STUDIES ON MICROPROPAGATION OF *DENDROCALAMUS STOCKSII* (MUNRO.) THROUGH NODAL EXPLANT

By

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STUDIES ON MICROPROPAGATION OF *DENDROCALAMUS STOCKSII* (MUNRO.) THROUGH NODAL EXPLANT

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by

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This is to certify that, the thesis entitled **"STUDIES ON MICROPROPAGATION OF *DENDROCALAMUS STOCKSII* (MUNRO.) THROUGH NODAL EXPLANT"** is a record of independent bonafied research work carried out by **Miss SAVITRI RAJAN INDULKAR (FDPM 14-45)** at this college, under my guidance and supervision for the degree of M. Sc. (Forestry) in Forest Biology and Tree Improvement of Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli. The said thesis has no previously formed the basis for the award of any degree, diploma, associate ship, fellowship or any other similar title.

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This is to be certified that the thesis entitled **"Studies on micropropagation of *Dendrocalamus stocksii* (munro.) through nodal explant"** is a record of independent bonafied research work carried out by **Miss. Savitri Rajan Indulkar** (Registration No. FDPM 14-45) at this college during the period of study from 2014 to 2017 under our guidance and supervision for the degree of Masters of Science (Forestry) in Forest Biology and Tree Improvement (Forest Biotechnology) of Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli. The said thesis has not previously formed the basis for the award of any degree, diploma, fellowship or any other similar title.

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DECLARATION OF STUDENT

I hereby declare that the experimental work and its interpretation of the thesis entitled **"Studies on micropropagation of *Dendrocalamus stocksii* (munro.) through nodal explant"** or part thereof has neither been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis / publication of any University or scientific organization. The source of materials used and all assistance received during the course of investigation have been duly acknowledged.

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Date: May, 2017

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C E R T I F I C A T E

"I have no objection if data / observations / findings generated by me during my PG research are utilized by major guide or minor guide in future preparing any project or making recommendation or publication in any research journal or nominating for award."

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CHAPTER I
INTRODUCTION

Bamboo is one of the most fascinating, versatile, multipurpose, fast growing group of plants belonging to the grass sub family Poaceae of family Gramineae. It is prominently found in the wet evergreen and moist deciduous forests of tropical, sub-tropical and temperate parts of the world. It is distributed throughout the world except Europe and West Asia which do not have native bamboos. Bamboo is a cultural feature of south-east Asia where no country is found without indigenous bamboo flora.

Bamboo is an important natural resource that plays a major role in the livelihood of rural people and as well as large scale industry. With the peculiar characteristic of short rotation, marketability of culms every year and immediate returns, bamboos are one of the fastest growing multipurpose plants of high economic and environmental value that converts solar radiation into useful goods and services better than most tree species (Kassahun, 2000). Bamboo is regarded as a sustainable resource from which raw material can be harvested on a regular basis. Bamboo is used for house construction, scaffolding, ladders, mats, baskets, fencing, garden support, fodder, fishing rods, walking sticks, tool- handles, pipes, toys, handicrafts etc., and for several other articles of everyday use (Tamang *et al.,* 2013). Other than this, it could be used as a substitute for wood. There is increasing interest for uses in ecological applications and as energy crops. Bamboos also warrant serious consideration for carbon farming, carbon sequestration and carbon trading (Nath *et al.*, 2015).

There are about 1250 species of bamboo in some 75 genera. Bamboo roughly covers 1 per cent of the global forest area and 3.2 per cent of the total forest area if bamboo outside forest land is included. Out of the 36 million hectares area of bamboo distributed throughout the world, around 24 million hectares of bamboo is reported in Asia alone.

Asia remains the richest continent, with about 65 per cent of total world bamboo resources. India accounts for roughly half the total area of bamboo reported from Asia. In India 16 % bamboo forest is privately owned whereas 84 % lies with the government or public. Approximately 30 per cent of the total area of bamboo in Asia is planted. In India 84.34 lakh ha is naturally regenerated and 29.27 lakh ha is planted. India has 3 million hectares of planted bamboo and approximately 25 per cent of the total bamboo resources (Lobovikov, 2007).

India has 119 native species and 25 introduced species making the total diversity of bamboo to 102. As per latest compilation, 23 genera and 128 species of bamboo have been reported in India (Seethalakshmi and Kumar, 1998). The 18 genera found in India are 1.*Arundinaria*, 2.*Bambusa*,3.*Chimonobambusa*, 4.*Dendrocalamus*, 5.*Dinochloa*, 6.*Gigantochloa*,7.*Melocanna*,8.*Ochlandra*,9.*Oxytenanthera*,10.*Phyllostachys*,11.*Pleioblastus*,12.*Pseudosasa*,13.*Pseudoxytenanthera*, 14.*Racemobambos*,15.*Schizoztachyum*,16.*Sinarundinaria*, 17.*Thamnocalamus*and 18.*Thyrsostachys*.About 2.5 billion people in the world depend economically on bamboo(IFAR/INBAR, 1991) and international trade in bamboo amounts to about US$2.5 million (Lobovikov, 2007).

The genus *Dendrocalamus*has over fifty species distributed in the tropical and subtropical regions of the world. It is characterized by sympodial rhizomes and large sized dense clumps. Most of these species are economically exploited by rural communities in south and south-east Asia.

*Dendrocalamusstocksii*is a strong, arborescent and thornlessbamboo species. It is naturally distributed in the Central Western Ghats of Maharashtra, Karnataka, Goa and Kerala. It is commonly known as Marihal, Manga, Mes, Chiva, etc. (Viswanath *et al.*, 2012). It is an

extremely manageable bamboo species with a great economic and ecological importance (Singhal *et al.,* 1999).

It is presently confined to the coastal tracts where it is cultivated in homesteads and in farm boundaries as live fence (Rane *et al.,* 2014). It is primarily planted around the arecanut gardens and paddy fields. It is observed to be performing well in humid, sub-humid and semi-arid zones which expand its scope for cultivation across Peninsular India. Although it's natural distribution is in lateritic soil it also comes up well in black and red soils with wide adaptability (Viswanath*et al.*, 2012).

Larger culms have demand in constructions, furniture, ladders and supports while smaller culms find use in agricultural implements, handicrafts, fencing material, etc. Two year old culms are used for preparing baskets and the matured ones in construction and other rural works (Singhal*et al.,* 1999) It can be stated as a substitute for cane and rattan in bamboo based furniture industry due to its typical anatomical characteristics like presence of pre-dominant nodes, solid nature and good culm wall thickness (Viswanath *et al.*, 2012).

About 35% of *D. stocksii*shoot is regarded as edible portion (Sowmya*et al.,* 2014). Besides edible shoots and in handicrafts, it is a component of various agricultural implements and stakes in agricultural fields. It is also used in scaffolding, pulp and paper, crafts, umbrella handles, walking sticks and as navigation tool in country boats, etc. (Somen *et al.,* 2011). Owing to its multifarious uses and perceived importance, National Bamboo Mission (NBM) has prioritized this species for large scale cultivation in Peninsular India.

In few villages of Maharashtra, cultivation of this species is a major source of income and livelihood where it gives a steady income to farmers. Farmers in Peninsular India are reluctant to adopt this species in agro-forestry practices. Though several bamboo species are found in Peninsular India, they are difficult to manage due to excessive branching

pattern, distorted shape and thorny habit. Contrastingly, culms of *D. stocksii* are loosely spaced thus easy to manage. It is a low cost natural resource, renewable and versatile. The conventional method of propagation is by rhizome and stem cuttings. Propagation through culm cuttings has been the practical and time tested method of propagation of this species (Viswanath *et al.,* 2012).

However, large scale cultivation is hampered by non-availability of planting stock (Sanjaya *et al.,* 2005). Propagation of bamboo by seeds is unreliable due to long and unpredictable flowering habit and also undesirable on account of large variation found in seedling propagation. Sterility in *D. stocksii*is attributed to the less quantity of pollen produced, viability of pollen, percentage of anthesis, short receptivity of stigma, etc. (Beena, 2011).

Due to poor seed setting and non-gregarious nature of this species, genetic diversity could be highly restricted and continuous vegetative propagation from a narrow genetic base could have serious implication for conservation of the species (Rane *et al.,* 2013). Lack of viable seeds and scalability issues in macro-propagation techniques have led to the nascent steps in micro-propagation protocol development. Micro-propagation through both axillary shoot proliferation and somatic embryogenesis has yielded good results (Viswanath *et al.,* 2012).

While improved vegetative propagation is available, only a limited number of plants can be raised using this method, which is inadequate to meet the growing demand. Therefore, in order to supplement the conventional methods, an efficient *in vitro* propagation method would offer a desirable alternative for large-scale multiplication of elite genotypes. There is also an immense potential for improving species through selection and breeding programs. It will lead to a method of collective benefits of easy to raise, cost effective, economic to adopt and easy to transport for selling purpose.

Therefore, the present investigation on **“**Studies on micropropagationof *Dendrocalamusstocksii*(Munro.)through nodal explant” was undertaken with the following objectives:

1. To standardize explant surface sterilization technique for *Dendrocalamusstocksii*.
2. To standardize media combination for *in vitro* shoot initiation and proliferation of *Dendrocalamusstocksii.*
3. To standardize media combination for *in vitro* root induction in *Dendrocalamusstocksii*.

CHAPTER II
REVIEW OF LITERATURE

The literature available on the various aspects of tissue culture has been reviewed and presented in this chapter.

4.1. Disinfection of Explants

For *in vitro* culture initiation, explants are normally collected from nursery grown plants, so there are chances of plant material liable to be contaminated by micro organisms which must be disinfected before the explants are transferred to *in vitro* conditions. Variations in sterilization procedure have been proposed by several researchers.

Prutpongse *et al.* (1992) studied *in vitro* propagation of 54 species of bamboo, in which the explants were sprayed with 70% ethanol, surface sterilized for 30 min in 1% sodium hypochlorite, and washed three times in sterile water.

Sanjaya *et al*. (2005) established contamination free cultures of *D. stocksii*by treating the nodal explants with 0.075% (w/v) mercuric chloride for 4-5 min followed by washing the explants thoroughly for six to seven times with sterile distilled water.

Ndiaye *et al.* (2006) used the method of sterilization in which nodal fragments of *Bambusa vulgaris* were abundantly washed in running tap water and surface disinfected by dipping them into $HgCl_2$ (0.1%) for 20 minutes followed by 4 times rinsing in sterile distilled water.

Mudoi *et al.* (2009) described a procedure for surface sterilization in which the sheath was removed and the node containing axillary bud was dipped in 5% (v/v) Tween 20 solution for 3 hours followed by thoroughly washing under running tap water for 20 minutes. Explants were then disinfected with 0.1% mercuric chloride solution for 5-7 min and rinsed thoroughly with sterile distilled water prior to culture. Pretreatment of the explants with a mixture of an antifungal and an

antibiotic (0.1% solution each of mancozeb and gentamycine) was done, depending upon the time of collection of the explants.

Mehta *et al.* (2010) obtained successful establishment of cultures by a pretreatment of surface sterilization in which the leaf sheaths were removed to expose the tender buds, which were surface cleaned with a liquid detergent (Tween 20) followed by continuous rinsing with water. Thereafter, the explants were treated with Bavistin (0.1% w/v) and streptomycin sulphate for 20-25 min with constant stirring. Finally, the explants were surface sterilized in a laminar hood with 70% ethanol for 1-2 min followed by treatment with an aqueous solution of mercuric chloride (0.04% w/v) containing 1-2 drops of liquid detergent for 5-6 min and again washing thoroughly with distilled water.

Bisht *et al.* (2010) used a sequence of surface sterilization treatments beginning with swabbing the nodal explants with alcohol soaked cotton and pretreating with 1% Bavistin (50% carbendazin WP) for 15-20 minutes. Later on the explants were surface sterilized with 0.1 % $HgCl_2$ solution for 12-14 minutes followed by 3-4 times washing with sterilized distilled water to remove the traces of steriliant.

Negi *et al.* (2011) described an efficient technique of sterilization by giving the explants a quick rinse of 70% ethanol, washed with Teepol (4-5 drops) and four to five drops of a germicide called savlon for 15 min followed by thorough washing under running tap water for 20 min. The nodal segments were then surface sterilized with 0.1% mercuric chloride for 10 minutes.

Sharma *et al.* (2011) reported an efficient protocol for large scale productions of *Bambusabalcooa*. For sterilization, leaf sheath of nodal segment were removed, sized and were surface sterilized using 70% ethanol and then disinfected with 0.2% $HgCl_2$ solution for 5 minutes. The disinfected explants were washed thoroughly under running tap water containing 1-2 drops of Tween 20 solution and then with sterile distilled

water. Pretreatment of the explants were carried out with aqeous solution of 0.5% of Bavistin a fungicide and bacteriomycine for 15 minutes.

Beena *et al.* (2012) reported *in vitro* cloning in *Bambusapallida*initiating the sterilization technique by removal of the sheath and swabbing the surface of shoots with 70% (w/v) ethanol. The nodal segments were immersed in 0.01% (v/v) liquid detergent (Tween 80) for 5 min to remove dust and dirt particles. It was followed by 5-6 washes of distilled water. Explants were later dipped in 0.2% (w/v) Bavistin for 5 min followed by 4-5 times washing with distilled water. At the laminar the explants were surface sterilized using 70% ethanol (w/v) for 30s followed by 4-5 times wash of distilled water. Later it was sterilized using 0.075-0.1 (w/v) mercuric chloride for 5 min followed by 6-7 times thorough wash.

Shroti *et al.* (2012) reported the use of sodium hypochlorite (4%) for 20 minutes followed by three to four rinses with sterile distilled water for establishing culture of *Dendrocalamusasper.*

Pandey*et al.* (2012) reported micropropagation of *Dendrocalamusstrictus*from mature nodal explants. The explants were washed in 5% labolene solution with few drops of Tween-20 for 15 minutes and rinsed with distilled water. Further surface sterilization was done under laminar airflow by treating the explants with 0.2% $HgCl_2$ solution for 10, 15 and 20 minutes. It was then given a 30 second dip in 70% ethanol and again rinsed.

Jha *et al.* (2013) reported the use of 70 percent ethanol for surface sterilization followed by 0.1 per cent $HgCl_2$ for 5 minutes. Disinfected plants were washed thoroughly under running tap water containing 1-2 drops of Tween 20 solution. After wash it was then given a 30 second dip in 70% ethanol and again rinsed. Pretreatment was carried out with aqueous solution of 0.5 percent of Bavistin, a fungicide for 15 minutes.

Sharma *et al.* (2013) developed an efficient micropropagation protocol from nodal segments of *Bambusatulda.* Leaf sheath of nodal segment were removed, sized and were surface sterilized by using cotton swab dipped in 70 percent ethanol. They were then disinfected with 0.1 percent (v/v) $HgCl_2$ solution for 5 minutes. Then the explants were dipped in 5 percent (v/v) Tween 20 solution for 2 hour and then washed thoroughly under running tap water for 30 min. The explants were then rinsed with sterile distilled water. Pretreatment of the explants were carried out with aqueous solution of 0.5 percent of Bavistin, a systemic carbendazim fungicide and gentamycine for 15 minutes.

Waikhom *et al.* (2014) gave an efficient method of sterilization in which the explants sheath were carefully removed and wiped with 70% (v/v) ethanol using sterilized cotton and were surface sterilized in 0.1 % (v/v) solution of mercuric chloride for 15 minutes and washed 4 times in double distilled water, with each washing step lasting 5 minutes.

Kapruwan *et al.* (2014) reported in *Dendrocalamusstrictus,* explants were washed thoroughly under running tap water followed by Tween 20 solution for 5 min and then pre-sterilized in a mixture of fungicide viz. 1% (w/v) Bavistin and 1% (w/v) Blitox for 15 min. They were then disinfected for 10 to 15 min in 0.1% or 0.2% (w/v) mercuric chloride to get best aseptic cultures and washed in sterile distilled water four to five times. For initiation of aseptic cultures various concentrations of mercuric chloride for different time period was studied and it was found that 0.2% $HgCl_2$ was most effective.

Sawant*et al.* (2016) reported presoaking of explants in 0.2% Bavistin and 0.2% Streptomycin for 1 hr. followed by 0.1% $HgCl_2$ for 10 min. (Washingwith sterile double distilled water 3-4 times after each step) obtained highest percentage of asceptic culture of cultivars of *Pseudoxytenantherastocksii.*

4.2. Nodal explant

Prutpongse *et al.* (1992) studied *in vitro* propagation of 54 species of bamboo, of which nearly every species produced multiple shoots from axillary buds on stem node segments cultured on MS medium containing BA whereas a very few species could be regenerated adventitiously from callus and was not very efficient or reliable.

Ramanayake *et al.* (1997) reported micropropagation of *Dendrocalamusgiganteus*(giant bamboo) from nodal explants of field grown culms.

Ravikumar *et al.* (1998) reported *in vitro* propagation of *Dendrocalamusstrictus*induced from seedling and axillary buds of mature plants on MS medium supplemented with BA and kinetin.

Sanjaya *et al.* (2005) described an efficient reproducible procedure for large-scale propagation of *Pseudoxytenantherastocksii*from nodal shoot segments (2.0-3.0 mm diameter, 2.5-3.5 cm length) on Murashige and Skoog's liquid medium supplemented with NAA and BAP.

Diab *et al.* (2008) developed a protocol for micropropagation of *Oxytenantheraabyssinica* by culturing nodal explants from 12 months seedlings on MS medium supplemented with different concentrations of BA in combination with NAA.

Mudoi *et al.* (2009) described *in vitro* procedure for micropropagation of *Bambusabalcooa*from excised tender node (12-18 mm in length) containing axillary bud isolated from secondary branches of $1^1/_2$ year old culms, when implanted on MS medium containing BAP (1.0 mg/l).

Mehta *et al.* (2010) reported *in vitro* establishment of multiple shoots from nodal segments (3-4 cm) of young branches of mature culms were established in MS medium supplemented with various concentrations of BAP or in combination with NAA or kinetin. February-

March and December were found to be the best seasons for establishment of the cultures.

Bisht *et al.* (2010) reported *in vitro* regeneration of complete plantlets of *Gigantochloaatroviolaceae*through nodal explants.

Negi *et al.* (2011) described an efficient protocol for *in vitro* propagation of *Bambusanutans*through axillary shoot proliferation.

Sharma *et al.* (2011) reported an efficient protocol for large scale productions of *Bambusabalcooa*from nodal explants from field grown culms culturing on MS medium supplemented with auxins and cytokinins.

Shroti *et al.* (2012) developed an efficient and reproducible procedure for the large-scale propagation of *Dendrocalamusasper*through internodal segment (1-2 cm).

Beena *et al.* (2012) reported *in vitro* cloning in *Bambusapallida*from the nodal shoot segments of the field-grown candidate plus clump explants on MS liquid medium with additives.

Pandey*et al.* (2012) reported micropropagation of *Dendrocalamusstrictus*from mature nodal explants.

Jha *et al.* (2013) reported micropropagation of *Dendrocalamushamiltonii*through nodal explants on White's medium supplemented with BA and Kinetin.

Sharma *et al.* (2013) developed an efficient micropropagation protocol from nodal segments (1.5-2.0 cm in length) with internodal portion were excised from young lateral branches of main culm of 10 to 20 year old field grown culms of *Bambusatulda* when cultured on MS basal medium.

Waikhom *et al.* (2014) developed an effective protocol for micropropagation of *Bambusatulda* and *Melocannabaccifera* through nodal explant (1.5-2 cm) in MS medium supplemented with BAP (3 mg/l).

Kapruwan *et al.* (2014) reported rapid *in vitro* propagation of *Dendrocalamusstrictus*through axillary shoot proliferation (2.5 to 3.0 cm in length).

Sawant*et al.* (2016) reported micropropagation of four different bamboo species through axillary buds and apical buds (5-7cm).

4.3. Culture medium

The choice of correct medium formulation plays a decisive role in development of protocols for *in vitro* culture.

Prutpongse *et al.* (1992) studied *in vitro* propagation of 54 out of 67 species of bamboo tested were successfully propagated. Nodal sections containing an axillary bud were placed on media containing MS salts and vitamins supplemented with 88 µM sucrose, 6 g agar/litre, NAA (2.7, 5.4, or 10.8 µM) and BA (2.2, 4.4, 8.8, 22.0 or 44.0 µM).

Ramanayake *et al.* (1997) reported micropropagation of *Dendrocalamusgiganteus*(giant bamboo) from nodal explants of field grown culms. Cultures of axillary shoots were initiated during peak bud-break periods in a semi-solid MS medium with BAP 2 (mg/l), kinetin (0.1mg/l) and Benlate (benomyl) 1g/l.

Ravikumur *et al.* (1998) studied *in vitro* propagation of *Dendrocalamusstrictus*induced from seedling and axillary buds of mature plants on MS medium supplemented with BA and kinetin.

Sanjaya*et al*. (2005) worked on *in vitro* propagation on *Pseudoxytenantherastocksii* on MS liquid medium supplemented with NAA and BAP fortified with additives like ascorbic acid, citric acid, cysteine and glutamine.

Ndiaye *et al.* (2006) studied *in vitro* regeneration of adult *Bambusa vulgaris* by collecting 1 to 2 cm long nodal fragments that were tested on 4 basal medias, of which modified MS medium showed highest rate of

regeneration (100%) which was added with 30 g/l sucrose and various concentrations of BAP and kinetin. pH was adjusted to 5.5- 5.6.

Diab *et al.* (2008) developed a protocol for micropropagation of *Oxytenantheraabyssinica* by culturing nodal explants from 12 months seedlings on MS medium supplemented with 5.0 mgl^{-1} BA and 0.2 mgl^{-1} NAA achieving highest shoot multiplication (4.4/shoot/explants). Proliferation media in liquid status showed better performance than solid media.

Bisht *et al.* (2010) reported *in vitro* regeneration of complete plantlets of *Gigantochloaatroviolaceae*through nodal explants. Axillary bud break was accomplished in full strength liquid MS medium fortified with BAP (25.0 µM).

Negi *et al.* (2011) described an efficient protocol for *in vitro* propagation of *Bambusanutans*through axillary shoot proliferation. MS medium supplemented with BAP (4.4 µM) and kinetin (2.32 µM) gelled with 0.2% gelrite yielded 80% aseptic cultures with 100% bud break.

Sharma *et al.* (2011) reported an efficient protocol for large scale productions of *Bambusabalcooa*from nodal explants from field grown culms culturing on MS medium supplemented with 100 mg/l myo-inositol, sucrose (3%) and 2.5 percent Gelrite. The pH of the medium was adjusted to 6.0.

Singh *et al.* (2012) studied micropropagation in *Dendrocalamusasper*and found that MS media along with BAP (15 µM) yielded maximum frequency of shoot initiation (4.83/explant).

Beena *et al.* (2012) reported *in vitro* cloning in *Bambusapallida* from the nodal shoot segments of the field-grown candidate plus clumps. Explants were inoculated on MS liquid medium with additives like ascorbic acid (50 mg/l) + citric acid (25 mg/l) + cysteine (25 mg/l) and

combined use of NAA (1.34 µM) and thidiazuron (1.125 µM) in two weeks.

Shroti *et al.* (2012) developed an efficient and reproducible procedure for the large-scale propagation of *Dendrocalamusasper*through internodal segment (1-2 cm) using modified MS medium supplemented with BAP (0.5 mg/l).

Pandey *et al.* (2012) reported micropropagation of *Dendrocalamusstrictus*from mature nodal explants. In solidified MS basal medium with BAP (2 mg/l).

Jha *et al.* (2013) reported micropropagation of *Dendrocalamushamiltonii*through nodal explants on White's medium supplemented with BA and Kinetin among which BA (0.50 mg/l, 1.0 mg/l) was a suitable growth hormone for shoot-induction.

Waikhom *et al.* (2014) developed an effective protocol for micropropagation of *Bambusatulda*and *Melocannabaccifera* through nodal explant in MS medium supplemented with BAP (3 mg/l), myo-inositol 100 mg/l and sucrose 30 g/l. pH 5.7.

Kapruwan *et al.* (2014) reported rapid *in vitro* propagation of *Dendrocalamusstrictus*through axillary shoot proliferation. The axillary shoots containing single axillary bud were inoculated in semisolid MS medium fortified with different concentrations of growth regulator, BAP

Sawant*et al.* (2016) reported micropropagation of *Pseudoxytenantherastocksii*in MS medium fortified with BAP (1 mg/l), NAA (0.5 mg/l), adenine sulphate (80 mg/l), ascorbic acid (50 mg/l), citric acid (25 mg/l) and cystiene (25 mg/l) (liquid).

4.4. Shoot initiation and shoot multiplication

Prutpongse *et al.* (1992) studied *in vitro* propagation of 54 species of bamboo, in which most species produced multiple shoots form the axillary bud on a stem node from young shoots when placed on medium

containing BA (22 µM). Other cytokinins including kinetin were inferior to BA in inducing proliferation whereas addition of auxins (NAA) had little effect on the multiplication rate in most of the species. Propagules containing minimum three shoots proliferated at a maximum rate whereas propagules containing single shoot did proliferate but at a much slower rate.

Ramanayake *et al.* (1997) reported micropropagation of *Dendrocalamusgiganteus*(giant bamboo) from nodal explants of field grown culms. Cultures of axillary shoots were initiated during peak bud-break periods in a semi-solid MS medium with BAP (2 mg/l), kinetin (0.1 mg/l) and 1g/l Benlate (benomyl). Continuous shoot proliferation for a period on 1 year was achieved in a liquid MS medium with BAP (6 mg/l), kinetin (0.1 mg/l) and 38% (v/v) coconut water.

Ravikumur *et al.* (1998) reported *in vitro* propagation of *Dendrocalamusstrictu*s induced from seedling and axillary buds of mature plants on MS medium supplemented with BA (0.25 to 2.0 mg/l) and coconut water (200ml/l) with or without kinetin (0.5 to 1.0 mg/l).

Sanjaya *et al.* (2005) suggested the best treatment to induce shoot multiplication was the combination of MS liquid medium fortified with NAA (2.68 µM) and BAP (4.40 µM).

Ndiaye*et al.* (2006) studied *in vitro* regeneration of adult *Bambusa vulgaris.* Optimum phytohormones for shoot multiplication were found to be BAP (2 mg/l) whereas kinetin had no significant influence. Same medium was optimum for shoot elongation with 56mm after 16 days.

Diab *et al.* (2008) developed a protocol for micropropagation of *Oxytenantheraabyssinica* by culturing nodal explants from 12 months seedlings on MS medium supplemented with 5.0 mgl^{-1} BA and 0.2 mgl^{-1} NAA achieving highest shoot multiplication (4.4 shoot/explants). Among MS basal media (full, half, quarter) strength, MS full strength achieved highest shoot length (7.6 cm).

Mudoi *et al.* (2009) described *in vitro* procedure for micropropagation of *Bambusabalcooa*from excised tender node (12-18 mm in length) containing axillary bud isolated from secondary branches of $1^{1}/_{2}$ year old culms, when implanted on MS medium containing BAP (1.0 mg/l). Continuous shoot proliferation, tenfold, every 4 weeks was achieved by sub-culturing shoot clumps (2-3 shoots/cluster) in BAP (1.0 mg/l) fortified medium.

Bisht *et al.* (2010) reported *in vitro* regeneration of complete plantlets of *Gigantochloaatroviolaceae*through nodal explants. Axillary bud break was accomplished in full strength liquid MS medium fortified with BAP (25.0 µM). Axillary shoots produced were multiplied on semi-solid MS medium supplemented with BAP (20 µM) + NAA (3.0 µM) giving a multiplication rate of 2.39.

Negi *et al.* (2011) described an efficient protocol for *in vitro* propagation of *Bambusanutans*through axillary shoot proliferation. MS medium supplemented with BAP (4.4 µM) and kinetin (2.32 µM) was used for initiation and was successfully multiplied in MS liquid medium supplemented with BA (13.2 µM), Kin (2.32 µM) and IBA (0.98 µM) giving 3.5 fold proliferation.

Sharma *et al.* (2011) reported an efficient protocol for large scale productions of *Bambusabalcooa*from nodal explants from field grown culms culturing on MS medium supplemented with auxins and cytokinins. Found *in vitro* axillary shoot formation successful in MS basal medium supplemented with 6-benzyle adenine (1.0 mg/l and 1.5 mg/l).

Beena *et al.* (2012) reported *in vitro* cloning in *Bambusapallida*from the nodal shoot segments (2.5-3.5 cm long and 3.4 mm wide) with dormant buds of the field-grown candidate plus clump explants on MS liquid medium with additives. Further shoot multiplication was achieved in MS liquid medium with additives + NAA (1.34 µM) + BAP (4.4 µM).

Shroti *et al.* (2012) developed an efficient and reproducible procedure for the large-scale propagation of *Dendrocalamusasper*through internodal segment (1-2 cm) using modified MS medium supplemented with BAP (0.5 mg/l). A multiplication rate of 15-16 fold was achieved on MS medium + BA (2.0 mg/l).

Singh *et al.* (2012) studied micropropagation in *Dendrocalamusasper*and found that MS media supplemented with 10 µM BAP and 75 µM Adenine sulphate to be optimum use. It was also reported that BAP was superior to Kin for both explants establishment, as well as, shoot multiplication.

Pandey*et al.* (2012) reported micropropagation of *Dendrocalamusstrictus*from mature nodal explants in solidified MS basal medium with BAP (2 mg/l). The buds, which started growing, were transferred to solidified MS basal medium with BAP (4 mg/l) and Ads (15 mg/l) to achieve about three fold multiplication.

Sharma *et al.* (2013) developed an efficient micropropagation protocol from nodal segments (1.5- 2.0 cm in length) with internodal portion were excised from young lateral branches of main culm of 10 to 20 year old field grown clumps of *Bambusatulda* when cultured on MS basal medium supplemented with BAP (1.0 mg/l).

Jha *et al.* (2013) reported micropropagation of *Dendrocalamushamiltonii*through nodal explants on White's medium supplemented with BA and Kinetin among which BA (0.50 mg/l, 1.0 mg/l) was a suitable growth hormone for shoot-induction. Shoot multiplication rate was maximum in corporation with BA (2.00 mg/l) and maximum shoot length with the use of BA (1.50 mg/l).

Waikhom *et al.* (2014) developed an effective protocol for micropropagation of *Bambusatulda*and *Melocannabaccifera* through nodal explant in MS medium supplemented with BAP (3 mg/l).

Combining kinetin (2 mg/l) with BAP (3 mg/l) produced a synergistic effect for shoot multiplication.

Kapruwan *et al.* (2014) reported rapid *in vitro* propagation of *Dendrocalamusstrictus*through axillary shoot proliferation. Maximum bud break and multiple shoot formation were observed in BAP (5mg/l).

Sawant*et al.* (2016) developed an effective protocol for *in vitro* regeneration *Pseudoxytenantherastocksii* in MS medium fortified with BAP (1 mg/l), NAA (0.5 mg/l), adenine sulphate (80 mg/l), ascorbic acid (50 mg/l), citric acid (25 mg/l) and cystiene (25 mg/l) (liquid) and achieved 33.3% response rate.

4.5. Root induction

Prutpongse *et al.* (1992) studied *in vitro* propagation of 54 species of bamboo, in which rooting occurred in media containing NAA (2.7 µM to 5.4 µM). rooting experiments were conducted with full-strength, half-strength and quarter-strength media supplemented with NAA (13.5, 27.0, or 54.0 µM), BA (0.44, 4.4, or 8.8 µM) or IBA (2.5,10.0, 12.5, or 25.0 µM) . Multiple shoot development occurred within 30 days, and roots appeared after 2 to 3 weeks.

Ramanayake *et al.* (1997) reported micropropagation of *Dendrocalamusgiganteus*(giant bamboo) from nodal explants of field grown clumps. Initiated shoots were rooted when transferred from the shoot proliferation medium containing IBA (3 mg/l) in the last two passages, to a rooting medium with MS modified to half strength major salts with IBA (3 mg/l) and coumarin (10 mg/l).

Sanjaya *et al.* (2005) found the best results of root induction on half-strength MS basal liquid medium supplemented with IBA(4.90 µM), BAP (0.44 µM) and additives.

Ndiaye *et al.* (2006) studied *in vitro* regeneration of adult *Bambusa vulgaris.* Explants from adult trees present difficulties for rooting;

addition of 5 mg/l of auxin did not induce rooting which was achieved after using high concentration of IBA (20 mg/l). There was no significant difference found between the auxins (IBA, NAA) on the nature of the roots.

Diab *et al.* (2008) developed a protocol for micropropagation of *Oxytenantheraabyssinica.* When the propagated shoots were transferred to a rooting medium (full, half, quarter strength) MS, supplemented with IBA (0, 0.5, 1.0, 2.0, 4.0, 8.0 mg L^{-1}) concentrations, the highest percentage (70%) was achieved in full strength MS media supplemented with IBA (8.0 mg/l).

Mudoi *et al.* (2009) described *in vitro* procedure for micropropagation of *Bambusabalcooa.* Seventy-five percent of shoots were rooted efficiently on excised propagules when transferred to MS medium supplemented with BAP (1.0 mg/l) and NAA (3.0 mg/l).

Bisht *et al.* (2010) reported *in vitro* regeneration of complete plantlets of *Gigantochloaatroviolaceae*through nodal explants. *In vitro* shoots were rooted on full strength MS medium supplemented with varying concentrations of auxins. Optimal rooting was achieved on medium supplemented with IBA (35.0 µM).

Negi *et al.* (2011) described an efficient protocol for *in vitro* propagation of *Bambusanutans*through axillary shoot proliferation. Shoot clumps containing three to five shoots were successfully rooted with 100% success on half-strength MS liquid medium supplemented with IBA (9.8 µM), IAA (2.85 µM), NAA (2.68 µM) and 3% sucrose.

Sharma *et al.* (2011) reported an efficient protocol for large scale production of *Bambusabalcooa.* Clumps of at least 3 shoots were used for root induction in MS medium with NAA (3.5 and 4.0 mg/l). Successful acclimatization and 100 per cent survival rate after field transfer was achieved.

Singh *et al.* (2012) studied micropropagation in *Dendrocalamusasper*and achieved optimal rooting in shoots cultured on $^{1}/_{2}$ strength MS medium supplemented with IBA and NAA (5 µM each) and achieved 92.34% success in hardening and 100% survival rate in the field.

Beena*et al.* (2012) reported *in vitro* cloning in *Bambusapallida.* Shoots were rooted within four weeks in MS $^{1}/_{2}$ basal medium with additives + 2% sucrose +1 % glucose, and 0.6% agar by pulse treatment of shoots with IBA (0.5 mg/ml) for 30 min. 95% survival after hardening.

Pandey*et al.* (2012) reported micropropagation of *Dendrocalamusstrictus*from mature nodal explants. Among 1, 3 and 5 mg/l of IAA, IBA and NAA, only 20% rooting was achieved in IBA (5 mg/l).

Sharma *et al.* (2013) developed an efficient micropropagation protocol for *Bambusatulda*. Clumps with minimum 3 shoots were used for root induction in MS medium with IAA, IBA and NAA in which response was found more in NAA (5.0 mg/l).

Waikhom *et al.* (2014) developed an effective protocol for micropropagation of *Bambusatulda*. Under optimized conditions in half-strength MS medium supplemented with IBA (3 mg/l), coumarin (10 mg/l) and 3 % sucrose, profuse production of dark-brown rhizome were achieved.

Kapruwan *et al.* (2014) reported rapid *in vitro* propagation of *Dendrocalamusstrictus*through axillary shoot proliferation. A maximum of 100% shoots were effectively rooted when transferred to MS liquid medium supplemented with BAP (2.5mg/l) and IAA (5mg/l). Effect of Phenyl-N'-(1,2,3-thiadiazol-5-yl) was also studied. Increase in TDZ concentration had negative impact on bud break and multiple shoot formation.

Sawant*et al.* (2016) developed an effective protocol for *in vitro* regeneration *Pseudoxytenantherastocksii* in which rooting was reported reported in $^{1}/_{4}$ MS medium supplemented with high concentration of NAA (2.5 mg/l) in 15-20 days with 2-3 roots per explant.

CHAPTER III
MATERIALS AND METHODS

The present investigation entitled “Studies on micropropagation of *Dendrocalamusstocksii*(Munro.) through nodal explants” was carried out in tissue culture laboratory of Plant Biotechnology Centre, Dr. BalasahebSawantKonkanKrishiVidyapeeth, Dapoli, Dist. Ratnagiri (M. S.) during the academic year 2014-2017.

5.1. MATERIALS

5.1.1. Explant

The present investigation was carried out with a species of Bamboo, *Dendrocalamusstocksii*. Nodal shoot segments sized 2.5-3.0 cm in length and 0.25-0.35 mm in diameter was taken as explant. The experimental material for the present investigation was collected from healthy clumps of the Germplasm bank and nursery of College of Forestry, Dapoli, Dist. Ratnagiri (Plate no.1).

5.1.2. Chemicals

The details of the various laboratory chemicals and biochemicals used in the present investigation for media preparation, surface sterilization of the explants etc., are given below:

a) Chemicals for surface sterilization

1) 70% ethyl alcohol
2) Tween 20/ Tween 80
3) Carbendazin
4) Sodium hypochlorite
5) Mercuric chloride
6) Cefotaxime

b) Chemicals for media preparation:

1) Salts of macro and micro elements of analytical grade
2) Vitamins and amino acids

3) Sucrose and myo-inositol as a carbon source
4) Anti-oxidizing agents
5) Agar agar as a gelling agent
6) 1N HCl and 0.5N NaOH for adjusting pH

c) Growth regulators

1) Auxins: NAA and IBA
2) Cytokinins: BAP, Kinetin and TDZ

5.1.3. Culture vessels and Culture apparatus

a) Conical flasks of capacity 100, 250, 500 and 1000 ml
b) Beakers of capacity 100, 250, 500 and 1000 ml
c) Petri plates of size 100 x 20 mm
d) Pipettes of capacity 5 and 10 ml
e) Micropipettes of capacity 20-100 µl and 200-1000 µl
f) Measuring cylinders of capacity 5, 100, 250, 500 and 1000 ml
g) Test tubes with cotton plugs
h) Culture bottles with polyurethane polyvinyl cap

All the culture vessels and culture apparatus were first soaked in detergent solution (Teepol 0.1%) overnight and were thoroughly washed under running tap water, then rinsed twice in double distilled water (DDW). They were then dried in an oven at 105^0 C. The petri plates and other glasswares were wrapped in plastic bags and then autoclaved at 15 lbs/ in^2 pressure for 20 minutes. Sterilized containers were later on stored in dust proof room for further use.

5.1.4. Laboratory Equipments

- Refrigerator – for storing stock solutions and plant growth regulator
- Hot air oven – drying glass wares

- Double distillation unit – obtaining sterilized water
- Electronic Digital balance – accurate weighing of chemicals
- Hot plate magnetic stirrer – mixing media constituents
- pH meter – testing pH of solutions
- Horizontal autoclave – autoclaving glass wares, ddw and nutrient media
- Laminar air flow bench – aseptic inoculation
- Glass bead sterilizer – sterilizing forceps and scalpels
- Incubator – maintenance of temperature and light
- Shaker

5.1.5. Others

Trays, caps, bags, para film, aluminum foil, non-absorbent cotton, absorbent cotton, spirit lamp or gas burner, forceps, scalpels, surgical blades and secateurs are needed for maintaining the aseptic culture conditions.

5.1.6. Experimental Conditions

All *in vitro* studies were carried out aseptically in laminar air flow cabinet. The experiments were conducted under well-defined conditions of culture room maintained at 25± 2^{o}C temperature, uniform light (2500 Lux) provided by fluorescent tubes (7200 K) over a light and dark cycle of 12 hours photoperiod.

5.1.7. Culture medium

The basal medium developed by Murashige and Skoog (1962) was used with certain addition of various concentration and combinations of plant growth regulators. The concentrations of different nutrients per litre of nutrient medium are given in Table 1.

Table No. 1: Composition of Murashige and Skoog medium with certain additives.

Name of stock solution	ChemicalCompositi	Quantity in mg/l	Quantity in g/l of stock solution	Strength of stock solution	Volume to be be taken for1lt ofmedia in ml
A	NH_4NO_3	1650	82.5	50X	20
B	KNO_3	1900	95.0	50X	20
C	KH_2PO_4	170	34.0	200X	5
	H_3BO_4	6.20	1.24		
	Na_2Mo_4. $2H_2O$	0.25	0.05		
	KI	0.83	0.166		
	$CoCl_2$. $6H_2O$	0.025	0.005		
D	$CaCl_2.2H_2O$	440	88.0	200X	5
E	$MgSO_4$. $7H_2O$	370	74.0	200X	5
	$MnSO_4$. $4H_2O$	16.90	3.38		
	$ZnSO_4.7H_2O$	8.60	1.72		
	$CuSO_4$. $5H_2O$	0.025	0.005		
F	$FeSO_4$. $7H_2O$	27.80	5.50	200X	5
	Na_2EDTA. H_2O	37.30	7.46		
Vitamins	Thiamine. HCl	0.5	1.0	2000X	0.5
	Pyridoxine. HCl	0.5	1.0	2000X	0.5
	Nicotinic acid	0.5	1.0	2000X	0.5
Amino Acid	Glycine	2.0	1.0	500X	2.0
Carbohydrate Source	Myo-inositol	100			30
	Sucrose		30		6
Gellingagent	Agar agar		6		
Additives	Ascorbic acid	50			
	Citric acid	25			
	Cystein	25			
	Glutamine	100-250			
Plant growth regulators	Auxins	Requisite amount			
	Cytokinins	Requisite amount			

5.2 METHODS

5.2.1. Preparation of stock solutions

At the time of media preparation, it is practically not possible to weigh each of the constituent chemicals added in the medium. Hence, for the sake of convenience, concentrated stock solutions of basal MS medium containing different stock solutions were prepared in sterilized water, stored in borosil reagent bottles and kept in refrigerator at 5-7^{0}C temperature.

1. Major salts (50X concentrated)

2. Minor salts (200X concentrated)

To make 1000ml of stock solution, weighed amount of required salts grouped as A to F were dissolved in 800ml of double distilled water and then made up the volume. The solution was filtered and labeled separately and stored in refrigerator at 5^{0}C.

3. Iron (200X concentrated)

Stock F was prepared by dissolving Na_2EDTA and $FeSO_4.7H_2O$ in separate containers with measured volume of half distilled water. $FeSO_4.7H_2O$ solution was slowly poured into Na_2EDTA solution with continuous stirring at 60^{0}C temperature to ensure proper mixing of Na_2EDTA as it functions as a chelating agent to $FeSO_4.7H_2O$. Stock F should always be stored in amber colour bottle at 5^{0}C.

5.2.1.1. Salts and Vitamins

The solutions of major and minor inorganic salts of MS medium were prepared as recommended and stored in a refrigerator. Similarly, vitamin stocks of MS were also prepared and stored in the refrigerator at 10^0 C until needed.

5.2.1.2. Auxins

Auxins like NAA and IBA were dissolved in 70% ethanol or 0.5M solution of NaOH (0.5-1.0). All auxin stock solutions were prepared for 1000 ppm (1mg/ml).

5.2.1.3. Cytokinins

Kinetin and BAP were first dissolved in 0.1N HCl (0.5-0.1 ml solution) and final 1000 ppm (1 mg/ml) strength was maintained. The quantity of cytokinin in stock solution was adjusted such that each ml of stock contained 1mg of cytokinin. In case of TDZ, it was dissolved in KOH.

5.2.2 Preparation of culture media

For preparing media, a balance sensitive to milligram quantities was used for weighing hormones and vitamins and a less sensitive balance was used for weighing agar and carbohydrates.

For preparing media for multiplication and rooting, 6g agar was added to the medium.

A combination of hot plate and magnetic stirrer was used for dissolving media stocks, PGRs, vitamins, etc. pH meter was used for adjusting the final pH of media.

All the contents of media were added to sterilized water and the pH was adjusted to 6.2 using 1N HCl and 0.5N NaOH.

The medium was poured in culture containers (bottles/flasks/ test tubes) and autoclaved using horizontal steam sterilizer at 121^0C and 15 psi for 20 min. Culture bottles were allowed to solidify at room temperature and stored in dust proof room for at least 1 day before use, to check for any contamination.

5.2.3. Aseptic techniques

The standard sterilization technique was followed as suggested by Street (1977) for inoculation and sub culturing of explants in culture

bottles. Inoculation of explants was carried out under aseptic conditions in laminar airflow bench. Laminar airflow bench was sterilized by introducing to UV light for 20 minutes and use of 70% ethanol to maintain sterile conditions while working. During the course of transfer of explants, all surgical instruments were sterilized in glass bead sterilizer.

5.2.4. Preparation of explant for culturing

Processing and surface sterilization of explant

Explants (nodal shoot segments) were collected from mature clump and one year old healthy culm, wiped with 70% alcohol and stored in ice box while transporting. The sheath enclosing the bud within was carefully removed. The explants were trimmed using stainless secateurs until the length of explant was about 3 to 4 cm. The explants were processed using bavistin and surface sterilized with different chemicals like 70% ethanol, $HgCl_2$, cefotaxime, sodium hypochlorite, etc. in different concentrations and for different time period (Table 2). Number of replicates for each treatment was 3 and each replicate consisted 5 explants. The data was recorded at the end of 4th week.

Table 2: Various treatments of surface sterilizing agents

Tr. no.	Treatment details	Time (min)
T_1	Control (DDW washing)	20
T_2	2% Tween 20	20
T_3	2% Bavistin	10
T_4	0.2 % $HgCl_2$	10
T_5	70% alcohol	1
T_6	1%Tween 20 + 0.1%bavistin + 1%sodium hypochlorite + 250mg/l cefotaxime	5+10+5+15
T_7	1%Tween 20 + 0.1%bavistin + 1%sodium hypochlorite + 250mg/l cefotaxime	5+15+15+30
T_8	1%Tween 20 + 1%bavistin + 1%sodium hypochlorite + 250mg/l cefotaxime	5+20+20+45
T_9	1%Tween 20 + 1%bavistin + 1%sodium hypochlorite + 250mg/l cefotaxime	5+20+20+60

T_{10}	1%Tween 20 + 1%bavistin + 1%sodium hypochlorite + 250mg/l cefotaxime	5+30+20+60
T_{11}	1%Tween 20 + 1%bavistin + 70% alcohol + 0.1% mercuric chlorite + 250mg/l cefotaxime	$5+20+^{1}/_{2}+20+45$
T_{12}	1%Tween 20 + 0.1%bavistin + 70% alcohol + 1%sodium hypochlorite	$5+15+^{1}/_{2}+5$
T_{13}	1%Tween 80 + 0.1%bavistin + 70% alcohol + 0.5%mercuric chloride	$5+10+^{1}/_{2}+5$

Observations recorded:

1) Number of Days for bud break
2) Per cent aseptic cultures

5.2.5. Inoculation of explants for *in vitro* multiple shoot induction

The nodal explants were inoculated in test tubes containing 10 ml of MS liquid medium supplemented with additives (ascorbic acid 50mg/l, citric acid 25mg/l, cysteine 25mg/l and glutamine 100mg/l) and suitable growth hormones by aseptic techniques. The cultures were incubated in the culture room for a period of 30 days while maintaining the media content by adding new media in the same cultures each week for a period of one month. Number of replicates for each treatment was 3 and each replicate consisted of 4 explants. The data was recorded at the end of 3 weeks.

To study the effect of PGRs on shoot initiation various concentration and -combinations of cytokinins were used (Table 3).

Table 3: *In vitro* shoot induction in various levels of growth regulators

Tr. No.	Composition of media
T_1	HF MS medium (control)
T_2	MS + add + IBA 1.0 mg/l
T_3	MS + add + IBA 2.5 mg/l
T_4	MS + add + NAA 1.0 mg/l
T_5	MS + add + NAA 2.5 mg/l
T_6	MS + add + Kin 1.0 mg/l + IBA 0.5 mg/l
T_7	MS + add + Kin 2.0 mg/l + IBA 0.5 mg/l
T_8	MS + add + Kin 3.0 mg/l + IBA 0.5 mg/l
T_9	MS + add + Kin 4.0 mg/l + IBA 0.5 mg/l
T_{10}	MS + add + BAP 1.0 mg/l + IBA 0.5 mg/l
T_{11}	MS + add + BAP 2.0 mg/l + IBA 0.5 mg/l

T_{12}	MS + add + BAP 3.0 mg/l + IBA 0.5 mg/l
T_{13}	MS + add + BAP 4.0 mg/l + IBA 0.5 mg/l
T_{14}	MS + add + BAP 1.0 mg/l + NAA 0.25 mg/l
T_{15}	MS + add + BAP 2.5 mg/l + NAA 0.25 mg/l
T_{16}	MS + add + TDZ 0.1 mg/l + NAA 0.25 mg/l
T_{17}	MS + add + TDZ 0.25 mg/l + NAA 0.25 mg/l
T_{18}	MS + add + Kin 1.0 mg/l + NAA 0.25 mg/l
T_{19}	MS + add + Kin 2.5 mg/l + NAA 0.25 mg/l

Observations recorded:

1) Number of days required for shoot initiation
2) Per cent response
3) Number of shoots per explant
4) Average length of shoots per explant after one month

5.2.6. Inoculation of initiated shoots for *in vitro* shoot multiplication

Explants with initiated shoots were transferred to the different multiplication medium (Table 4) in bottles after 28 days. For multiplication, MS basal medium supplemented with additive (ascorbic acid 50mg/l, citric acid 25mg/l, cysteine 25mg/l and glutamine 100mg/l) and plant growth regulators with different concentrations were tested. Shoot clumps were detached from the explant when the shoots had completely used stored nutrients from the explant and were capable for intake of nutrients from the nutrient medium on its own. Sub culturing was carried out every 1-2 weeks of interval for 2-3 months. During every subculture, the dried leaves, detoriated cells/material, shoots and sheath were removed, encouraging the underneath buds with space to grow. Number of replicates for each treatment was 3 and each replicate consisted of 4 explants. Data was recorded at the end of 8^{th} week.

Table 4: Effect of plant growth regulators on shoot multiplication

Tr. No.	Treatments (PGRs, mg/l)
T1	MS + add + BAP 1.0 mg/l + NAA 0.25 mg/l
T2	MS + add + BAP 2.5 mg/l + NAA 0.25 mg/l

T3	MS + add + TDZ 0.1 mg/l + NAA 0.25 mg/l
T4	MS + add + TDZ 0.25 mg/l + NAA 0.25 mg/l
T5	MS + add + Kin 1.0 mg/l + NAA 0.25 mg/l
T6	MS + add + Kin 2.5 mg/l + NAA 0.25 mg/l

Observations recorded:

1) Number of new shoots emerged
2) Length of shoots

5.2.7. Inoculation of established shoot clumps for *in vitro* root induction

For induction of roots, clumps with 2-3 shoots were inoculated on different rooting media combinations (Table 5). Number of replicates for each treatment was 3 and each replicate consisted of 4 explants and the data was recorded at the end of 1 month.

Table 5: *In vitro* induction of roots on various levels of growth regulators

Tr. No.	**Composition of media**
T_1	HF (Control)
T_2	MS/2 + NAA 0.5 mg/l
T_3	MS/2 + NAA 1.0 mg/l
T_4	MS/2 + NAA 1.5 mg/l
T_5	MS/2 + NAA 2.0 mg/l
T_6	MS/2 + NAA 2.5 mg/l

Observations recorded:

1) Per cent response
2) Number of roots emerged
3) Length of roots

5.2.9 Statistical analysis:

As all the studies were done in laboratory under well-defined aseptic conditions of the medium, growth, temperature, and light, Factorial Completely Randomized Design (FCRD) as described by Panse and Sukhatme (1995) was employed for the experiment and the data was analyzed using SAS 9.3 (Statistical Analysis System V 9.3).

CHAPTER IV
RESULTS

6.1. Surface sterilization technique for nodal explants

The results obtained from the surface sterilization treatments for *D. stocksii*are given in the Table 6.

Explants used throughout this experiment were excised from mature clumps of *D. stocksii* from Germplasm bank, College of Forestry, Dapoli. Contamination rate is often high when the explants are excised from field conditions. To reduce the incidence of fungal and bacterial contamination, various sterilization treatments were used.

Out of the various treatments tested, T_{13} (1%Tween 80 for 5 minutes + 0.1%bavistin for 10 minutes + 70% alcohol for 30 seconds + 0.5%mercuric chloride for 5 minutes) recorded maximum (100 %) aseptic culture establishment with 100% survival rate followed by treatment T_{12} (1%Tween 20 for 5 minutes + 0.1%bavistin for 15 minutes + 70% alcohol for 30 seconds + 1%sodium hypochlorite for 10 minutes) with 86.67% success rate. From the results recorded, it is evident that, production of aseptic cultures ranged from 0 to 100 per cent (Plate no.2 and Fig. 1).

Table 6: Effect of surface sterilizing agents on aseptic culture establishment of *D. stocksii*nodal explant

Tr. no.	Treatment details	Time (min)	Per cent survivability	Per cent aseptic culture
T_1	Control (DDW washing)	20	100.00	0.00
T_2	2% Tween 20	20	100.00	0.00
T_3	1% Bavistin	10	100.00	0.00
T_4	0.2 % $HgCl_2$	10	100.00	0.00
T_5	70% alcohol	1	100.00	0.00
T_6	1%Tween 20+ 0.1%bavistin+ 1%sodium hypochlorite+ 250mg/l cefotaxime	5+10+5+15	85.00	13.33
T_7	1%Tween 20+ 0.1%bavistin+ 1%sodium hypochlorite+ 250mg/l cefotaxime	5+15+15+30	75.00	26.67
T_8	1%Tween 20+ 1%bavistin+ 1%sodium hypochlorite+ 250mg/l cefotaxime	5+20+20+45	75.00	46.67
T_9	1%Tween 20+ 1%bavistin+ 1%sodium hypochlorite+ 250mg/l cefotaxime	5+20+20+60	65.00	66.67
T_{10}	1%Tween 20+ 1%bavistin+ 1%sodium hypochlorite+ 250mg/l cefotaxime	5+30+20+60	55.00	73.33
T_{11}	1%Tween 20+ 1%bavistin+ 70% alcohol+ 0.1% mercuric chlorite + 250mg/l cefotaxime	$5+20+{}^{1}/_{2}+20+45$	70.00	80.00
T_{12}	1%Tween 20+ 0.1%bavistin+ 70% alcohol+ 1%sodium hypochlorite	$5+15+{}^{1}/_{2}+10$	90.00	86.67

T_{13}	1%Tween 80 + 0.1%bavistin + 70% alcohol + 0.5%mercuric chloride	$5+10+^{1}/_{2}+5$	**100.00**	**100.00**
	SE (m)±	0.26		
	CD (at 1%)	1.04		

Among all the 13 treatments, all sterilizing agents when used individually showed no control over fungal and bacterial contamination the same was followed by combination treatment of 1%Tween 20 + 0.1%bavistin + 1%sodium hypochlorite + 250mg/l cefotaxime (T_6) which also reduced the survival rate with increased control over contamination.

6.2. Effect of media combination on shoot initiation

According to the previous studies, MS media was found to be best responding in *in vitro* regeneration of bamboos. Thus, all the following studies on *D. stocksii* were undertaken keeping MS media constant with additives (ascorbic acid 50 mg/l + citric acid 25 mg/l + cysteine 25 mg/l + glutamine 100 mg/l).

6.2.1. Effect of plant growth regulators on shoot initiation response

Different response in shoot initiation was observed in 19 different treatments of PGRs (Table 7, Plate no.3 and Fig. 2). Breaking of nodal buds and sprouting of shoot depends on the thickness of explant, tenderness of explant, season of the year and culture conditions.

Number of days required for shoot initiation ranged from 2 to 14 days. The response of nodal shoot segments to the various combinations of shoot initiation is given in table 7.

Treatment T10 to T17 showed 100% bud break which indicates that cytokinins BAP and TDZ along with small amount of auxins, either IBA or NAA is best treatment for shoot induction in nodal shoot segments of *D. stocksii*. Whereas, good response (91.7%)was observed in controlled

conditions. Comparatively, kinetin showed less (66.7-91.7%) response rate.

Auxins (IBA&NAA) when used individually showed least (33.3 and 50% respectively) response rate.

Table 7: Effect of plant growth regulators on of frequency shoot initiation

Tr. No.	Treatments (PGRs, mg/l)	Frequency (%)
T1	HF MS (Control)	91.67
T2	MS + add + IBA 1.0 mg/l	58.33
T3	MS + add + IBA 2.5 mg/l	66.67
T4	MS + add + NAA 1.0 mg/l	66.67
T5	MS + add + NAA 2.5 mg/l	66.67
T6	MS + add + Kin 1.0 mg/l + IBA 0.5 mg/l	66.67
T7	MS + add + Kin 2.0 mg/l + IBA 0.5 mg/l	75.00
T8	MS + add + Kin 3.0 mg/l + IBA 0.5 mg/l	83.33
T9	MS + add + Kin 4.0 mg/l + IBA 0.5 mg/l	91.67
T10	MS + add + BAP 1.0 mg/l + IBA 0.5 mg/l	100.00
T11	MS + add + BAP 2.0 mg/l + IBA 0.5 mg/l	100.00
T12	MS + add + BAP 3.0 mg/l + IBA 0.5 mg/l	100.00
T13	MS + add + BAP 4.0 mg/l + IBA 0.5 mg/l	100.00
T14	MS + add + BAP 1.0 mg/l + NAA 0.25 mg/l	100.00
T15	MS + add + BAP 2.5 mg/l + NAA 0.25 mg/l	100.00
T16	MS + add + TDZ 0.1 mg/l + NAA 0.25 mg/l	100.00
T17	MS + add + TDZ 0.25 mg/l + NAA 0.25 mg/l	100.00
T18	MS + add + Kin 1.0 mg/l + NAA 0.25 mg/l	83.33
T19	MS + add + Kin 2.5 mg/l + NAA 0.25 mg/l	83.33
SE (m)±		0.33
CD (at 1%)		1.34

6.2.2. Effect of plant growth regulators on number of shoots

The results showed statistically significant effect of treatments on shoot initiation rate (Table 8, Plate no.3 and Fig. 3). Among the three cytokinins: BAP, TDZ and Kinetin used in different concentrations with two different auxins, TDZ, a high potent cytokinin showed better response in terms of multiple shoot induction.

Among the 19 concentrations of various plant growth regulators tested for multiple shoot initiation from the nodal shoot segments, MS liquid medium fortified with additives: ascorbic acid (50 mg/l) + citric acid (25 mg/l) + cysteine (25 mg/l) + glutamine (100mg/l)TDZ (0.25 mg/l) + NAA (0.25 mg/l), proved the best treatment (4.25 shoots/explant).

Table 8: Effect of plant growth regulators on number of shoots

Tr. No.	Treatments (PGRs, mg/l)	Number of shoots
T1	HF MS (Control)	2.83
T2	MS + add + IBA 1.0 mg/l	2.50
T3	MS + add + IBA 2.5 mg/l	2.07
T4	MS + add + NAA 1.0 mg/l	2.70
T5	MS + add + NAA 2.5 mg/l	2.42
T6	MS + add + Kin 1.0 mg/l + IBA 0.5 mg/l	2.63
T7	MS + add + Kin 2.0 mg/l + IBA 0.5 mg/l	2.64
T8	MS + add + Kin 3.0 mg/l + IBA 0.5 mg/l	3.07
T9	MS + add + Kin 4.0 mg/l + IBA 0.5 mg/l	3.05
T10	MS + add + BAP 1.0 mg/l + IBA 0.5 mg/l	2.50
T11	MS + add + BAP 2.0 mg/l + IBA 0.5 mg/l	2.55
T12	MS + add + BAP 3.0 mg/l + IBA 0.5 mg/l	3.00
T13	MS + add + BAP 4.0 mg/l + IBA 0.5 mg/l	2.94
T14	MS + add + BAP 1.0 mg/l + NAA 0.25 mg/l	2.50
T15	MS + add + BAP 2.5 mg/l + NAA 0.25 mg/l	3.25
T16	MS + add + TDZ 0.1 mg/l + NAA 0.25 mg/l	3.83
T17	MS + add + TDZ 0.25 mg/l + NAA 0.25 mg/l	**4.25**

T18	MS + add + Kin 1.0 mg/l + NAA 0.25 mg/l	2.53
T19	MS + add + Kin 2.5 mg/l + NAA 0.25 mg/l	3.04
SE (m)±		0.29
CD (at 1%)		1.73

6.3. Shoot multiplication

Initiated shoots were multiplied on MS liquid basal media fortified with additives (ascorbic acid 50mg/l, citric acid 25mg/l, cysteine 25mg/l and glutamine 100mg/l) in order to strengthen the shoots to withstand stress conditions in rooting. Among the two cytokinins (TDZ and BAP) tested, MS basal medium supplemented with additives and BAP (2.5 mg/l) + NAA (0.25mg/l) was found optimum for multiplication with 32.23 shoots/ explant with 6.58 cm length (Table 10, Plate no.4 and Fig. 4).

Table 9: Effect of plant growth regulators on shoot multiplication

Tr. No.	Treatments (PGRs, mg/l)	Shoot frequency	Shoot elongation
T1	MS + add + BAP 1.0 mg/l + NAA 0.25 mg/l	26.45	4.79
T2	MS + add + BAP 2.5 mg/l + NAA 0.25 mg/l	**32.23**	**6.58**
T3	MS + add + TDZ 0.1 mg/l + NAA 0.25 mg/l	26.90	3.21
T4	MS + add + TDZ 0.25 mg/l + NAA 0.25 mg/l	28.49	3.29
T5	MS + add + Kin 1.0 mg/l + NAA 0.25 mg/l	7.21	2.12
T6	MS + add + Kin 2.5 mg/l + NAA 0.25 mg/l	8.36	2.43
SE(m)±		0.59	0.21
CD (at 1%)		2.37	0.83

6.4. *In vitro* rooting

6.4.1. Effect of plant growth regulators and media modification on root induction response

A clump of 2-3 shoot was found essential for *in vitro* rooting in *D. stocksii*. Sub-culturing of shoots on MS hormone free agar gelled medium for one week prior to rooting was tried for improving the quality of shoots and to obtain high rate of rooting. In this case, root initiation was observed but the shoots completely dried off followed by no growth in roots.

Half strength MS basal medium with combinations of NAA and small amount of cytokinin (BAP 0.1 mg/l) was used to induce rooting. The results showed statistically significant effect of different concentrations (0.5 mg/l to 2.5 mg/l) of NAA on root induction. The influence was significant in terms of per cent response, root number and root length. MS/2 basal salt agar gelled medium containing NAA (1.0 mg/l) (T3) proved best with maximum (100%) root induction followed by NAA (1.5 mg/l) (88.89%) (T4) after five weeks period at 25± 2^0 C temperature and 2500 lux intensity of light for 12 h photoperiod. Least response rate was observed in MS/2 basal salt agar gelled medium fortified with NAA (2.5 mg/l) as shown in Table 11 and Fig. 5.

Table 10: Effect of plant growth regulators on root induction response

Tr. No.	Treatments (PGRs, mg/l)	Per cent rooting response
T1	HF MS (Control)	11.11
T2	MS/2 + NAA 0.5 mg/l	55.55
T3	MS/2 + NAA 1.0 mg/l	**100.00**
T4	MS/2 + NAA 1.5 mg/l	88.89
T5	MS/2 + NAA 2 mg/l	77.77
T6	MS/2 + NAA 2.5 mg/l	0.00
SE(m)±		0.18

CD (at 1%)	0.73

6.4.2. Effect of plant growth regulators on root initiation frequency

The results showed statistically significant effect of treatments on root initiation rate (Table 12, Plate no.5 and Fig. 6). Among the five different concentrations of NAA used in MS/2 basal agar gelled medium, NAA (1 mg/l) showed maximum number of roots (5.78 roots/explant) initiated per explant followed by NAA (2 mg/l) (4.89 roots/explant).

MS/2 basal agar gelled fortified with NAA (2.5 mg/l) showed no emergence of roots followed by hormone free medium with least frequency of roots (0.11 roots/explant).

Table 11: Effect of plant growth regulators on number of roots

Tr. No.	Treatments (PGRs, mg/l)	Number of roots
T1	HF MS (Control)	0.11
T2	MS/2 + NAA 0.5 mg/l	2.44
T3	MS/2 + NAA 1.0 mg/l	**5.78**
T4	MS/2 + NAA 1.5 mg/l	4.44
T5	MS/2 + NAA 2 mg/l	4.89
T6	MS/2 + NAA 2.5 mg/l	0.00
SE (m) ±		0.49
CD (at 1%)		1.97

CHAPTER V
DISCUSSION

A systematic study on standardization of *in vitro* regeneration technique for a valuable species of bamboo, *Dendrocalamusstocksii,* for mass propagation has been undertaken during the training.

Disinfection of explants

During the growing period in the green house or in the field, plants are exposed to numerous contaminants, insects and pests. Hence, the plant material to be used as an explant for culture is treated with sterilizing agents to inactivate the microbes present on the surface of explants. Further, it is observed that long time exposure to surface sterilization of tissues has resulted in explant browning and subsequent explant death. This is usually due to phenolic compounds produced in tissues damaged by surface sterilization and during dissection process. As the sterilization agents used are toxic to the plant tissues, it is necessary to optimize the treatment duration and concentration of sterilizing agents in order to cause minimum tissue death. Hence, the duration and concentration of sterilizing agents is needed to be standardized (Kamble S. R., 2014).

In the present investigation, nodal shoot segments were exposed to various sterilizing agents. Results clearly indicate that treatment involving Tween 80 (1%) for 5 min, Bavistin (0.1%) for 10 min, alcohol (70%) for 30 sec and mercuric chloride (0.5%) for 5 min duration recorded maximum aseptic culture establishment (100%) with 100% survival rate. These results are in accordance with those reported earlier by Sanjaya*et al.* (2005) except the concentration of mercuric chloride used, which was 0.075%. Sawant*et al.* (2016) reported a presoaking treatment of explants in 0.2% Bavistin and 0.2% Streptomycin for 1 hr. followed by 0.1% $HgCl_2$ for 10 min.

Effect of media combinations on *in vitro* shoot initiation

Optimal growth and morphogenesis of tissue may vary for different plants according to their nutritional requirements. Moreover, tissues from different parts of plants may also have different requirements of nutrients for satisfactory growth. Tissue culture media were first developed from nutrient solutions used for culturing whole plant.

High frequency multiple shoot initiation and subsequent growth of the shoots from the explants of mature plants is more difficult than seedlings of tropical bamboos due to the problems associated with endogenous contamination, hyperhydricity and instability of multiplication rate at initial stage (Gielis*el al.,* 2002). The balance between particular auxin and cytokinin is essential for successful growth and differentiation in the tissues (Skoog and Miller, 1957).

Response in auxins (IBA and NAA) was studied separately for selecting appropriate auxin to be used in combination with cytokinins. It was observed that NAA (1mg/l) in addition to MS basal medium supplemented with additives responded better (2.70 shoots/explant) compared to higher (2.5mg/l) concentr/ation of NAA and IBA. Similar reports of use of lower (0.25mg/l) concentration of NAA have been reported in *D. stocksii*by Somashekar*et al.* (2008) and Muyeed (2011).

In the present study, significant effect of cytokinins (BAP, TDZ and Kinetin) in combination with auxins (NAA and IBA) on multiple shoot induction and shoot length was observed. Among the cytokinins used, TDZ (0.25mg/l) proved best (4.25 shoots/explant) for multiple shoot induction.

Contrary to present results, Nasreen (2015) reported that NAA and TDZ, when used separately gave optimum growth in *D. stocksii.*

Superiority of TDZ over BAP in multiple shoot induction has been proved in other bamboo species such as; *D. strictus*(Kabade, 2009), *B.*

oldhamii(Lin *et al.*, 2007) and *B. edulis*(Lin and Chang, 1998). Similar to these observations, Sanjaya*et al.* (2005), Somashekar*et al.* (2008) and Muyeed (2011) observed the best results in terms of shoot number and shoot length by the use of NAA (0.25 mg/l) + TDZ (0.25 mg/l) in MS liquid medium in *D. stocksii.* Similar response was observed by Sawant*et al*. (2016) using MS medium fortified with BAP (1 mg/l), NAA (0.5 mg/l), adenine sulphate (80 mg/l), ascorbic acid (50 mg/l), citric acid (25 mg/l) and cystiene (25 mg/l) (liquid).

Effect of media combinations on *in vitro* shoot multiplication

To promote the growth of axillary buds and reduce apical dominance during shoot multiplication, cytokinins are usually incorporated in the medium. Optimum requirement of growth hormones for shoot multiplication varies with the plant species.

In the present study, it was revealed from the results that, among the cytokinins (BAP, TDZ and Kin) used, medium consisting BAP proved better for shoot multiplication and growth. MS liquid medium with additives + NAA (0.25 mg/l) + BAP (2.5 mg/l), proved the best for shoot multiplication (32.23 shoots) with maximum shoot length (6.58 cm). In accordance to our results, combined use of NAA (0.25mg/l) + BAP (2.5 mg/l) in MS liquid medium with additives proved the best (30.22 shoots/clump) for shoot multiplication by Muyeed (2011) but the maximum shoot length (3.87cm) was lesser compared to present results.

Contrary to the above observations, lower concentration of BAP (1.0 mg/l) was reported the best for shoot multiplication by Somashekar*et. al*(2008).

Effect of media combinations on *in vitro* root induction

Rooting is the most crucial step particularly for the shoot originated from the mature woody plants including bamboo species. High frequency root induction is of prime concern for large scale production of

clonal planting material. Initial medium of shoot cultures, quality of shoots, shoot length, nutrient medium, auxin and its concentrations are important factors which influence the rooting frequency, root number, length and subsequent shoot growth after rooting.

In the present study, it was revealed that different concentrations of NAA with small amount of BAP (0.1 mg/l) had significant effect on rooting in *D. stocksii*. Among the different concentrations of NAA (0.5mg/l to 2.5mg/l) tested for *in vitro* rooting, half strength MS basal medium containing NAA (1 mg/l) proved best with 100% response and favored high (5.78 roots) frequency rooting.

Similarly, high frequency rooting in *D. stocksii*is reported by Somashekar*et. al.* (2008) and Muyeed (2011) in NAA (1.0 mg/l) but is contrary with the present study in terms of strength of MS basal agar gelled medium used. Somashekar*et. al.* (2008) and Muyeed (2011) had observed best rooting results in quarter strength MS basal agar gelled medium whereas the present study states half strength MS basal agar gelled medium as optimum for rooting. Use of MS/2 basal agar gelled medium in *D. stocksii*by Sanjaya*et. al.* (2005) is in accordance with the present results, but the PGR's used (IBA 1.0 mg/l and BAP 0.1 mg/l) by them gave contrary results. In contrary, Sawant*et al.* (2016) reported rooting in $^{1}/_{4}$ MS medium supplemented with high concentration of NAA (2.5 mg/l).

CHAPTER VI
SUMMARY AND CONCLUSION

The experiment entitled “Studies on micro-propagation of *Dendrocalamusstocksii*(Munro.)through nodal explant” was conducted at Plant Biotechnology Centre, Dr. BalasahebSawantKonkanKrishiVidyapeeth, Dapoli. The experiment was laid out in FactorialCompletely Randomized Design (FCRD) with three replications to standardize *in vitro* regeneration technique of *D. stocksii*using nodal explant. Study was carried out on standardization of sterilization treatment, *in vitro* shoot initiation and proliferation medium and *in vitro* root induction medium. The results obtained during the period of investigation are summarized in this chapter.

Among the different surface sterilization treatments deployed on nodal explant, treatment involving 1%Tween 80 for 5 minutes, 0.1%bavistin for 10 minutes, 70% alcohol for 30 seconds and 0.5%mercuric chloride for 5 minutes recorded maximum (100 %) aseptic culture establishment with 100% survival rate followed by treatment involving 1%Tween 20 for 5 minutes, 0.1%bavistin for 15 minutes, 70% alcohol for 30 seconds and 1%sodium hypochlorite for 10 minutes with 86.67% success rate.

Different media combinations created variable response in shoot induction in the nodal explants. MS liquid medium fortified with additives: ascorbic acid (50 mg/l) + citric acid (25 mg/l) + cysteine (25 mg/l) + glutamine (100mg/l) + TDZ (0.25 mg/l) + NAA (0.25 mg/l), proved to be the best treatment(4.25 shoots/explant).

Cytokinins have shown significant effect on shoot multiplication and among the three cytokinins (BAP, TDZ and Kin) tested, BAP proved better than TDZ and Kin for shoot multiplication. MS liquid medium with additives + NAA (0.25 mg/l) + BAP (2.5 mg/l) proved the best for shoot multiplication (32.23 shoots/clump).

MS/2 basal salt agar gelled medium containing NAA (1.0 mg/l) proved best for maximum (100%) root induction followed by NAA (1.5 mg/l) (88.89%) after five weeks period. Among the five different concentrations of NAA in MS/2 basal agar gelled medium, NAA (1 mg/l) showed maximum number of roots (5.78) per explant followed by NAA (2 mg/l) with 4.89 roots per explant.

Conclusion

For refinement of *in vitro* regeneration protocols through axillary shoot proliferation, studies on the effect of plant growth regulators on shoot initiation and shoot multiplication and effect of auxins on *in vitro* rooting were carried out in *D. stocksii.*

- From the present investigation, it can be concluded that, proper sterilization treatment prior to inoculation is very necessary to completely inhibit contamination in the cultures. The best surface sterilization treatment found was 1% Tween 80 for 5 minutes, 0.1% bavistin for 10 minutes, 70% alcohol for 30 seconds and 0.5% mercuric chloride for 5 minutes.
- Cytokinins have significant effect on shoot initiation from the nodal segments. Among the three cytokinins (BAP, TDZ and kinetin) tested in different concentrations, MS liquid medium fortified with additives (ascorbic acid 50mg/l + citric acid 25mg/l + cysteine 25mg/l + glutamine 100mg/l) + TDZ (0.25 mg/l) + NAA (0.25 mg/l) proved best in terms of per cent response, shoot number and shoot length.
- Sub culturing the induced shoot clumps for 2 months prior to rooting was found essential.
- Cytokinins have shown significant effect on shoot multiplication and among the two cytokinins (BAP and TDZ) tested, BAP proved better than TDZ for shoot multiplication. MS liquid medium with

additives + NAA (0.2 mg/l) + BAP (2.5 mg/l) proved best (32.23 shoots/explant) for shoot multiplication.

- Among the different concentrations (0.5-2.5 mg/l) of NAA tested for *in vitro* root induction, 100% root induction was induced in 2-3 shoot clumps using half strength MS agar gelled basal salt fortified with NAA (1 mg/l) and BAP (0.1 mg/l).
- The results obtained from the present investigation clearly indicate that, the standardized *in vitro* regeneration technique of *D. stocksii*from nodal explants will provide a successful and rapid regeneration technique that can be successfully used for mass propagation of elite genotypes.

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THESIS ABSTRACT

Title of Thesis	**:**	Studies in Micropropagation of *Dendrocalamus stocksii* (Munro.) through nodal explant
Name of the student	**:**	Ms. SavitriRajanIndulkar
Regd. No.	**:**	FDPM-14-45
Name of Research Guide	**:**	Dr. A. D. Rane

ABSTRACT

Present investigation was aimed to produce disease and virus free plantlets by standardizingnodal explant sterilization treatment and media combinations for *in vitro* initiation, multiplication and root induction of *Dendrocalamus stocksii* was conductedin Factorial Completely Randomized Designat Plant Biotechnology Centre, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (M.S.) during the academic year 2014-2017.

Surface sterilization treatment involving Tween 80 (1%) for 5 min, Bavistin (0.1%) for 10 min, alcohol (70%) for 30 sec and$HgCl_2$ (0.5%) for 5 min duration recorded maximum aseptic culture establishment (100%) with 100% survival rate followed by treatment involving 1%Tween 20 for 5 minutes, 0.1%bavistin for 15 minutes, 70% alcohol for 30 seconds and 1%sodium hypochlorite for 10 minutes with 86.67% success rate.Among the different media combinations experimented to establish multiple shoot induction from nodal explant, MS liquid medium fortified with additives: ascorbic acid (50 mg/l) + citric acid (25 mg/l) + cysteine (25 mg/l) + glutamine (100 mg/l) + TDZ (0.25 mg/l) + NAA (0.25 mg/l), proved to be the best treatment in terms of number of shoots/explant induced.MS liquid medium with additives + NAA (0.25 mg/l) + BAP (2.5 mg/l) proved best for shoot multiplication rate(32.23 shoots/clump) and maximum shoot length (6.58 cm).MS/2 basal salt agar gelled medium containing NAA (1.0 mg/l) proved best for maximum (100%) root induction followed by NAA (1.5 mg/l) (88.89%) after five weeks period. Whereas, MS/2 basal agar gelled medium fortified with NAA (1 mg/l) showed maximum number of root (5.78) per explant followed by NAA (2 mg/l) with 4.89 roots per explant. MS/2 basal agar gelled medium fortified with NAA (1.5 mg/l) showed maximum elongation (4.25 cm) of root per explant followed by NAA (1 mg/l) (4.12 cm).

Key words: *Dendrocalamus stocksii*, micro-propagation, nodal explant.

APPENDIX I

ABBREVATIONS

/	**:**	Per
^{0}C	**:**	Degree celcius
%	**:**	Percentage
BAP	**:**	6 Benzyl amino purine
cm	**:**	Centimeter (s)
DDW	**:**	Double distilled water
et al.	**:**	and others
etc.	**:**	et cetera
ha	**:**	Hector
HCl	**:**	Hydrochloric acid
$HgCl_2$	**:**	Mercuric chloride
hrs.	**:**	Hours
i.e.	**:**	id est. (that is)
IBA	**:**	Indole-3-butyric acid

Kin	**:**	Kinetin
mg	**:**	Milligram
mg/l	**:**	Milligram per litre
ml	**:**	Millilitre
MS	**:**	Murashige and Skoog basal medium
NAA	**:**	1-Naphthaleneacetic acid
NaOH	**:**	Sodium hydroxide
ppm	**:**	Parts per million
TDZ	**:**	Thiadiazuron
viz.	**:**	Namely

Plate No. 1

a) **Young clump of *D. stocksii* used for explant collection**
b) **Nodal shoot segments sized 2.5-3.0 cm in length and 0.25-0.35 mm in diameter used as explant.**

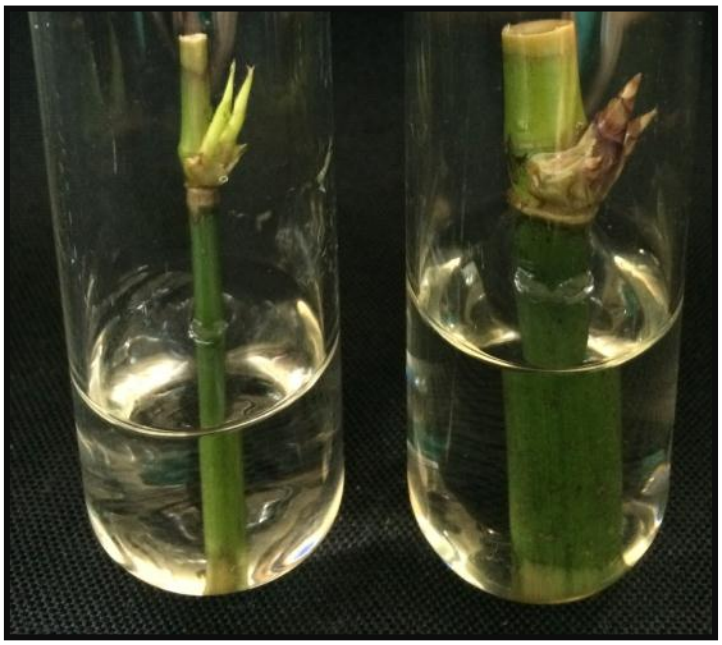
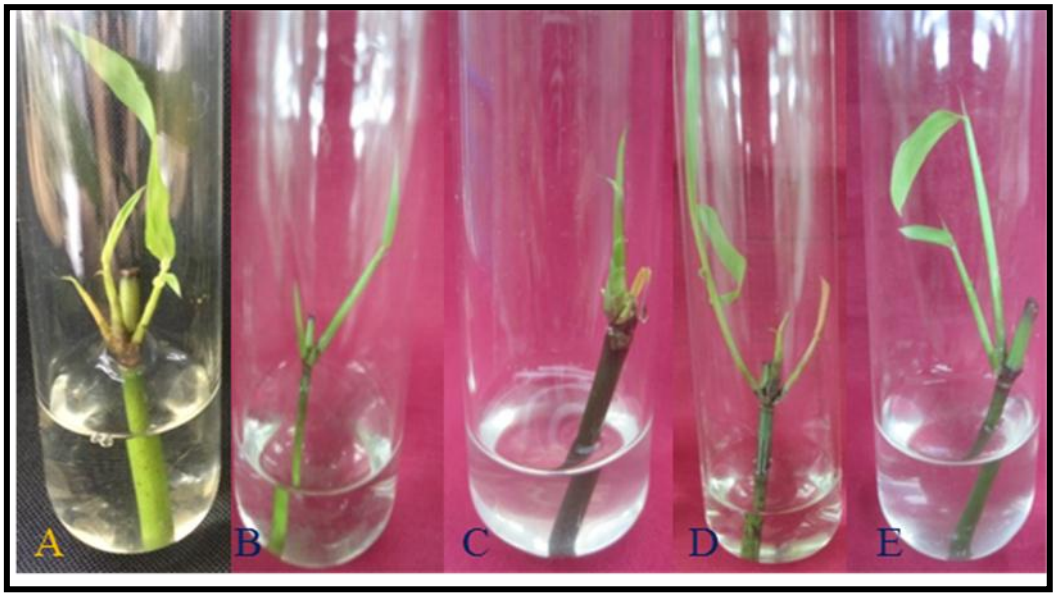


Plate No. 2

a) **Fungal contamination in nodal explants of *D. stocksii***
b) **Established contamination free cultures of *D. stocksii***



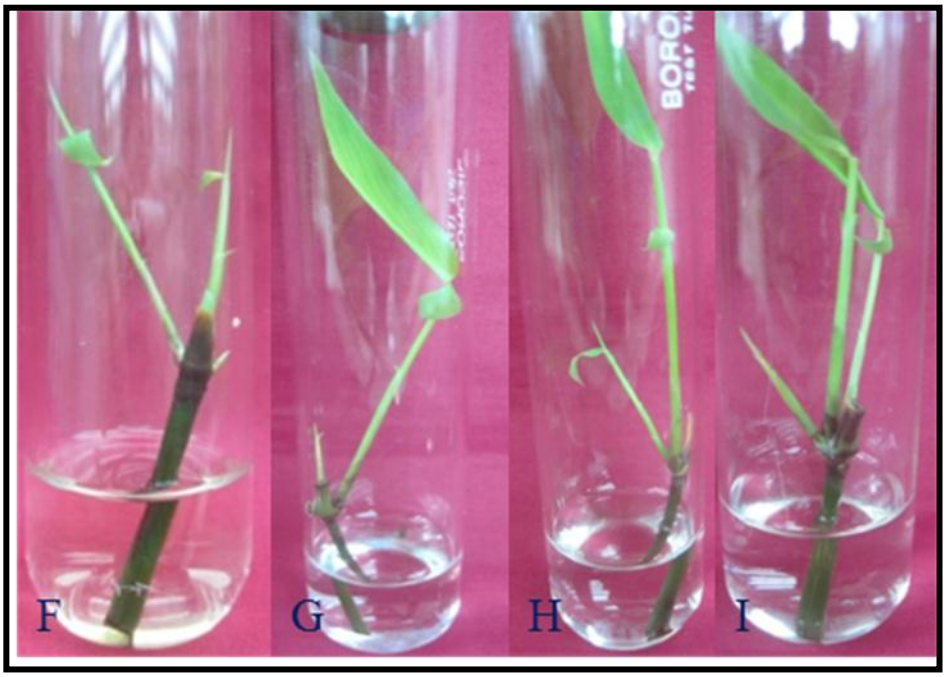


Plate No. 3 (a)

Effect of PGR's on *in vitro* shoot initiation in *D. stocksii*

a) Control (HF MS)

b) MS + add + IBA 1.0mg/l

f) MS + add + Kin 1.0mg/l + IBA 0.5mg/l

g) MS + add + Kin 2.0mg/l + IBA 0.5mg/l

c) MS + add + IBA 2.5mg/l

d) MS + add + NAA 1.0mg/l

e) MS + add + NAA 2.5mg/l

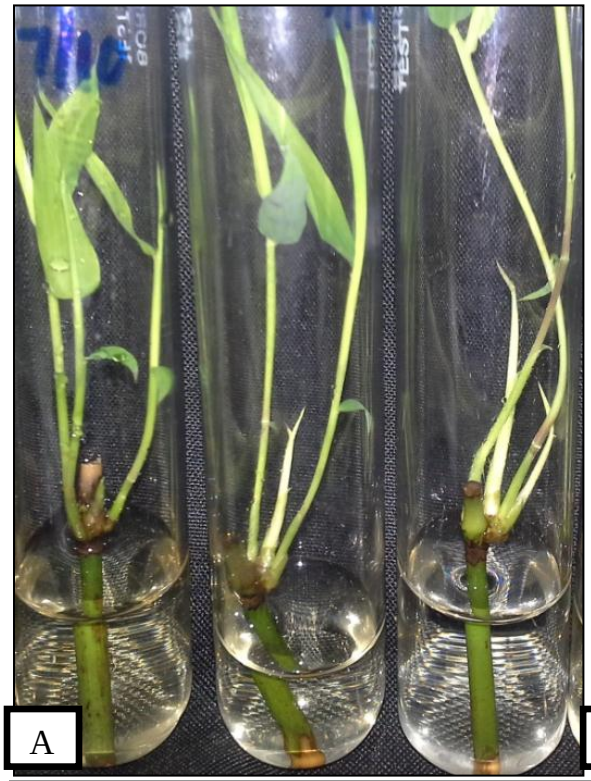
h) MS + add + Kin 3.0mg/l + IBA 0.5mg/l

i) MS + add + Kin 4.0mg/l + IBA 0.5mg/l

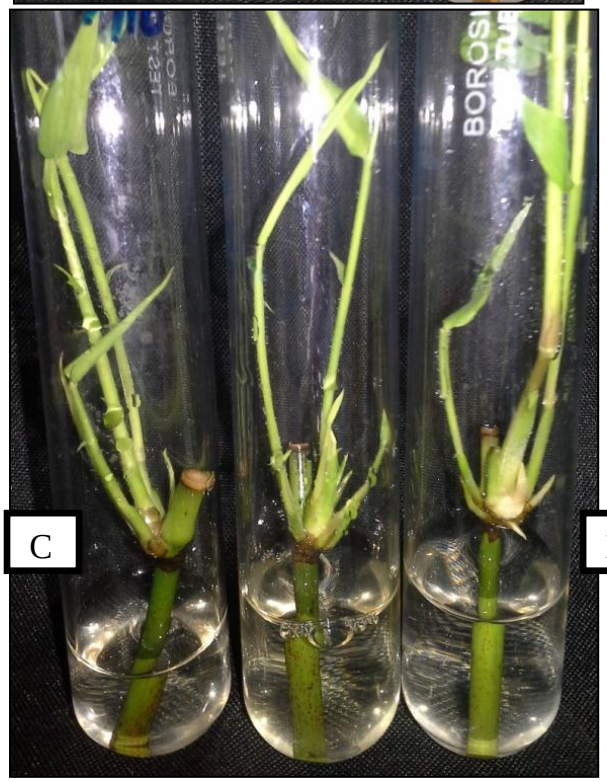
Plate No. 3 (b)

Effect of PGR's on *in vitro* shoot initiation in *D. stocksii*

a) MS + add + BAP 1.0mg/l + IBA 0.5mg/l
b) MS + add + BAP 2.0mg/l + IBA 0.5mg/l
c) MS + add + BAP 3.0mg/l + IBA 0.5mg/l
d) MS + add + BAP 4.0mg/l + IBA 0.5mg/l
e) MS + add + Kin 1.0mg/l + NAA .25mg/l
f) MS + add + Kin 2.0mg/l + NAA 0.25mg/l



A B



C D

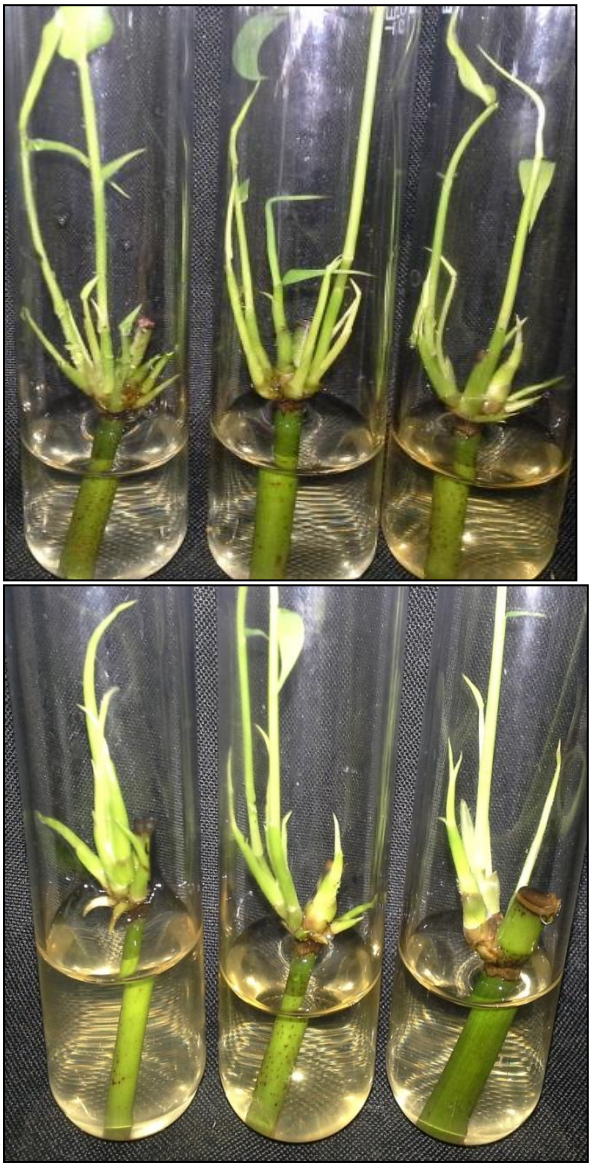
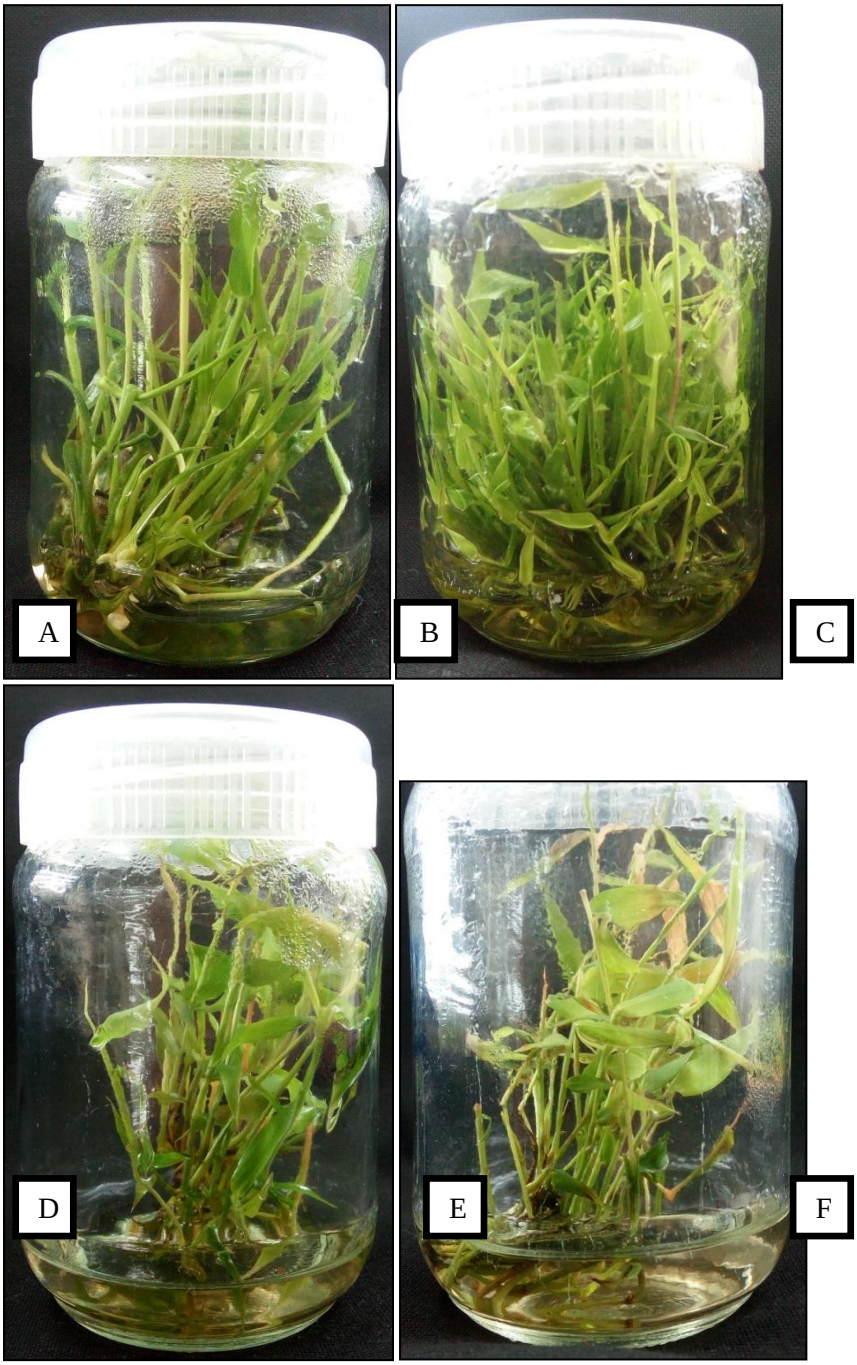


Plate No. 3 (c)

Effect of PGR's on *in vitro* shoot initiation in *D. stocksii*

a) MS + add + BAP 1.0mg/l + NAA 0.25mg/l
b) MS + add + BAP 2.5mg/l + NAA 0.25mg/l
c) MS + add + TDZ 0.1mg/l + NAA 0.25mg/l
d) MS + add + TDZ 0.25mg/l + NAA 0.25mg/l


A
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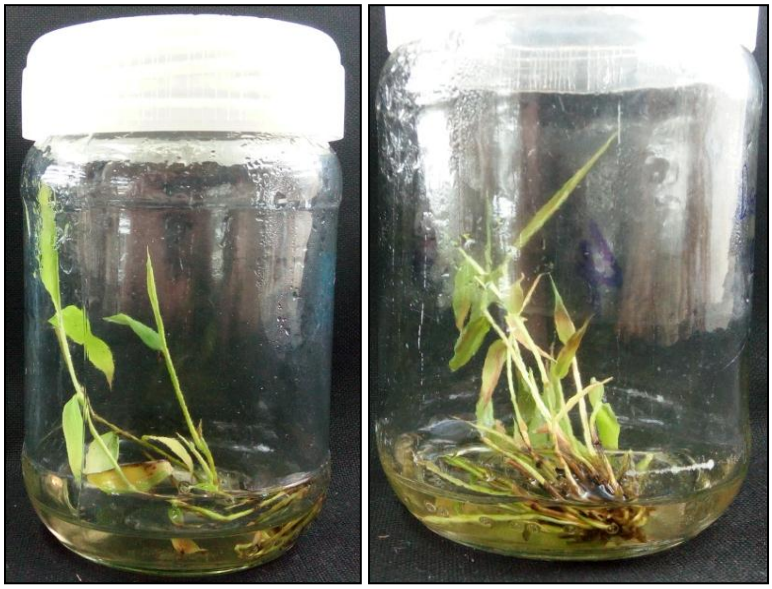

Plate No. 4

Effect of PGR's on *in vitro* shoot multiplication in *D. stocksii*

a) MS + add + BAP 1.0 mg/l + NAA 0.25 mg/l
b) MS + add + BAP 2.5 mg/l + NAA 0.25 mg/l
c) MS + add + TDZ 0.1 mg/l + NAA 0.25 mg/l
d) MS + add + TDZ 0.25 mg/l + NAA0.25 mg/l
e) MS + add + Kin 1.0 mg/l + NAA 0.25 mg/l
f) MS + add + Kin 2.5 mg/l + NAA 0.25 mg/l

A

B

C

D

E

F

Plate No. 5

Effect of PGR's on *in vitro* root induction in *D. stocksii*

a) Control (HF)
b) MS/2 + NAA 0.5mg/l
c) MS/2 + NAA 1.0mg/l
d) MS/2 + NAA 1.5mg/l
e) MS/2 + NAA 2.0mg/l
f) MS/2 + NAA 2.5mg/l

Fig-4 Effect of plant growth regulators on shoot multiplication

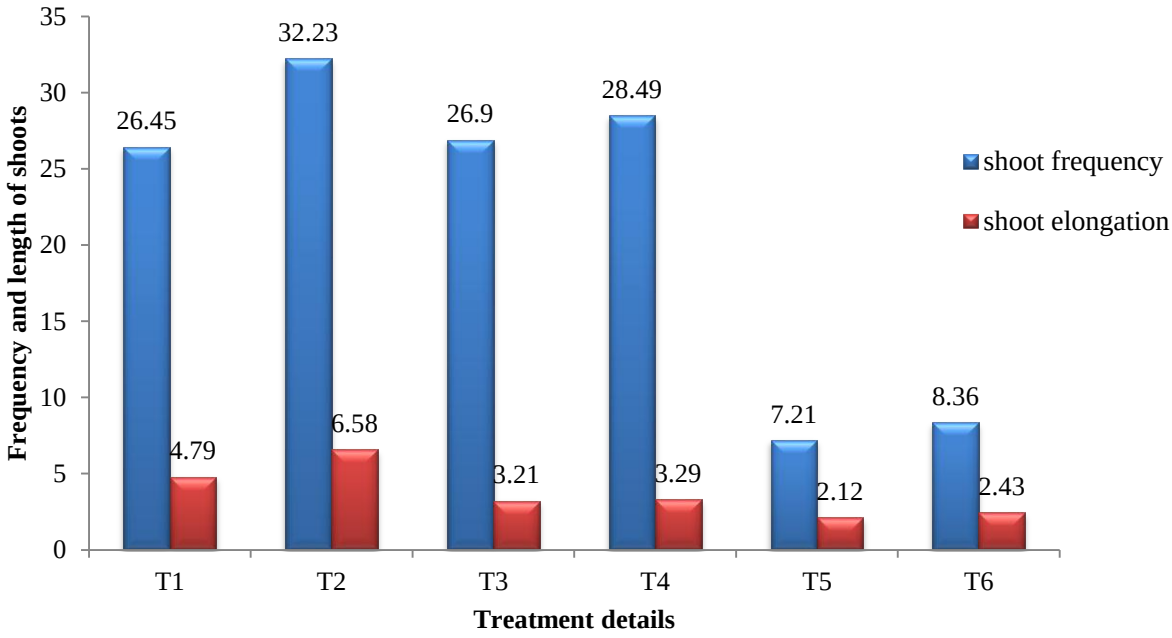

Fig-5 Effect of plant growth regulators on root induction response

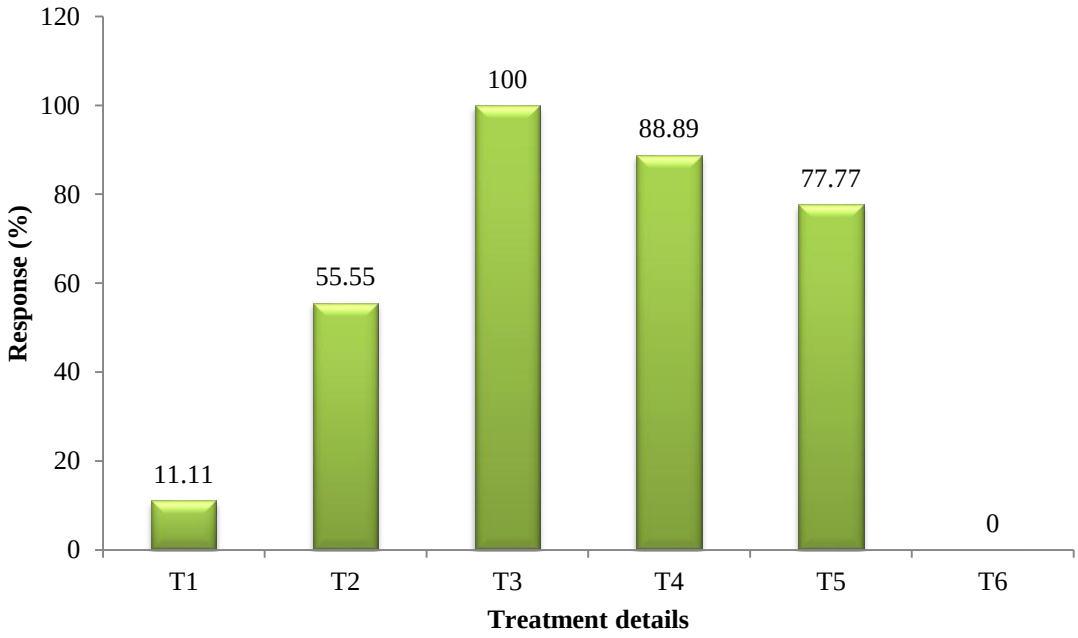

Fig-6 Effect of plant growth regulators on root induction