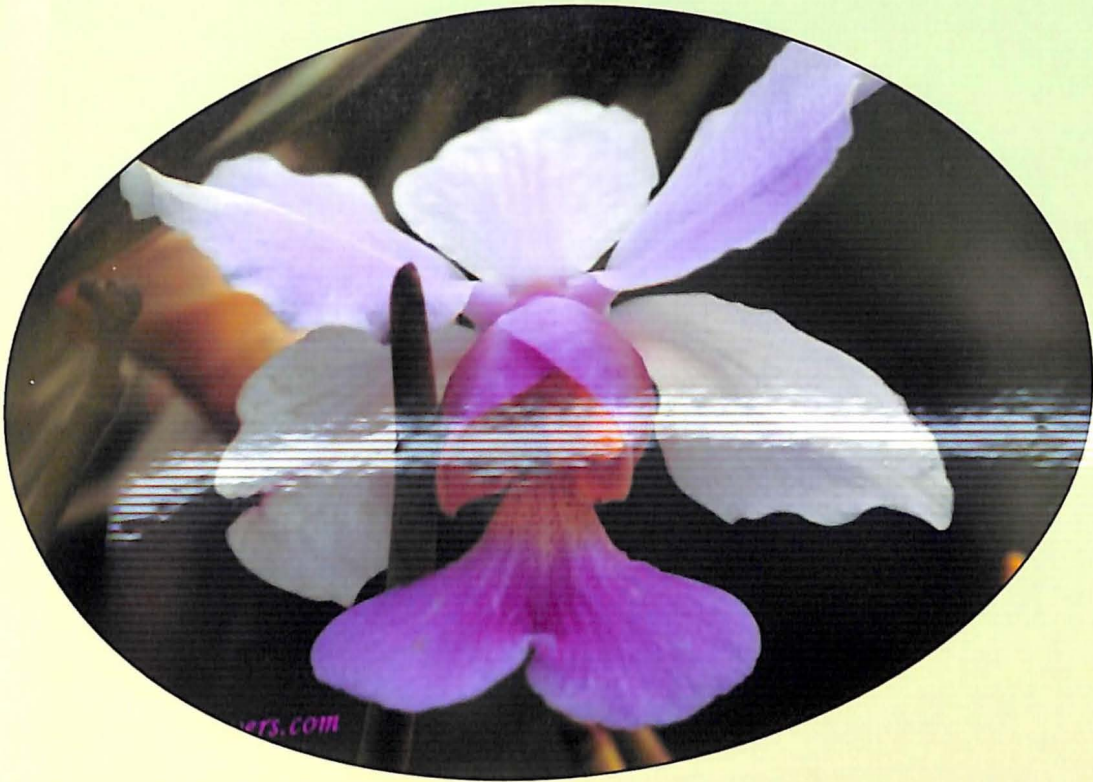
**Plate 28.** *In vitro* hardening of *Dendrobium* cv. Sonia-17 in pots in media combination (Brick pieces + charcoal + cocopeat + sphagnum moss + decayed wood) after 45 days of transplanting



**Plate 29.** *In vitro* hardening of *Dendrobium* cv. Sonia-17 in pots in media combination (Brick pieces + charcoal + cocopeat + sphagnum moss + decayed wood) after 60 days of transplanting



Plate 30. *In vitro* hardening of *Dendrobium* cv. Sonia-17 in pots inside the polyshade net structure



## CHAPTER V

# *Discussion*

The aim of discussion, should not be  
victory but progress.....

## DISCUSSION

Conventionally, Orchids are propagated both by sexual and asexual means. Orchid capsule contains 2-3 millions seeds which look just like dust. The capsule usually takes 4-17 months to mature. Due to lack of any metabolic machinery and functional endosperm though the seeds are functional they are not able to germinate properly. However these seeds need a symbiotic relationship with a fungus called as Orchid mycorrhiza and about 0.2-0.3 % of seeds in a capsule germinate in nature. Asexually Orchids propagate by division of rhizomes, air layering, separation of offshoots, keikis. Considering the aforementioned propagation methods, it has been seen that both the processes of propagation are very slow, tedious and time consuming. These problems necessitate an alternate method of propagation which helps in bypassing the slow rate of multiplication, bulking of true to type disease free stocks and production of huge quantity of planting materials in shorter span of time. Though *in vitro* techniques have been standardized for different Orchid species by various workers (Indhumati *et al.*(2003), Parvin *et al.* (2009), Vyas *et al.*(2011), ). The work on standardization of media, plant bioregulators, adjuvants and their combinations respond to shoot proliferation, rooting, hardening for a specific Orchid species or varieties is neagre. Therefore in present investigation attempts are made to standardize the media combinations for shoot proliferation, root initiation and development & hardening of Orchids (*Papilionanthe teres* and *Dendrobium* cv. Sonia-17) and the results obtained so far have been discussed in this chapter.

## **5:1: STANDARDIZATION OF MEDIA SUPPLEMENTS FOR SHOOT PROLIFERATION OF ORCHIDS (*PAPILIONANTHE* AND *DENDROBIUM*)**

### **5:1:1: Standardization of media supplements for shoot initiation of *Papilionanthe teres***

In the present investigation, protocorm like bodies (PLBs) derived from the immature seeds used for shoot initiation and MS medium alone or fortified with BAP 2-3 mg/l, NAA 0.5 mg/l and AdS 100 mg/l are used for shoot proliferation study. After 30 days of culture it has been noted that in *Papilionanthe teres*, MS medium supplemented with 2 mg/l BAP along with 100 mg/l AdS produced shoots with longer and prominent leaves along with calli mass and PLBs. On the other hand least number of leaves were formed with BAP 3.0 mg/l and NAA 0.5 mg/l. 100 mg AdS when added to BAP @ 2 mg/l produced more number of rudimentary leaves with more calli and in combination with BAP 3mg/l produced shoots with prominent leaves, PLBs and greenish yellow calli mass in *Papilionanthe teres*.

The result is in confirmity with the finding obtained by Rasmussen (1995) who reported a positive effect of adding cytokinins to the media. It might be due to enhancement of cell division normally observed by action of cytokinin. Similar effect of BAP on stimulation of shoot growth *Oncidium* was reported by Kalimuthu *et al.* (2007) who observed that culture of immature seeds of *Oncidium spp* in MS medium supplemented with BAP at 2.0 mg/l stimulated shoot multiplication and recorded maximum number of shoots/bottle.

### **5:1:2: Standardization of media supplements for shoot proliferation of Orchids *Dendrobium* cv. Sonia-17**

As far as shoot proliferation of *Dendrobium* cv. Sonia-17 is concerned MS medium supplied with BAP 2.0 mg/l significantly enhanced plant height after 30,45 and 60 days of culture. MS medium supplemented with BAP @ 2.5 mg/l, NAA 0.5 mg/l and AdS 100 mg/l significantly increase the number of leaves after 30, 45 and 60 days of culture where as MS medium supplied with BAP @ 2.0 mg/l, NAA 0.5 mg/l and AdS 100 mg/l enhanced the total number of suckers. However, variation is recorded in various treatments when the character like leaf length is concerned. As the effect was not spectacular after 30 and 45 days of culture importance is given on 60 days of culture. The longest leaf (1.75 cm) was observed in MS medium supplemented with BAP 2.0 mg/l and NAA 0.5 mg/l.

BAP alone (2.0 mg/l) increased plant height, with NAA (0.5 mg/l) produced longer leaves and along with 100 mg/l AdS increased number of suckers/plant. BAP (2.5mg/l), NAA (0.5 mg/l) and AdS (100mg/l) increased number of leaves/ plant.

The result corroborated with the findings of Talukder *et al.* (2003) who observed improvement in shoot proliferation, leaf number and shoot length in *Dendrobium* Orchid in MS medium supplemented with BAP @ 1.0 mg/l and 2.5 mg/l as compared to control i.e. 0 mg/l BAP and the most effective treatment was 2.5 mg/l BAP. Salam (2010) reported similar result with application of BAP 2.0 mg/l to MS medium. Addition of adjuvant like increased number of suckers as well as number of leaves. The result obtained in this study was similar as that of Aswath and Choudhury (2001), Patra and Beura where shoot organogenic capacity was higher with BAP and AdS in

Gerbera. In potato addition of AdS lead to shoot proliferation from potato sprouts was reported by Beura (2002) and in tobacco, application of Adenine promoted bud differentiation and development (Chawala, 2002). BAP, a potent cytokinin along with adjuvant ADS was most effective combinations for shoot proliferation in Gerbera. Shailaja *et al.* (2004) reported that suppression of apical dominance leads to production of more number of multiple shoots in *Gerbera jamesonii* Bolus.

### **5:1:3: Impact of plant bioregulators on shoot proliferation of *Dendrobium* cv Sonia-17**

MS medium fortified with 5 mg/l kinetin increased the shoot height after 30, 45 and 60 days of inoculation throughout the period of investigation and significantly increased the number of leaves up to 45 days where as MS medium supplemented with kinetin 6 mg/l significantly increase the leaf length throughout the period of investigation. Kinetin @ 4mg/l when added to the MS medium significantly enhance the number of suckers upto 45 days of inoculation. A very interesting result was obtained in the culture within 45-60 days of inoculation. After 45 days of inoculation obtained in lower concentrations of kinetin i.e. 3 mg/l shown a spectacular effect on the number of leaves/ clump after 45-60 days of inoculation. The number of suckers remained constant (3.45) in MS medium alone after 45 as well as 60 days of inoculation. After 45 days of inoculation MS medium supplied with kinetin degenerated some of the suckers thereby decreasing the number of suckers. MS medium supplemented with higher concentration of kinetin or all the concentrations of kinetin with NAA reduced the number of leaves/clump as the leaves touching the medium degenerated. So kinetin supplements increase the

plant height, number of leaves, number of suckers, leaf length up to 45 days of culture where as MS medium alone shows good results after 45-60 days of culture.

The results corroborated with the findings of Patra (2009) who reported that kinetin 2.0 or 2.5 with ads 75 or 100 mg/l produce longer shoots in Gerbera. In Tobacco, the presence of adenine or kinetin in the medium leads to promotion of bud differentiation and development (Chawala 2002). Pattnaik (2007) reported that the longer shoots in cv. common red of gerbera. Pyati *et al* 2002 stated that axillary shoots are induced directly in medium containing BA, kinetin and coconut water.

## **5:2: STANDARDIZATION OF MEDIA SUPPLEMENTS FOR ROOT INITIATION AND DEVELOPMENT**

### **5:2:1: Standardization of media supplements for root initiation and development of *Papilionanthe teres***

In the present study, the existing 3-4 cm height shoots were used for root formation study. Various combinations of IBA and NAA were added to the half MS medium to study the number of roots/bottle, roots/plantlet, root length (cm) on 30, 60, 90 days after inoculation. The treatment produced the maximum number of roots/bottle (2.83), number of roots/ plantlet (0.83) and root length (2.83 cm).

The results corroborated with the findings of Raju *et al.* (2003) who obtained maximum number of rooting *Dendrobium osteroholt* in IBA @ 1.0 mg/l and NAA @ 1.0 mg/l. Hussain (1995) reported that lower levels of auxins (0.5 or 1.0 mg/l NAA) induced early rooting. The result was partially in agreement of Kusumotto (1978) who observed that basal medium supplemented with combinations of 1.0 mg/l GA<sub>3</sub> and 0.1 mg/l NAA was most

satisfactory for root formation. Epstein and Ludwig-Muller (1993) reported that during the process root induction, there were two major pathway of conversion: oxidation and conjugation. Exogenous auxins applied to micro-cuttings get oxidized, resembled the enzymes involved in the wounding reaction as reported by De Klerk *et al.* (1999). They also indicated that IAA or IBA oxidation caused by non-specific peroxidase was related to the rooting response.

### **5:2:2: Standardization of media supplements for root initiation and development of *Dendrobium* cv. Sonia-17**

*In vitro* root formation study in *Dendrobium* cv. Sonia-17 has shown very interesting results as far as plant bio-regulators are concerned. the result clearly indicated that MS medium fortified with most potent auxin IBA @ 1.0 mg/l significantly increased the number of roots/bottle, number of roots/plantlet and root length (cm) on 30, 60, 90 days of inoculation. Being highest after 90 days.

The results corroborated with the findings of Raju *et al.* (2003) who obtained maximum number of rooting *Dendrobium osteroholt* in IBA 1.0 mg/l and NAA 1.0 mg/l. The result was in agreement with Salam (2010) who reported that MS medium supplemented with IBA 1.0 mg/l on rooting of cv. banayat pink. Ancora *et al.* (1981) reported the effectiveness of IBA and NAA in root induction of *in vitro* produced plants in Globe artichoke. Sit and Tiwari (1997-98) stated that half MS medium supplemented with IBA successfully produced roots in Turmeric. MS medium supplemented with NAA developed healthy roots in Turmeric (Meenakhi *et al.*, 2001).

Treatments with auxins stimulate and show an increase in peroxidase activity, as observed by Palai (2001). It was suggested that the auxin entered through the cut surfaces of the proliferated shoots and rapidly absorbed in the

cell walls by pH trapping (Rubery and Sheldrake, 1973) and by influx carriers (Delbarre *et al.*, 1996). Epstein and Ludwig-Muller (1993) reported that during the process root induction, there were two major pathway of conversion: oxidation and conjugation. Exogenous auxins applied to micro-cuttings get oxidized, resembled the enzymes involved in the wounding reaction as reported by De Klerk *et al.* (1999). They also indicated that IAA or IBA oxidation caused by non-specific peroxidase was related to the rooting response.

### **5:3: STANDARDIZATION OF MEDIA MIXTURE FOR HARDENING OF THE *IN VITRO* PLANTLETS**

The success of tissue culture depends on the sustainability of the *in vitro* produced plants under the natural conditions. Under *in vitro* conditions, the plantlets are heterotrophs. So they have to be gradually converted into autotrophs. The pre- hardening and hardening are the processes for making the *in vitro* raised plantlets into autotrophs and adapt them to the outside environment (Beura, 1998). The existing rooted plantlets varying from height 5-7.5 cm were taken for hardening study in poly shade net structure. For hardening different media such as brick pieces, charcoal, cocopeat alone or in combination with sphagnum moss and decayed wood were used in nineteen category i.e. under nineteen treatments. The plants were hardened up to 60 days. . The half MS liquid medium was fertigated to the seedlings at an interval of two days up to two weeks and then one fourth MS liquid medium was applied at an interval of two days the next two weeks. Different observations such as plant height, leaf number, leaf length and leaf width have been recorded after 30 and 60 days of hardening. the data indicated very specific results that the media combinations such as brick pieces: charcoal: cocopeat: sphagnum moss: decayed wood in 1:1:1:1:1 proportion significantly increase plant height, leaf

number, leaf length and leaf width after 30 as well as 60 days of hardening followed by the media combinations such as brick pieces: charcoal: cocopeat: decayed wood as far plant height, leaf number, leaf length concerned.

The results of the present investigation are in partially agreement with that of Sharma (1995) who reported that the media combination comprising of brick: charcoal: tree fern: bark pieces: leaf mould: dry sphagnum moss 1:1:1:1:2 in the ratio was considered suitable for maximum survival of *Dendrobium chrysanthemum* seedlings. Indhumati *et al.*, (2003) reported that charcoal+brick+cocopeat in the ratio 1:1:1 was found suitable for establishment of plantlets of *Dendrobium* Hybrid Sonia-17. Pure charcoal increased the root number where half KC nutrient solution improved the growth and establishment during the investigation period. According to Pathania *et al.* (1998) hardening medium containing bark pieces, brick pieces, moss, charcoal pieces (1:1:1:1) proved to be suitable for the establishment of *in vitro* rooted plantlets of Sonia-17.





## CHAPTER VI

# *Summary and Conclusion*

**The end justifies  
The meaning....**

## SUMMARY AND CONCLUSION

The present investigation entitled “**High frequency multiplication of Orchids through *in vitro* culture**” was carried out in the Plant Tissue Culture Laboratory, Department of Floriculture and Landscaping, Orissa University of Agriculture and Technology, Bhubaneswar during the year 2011-12. The following steps were undertaken for conducting the whole experiment:

1. Standardization of media supplements for shoot proliferation of Orchids (*Papilionanthes* and *Dendrobium*).
2. Standardization of media supplements for root initiation and development.
3. Standardization of media mixture for hardening of the *in vitro* plantlets.

The results of the experiments are summarized as follows.

### **6:1: STANDARDIZATION OF MEDIA SUPPLEMENTS FOR SHOOT PROLIFERATION OF ORCHIDS (*PAPILIONANTHE* AND *DENDROBIUM*).**

#### **6:1:1: Effect of Plant Bio Regulators and Adjuvant on shoot initiation in *Papilionanthe teres***

MS medium supplemented with 3.0 mg/l BAP and 100 mg/l AdS was found to be the most appropriate combination for shoot initiation in *Papilionanthe teres*.

**6:1:2: Standardization of media supplements for shoot proliferation of *Dendrobium cv. Sonia-17***

- i. After 30 days of culture MS medium supplemented with 2.0 mg/l BAP increased the shoot height (2.17 cm). MS medium supplemented with 0.5 mg/l NAA, 100 mg/l AdS along with three different concentrations of BAP separately has given very interesting results, as when BAP @ 2.5 mg/l was supplemented significantly increased the number of leaves (7.11) ; BAP when supplemented @ 3.0 mg/l significantly increased the leaf length (1.15 cm) and BAP at 2.0 mg/l significantly enhanced the number of suckers (3.53) per clump in *Dendrobium cv. Sonia-17*.
- ii. After 45 days of culture MS medium fortified with 2.0 mg/l BAP increased the shoot height (2.27 cm). The leaf length (1.37 cm) was best observed in MS medium fortified with BAP 2.0 mg/l and AdS 100 mg/l. MS medium fortified with 0.5 mg/l NAA, 100 mg/l AdS. BAP @ 2.5 mg/l was supplemented significantly increase the number of leaves (9.43) and @ 2 mg/l significantly increased the number of suckers (3.73) in *Dendrobium cv. Sonia-17*.
- iii. MS medium supplemented with 2.0 mg/l BAP increased the shoot height (2.45cm). The number of leaves (12.39) was best observed in MS medium fortified with BAP 2.5 mg/l, 0.5 mg/l NAA and AdS 100 mg/l. Longer leaf (1.75 cm) was observed in MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA. MS medium fortified with 3 .0 mg/l BAP, 100 mg/l AdS gave the highest number of suckers (3.91) in *Dendrobium cv. Sonia-17* after 60 days of culture.

### 6:1:2: Standardization of media supplements for shoot proliferation of *Dendrobium cv. Sonia-17*

- i. After 30 days of culture MS medium supplemented with 2.0 mg/l BAP increased the shoot height (2.17 cm). MS medium supplemented with 0.5 mg/l NAA, 100 mg/l AdS along with three different concentrations of BAP separately has given very interesting results, as when BAP @ 2.5 mg/l was supplemented significantly increased the number of leaves (7.11) ; BAP when supplemented @ 3.0 mg/l significantly increased the leaf length (1.15 cm) and BAP at 2.0 mg/l significantly enhanced the number of suckers (3.53) per clump in *Dendrobium cv. Sonia-17*.
- ii. After 45 days of culture MS medium fortified with 2.0 mg/l BAP increased the shoot height (2.27 cm). The leaf length (1.37 cm) was best observed in MS medium fortified with BAP 2.0 mg/l and AdS 100 mg/l. MS medium fortified with 0.5 mg/l NAA, 100 mg/l AdS. BAP @ 2.5 mg/l was supplemented significantly increase the number of leaves (9.43) and @ 2 mg/l significantly increased the number of suckers (3.73) in *Dendrobium cv. Sonia-17*.
- iii. MS medium supplemented with 2.0 mg/l BAP increased the shoot height (2.45cm). The number of leaves (12.39) was best observed in MS medium fortified with BAP 2.5 mg/l, 0.5 mg/l NAA and AdS 100 mg/l. Longer leaf (1.75 cm) was observed in MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA. MS medium fortified with 3 .0 mg/l BAP, 100 mg/l AdS gave the highest number of suckers (3.91) in *Dendrobium cv. Sonia-17* after 60 days of culture.

### **6:1:3: Impact of Plant Bio Regulators on shoot proliferation of *Dendrobium cv. Sonia-17***

- i. Relating to shoot proliferation study the results after 30 days of culture MS medium supplemented with kinetin 5.0 mg/l increased the shoot height (1.35 cm) and number of leaves (7.40). The best leaf length (0.74 cm) was observed in MS medium fortified with 6.0 mg/l kinetin. The highest number of suckers (3.33) observed in MS medium fortified with 4.0 mg/l kinetin in *Dendrobium cv. Sonia-17*.
- ii. After 45 days of culture, MS medium supplemented with kinetin 5.0 mg/l increased the shoot height (1.66 cm) and number of leaves (9.60). The best leaf length (0.87 cm) was observed in MS medium fortified with 6.0 mg/l kinetin. The highest number of suckers (3.82) observed in MS medium fortified with 4.0 mg/l kinetin in *Dendrobium cv. Sonia-17*.
- iii. After 60 days of culture revealed that MS medium supplemented with kinetin 5.0 mg/l increased the shoot height (2.54 cm). The maximum number of leaves (13.07) and highest number of suckers (3.45) were observed in basal MS medium after 60 days of culture.. The leaf length (1.15 cm) was observed in MS medium fortified with 6.0 mg/l kinetin in *Dendrobium cv. Sonia-17* .

### **6:2: STANDARDIZATION OF MEDIA SUPPLEMENTS FOR ROOT INITIATION AND DEVELOPMENT**

#### **6:2:1: Impact of Plant Bio Regulators on root initiation and development of *Papilionanthe teres***

- i. Half MS medium supplemented with 0.5 mg/l IBA along with 0.5 mg/l or 1.0 mg/l NAA has given best results in number of roots per

culture bottle (1.33). The number of roots per plantlet was highest (0.33) and the root length was maximum (1.43 cm) in half MS medium supplemented with 0.5 mg/l IBA and 0.5 mg/l NAA in *Papilionanthe teres* after 30 days of inoculation and the result was non-significant.

ii. Half MS medium enriched with 0.5 mg/l IBA alone or in combination 0.5 mg/l NAA increased the number of roots per culture bottle (2.0). IBA @ 0.5 mg/l alone or in combination with NAA 1.0 mg/l increased the number of roots per plantlet (0.5). Root length was found to be maximum (1.60 cm) in half MS medium supplemented with 0.5 mg/l IBA, 0.5 mg/l NAA in *Papilionanthe teres* after 60 days of culture. All the parameters under study have given non-significant results.

iii. Half MS supplemented IBA @ 0.5 mg/l resulted higher number of roots per bottle (3.67) and with 1.0 mg/l resulted maximum number of roots per plantlet. MS medium fortified with IBA (1.0 mg/l) along with NAA (1.0 mg/l) resulted maximum root length after 90 days of culture in *Papilionanthe teres*. However the result was non-significant for all the characters after 90 days of culture.

#### **6:2:2: Impact of Plant Bio Regulators on root initiation and development of *Dendrobium* cv. Sonia-17**

i. Half MS medium enriched with 1.0 mg/l IBA significantly produced more number of roots/bottle (3.33) and roots/plantlet (0.83) was most suitable treatment for root primordia initiation in the *Dendrobium* cv. Sonia-17 after 30 days of culture. However longer roots were produced in half MS alone (1.72 cm).

- ii. Half MS medium fortified with 1.0 mg/l IBA was found to be best treatment for recording highest number of roots/ bottle (12.33), number of roots/plantlet (3.08) and root length (3.33 cm) in *Dendrobium* cv. Sonia-17 after 60 days of culture.
- iii. After 90 days of culture, half MS medium enriched with 1.0 mg/l IBA was most suitable for further root growth and development in *Dendrobium* cv. Sonia-17 in terms of number of roots/bottle (15.00), number of roots per plantlet (3.75) and root length (5.44 cm).

**6:3: Standardization of media mixture for hardening of the *in vitro* plantlets.**

- i. The media combinations such as Brick pieces: Charcoal: Cocopeat: Spahgnum moss: Decayed wood (1:1:1:1:1) was found to be the most suitable for hardening of *Dendrobium* cv. Sonia-17 after 30 days of planting, as the combination significantly enhanced the plant height (10.2 cm), leaf number (8.00), leaf length (9.43 cm) and leaf width (0.70 cm)
- ii. The media combinations i.e. Brick pieces: Charcoal: Cocopeat: Spahgnum moss: Decayed wood (1:1:1:1:1) was most spectacular treatment for hardening of the tissue culture plantlets of *Dendrobium* cv. Sonia-17 after 60 days of planting, as the combination had profound effect on enhancing significantly the plant height (12.63 cm), leaf number (8.33), leaf length (9.73 cm) and leaf width (0.80 cm).

## CONCLUSION

The findings of the present investigations are expected to give a new orientation to the thoughts of the future research workers for the large scale multiplication of the precious Orchids through *in vitro* culture. The work on *in vitro* culture *Papilionanthe teres* appears to be quite innovative as there has been no work, reported so far on this prized species. Earlier work in the department of Horticulture some work has been done on *Papilionanthe teres* which obtained spectacular variations in respect of colour , size and shape of the flower. Hence, more research work on *in vitro* culture to fine-tune the outcome of the present investigation will be highly appreciated.

The work on *in vitro* culture of *Dendrobium* has given very promising results in respect of shoot proliferation and initiation and development of roots which can go in form of standard recommendation. The media combinations such as Brick pieces: Charcoal: Cocopeat: Spahgnum moss: Decayed wood (1:1:1:1:1) was found to be the most suitable for hardening of the *Dendrobium spp* cv. Sonia-17. The outcome of the observations made during the hardening studies of the *Dendrobium* cv. Sonia-17 gives the author enough confidence to recommend that the suitable medium used for hardening could also be used for growing *Dendrobiums* on large scale as the plantlets exhibited luxuriant growth with cent percent success. This, however, again needs some fine-tuning before giving a commercial recommendation for cultivation in this line.





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