MICROPROPAGATION OF TEAK
(*Tectona grandis* Linn.) THROUGH
In vitro TECHNIQUES

By

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THESIS
Submitted in partial fulfilment of the
requirement for the degree of

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DECLARATION

I hereby declare that this thesis entitled "**Micropropagation of teak** (*Tectona grandis* **Linn.**) **through** *in vitro* **techniques**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.
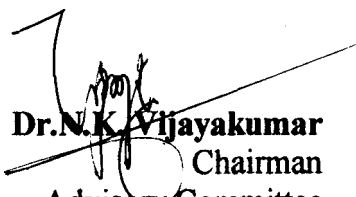
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Certified that this thesis, entitled **"Micropropagation of teak (***Tectona grandis* **Linn.) through** *in vitro* **techniques"** is a record of research work done independently by **Mr.Sandeep Sharma,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.
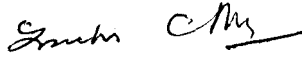
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We, the undersigned members of the Advisory Committee of **Mr.Sandeep Sharma,** a candidate for the degree of **Master of Science in Forestry**, agree that the thesis entitled *"***Micropropagation of teak (***Tectona grandis* **Linn.) through** *in vitro* **techniques"** may be submitted by Mr.Sandeep Sharma, in partial fulfilment of the requirement for the degree.

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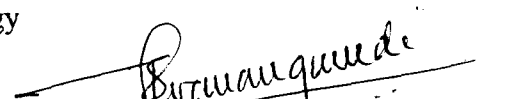College of Forestry, Vellanikkara

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To my loving parents

ACKNOWLEDGEMENT

CONTENTS

ABBREVIATIONS

ABA	-	Abscissic acid
AC	-	Activated charcoal
AdS	-	Adenine sulphate
BA	-	Benzyl adenine
CH	-	Casein hydrolysate
cm	-	centimeter
cv	-	cultivar
CW	-	Coconut water
dia	-	diameter
h	-	hour
$HgCl_2$	-	Mercuric chloride
IAA	-	Indole-3-acetic acid
IBA	-	Indole-3-butyric acids
Kn	-	Kinetin
m	-	meter
mg	-	milligram
min	-	minute
mm	-	millimeter
µm	-	micrometer
MS	-	Murashige and Skoog (1962) medium
½ MS	-	half-strength MS medium
NAA	-	Napthalene acetic acid
ppm	-	part per million
PVP	-	Polyvinyl pyrrolidone
Spp	-	species
UV	-	Ultra Vilot
v/v	-	volume by volume
w/v	-	weight by volume
WPM	-	Woody Plant Medium of Lloyd and McCown (1980)
yr	-	year
2ip	-	N-6-(2-isopentyl) adenine
2,4-D	-	2,4-Dichlorophenoxy acetic acid

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Introduction

INTRODUCTION

Teak (*Tectona grandis* Linn. f) is an important deciduous hardwood tree. It belongs to family Verbenaceae. For long Burma Teak has been considered the best timber available in the world. Teak from central India is very much similar to Burma Teak. It is the principal timber tree of peninsular India. It is referred as the Paragon among Indian timbers. Teak timber is a standard against which other timbers are compared with. It is widely distributed in Peninsular India, Burma, Indonesia and Thailand. It is aptly said that "There is virtually no use to which timber can be put for which teak cannot be employed". To ship building, teak primarily owes its centuries old and worldwide reputation. In its home countries it is a major timber for buildings, bridge and wharf construction, piles, furniture, cabinet work, railcar, wagon, wheelspokes and general carpentry. The reputation of teak is due to its matchless combination of qualities viz. termite, fungus and weather resistance, lightness with strength, attractiveness, workability and seasoning capacity without splitting, cracking, wrapping or materially altering shape. In India, mechanical properties, physical properties, durability etc. of teak are taken as standard to compare other wood with respect to these qualities.

Teak is normally seed propagated. Weight of seed varies widely with locality. The major barrier in teak propagation is the poor rate of germination and seedling mortality. Fluting and twist defects are also common in teak trees in some localities and they are probably inherited. Teak of some localities are sensitive to attack by fungus (*Corticium salmonicolor*), defoliator (*Hyblea puera*) and teak skeletonizer (*Hapalia machaeralis*). There are also certain genotypes resistant to all the defects, diseases and pest attack and possess all favourable qualities of a timber. They need true to the type multiplication in large number and production of the same in short rotation. This is an arduous task to be performed through conventional method and tissue culture is the viable and economically feasible alternative.

Already tissue culture techniques have been standardized for micropropagation of teak both from seedling and mature tree by Gupta *et al.* (1980), Lakshmisita *et al.* (1986) have reported the development of embryo like structures in shoot and endosperm explant derived callus culture of teak. Mascarenhas *et al.* (1993) have obtained globular callus from different explants (apical meristem, *in vitro* developed leaves, stem shoot tips and axillary buds). Lately Devi *et al.* (1994) have reported a method of rapid cloning of 30 year old elite teak tree by *in vitro* multiple shoot production.

In teak, high degree of inter and intra population variation in morphology, ecology, floral biology, seed biology and, bole and timber character are known. Availability of wide genotypic variability stresses the need to standardize the technique of micropropagation of different superior genotype of teak individually.

In this backdrop the present study was chosen with the objective to standardize the procedure for micropropagation of teak (*Tectona grandis* Linn.) through the technique of tissue culture.

Review of Literature

REVIEW OF LITERATURE

Science of tissue culture has the potential to provide a pragmatic solution to rapid cloning of elite clones of individuals with a particular trait that enables them to flourish in a particular environment. The great German botanist Gottlieb Haberlandt (1854-1945) is regarded as the father of plant tissue culture. In India micropropagation of tree species began in 1960's when culture of *Pinus gerardiana* were established and studied by Konar (1963). Since then micropropagation protocol for large number of tree species have been worked out. Vegetative propagation is a very important for producing large quantities of good quality planting material for forest tree species. Whitehead and Giles (1977) estimated that more than 10^6 rooted plantlets could be produced per year from a culture of a single bud of *Populus nigra, Populus chinnanesis* and *Populus hybrid*. Govil and Gupta (1997) estimated that global market is dealing with 15 million US dollar per annum of tissue culture products. Indian industry has expended from 5 million annual capacity in 1988 to 190 million in 1996. Micropropagation of forest tree is difficult to work out, though it is acquiring popularity. It has the potential to reproduce the crop that are other wise difficult to propagate conventionally by seeds or by other vegetative means. Considerable amount of work has been done in *in vitro* propagation of softwood species like *Pinus pinaster* (David *et al.*, 1982), *Cedrus deodara* (Bhatnagar *et al.*, 1983), *Pinus nigra* (Kolvesta-Pletikapas *et al.*, 1983), *Euphedra foliata* (Bhatnagar and Singh, 1984), different species of *Picea* (Arnold and Erikson, 1985; Robert *et al.*, 1992), *Pinus radiata* (Smith, 1986), *Juniperus oxycedrus* (Gomez and Segura, 1994), *Pinus eldarica* (Sen *et al.*, 1994).

Workable protocol for the *in vitro* propagation has been worked out for many broad leaves species. The review of recent literature pertaining to the micropropagation of broad leaves species have been highlighted in detail below:

2.1 Micropropagation

2.1.1 *Acacia* species

Plant regeneration via somatic embryogenesis was achieved from callus derived from immature cotyledons of *Acacia catechu* Wild. The callus was cultured on WPM supplemented with 13.9 µM Kn and 2.7 µM NAA. The addition of 0.9-3.5 µM L.proline to medium influenced the development of somatic embryo and also promoted secondary embryogenesis (Rout *et al.*, 1995). Bhaskar and Subash (1996) obtained *in vitro* shoots in *Acacia mangium* by culturing nodal explants from 8 year old elite trees on MS medium supplemented with 3 mg l^{-1} BA and 0.1 mg l^{-1} NAA. Beck *et al.* (1998) reported about induction of multiple shoots of *Acacia mearnsii* when nodal explants were cultured on MS medium supplemented with 2.0 mg l^{-1} BA. In *Acacia senegal* maximum number of shoot buds were obtained when nodal segments were cultured on MS medium supplemented with BA 4.0 mg l^{-1} and 0.5 mg l^{-1} NAA. Addition of AdS (Adenine sulphate) at the rate of 25.0 mg l^{-1}, 100.0 mg l^{-1} ascorbic acid and 146.0 mg l^{-1} glutamine to the medium were found beneficial. Rooting was found best in MS medium fortified with 5.0 mg l^{-1} IAA (Kaur *et al.*, 1998).

2.1.2 *Aegle marmelos*

More than 12,376 shoots (in 5 months after repeated subculturing) of *Aegle marmelos* were obtained when cotyledonary node explants, excised from 15 days old seedlings were cultured on MS medium supplemented with 3 mg l^{-1} BAP. Rooting was obtained on MS medium containing 4.0 mg l^{-1} IBA (Arumugam and Rao, 1996). A high frequency of adventitious shoot regeneration was obtained from the radical tissue of same species, when explant was cultured on MS medium supplemented with 1.0 mg l^{-1} BA and 0.2 mg l^{-1} NAA. The shoots grown in medium containing 0.5 mg l^{-1} IBA for one week and than transferred to basal medium produced roots (Islam *et al.*, 1996).

2.1.3   *Albizia* species

Ahlawat (1997) reported that, in *Albizia procera,* maximum number of shoots per explant were obtained on MS medium containing 2 mg l^{-1} BA and 0.5 mg l^{-1} NAA in 60 days. Rooting of microshoots were achieved in ½ MS supplemented with 2.0 mg l^{-1} IBA + 0.1 per cent Activated charcoal. Majumdar *et al.* (1998) reported *in vitro* differentiation of adventitious shoots from cotyledonary explants of *Albizia procera* on MS media containing 4.0 mg l^{-1} BA and coconut water 15 v/v. A higher frequency of shoot bud multiplication and elongation was observed on medium with BA at lower concentration.

2.1.4   *Ailanthus* species

Natesha (1999) obtained maximum number of bud initiation in *Ailanthus triphysa* when nodal explant was cultured on MS medium supplemented with 2.0 mg l^{-1} BA and 2.0 mg l^{-1} Kn. Rooting was obtained on half strength MS medium supplemented with 4.0 mg l^{-1} IAA and 0.4 mg l^{-1} IBA.

2.1.5   *Alnus cremastogyne*

Tang *et al.* (1996) reported that multiple shoots were grown from seedling explants of *Alnus cremastogyne* Burk. by two stage procedure. Bud initiation was observed on WP medium supplemented with 2-8 µM BA for 6 weeks thereafter subcultured on the same medium with 1 µM BA. Rooting was obtained on half strength WP medium either with IBA or without any growth regulators.

2.1.6   *Annogeissus pendula*

Multiple shoots ranging from 15-20 and 4-6 shoots were obtained from cotyledonary and epicotyledonary explants, respectively, when cultured on MS medium supplemented with 1.0 mg l^{-1} BA and 0.1 mg l^{-1} IAA. Rooting of *in vitro* shoots were obtained on half strength MS medium containing 1.5 mg l^{-1} BA + 0.1 mg l^{-1} Kn (Joshi *et al.,* 1991).

2.1.7   *Annona squamosa*

Lemos and Blake (1996) reported establishment and proliferation of *Annona squamosa* (sugar apple) culture on woody plant medium supplemented with 9 µM BA and 10 g l^{-1} activated charcoal. Rooting was reported on WPM supplemented with 43 µM NAA or 39 µM IBA.

2.1.8   *Artocarpus*

Establishment of culture and shoot proliferation were obtained from explant treated with 0.2 per cent $HgCl_2$ for 4 minute or with 0.1 per cent $HgCl_2$ for 10 minutes, when cultured on MS medium and woody plant medium supplemented with 10 mg l^{-1} BA, 0.5 mg l^{-1} NAA, GA_3 5.0 mg l^{-1} and gelrite 25 per cent. Large explants were reported to be better than small explants (Charan *et al.*, 1996). Roy *et al.* (1996) successfully obtained multiple shoot from shoot tip and nodal segments of *Artocarpus heterophyllus* on MS medium supplemented with 2.5 mg l^{-1} BA and 0.5 mg l^{-1} NAA. Coconut milk addition to medium had a synergistic effect on number of shoot production. Rooting excised shoots were obtained on half strength MS containing NAA and IBA each at 1.0 mg l^{-1}.

2.1.9   *Azadirachta*

Eeswara *et al.* (1997) reported that shoots of *Azadirachta indica* can be induced from leaf explants when cultured on MS medium containing BA 1.0 mg l^{-1}, Kn 0.8 mg l^{-1} and AdS (Adenine sulphate) 6.0 mg l^{-1}. In 32 weeks 80 shoots were produced from single leaf of 10 x 10 mm. The induction of light yellow friable callus and shoots of *Azadirachta indica* on MS medium supplemented with 0.5 mg l^{-1} IAA and 1 mg l^{-1} BA were reported by Zypman *et al.* (1997).

2.1.10   Bamboo

Lin and Chang (1998) reported that nodal explant from 10 year old *Bambusa eludis* produce multiple shoots on MS medium supplemented with

0.1 mg l^{-1} thiodiazuron (TDZ) hundres of regenerated shoots rooted well on the medium supplemented with 0.5 mg l^{-1} 2,4-dichlrophenoxy acetic acid and were successfully transferred to soil. Ravikumar *et al.* (1998) observed induction of multiple shoots from seedlings and axillary buds of *Dendrocalamus strictus* Nees. on MS medium with 0.5 mg l^{-1} BA and 0.5 mg l^{-1} Kn supplemented with 200 ml l^{-1} coconut water. Rooting was induced on MS medium containing 0.25 mg l^{-1} IBA.

2.1.11   *Bauhinia variegata* Linn

Dasgupta and Bhattacharya (1995) observed that callus formation was best from leaf apex and leaf base explant. *Bauhinia variegata* on MS medium supplemented with 2.0 mg l^{-1} 2,4-D. Plantlet regeneration was achieved on medium supplemented with 2.0 mg l^{-1} 2,4-D and 0.5-1.0 mg l^{-1} BA.

2.1.12   Betula

Katayase (1995) successfully obtained plantlets from winter bud explants of 30 year old *Betula maximowicziana* tree by culturing on woody plant medium containing 0.02-0.2 mg l^{-1} NAA and BA. The basal portion of the regenerated shoots were dipped in 50 ppm IBA solution and roots were regenerated on half strength MS medium containing 0.2 mg l^{-1} NAA. In *Betula pendula* var. Carelica with best growth occurred in explant with removed apices on MS medium supplemented with exogenously added cytokinin (BA 0.5 mg l^{-1}) (Durkovic, 1997).

2.1.13   Boswellia

Induction on callus from anther of *Boswellia serrata* was reported by Prakash *et al.* (1999) on BNM (Bown and Nitsch medium) supplemented with 0.5 mg l^{-1} 2,4-D, 1.0 mg l^{-1} BA, 200 mg l^{-1} casein hydrolysate and 3 per cent sucrose. Phenolic exudation from cultured anthers was checked by use of PVP (polyvinyl polypyrrolidone) and other antioxidants like ascorbic acids addition to

the medium. Besides dark treatment to cultures for 24 h was found suitable to minimize phenol.

2.1.14   Cashew nut

Ananthakrishnan *et al.* (1999) reported that maximum number of multiple shoots, in cashew nut were obtained from embryonic axis with cotyledon segment, when cultured on woody plant medium supplemented with 5 mg l^{-1} BA and adenine sulphate. Well developed shoots were rooted on WPM containing IAA and IBA (1 mg l^{-1}) each.

2.1.15   Casuarina

Shoot apices (8-10 mm long) excised from 10 yr old tree formed bud like structure with in 4-5 weeks. When cultured on MS on Gamborg and Nitch (NB) media without growth regulators. Supplementation with cytokinin, BA (1.5-2.5 mg l^{-1}) enhanced shoot proliferation. Activated charcoal (3.0 per cent) promoted shoot elongation. Rooting was obtained on NB medium supplemented with 1-3 mg l^{-1} IBA (Nanda and Gupta, 1991).

2.1.16   *Cinnamomum camphora*

A micropropagation protocol was developed for *Cinnamomum camphora* using 3 to 5 mm shoot tips from newly emerged laterals of 2 year old trees as initial explants. Nine shoots per nodal segments were obtained on MS medium supplemented with 4.4 µM BA. Rooting of shoots occurred best on medium supplemented with 0.54 µM NAA (Huang *et al.*, 1998).

2.1.17   *Citrus jambhiri*

Singh *et al.* (1999) observed maximum shoot proliferation of *Citrus jambhiri*, when shoot tip explant was cultured on MS medium supplemented with 1.0 mg l^{-1} BA, 1.0 mg l^{-1} AdS (adenine sulphate) and 0.25 mg l^{-1} NAA. Rooting

was best obtained on MS medium supplemented with 0.1 mg l^{-1} BA, 0.1 mg l^{-1} AdS, 0.5 mg l^{-1} NAA and 1.0 mg l^{-1} IBA.

2.1.18   Dalbergia

Datta and Datta (1983) successfully cultured the nodal segments of *Dalbergia sissoo* on MS medium and shoot multiplication was obtained when medium was supplemented with high BA and low auxin. Mahato (1992) obtained multiple shoots from nodal segments from two and half year seedlings as well as twelve year old trees of *Dalbergia latifolia* on MS and WPM were found suitable for primary culture establishment. Kannan (1995) obtained enhanced release of axillary buds of *Dalbergia latifolia* on WPM supplemented with 1.0 mg l^{-1} Kn and 0.1 mg l^{-1} IAA. Multiple shoots were induced best on MS medium in combination with 2.0 mg l^{-1} BA. Buds of young trees could produce roots when cultured, after pulse treatment with 1000.0 mg l^{-1} IBA solution at the base of shoot, on half strength basal medium. Direct roots and shoots were also obtained from nodal explants of young trees on WPM supplemented with 1.0-2.0 mg l^{-1} IAA. Das *et al.* (1996) found that shoot bud regeneration from different explants of *Dalbergia latifolia, D. sissoo* and *D. penniculata* was most frequent in MS medium supplemented with BA (1.0-2.0 mg l^{-1}) and NAA (0.5-1.0 mg l^{-1}). The rooting was obtained on half strength MS medium with BA (0.25 mg l^{-1}). Somatic embryogenesis was achieved in callus culture derived from 40 days old semi mature zygotic embryo of *D. sissoo* on semisolid medium supplemented with 0.46-1.16 μM Kn, 6.78-9.04 μM 2,4-D and 30 gm sucrose.

2.1.19   *Daubanga grandiflora*

Kumar and Kumar (1997) obtained multiple shoots by culturing coteledonery nodes on MS medium supplemented with BA at range of concentration 0.5-5.0 mg l^{-1}. Isolated microshoots rooted on MS medium containing 0.1 mg l^{-1} each of IBA and NAA.

2.1.20   *Delonix regia*

Hoque *et al.* (1992) achieved shoot regeneration from cotyledonary nodes cultured on MS medium with 1.0 mg l^{-1} BA and 1.0 mg l^{-1} IAA and also from shoot tip cultured on MS medium containing 0.5 mg l^{-1} BA and 0.5 mg l^{-1} Kn. Half strength MS with 0.2 mg l^{-1} IBA was used for root induction. Gupta *et al.* (1996) observed that up to 25 shoots per culture were obtained from various explants (hypocotyl, nodal region, transition zone, leaflet and petiole) on MS medium supplemented with 50 mg l^{-1} asparagine, 50 mg l^{-1} arginine, 50 ppm glutamine, 5.0 mg l^{-1} cystine hydrochloride, Alpha NAA and BA.

2.1.21   *Elaeocarpus robusta*

Roy *et al.* (1998) obtained multiple shoots from shoot tip and nodal explant of 20 year old trees of *Elaeocarpus robusta* on MS medium supplemented with 0.5 mg l^{-1} of BA and 0.5 mg l^{-1} Kn. The addition of coconut milk (10%) and casein hydrolysate 100 mg l^{-1} to the medium enhanced the number of shoots up to 20 per cent per subculture and enhanced the length of shoots. Rooting was obtained on half strength MS medium containing 1.0 mg l^{-1} IBA and 0.5 mg l^{-1} IAA.

2.1.22   *Emblica officinalis*

In *Emblica officinalis* three to four shoots per explant (juvenile and mature explants) were obtained when cultured on MS medium supplemented with 5.0 mg l^{-1} BAP and 0.5 mg l^{-1} IAA along with antioxidants. The shoots were elongated on hormone free MS medium. Rooting was obtained on half strength MS fortified with IBA 2.0 mg l^{-1} and sucrose 1.5 per cent (Kant *et al.*, 1999).

2.1.23.   Eucalyptus:

Short bud initiation was reported best in Eucalyptus hybrid FRI-4 when explant was cultured on medium supplemented with 1.0 and 2.0 mg l^{-1} BA and best-shoot elongation was obtained with MS medium supplemented with

BA 0.1 mg l⁻¹. The shoots were rooted on half strength MS medium supplemented with 0.5 mg l⁻¹ IBA. Rooted plantlets were acclimatized in pots filled with sand. (Chauhan et. al, 1996). Pattanaik and vijayakumar (1997) induced multiple shoots in *Eucalyptus globulus*. On MS medium supplemented with 0.5 mg l⁻¹ BA, rooting was reported on half strength MS medium supplemented with 0.5 mg l⁻¹ IAA and 0.5 mg l⁻¹ IBA.

2.1.24 *Ficus*

Multiple roots were obtained when dormant axillary buds of nodal segments of *Ficus religiosa* L. were cultured on MS medium supplemented with 1.5 mg l⁻¹ BA and 1.5 mg l⁻¹ adenine sulphate (Deshpande, 1998). Elongations of shoots were maintained on MS medium containing 2.0 mg l⁻¹ BA, 0.5 mg l⁻¹ NAA and 0.3 w/v activated charcoal. Rooting was induced on MS supplemented with 2.0 mg l⁻¹ IBA and 0.1 mg l⁻¹ NAA for one week there after transferred to half strength basal medium. Kumar *et al.* (1998) obtained multiple shoots in *Ficus carica* by taking buds from 7 to 8 years old trees. On MS medium supplemented with 2.0 mg l⁻¹ BA and 0.2 mg l⁻¹ NAA. Rooting was obtained on half strength MS medium supplemented with 2.0 mg l⁻¹ IBA and 0.2 per cent activated charcoal.

2.1.26 *Gmelina arborea*

Sen *et al.* (1992) reported successful propagation of juvenile plants of *Gmelina arborea* roxb. by *in vitro* methods. Multiple shoots formation was obtained by culturing shoot life and nodal explants of Juvenile source on MS medium containing 1.0 mg l⁻¹ BA and 0.1 mg l⁻¹ NAA. Yong *et al.* (1993) reported *in-vitro* clonal propagation of cell suspension culture of *Gmelina arborea* (Kannan and Jasrai, 1996). Multiple shoots were obtained from single explants when cultured on MS medium supplemented with 1.0 µM BA. For root initiation cut ends of microshoots when pulsed for 5 minutes with 246 µM IBA and transferred to a plastic cup. Containing sterile vermiculite. For hardening the plantlets were transferred to earthen pots containing a mixture of garden soil compost.

Thirunavoukkarasu and Debata (1998) successfully induced shoots of *Gmelina arborea* by culturing axillary buds on McCown's medium for wood plants supplemented with different concentrations of BA (0.25 - 0.5 mg l^{-1}). Higher concentration of BA (1.0-1.5 mg l^{-1}) in combination with NAA o.5 mg l^{-1} resulted in few or no shoots and formed callus.

2.1.27   *Grewia optiva*

Sehgal and Handa (1991) reported establishment of *Grewia optiva* culture on woody plant medium supplemented with 0.5 mg l^{-1} BA alone.

2.1.28   *Hevea brasiliensis*

Mendanha *et al.* (1998) obtained shoots from axillary buds of *Hevea brasiliensis* when cultured on MS medium supplemented with 1.0 mg l^{-1} kinetin, 1.0 mg l^{-1} 2,4-D, 20 mg l^{-1} sucrose and 4 g l^{-1} Difco agar. Formation of a root similar to tap roots was induced on MS medium supplemented with 5.0 mg l^{-1} NAA and 3.0 mg l^{-1} IBA, 50 g l^{-1} sucrose and 4.0 g l^{-1} Difco agar.

2.1.29   *Mangifera indica*

Somatic embryogenesis was induced from nucellus explants of *Mangifera indica* by culturing on modified MS medium consisting of half strength major salt, full strength minor and organics, 400 mg l^{-1} L. glutamine, 100 mg l^{-1} ascorbic acid, 6.0 per cent w/v sucrose, 1.0mg l^{-1} 2,4-D (2,4-dichloro phenoxyacetic acid) gelled with 0.8 per cent (w/v) agar for 8-10 weeks in dark. The somatic embryos were matured on 0.8 per cent agar gelled medium with 6.0 per cent sucrose and converted into plantlets in liquid medium with 3.0 per cent sucrose + 1.0 mg l^{-1} GA$_3$. Maximum rooting was obtained in medium supplemented with 5.0 mg l^{-1} of IBA (Ara *et al* 1998).

2.1.30   *Morus* spp.

Kanwar *et al.* (1991) reported successful establishment of *Morus alba* cultures on MS medium containing 0.1 mg l^{-1} BA. For further proliferation MS medium with 1.0 mg l^{-1} BA was used. Pattanaik *et al.* (1996) successful produced multiples shoots from nodal explant of *Morus australis* on MS medium supplemented with BA 1.0 g l^{-1}. The shoots were successfully rooted on half strength MS containing combination of IAA, IBA, and IPA each at 1.0 mg l^{-1}. Pattanaik and chand (1997) successfully established a method of induction of multiple shoots from apical shoot bud and nodal explant of *Morus cathayana, Morus ihou* and *Morus serrata* on MS medium containing 0.5-1.0 mg l^{-1} BA. Addition of gibberellic acid 0.4 mg l^{-1} along with BA induced faster bud break. Shoot culture initiation was greatly influenced by explant type, age and explanting season. Shoots were successfully rooted on half strength MS medium containing IAA, IBA, IPA each at 1.0 mg l^{-1}.

2.1.31   *Robinia pseudoacacia*

Barghchi and chi (1998) observed that all explant type from *in vitro* grown seedlings of *Robinia pseudoacacia* developed callus and adventitious shoots in MS basal medium containing various concentration of BA and 2,4-D. Shoot multiplication was achieved readily on medium supplemented with 0.25 - 1.0 mg l^{-1} BA shoots produced roots in MS medium containing 1.0 mg l^{-1} IBA.

2.1.32   *Salmalia malbarica*

Das (1996) reported that maximum number of shoot buds in a *Salmalia malbarica (Bombax malbaricum)* was induced on MS medium supplemented with 1.0 mg l^{-1} BA along with NAA and IBA. Continuous growth in the same medium containing NAA causes callus. Replacement of NAA by IBA (0.01 mg l^{-1}) reduced the callus problem and enhance the regeneration and multiplication of shoots. Rooting was obtained on medium containing 1.0 mg l^{-1} IBA.

2.1.33   Sandal

Endosperm tissue of *Santalum album* when cultured on modified MS with 2,4-D (1-2 mg l⁻¹), Kn (0.1-0.2 mg l⁻¹), BA (0.5-2.0 mg l⁻¹) and NAA (1.0 mg l⁻¹) induced callus formation followed by differentiation (Sita *et al.*, 1980).

Parthiban *et al.* (1998) reported that axillary shoot multiplication was achieved in sandal when explant (nodal segments, shoot tips, inflorescence and seeds) were inoculated on to MS medium containing various amount of BA or Kn.

2.1.34   *Sapium sebiferum*

Siril and Dhar (1997) reported the induction of multiple shoots in *Sapium sebiferum* when nodal explant was cultured on MS medium supplemented with 2.5 µM BA and 0.25 µM NAA. Shoots were rooted on half strength, growth regulator free, agar gelled MS medium, after 48 hour treatment on half strength MS liquid medium with 10 µM IBA. The effect of seasonal variation on shoot proliferation was also reported.

2.1.35   *Sterculia urens*

On an average minimum 6 adventitious shoots were regenerated from a nodal explant from selected trees of *Sterculia urens* on MS medium supplemented with 6.22 µM BA. Rooting was obtained *in vitro* on ¼ strength MS medium containing 9.82 µM IBA (Sunnichan *et al.*, 1998).

2.1.36   *Stryphnodendron polyphythum*

Franca *et al.* (1995) observed that cotyledonary node segment when inoculated in MS medium supplemented with 13.3 µM BA gave best shoot proliferation. However, enhanced shoot length was observed on MS medium supplemented with 0.04 µM BA and 0.005 µM IAA. Rooting was reported on half strength MS medium supplemented with 5.37 µM NAA and 80 mg l⁻¹ phloroglucinol.

2.1.37   *Syzygium*

Multiple shoots were obtained from nodal explants of 10 year old trees of *Syzygium cumminii* on MS medium supplemented with 2.5 mg l^{-1} Kn. Incorporation of casein hydrolysate 100 mg l^{-1} and 15 per cent v/v coconut water to medium increase shoot length (Roy *et al.*, 1996). Khan *et al.* (1997) observed that in *Syzygium alternifolium* a combination of BA with Auxin in MS medium produced more shoots from both type of explants (shoot tip and nodal bud) than medium containing only cytokinin. The highest multiplication rate was achieved with nodal bud explant in presence of 17.7 µM BA and 26 µM NAA. Excised shoots were rooted on half strength MS with either IAA or IBA.

2.1.38   *Tecomella undulata*

Rathore *et al.* (1991) established a method to produce *in vitro* shoots from nodal segments of trees of *Tecomella undulata* by culturing on MS medium supplemented with 0.05 mg l^{-1} IAA and 2.0 mg l^{-1} BA. Shoots were rooted on half strength MS liquid medium containing 2.5 mg l^{-1} IBA for 48 hours and than transferring to harmone free half strength MS medium. Nandwani *et al.* (1996) obtained multiple shoots from callus, on MS medium supplemented with IAA 0.1 mg l^{-1} and 2.5 mg BA l^{-1}. Soft and brown callus obtained from seedling explants regenerated more profusely. Regenerated shoots were pretreated with mixture of auxin (IAA, NAA, IBA) 5.0 mg l^{-1} for 24 h before transferring to MS medium (half strength devoid of growth regulators) for rooting.

2.1.39   *Tectona grandis*

Narasimhan and Dhruva (1970) during their study of different methods of culturing tissues of various tree species, found that teak tissues grow well on MS medium containing 1.0 mg l^{-1} glycine. Addition of gibberellic acid to medium resulted in good pigmentation. Gupta *et al.* (1980) successfully induced multiple shoots from excised seedling explant and terminal buds of 100 year elite *Tectona grandis* in 10-15 days on MS medium supplemented with 0.1 mg l^{-1} each of BA

and Kn (seedling explant) and on MS + Kn 0.5 mg l^{-1} and BA 1.0 mg l^{-1} (explant from mature tree). Rooting was obtained on low salt white's liquid medium supplemented with IAA, IBA and IPA at two concentration of each (0.1 mg l^{-1} and 2.0 mg l^{-1}). Addition of polytar (Polyvinylpyrrolidone), ascorbic acid, H$_2$O$_2$ etc. were used to reduce the blackening of explant and contained phenol. Lakshmisita and Chatopadhya (1986) have reported the development of embryo like structure in shoot and endosperm explant derived callus culture of teak.

Tiwari and Dharia (1989) based on the five year data on experimental plantation of tissue culture grown plants of teak at State Forest Research Institute, Jabalpur, found that the tissue culture grown plants are better in respect of survival percentage, collar diameter and height increment, when compared with seedling plants. Devi et al. (1994) collected shoot buds of different sizes from elite teak plants in different months round the year. Initially, shoot buds were cultured in agar gelled medium for three successive passages (24 hour each) to allow brown exudates to leach out. Finally shoot buds were transferred to establishment medium containing MS + 1.0 mg l^{-1} Kn and 1.0 mg l^{-1} BA along with adenine sulphate. Apical shoots of 30 year old elite teak plant, collected in December proved best to initiate culture in establishment medium. For multiplication shoots were initially grown in M-2 medium (MS + 1.0 mg l^{-1} Kn + 5.0 mg l^{-1} BA) for 7 days. During this period they formed 5-6 axillary shoot initials. Individual shoots from the cultures were then transferred to MS + 1.0 mg l^{-1} Kn + 1.0 mg l^{-1} BA medium for continued shoot elongation. Using this two step protocol, 216 shoots were obtained from an established explant in 81 days of culture. Shoots (2-3 cm) rooted (100%) when they were cultured for 72 hr on white liquid medium supplemented with 2.0 mg l^{-1} IBA followed by 21 days on white's salt solution without auxin.

Tiwari and Pandey (1995) established shoot cultures of teak from apical and seedling explants, cultures were raised on especially developed (revised) MS medium. Morphogenetic response of the shoot bud induction got boosted up significantly with addition of Kn 4.0 mg l^{-1} to 5.0 mg l^{-1} + IAA 2.0 mg l^{-1} to the

medium. Optional shoot differentiation has been achieved with 5.0 mg l^{-1} Kn and 2.0 mg l^{-1} of IAA in culture medium. Callus induction from leaf explants was observed when medium was supplemented with 0.5 mg l^{-1} 2,4-D but the shoot induction from callus were not observed on shoot induction medium. Root induction was maximum when medium contained IBA at the rate of 2.0 mg l^{-1}. Bo and Monteuuis (1996) developed protocol, giving rise to the production and acclimatization of several thousand teak plantlets. A mass propagation techniques was established for mass producing cloned tissue culture plantlet from mature genotype. Kushalkar and Sharan (1996) produced callus from apical buds, with globular and heart shaped somatic embryo on MS medium supplemented with BA 1.0 mg l^{-1} + 0.01 mg l^{-1} NAA and 3 per cent sucrose. Reddy *et al.* (1997) observed that vermiculite is more suitable for the germination of teak seed than MS medium under *in vitro* condition.

2.1.40   *Terminalia ivorensis*

Belaizi *et al.* (1992) cultured explant excised from nodules of *Terminalis ivorensis* on MS medium and growth and multiple shoots were obtained with 1.0 mg l^{-1} BA, 0.1 mg l^{-1} GA$_3$ and 0.01 mg l^{-1} IBA. Rooting was obtained on MS medium containing 5.0 mg l^{-1} IBA for first 7 days than shoots were transferred to the same medium without auxin.

2.1.41   *Vateria indica*

Divatar (1994) attempted for enhanced release of axillary buds, organogenesis and embryogenesis of Malabar White pine (*Vateria indica* L.) using axillary bud explants. Bud break and shoot production was mostly observed on media with 2ip and IBA. Callusing was obtained from leaf and internodal segments on MS and half strength MS containing 2ip and 2,4-D or 2ip and IBA respectively.

2.1.42   *Ziziphus*

Mathur *et al.* (1995) reported that stem explants from a mature tree of *Ziziphus maurtiana* when cultured on modified MS medium containing 3800 mg l^{-1} potassium nitrate and 2478 mg l^{-1} ammonium nitrate, 11 μM BA and 0.5 μM IAA produces 15-20 shoots per inoculam. Rooting was induced by pretreatment with 50 μM IBA or NAA for 24 hours followed by transfer to auxin free White's medium, plantlet grew well on soil and vermiculite.

2.2   Controlling factors in micropropagation

2.2.1   Culture medium

The nature and type of a cultural medium is a prominent factor that influence the success rate in the plant tissue culture. Nutritional requirement for optimal growth of a tissue *in vitro* may vary with the species. Even tissues from different parts of a plant may have different requirement for satisfactory growth (Murashige and Skoog, 1962). Selection of culture medium depends upon the tissue we are dealing with. This has given rise to large numbers, of media formulations during the past 25 years. A proper media should contain not only adequate quantity of major plant nutrients like salts of nitrogen, potassium, calcium, phosphorus, magnesium and sulphur as well as minor nutrients like salts of Iron, Manganese, Zinc, Boron, Copper, Molybdenum and Cobalt but also carbohydrate, usually sucrose, trace amount of organic compounds like vitamins, amino acids and plant growth regulators. Some culture perform better when supplemented with undefined organic compounds like coconut water, fruit juice, yeast extract and casein hydrolysate.

2.2.2   Basal media

Most of the species show considerable quality of growth differences, when grown on different basal media. So it become imperative to work out a medium that would fulfil the specific requirement of that species. Presently a vast number of media formulations for the culture of woody plants including forest

trees can be obtained (McCown and Sellmer, 1987). Some of the earliest plant tissue culture media eg. root culture medium of White (1943) and callus culture medium of Gautheret (1939), were developed from nutrient solutions previously used for whole plant culture. White evolved the medium from Uspenski and Uspenskaia's medium (1925) for algae and Gautheret's medium is based on Knop's (1865) salt solutions. All subsequent media formulations are based on White's and Gautheret's media (Bhojwani and Razdan, 1983). The typical features, which characterised the earlier media, were low concentration of inorganic ions especially potassium and nitrate and proving nitrogen solely in the form of nitrate. Later, the media developed for germination of orchid seeds were similar except that they contained ammonium ions (Knudson, 1946; Vacin and Went, 1949). Many workers have contributed by developing different formulations for different media. These media are named after them such as Hilderbrandt *et al.* (1946), Nitsch (1951), Heller (1953), Reinert and White (1956), Murashige and Skoog (1962), Linsmaier and Skoog (1965), Gamborg *et al.* (1968), Schenk and Hildebrandt (1972) etc.

A medium specially designed for tree species is the woody plant medium (WPM) of Llyod and McCown (Llyod and McCown, 1980). Compared with MS it is low in ammonium nitrate, potassium, chloride and high in sulphate. The B_5 medium originally designed for cell suspension or callus culture has, with modification proved valuable for protoplast culture (Gamborg and Shyluk, 1981). The SH medium is similar to B_5 but with slightly higher levels of mineral salts. The medium designated N_6 was developed for cereals anther culture and is used with success in other types of cereal tissue culture also (Chu, 1978). The E_1 medium support rapid growth of cells for embryogenesis and for the culture of protoplast (Gamborg *et al.*, 1983). In experiments with anther culture the medium devised by Nitsch and Nitsch (1969) is frequently used. After 1980, the most popular media have been DCR (Gupta and Durzan, 1985) and WPM (Lloyd and McCown, 1980) especially for woody species.

2.2.3   Growth regulators

In addition to the nutrient, it is generally necessary to add one or more growth substances. Proper selection and addition of growth regulator at an optimum level is one of the important factors for successful plant tissue culture (Karikarian, 1982). Plant growth regulators include naturally occurring hormones such as IAA, GA_3, BA, ABA etc. and also a number of synthetic chemicals that affect or control plant growth and development (Minocha, 1987). Skoog and Miller (1957) proposed the concept of hormonal control. Their classic experiments on tobacco pith cultures showed that root and bud initiation were conditioned by balance between auxin and cytokinin. High concentration of auxin promoted rooting where as proportionately more cytokinin initiated bud or shoot formation.

Following the discovery of auxin by Went (1926) and its chemical characterisation by Kogl et al. (1934) several workers found out its use in cell cultures for purposes like callus induction rooting etc. In Ceratonia siliqua the in vitro callusing behaviour of explants and exogenous content of some secondary metabolites in the callus were significantly effected by various types of auxins (NAA, 2,4-D, etc.) and Cytokinin (BA and Kn) which were incorporated to MS media. Using 2,4-D in combination with BA resulted in highest callusing frequency and heaviest mass of fresh callus, 2,4-D and Kn produced dry callus where as incorporation of NAA to the media produced green callus (Yousef, 1997). Media are mainly supplemented with various auxins such as IH-indole-3-acetic acid (IAA), 1,naphthalene acetic acid (NAA), IH-indole-3-butyric acid (IBA), 2,4-dichlorophenoxy acetic acid (2,4-D), naphthoxy acetic acid (NOA), 4-chlorophenoxy acetic acid (4-CPA), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2-methyl-4-chlorophenoxy acetic acid (MCPA), 4-amino-3,5,6-trichloro picolinic acid (Picloram) and 3,6-dichloro-2-methoxy benzoic acid (Dicamba).

Cytokinins and adenine derivatives are frequently used for shoot production, proliferation and elongation. In combination with auxin they produce

callus (Yousef, 1997). However, extent of effects of these growth regulators vary from species to species (Bon *et al.*, 1998). Singh and Mangia (1998) reported that multiple shoot formation did not occur without growth regulators in *Acacia tortilis*. The most frequently used cytokinins are 6 Furfuryl-aminopurine (Kinetin), N-6-Benzyl-adenine (BA), 6-(4 hydroxy-3 methyl-trans-2-butanyl amino) purine (zeatin) and N-6 (2 isopentyl) adenine (2ip). It was also discovered that N,N.diphenyl-urea (D.P.U), N-2 chloro-4-pyridyl-N-Phenyl urea (CPPU), N.phenyl-N-1,2,3-thiadiazol-5-yl urea (thidiazuron or TDZ) and other derivatives of diphenyl urea show the cytokinin type activity (Pierik, 1989). Shankla *et al.* (1994) found that TDZ in *Albizia julibrissin* is must for callus and shoot formation.

Gibberellins and abscisic acids are not in frequent use in tissue culture there are over 20 known gibberellins of these generally GA_3 is used. It is reported to stimulate normal development of plantlet from *in vitro* formed adventive embryo (Bhojwani and Razdan, 1983). Ethylene is also an important growth regulator but its specific role has not been described fully in the field of tissue culture.

2.2.4   Other media supplements

Extracts and preparation from different sources are often utilized as supplements to plant tissue culture media. Earlier their use in tissue culture media was common. The role of these organic compounds are unpredictable and therefore it has been recommended to avoid their use as far as possible (Gamborg and Shyluk, 1981). Much has been known about the behaviour of growth regulators their mode of action and the degree of compatibility with inorganic salt in the media still the supplements are commonly used. Most common supplements include casein hydrolysate, yeast, coconut water, tomato juice, corn endosperm and orange juice besides it also include adenine, adenine sulphate, pepetone etc. Skoog and Tsui (1948) found that AdS (Adenine Sulphate) when added to medium often enhance growth and shoot formation. Minocha (1987) has reported that the

addition of casein hydrolysate at 500 mg l^{-1} to the medium for the culture of shoot tip of paper bark birch cause significant increase in growth as measured by the number of leaves produced per plant. Einset (1978) found that the *in vitro* growth of explants from several citrus species was greatly stimulated by the addition of orange juice to the medium. Coconut water is reported to be having promoting effect on growth and differentiation in a wide variety of excised tissues (Vanoverbeek *et al.*, 1943). Hawker *et al.* (1973) reported that replacement of casein with coconut milk could double growth rate of callus in grape berries. Polyphenolic compounds like phloroglucinol in the medium has been found to be have a beneficial effect on organogenesis and growth (Hunter, 1979; Mallika *et al.*, 1992).

Use of activated charcoal in tissue culture media may be harmful on beneficial. There are contradictory reports which bolster this very facts. Growth, rooting, organogenesis and embryogenesis are reported to be stimulated in a wide variety of species and tissues (Wang and Huang, 1976).

Harmful effects of activated charcoal include binding of plant growth regulators and other metabolites (Weatherhead *et al.*, 1978). Activated charcoal added to liquid MS reduced IAA and IBA concentration by over 97 per cent (Scott *et al.*, 1990). Wann *et al.* (1997) reported that activated charcoal does not catalyse sucrose hydrolysis in tissue culture media during autoclaving. Although beneficial effects of activated charcoal have been documented, a complex substance and the entire range of its effects on tissue culture media and subsequent growth and morphogenesis of tissue culture is unknown.

2.2.5   Carbon energy source

Cultured plant cells require a source of carbohydrate. So there occurs a need for an exogenous supply of carbohydrate as a carbon and energy source in culture medium for the cultured cells. The early studies (Gautheret, 1941; Gautheret, 1945; Hilderbrandt *et al.*, 1949) had documented that a number of

carbohydrates could support growth, but over the years it has become apparent that sucrose is generally the best carbon and energy source (Street, 1969; Thorpe, 1982). It has been found that sucrose was the only sugar necessary for bud induction in *Pinus contorta* (Von and Eriksson, 1981). Despite the widespread use of sucrose, this compound is not always the most effective carbohydrate for shoot initiation. Fructose and glucose were found to be the best source for mulberry bud culture (Oka and Ohyama, 1982). Carbohydrate not only functions as a carbon source in metabolism but they also play an important role in the regulation of external osmotic potential (Brown and Thorpe, 1980). Lemos and Baker (1998) have reported the effect of sorbitol in *de nova* shoot from internodal explant of mature tree of *Annona muricata*. The choice and concentration of the sugar to be used depends mainly on the plant tissue to be cultured and the purpose of the experiment (Dodds and Robert, 1985). In *Alnus crimastogyne* type and concentration of sugar used in the multiplication medium were observed to be a critical factors for both multiple shoot induction and bud elongation (Tang *et al.*, 1995).

2.2.6   Vitamins

Vitamins are required in trace amount, though they are essential for the proper growth and development of the tissues. It is imperative to add the medium with appropriate vitamins and amino acid to achieve good growth of the tissue. The more frequently used vitamins include thiamine (B_1), nicotinic acid (B_3), Pyridoxine (B_6), Calcium pentothenate (B_5) and myo-inositol. Some consider that thiamine (vitamin B_1) may be the only essential vitamin for nearly all plant tissue cultures, whereas nicotinic acid (niacin) and pyridoxine (vitamin B_6) may stimulate growth (Ohira *et al.*, 1976). Linsmaier and Skoog (1965) demonstrated that most vitamin are not essential for callus growth in tobacco. Pyridoxine, biotin and nicotinic acid could be deleted from medium without serious impact on growth. Ascorbic acid which may be employed with other organic acids, is useful as an antioxidant to alleviate tissue browning (Reynold and Murashige, 1979).

2.3   Explant

2.3.1   Explant size and its position on the mother plant

The surface size, volume and cell number of an explant cell can affect the result obtained by using tissue culture. Intact plantlet could be regenerated only from *Manihot esculenta* shoot tip measuring over 2 mm in light, although tips less than 2 mm long produce only either callus or roots. In contrast, when eradication of viral infection is one of the culture objective, small explant should be used. When shoot tip explants of *Dianthus caryophyllus* were less than 2.0 mm only roots were induced. If explants measuring 7.5 mm long were used, virus could not be removed. So the optimum explants are 2-5 mm in length (Bhan, 1998). Pseudoterminal buds of *Betula uber*, approximate 5 mm long opened after 4 to 5 days of culturing and produced 3 leaves in a week where as 3-4 mm axillary bud too 10-20 days to increase in the same size (Vijayakumar *et al.*, 1990). Norton and Norton (1986) studied the effect of explant length (2.5 to 20 mm) axillary bud number (0 to 6) presence or absence of apex and explant derivation (top, middle or base of plant canopy) in prunus and spicea. The number of shoots formed after four weeks increased with the number of buds present.

The *in vitro* response may also vary with the position of the explant of the parent plant (Dhawan, 1993). In shoot tip culture of *Rosa rugosa* the success rate of terminal bud culture was higher than using laterals (Bhan, 1998). Culture of buds taken from stem part located close to tip yielded more callus than shoot where as axillary buds at distant position from apical buds yielded more shoots (Periera *et al.*, 1995). Explant taken from the top of the canopy produced most shoots but removal of apex did not affect the shoot number (Norton and Norton, 1986). Rahman and Blake (1988) observed in the jack that nodal explant proliferate more vigorously than shoot tip.

2.3.2   Age of explant

Ease of *in vitro* cloning of many species is a function of degree of juvenility of the explanted tissue (Bonga and Durzan, 1987). If the explant is more juvenile it is easy to propagate by vegetative means. Culture derived from explants of seedlings, terminal twigs of a 50 year old tree and basal sprout of other 50 year old had shown significant variations in response at establishment, shoot proliferation and rooting stages when cultured on the same media. Seedling and basal explant had shown maximum shoot length of *Lagerstromia parviflora* (Quraishi, 1997) in *Sorbus domestica* the shoot tip from either juvenile or adult plants were more responsive than axillary bud explant (Arrilaga *et al.*, 1991). Often some part of the tree may be mature or senescent, while other portion still display juvenile characteristics. Slow growth, low propagation rate and weak *in vitro* performance of mature explants as compared to juvenile shoot has been discussed in different tree species. Various forest tree species have been cloned by successfully by taking nodal and terminal bud explants from mature tree species such as *Azadirachta indica* (Eeswara *et al.*, 1997), *Tectona grandis* (Gupta *et al.*, 1980), *Dalbergia sissoo* (Datta and Datta, 1983) etc.

2.3.3   Season of collecting the explants

The season during which the explant is collected is one of the vital factor to be taken into consideration. Both the dormant period and the sprouting time of *in vitro* cultured citrus buds correspond to that occurring in the field conditions suggesting that buds about to sprout are suitable explant (Bhan, 1998). In teak the explants have to be collected in sprouting season (when they are fresh and green). The season when the explants are collected is very critical for the success of micropropagation of teak and explants can be collected only once in a year. This time vary from place. In Maharastra (Allappally forest) the sprouting season in April-June (John *et al.*, 1997). The nodal segments explants harvested during March-April and August-September-October were found to be the best for establishment of cultures of *Capparis decidua* (Deora and Shekhawat, 1995). The

explants from actively growing shoots at the beginning of the growing season generally give best result (Anderson, 1980). Yu (1991) observed that the test material from *Litchi chinensis* taken after 10 continuous rainy days had a contamination rate of cent per cent and that taken after 15 continuous sunny days had a low contamination rate (20 per cent). In *Litchi chinensis* contamination rate was found to be higher for the culture initiated during July-August and September-October. Successful plantlet production in chestnut was obtained with shoots taken during mid May (Chauvina and Salesser 1988).

2.3.4   Genotype

Successful application of *in vitro* technology to the production of clone depends upon inducibility of growth and differentiation in tissue of woody plants and the regeneration of true to the type viable plants in selected genotypes. Great differences exist in organogenesis, embryogenesis, and regeneration of plantlets among plant species, varieties and even individuals of the same varieties (Ahuja, 1983). In woody plants some materials are easier to regenerate *in vitro* than others (Chen and Ahuja, 1993). A strong genetic component has been implicated in regeneration capacity of tree species (Ahuja, 1983). McComb and Bennet (1982) observed that there was large difference in the capacity of explants from different selection of mature *Eucalyptus marginata* tree to survive in culture. Specific effect of genotype have been reported for *Sequoia sempervirens* (Sul and Korban, 1994).

Kallak *et al.* (1997) observed differences in shoot and root regeneration efficiency of carnation calli depending on genotype. The calli from different cultivars revealed significant differences in growth colour and structure.

2.3.5   Surface sterilization

Plant tissue culture media is rich in inorganic salts and organic nutrients including sucrose. This media is good substrate for the growth of many saprophytic bacteria and fungi. The potential sources of contamination in tissue

cultures are culture vials, nutrient media, plant tissue, instruments, and environment of transfer area, technicians and incubation room. The procedure for preparing sterile explants varies with the nature of the explant tissues. Lakshmisita and Raghava Swamy (1992) surface sterilized the nodal segments of rose wood (*Dalbergia latifolia*) with 0.1 per cent $HgCl_2$ for 15 minutes followed by three to four washes with sterile distill water. Harada and Murai (1996) surface sterilized the buds of *Prunus mume* (Japanese apricot) with 70 per cent ethyl alcohol in one minute and than with 0.25 per cent sodium hypochloride solution containing 0.05 per cent, Tween-20 for 15 minutes followed by three washes with sterile distill water. Ethyle alcohol, mercuric chloride, chlorine water, bromine water, silver nitrate, sodium hypochlorate, calcium hypochlorite, commercial bleach etc. are some common surface sterilants used in tissue culture. Addition of Tween-80 increases the efficiency of the sterilant and rinsing the tissue with 70 per cent ethanol serve as a good surfactant (Dhawan, 1993).

2.3.6   Systemic contaminant

The contamination can be caused by bacteria fungi or virus present on the surface of bark, glandular hairs at the nodes and internal tissues. It is easy to eliminate and contain the micro-organism present on the surface but contamination due to endogenously present microorganisms is hard to wipe out. It is a serious problem with woody plants. Perciea *et al.* (1995) watered the plant before 15 days of explant removal with 1 per cent w/v benomyl solution. Mallika *et al.* (1990) suggested that fungal infection of field explant of cocoa could be substantially controlled by prior fungicidal treatments of mother plants. Freshly prepared chlorine water was found to be an effective sterilant of the explant. Mallika *et al.* (1992) stressed that growing plants under controlled condition and regularly spraying the plant with systemic and contact fungicide can reduce or avoid contamination problems to certain extent. Addition of propamocrab hydrochloride to media yielded good result for eradication of fungal infection in *Eucalyptus grandis* (Watt *et al.*, 1995). Dodds and Roberts (1985) observed that it is preferable

to avoid the use of antibiotics for sterilization because they or their degradation product may metabolized by plant tissue with unpredictable result.

2.3.7 Browning of the medium

A serious problem associated with the culture of adult tissues from woody species is the oxidation of phenolic substances leached from the cut ends of the explants. Phenolics occur in plants in a variety of forms and constitute an exceedingly important group of secondary plant products. The oxidised products of phenols are brown to black in colour and are toxic to living tissues. Depending upon plant species the problem may be restricted to the time of establishing fresh culture or may perpetuate at each subculture. When the problem of browning occur only at the culture initiation stage explants are cultured in liquid medium containing antioxidant solution for few days and then transferred to semisolid medium (Dhawan, 1993). The shoot tip explant of *Psidium* (Amin and Jaiswal, 1987) and *Vaccinium angustifolium* were soaked in a solution containing 150 mg l^{-1} of citric acid and ascorbic acid prior to surface sterilization. Cysteine at 250 mg l^{-1} prevent meristmatic tip necrosis in pyrus (Dhawan, 1993). In *Tectona grandis* terminal and axillary buds were suspended in 2.0 per cent sucrose solution and 0.7 per cent soluble polyvinylpyrrolidone (PVP) in 100 ml conical flask on shaker at 100 rpm before planting in a 100 ml conical flask on a semisolid medium (Gupta *et al.*, 1980). For *Eucalyptus citridora*, the explants were first incubated in liquid medium in continuous light for 3 days and then transferred to fresh media of similar composition and maintained on a shaker for two days. The individual shoot were then excised and transferred to semisolid media for further multiplication (Gupta *et al.*, 1981). A combination of P.V.P. (0.5%) with citric acid (75 mg l^{-1}) and ascorbic acid 50 mg l^{-1} resulted in 80 per cent survival of gauva shoot explants (Amin and Jaiswal, 1988). In *Tectona*, insoluble P.V.P. proved superior to soluble P.V.P. (Gupta *et al.*, 1981). Incorporation of 2-3 per cent activated neutralized charcoal in the medium reduces the problem of browning by absorbing the toxic metabolites that are released to the medium. Repeated subculturing on a fresh

medium also help in avoiding browning problem in *Pyrus* (Banno *et al.*, 1989) and *Dalbergia latifolia* (Rai and Chandra, 1989).

2.4.   Culture environment

The physical form of the medium whether liquid or semisolid medium, pH, other environmental factors like light, temperature, R.H. and season of culture etc. play a vital role in *in vitro* growth and differentiation. Light requirement for differentiation involve a combination of several components namely intensity, quality and duration (Murashige, 1974). According to Murashige (1977) the optimum day light period required is 16 hours for wide range of plants. Changing the concentration of the gelling agent in the medium brings about changes in the physical condition of the medium. Importance of physical condition on culture is evident in case of *Dalbergia latifolia* (Mahato, 1992). In plant cell media the pH is generally adjusted to 5.7-5.8. In teak the optimum temperature was found to be 28±2°C at lower temperature suppression of growth and at higher temperature browning of shoots was observed. A 16 h protoperiod (1000 lux) was found suitable. Higher multiplication rate and healthy vigorous growth of shoot could be obtained under a continuous light region (1000 lux) although percentage of rooting and survival of these shoots were low (Mascarenhas *et al.*, 1993). Relative humidity is rarely a problem except in climates, where rapid drying occurs. The humidity of air is rarely controlled and when controlled 70 per cent has been found to be most frequent setting (Hu and Wang, 1983).

2.5   Rooting of *in vitro* formed shoots

Axillary and adventitious shoots are formed on cytokinin enriched media that favours shoot induction/multiplication. To obtain complete plantlets the roots are obtained either *in vitro* or *ex vitro*. For *in vitro* rooting the shoots are transferred to a rooting medium supplemented with auxins. Cold storage of kiwi fruit shoots improves rooting (Monette, 1987). Often where shoot multiplication was induced on full strength MS medium, the salt concentration was reduced to

half (Garland and Stoltz, 1981, Zimmerman and Broome, 1981) or a quarter (Skirvin and Chu, 1979) for rooting. Lowering nitrogen content has been observed to promote rooting (Rucker, 1982). root induction is always controlled by hormonal treatments. Among auxins NAA is most effective for rooting (Ancora *et al.*, 1981). Phloroglucinol improves rooting in apple (Zimmerman *et al.*, 1987) and Robinia (Donnelly and Daubeny, 1986) and arginine in cinchona (Sharma and Chaturvedi, 1988). Quraishi *et al.* (1997) obtained rooting in microshoot of *Lagerstromia parviflora*, obtained from seedling and basal sprout explant on MS medium with 4.9 μM IBA. Mallika (1990) used pulse treatment with 1000 mg l^{-1} IBA to the excised *in vitro* shoots before culturing them in half MS medium containing 1.0 per cent activated charcoal. Gupta *et al.* (1980) reported rooting in teak on low salt White's liquid medium containing IAA, IBA and NAA 0.1 mg l^{-1} each for 48 hours and transferred the excised shoots to auxin free White's liquid medium. Tang *et al.* (1995) obtained rooting in *Alnus cremastogyne* on half strength WPM either with IBA or without any growth regulators.

In some plants it has been possible to treat the shoot formed in culture as minicuttings and to root them *in vivo*. The cut end of the shoot is treated in a standard rooting powder (such as IBA in talc) and planted in the potting mix (Dhawan, 1993). In *Rhododendron* and blueberry the shoots can be rooted either *in vitro* or *in vivo* (Kyte and Briggs, 1979; Cohen and Elliott, 1979). *In vivo* rooting is reported for many more species eg. *Castanea sativa* (Mullins, 1987), *Actinidia chinensis* (Monette, 1987), *Lagerstromia indica* (Zhang and Davies, 1986) and *Alnus* sp. (Cremeire *et al.*, 1987). *In vivo* rooting is highly economical and simple but it is not always successful for many species such as *Leuceana*. Conventionally easy to root species such as bamboo and eucalyptus *in vivo* roots has proved unsuccessful (Dhawan, 1993). In teak application of commercial rooting hormone with fungicide (Bavistin) and talcum powder is very effective for rooting (Mascarenhas *et al.*, 1993).

2.6 Hardening and planting out

Transferring plants from the culture vessel to the potting mix require careful and step by procedure. The plantlets formed in the culture are deficient in photosynthetic efficiency and mechanism to control water loss. The humidity inside the culture vessel is very high (close to 95%) thus the plants lack the protective cuticle. Success in acclimatization depends upon not only post transfer condition but the pre-transfer culture conditions also (Ziv, 1986). The culture media is enriched with sucrose and other organic nutrients consequently plantlet though they appear green lack photosynthesis. Schacket *et al.* (1990) reported that stomata of apple shoots did not close after being removed from the culture, so it become imperative to harden such plant for success in planting out. Chalupa (1983) transferred rooted plantlets of *Robinia pseudoacacia* into the mixture of peat and perlite (1:1) and were grown under intermittent mist for 2-3 week. After hardening they were planted out. Wang *et al.* (1985) hardened the *in vitro* raised plantlets of *Robinia pseudoacacia* by removing the jar stopper for 3-4 days there after they were transferred to sandy soil. The field survival of these was 97.9 per cent. Mulberry (*Morus alba*) plantlets with well developed roots were washed thoroughly with tap water planted in pots filled with damp mixture of peat and sand (1:1) covered with polythene bags and acclimatized for a week by gradual lowering the humidity (Kanwar *et al.*, 1991). Hardening of *Robinia pseudoacacia* was done successfully in stratified potting mixture of sand and soil (1:1). Kanwar *et al.*, (1996) and Lemos and Blake (1996) reported successful transfer of *in vitro* raised plantlets of *Annona sequmosa* with 80 per cent survival. In teak *in vitro* rooted shoots were planted in a 3:3:1 soil:sand:compost mixture in small polybags covered with glass sheets (John *et al.*, 1997).

Materials and Methods

MATERIALS AND METHODS

The present investigation was carried out in the Tissue Culture Laboratory, Department of Tree Physiology and Breeding, College of Forestry, Vellanikkara from 1998 to 2000. The materials used and methods followed during the study are presented here.

3.1   Plant material

Axillary and terminal buds from one to two year old teak seedlings, which were established and maintained in the College nursery were used as explants.

3.2   Collection and preparation of explants

The mother plants growing under open field conditions are more vulnerable to microbial inoculam, which in turn contribute tremendously for high rate of contamination in micropropagation. Therefore, prophylactic sprays of a mixture of the systemic fungicide, Bavistin 50 per cent W.P. (Carbendazim) and contact fungicide, Indofil M-45 (Mancozeb) each at 0.1 per cent were given on alternate days to the source material except in rainy season.

Stem segments with 3-4 nodes were excised from mother plant with the help of secateurs. The stem cuttings were brought to the lab, defoliated and converted into nodal segment of different dimension (0.5-1.5 cm). The segments were then washed under running water for 2-3 minutes. After this they were washed thoroughly with teepol to remove all extraneous materials adhered to the segments followed by a dip in the fungicidal solution of contact and systemic fungicide (Bavistin 50 per cent W.P. and Indofil M-45 each at 0.1%) for 30 minutes. The material was later washed under running water and taken for surface sterilization.

3.3   Surface sterilization of explants

The process of surface sterilization was carried out in a Klenzaids laminar airflow cabinet, which was made sterile by the incessant exposure of germicidal U.V. rays for half an hour before use. The pretreated explants were taken in sterilized conical flask and immersed in the solution of mercuric chloride of different concentration (0.10, 0.15, 0.20%) and for different time duration (5, 8, 10, 12, 15, 20 minutes). A drop of teepol was added to the mercuric chloride as a wetting agent. The liquid was stirred by swirling to give proper contact of the chemical to the explant. The explants were washed three times with sterile water after they were taken out of the mercuric chloride solution. After thorough wash with sterile water the explants were spread on the presterilized petridishes lined with blotting paper for drying.

3.4   Culture media used

3.4.1   Composition of culture medium

The explants were cultured on agar solidified Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), half strength MS medium and woody plant medium (WPM) (Llyod and McCown, 1980). The chemical compositions of the different media are given in Table 1.

Various plant growth regulators and other media additives were supplemented to the basal media in different experiments. To check the fungal growth 2 ml l^{-1} Grisovin solution (1000 mg l^{-1}) was also added.

Besides auxins (IAA, NAA, IBA) and cytokinin (BA, Kn) individually or in combination at different concentrations, various other supplements like adenine sulphate (1-2 mg l^{-1}), casein hydrolysate (100-1500 mg l^{-1}), coconut water (10, 20%) and charcoal (0.25-1%) were also added to the basal media. Antioxidants like citric acid (150 mg l^{-1}) and ascorbic acid (150 mg l^{-1}), individually or in combination were also added.

Table 1. Chemical composition of Murashige and Skoog (MS) medium and
Woody Plant Medium (WPM)

Compound	Quantity (mg l^{-1})	
	Murashige and Skoog (MS)	Woody Plant Medium (WPM)
Inorganic		
Ammonium nitrate	1650	400.00
Boric acid	6.20	6.20
Calcium chloride-2- hydrate	440.00	96.00
Calcium nitrate-4- hydrate	0.00	556.00
Cobalt chloride-6-hydrate	0.025	0.00
Copper sulphate-5-hydrate	0.025	0.025
Ferrous sulphate-6-hydrate	27.80	27.80
Manganese sulphate-1-hydrate	22.30	22.30
Magnesium sulphate-7-hydrate	370.00	370.00
Na$_2$EDTA-2 hydrate	37.30	37.30
Potassium dihydrate phosphate	170.00	170.00
Potassium iodide	0.83	0.00
Potassium nitrate	190.00	0.00
Potassium sulphate	0.00	990.00
Sodium molybdate-2 hydrate	3.25	0.25
Zinc sulphate-7 hydrate	0.60	8.60
Organic		
Inositol	100.00	100.00
Nicotinic acid	0.50	0.50
Thiamine HCl	0.10	0.10
Pyridoxine HCl	0.50	0.10
Glycine	2.00	2.00
Others		
Sucrose (in percent w/v)	3.00	2.00
Agar (in per cent w/v)	0.70	0.70

½ MS denotes half amounts of the organic constituents, full amount of organic and
other constituents per litre of MS medium

3.4.2   Preparation of culture medium

Standard procedure as given by Gamborg and Shyluk (1981) was followed for the preparation of media. The chemicals used for preparation of media were of analytical grade from SISCO Research Laboratories (S.R.L.), Merck or Sigma. Stock solution of major and minor nutrients were prepared by dissolving the required quantity of the chemicals in distilled water and were stored in amber coloured bottles under refrigerated conditions. The stock solutions of nutrients were prepared freshly every four weeks and that of vitamin and growth regulators once every week.

The stock solutions in the required quantity were pipetted out into a steel vessel containing distilled water. Inositol and sugar were than dissolved in the solution in appropriate concentrations. The whole solution was made up to the required volume. The pH of the solution was adjusted to the range 5.7-5.8 (using 1N NaOH or 1N HCl) with the help of pH meter. Weighed quantity of agar was added to this and mixed well.

The solution was then heated on a gas burner or in a microwave oven to dissolve the agar. The hot media (approximately 20 ml/tube) was poured into the well washed, oven dried culture tubes (150 mm x 25 mm). The culture tubes were than plugged tightly with non-absorbent cotton wool plugs.

3.4.3   Sterilization of culture medium

The sterilization of the culture medium was carried out in an autoclave at pressure of 1.06 kg cm^{-2} for 15 minutes at 121°C (Dodds and Roberts, 1988). After sterilization, the culture tubes were stored in an air-conditioned culture room until further use.

3.5 Sterilization of equipments

All metal and glass instruments and other accessories used in the transfer cabinet were wrapped in aluminium foil and sterilized in an autoclave at 1.06 kg cm^{-2} pressure for 15-20 minutes at 121°C temperature. Scalpels, scissors, forceps etc. used were again dipped in alcohol and flamed on a sprit lamp at the time of use.

3.6 Inoculation of explants

The inoculation was carried out under strict aseptic condition inside the transfer cabinet. The cotton plug of the culture tube was removed in front of the flame of a gas burner, kept inside the cabinet. The tube neck was thoroughly flamed. The sterile explant was quickly transferred into the medium with the help of a sterile forceps. The neck of the culture tube was again flamed and the cotton plug replaced. In order to curtail contamination during drying and inoculation only few explants were treated at a time.

3.7 Culture conditions

The cultures were incubated in a culture room provided with cool, white fluorescent (Philips) lamps to give a light intensity of 2000 lux for 16 hours light period or in complete darkness for the initial eight to ten days of the culture to over come the phenol problem. The temperature was maintained at 25°C±2°C.

3.8 Treatments to contain, eliminate or neutralize polyphenols

Exudation of polyphenols from the explants as indicated by the browning of the culture medium was noticed in most of the cultures. In order to check this problem, the following treatments were carried out.

a) Addition of ascorbic acid (150 mg l^{-1}) into the media.

b) Addition of citric acid (150 mg l^{-1}) into the media.

c) Addition of citric acid and ascorbic acid (150 mg l^{-1}) into the medium.

d) Addition of charcoal (0.25 – 1.0 g l^{-1}) in the media.

e) Dark incubation of cultures for two weeks.

3.9 Shoot induction and growth

For induction of shoots various treatment combinations (180) were tried. Details of the combinations are given in the Table 2 and 3.

3.10 Rooting of shoots

3.10.1 Rooting *in vitro*

The *in vitro* regenerated shoots were cultured in certain selected growth regulator combinations in ½ MS and WPM. The rooting treatments attempted are listed in table 4. Activated charcoal (0.25% w/v) was added to the media for providing dark conditions. Pulse treatment was given by dipping the cut end of freshly excised shoot in sterile 1000 mg l^{-1} IAA solution for 2-3 min. before inoculation. Shoots were transferred to auxin free media after 7 days.

3.10.2 *Ex vitro* rooting

Efforts were made to induce *ex vitro* rooting in the *in vitro* induced shoots. Various media tried for *ex vitro* rooting were

1) Sand
2) Cocopeat
3) Vermiculite

All these media were sterilized and taken in small paper cups. The bases of in vitro shoots were cut with a sharp blade. The shoots were given a dip for few minutes in a fungicidal solution. After washing with distilled water, the shoots were given dip in 1000 mg l^{-1} IAA solution for two minutes followed by a dip in IAA powder. The shoots were planted in different media and pots were covered with polythene cover to provide 100 per cent humidity. After 15 days a few holes were made in the polythene bags. Then the polythene bags were completely removed for four to five hours per day over a period of one week. Later on, the polythene bags were completely removed and the plantlets were kept open.

Table 2. Growth regulator combination tried for bud break and shoot growth from axillary and terminal buds of *Tectona grandis* L. in MS

Basal media	Growth regulators combination									
	In mg l⁻¹								In %	
	BA	Kn	2ip	NAA	IAA	IBA	CH	AdS	AC	CW
MS	-	-	-	-	-	-				
MS	0.1, 05 1.0, 1.5 2.0, 2.5 3.0, 4.0	-	-	-	-	-	-	-	-	-
MS	-	0.1, 05 1.0, 1.5 2.0, 2.5 3.0, 3.5	-	-	-	-	-	-	-	-
MS	-	-	-	0.1, 0.5 1.0, 1.5	-	-	-	-	-	-
MS	-	-	-	-	0.1, 0.5 1.0, 1.5	-	-	-	-	-
MS	-	-	-	-	-	0.1, 0.5 1.0, 1.5	-	-	-	-
MS	1.0, 1.5 2.0	-	-	0.1, 0.5 1.0, 1.5	-	-	-	-	-	-
MS	1.0, 1.5 2.0	-	-	-	0.1, 0.5 1.0, 1.5	-	-	-	-	-
MS	1.0, 1.5 2.0	-	-	-	-	0.1, 0.5 1.0, 1.5	-	-	-	-
MS	-	0.5, 1.0 1.5	-	0.1, 0.5 1.0, 1.5	-	-	-	-	-	-

MS	-	0.5, 1.0 1.5	-	-	0.1, 0.5 1.0, 1.5	-	-	-	-	-
MS	-	0.1, 1.0 1.5	-	-	-	0.1, 0.5 1.0, 1.5	-	-	-	-
MS	1.0	-	0.25, 1.00 3.0, 5.0	-	0.1	-	-	-	-	-
MS	-	1.0	0.25, 1.0 3.0, 5.0	-	0.5	-	-	-	-	-
MS	0.05, 0.1 0.2, 0.3	0.05, 0.1 0.2, 0.3	-	-	-	-	-	-	-	-
MS	1.0	-	-	-	0.1	-	100, 500, 1000, 1500	-	-	-
MS	1.0				0.1	-	-	-	0.25, 1.0	
MS	1.0	-	-	-	0.1	-	-	1.0, 4.0, 8.0, 12.0	-	-
MS	1.0	-	-	-	0.1	-	-	-	-	10.0, 20.0
MS	-	1.0	-	-	0.5	-	100, 500, 1000, 1500	-	-	-
MS	-	1.0	-	-	0.5	-	-	-	0.25, 1.0	-
MS	-	1.0	-	-	0.5	-	-	1.0, 4.0, 8.0, 12.0	-	-
MS	-	1.0	-	-	0.5	-	-	-	-	10.0, 20.0

Table 3. Growth regulator combination tried for bud break and shoot growth from axillary and terminal buds of *Tectona grandis* L. in WPM

Basal media	Growth regulators combination mg l^{-1}					
	BA	Kn	2ip	NAA	IAA	IBA
WPM	-	-	-	-	-	-
WPM	0.1, 05 1.0, 1.5 2.0, 2.5 3.0, 4.0	-	-	-	-	-
WPM	-	0.1, 0.5 1.0, 1.5 2.0, 2.5 3.0, 4.0	-	-	-	-
WPM	-	-	-	0.1, 0.5 1.0, 1.5	-	-
WPM	-	-	-	-	0.1, 0.5 1.0, 1.5	-
WPM	-	-	-	-	-	0.1, 0.5 1.0, 1.5

Table 4. Media combination tried for *in vitro* roots induction from microshoots
of *Tectona grandis*

Basal media	Auxins (mg l⁻¹)			AC (%)	Pulse treatment
	IBA	NAA	IAA		
½ MS (liquid)	2.0	-	2.0	-	+
½ MS	2.0	-	2.0	0.25	+
½ MS	2.0	-	2.0	0.25	-
½ MS	-	0.4	4.0	0.25	-
½ MS	-	0.4	4.0	0.25	+
½ MS	0.4	-	4.0	0.25	+
½ MS	0.4	-	4.0	0.25	-
½ MS	0.1	0.1	0.1	0.25	+
½ MS	0.1	0.1	0.1	0.25	-
WPM	0.5	-	0.5	-	+
WPM	0.5	-	0.5	-	-
WPM	0.1	0.1	0.1	-	+
WPM	0.1	0.1	0.1	-	+

3.11   Acclimatization

In vitro raised plantlets were carefully taken out of the test tubes without damaging their roots. The roots were washed gently under running tap water to remove the adhering medium. The roots were dipped in 0.1 per cent solution of Bavistin and Indofil M-45 to avoid fungal attack and plantlets were transferred into pots containing sterile sand, cocopeat or vermiculite. The pots were then covered with polythene bags to maintain humidity. After one week, holes were made in polythene bags to reduce the humidity. The plantlets were gradually exposed to natural milieu by keeping them open (without polythene cover) for few hours a day. Later the polythene covers were completely removed.

3.12   Observations

Each trial was carried out with minimum 10 tubes replicated three times. The observations were recorded for 2 months after inoculation. The data were calculated based on cultures that remained uncontaminated after the required period of incubation. The following observations were recorded from various experiments.

(1) Number of cultures uncontaminated

This was recorded for different surface sterilization treatments. Numbers of cultures free from contamination were expressed as percentage of total number of cultures.

(2) Number of explants showing bud break

Number of cultures showing bud initiations were expressed as a percentage of total number of surviving cultures. A culture was said to have bud initiation when the dormant axillary bud has just emerged.

(3) Time taken for bud break

The time taken (in days) for bud initiation was noted.

(4) Number of explants showing leaf production

Number of cultures that produced leaves was expressed as percentage of total surviving culture that produce bud.

(5) Time taken for leaf production

This was recorded in days.

(6) Number of explants with shoot development

Numbers of explants with shoot development were expressed as percentage of total number of surviving cultures.

(7) Number of shoots per culture

Average numbers of shoots per culture were expressed as average of the number of shoots produced in different replications.

(8) Shoot length

This was expressed as average of all shoots produced in different replications.

(9) Number of cultures rooted *in vitro*

Number of cultures that produced roots *in vitro* was expressed as percentage of total cultures in a particular combination tried.

(10) Number of shoots rooted *ex vitro.*

This *was* expressed as the percentage of total shoots tried for *ex vitro* rooting in a treatment.

(11) Number of roots

Numbers of roots were expressed as an average number of roots in the related cultures.

(12) Production of callus

Observations on production of callus at the base of nodal segment were made.

3.13   Statistical analysis

Wherever necessary the data were statistically analysed by CRD (Snedecor and Cochron, 1967) with one factor or two factors as the case may be. Treatment means were compared using Duncan's Multiple Range Test (Duncan, 1955).

Results

RESULTS

The various experiments on micropropagation of teak (*Tectona grandis*) were conducted at the tissue culture laboratory of College of Forestry, Vellanikkara during 1998-2000. The obtained at present in this chapter.

4.1   Surface sterilization of explants

The explants of teak were subjected to various surface sterilization treatments to reduce the culture contamination. The results obtained are tabulated in Table 5. It was found that the treatment effect did not differ significantly. However, a 15 minute sterilization treatment in 0.15 per cent mercuric chloride preceded by prophylactic spray of fungicide (0.1% Bavistin and 0.1% Indofil) to the mother plants in the field and 30 min dip of the explant in the fungicidal mixture of Bavistin (0.1%) and Indofil (0.1%) was found to be better than all other treatments. The culture contamination was as high as 91.65 per cent after surface sterilization with 0.15 per cent $HgCl_2$ for 5 minutes.

It was observed that size of the explants had a pronounced effect on culture contamination. The percentage of contamination in different size of explants, treated with 0.15 per cent mercuric chloride for 15 min is presented in Table 6 and Fig.1. The contamination was found as high as 68.78 per cent when 2.0 cm long (below node) explants were used. The percentage of contamination was 46.00 per cent in cultures with 1.0 cm long explants.

4.2   Phenol exudation

A serious problem associated with the teak tissue culture is excessive exudation of phenolic substances from the cut ends of the explants. Various treatments were tried to contain phenol exudation. The results obtained are presented in Table 7 and Fig. 2 (Plate 4). It was found that 94.44 per cent of cultures exhibited phenol exudation when explants of different sizes were cultured

Table 5. Effect of various surface sterilization treatments on culture contamination of *Tectona grandis*

Duration of treatment (min)	Percentage of contamination with different treatments			
	HgCl$_2$ 0.10	HgCl$_2$ 0.15	HgCl$_2$ 0.20	Bavistin (0.1%) + Indofil (0.1% dip for 30 min + (HgCl$_2$ 0.15%)
5	87.50	91.65	89.575	-
8	91.50	75.00	85.42	-
10	83.00	85.40	85.40	-
12	81.25	62.52	70.82	45.00
15	86.50	85.42	79.17	20.00
20	70.50	66.67	72.90	-

Table 6. Effect of mercuric chloride (0.15%) on culture contamination of three different sizes of explants of *Tectona grandis*

Size and characteristics of explant	Percentage of contamination
1.0 cm below the node	46.00
1.0 cm above and 1.0 cm below the node	54.00
2.0 cm below the node	68.78
SEm±	7.19
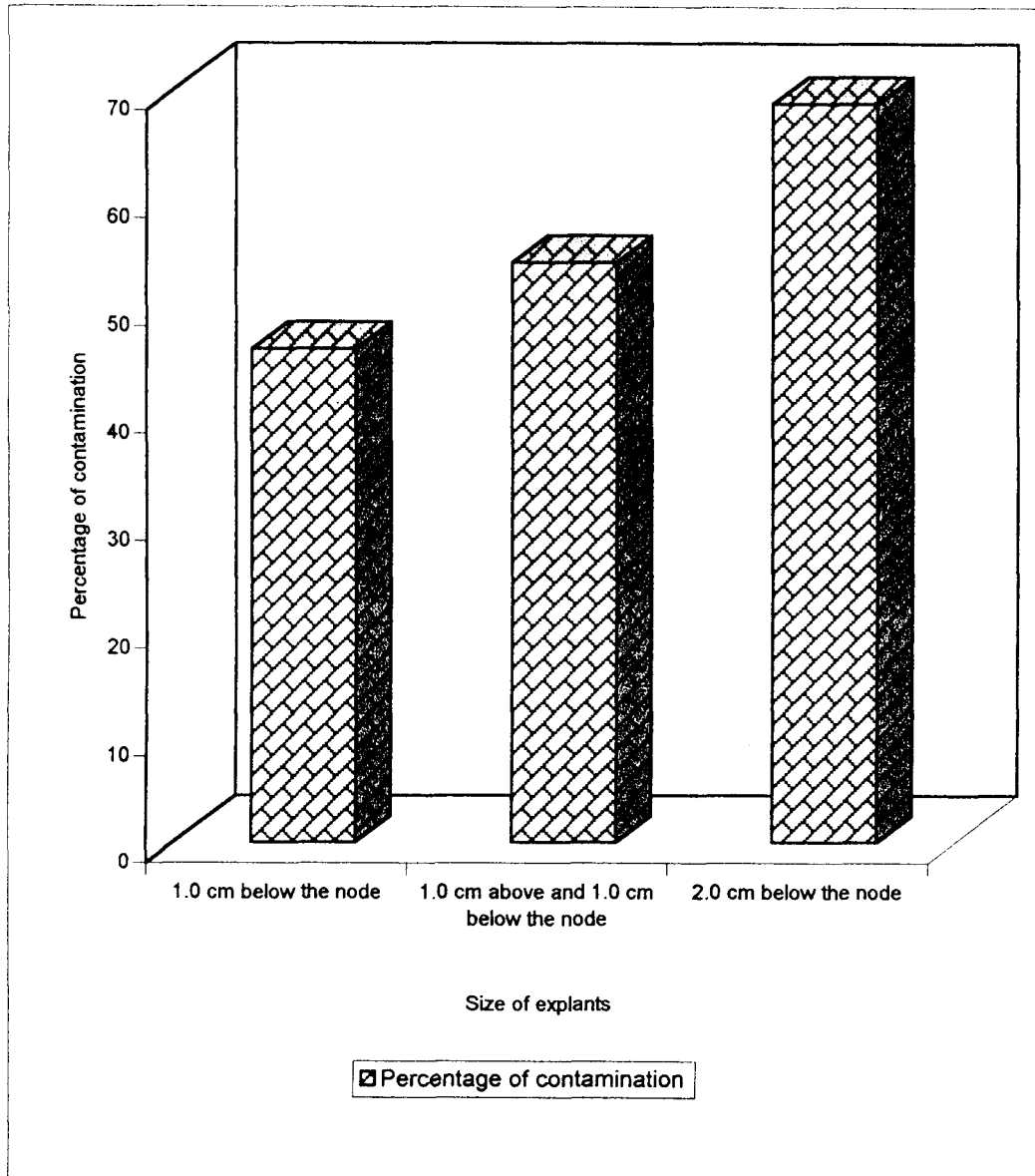
Fig.1 **Effect of mercuric chloride (0.15%) on culture contamination of three different sizes of explants of *Tectona grandis***

Table 7. Effect of various treatments on phenol exudation in three different sizes of explants

Treatment	Percentage of culture showing phenol exudation in different explant sizes			
	1.0 cm long below the node	1.0 cm above and 1.0 cm below the node	2.0 cm below the node	Mean
150 mg l^{-1} ascorbic acid in media	33.33 (+)	60.00 (+)	100.00 (++)	64.00
150 mg l^{-1} citric acid in media	83.33 (+)	100.00 (++)	100.00 (++)	94.44
150 mg l^{-1} citric acid and 150 mg l^{-1} ascorbic acid in media	14.28 (+)	50.00 (+)	100.00 (++)	54.76
1.0 gm l^{-1} activated charcoal in media	40.00 (+)	80.00 (+)	80.00 (+)	66.66
Keeping cultures in dark for initial 20 days	0.00 (-)	0.00 (-)	20.00 (+)	20.00

-      Negligible quantity
+   Low quantity
++   High quantity
+++   Very high quantity
Quantity of phenol exudation is shown in parenthesis
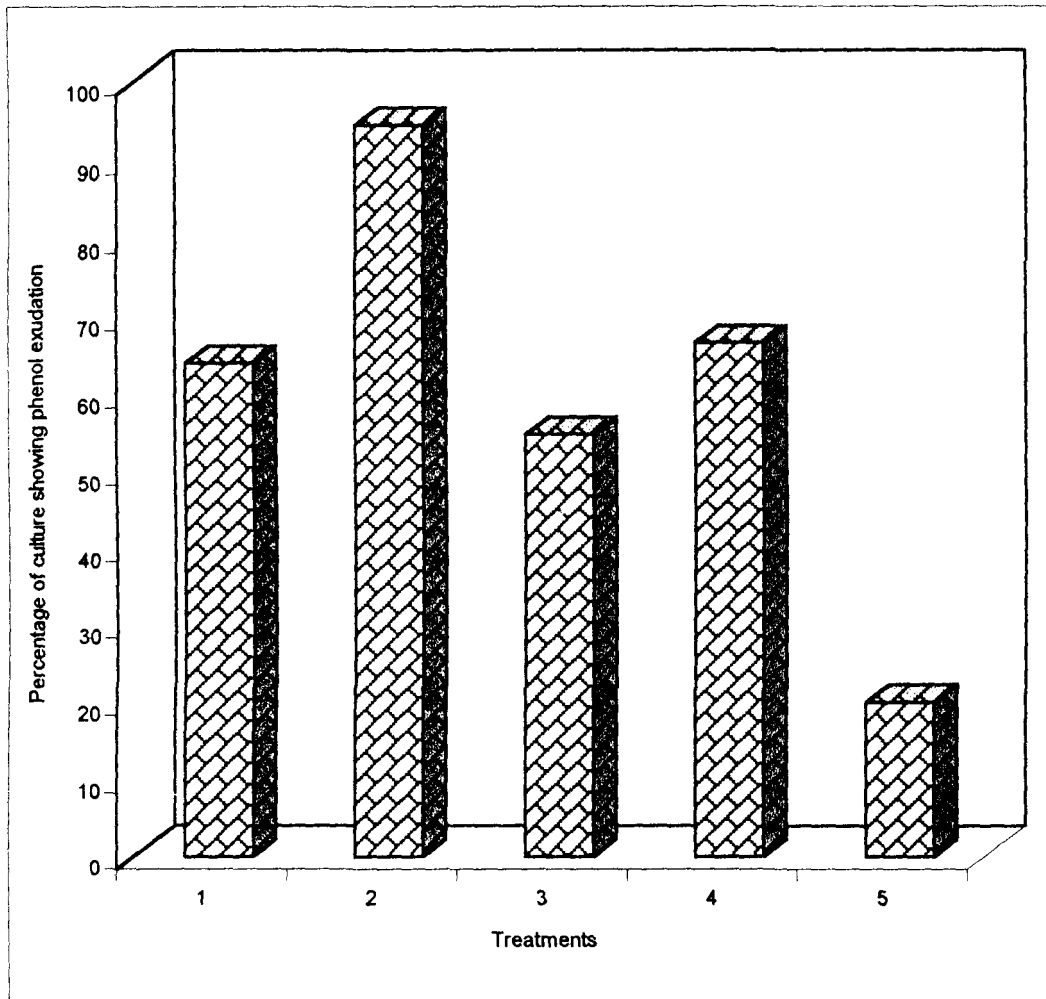
1 - 150 mg I-1 ascorbic acid in media
2 - 150 mg I-1 citric acid in media
3 - 150 mg I-1 citric acid and 150 mg I-1 ascorbic acid in media
4 - 1.0 gm I-1 activated charcoal in media
5 - Keeping cultures in dark for initial 20 days

Fig. 2   Effect of various treatments on phenol exudation

on the media containing 150 mg l^{-1} citric acid. However, when explants were given dark incubation in the initial 20 days, the percentage of cultures showing phenol exudation went all time low to 20.0 per cent. In this all treatments the cultured explants turned dark without phenol exudation and did not show any morphogenic or callogenic response. A comparison of explants of different sizes has revealed that cultures with smallest size (1.0 cm) exhibited the minimum amount of phenol exudation in all types of treatments.

Percentage of cultures showing phenol exudation was also lowest in the small explants. The percentage of cultures with phenol exudation was as low as 14.28 per cent when explants of size 1.0 cm long (below node) were cultured on MS media supplemented with ascorbic acid and citric acid at 150 mg l^{-1} each. Negligible quantity of phenol was found when cultures were given dark incubation in the initial period.

4.3 Effect of different basal media on culture establishment and growth in axillary and terminal buds

Three basal media namely MS, WPM and half strength MS were used in the present study to evaluate their suitability for culture establishment in *Tectona grandis*. Their comparative effects on various parameters of growth and development of explants are presented in Table 8 and Fig.3 (Plates 1, 2 & 3). The treatment effect was found to be non-significant. However, the percentage of bud initiation was found to be the highest in WPM (49.30) followed by MS (47.65) and lowest in ½ MS (33.48).

Percentage of leaf initiation was also higher in WPM (49.97) followed by ½ MS (46.66). The lowest was recorded in MS (31.11%). The bud initiation was quite early in the explants cultured on MS (9.0 days) followed by WPM (12.00 days). Maximum mean number of leaves were found to be 9.33 in MS where as it was 8.33 and 6.53 in case of WPM and ½ MS respectively. After a comparitive

Table 8. Effect of different basal media on culture establishment and growth in axillary ad terminal buds of *Tectona grandis*

Basal media	Percentage of		Period (days)		Mean no. of leaves
	Bud initiation	Leaf initiation	Bud initiation	Leaf initiation	
WPM	49.30	49.97	12.00	21.00	8.33
½ MS	33.48	46.66	19.00	27.00	6.53
MS	47.65	31.11	9.00	31.00	9.33
SEm±	4.82 NS	8.61 NS	2.28 NS	2.28 NS	0.66 NS
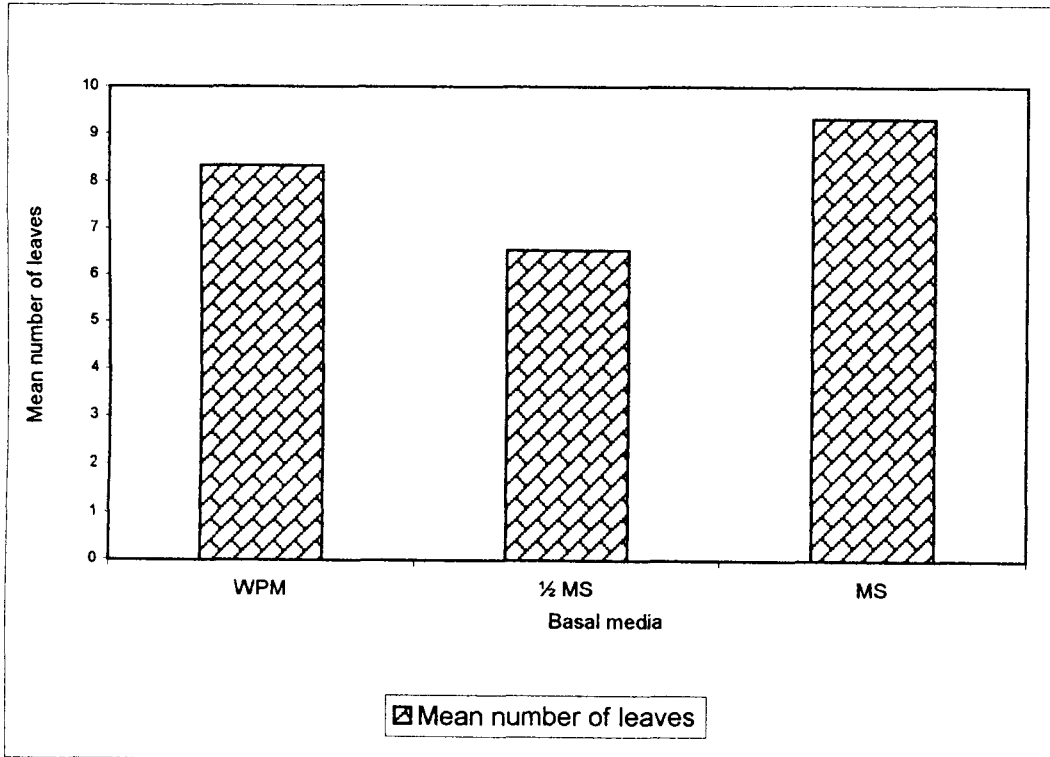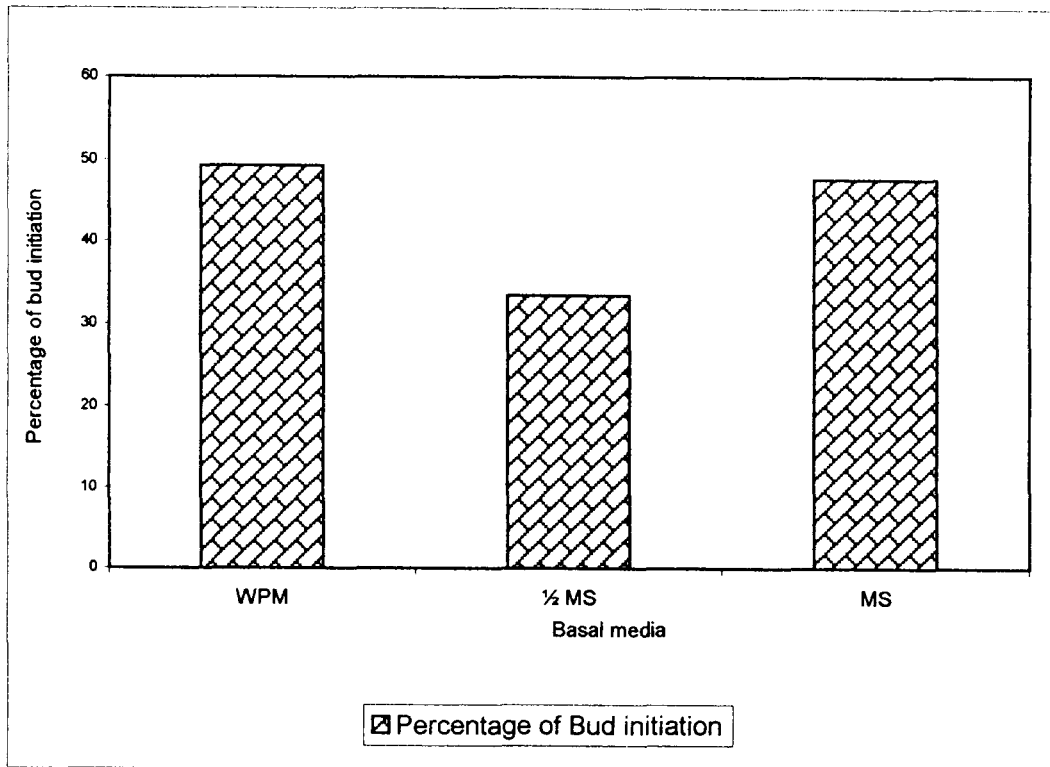
Fig. 3 Effect of various basal media on bud initiation/
production of number of leaves

perusal of various growth parameters in different media, two basal media, MS and WPM, were selected for further studies (Plate 5.)

4.4 Effect of growth regulators

Medium MS and WPM were selected and these media were supplemented with various growth regulators such as cytokinin, auxins and other growth supplements (adenine sulphate, casein hydrolysate, activated charcoal, coconut water) at different concentration either separately or in combination for further studies.

4.4.1 Effect of different concentration of BA on culture establishment and growth in MS media

The MS media was supplemented with eight different concentrations of BA to study their effect on different growth parameters. The result obtained is presented in Table 9 and Fig.4.

Percentage of bud initiation was 66.66 per cent when MS medium was supplemented with BA at 0.1 or 2.0 mg l^{-1}. However, in general, bud initiation percentage was higher when MS medium was supplemented with low concentration whereas it decreased to 22.22 per cent when MS was supplemented with 3.0 mg l^{-1} BA.

Percentage of leaf initiation also followed the same trend as bud initiation. It was 83.33 per cent in MS + 0.1 mg l^{-1} BA, whereas there was no leaf initiation when MS was supplemented with higher concentration of BA (3.0 and 4.0 mg l^{-1}).

Treatment effect on the time taken for bud initiation was found non-significant. However explants on media with higher concentration of BA took generally less time for bud initiation as compared to explants on media with low concentration of BA. Days taken for bud initiation were as high as 19 when MS

Table 9: Effect of different concentration of BA on culture establishment and growth in axillary and terminal bud cultures of *Tectona grandis* in MS media

BA mg l^{-1}	Percentage of		Period (days) for		Mean no. of leaves per explant	Mean no. of shoots/ bud sprouts per explant	Mean shoot length (cm)	Maxi- mum no. of shoots
	Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
0.1	66.66	83.33	15.00	23.00	9.20	1.60	4.00	2.00
0.5	57.11	62.50	19.00	26.00	10.40	1.80	1.25	2.00
1.0	56.25	55.55	17.00	35.00	8.80	2.20	1.93	5.00
1.5	62.50	60.00	16.00	19.00	7.66	1.83	2.70	2.00
2.0	66.66	78.57	13.00	27.00	7.30	2.00	1.80	3.00
2.5	57.14	25.00	11.00	14.00	2.00	2.00	0.00	2.00
3.0	22.22	00.00	10.00	0.00	0.00	2.00	0.00	2.00
4.0	28.57	00.00	10.00	0.00	0.00	2.00	0.00	2.00
SEm±			1.21 NS	2.59 NS	0.98 NS	0.12 NS		
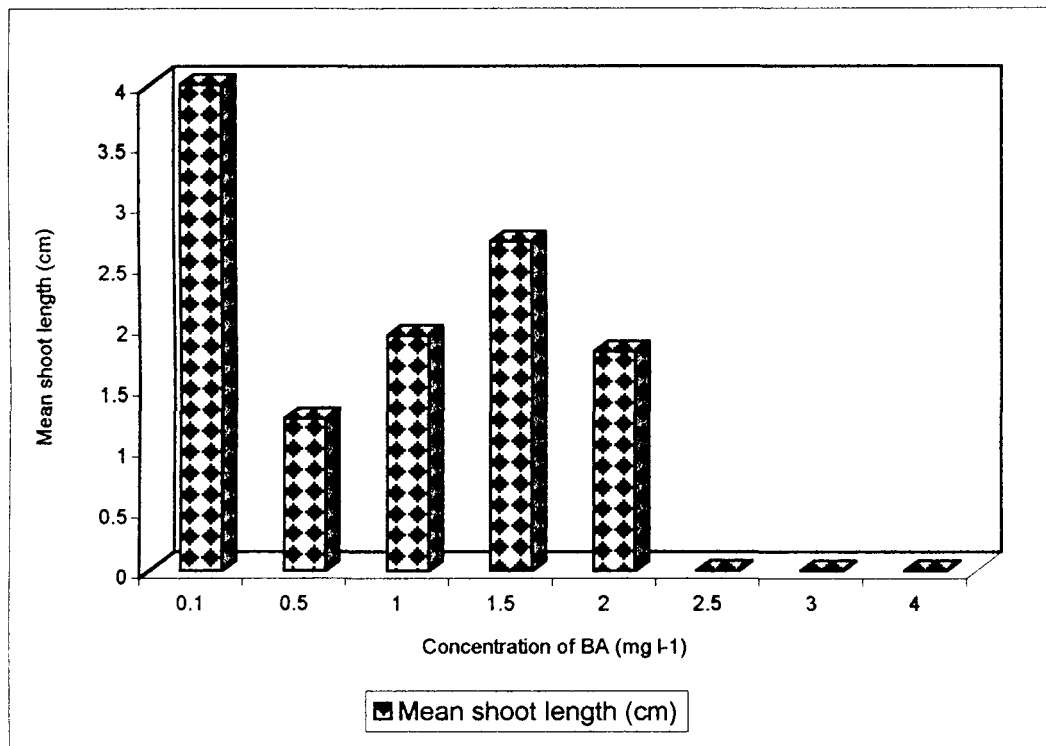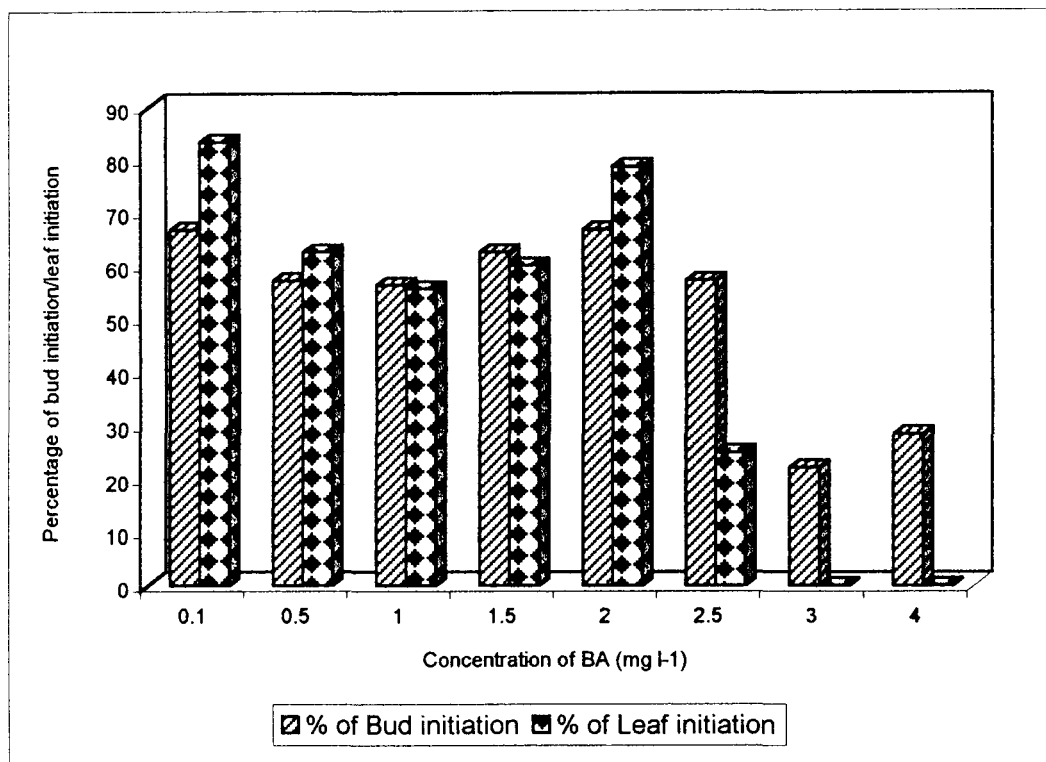
Fig. 4   Effect of different concentrations of BA on bud/leaf initiation
and shoot elongation in MS medium

media was supplemented with 0.5 mg l^{-1} BA and as low as 10 days when BA at the levels of 3.0 and 4.0 mg l^{-1} were used. Leaf initiation were delayed upto 35 days, when MS media was supplemented with 1.0 mg l^{-1} BA followed by 27 days at 2.0 mg l^{-1} BA. With reference to leaves, the mean number per explant was more when BA was added to media at lower concentration. Treatment effect on mean number of leaves was however non significant. Maximum leaves (10.40) were found when media contained BA at 0.5 mg l^{-1}, whereas no leaf formation took place on MS + 4.0 mg l^{-1} BA.

Treatment effect on mean number of shoots was found to be non significant. The media with 1.0 mg l^{-1} BA produced a mean number of 2.20 shoots per shoot. Maximum mean height (2.70 cm) was found on media containing 1.5 mg l^{-1} BA, whereas no elongation in the shoots in media with higher concentration of BA, was noted. Maximum number of shoots per explant was recorded as 5.0 in MS + 1.0 mg l^{-1} BA (Plate 7).

4.4.2 Effect of different concentration of Kn on culture establishment and growth in MS media

Eight levels of Kn were supplemented to MS medium. Their effect on growth and culture establishment is presented in Table 10. Percentage of bud initiation was found to be higher when Kn was used at lower concentrations. Maximum of 62.50 per cent of bud initiation was noted on media with 1.0 mg l^{-1} Kn. Hundred per cent of explants showing bud initiation produced leaves in MS + 3.5 mg l^{-1} Kn, whereas only 40 per cent of total explants showing bud initiation could produce leaves on media containing 0.1 mg l^{-1} Kn.

Effect of treatment on time taken for bud initiation and leaf initiation was found non significant. Time taken for leaf initiation was also non significant and ranged between 20 days in media containing 20.0 mg l^{-1} Kn to 12 days in media with 1.0, 1.5 and 3.5 mg l^{-1}.

Table 10. Effect of different concentration of Kn on culture establishment and growth in axillary and terminal bud cultures of *Tectona grandis* in MS media

Kn mg l^{-1}	Percentage of		Period (days) for		Mean no. of leaves per explant	Mean no. of shoots/ bud sprouts per explant	Mean shoot length (cm)	Maxi-mum no. of shoots
	Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
0.1	26.31	40.00	12.00	13.00	9.00	1.50	0.50	2.00
0.5	50.00	50.00	12.00	14.00	3.50	1.75	1.75	2.00
1.0	62.50	40.00	8.00	12.00	3.00	1.50	1.50	2.00
1.5	55.55	60.00	11.00	12.00	7.33	1.66	1.66	2.00
2.0	22.00	00.00	28.00	00.00	0.00	2.00	2.00	2.00
2.5	37.50	86.66	10.00	15.00	4.00	2.00	2.00	2.00
3.0	20.00	100.00	8.00	12.00	4.00	2.00	2.00	2.00
4.0	33.33	50.00	12.00	20.00	6.00	2.00	2.00	2.00
SEm±			1.31 NS	1.34 NS	1.33 NS	0.11 NS		

Maximum number of leaves per shoot (9.00) were recorded on media with 0.1 mg l^{-1} Kn and the least were found on media with 1.0 mg l^{-1} Kn. Mean height was found to be maximum (0.75 cm) on media with 0.5 and 2.5 mg l^{-1} Kn.

4.4.3   Effect of various concentration of Kn on culture establishment and growth in WPM

Eight different levels of Kn were added to WPM. Their effect on culture establishment and growth parameters are presented in Table 11. Percentage of bud initiation was found high in media with lower concentration of Kn. Highest percentage (66.66) of cultures exhibited bud initiation in media containing 0.5 mg l^{-1} Kn. The least cultures i.e. 25.00 per cent and 27.00 per cent, initiated buds on media with 1.0 and 4.0 mg l^{-1} Kn, respectively. Same trend was noted in leaf initiation. Seventy five per cent of explants producing buds produced leaves on media containing 0.1 mg l^{-1} Kn. There was no leaf initiation on media with 3.0 mg l^{-1} Kn and only 33.33 per cent of explants exhibiting bud initiation, produced leaves on media supplemented with 4.0 mg l^{-1} and 2.5 mg l^{-1} Kn. While explants on media containing 2.5 and 2.0 mg l^{-1} Kn produced buds in 11 and 13 days respectively, it took 27 days on media containing 3.5 mg l^{-1} kn. Leaf initiation was observed after 16 days on media with 2.0 and 2.5 mg l^{-1} Kn. It took 53 days in explants cultured on media containing 3.5 mg l^{-1} Kn.

Effect of treatment on mean number of leaves per explant was found non significant. However, highest number of leaves i.e. 10.67 and 10.00 were recorded on media containing 3.5 and 1.0 mg l^{-1} Kn, respectively. Effect of the treatment on mean number of shoots were also found non significant. A maximum of 2.0 shoots were obtained. Mean height was recorded highest in cultures on media containing 1.5 mg l^{-1} Kn in contrary there was no elongation of shoots in media with higher concentration of Kn.

Table 11. Effect of different concentration of Kn culture establishment and growth in axillary and terminal bud cultures of *Tectona grandis* in WPM

Kn mg l^{-1}	Percentage of		Period (days) for		Mean no. of leaves per explant	Mean no. of shoots/ bud sprouts per explant	Mean shoot length (cm)	Maxi- mum no. of shoots
	Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
0.1	60.00	75.00	24.00	37.00	7.33	1.66	2.50	2.00
0.5	66.66	50.00	23.00	48.00	9.00	1.50	1.75	2.00
1.0	25.00	50.00	16.00	18.00	10.00	2.00	3.00	2.00
1.5	28.57	50.00	13.00	16.00	2.00	2.00	0.00	2.00
2.0	42.85	33.33	11.00	16.00	2.00	2.00	0.00	2.00
2.5	33.33	00.00	23.00	0.00	0.00	1.00	0.00	2.00
3.0	50.00	60.00	27.00	53.00	10.57	1.67	2.33	2.00
4.0	27.00	33.33	24.00	28.00	28.00	2.00	0.00	2.00
SEm±			1.21 NS	2.59 NS	0.98 NS	0.12 NS		

A perusal of the Table 11 reveals that low concentration of Kn in WPM has pronounced effect on enhancement of growth as compared to higher concentration.

4.4.4   Effect of different concentration of BA on culture establishment and growth in WPM

The data on the effect of different concentration of BA in WPM is given in Table 12 and Fig.5. Highest bud initiation (66.66%) and leaf initiation (83.33%) was recorded in media supplemented with 0.1 mg l^{-1} BA. While least bud initiation (33.33%) in media containing 2.0 mg l^{-1} BA.

Effect of treatment on time taken for bud initiation was found significant. While BA at 0.1 and 4.0 mg l^{-1} lead to a significant delay in bud initiation the rest of the levels were on par with each other and ranged between 11 to 17 days. The lowest time taken for bud initiation was recorded as 17 days on media containing 1.5 mg l^{-1} BA. In media containing 0.1 and 0.4 mg l^{-1} BA the leaf initiation was delayed upto 65 and 66 days respectively. Highest mean number of leaves (8.40) were observed in media containing 0.1 mg l^{-1} BA, while least (2 leaves) were recorded in 3.5 mg l^{-1}. Maximum mean height of the shoot was 3.0 cm on media containing 0.5 mg l^{-1} BA and no elongation was recorded on 3.5 and 4.0 mg l^{-1} BA.

4.4.5   Effect of various concentration of IAA, NAA and IBA on culture establishment and growth in WPM

Four levels each of IAA, NAA and IBA were added to WPM individually and their effect on growth were observed. The result is presented in Table 13. Among the 12 media combination the percentage bud initiation was found to be highest (92.85) in media containing 1.5 mg l^{-1} IAA and the least (25) was in 0.5 mg l^{-1} IAA. All the cultures showing bud initiation exhibited leaf

Table 12. Effect of different concentration of BA on culture establishment and growth in axillary and terminal bud cultures of *Tectona grandis* in WPM

BA mg l^{-1}	Percentage of		Period (days) for		Mean no. of leaves per explant	Mean no. of shoots/ bud sprouts per explant	Mean shoot length (cm)	Maximum no. of shoots
	Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
0.1	66.66	83.33	47.00	65.00	8.40	2.00	2.00	2.00
0.5	42.85	66.66	16.00	30.00	7.00	1.50	3.00	2.00
1.0	42.85	33.33	11.00	22.00	2.00	2.00	0.00	5.00
1.5	46.66	28.57	15.00	17.00	3.00	1.50	0.00	2.00
2.0	33.33	50.00	17.00	22.00	8.00	1.00	2.50	3.00
3.5	40.00	50.00	17.00	22.00	2.00	2.20	0.00	2.00
4.0	40.00	25.00	57.00	66.00	4.00	1.00	0.00	2.00
SEm± CD (0.05)			3.61 20.6	5.75 NS	4.05 NS	0.12 NS		
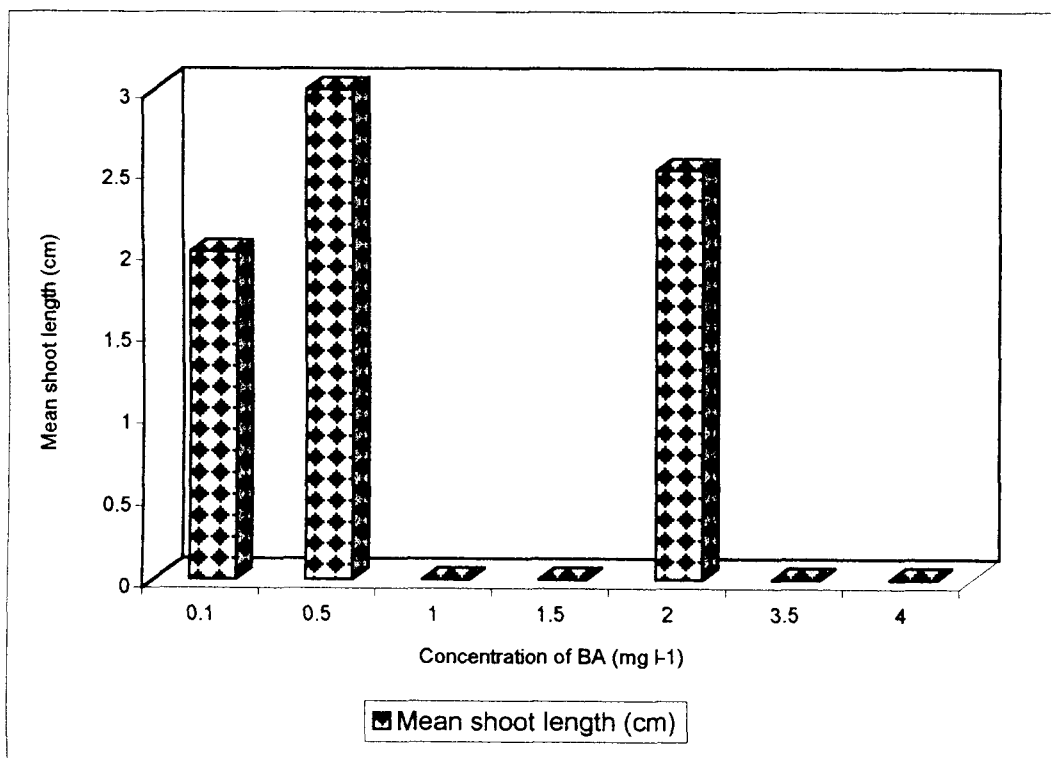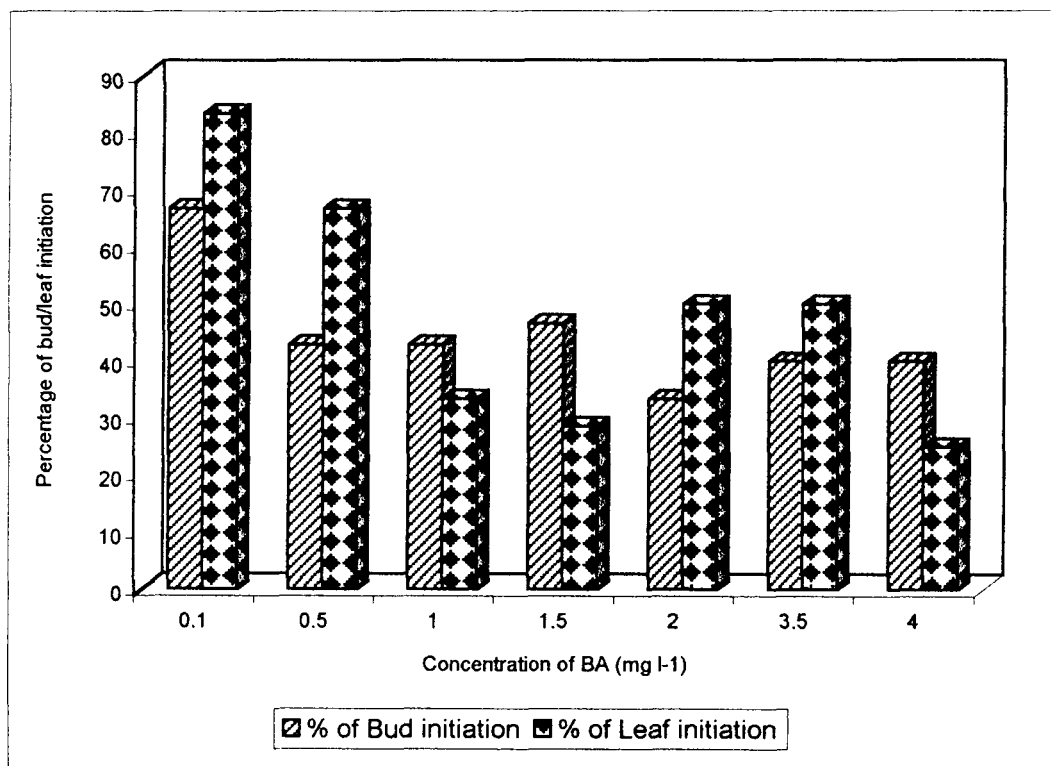
Fig. 5 Effect of different concentration of BA on bud/leaf initiation and shoot elongation in WPM

Table 13. Effect of different concentration of IAA, NAA and IBA on culture establishment and growth in axillary and terminal buds of *Tectona grandis* in WPM

Growth regulator	Concent-ration mg l^{-1}	Percentage of		Period (days) for		Mean no. of leaves per explant	Mean no. of shoots/bud sprouts per explant	Mean shoot length (cm)	Maximum no. of shoots
		Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
IAA	0.1	60.00	0.00	18.00	-	0.00	1.00	0.00	1
	0.5	25.00	10.00	25.00	33.00	2.00	2.00	0.00	2
	1.0	90.00	66.66	17.00	28.00	8.00	1.66	2.20	2
	1.5	92.85	76.92	15.00	29.00	7.00	1.50	2.43	2
NAA	0.1	33.33	66.66	24.00	32.00	5.00	1.00	0.00	1
	0.5	66.66	75.00	22.00	24.00	5.00	1.50	0.00	2
	1.0	75.00	88.88	24.00	35.00	5.50	1.87	1.70	2
	1.5	85.71	91.66	33.00	38.00	7.45	2.00	1.25	2
IBA	0.1	76.71	92.30	30.00	37.00	5.33	1.58	1.85	2
	0.5	70.05	83.33	27.00	40.00	8.22	1.55	1.70	2
	1.0	63.63	28.57	20.00	42.00	5.00	2.00	0.00	2
	1.5	44.44	75.00	18.00	29.00	8.50	1.50	0.00	2
SEm±				1.10	1.49	0.48	0.06		
CD (0.05)				10.65	NS	NS	0.51		

initiation on media with 0.5 mg l^{-1} IAA. In media supplemented with 1.0 mg l^{-1} NAA, only 28.57 per cent cultures exhibited leaf initiation.

The effect of treatment on time taken for bud initiation differed significantly. Treatment combination WPM + 1.5 mg l^{-1} IAA and WPM + 1.0 mg l^{-1} IAA were found significantly superior to WPM + 1.5 mg l^{-1} NAA and WPM + 0.1 mg l^{-1} IBA. The time taken for bud initiation was 15 days on media containing 1.5 mg l^{-1} IAA as compared to 33 days on media with 1.5 mg l^{-1} NAA.

The treatment effect on time taken for leaf initiation was non significant. However, after 24 days leaf initiation was observed on media containing 0.5 mg l^{-1} NAA. While leaf initiation was prolonged upto 40 days on media containing 0.5 mg l^{-1} IBA.

Maximum mean number of leaves per explant (8.50) were observed on media with 1.5 mg l^{-1} IBA while there was no leaf formation on WPM + 0.1 mg l^{-1} IAA.

The mean number of shoots per explant was found to differ significantly with different treatments. Treatment combination WPM + 0.5 mg l^{-1} IAA, WPM + 1.0 mg l^{-1} IBA and WPM 1.5 mg l^{-1} NAA were significantly superior to WPM + 0.1 mg l^{-1} IAA and WPM + 0.1 mg l^{-1} NAA. Maximum mean height was found on WPM + 1.5 mg l^{-1} IAA. No elongation was observed at lower concentration of IAA and NAA (0.1 and 0.5 mg l^{-1}) and at higher level of IBA (1.0 and 1.5 mg l^{-1}). Multiple shoots were not produced by any of the treatment combinations. However, few explants in WPM + 0.1 mgl^{-1} NAA induced direct rooting from the explants along with shoot morphogensis (Plate 8).

4.4.6   Effect of various concentration of IAA, NAA and IBA on culture establishment and growth in MS media

The data on the effect of various concentration of IAA, NAA and IBA, added to MS media individually, on growth and culture establishment is tabulated in Table 14. Bud initiation percentage was 92.30 on media containing 1.5 mg l^{-1} IAA and least (18.18) on media with 0.5 mg l^{-1} NAA. All the explants showing bud initiation exhibited leaf initiation on media containing 0.1 mg l^{-1} IAA. Least leaf initiation (25.0%) was observed on two media combination viz., MS + 1.0 mg l^{-1} IAA and MS + 1.0 mg l^{-1} NAA.

The effect of treatments on time taken for bud initiation and leaf initiation were found significant. Medium MS + 1.5 mg l^{-1} IBA was significantly superior to MS supplemented with IAA 0.1, 1.5 or NAA 0.1 mg l^{-1}. In the former treatment, the time taken for bud initiation was 10 days while in latter treatments it was delayed upto 38, 28 and 27 days, respectively. For leaf initiation media combination MS + 1.0 mg l^{-1} IBA was found significantly superior (17 days).

Effect of treatment on mean number of leaves was non significant. However, media containing 1.0 mg l^{-1} IAA produced more leaves (18 leaves) per explant as compared to 3.0 leaves per explant on MS + 1.5 mg l^{-1} NAA and MS + 0.1 mg l^{-1} NAA. Mean number of shoots per explant was not more than 2.0. No multiple shoots were obtained. Maximum mean height was found to be 3.0 cm on 0.5 mg l^{-1} IBA + MS whereas no elongation took place on MS + 1.0 mg l^{-1} NAA, MS + 1.5 mg l^{-1} NAA and MS + 1.0 mg l^{-1} IBA .

4.4.7   Effect of BA and IAA on culture establishment and growth in MS medium

Effect of various concentration of BA with IAA on culture establishment and growth is presented in Table 15 and fig 6. Percentage of bud initiation was highest (87.75) on media containing 1.0 mg l^{-1} BA + 0.1 mg l^{-1} IAA

Table 14. Effect of different concentration of IAA, NAA and IBA on culture establishment and growth in axillary and terminal buds of *Tectona grandis* in MS media

Growth regulator	Concent-ration mg l⁻¹	Percentage of		Period (days) for		Mean no. of leaves per explant	Mean no. of shoots/bud sprouts per explant	Mean shoot length (cm)	Maximum no. of shoots
		Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
IAA	0.1	60.00	100.00	14.00	38.00	6.00	1.33	2.00	2
	0.5	50.00	33.33	17.00	39.00	12.00	1.00	2.50	2
	1.0	40.00	25.00	38.00	52.00	18.00	2.00	1.75	2
	1.5	92.30	91.66	28.00	38.00	10.42	1.85	2.15	2
NAA	0.1	71.42	80.00	19.00	34.00	3.00	1.25	0.00	2
	0.5	18.18	50.00	19.00	39.00	6.00	2.00	0.50	2
	1.0	66.66	25.00	20.00	24.00	6.00	2.00	0.00	2
	1.5	50.00	66.66	24.00	25.00	3.00	1.00	0.00	1
IBA	0.1	75.00	50.00	27.00	32.00	4.00	1.66	1.50	2
	0.5	60.00	66.66	22.00	45.00	5.00	1.50	3.00	2
	1.0	42.85	66.66	13.00	17.00	8.00	2.00	0.00	2
	1.5	83.33	60.00	10.00	22.00	6.00	2.00	0.50	2
SEm±				1.51	2.31	0.92	0.08		
CD (0.05)				11.61	14.57	NS	NS		

Table 15. Effect of different concentration of BA and IAA on culture establishment and growth in axillary and terminal buds of *Tectona grandis* in MS media

BA (mg l⁻¹)	IAA (mg l⁻¹)	Percentage of		Period (days) for		Mean no. of leaves per explant	Mean no. of shoots/bud sprouts per explant	Average shoot length (cm)	Maximum no. of shoots
		Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
1.0	0.1	87.75	100.00	22.00	36.00	12.85	1.86	2.35	2
1.0	0.5	66.66	83.33	21.00	26.00	12.80	2.00	3.08	2
1.0	1.0	66.66	75.00	14.00	17.00	21.33	2.00	4.05	2
1.0	1.5	57.14	50.00	14.00	24.00	16.00	2.50	3.25	3
1.5	0.1	75.00	66.66	16.00	32.00	6.00	2.00	0.00	2
1.5	0.5	50.00	100.00	27.00	46.00	10.67	2.00	3.00	2
1.5	1.0	80.00	75.00	20.00	31.00	10.00	2.00	3.50	2
1.5	1.5	66.66	100.00	38.00	56.00	1.00	2.00	0.00	2
2.0	0.1	66.66	75.00	21.00	26.00	20.00	2.00	1.50	2
2.0	0.5	87.50	100.00	35.00	42.00	16.00	2.00	2.45	2
2.0	1.0	81.81	66.66	20.00	33.00	14.67	1.67	3.11	2
2.0	1.5	66.70	75.00	31.00	44.00	6.67	2.00	3.25	2
SEm±				1.59	1.91	1.36	0.04		
CD (0.05)				12.16	12.17	NS	NS		
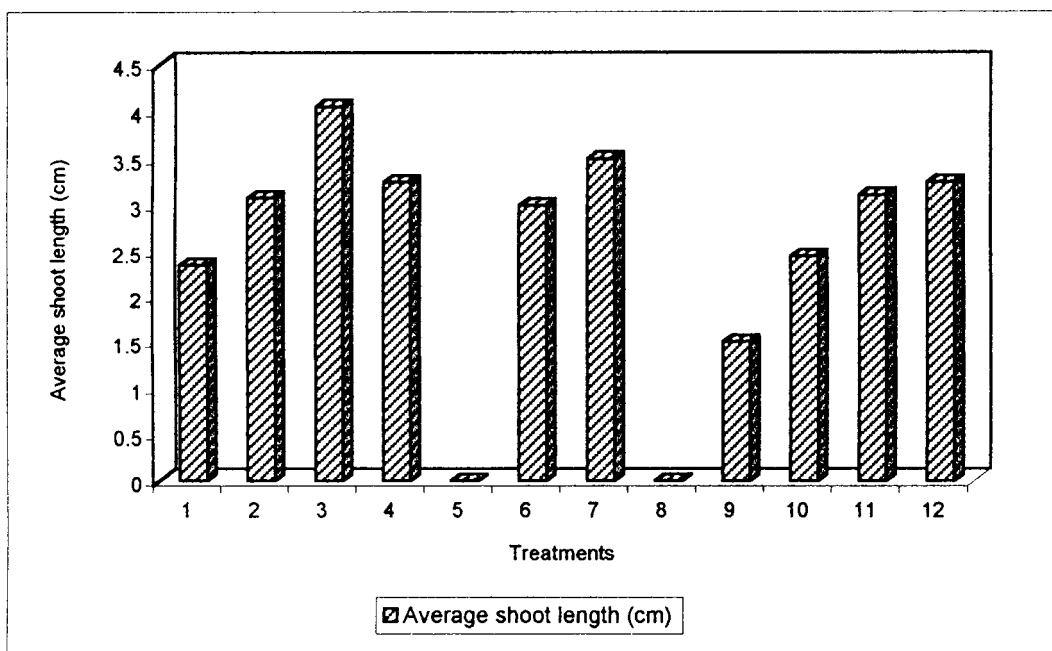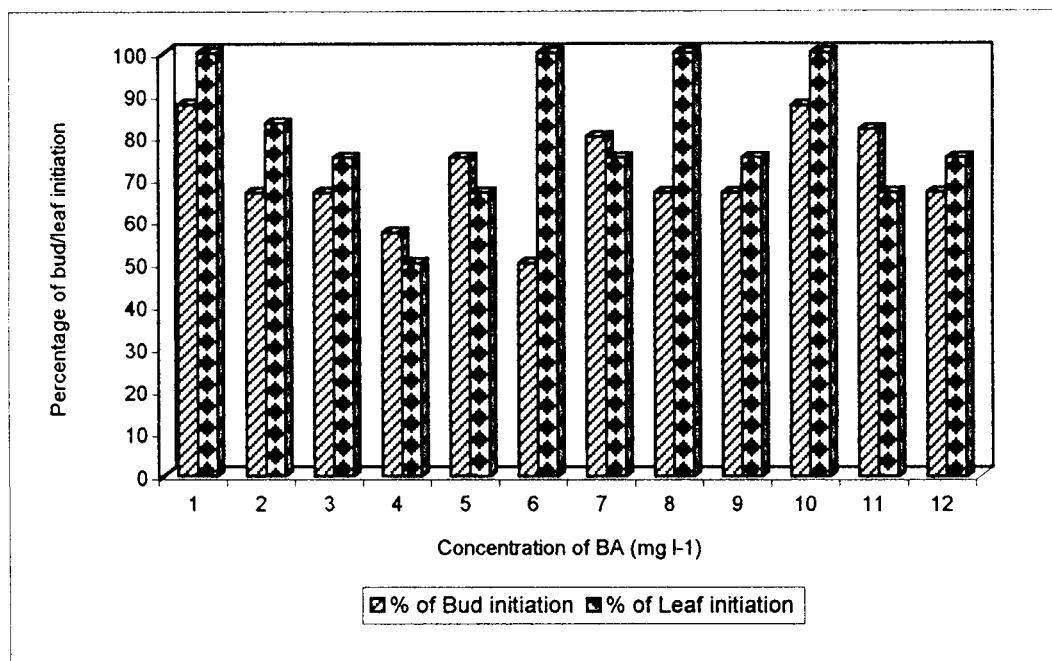
1 - MS + 0.5 mg l⁻¹ BA + 0.1 mg l⁻¹ IAA   7 - MS + 1.0 mg l⁻¹ BA + 1.0 mg l⁻¹ IAA
2 - MS + 0.5 mg l⁻¹ BA + 0.5 mg l⁻¹ IAA   8 - MS + 1.0 mg l⁻¹ BA + 1.5 mg l⁻¹ IAA
3 - MS + 0.5 mg l⁻¹ BA + 1.0 mg l⁻¹ IAA   9 - MS + 1.5 mg l⁻¹ BA + 0.1 mg l⁻¹ IAA
4 - MS + 0.5 mg l⁻¹ BA + 1.5 mg l⁻¹ IAA   10 - MS + 1.5 mg l⁻¹ BA + 0.5 mg l⁻¹ IAA
5 - MS + 1.0 mg l⁻¹ BA + 0.1 mg l⁻¹ IAA   11 - MS + 1.5 mg l⁻¹ BA + 1.0 mg l⁻¹ IAA
6 - MS + 1.0 mg l⁻¹ BA + 0.5 mg l⁻¹ IAA   12 - MS + 1.5 mg l⁻¹ BA + 1.5 mg l⁻¹ IAA

Fig. 6   Effect of different concentration of BA and IAA on bud/leaf initiation and shoot elongation in MS medium

which was followed by 87.50 per cent on MS + 2.0 mg l^{-1} BA + 0.5 mg l^{-1} IAA. Least bud initiation (50.0%) was recorded on MS + 1.5 mg l^{-1} BA + 0.5 mg l^{-1} IAA. All the explants showing bud initiation exhibited leaf initiation on MS + 1.0 mg l^{-1} BA + 0.1 mg l^{-1} IAA, MS + 1.5 mg l^{-1} BA + 0.1 mg l^{-1} IAA, MS + 1.5 mg l^{-1} BA + 1.5 mg l^{-1} IAA and MS + 2.0 mg l^{-1} BA + 0.5 mg l^{-1} IAA. where as minimum of 50 per cent leaf initiation was recorded on MS + 1.0 mg l^{-1} BA + 1.5 mg l^{-1} IAA.

Treatment effect on time taken for bud initiation and leaf initiation was found to be significant. For both of these, media containing 1.0 mg l^{-1} IBA + 1.0 mg l^{-1} IAA was found to be significantly superior to MS + 1.5 mg l^{-1} BA + 1.5 mg l^{-1} IAA. In the former treatment the time taken for bud initiation and leaf initiation was found to be 14 and 17 days, respectively, while in latter treatment that was 37 and 56 days.

Treatment effect on mean number of leaves per explant and mean number of shoots per explant was found to be non significant. However, maximum mean leaves (21.33) were observed on media containing 1.0 mg l^{-1} BA + 1.0 mg l^{-1} IAA. Whereas least (1.0) leaf was found on media containing BA 1.5 mg l^{-1} + 1.5 mg l^{-1} IAA.

4.4.8   Effect of Kn and IAA on culture establishment and growth in MS media

Effect of 12 different combinations of Kn and IAA (Kn at three levels and IAA at four levels) in MS medium was assessed on various culture growth parameters of *Tectona grandis*. The result obtained is presented in Table 16 and Fig 7. The results revealed that 92.30 and 91.66 per cent of cultures lead to bud initiation on MS + 1.0 mg l^{-1} Kn + 1.0 mg l^{-1} IAA and MS + 0.5 mg l^{-1} Kn + 0.5 mg l^{-1} IAA, respectively. Minimum percentage of bud initiation (42.85) was observed on MS + 1.5 mg l^{-1} Kn + 0.1 mg l^{-1} IAA.

Table 16. Effect of different concentration of Kn and IAA on culture establishment and growth in axillary and terminal buds of *Tectona grandis* in MS media

Kn (mg l⁻¹)	IAA (mg l⁻¹)	Percentage of		Period (days) for		Mean no. of leaves per explant	Mean no. of shoots/ bud sprouts per explant	Average shoot length (cm)	Maximum no. of shoots
		Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
0.5	0.1	80.00	87.50	22.00	33.00	9.36	1.81	1.50	2
0.5	0.5	21.66	100.00	24.00	37.00	20.20	2.00	2.86	2
0.5	1.0	83.33	100.00	22.00	34.00	9.14	1.85	3.16	2
0.5	1.5	80.00	62.50	27.00	43.00	13.20	1.60	1.06	3
1.0	0.1	50.00	75.00	24.00	37.00	16.66	2.00	1.08	2
1.0	0.5	85.00	76.47	18.00	27.00	16.00	1.31	2.41	2
1.0	1.0	92.30	83.33	18.00	28.00	20.80	1.80	2.80	2
1.0	1.5	80.00	87.50	37.00	43.00	8.85	1.71	1.56	2
1.5	0.1	42.85	100.00	30.00	35.00	12.66	2.00	1.12	2
1.5	0.5	87.50	100.00	22.00	37.00	18.14	1.86	3.21	2
1.5	1.0	84.61	91.91	24.00	32.00	17.11	1.67	2.79	2
1.5	1.5	71.42	100.00	21.00	41.00	13.20	1.80	1.50	2
SEm±				0.89	1.14	0.84	0.04		
CD (0.05)				9.729	12.2	8.77	0.45		
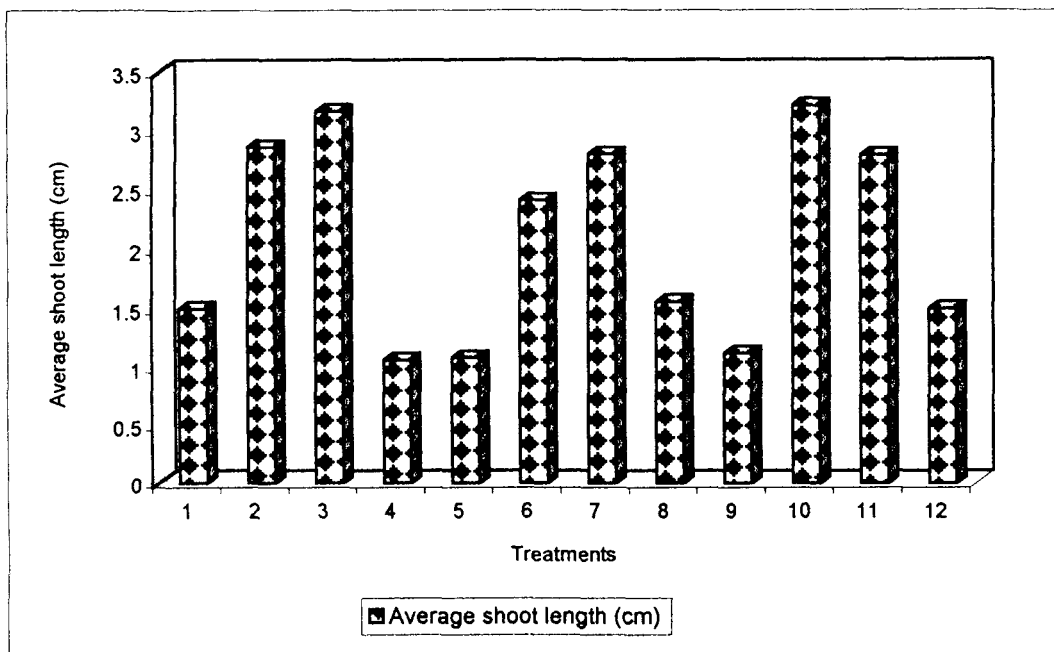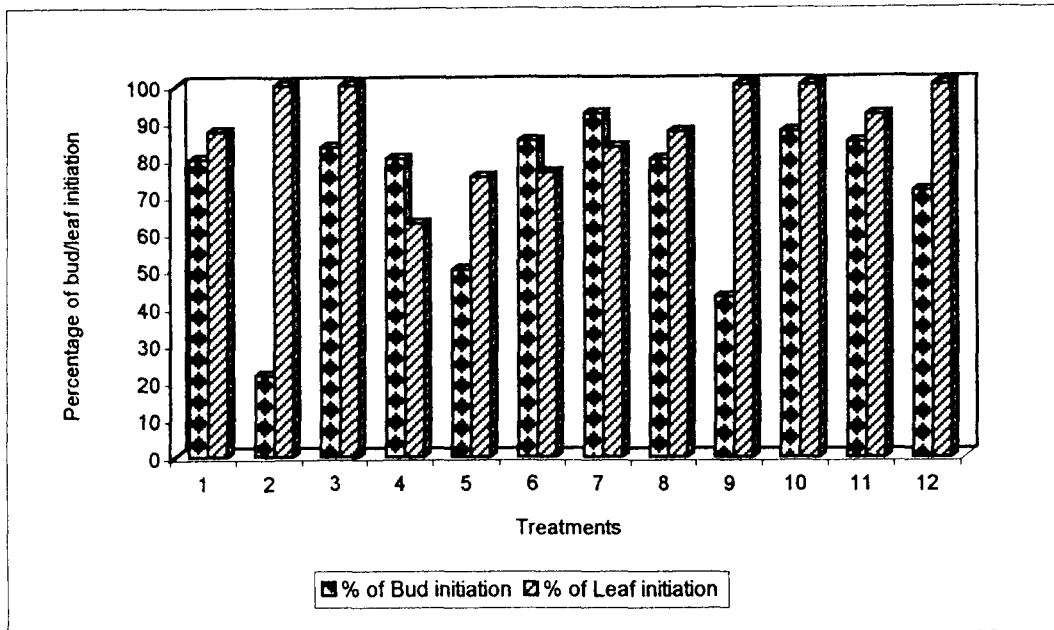
1 - MS + 0.5 mg l⁻¹ Kn + 0.1 mg l⁻¹ IAA
2 - MS + 0.5 mg l⁻¹ Kn + 0.5 mg l⁻¹ IAA
3 - MS + 0.5 mg l⁻¹ Kn + 1.0 mg l⁻¹ IAA
4 - MS + 0.5 mg l⁻¹ Kn + 1.5 mg l⁻¹ IAA
5 - MS + 1.0 mg l⁻¹ Kn + 0.1 mg l⁻¹ IAA
6 - MS + 1.0 mg l⁻¹ Kn + 0.5 mg l⁻¹ IAA

7 - MS + 1.0 mg l⁻¹ Kn + 1.0 mg l⁻¹ IAA
8 - MS + 1.0 mg l⁻¹ Kn + 1.5 mg l⁻¹ IAA
9 - MS + 1.5 mg l⁻¹ Kn + 0.1 mg l⁻¹ IAA
10 - MS + 1.5 mg l⁻¹ Kn + 0.5 mg l⁻¹ IAA
11 - MS + 1.5 mg l⁻¹ Kn + 1.0 mg l⁻¹ IAA
12 - MS + 1.5 mg l⁻¹ Kn + 1.5 mg l⁻¹ IAA

Fig. 7   Effect of various concentration of Kn and IAA on bud/leaf initiation and shoot elongation in MS media

In most of the treatments 75.0-100.0 per cent explants showing bud initiation exhibited leaf initiation except on MS + 0.5 mg l^{-1} Kn + 1.5 mg l^{-1} IAA. In this only 62.50 per cent explants exhibiting bud initiation produced leaves.

Treatment effect on time taken for leaf initiation/bud initiation, mean number of leaves per explant and mean number of shoots per explant was found significant. In time taken for bud initiation and leaf initiation media containing 1.0 mg l^{-1} Kn + 0.5 mg l^{-1} IAA was found significantly superior to MS + 1.0 mg l^{-1} Kn + 1.5 mg l^{-1} IAA. In former media the time taken for bud initiation and leaf initiation were recorded as 18 and 27 days respectively whereas in latter they were 37 and 43 days, respectively.

On media containing 1.0 mg l^{-1} each of Kn and IAA maximum mean number of leaves per explant (20.80 leaves) were produced and it was found significantly superior to MS + 1.0 mg l^{-1} Kn + 1.5 mg l^{-1} IAA. For maximum mean number of shoots per explant treatment MS + 0.5 mg l^{-1} Kn + 0.5 mg l^{-1} IAA, MS + 1.5 mg l^{-1} Kn + 0.1 mg l^{-1} IAA and MS + 1.0 mg l^{-1} Kn + 0.1 mg l^{-1} IAA were found significantly superior to MS + 1.0 mg l^{-1} Kn + 0.5 mg l^{-1} IAA on which only 1.3 shoots were recorded. No multiple shoots were observed. Maximum mean height (3.21 cm) was observed on MS + 1.5 mg l^{-1} Kn + 0.5 mg l^{-1} IAA (Plate 6). In contrary least was recorded (1.06 cm) and MS + 0.5 mg l^{-1} + 1.5 mg l^{-1} IAA.

4.4.9   Effect of BA and NAA on culture establishment and growth in MS media

Different combination of BA and NAA were used to find out their effect on growth. The result obtained are presented in Table 17. Percentage of bud initiation was highest (80.00) on MS + 1.0 mg l^{-1} BA + 1.0 mg l^{-1} NAA and MS + 1.0 mg l^{-1} BA + 0.5 mg l^{-1} NAA. Least percentage of bud initiation (33.33) was recorded on MS + 1.5 mg l^{-1} BA + 0.1 mg l^{-1} NAA. All the explants exhibiting bud initiation produced leaves on MS + 1.5 mg l^{-1} BA + 0.1 mg l^{-1} NAA and MS +

Table 17. Effect of different concentration of BA and NAA on culture establishment and growth in axillary and terminal buds of *Tectona grandis* in MS media

BA (mg l⁻¹)	NAA (mg l⁻¹)	Percentage of		Period (days) for		Mean no. of leaves per explant	Mean no. of shoots/ bud sprouts per explant	Mean shoot length (cm)	Maximum no. of shoots
		Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
1.0	0.1	66.66	50.00	30.00	35.00	3.00	2.00	0.00	2
1.0	0.5	80.00	75.00	31.00	35.00	9.33	2.00	2.50	2
1.0	1.0	80.00	87.50	31.00	36.00	15.28	1.714	2.40	2
1.0	1.5	50.00	66.66	36.00	46.00	16.00	2.00	2.25	2
1.5	0.1	33.33	100.00	16.00	34.00	4.00	2.00	0.00	2
1.5	0.5	50.00	0.00	34.00	0.00	0.00	2.00	0.00	2
1.5	1.0	50.00	50.00	21.00	25.00	2.00	2.00	0.00	2
1.5	1.5	60.00	66.66	20.00	31.00	7.00	2.00	0.50	2
2.0	0.1	57.71	100.00	12.00	25.00	5.00	1.75	1.50	2
2.0	0.5	66.66	83.33	23.00	33.00	12.60	1.60	1.85	2
2.0	1.0	66.66	75.00	28.00	29.00	6.67	2.00	0.00	2
SEm±				2.02	2.46	1.38	0.08		
CD (0.05)				NS	NS	NS	NS		

2.0 mg l^{-1} BA + 0.5 mg NAA. No leaf initiation was recorded on MS + 1.5 mg l^{-1} BA + 0.5 mg l^{-1} NAA.

The treatment effect on time taken for bud initiation/leaf initiation, mean number of leaves per explant and mean number of shoots per explant was found non significant. However, on MS + 2.0 mg l^{-1} BA + 0.5 mg l^{-1} NAA it took only 12 days and 25 days for bud initiation and leaf initiation respectively. On contrary they were delayed upto 36 and 46 days on MS + 1.0 mg l^{-1} BA + 1.5 mg l^{-1} NAA. Maximum mean number of leaves (16.00) per explant were recorded on MS + 1.0 mg l^{-1} BA + 1.5 mg l^{-1} NAA. while no leaf initiation took place on MS + 1.5 mg l^{-1} BA + 0.5 mg l^{-1} NAA. No multiple shoots were observed. Maximum mean height (2.50 cm) was recorded on media containing 1.0 mg l^{-1} BA + 0.5 mg l^{-1} NAA.

4.4.10   Effect of Kn and NAA on culture establishment and growth in MS media

Various combination of Kn and NAA were used to find out their effect on growth and culture establishment. The result obtained are presented in Table 18. In 80.00 per cent cultures bud initiation was recorded on MS + 1.5 mg l^{-1} Kn + 1.0 mg l^{-1} NAA. Only 33.40 per cent of explants exhibited bud initiation on MS + 1.5 mg l^{-1} Kn + 0.5 mg l^{-1} NAA. All the explants showing bud initiation produced leaves on MS + 1.0 mg l^{-1} Kn + 0.5 mg l^{-1} NAA and MS + 1.5 mg l^{-1} Kn + 0.5 mg l^{-1} NAA. Only 50.00 per cent leaf initiation was recorded when 1.5 mg l^{-1} Kn + 0.1 mg l^{-1} NAA were supplemented to MS medium.

Treatment effect on time taken for bud initiation and leaf initiation was found significant. For bud and leaf initiation MS + 0.5 mg l^{-1} Kn + 0.1 mg l^{-1} NAA (12 days for bud initiation and 14 days for leaf initiation) and MS + 1.0 mg l^{-1} Kn + 1.0 mg l^{-1} NAA (19 days for bud initiation and 28 days for leaf initiation) were found significantly superior to MS + 0.5 mg l^{-1} Kn + 1.5 mg l^{-1} NAA (34 days for

Table 18. Effect of different concentration of Kn and NAA on culture establishment and growth in axillary and terminal buds of *Tectona grandis* in MS media

Kn (mg l^{-1})	NAA (mg l^{-1})	Percentage of		Period (days) for		Mean no. of leaves per explant	Mean no. of shoots/ bud sprouts per explant	Mean shoot length (cm)	Maximum no. of shoots
		Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
0.5	0.1	60.00	66.66	12.00	14.00	10.00	2.00	1.00	2
0.5	1.0	77.77	71.42	20.00	29.00	9.20	1.80	1.50	2
0.5	1.5	72.72	75.00	34.00	40.00	8.00	1.50	0.83	3
1.0	0.1	42.85	67.00	31.00	40.00	5.00	2.00	3.00	2
1.0	0.5	34.00	100.00	32.00	61.00	8.00	2.00	2.00	2
1.0	1.0	36.36	75.00	19.00	28.00	4.67	1.67	0.83	2
1.0	1.5	57.14	75.00	28.00	36.00	6.66	2.00	1.33	2
1.5	0.1	66.70	50.00	21.00	32.00	22.0	2.00	2.00	2
1.5	0.5	33.40	100.00	22.00	40.00	12.00	2.00	1.50	2
1.5	1.0	80.00	62.50	27.00	39.00	8.00	1.60	1.85	2
1.5	1.5	76.92	80.00	25.00	32.00	9.50	1.62	1.50	2
SEm±				1.26	1.80	0.89	0.07		
CD (0.05)				8.38	10.51	NS	NS		

bud initiation and 40 days for leaf initiation) and MS + 1.0 mg l^{-1} Kn + 0.5 mg l^{-1} NAA (32 days for bud initiation and 61 days for leaf initiation).

The effect of treatment on maximum mean number of leaves per explant and mean number of shoots per explant was found non significant. However, maximum number of leaves (22 leaves) were observed on MS + 1.5 mg l^{-1} Kn + 0.1 mg l^{-1} NAA and lowest 5 leaves/explant were found on MS + 1.0 mg l^{-1} Kn + 0.1 mg l^{-1} NAA. No multiple shoots were observed. Maximum 2.0 shoots were found (one from each bud). Maximum mean height per shoot was found to be 3.00 cm on MS + 1.0 mg l^{-1} Kn + 0.1 mg l^{-1} NAA and minimum (0.83 cm) on MS + 0.5 mg l^{-1} Kn + 1.5 mg l^{-1} NAA and MS + 1.0 mg l^{-1} Kn + 1.0 mg l^{-1} NAA.

4.4.11   Effect of BA and IBA on culture establishment and growth in MS media

Various combinations of BA and IBA (Three levels of BA and Four levels of IBA) were used to find their effect on culture establishment and growth. The data obtained is tabulated in Table 19. Maximum percentage of bud initiation (86.00) was found on MS + 1.5 mg l^{-1} BA + 1.5 mg l^{-1} IBA, whereas on MS + 1.0 mg l^{-1} BA + 1.5 mg l^{-1} IBA and MS + 2.0 mg l^{-1} Kn + 0.5 mg l^{-1} IBA only 50 per cent of bud initiation were noted. All the explants exhibiting bud initiation produced leaves on MS + 1.0 mg l^{-1} BA + 0.5 mg l^{-1} IBA, MS + 1.0 mg l^{-1} BA + 1.5 mg l^{-1} IBA and MS + 1.5 mg l^{-1} BA + 0.1 mg l^{-1} IBA. No leaf initiation was observed on MS + 2.0 mg l^{-1} BA and 0.5 mg l^{-1} IBA.

Effect of treatment on time taken for bud/leaf initiation, mean number of leaves per explant and mean number of shoot was found non significant. However, it took only 14 days for bud initiation on MS + 1.0 mg l^{-1} BA + 1.5 mg l^{-1} IBA while it was delayed upto 22 days on MS + 1.5 mg l^{-1} BA + 0.1 mg l^{-1} IBA. Leaf initiation was recorded as early as 20 days after culturing on MS + 1.5 mg l^{-1} BA + 0.5 mg l^{-1} IBA whereas on MS + 1.5 mg l^{-1} BA + 0.1 mg l^{-1} IBA and MS + 1.5 mg l^{-1} BA + 1.0 mg l^{-1} IBA it was delayed upto 28 days.

Table 19. Effect of different concentration of BA and IBA on culture establishment and growth in axillary and terminal buds of *Tectona grandis* in MS media

BA (mg l^{-1})	IBA (mg l^{-1})	Percentage of		Period (days) for		Mean no. of leaves per explant	Mean no. of shoots/ bud sprouts per explant	Mean shoot length (cm)	Maximum no. of shoots
		Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
1.0	0.1	75.00	66.70	18.00	26.00	13.50	2.00	1.90	2
1.0	0.5	25.00	100.00	20.00	24.00	2.00	1.00	0.00	1
1.0	1.0	71.42	80.00	19.00	27.00	11.00	1.75	1.66	3
1.0	1.5	50.00	100.00	14.00	25.00	17.00	2.00	2.75	2
1.5	0.1	57.14	100.00	22.00	28.00	11.00	2.00	2.00	2
1.5	0.5	85.71	83.33	18.00	20.00	17.60	1.60	2.68	2
1.5	1.0	85.71	50.00	20.00	28.00	15.33	1.20	2.10	2
1.5	1.5	86.00	83.30	18.00	24.00	19.20	1.80	2.94	2
2.0	0.1	62.50	80.00	17.00	26.00	13.50	1.75	2.75	2
2.0	0.5	50.00	0.00	20.00	0.00	0.00	1.33	0.00	2
2.0	1.0	83.33	20.00	17.00	21.00	4.00	2.00	0.00	2
2.0	1.5	85.71	83.40	16.00	21.00	12.80	1.60	1.92	
SEm±				0.89	1.35	1.43	0.08		
CD (0.05)				NS	NS	NS	NS		

Maximum mean number of leaves per explant (19.20 leaves) were found on MS + 1.5 mg l^{-1} BA + 1.5 mg l^{-1} IBA and minimum of 2.0 leaves were recorded on MS + 1.0 mg l^{-1} BA + 0.5 mg l^{-1} IBA. No multiple shoots were observed. Maximum mean height was found to be 2.99 cm on MS + 1.5 mg l^{-1} BA and 1.5 mg l^{-1} IBA. No elongation was observed on MS + 1.0 mg l^{-1} BA + 0.5 mg l^{-1} IBA, MS + 2.0 mg l^{-1} BA + 0.5 mg l^{-1} IBA and MS + 2.0 mg l^{-1} BA + 1.5 mg l^{-1} IBA.

4.4.12   Effect of Kn and IBA on culture establishment and growth in MS media

The data on the effect of different concentration of Kn and IBA on culture establishment and growth in MS media is presented in Table 20. On MS + 1.5 mg l^{-1} Kn + 0.5 mg l^{-1} IBA highest percentage (90.00) of bud initiation was recorded, whereas least (70.0%) bud initiation was found on MS + 0.5 mg l^{-1} Kn + 0.1 mg l^{-1} IBA.

All the explants in which bud initiation was observed produced leaves on MS + 1.0 mg l^{-1} Kn + 0.1 mg l^{-1} IBA. Whereas only 40.0 per cent of leaf initiation was observed on MS + 1.0 mg l^{-1} Kn + 1.5 mg l^{-1} IBA.

Treatment effect on time taken for bud initiation/ leaf initiation, mean number of leaves per explant and mean number of shoots per explant was found non significant. However, it took minimum days (18 days) for bud initiation on MS + 1.0 mg l^{-1} Kn + 0.1 mg l^{-1} IBA where it was delayed upto 25 days. On MS + 0.5 mg l^{-1} Kn + 0.1 mg l^{-1} IBA. Leaf initiation was observed after 25 days. On MS + 1.5 mg l^{-1} Kn + 0.1 mg l^{-1} IBA. On the other hand it was as late as after 39 days on media containing 0.5 mg l^{-1} Kn + 0.1 mg l^{-1} IBA.

Maximum mean number of leaves (17 leaves) were found on MS + 1.5 mg l^{-1} Kn + 1.5 mg l^{-1} IBA and minimum mean number of leaves (5.50 leaves) were observed on media containing 1.5 mg l^{-1} Kn + 0.1 mg l^{-1} IBA. No multiple shoots were observed. Maximum mean height per shoot (2.50 cm) was found on

Table 20. Effect of different concentration of Kn and IBA on culture establishment and growth in axillary and terminal buds of *Tectona grandis* in MS media

Kn (mg l^{-1})	IBA (mg l^{-1})	Percentage of		Period (days) for		Mean no. of leaves per explant	Mean no. of shoots/ bud sprouts per explant	Mean shoot length (cm)	Maximum no. of shoots
		Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
0.5	0.1	70.00	85.71	25.00	39.00	11.00	1.66	1.40	2
0.5	0.5	80.00	50.00	22.00	38.00	7.00	1.50	0.50	2
0.5	1.0	80.00	50.00	23.00	33.00	11.00	2.00	1.62	2
0.5	1.5	75.00	66.66	24.00	35.00	14.00	1.75	1.37	3
1.0	0.1	83.33	100.00	18.00	29.00	10.00	2.00	1.50	2
1.0	0.5	80.00	50.00	27.00	41.00	7.00	2.00	0.50	2
1.0	1.0	75.00	66.70	22.00	34.00	8.00	1.50	1.25	2
1.0	1.5	83.33	40.00	19.00	27.00	8.00	1.00	2.50	1
1.5	0.1	87.50	57.14	20.00	25.00	5.50	1.25	0.83	2
1.5	0.5	90.00	88.88	24.00	27.00	11.71	1.57	1.66	2
1.5	1.0	81.81	88.98	22.00	31.00	11.33	1.66	1.53	2
1.5	1.5	84.61	72.72	22.00	30.00	17.18	1.87	1.56	2
SEm±				0.72	1.19	0.86	0.07		
CD (0.05)				NS	NS	NS	NS		

MS + 1.0 mg l⁻¹ Kn + 1.5 mg l⁻¹ IBA whereas minimum (0.50 cm) was found on MS + 0.5 mg l⁻¹ Kn + 0.5 mg l⁻¹ IBA and MS + 1.0 mg l⁻¹ Kn + 0.5 mg l⁻¹ IBA.

4.4.13   Effect of BA, IAA, Kn and 2iP on culture establishment and growth in MS medium
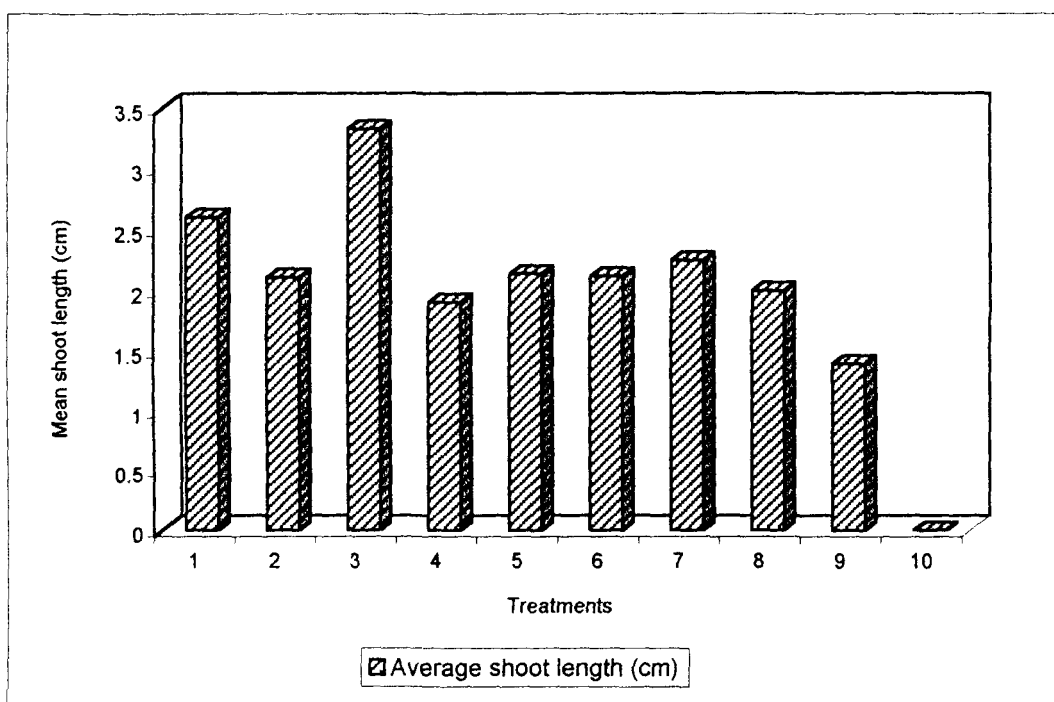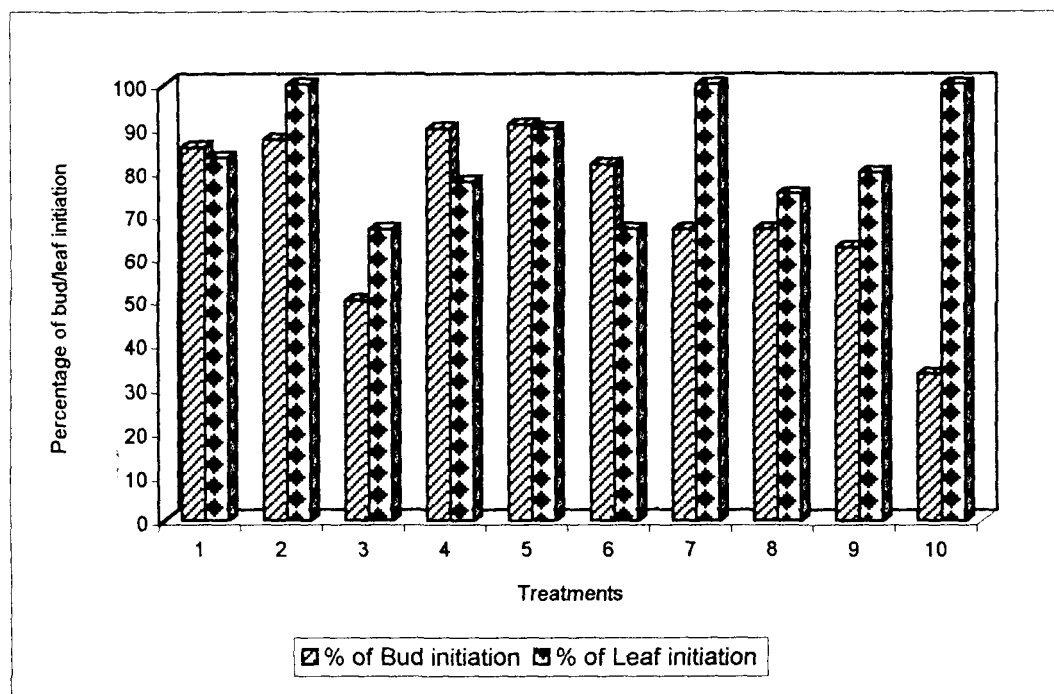
Different concentration of BA, IAA, Kn and 2ip were supplemented to MS medium and their effect on culture establishment and growth was studied. The data obtained are tabulated in Table 21. It was observed that percentage of bud initiation was highest (90.90) on MS + 1.0 mg l⁻¹ kn + 0.1 mg l⁻¹ IAA + 1.0 mg l⁻¹ 2ip where as least (33.33%) was found on MS + 1.0 mg l⁻¹ kn + 0.5 mg l⁻¹ IAA + 5.0 mg l⁻¹ 2ip. All the cultures which exhibited bud initiation produced leaves on MS + 1.0 mg l⁻¹ + BA 0.1 mg l⁻¹ IAA + 1.0 mg l⁻¹ 2ip, MS + 1.0 mg l⁻¹ kn + 0.1 mg l⁻¹ IAA + 5.0 mg l⁻¹ 2ip and MS + 1.0 mg l⁻¹ Kn + 0.5 mg l⁻¹ IAA + 6.0 mg l⁻¹ 2ip.

Effect of treatments on time taken for bud initiation/leaf initiation, mean number of leaves per explant and mean number of shoots per explants was found non significant. However, bud initiation was recorded as early as after 15 days on MS + 1.0 mg l⁻¹ BA + 0.1 mg l⁻¹ IAA + 0.25 mg l⁻¹ 2ip and as late as after 27 days on MS + 1.0 mg l⁻¹ kn + 0.5 mg l⁻¹ IAA + 0.25 mg l⁻¹ 2ip. Time taken for leaf initiation was minimum (21 days) on MS + 1.0 mg l⁻¹ BA + 0.1 mg l⁻¹ IAA + 3.0 mg l⁻¹ 2ip and MS + 1.0 mg l⁻¹ kn + 0.5 mg l⁻¹ IAA + 1.0 mg l⁻¹ 2ip. Maximum time (36 days) taken for leaf initiation was observed on MS + 1.0 mg l⁻¹ kn + 0.1 mg l⁻¹ IAA and 0.25 mg l⁻¹ 2ip.

Maximum mean number of leaves (17 leaves) per explant were found on MS + 1.0 mg l⁻¹ BA + 0.1 mg l⁻¹ IAA + 3.0 mg l⁻¹ 2ip. Whereas only 4 leaves per explant were found on MS + 1.0 mg l⁻¹ Kn + 0.5 mg l⁻¹ + IAA + 5.0 mg l⁻¹ 2ip. Maximum 5 shoots on an explant were observed on MS + 1.0 mg l⁻¹ BA + 0.1 mg l⁻¹ IAA + 1.0 mg l⁻¹ 2ip. Maximum mean height of the shoot (3.33 cm) was

Table 21. Effect of different concentration of BA, Kn, IAA and 2ip on culture establishment and growth in axillary and terminal buds of *Tectona grandis* in MS media

Kn (mg l⁻¹)	BA (mg l⁻¹)	IAA (mg l⁻¹)	2ip (mg l⁻¹)	Percentage of		Period (days) for		Mean no. of leaves per explant	Mean no. of shoots/ bud sprouts per explant	Average shoot length (cm)	Maximum no. of shoots
				Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
0.00	1.00	0.1	0.25	85.71	83.33	15.00	26.00	11.20	1.40	2.60	2
0.00	1.00	0.1	1.00	87.50	100.00	18.00	26.00	14.28	1.85	2.11	5
0.00	1.00	0.1	3.00	50.00	66.66	17.00	21.00	17.00	1.50	3.33	2
1.00	0.00	0.1	0.25	90.00	77.77	21.00	36.00	7.14	1.00	1.91	1
1.00	0.00	0.1	1.00	90.90	90.00	18.00	28.00	7.33	1.11	2.14	2
1.00	0.00	0.1	3.00	81.81	66.70	20.00	24.00	6.66	1.00	2.12	1
1.00	0.00	0.1	5.00	66.66	100.00	22.00	29.00	11.66	1.50	2.25	2
1.00	0.00	0.5	0.25	66.70	75.00	27.00	36.00	12.66	1.66	2.00	2
1.00	0.00	0.5	1.00	62.50	80.00	17.00	21.00	7.20	2.00	1.40	2
1.00	0.00	0.5	5.00	33.33	100.00	19.00	29.00	4.00	1.00	0.00	1
SEm±						0.49	0.96	0.86	0.07		
CD (0.05)						4.26	NS	NS	NS		

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1 - MS + 1.0 BA + 0.1 mg l⁻¹ IAA + 0.25 mg l⁻¹ 2ip   6 - MS + 1.0 Kn + 0.1 mg l⁻¹ IAA + 3.0 mg l⁻¹ 2ip
2 - MS + 1.0 BA + 0.1 mg l⁻¹ IAA + 1.0 mg l⁻¹ 2ip   7 - MS + 1.0 Kn + 0.1 mg l⁻¹ IAA + 5.0 mg l⁻¹ 2ip
3 - MS + 1.0 BA + 0.1 mg l⁻¹ IAA + 3.0 mg l⁻¹ 2ip   8 - MS + 1.0 Kn + 0.5 mg l⁻¹ IAA + 0.25 mg l⁻¹ 2ip
4 - MS + 1.0 Kn + 0.1 mg l⁻¹ IAA + 0.25 mg l⁻¹ 2ip   9 - MS + 1.0 Kn + 0.5 mg l⁻¹ IAA + 1.0 mg l⁻¹ 2ip
5 - MS + 1.0 Kn + 0.1 mg l⁻¹ IAA + 1.0 mg l⁻¹ 2ip   10 - MS + 1.0 Kn + 0.5 mg l⁻¹ IAA + 5.0 mg l⁻¹ 2ip

Fig. 8   Effect of different concentration of BA, Kn, IAA and 2ip on bud/leaf initiation and shoot elongation in MS medium

found on MS + 1.0 mg l^{-1} BA + 0.1 mg l^{-1} IAA + 3.0 mg l^{-1} 2ip in contrary no elongation was found an MS + 1.0 mg l^{-1} Kn + 0.5 mg l^{-1} IAA + 5.0 mg l^{-1} 2ip.

4.4.14   Effect of various concentration of BA and Kn on culture establishment and growth in MS medium

Various treatment combination of BA and Kn were added to MS medium to find out their effect on culture establishment on growth. The result obtained is presented in Table 22 and Fig 9.

Highest percentage (77.78) of bud initiation was observed on MS + 0.5 mg l^{-1} BA + 0.05 mg l^{-1} Kn. Only 16.67 per cent cultures exhibits bud initiation though, all of them produced leaves on MS + 0.3 mg l^{-1} BA and 0.3 mg l^{-1} Kn. No leaf initiation was observed on MS + 0.1 mg l^{-1} BA + 0.2 mg l^{-1} Kn.

Bud and leaf initiation were found as early as after 8 and 10 days respectively on MS + 0.1 mg l^{-1} BA + 0.05 mg l^{-1} Kn. On the other hand bud and leaf initiation were protracted upto 23 and 45 days, respectively on MS + 0.05 mg l^{-1} BA + 0.3 mg l^{-1} Kn.

Maximum mean number of leaves per explant (16.00) were observed on MS + 0.2 mg l^{-1} BA + 0.2 mg l^{-1} Kn and minimum mean number of leaves (1.00) was found on MS + 0.05 mg l^{-1} + 0.3 mg l^{-1} Kn. Multiple shoots were not observed on any media combination. Maximum height per shoot (1.00 cm) was found on MS + 0.2 mg l^{-1} BA + 0.2 mg l^{-1} Kn, MS + 0.3 mg l^{-1} BA + 0.2 mg l^{-1} Kn and MS + 0.3 mg l^{-1} BA and 0.3 mg l^{-1} Kn. No elongation of shoots was observed on MS + 0.05 mg l^{-1} BA + 0.1 mg l^{-1} Kn, MS + 0.05 mg l^{-1} BA + 0.05 mg l^{-1} Kn and MS + 0.1 mg l^{-1} BA + 0.2 mg l^{-1} Kn.

Table 22. Effect of different concentration of BA and Kn on culture establishment and growth in axillary and terminal buds of *Tectona grandis* in MS media

BA (mg l^{-1})	Kn (mg l^{-1})	Percentage of		Period (days) for		Mean no. of leaves per explant	Mean no. of shoots/ bud sprouts per explant	Mean shoot length (cm)	Maximum no. of shoots
		Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
0.05	0.05	77.78	57.14	18.00	24.00	3.55	1.00	0.50	2
0.05	0.1	50.00	50.00	16.00	23.00	2.00	1.00	0.00	1
0.05	0.3	66.67	50.00	23.00	45.00	1.00	1.00	0.50	1
0.1	0.05	50.00	50.00	28.00	10.00	4.00	2.00	0.00	2
0.1	0.1	45.46	60.00	13.00	18.00	6.00	1.00	0.83	2
0.1	0.2	25.00	0.00	9.00	0.00	0.00	1.00	0.00	1
0.1	0.3	50.00	66.70	14.00	21.00	2.00	1.33	0.50	2
0.2	0.05	50.00	75.00	9.00	20.00	3.33	1.33	0.75	2
0.2	0.1	33.34	66.67	9.00	14.00	5.00	1.00	0.66	2
0.2	0.2	33.34	50.00	16.50	20.00	16.00	1.50	1.00	2
0.3	0.1	27.27	66.67	19.00	28.00	5.00	1.50	0.75	2
0.3	0.2	42.85	66.66	14.00	16.00	1.55	1.00	1.00	1
0.3	03	16.67	100.00	15.00	41.00	2.00	1.00	1.00	1
SEm± CD (0.05)									

1 - MS + 0.05 mg l⁻¹ BA + 0.05 mg l⁻¹ Kn
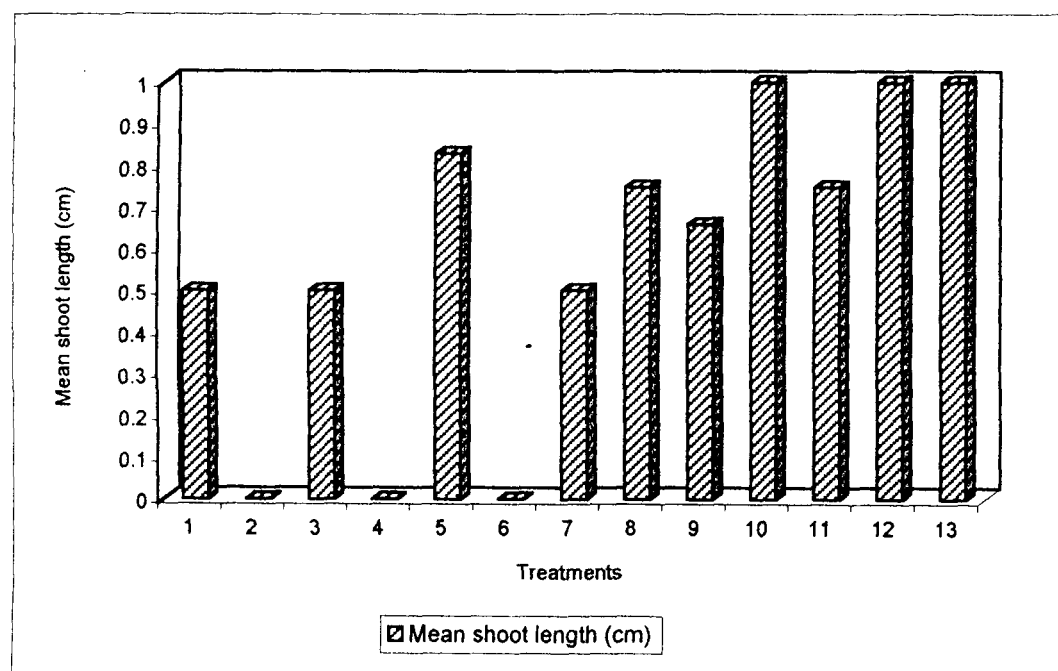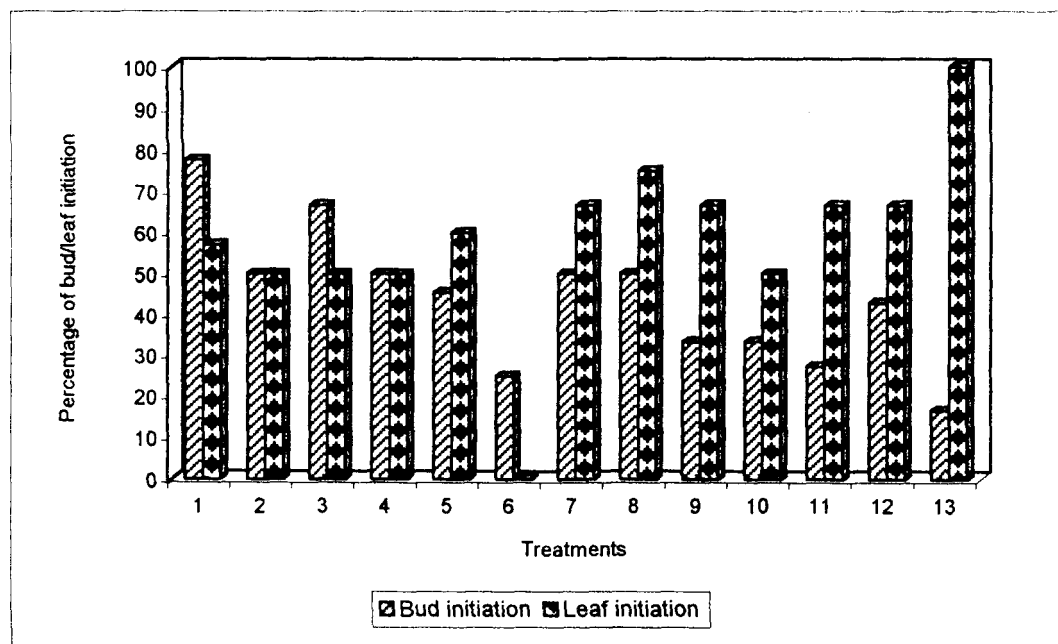
Legend:
1 - MS + 0.05 mg l^{-1} BA + 0.05 mg l^{-1} Kn
2 - MS + 0.05 mg l^{-1} BA + 0.1 mg l^{-1} Kn
3 - MS + 0.05 mg l^{-1} BA + 0.3 mg l^{-1} Kn
4 - MS + 0.1 mg l^{-1} BA + 0.05 mg l^{-1} Kn
5 - MS + 0.1 mg l^{-1} BA + 0.1 mg l^{-1} Kn
6 - MS + 0.1 mg l^{-1} BA + 0.2 mg l^{-1} Kn
7 - MS + 0.1 mg l^{-1} BA + 0.3 mg l^{-1} Kn

8 - MS + 0.2 mg l^{-1} BA + 0.05 mg l^{-1} Kn
9 - MS + 0.2 mg l^{-1} BA + 0.1 mg l^{-1} Kn
10 - MS + 0.2 mg l^{-1} BA + 0.2 mg l^{-1} Kn
11 - MS + 0.3 mg l^{-1} BA + 0.1 mg l^{-1} Kn
12 - MS + 0.3 mg l^{-1} BA + 0.2 mg l^{-1} Kn
13 - MS + 0.3 mg l^{-1} BA + 0.3 mg l^{-1} Kn

Fig. 9   Effect of different concentration of BA and Kn, on bud/leaf initiation and shoot elongation in MS medium

4.5   Effect of other media supplements on culture establishment and growth in MS medium

Various growth supplements like coconut water, activated charcoal casein hydrolysate and adenine sulphate along with other growth regulators were added in different concentration to find out their effect on culture establishment and growth. The result obtained are mentioned below.

4.5.1   Coconut water

Effect of two concentrations (10 and 20%) of coconut water along with other growth regulators, incorporated in MS medium, on culture establishment and growth was recorded. The result obtained is presented in Table 23(a) and 23(b).

Both the Table 23(a) and 23 (b) reveals that none of the two concentration (10 and 20%) of coconut water had any favourable effect on percentage of bud initiation and leaf initiation. However, in both tables the time taken for bud initiation and leaf initiation was short. On MS + BA 1.0 mg 1 + 0.1 mg l^{-1} IAA + 20% coconut water the time taken for bud initiation and leaf initiation was shortest (10 and 14 days, respectively). Shoot elongation was not observed in any level of coconut water with any of growth regulator combinations used.

4.5.2   Activated charcoal

Two levels of activated charcoal (0.25 and 1.0%) were added along with other growth regulators to the MS media. Data on culture establishment and growth are presented in Table 24(a) and 24(b).

Activated charcoal did not had any favourable effect on the percentage of bud initiation and leaf initiation. Both were more (87.75 and 100.00%) in BA and IAA supplemented media and (91.6 and 100%) in Kn and IAA supplemented media (without activated charcoal). However, bud initiation and leaf initiation were observed earlier (13 and 14 days) on MS + BA 1.0 mg l^{-1} + 0.1 mg l^{-1} IAA +

Table 23. Effect of coconut water on establishment and growth in axillary and terminal buds of *Tectona grandis* in MS media

Table 23a.

Treatment	Percentage of		Period (days) for		No. of leaves per explant	No. of shoots/ bud sprouts per explant	Mean shoot length (cm)	Maximum no. of shoots
	Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
BA 1.0 mg l^{-1} + 0.1 mg l^{-1} IAA	87.75	100.00	22.00	36.00	12.85	1.85	2.35	2.0
BA 1.0 mg l^{-1} + 0.1 mg l^{-1} IAA + 10% coconut water	28.57	50.00	13.00	14.00	4.00	1.50	0.00	2.0
BA 1.0 mg l^{-1} + 0.1 mg l^{-1} IAA + 20% coconut water	50.00	50.00	10.00	14.00	2.00	1.50	0.00	2.0

Table 23b.

Treatment	Percentage of		Period (days) for		No. of leaves per explant	No. of shoots/ bud sprouts per explant	Mean shoot length (cm)	Maximum no. of shoots
	Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
Kn 1.0 mg l^{-1} + 0.5 mg l^{-1} IAA	91.66	100.00	24.00	37.00	20.20	2.00	2.86	2.0
Kn 1.0 mg l^{-1} + 0.5 mg l^{-1} IAA + 10% coconut water	33.33	50.00	11.00	14.00	2.00	1.00	0.00	1.0
Kn 1.0 mg l^{-1} + 0.5 mg l^{-1} IAA + 20% coconut water	20.00	00.00	34.00	0.00	0.00	1.00	0.00	1.0

Table 24. Effect of activated charcoal on establishment and growth in axillary and terminal buds of *Tectona grandis* in MS media

Table 24a.

Treatment	Percentage of		Period (days) for		No. of leaves per explant	No. of shoots/bud sprouts per explant	Mean shoot length (cm)	Maximum no. of shoots
	Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
BA 1.0 mg l⁻¹ + 0.1 mg l⁻¹ IAA	87.75	100.00	22.00	36.00	12.85	1.85	2.35	2.0
BA 1.0 mg l⁻¹ + 0.1 mg l⁻¹ IAA + 0.25% activated charcoal	60.00	66.67	13.00	14.00	6.00	1.00	3.00	1.0
BA 1.0 mg l⁻¹ + 0.1 mg l⁻¹ IAA + 1.0% activated charcoal	50.00	100.00	16.00	20.00	6.00	1.50	2.50	2.0

Table 24b.

Treatment	Percentage of		Period (days) for		No. of leaves per explant	No. of shoots/bud sprouts per explant	Mean shoot length (cm)	Maximum no. of shoots
	Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
Kn 1.0 mg l⁻¹ + 0.5 mg l⁻¹ IAA	91.66	100.00	24.00	37.00	20.20	2.00	2.86	2.0
KN 1.0 mg l⁻¹ + 0.5 mg l⁻¹ IAA + 0.25% activated charcoal	33.33	100.00	17.00	30.00	2.00	1.00	0.50	1.0
Kn 1.0 mg l⁻¹ + 0.5 mg l⁻¹ IAA + 1.0% activated charcoal	75.00	66.67	15.00	19.00	4.00	1.00	1.00	1.0

Table 25a. Effect of Adenine sulphate on establishment and growth in axillary and terminal buds of *Tectona grandis* in MS media

Treatment	Percentage of		Period (days) for		No. of leaves per explant	No. of shoots/bud sprouts per explant	Mean shoot length (cm)	Maximum no. of shoots
	Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
BA 1.0 mg l^{-1} + 0.1 mg l^{-1} IAA	87.75	100.00	22.00	36.00	12.85	1.85	2.35	2.00
BA 1.0 mg l^{-1} + 0.1 mg l^{-1} IAA + 1 mg l^{-1} Adenine sulphate	50.00	100.00	21.00	43.00	2.00	1.00	0.50	1.00
BA 1.0 mg l^{-1} + 0.1 mg l^{-1} IAA + 4 mg l^{-1} Adenine sulphate	60.00	33.34	32.00	38.00	4.00	2.00	0.50	2.00
BA 1.0 mg l^{-1} + 0.1 mg l^{-1} IAA + 8 mg l^{-1} Adenine sulphate	50.00	66.67	15.00	21.00	14.00	3.50	0.70	5.00
BA 1.0 mg l^{-1} + 0.1 mg l^{-1} IAA + 12 mg l^{-1} Adenine sulphate	16.66	0.00	16.00	0.00	0.00	1.00	0.00	1.00

Table 25b.

Treatment	Percentage of		Period (days) for		No. of leaves per explant	No. of shoots/bud sprouts per explant	Mean shoot length (cm)	Maximum no. of shoots
	Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
Kn 1.0 mg l^{-1} + 0.5 mg l^{-1} IAA	91.66	100.00	24.00	37.00	20.20	2.00	2.86	2.00
Kn 1.0 mg l^{-1} + 0.5 mg l^{-1} IAA + 1 mg l^{-1} Adenine sulphate	33.33	50.00	9.00	30.00	2.00	1.00	0.00	1.00
Kn 1.0 mg l^{-1} + 0.5 mg l^{-1} IAA + 4 mg l^{-1} Adenine sulphate	50.00	33.34	13.00	23.00	2.00	1.00	0.00	1.00
Kn 1.0 mg l^{-1} + 0.5 mg l^{-1} IAA + 8 mg l^{-1} Adenine sulphate	60.00	66.67	10.00	18.00	4.00	1.00	0.75	1.00
Kn 1.0 mg l^{-1} + 0.5 mg l^{-1} IAA + 12 mg l^{-1} Adenine sulphate	33.33	0.00	14.00	0.00	0.00	1.00	0.00	1.00

Table 26a. Effect of Casein hydrolysate on establishment and growth in axillary and terminal buds of *Tectona grandis* in MS media

Treatment	Percentage of		Period (days) for		No. of leaves per explant	No. of shoots/bud sprouts per explant	Mean shoot length (cm)	Maximum no. of shoots
	Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
BA 1.0 mg l^{-1} + 0.1 mg l^{-1} IAA	87.75	100.00	22.00	36.00	12.85	1.85	2.35	2.00
BA 1.0 mg l^{-1} + 0.1 mg l^{-1} IAA + 100 mg l^{-1} Casein hydrolysate	18.18	50.00	14.00	24.00	10.00	2.00	1.00	2.00
BA 1.0 mg l^{-1} + 0.1 mg l^{-1} IAA + 500 mg l^{-1} Casein hydrolysate	20.00	50.00	13.00	24.00	4.00	1.00	1.00	1.00
BA 1.0 mg l^{-1} + 0.1 mg l^{-1} IAA + 1000 mg l^{-1} Casein hydrolysate	16.66	0.00	19.00	0.00	0.00	1.00	0.00	1..00
BA 1.0 mg l^{-1} + 0.1 mg l^{-1} IAA + 1500 mg l^{-1} Casein hydrolysate	42.85	0.00	26.00	0.00	0.00	1.00	0.00	1.00

Table 26b.

Treatment	Percentage of		Period (days) for		No. of leaves per explant	No. of shoots/bud sprouts per explant	Mean shoot length (cm)	Maximum no. of shoots
	Bud initiation	Leaf initiation	Bud initiation	Leaf initiation				
Kn 1.0 mg l^{-1} + 0.5 mg l^{-1} IAA	91.66	100.00	24.00	37.00	20.0	2.00	2.86	2.00
Kn 1.0 mg l^{-1} + 0.5 mg l^{-1} IAA + 100 mg l^{-1} Casein hydrolysate	33.34	33.34	9.00	20.00	4.00	1.30	0.50	2.00
Kn 1.0 mg l^{-1} + 0.5 mg l^{-1} IAA + 500 mg l^{-1} Casein hydrolysate	33.34	25.00	14.00	22.00	8.00	1.00	1.00	1.00
Kn 1.0 mg l^{-1} + 0.5 mg l^{-1} IAA + 1500 mg l^{-1} Casein hydrolysate	18.18	100.00	16.00	18.00	2.00	1.00	0.00	1.00

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time taken for bud initiation and leaf initiation was least (13 and 24 days). In MS + BA 1.0 mg l^{-1} + 0.1 mg l^{-1} IAA + 500 mg l^{-1} casein hydrolysate.

Mean shoot length was maximum (2.35 cm) in control while no elongation took place when MS + BA 1.0 mg l^{-1} + 0.1 mg l^{-1} IAA was supplemented with 1000 and 1500 mg l^{-1} Casein hydrolysate.

It took only 9 days for bud initiation on MS + Kn 1.0 mg l^{-1} + 0.1 mg l^{-1} IAA + 100 mg l^{-1} casein hydrolysate and no elongation of shoots was observed a MS + Kn 1.0 mg l^{-1} + 0.1 mg l^{-1} IAA + 1500 mg l^{-1} Casein hydrolysate.

4.6   Root induction from *in vitro* produced shoots

4.6.1   *In vitro* rooting

Various treatment combinations were tried for root induction in the *in vitro* produced shoots. Result obtained are presented in Table 27 and Fig 10. Maximum percentage of rooting (56.25) was observed on ½ MS + 0.4 mg l^{-1} NAA + 4.0 mg l^{-1} IAA + 0.25% activated charcoal supplemented to ½ MS differ from the shoots which were given pulse treatment in sterilized 1000 mg l^{-1} IAA solution.

Treatment effect on mean number of roots per explant and mean length of the root was found non significant. However, maximum mean number of roots per shoot (2.0) were found on ½ MS + 0.4 mg l^{-1} IBA + 4.0 mgl^{-1} IAA + 0.25 per cent activated charcoal (with pulse treatment) and WPM + 0.1 mg l^{-1} NAA + 4.0 mg l^{-1} IAA (with pulse treatment). In the latter treatment callus at the base of shoot was the main problem (Plate 9). Maximum mean length of roots (4.15 cm) was found on ½ MS + 0.4 mg l^{-1} IBA + 4.0 IAA + 0.25% activated charcoal (with pulse treatment) (Plate 10).

4.6.2   *Ex vitro* rooting

Three different media were tried for *ex vitro* rooting in *in vitro* produced shoots. The data obtained is tabulated in Table 28. Maximum rooting percentage

Table 27. Effect of various media combination on *in vitro* rooting of microshoots of *Tectona grandis*

Media	Treatments						Percentage of rooting	Mean nos. of roots per explant	Maximum nos. of roots	Mean length of roots (cm)	Max. length of roots (cm)
	Corresponding supplements				Pulse* treatment	State of medium					
	IAA (mg l^{-1})	IBA (mg l^{-1})	NAA (mg l^{-1})	AC (%)							
½ MS	0.1	0.1	0.1	0.25	-	Semisolid	0.00	0.00	0.00	0.00	0.00
	0.1	0.1	01	0.25	+	Semisolid	0.00	0.00	0.00	0.00	0.00
	2.0	2.0	-	-	-	Liquid	0.00	0.00	0.00	0.00	0.00
	2.0	2.0	-	-	+	Liquid	0.00	0.00	0.00	0.00	0.00
	2.0	2.0	-	0.25	-	Semisolid	0.00	0.00	0.00	0.00	0.00
	4.0	-	0.4	0.25	-	Semisolid	36.36	1.25	2.00	2.30	3.00
	4.0	-	0.4	0.25	+	Semisolid	56.25	1.55	2.00	2.00	5.00
	4.0	0.4	-	0.25	-	Semisolid	35.71	1.20	2.00	0.60	1.00
	4.0	0.4	-	0.25	+	Semisolid	55.55	2.00	4.00	4.15	6.00
WPM	0.1	0.1	0.1	-	-	Semisolid	33.33	1.00	1.00	2.50	2.50
	0.1	0.1	0.1	-	+	Semisolid	33.33	1.00	1.00	0.50	0.50
	0.5	0.5	0.5	-	-	Semisolid	33.33	1.00	1.00	0.50	0.50
	0.5	0.5	0.5	-	+	Semisolid	0.00	0.00	0.00	0.00	0.00
	4.0	-	0.1	-	-	Semisolid	33.33	2.00	2.00	2.00	2.00
	4.0	-	0.1	-	+	Semisolid	33.33	1.00	1.00	0.50	0.50
SEm±								0.15 NS		0.07 NS	

Pulse treatment is a dip of the base microshoots in 1000 mg l^{-1} IAA solution
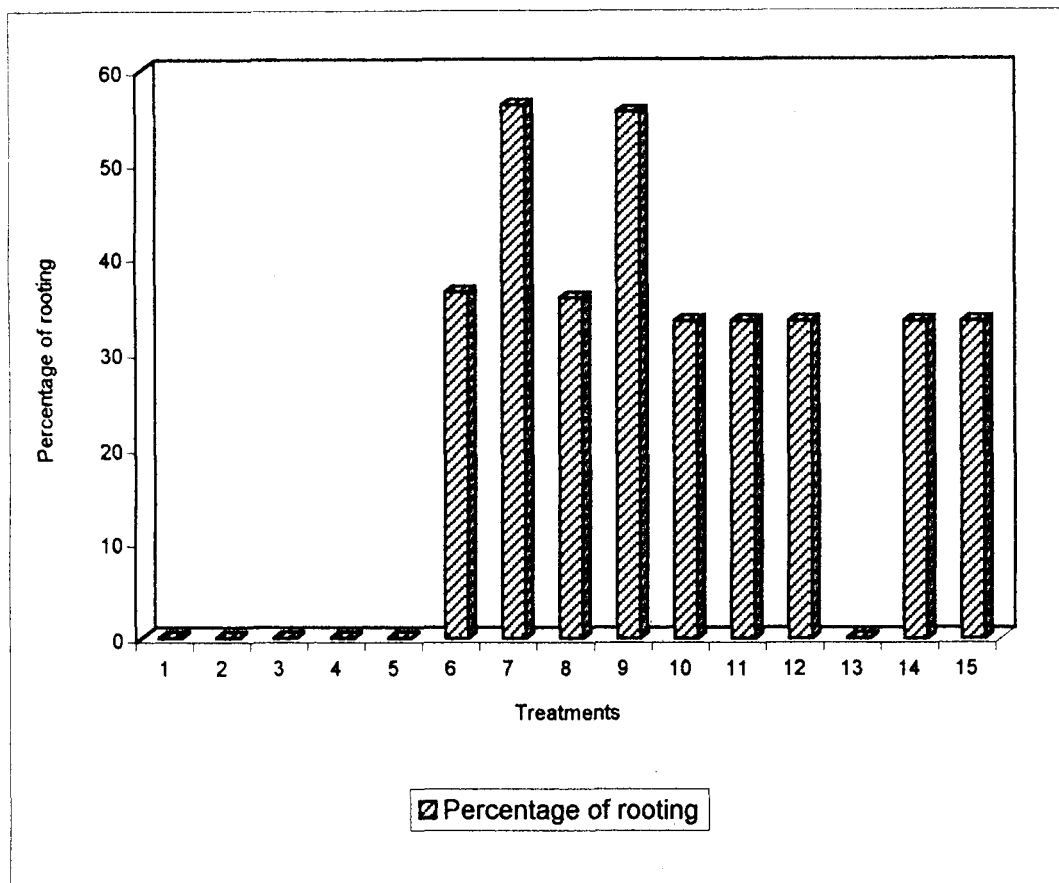
1 - ½MS + 0.1 mg l^{-1} IAA + 0.1 mg l^{-1} IBA + 0.1 mg l^{-1} NAA + 0.25% AC

2 - ½MS + 0.1 mg l^{-1} IAA + 0.1 mg l^{-1} IBA + 0.1 mg l^{-1} NAA + 0.25% AC*

3 - ½MS + 2.0 mg l^{-1} IAA + 2.0 mg l^{-1} IBA

4 - ½MS + 2.0 mg l^{-1} IAA + 2.0 mg l^{-1} IBA + 0.25% AC

5 - ½MS + 2.0 mg l^{-1} IAA + 2.0 mg l^{-1} IBA*

6 - ½MS + 4.0 mg l^{-1} IAA + 0.4 mg l^{-1} NAA + 0.25% AC

7 - ½MS + 4.0 mg l^{-1} IAA + 0.4 mg l^{-1} NAA + 0.25% AC*

8 - ½MS + 4.0 mg l^{-1} IAA + 0.4 mg l^{-1} IBA + 0.25% AC

9 - ½MS + 4.0 mg l^{-1} IAA + 0.4 mg l^{-1} IBA + 0.25% AC*

10 - WPM + 0.1 mg l^{-1} IAA + 0.1 mg l^{-1} IBA + 0.1 mg l^{-1} NAA

11 - WPM + 0.1 mg l^{-1} IAA + 0.1 mg l^{-1} IBA + 0.1 mg l^{-1} NAA*

12 - WPM + 0.5 mg l^{-1} IAA + 0.5 mg l^{-1} IBA + 0.5 mg l^{-1} NAA

13 - WPM + 0.5 mg l^{-1} IAA + 0.5 mg l^{-1} IBA + 0.5 mg l^{-1} NAA*

14 - WPM + 4.0 mg l^{-1} IAA + 0.1 mg l^{-1} NAA

15 - WPM + 4.0 mg l^{-1} IAA + 0.1 mg l^{-1} NAA*

Fig. 10   Effect of various media combinations in vitro rooting of microshoots of Tectona grandis

Table 28. Effect of various media on *ex vitro* rooting of *in vitro* regenerated microshoots of *Tectona grandis*

Media	Percentage of *ex vitro* rooting	Mean no. of roots per explant	Maximum no. of roots	Mean length of roots (cm)	Maximum length of the roots (cm)
Sand	25.00	3.33	5.00	1.16	2.00
Vermiculite	87.50	4.28	12.00	1.70	3.00
Cocoa peat	55.55	2.20	3.00	0.70	1.00
SEm±		0.72 NS		0.19 NS	

* All the microshoots were given dip of 1000mgl^{-1} IAA solution (at the base for 2 minutes) followed by dip in IAA powder before transferring to *ex vitro rooting media*

(87.5) was found in vermiculite (Plates 10,11), where as least (25) was observed on sand.

Treatment effect on mean number of roots per explant and mean length of root was found non significant. However, maximum mean number of roots per explant (4.28), maximum number of roots per shoot (12) and maximum mean shoot length (1.70 cm) was observed on vermiculite. Although cocopeat induced root initiation in 55.5 per cent of cultures, further growth of the roots were not satisfactory(Plate 12).

4.7   Planting out and acclimatization

Various media combinations were tried for acclimatization of *in vitro* produced plantlets. The result obtained is tabulated in Table 29 (Plates 14,15 & 16). The *in vitro* raised plantlets were transferred to pots containing different potting media. The pots were then covered with polythene bags to maintain the humidity. After one week some holes were made in the polythene bags to reduce the humidity. The plantlets were gradually exposed to ambient condition by keeping them open (without polythene covers) for few hour a day, later polythene covers were completely removed.

All the plants survived on sand and vermiculture after 2 months. In all other treatment the plantlets died due to fungal infection at the basal portion.

Table 29. Effect of various media on acclaimatization of the *in vitro* regenerated plantlet of *Tectona grandis*

Media	Survival percentage
Sand	100.00
Vermiculite	100.00
Cocoa peat	0.00
50% sand + 50% vermiculite	0.00
50% sand + 25% vermiculite + 25% cocoa peat	0.00
33% sand + 33% cocoa peat + 33% vermiculite	0.00

Discussion

DISCUSSION

Teak (*Tectona grandis* Linn. f.), one of the most important commercial timbers of the country as well as world, with wide genotypic variation from region to region, aptly need a viable method for true to the type multiplication. The present investigation was undertaken to develop a workable protocol for *in vitro* propagation of *Tectona grandis* by using explants from seedling. The result of the study are discussed below.

5.1   Culture contamination

In the present investigation culture contamination was a serious problem since the mother plants were growing in the open field. It was observed that there was almost 100.0 per cent contamination during rainy season. This may be due to the congenial and conducive weather conditions which favour the rampant proliferation of microbial innoculam in the field.

Among the various measures taken to control culture contamination during the present study, a substantial reduction in contamination could be obtained by immersing the explants in 0.15 per cent mercuric chloride. A further enhancement in the number of contamination free culture was noticed when the mother plants were given prophylactic spraying of fungicide before the explants were collected. The most ideal treatment combination was found to be periodic spraying of mother plants with Bavistin and Indofil M-45 (both at 0.1 per cent), dipping the explant from source plant in the fungicide solution for 30 min and finally surface sterilization by 0.15 per cent mercuric chloride for 15 min. Use of mercuric chloride as a sound surface sterilant was reported by a number of authors in many tree species such as *Gmelina arborea* (Kannan and Jasrai, 1996), *Lagerstromia parviflora* (Quraishi *et al.*, 1997), *Vateria indica* (Divatar, 1994), *Dalbergia latifolia* (Kannan, 1995), *Ailanthus triphysa* (Natesha, 1999) etc.

Plant tissue culture media is rich in inorganic salts and organic nutrients including sucrose. This media is a good substrate for growth of many saprophytic bacteria and fungi (Dhawan, 1993). The problem of contamination get aggravated further if the inoculate or explant used is not from properly disinfected plant material (Razdan, 1993). Leifert and Woodward (1998) reported that microbial contamination is the single most important cause of losses in commercial and scientific plant tissue culture laboratories. The percentage of contamination was more than 90 for field explant irrespective of procedure used for their sterilization (Doublin, 1984). Many times, control of contamination is extremely difficult and many contaminants impossible (Leifert and Woodward, 1998). The preculture treatment of the explant source with fungicidal spray for control of contamination was suggested by many authors. Legrand and Mississo (1986); Mahato (1992); Santhoshkumar (1993); Divatar (1994); Kannan (1995); Natesha (1999).

The size of explants appears to have its effect on manifestation of culture contamination. This is indicated by the observation that 2.0 cm long explants showed a higher contamination (68.78%) in comparison to the 1.0 cm long explants (46.88%). The obvious reason for this differences can be that the large sized explants have larger surface area and may harbour more inoculate as compared to smaller sized explant. The effect of size of explants on contamination was documented by Natesha (1999) in *Ailanthus triphysa*.

It is always desirable for economic feasibility of tissue culture technique to have minimum percentage of contamination. In many cases the contamination was found as late as after 15 days of culturing and also with bud sprout. This may be due to the presence of latent spores of fungus or other microorganism deep inside the tissues which easily get survived in the surface sterilization treatment and later when metabolic process starts their growth also is favoured and get expressed after a long gap.

5.2   Polyphenol exudation

A serious problem associated with the culture of teak tissue culture is incessent exudation of phenolic substances from cut ends of explant (Plate 4). These phenols subsequently get oxidised to dark coloured quinol which are toxic to the explants thus leading to their death. Depending on the plant species the problem may be restricted to time of establishing fresh culture or may perpetuate at each subculture (Dhawan, 1993). Survival of explants was reduced to 20 per cent when polyphenol exudation was left unchecked (Razdan, 1993). Polyphenol can be oxidised either by peroxidases or oxidases (Mayer and Harel, 1979). The oxidised compounds are highly toxic, they form covalent bonds with the plant protiens thus inhibiting enzyme activity (Hu and Wang, 1983) causing browning and death of the explant.

In the present study, 20.0 per cent cultures exhibited phenol exudation when cultures were given dark incubation for initial 20 days. However, all explants died. This result suggest that light had pronounced effect on phenol exudation. Reduction in phenol exudation when cultures were kept in darkness is reported by Preper and Zimmer (1986).

A substantial decrease in percentage of culture exhibiting phenol exudation and quantity, without death of explants, recorded in media supplemented with citric acid and ascorbic acid at 150.0 mg l^{-1} each. In all the treatments phenol exudation from smallest size of explants was the least. Use of various antioxidants in media as well as their treatment to explant before sterilization has been reported by many authors. The shoot tips of *Psidium* were soaked in a solution containing 150.0 mg l^{-1} of citric acid and ascorbic acid prior to surface sterilization (Amin and Jaiswal, 1987). Gupta *et al.* (1980) reported that use of PVP for the control of phenol in teak. Dhawan (1993) has also documented that incorporation of antioxidants in media become obligatory to overcome browning problem. If it occur at each subculture. Activated charcoal (A.C.) at 1.0 g l^{-1} reduced the quantity

of phenol in all sizes of explants. Weatherhead *et al.* (1979) reported that A.C. absorb substances inhibitory to growth and thereby promote growth.

5.3 Basal media and effect of various growth regulators on culture establishment and growth

Widely used culture media for micropropagation of trees are MS (Murashige and Skoog, 1962), WPM (Lloyd and McCown, 1980) and half strength MS media. A comparitive study of the effect of various media on culture establishment and growth of axillary and terminal buds was carried out. The percentage of bud initiation and mean number of leaves per explants were found highest in WPM and MS. The number of days taken for bud initiation was also lesser in these media.

Both MS and WPM were supplemented with various concentration of Kn and BA individually. It was observed that lower concentration of BA or Kn leads to higher percentage of bud initiation, mean shoot length and mean number of leaves per explant (Tables 9, 10, 11, 12). Though BA (0.1 mg l^{-1}) in both MS and WPM and Kn (0.5 mg l^{-1}) in WPM produced the highest percentage of bud initiation (66.66%). Yet it was found that BA (at 0.1 mg l^{-1}) performed better in terms of percentage of leaf initiation and mean shoot length (4.0 cm).

Ahmed (1990) reported that in general BA has been frequently used to induce better shoot growth and multiplication than other cytokinins particularly in tree species. Kannan (1995) had documented that increase in BA concentration brought down the number of responding cultures and shoot length both in MS and WPM. Natesha (1999) reported that BA was found to yield better result in MS based media in comparison to Kn. Lower concentration of BA leads to the best growth of shoots and leaves was documented by many authors in species like *Eucalyptus globulus* (Pattanaik and vijayakumar, 1997), *Morus alba* (Kanwar *et al.*, 1991), *Daubanga grandiflora* (Kumar and Kumar, 1997), *Ailanthus triphysa* (Natesha, 1999) and *Betula pendula* (Durkovic, 1997).

Out of the different combinations of BA and Kn attempted in MS and WPM, 1.0 mg l⁻¹ BA in MS induced upto 5 shoot per culture. This indicate the potentiality of BA for induction of multiple shoots in axillary and terminal buds. Multiple shoots induction on media supplemented with BA has been reported by many authors in species like *Acacia mearnsii* (Beck *et al.*, 1998) and *Aegle marmelos* (Arumugam and Rao, 1996).

In plant tissue culture auxins are exclusively used to exploit its potentiality for stimulation of cell division and elongation. However, the requirement of these growth harmones vary considerably with reference to the types and its concentration. The response of the tissues to the various auxins is believed to vary depending upon their endogenous levels in the tissue. In the present study 4 levels of IAA, NAA and IBA were supplemented to MS and WPM. A comparison between the three auxins, points out that IAA has a better response in both the medium with reference to bud and leaf initiation. In both the media IAA could induce leaf formation in 100.0 per cent of cultures sharing bud break. The superior response of IAA was evident in case of the mean number of leaves produced as well as average length of the shoot. This response was however better when IAA was added to MS media.

Among the two basal media attempted in the investigation, medium MS by itself and also in combination with the cytokinin BA as well as auxin IAA was showing a better performance on the axillary and terminal bud cultures of *Tectona grandis* (Plate 5). This could be seen with respect to percentage of bud initiation, leaf initiation, mean number of leaves as well as shoot length (Plates 2 & 3). Medium MS is reported to be widely used in a number of species like *Eucalyptus globulus* (Pattanaik and Vijayakumar, 1997), *Gmelina arborea* (Yaung *et al.*, 1993), *Robinia pseudoacacia* (Barghchi and Chi, 1998), *Tectona grandis* (Narsimhan and Dhruva, 1970), *Betula pendula* (Durkovic, 1997). Based on these observation MS was selected for further studied and was supplemented with auxins, cytokinins as well as other growth supplements.

The interaction of auxin and cytokinin is complex. Kaur *et al.* (1998) reported that in *Acacia senegal* maximum number of shoot buds were formed on MS medium supplemented with auxin and cytokinins. The inhibitory effect of higher concentration of cytokinin alone in media have been observed in earlier experiments. Lundergan and Janick (1980) reported that the auxin can nullify the suppressive effect of high cytokinin. A synergistic effect of cytokinin and auxin combination on shoot production has been reported in *Syzygium alternifolium* by Khan *et al.* (1997).

In the present study, a combination of BA and IAA at the lower concentration (1.0 mg l^{-1} BA + 0.1 mg l^{-1} IAA) were found to have synergistic effects in bud as well as leaf initiation from the cultures. Higher concentration of BA (1.5 mg l^{-1}) which was showing a relatively lower percentage of bud and leaf initiation when supplemented alone could give upto 75.0 per cent bud initiation with additional supplementation of 0.1 mg l^{-1} IAA. In combination of Kn and IAA also a similar trends could be envisaged. Upto 92.30 per cent of bud initiation has been noted in MS + 1.0 mg l^{-1} Kn + 1.0 mg l^{-1} IAA. However, leaf initiation was found to be better at lower levels of auxins (Table 16 and Plate 6).

Combination of other two auxins, namely, IBA and NAA along with BA and Kn seems to be not as effective as the IAA combination. Eventhough upto 90.0 per cent bud initiation could be obtained in some of the combinations, leaf formation as well as shoot growth was relatively less in almost all these levels. Das *et al.* (1996) has reported that maximum number of shoots of *Salmalia malbarica* were obtained on MS media containing cytokinin (BA) and auxins (NAA, IBA).

The naturally occurring cytokinin, 2ip has been reported to be more effective than BA or Kn in number of species like *Rhododendron* (Anderson, 1975), *Kalmia latifolia* (Lloyd and McCown, 1980). Considering the potential of 2ip for shoot production, an experiment was carried out to find out the effect of

2ip, on culture establishment and growth of teak, when supplemented to some of the promising levels of BA, Kn and IAA. With the incorporation of 2ip into these media combinations the percentage of bud initiation increased considerably. In MS + 1.0 mg l^{-1} Kn + 0.1 mg l^{-1} IAA + 1.0 mg l^{-1} 2ip highest percentage of bud initiation (90.90) in 18 days with 90.00 per cent leaf initiation was observed as compared to only 50.00 per cent bud initiation (in 24 days) and 75 per cent leaf initiation on the same media devoid of 2ip. The mean shoot length was observed to be high in the former media combination than latter. It was also observed that with the increase in concentration of 2ip the percentage of bud initiation followed a decreasing trend.

Thirteen different treatment combination of Kn and BA were tried (Table 22). With the increase in concentration of cytokinins a decrease in percentage of bud initiation was recorded. Highest percentage of bud initiation (77.78) was observed on media with 0.05 mg l^{-1} BA and 0.05 mg l^{-1} Kn with 54.14 per cent of leaf initiation in 18 days. However, on comparison with the result of earlier experiments it was found that media containing BA or Kn individually or in combination with auxin could induce better bud initiation, leaf initiation and shoot elongation. It suggest that in general, combination of cytokinins (BA and Kn) in MS do not have any synergistic effect on any of the growth parameter. Our results were not in confirmity with that of Ravikumar *et al.* (1998) (*Dendrocalamus strictus*) and Sita *et al.* (1992) have reported that combination of BA and Kn was better to increase shoot number in Red sandal wood. However, Minocha (1987) suggested that some morphogenetic responses are caused by combination of two or more growth regulators at certain concentration or in certain ratios, where as other concentration of some growth regulators either have no effect or have qualitatively different effect.

5.4   Other growth supplements

5.4.1   Coconut water

Positive effect of coconut water on bud break and shoot formation have been reported by many authors in a number of species such as *Artocarpus heterophyllus* (Roy *et al.*, 1996), *Dendrocalamus strictus* Nees. (Ravikumar *et al.*, 1998), *Elaeocarpus robusta* (Roy *et al.*, 1998), *Syzygium cumminii* (Roy, 1996). Straus and rodney (1960) have reported that the favourable effect of coconut water in plant tissue culture is due to the presence of cytokinin and gibberellin like substances in it.

In the present investigation coconut water did not exhibit any additional effect on bud initiations and shoot elongation. Among the various combinations of CW tried in MS medium, maximum percentage of shoot initiation (50.00) was found on MS with BA (1.0 mg l^{-1}), Kn (0.1 mg l^{-1}) and 20 mg l^{-1} coconut water. Where as in media without coconut water, the values corresponding to above mentioned parameter were much better. Since coconut water is a natural product containing various chemicals, the concentration of its active ingredients vary from fruit to fruit, tree to tree and locality to locality. It is therefore considered as not a reliable media supplement. Lack of beneficial effect of coconut water on shoot formation and leaf production has been noticed in *Dalbergia latifolia* (Mahato, 1992), *Vateria indica* (Divatar, 1994)and *Dalbergia latifolia* (Kannan, 1995).

5.4.2   Activated charcoal

In the present study, it has been observed that activated charcoal had an inhibitory effect on shoot initiation and growth of axillary and terminal buds. Highest per cent of bud initiation (75.00) was found on MS supplemented with 1.0 mg l^{-1} Kn + 0.5 mg l^{-1} IAA + 1.0 per cent A.C. as compared to 91.66 per cent in control. Inhibitory effect of A.C. may be due to its binding with plant growth regulator and other metabolites (Weatherhead *et al.*, 1978). Addition of activated charcoal to liquid MS media reduced IAA and IBA concentration over 97 per cent

(Scott *et al.*, 1990). Various other authors have also reported this effect in many species like in *Dalbergia latifolia* (Mahato, 1992), *Pterocarpus marsupium* (Santhoshumar, 1993), *Dalbergia latifolia* (Kannan, 1995).

5.4.3   Adenine sulphate

Favourable effect of adenine sulphate on shoot initiation and growth have been reported in *Acacia senegal* (Kaur *et al.*, 1998), *Azadiracta indica* (Eeswara *et al.*, 1997), Cashewnut (Ananthakrishnan *et al.*, 1999), *Citrus jambhiri* (Singh *et al.*, 1999), *Ficus religiosa* (Deshpande, 1998). The beneficial effect of adenine may be due to its cytokinin like activity (Skoog and Tsui, 1948). Various levels (4) of adenine sulphate were tried with BA and IAA or Kn and IAA in *T. grandis* during the present investigation (Tables 25a and 25b). Highest percent of bud initiation and leaf initiation (60.00 and 66.67, respectively) were found on MS + Kn 1.0 mg l^{-1} + 0.5 mg l^{-1} IAA + 8 mg l^{-1} adenine sulphate in minimum number of days (10 and 18, respectively) with maximum mean shoot length (0.75 cm). However, the maximum of various growth parameter obtained were lower than in control. The inhibitory effects of adenine sulphate have been documented in an earlier study to potato by Jerret *et al.* (1980).

5.4.4   *Casein Hydrolysate*

Casein hydrolysate is a complex mixture of several amino acids. Beneficial effect of CH in micropropagation has been reported in *Elaeocarpus robusta* (Roy *et al.*, 1998), *Boswellia serrata* (Prakash *et al.*, 1999), *Syzygium cumminii* (Roy, 1996).

In the present study it was observed that CH had a high degree of inhibitory effect on shoot initiation, elongation and leaf formation as compared to all other growth supplement used. This may be due to excess of nitrogen in the form of ammonium in casein hydrolysate. Many workers have reported ammonium to be toxic to plant cell as it cannot be accumulated in the cytoplasm (Kirby *et al.*,

1987). Out of the different combinations tried (Tables 26a and 26b), MS with Kn (1.0 mg l^{-1}) + IAA 90.5 mg l^{-1}) + 100.0 mg l^{-1} CH was found better with high percentage of leaf initiation and bud initiation in minimum time. In some earlier studies by Divatar (1994) in *Vateria indica* and Kannan (1995) in *Dalbergia latifolia* also, beneficial effect of CH was not found.

5.5   Multiple shoot

In commercial tissue culture, production of one shoot from one bud is neither economical nor desirable. So to decrease the cost of production of tissue cultured plants and to produce large number of planting material from scarce resource available, it is imperative to develop a procedure to obtain multiple shoots. These shoots can be obtained simultaneously or by subsequent subculturing of single explant.
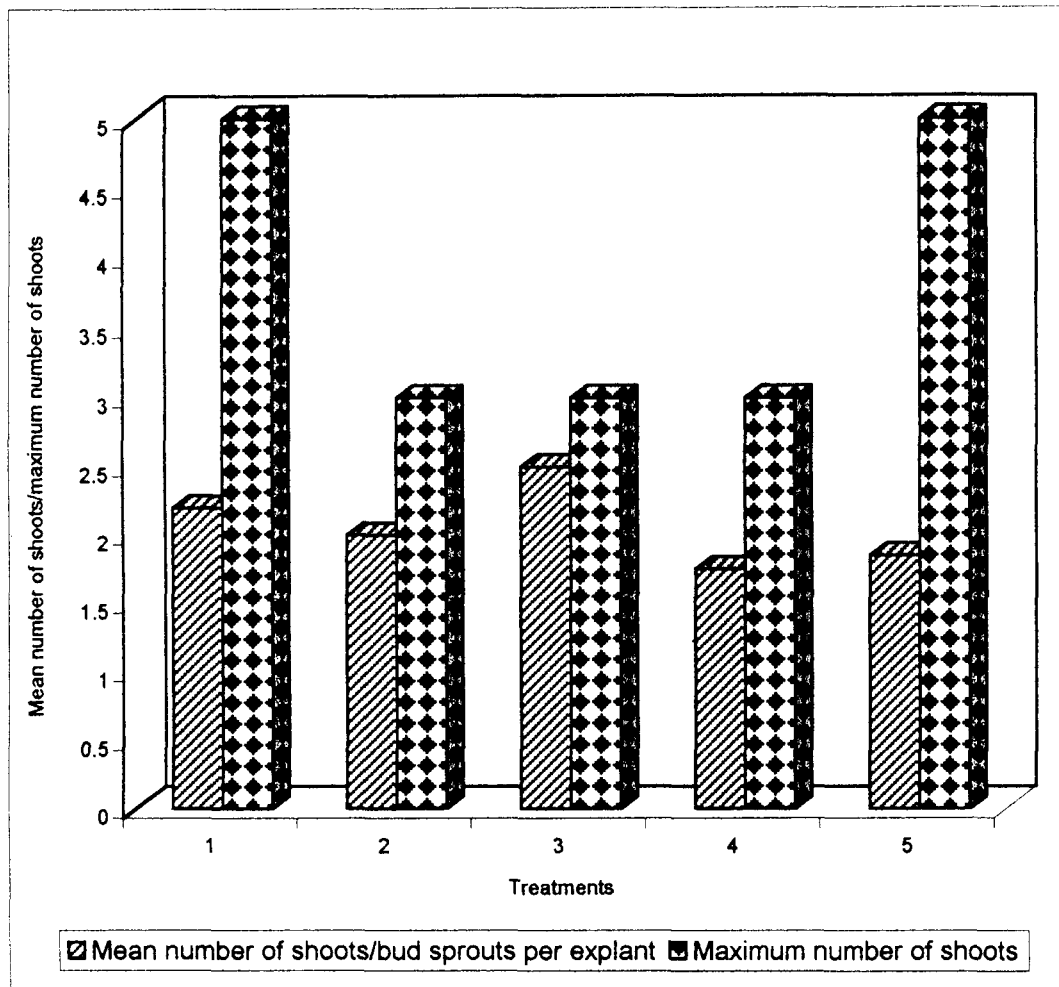
In the present study a normal explant (nodal segment) of teak have two buds owing to the opposite leaf arrangement. Thus two shoots from one explants were considered as normal (Plate 1). In some of the growth regulator combination multiple shoots were obtained (Table 30 and Plate 7). Highest numbers of shoots per explant (5) were found in MS media supplemented with Kn 1.0 mg l^{-1}. Induction of multiple shoots have been reported in many species like *almus cremastogyna* (Tang *et al.*, 1996), *Annogeissus pendula* (Joshi *et al.*, 1991), cashewnut (Ananthakrishnan *et al.*, 1999).

5.6   Rooting

The formation of adventitious roots on microcutting is a crucial step in commercial micropropagation (Monocusin, 1991). Roots may be generated *in vitro* by placing excised microshoots from proliferation cultures into auxin supplemented media alternatively microshoots may be placed into soil less rooting medium for *ex vitro* root initiation and concurrent gradual acclimatization (George and Sherrington, 1984). The type of root system formed depends on the physical

Table 30. Some promising media combinations which induced multiple shoots.

Basal media	Growth regulators (mg l^{-1})					Mean shoot length (cm)	Mean number of shoots/bud sprouts per explant	Maxi mum numb er of shoots
	BA	Kn	IBA	IAA	2ip			
MS	1.5	-	-	-	-	1.93	2.20	5.0
MS	2.0	-	-	-	-	1.80	2.00	3.0
MS	1.0	1.5	-	-	-	3.25	2.50	3.0
MS	1.0	-	1.0	-	-	1.66	1.75	3.0
MS	1.0	-	-	0.1	1.0	2.11	1.85	5.0

1 - MS + 1.5 mg l^{-1} BA
2 - MS + 2.0 mg l^{-1} BA
3 - MS + 1.0 mg l^{-1} BA + 1.5 mg l^{-1} Kn
4 - MS + 1.0 mg l^{-1} BA + 1.0 mg l^{-1} IBA
5 - MS + 1.0 mg l^{-1} BA + 0.1 mg l^{-1} IAA + 1.0 mg l^{-1} 2ip

Fig. 11   Some promising media for multiple shoot induction

characteristics of rooting environment (Monocusin, 1991) as well as the species and quality of the microcutting (George and Sherrington, 1984).

5.6.1   *In vitro* rooting

In our study, it was observed that treatment combination containing lower levels of IAA or IBA in ½ MS could not induce any root from microshoots. But ½ MS + IAA at 4.0 mg l^{-1} with 0.4 mg l^{-1} IBA or NAA has induced rooting (Plate 10). Pulse treatment with high concentration auxin to the cut ends of microshoots further enhanced root production as well as elongation. Highest per cent of rooting (56.25) with average length of 5.0 cm was obtained on ½ MS + 0.4 mg l^{-1} NAA + 4.0 mg l^{-1} IAA + 0.25 per cent A.C. with pulse treatment. Minocha (1987) had proposed that generally auxins promote rooting of microshoots especially at lower concentration. Use of half strength basal media with various concentration of auxin and A.C. as rooting medium is reported by many workers in species like *Albizia lebbeck* (Majumdar *et al.*, 1998), *Elaeocarpus robusta* (Roy *et al.*, 1998) and *Eucalyptus globulus* (Pattanaik and Vijayakumar, 1997).

Woody Plant Medium was not a favourable basal medium for rhizogenesis in the microshooting of teak. At all levels of auxin supplementation in this medium, percent root induction, number of roots per shoot as well as length of roots were relatively less than that obtained in ½ MS media combinations (Plate 9). This observation can tentatively be attributed to the lack of A.C. in the combinations. Other probable reason may be the higher salt concentration in WPM in comparison to ½ MS which might had inhibitory effect on root induction. Pulse treatment also did not have any effect.

Out of two media tried, ½ MS along with various levels of auxins were found better for root induction. In this media it was noted that use of pulse treatment, addition of charcoal to media and transfer of the shoots into auxin free media after 7 days yielded best results. In cultures where the shoots were not transferred to auxin free media, no root elongation was observed. The further root

elongation in such media may however be inhibited by auxin. Transfer of microshoots to auxin free media as a requirement for root elongation was also documented in *Tectona grandis* (Gupta *et al.*, 1980), *Dalbergia latifolia* (Kannan, 1995), *Tecomella undulata* (Nandwani *et al.*, 1996), *Terminalia ivorensis* (Belaizi *et al.*, 1992).

5.6.2 *Ex vitro rooting*

Ex vitro rooting helps in reducing the number of steps involved in micropropagation. Peat, perlite, vermiculite, pumice, sand, soil, rock wool etc. are commonly used as rooting substrate (John *et al.*, 1997).

Among the three different media tried for *ex vitro* rooting (Table 28 and Plates 11, 12 & 13), vermiculite was found best with 87.5 per cent of rooting with highest number of roots per explants (4.28) and maximum mean length of root (1.70 cm). It may be attributed to high level of nutrient content and good water holding capacity of vermiculite. Kannan and Jasrai (1996) reported 100 per cent root initiation of *Gmelina arborea* when cut ends of microshoots were pulsed for 5 minutes with 246 µm Indole-3-butric acid and transferred to plastic cup containing sterile vermiculite. Schwarz *et al.* (1988) advocated the direct soil rooting approach which may provide a simple, highly efficient and more economic methodology in plant regeneration.

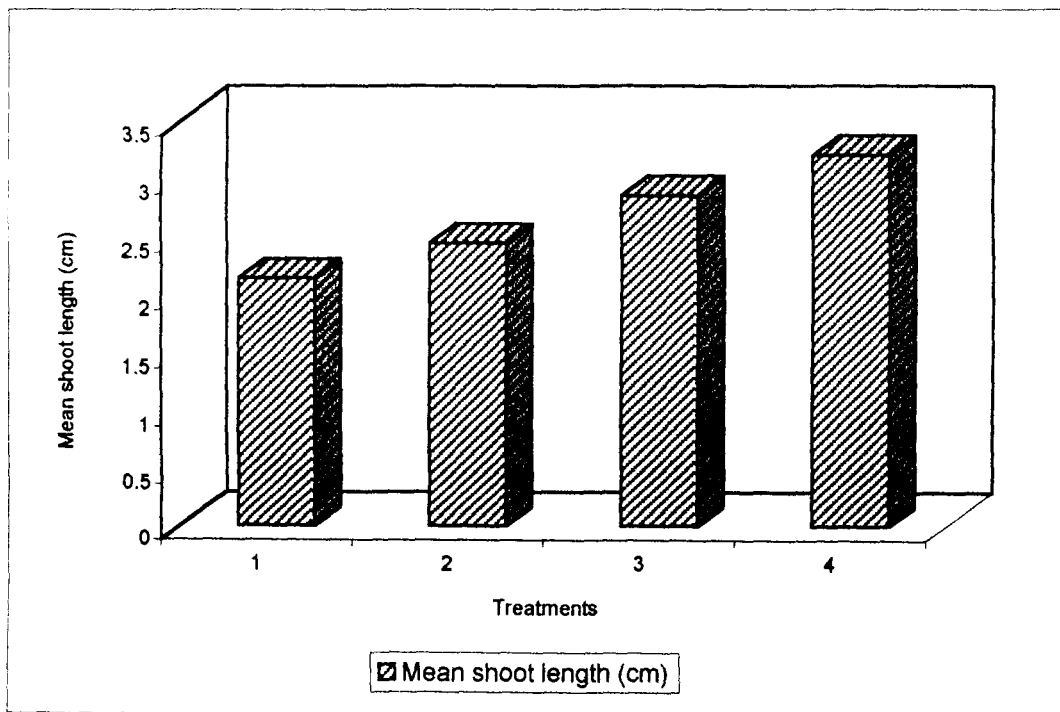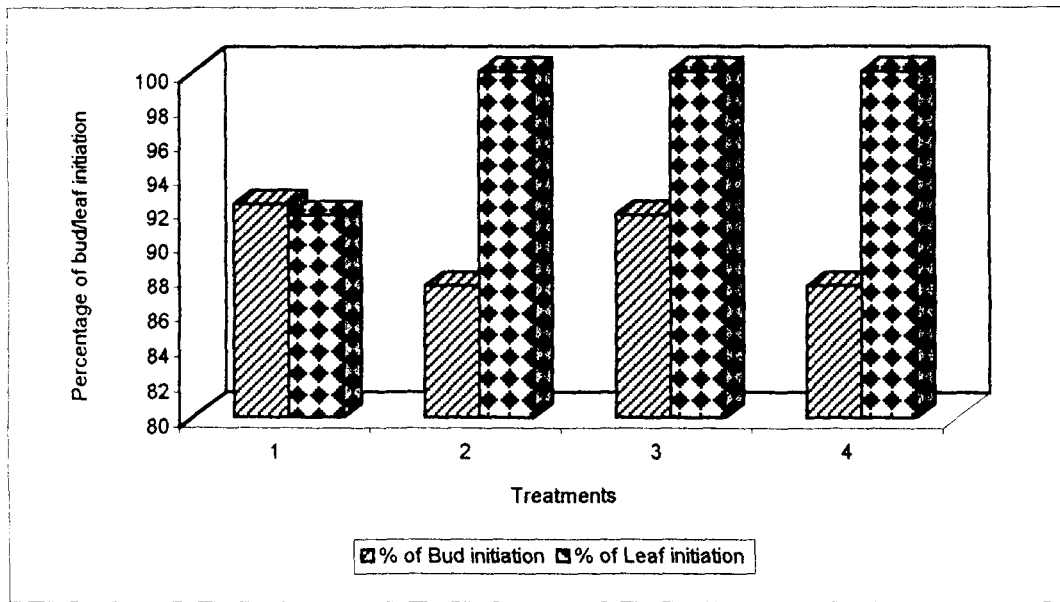## 5.6 Acclimatization and planting out

Hardening of tissue cultured plants is a critical factor for subsequent survival as these plantlets are very delicate and generally lack epicuticular development (Murashige, 1977). Transferring of plants from the culture vessel to the potting mixture require careful and stepwise procedure. Success in acclimatization depends upon not only post transfer condition but pretransfer culture conditions also (Ziv, 1986).

The culture media is enriched with sucrose and other organic nutrients and consequently plantlets, though they appear green, lack photosynthesis. The humidity inside culture vessel is very high (close to 95.0%) and therefore the plant lack the protective cuticle. In the present study, higher level of humidity was maintained initially by covering planted plantlets with polythene bags and spraying water regularly (Plate 14). The exposure time was increased with each day and later the covering was removed completely (Plate 15). Various media combination were tried (Table 29) for hardening. All the plantlets survived on sand as well as on vermiculite. This hardening techniques had also been documented in *Robinia pseudoacacia* (Wang *et al.* 1985), *Morus alba* (Kanwar *et al.*, 1991), teak (John *et al.*, 1997. Use of sand as a acclimatization medium was also reported in *Eucalyptus hybrid* FRI-4 (Chauhan *et al.*, 1996). Among the more than 180 media combination attempted during the course of the present investigation in the micropropagation of teak using axillary and terminal buds, the most promising ones for shoot morphogenesis have been identified and presented in Table 31.

The best rooting treatment that can be used is the *ex vitro* rooting in vermiculite medium (Plate 13).

1 - MS + 1.5 mg l⁻¹ IAA
2 - MS + 2.0 mg l⁻¹ BA + 0.5 mg l⁻¹ IAA
3 - MS + 0.5 mg l⁻¹ Kn + 0.5 mg l⁻¹ IAA
4 - MS + 1.5 mg l⁻¹ Kn + 0.5 mg l⁻¹ IAA

Fig. 12   Some promising media combinations for high percentage of bud/leaf initiation and shoot elongation

Summary

SUMMARY

The research programme entitled "Micropropagation of teak (*Tectona grandis* Linn.) through *in vitro* techniques" was undertaken in the Tissue Culture Laboratory of the College of Forestry, Vellanikkara during the period 1998-2000. The salient findings from the study are highlighted below:

1. The culture contamination was noted to be one of the major problems confronted in teak tissue culture. Prophylactic spray of fungicidal mixture of 0.1 per cent each of Bavistin (Carbendazim) and Indofil M-45 (Mancozeb) twice a week followed by a dip of explants in the same mixture for 30 minutes and 15 minutes surface sterilization with 0.15 per cent $HgCl_2$ reduced the percentage of culture contamination to 20.0 per cent. However, this percentage was higher during the rainy season.

2. Bigger the size of explant more was the percentage of contamination. The highest culture contamination (68.78%) was found when explants (2.0 cm long below node) were surface sterilised with 0.15 per cent $HgCl_2$ for 15 minutes.

3. Incessant flow of phenol from the cut ends and subsequent oxidation into quinones is the main hitch in micropropagation of teak. No phenol exudation was observed when the culture with explant of sizes 1 cm and 2 cm (1 cm below the node 1 cm above the node) were kept in dark, but all the explant did not survive. The phenol exudation could be reduced considerably when the media was supplemented with a mixture of citric acid and ascorbic acid at the rate of 150.0 mg l^{-1} each.

4. Out of three basal media tried, MS and WPM was found to be better than half strength MS and were used for further studies.

5. When MS and WPM were supplemented with auxins (IAA, NAA, IBA) and cytokinins (BA, Kn) at different levels, it was found that MS was better than WPM.

6. It was also observed that increase of the BA concentration in MS had adverse effect on growth and culture establishment. No elongation of shoots was found at high concentration of BA in MS.

7. Addition of BA in MS media at lower concentration had pronounced effect on culture establishment and growth.

8. Among the auxins (IAA, NAA and IBA), IAA was found to be better in terms of culture establishment and growth in both MS and WPM media.

9. Combinations of MS + 1.0 mg l^{-1} Kn + 1.0 mg l^{-1} IAA and MS + 0.5 mg l^{-1} Kn + 0.5 mg l^{-1} IAA were found better than all other treatment combinations in terms of high percentage of bud initiation, leaf initiation and shoot length.

10. Addition of 2iP at lower concentration to MS media containing low amount of cytokinin (BA, Kn) and auxin (IAA) had a synergistic effect on percentage of bud initiation, leaf initiation and mean shoot length.

11. Low concentration of BA and Kn in MS was found better than high concentration of both the cytokinins. Media with BA and Kn at 0.05 mg l^{-1} each was found best among all other treatment tried.

12. Coconut water, activated charcoal, casein hydrolysate and adenine sulphate added to media showed very little effect on bud initiation, leaf initiation and shoot elongation.

13. Among the cytokinin-cytokinin and cytokinin auxin combinations in MS, the later was found to be better.

14. Multiple shoots (5 number) were also obtained in MS + 1.0 mg l^{-1} BA and MS + 1.0 mg l^{-1} BA + 0.1 mg l^{-1} IAA + 1.0 mg l^{-1} 2ip.

15. Half strength MS media supplemented with 0.4 NAA + 4.0 IAA (in mg l^{-1}) + 25.0 per cent AC with pulse treatment was found to be the best for root induction.

16. Pulse treatment (dip of excised shoot in 1000 mg l^{-1} IAA solution for 2 minute) and transferring the shoot to auxin free media after 7 days lead to high percentage of rooting.

17. Roots with callus at the base of excised shoots were recorded on WPM supplemented with Auxins.

18. *Ex vitro* rooting was high (87.5%) in vermiculite, when the excised shoots were given pulse treatment for 2 minutes in liquid IAA followed by touch of IAA powder at cut end.

19. Planting them in sterile sand or vermiculite at high humidity condition and gradually bringing to ambient condition did acclimatization of *in vitro* plantlets. Such plantlets were further transferred to polybags containing ordinary potting mixture and treated like normal seedling.

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*Originals not seen

Plates

Plate 1. Bud sprout in nodal segment of teak

Plate 2. Initiation of leaves from axillary bud of teak

Plate 3. Leaf enlargement and internodal elongation

Plate 4. Phenol exudation in control and ascorbic acid + citric acid
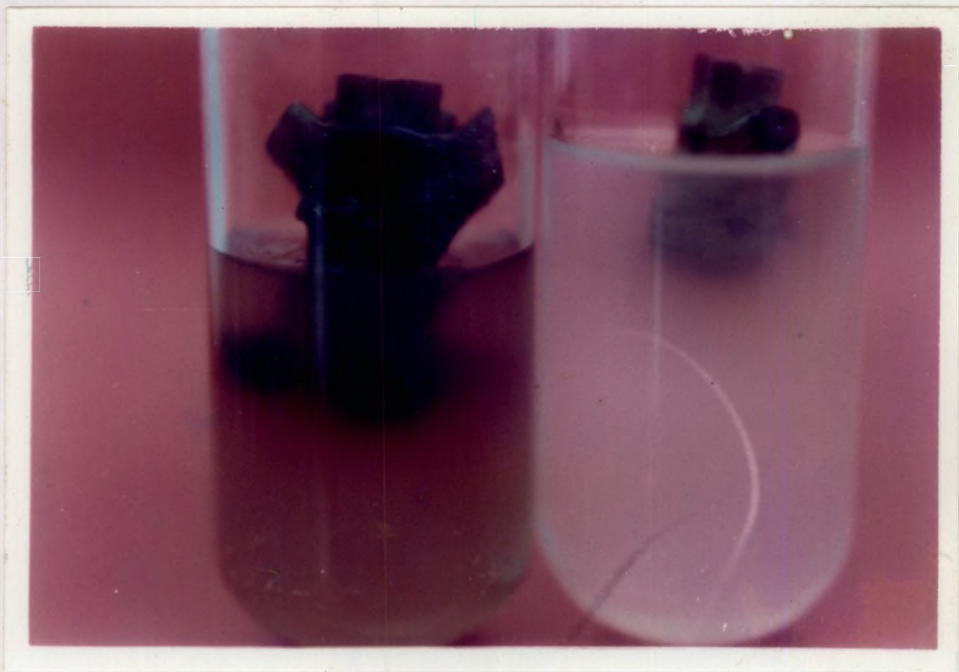supplemented media

Plate 5. Shoot characteristics in MS and ½ MS medium

Plate 6. Shoot elongation in MS + 1.5 mg l^{-1} Kn + 0.5 mg l^{-1} IAA

Plate 7. Multiple shoots production from axillary buds on MS + 1.0 mg l^{-1} BA

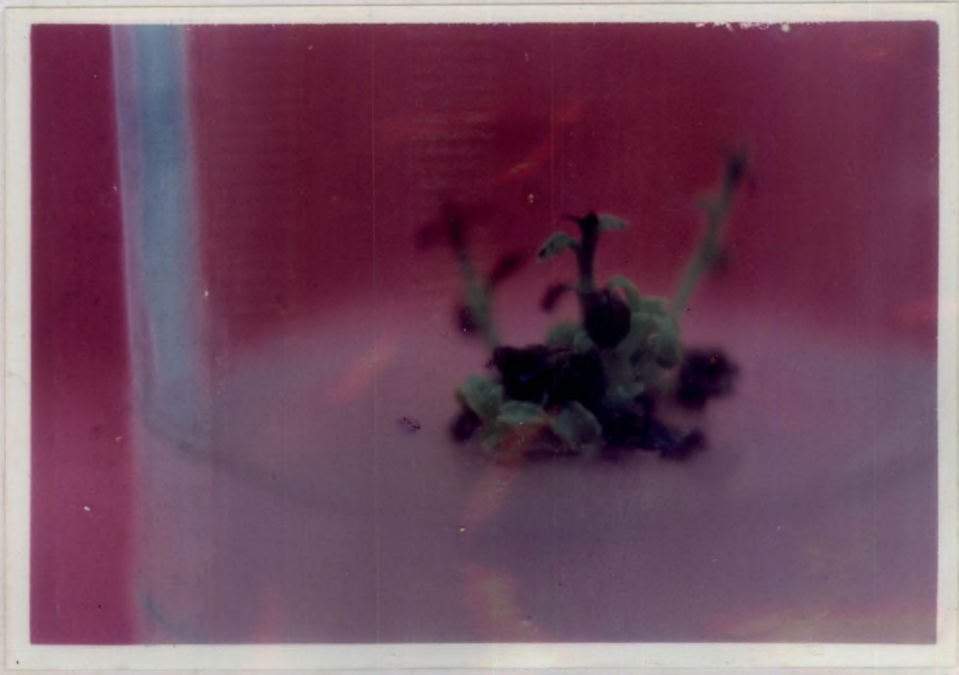Plate 8. Direct root formation from explants along with shoot morphogenesis in WPM + 0.1 mg l^{-1} NAA

Plate 9. *In vitro* rooting from microshoots of teak in WPM + 4.0 mg l^{-1} IAA + 0.1 mg l^{-1} NAA

Plate 10. Rooting of microshoots of teak in ½ MS + 4.0 mg l-1 IAA + 0.4 mg l-1 NAA + 0.25% AC + Pulse treatment

Plate 11. *Ex vitro* rooting of microshoot in vermiculite

Plate 12. Plantlets with *ex vitro* rooting in cocopeat

Plate 13. Plantlets with *ex vitro* rooting in vermiculite

Plate 14. Acclimatization of *in vitro* rooting plantlets

Plate 15. *In vitro* rooted plantlet after acclimatization

Plate 16. *In vitro* rooted plantlets planted into polythene bag containing potting mixture (two month old)

MICROPROPAGATION OF TEAK
(*Tectona grandis* Linn.) THROUGH
In vitro TECHNIQUES

By
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ABSTRACT OF THE THESIS
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ABSTRACT

The study under the title 'Micropropagation of teak (*Tectona grandis* Linn.) through *in vitro* techniques' was carried out at College of Forestry Vellanikkara during year 1998-2000. The objective of the programme is to standardize the method of clonal propagation of teak through teak tissue culture technique. The explants used were from one to two year old seedlings

Culture contamination mainly due to fungus was prominent in the rainy season. Prophylactic spraying of a mixture of fungicide (Bavistin and Indofil M-45 (0.1% each) on source material followed by fungicide dip to explants in similar solution for 30 min and surface sterilization treatment in 0.15 per cent mercuric chloride for 15 min controlled the contamination. Phenol exudation was contained effectively by supplementation of citric acid and ascorbic acid (150 mgl^{-1} each), to the media. It was also in low quantity when explants of smallest size (1 cm long below the node) were used.

Murashige and Skoog (MS) medium was found to be better than ½ MS and WPM. Individual supplementation of BA to MS media was found effective than other cytokinins. However, MS media supplemented with Kinetin and IAA 0.5mgl^{-1} each was found to be the best media for shoot proliferation. A maximum of 2.20 and 2.11 number of shoot shoots per explant could be induced in MS + 0.5 mg l^{-1} BA and MS + 1.0 mg l^{-1} BA + 0.1 mg l^{-1} IAA + 1.0 mg l^{-1} 2ip respectively. Addition of growth supplements like coconut water, activated charcoal, adenine sulphate, and casein hydrolysate did not have any favourable effect on growth and establishment.

Maximum *in vitro* rooting (56.25%) was obtained on ½ MS + 0.4 mg l^{-1} NAA + 4.0 mg l^{-1} IAA +0.25 per cent AC with pulse treatment (dip to excised shoot base in 1000 mg l^{-1} IAA solution for 2 min) and after transferring to auxin free media after 7 days. Under *ex vitro* rooting trials the maximum

percentage of rooting (87.50) with highest root length (3.0 cm) was obtained on vermiculite. Sand and vemiculite were found to be the best media for hardening of *in vitro* raised plantlets. The hardened plants were transplanted to polybags containing ordinary potting mixture and treated like a normal conditioned seedling. Hardened seedlings were out planted in the field.

The technique developed for the micropropagation of teak during present investigation can be used for large scale clonal multiplication of the species.