IN VITRO PROPAGATION OF SANDAL
(*Santalum album* L.)

By

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(2011-17-103)

THESIS

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DECLARATION

I hereby declare that this thesis entitled ***In vitro* propagation of sandal (*Santalum album L.*)** is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree diploma fellowship or other similar title of any other University or Society

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABBREVIATIONS

ABA	Abscisic Acid
BA	Benzyl Adenine
B_5	Gamborg s media
Cm	Centi meter
°C	Degree Celsius
Fig	Figure
et al	Co workers
GA_3	C bberelic Acid
G	Gram
$HgCl_2$	Mercuric Chloride
IAA	Indole 3 Acetic Acid
IBA	Indole 3 Butyric Acid
KIN	Kinetin
Mg	M lligram
mg l^{-1}	Milligram per liter
mM	Millimolar
MS	Murashige and Skoog medium
NAA	µ α Naphthalene Acetic Acid
No	Number
ppm	Parts per million
TDZ	Thidiazuron
WPM	Woody Plant Medium
2 iP	2 isopentenyladenine
2 4 D	2 4 Dichloro phenoxy acetic acid
AdS	Adenine Sulphate

DEDICATED TO

MY PARENTS & TEACHERS

Introduction

INTRODUCTION

Santalum album L. belonging to the family santalaceae is a prized gift of the plant kingdom woven into the cultural heritage of India (Kumar *et al.* 2012). It is one of the most valuable trees in the world (Fox 2000) and a medium sized evergreen hemi root parasite attaining 10-15 m height and 1-2 m girth at full maturity when it reaches the age of 60-80 years (Ghosh *et al.* 1985; Srinivasan *et al.* 1992; Jain *et al.* 1999). Flowering generally occurs twice a year from March to May and September to December. Sandal is a highly polymorphic species (Kulkarni 1995). Sandalwood plants exhibit significant variability for many traits like bark, leaf (Kulkarni and Srimathi 1982), seeds (Annapurna *et al.* 2005), colour of the heartwood and oil content (Kushalappa 1983; Bagchi and Veerendra 1985). Srimati *et al.* (1983) recorded three plant phenotypes in sandal: Thindlu, Chikkaballapur and Robust.

The tree flourishes well from sea level up to 1200 m altitude in regions with different soil type, varying climatic conditions and an annual precipitation of 600-1600 mm. The genus *Santalum* is distributed in tropics extending between 30°N and 40°S and is indigenous to peninsular India with a natural distribution of 9600 km^2 (Srinivasan *et al.* 1992; Radomiljac 1998) extending from Kerala in the South to Uttar Pradesh in the North in regions with varying ecoclimatic conditions and edaphic factors (Jain *et al.* 1998). More than 90 per cent of sandal is distributed in Karnataka (5245 km^2) and in Kerala. Sandal is spread over 15 km^2 mainly in Marayur in Idukki district, Wayanad district and Tirunelmalai in Kollam district (Srimathi *et al.* 1995).

Sandal is the second most expensive wood in the world next to the African Blackwood (*Dalbergia melanoxylon*) which is highly valued for its fragrant heartwood which yields oil preferred for perfumeries, cosmetics and medicines (Sanjaya *et al.* 1998). The quality of oil and wood of *Santalum album* L. is superior to those of other species in this genus which on steam distillation yields average 57% oil (McKinnell 1990; Srinivasan *et al.* 1992). The heartwood and oil content vary with locality and from tree to tree and increase with girth and age of the tree.

Global demand for sandalwood is about 5000 6000 tons/year and that of oil is 100 tons/year (Joshi and Kumar 2007) Out of this 80% 90% in the international market has been fu filled by Indian sandalwood for decades There is a decline in production from 5176 tons/year during 1960 65 to 1500 tons/year in 1997 98 and to 500 tons/year in 2007 Ma ket trend indicates that sa idal heartwood prices have increased from Rs 365 ton in 1900 to Rs 6 5 lakhs/ton in 1999 2000 and to Rs 37 lakhs/ton in 2007 (Jain *et al* 2003 Gairola *et al* 2007)

Since the oil percentage is higher in the heartwood of root as compared to stem the tree is invariably harvested by uprooting This removals spike disease and widespread smuggling have left India s sandalwood stands dangerously depleted Sandal has now been enlisted as a vulnerable species by IUCN (IUCN 2013) Since much of the sandal wealth and natural sandal bear ng areas have been lost the remaining sandal trees are to be protected effectively and natural sandal bearing areas are to be preserved Efforts are now needed to increase the area of cultivation and to improve productivity with the aim of sustainable supply (Srinivasan *et al* 1997 Swaminathan *et al* 1998)

The world requirement for sandalwood oil is 600 tonnes of which only 100 tonnes is met by natural resources Unlike the situation with major commercial timber tree species sandalwood stands out as one species for which no organized plantations have been established The policy of the Governments of Karnataka and Tamil Nadu to abolish their monopoly on sandalwood has generated interest in public and private sectors to raise sandalwood plantations Hence there is a need of mass distribution of seedings to these sectors (Kumar *et al* 2012)

Natural regeneration in sandal occurs mainly by the dispersal of seeds by birds and normally takes 4 to 8 weeks to germinate and under normal conditions they retain their viability up to six months and then gradually diminish However fresh seeds exhibit dormancy for 2 months and seedlings are extremely heterozygous due to outcrossing (Srinivasan *et al* 1992 Venkatesan 1995) In spite of good percentage of fruit initiation lower percentage of mature fruits were observed due to the presence of genotypic barriers for embryo development (Sindhuveerendra *et al* 1999)

On the other hand vegetative propagation is achieved through rooting of stem cuttings grafting and air layering or through root suckers However rooting of stem cuttings rooting has been achieved only in 15 20 per cent of cuttings (Rao and Srimath 1976 Unyal *et al* 1985 Balasundaran 1998 Sanjaya *et al* 1998) In one of the seed stands in Marayur that had established in Nachivayal Reserve II during 1980 1981 it has been reported that expected quantity of seeds are unavailable from these seed stands Root sucker induction had been adopted as a method of sandal regeneration for the last few decades in Marayur trees are grown in clusters and are confined to an area around the mother tree Though the trees flowered little or no fruit production occurred within these small populations (Ramya 2010)

The conventional breeding of sandalwood for new genetic information can be an expensive and difficult task because of their long generation time sexual incompatibility and heterozygous nature (Rugkhla 1997) *In vitro* regeneration techniques can be used to encounter difficulties of traditional propagation methods by microclonning of superior lines In vitro regeneration techniques can be used to clone superior lines In order to develop mass propagation methods for desirable qualities such as disease resistance and good heart wood containing plants tissue culture methods were employed *In vitro* propagation of sandalwood was attempted as by using various explants like embryo (Rangaswamy and Rao 1963) hypocotyls (Bapat and Rao 1979 Lakshmi Sita *et al* 1979) endosperm (Lakshmi Sita *et al* 1979 Bapat and Rao 1979 Rao and Bapat 1992) shoot tip (Lakshmi Sita and Raghava Ram 1995) nodal segment (Bapat and Rao 1979 Lakshmi Sita *et al* 1979 Rao and Bapat 1992 Rugkhla and Jones 1998 Sarangi *et al* 2000 Sangham tra and Chandn 2010) leaf disc (Mujib 2005) and cell suspension cultures (Dey 2001) with varying degree of success *Santalum album* is recalcitrant to *in vivo* and *in vitro* propagation for which only limited success has been achieved so far (Sanjaya *et al* 2003) A systematic study on the effects of combinations of plant growth regulators on morphogenesis is still insufficient

So there is an urgent need to develop clonal techniques to produce disease resistant and high oil yielding clones of superior trees Thus the present study titled *In vitro* propagation of sandal (*Santalum album* L) has been undertaken to develop

a potent al system of *r in o* regencrat on of plus trees of *S alb n* thiough ax llary shoot proliferation and somatic embryogenesis

Review of Literature

P ant tissue culture s def ned as the procedure of cult vating cells t ss es or organs of plants on art f c al med a under asept c cond t ons Pla t t ssue culture begins w th the selectio of a genotype on the bas s of hav n a problem in ts regenerat on to be solved and by determ ning appropr ate type of protocol to deal with t (Garc a Gonzales *et al* 2010) Tissue culture techn que s based upon two propert es of plant cells cell tot potency (Vasil and H ldebra dt 1965) and cell plasticity (Tho pe 2007) Cell tot potency s the genet cally reta ned capac ty that all l v ig cells posses to origi ate a new genet cally dent cal cell a d to for n t ss es orga s systems a id complete ind v duals after cell lar d v s on and d ffere t it on process s (Takebe *et al* 1971) Cellular plastic ty is the character stic which marks the d fference between pla it and an mal cells n tl e r capacity of mult plicat on d v sion d fferent ation and fo mat o of a new ndividual Hussa n *et al* 2012)

T ssue culture techniques for plant micropropagation rest on two fundamental morphogenesis processes organogenesis a d somat c e bryogenes s Organogenes s s the formation of plant organs from a selected t ssue n order to form complete plan s In th s process only one aerial organ or root s formed and f om th s a new co nplete plant s regenerated (V jaya and G r 2003) Somat c e nbryogenes s s the product on of embryos from somat c cells to obta n a complete plant by undergo ng following stages e nbryo formation and p oliferat on e nb yo maturat on and embryo germinat on At the sa ne t me the embryos may pass throug four stages n the r development tl e globular form the heart form tl e torpedo and the cotyledonary forms Unl ke organogenesis the aer al structures and roots of the plants are obtained from the somat c embryo itself (Ammirato 1983) Both the processes may be d rect f it or ginates directly from the init al explants or ind rect f it occurs from prev ously formed callus n the n tial explants

2 1 PL \NT TISSUE CUL TURE HISTORY

P oneer ng exper n ents on wo nd heal ng n plants l ave demonstrated spontai eous callus format on on the de ort cated region of el n pl ts Accord ng to Gautheret (1955) these stud es could be cons dered as forward for tl e development of plant t ssue ci lture as a sc ence The sc ence of plant t ssue cultuie l as ts foundatioi on the discove y of cell followed by the propounding of cell theo y wh cn states that cell s the basic structural i t of all v ig organ sms They visual zed t at cell s capable of auto iomy and the efore t sl ould be poss ble for each cell f g ven an env roi ment to regei erate nto whole plant The f rst repo ts ega d ng t ssue culture date back to the beg inmg of the 20th centuiy when Haberlandt (1902) developed exper ments to ma itain mesophyll cells i f ist t me attempted to culture solated s ngle pal sade cells from leai es i knop s salt solut on enr ched w th sucrose based on postulates which established the tot potent al ty of plant cells The cells rema ned al ve for up to one moi th ncreased in s ze accu nulated starch but fa led to d v de Though he was unsuccessful but this led to the development of t ssi e culture technology for wh ch he is regarded as the father of plant tissue culture (Hussa n *et al* 2012) After that some of tl e landmark discover es took place in t ssue cultu e wh ch s sum nar zed below

- 1904 Hann g cultured embryos from several cruc fers
- 1922 Knudson d d asymbiot c germ nation of orch d seeds
- 1972 Kolte and Robb ns cultured root tips *n v tro* separately
- 1925 La bacl appl ed embryo culture in nterspec fic crosses of *L num*
- 1934 Wh te ntroduced vitam n B as growth supplement n t ssue culture med a for tomato root t p
- 1934 Kogl dentif ed the f rst known plant growth regulator IAA
- 1939 Gautheret Wh te and Nobecourt established cont nuous prol ferat o i of callus cultures

- 1941 Van Overbeek fo the f t t me added coconut mill fo the culture of *Dati ra* embryos
- 1944 Sko g sed *n v t o* cultured tobacco to study advent t ous sl oot format on
- 1946 Bali raised wl ole pla ts of *Lup m s* a id *T opacoli* fiom shoot t ps
- 1948 Skoog and Tsu formed adve t t ous shoots and roots of tobacco determ ned by the rat o of aux i adenin
- 1950 Bal ege nerated organs from callus tissue of *Seqt o a scn pe v cns*
- 1952 Morel and Martin cultured v us free dahl as tl rough meristem culture
- 1953 Tulecke produced haplo d plants of *G ako b loba*
- 1954 Muir vas first to break callus t ssues nto s ngle cells
- 1955 Skoog and Miller d scovered kinetin as cell div s on hormone
- 1957 Skoog and M ller gave concept of hormonal control to egulate (aux n cytok n)
- 1959 Re ne t n id Steward regenerated embryos from callus clumps and cell suspens on of carrot (*Daucus carota)*
- 1960 Cock ig as f rst to isolate protoplast by enzymat c degradat on of cell wall
- 1960 Berg nann filtered cell suspension and solated s ngle cells by plating
- 1960 Kanta and Maheshwar developed test t be fertil zat on techn que
- 1962 Mu ash ge and Skoog developed MS med um w th h gher salt concentrat on
- 1964 Guha and Mal eshwar produced f rst haploid pla ts fro n pollen grains of Datura (anther culture)
- 1966 Stewa d demonstrated tot potency by regenerat ng ca rot plants from s ngle cells of tomato
- 1970 Powe i *et al* successfully achieved protoplast fus on
- 1971 Takebe *et al* regenerated f rst plants from protoplasts
- 1972 Carlson produced f rst nterspec f c hybrid of *N cot ana tabacu n* by protoplast fus on

- 1978 Melchers *et al* carried out somatic hybridization of tomato and potato resulting in pomato
- 1981 Larkin and Scowcroft introduced the term somaclonal variation
- 1983 Pelletier *et al* conducted intergeneric cytoplasmic hybridization in Radish and Grape

2.2 CONTROLLING FACTORS IN MICROPROPAGATION

The controlled conditions provide the culture an environment conducive for their growth and multiplication These conditions include proper supply of nutrients pH of medium adequate temperature and proper gaseous and liquid environment (Hussain *et al* 2012) From a practical point of view the mechanisms which trigger the development of a plant from a cell or a tissue section depend on factors which vary according to the species the type and the age of the tissue the environmental conditions and the composition of the culture media which are generally managed empirically on a case basis (Garcia Gonzales *et al* 2010)

2.2.1 Nutrient Medium for *in vitro* Cultures

Growth of plants under *in vitro* conditions is largely determined by the composition of the culture medium The importance of nutrition in plant tissue culture has been reported by Gautheret (1955) The main components of plant tissue culture media are mineral salts sugar as carbon source and water Other components may include organic supplements growth regulators and gelling agent (Gamborg *et al* 1968 and Gamborg and Phillips 1995)

Successful culture establishment has been achieved by standardizing different nutrient combinations MS medium (Murashige and Skoog 1962) White's medium (White 1963) B_5 medium (Gamborg *et al* 1976) Linsmaier and Skoog's medium (Linsmaier and Skoog 1965) Woody Plant Medium (Llyod

and McCown 1980) and Nitsch medium (Nitsch 1951) are some commonly used media plant tissue culture Among these nutrient media the following will be a particular one suited for a given species and also for a specific purpose

Differential Response on Media Types and its Strength

Each species responded differentially in a particular medium and also response of a particular species vary according to the media used Apart from that solid or liquid nature of media affects its response According to Monira (2012) for the mass production of *Swietenia macrophylla* through *in vitro* technique the explants cultured on MS full strength induced the highest number of shootlets/explant and the longest shootlet among the four types of culture medium (MS QL B_5 and WPM) In the case of *Acacia nilotica* WPM media which produced robust plants with good internodes was the best media compared to B where internodes were stunted (Sanake *et al* 2011) Multiplication of axillary shoots in *Acacia meansii* under B_5 MS SP and WPM MS promoted the best result plication of axillary shoots (3 7 buds explants) on the thirteenth day of culture (Dasarz and Corder 2009) Mehta *et al* (2008) reported that in the micropropagation of *Populus alba* × *P berolinensis* MS medium exhibited high efficiency for shoot regeneration followed by WPM medium while B_5 medium inhibited shoot regeneration Highest shoot multiplication rates in *Eucalyptus citriodora* were obtained when cultured on MS medium supplemented with 0 5 mg l BA compared to WPM and SH supplemented with BA (Koresh *et al* 2003) Britim *et al* (2003) cultured *Fraxinus caroliniana* in MS B Knudson or WPM The number of shoots with a diameter of more than 1 cm and fresh weight of aerial parts were highest in the WPM medium

The strength of the macro or micro elements in the media also affects the culture response The best rooting was observed on treatments with ½ MS medium In *Swietenia macrophylla* out of full and half strength norganic salts media MS full strength induced the highest number of shootlets explant and the

longest shootlet (Mona 2012) In *Terminalia arjuna* best shoot multiplication from nodal expal nts and furthei root induction was achieved on ½ MS medium by Pandey *et al* (2006) Khan *et al* (1999) investigated the effect of MS basal medium (0 ¼ ½ / or full) strength on *n vitro* rooting of *Syzyg m alterni fol n* The best rooting was observed on ½ MS + l mg l IBA + 2 pe cent sucrose + 10 µM spermine + 0 8% agar

In some species for each stage of *n vitro* propagation d fferent media were suited Bhargava *et al* (2003) observed that in *Phoenix dactylifera* globular proembryonic mass of callus was formed on MS media and when that callus were transferred to B_5 medium fragile snowy callus was formed According to Sharada *et al* (2003) MS medium was suited for shoot development and B_5 or WPM med um for root nduct on n *Celastrus paniculatus* Axillary bud ii itiation in *Dalberg a latifol a* was better on the MS medium while for the mult ple sl oot induction WPM media was the best (Swamy *et al* 1992)

In *Azadirachta indica* for the generation of embrvogenic callus cultures and nduction of somatic embryos MS media was used but maturation and germination of somatic embryos was achieved on ½ MS media (Rout 2005) Rathore *et al* (2008) used full MS for culture in tiation and multiple shoot induction in *Terminalia bellerica* However root induction was found to be better n ¼ MS

Plant Growth Regulators

Growth regulators are organ c compounds which in small amounts promote inhibit or qualitatively mod fy growth and development (Moore 1979) There are five known major classes of compounds with plant growth regulatory activity These are auxins cytokinins gibberellins abscisic ac d and ethylene Among them auxins (NAA IAA IBA and 2 4 D) and cytokin ns (BA Kinetin and Zeatin) are commonly used Different plant growth regulators have different effects and they vary with the type and quantity to be appl ed As stated by

Kr korian *et al* (1981) prope select o and ıdd t on of growth egulator at a opt mu n leve s one of tl e ımpoıtant factors for successf l pla t tıssue c.lture Bl ojwan and Razdan (198ɔ) eported tl at t s generall necessa y to ıdd o ıe or more ot tl ese plant g owth regulators to support good growtl of tıssues and organs

Auxıns have an essentıal role n shoot ınductıon and plant regcnerat on ı most plant specıes Aux ns are ma nly used to nduce callus ın var ous explants as well as for root ng of shoots 2 4 D (3 4 mg l) ınduced callus n *Te nal a ɹı na* (Arumugam and Gop natl 2011) and *O oxyl n nd cı n* leaf n drib explant (Rajurkar 2011) Roots were ınduced bv NAA ın *Syzyg u cı ı n* (Randr amamp ono ıa *et al* 2008) a ıd IBA n *Sw eten a nacroɻ l ylla* (Mo ıa 2012) *Sa aca asoca* (Subbu *et al* 2008) and *C nnc no ı u n campl o a* (Sha ıa and Vas stl a ?010) ı the concentrat ons l to 4 mg l IBA s the n ost su table root nduc ıg aux ı A ɤ ns also ndı ce somat c embrɔ genesıs from the cıllus of *C trus s nensıs* (Kochba and Sp eyel 1973)

Among the varıous cytokınıns BA ıs found to be best for aux llary bud prol ferat on n *Te m nal a catappa* (Phulwarıa *et al* 2012) *G el a a bo ca* (M shra and Sh n 2009) *Hola hena ant dɻ senter ca* (Kumar *et al* ?005) when used n concentratıons 10 15 µM l It was also most effectıve for shoot mult plıcat on n *O oxyl ı m nd c n* (Rajurkar 2011) *C nnamo ı n can pho a* (Shar ıa and Vash stha 2010) *Syzyg ı m cı ı n* (Randr ama nplonona *et al* 2008) *Term nal a belle ca* (Rathore *et al* 2008) *Ter n nal a arjı n* (Tho nas ct *al* 2003) and *Ste cul a cns* (Puroh t and Dave 1996) n the co ıce ıtrat o s l 2 mg l TDZ ıs a cytokın n suıted for regenerat on fron leaf explants (*Jat opha c cas* Kumar *et al* 2012) However hıgher concentrat ons of BA resulted n hyperhydr c and malformed shoots ın *Sa aca asoca* (Subbu *et al* 2008)

Spec fic comb nat on of growth regulators s found to be effect ve for development of certa n organs In *Tɔ ı m nal a arjı ı a* callus cultures showed the

shoot and root 1 at o n MS basal m d ur 1 supple 1e ted w th 5 ng l 2 4 D + 0 0 mg l K 1 et n and 1 0 mg l GA (Arumugam and Cop nath 2011) Gad dasu *et al* (20 1) reported that tl e combi 1at o of Kinet n (4 60 µM) v th BA (4 44 µM) evo ed an opti nu n response towards shoot prol ferat on whe eas rred um conta n 1 g K net n (4 60 µM) TDZ (4 54 µM) 1duced mult ple shoot formation n *St ebli s asper* Nodal seg nents of *Term r al a c tappa* developed opt mal number of shoots and shoot length on MS + 0 25 mg l BA + 0 25 mg l of Kinetin (Phulwar a *et al* 2012) Mona (2012) reported that n *Sw eten a mac ophylla* the h ghest number of shootlets shootlet length nu nber of eaves as well as fresl a d dry ve ghts were obta ed by apply ng 4 0 mg l BA + 0 4 ng l 2 D

Mo eover th natu e of organogenet c different at on is detern ined by the elat ve concen rat n of aux ns and cytok n ns Higher cytok ins to aux n rat o p omote shoot format on wh le l gher auxins to cytokinins ratio favours root d fferent at on Therefore an aux n/cytokin n rat o plays a cr tical ole in the nduction of roots and shoots (Skoog and Miller 1957) Kumar *et al* (2011) transferred the regenerated shoot buds of *Jatropha cu cas* to MS +10 µM Kinet n + 4 5 µM BA + 5 5 µM NAA for shoot proliferat on The prol fe ated sl oots were elongated on MS + 2 25 µM BA + 8 5 µM IAA Maharana *et al* (2012) reported that MS + 8 µM BA + 2 µM IBA was most su table for both call s nediated organogenes s a d elongat on of shoots of *Jatropha cu cas* The best shoot nult pl cat on espo 1se from nodal explants of *Ter n nal a a j n a* was obta ned o 1 4 44 µM BA and 0 53 µM NAA (Pandey *et al* 2006) I 1 tl e case of root ng of *Nyctant es arbo t st s* naximun response was obta ned on the med u n hav ng 0 25 mg dm ^{3} IBA and 0 1 mg dm ^{3} IAA (Rout *et al* 2008)

Carbon Energy Source

During culture carbohydrates play an important role and act as an energy source requ red for growth ma ntenance and for synthesis of cell co stituents

The most commonly used ca bohydrate source ıs sucıose but othei sugar like glucose fructose dextrose ma nitol and soıbitol are also occasionally used Meanwh le sucrose also has an ımportant role as ıt serves as a source of carbon and energy Sucıose ıs also required for differentiation of xylem and phloem elements ın the cultured cells (Alonı 1980) Glucose and fructose are also known to support good growth of some tıssues and are occasionally used Sucrose represents the major osmotic component of the medium and ıs necessary for various metabolic activities

Lopes *et al* (2012) found that ı ı *Jatı opha curcas* sucrose ınfluenced tl e development of embryos such that the range of 15 to ɔ0 g l of exogenous supplementation with sucrose pro notes the best shoot elongat on of plants however rhizogenesis ıs nore vigorous ın the range from 30 to 60 g l ın wh ch a signıf cant ı crease of the number of roots occurs The best sucrose concentration for plant v gor and speed ın obtaınıng explants ıs 30 g l Khan *ct al* (1999) found that ın *Syzygium alternifolium* sucrose concentrat on (2%) was positively correlated with rootıng percentage root number per shoot and root length For *Eucalyptus g andis* sucrose at 30 g l gave superior growth compared with the other three carbon sources tested such as maltose glucose and fructose (Wachira 1997) Among the various saccharides tested the best calogenic response was afforded by sucrose both ın terms of explant response and shoot develop ng potential of ep cotyl ın *Syzygium cumini* Sucrose at concentration 4 per cent proved to be the best ın developıng 4 2 shoots per explant (Jaın and Babbar 2004) Fructose (20 mg l) was found to be nore su table addıt ve n controllıng the neciosis ın *Lagerstroemia indica* (Niranjan *et al* 2008)

Additives in the Medium

There are some complex substances like coconut m lk (CM) case n hydrolysate (CH) adenine sulphate (AdS) actıvated charcoal (AC) which are sometimes required ın addıt on to growth hormones for callus ınduction and

regeneration For instance the coconut milk of green nut s ve y effect ve n prov d ng an undef ned mixture of organ c nutr e nts and growth factors (Gamborg and Phillips 1995)

Maharana *et al* (2012) used 45 µM AdS 15 µM gluta mine and 10 µM prol ne to enh ance the number of mult ple shoot prol fe at on pe explant a d elongat o n in *Jat opl a cr cas* Lava nya *et al* (2006) found that n *F cus bcnja n a* max mu n nu nber of multiple shoots was de eloped ov th e add t on of AdS (50 g l) Jain a d Babbar (2004) observed that n *Syzyg n c n* elongat on of the shoot buds was fac l tated when supplemented w th casein hydrolysate (1 5 g l) or glutam ne (?00 mg l) The comb nation of poly a n nes + IBA ncreased rooting percen tage co npared with the med a co nta n ng only IBA in *Syzyg m a te n fol* (Khan 1999) Swamy *et al* (1992) reported that the growth adjuvants like coconut m lk casein hydrolysate and AdS were also supplemented to the med a for direct organogenes s and somatic embryogenes s in *Dalber g a latifolia*

Fr dberg *et al* (1978) reported that charcoal had an nporta nt role dur ng culture by absorbing tox c compounds released by noculated expla nts P er k (1987) showed that th e add t on of AC often has a promot ng effect on growth and organogenes s n plant spec es Charcoal has been used n regenerat on medium for trees l ke *Dalber g a s ssoo* (Gulat and Jaiwal 1996) and *A eca catechu* (Mathew and Pl l p 2000) to prevent brown ng of cultu e due to phenol c exudatio released by th e explants The benef c al effects of act vated charcoal were also found on mult ple shoot nduct on f om nodal expla nts of *Wattakaka volu bil s* (Chakradhar and Pullaiah 2006)

Vitamins

V ta nins have catalyt c functions in enzyme systems and are requ red n trace amounts Thiami ne may be the only essential v tam n for nea ly all plant

tissue cultures here as niac n ai d pyridoxine may stimi late g owth (Gamborg *et al* 1976 and Oh ra *et al* 1976) Some othei v tam ns that nave been used in pla it t ssue culture med a nclude ascorbic acid tocopheiol b otin cya iocobalam n fol c acid and r boflavin (Huang and Murashige 1977 and Ga nborg and Shyluk 1981)

2 2 2 Explant

Genotype

B n *et al* (2009) reported the regeneration of adventitious buds and the rooting of shoots from the leaves of hybr d 717 from *Populus t emula* × *Populus alba* and 353 fioi i *Populus tiemula* × *Populi s t emulo des* 20 n g kanamycim could inhib the bud iegenerat on of the hybrid 717 and 35ɔ 40 mg l kanamycim could inhibit the root regeneration of the hyb id 717 and 353 from the shoots Prabhakaran *et al* (2003) stud ed the performance of *in vitro* cultured tamarind genotypes (PKM 1 Urigam Pollachi 2 Asanoor H 1 and Salem 144) Among tl e genotypes Ur gam gave the highest survival per entage (90 50%) bud break percentage (93 63) mean length of multiple shoots (1 40 cm) and mean number of shoots per bud (3 34) Urigam also gave the earliest days taken for bud break (29 94) Axillary shoot elongation formation of multiple shoots and rooting of shoots were compared in nodal segment cultures of *Gmelina ai boi ea* Roxb from seedl ngs obtained from s x provenances over seve al subcultures Provenance dependent variat on was observed with respect to these parameters (Naik *et al* 2003)

Explant Type

According to the type of explant used type of regeneration varies Also different explants have vary ng regeneration capacity Rajurkar *et al* (2011) carried out *in vitro* shoot induction and callus induction of *Oroxylum indicum* by

us g apical and ax illa y bud and leaf m dr b explants Ax illary b d showed s gnificantly l gh shoot ault pl cat o whereas leaf m dr b explants was found to be more effect ve for callus nduct o n Subbu *et al* (2008) attempted he clonal p opagat on of *Sa aca asoca* through shoot tip nodal a d n ernodal explants Regeneration was observed on all types of explants but shoot regeneration was most pronounced for nodal and nternodal explants Nodal expla ts produced more shoots than term nal apex n *Te r nal a a juna* (Thomas *et al* 2003) When tl e germ nat on f *D ospy os kak* do mant buds sprout buds and sl oot t ps are co mpared dor na t bud showed the h ghest response and ts prol ferat on ab lity s stronger (Kun *et al* 2010) Cotyledonary node expla ts of *Pcltopho r m pte oca pr m* gave best shoot mult pl ca on compared to shoot t p a d nodal seg nent (Udd n *et al* 2005) In *Pte oca pus sa tal nus* also coty ledo ary nodes showed s gn ficantly h gl er shoot mult pl cation rate a d shoot le gtl than leaf nodes (Rajeswar and Pal wal 2008) Cavusoglu *et al* (2012) repo ted that when node nternode nd leaf explants of t ssue culture rege erated *Popul s delto des* plantlets were used for d rect and nd rect somat c embryogenesis The best somat c embryogenes s observed n nter nodes and also gave the best result for embryogen c call formation Accord ng to Lu *et al* (2007) n *Eu c alypt s s th* the ability to form call s followed the order cotyledons and leaves young stems a d hypocotyls stems from old trees seeds and roots

Size of Explant

Vyas and Ba sal (2004) reported n *Bo bax ce ba* that the 2 5 mm s zed zygot c embryo exhibited optimum response w th respect to frequencies of explant swell ng callusing e nbryogenes s and green ng In *Artocarpu s alt l s* explants less than 10 n m rooted n 1 2 months compared to explants greater than 10 mm where roots developed after 3 4 months n culture (Tu a *et a l* 2007) In *C n amomu m campho a* when the performance of small shoot tips was compared w th that of 2 0 cm nodal segments during subculture cytok n ns nduced

hyperhydr c ty n s n ll shoo ps Hypc hvdr c ty was avoided s ibcultures by us g larger nodal segment (Chu ı *et al* 1998)

Influence of Γ\p ınt n the Mothcı Plant

B ı *et al* (2009) fou d that the best eaf explai t foı advent t ous buds regenerat o ı cre on the tip l 3 d leaf of stems poplar nybr d /17 and 35ɔ Maximum bud break (78 6 81%) was obta ned ın *Az ıd ıcl ta n l c* wl e ı m ddle order nodes (3 d or 4 h node from apeɣ) were taken (A ora *et al* 2010) Leaf d scs from the th rd expand ng leaf exl ıb ted h ghe regenerat on potent al than those from the fourth leaf n *Jatı oj ha cı cas* (Sı j tha and Mukta 1996) In *Lage st oe ı a pa v flo a* c ltures der ved f om explants of seedl ngs term nal tw gs and basal sprouts of 50 year old trees showed s gı ificınt var at on n responses at establ sl ment shoot proliferation and root g stages Cultures dei ved from seedling and basal sprout explants were successfully naınta ned for up to 6 success ve traı sfers whereas those der ved from t ee explants dıed after 3 transfers (Qura sh *et al* 1997)

Age of Explant

Mazu ndar *et al* (2010) observed that ın *Jat opha cı cas* the callus ınduction and shoot regenerat on capac ty of cotyledona y leaf segments were found related to the age of the explants and their or entatıon ı culture ned m Accord ng to Pasha and Irfan (2011) soft explants had faster shoot nitiat on than hard ones n *Eucalypti s c t oda a* In the micropropagat on of *A ad achta ıd ca* us ng nodal seg nents f om mature trees a d green house gıowı juvenile seedlings the cultures establ shed from the explants collected from the juven le seedl ngs were supe ıor to those from mature trees (Sr n dh *et al* 2008) Na r and Seen (2003) cultured young shoots such as red (1 2 weeks) p nk sh ed (3 5 weeks) pale green (6 8 weeks) and dark green (9 10 weeks) collected fro n mature trees of *Calophyllu apetalı m* All the shoot tıp and s ngle node expla ts

of the youngest 1 2 week old shoots were lost due to excessive browning and necros s nodes of the 6 8 week old shoots responded tl e r ost (68% of explants) w th tl e format o of 3 2 shoots per explant n 7 weeks Nine month old seedlings were observed to be the best source of explants of *Lage stroe n a reg nae* and the egenerat on response declined with an increase n age of the plants (Sumana and Kaver appa 2000)

According to Goodger *et al* (?008) n *Ei cal) ptus poly b actea* tl e age of the explant source also d d not influenced the success of m cropropagation a d as a result older plants (for wh ch key oil traits are known) can be selected as el te plants for multiply ng selected genotypes *v a* m cropropagat on

Season of Explant Collection

Kesar *et al* (2012) reported that percentage response from f eld grown mature nodal segme ts of *Pongam a pinnata* were h ghly dependant or the season w th greater than 68 per cent of culture develop ng adventit ous shoots dur ng spring Nodal sector explants of *Gmelina a borea* showed seasonal va at on n the sprouting of axillary buds *n v t o* (Thakar and Bhargava 1999) Garc a Ramirez *et al* (2010) stated that the season influenced on the *n v tro* establ sh nent of *B vulga is* var vulgar s such that h ghest numbers of buds sprouted and explants free of microbial contaminants was ach eved between January to April and November to December Accord ng to Saha *et al* (2013) the seasonal influence on bud emergence heavy microb al contam nat ons and phenolic exudat ons are the important factors that l m t the establish ient of ax llary bud cultures in *Schle chc a oleosa* Nodal stem segments collected dur g the month of Apr l gave best response *Azad achta ind ca* (Arora *et al* 2010) In the summer months of March to May 83 19 per cent ster le cultures were obta ned out of which 45 65 per cent sl owed ax llary bud sprout ng *n v t o* establishment of *Csi a na eqi setifol a* (Seth *et al* 2007)

Surface Steri li ti n of Explant

Tl e fungal contam nat o ı *Santal n albı* was el nınated by the use of Bav stın (a sy stem c fung cıde) n the ster l zat o ı procedure (Reddy and Subraman an 1998) Chandra *et al* (2004) reported that mango sl oot bud explants taken d rectly from f eld grown mature tree face najor problems of phenol c exudat on and deep seated contam nat on n the estabı sl ment of asept c cultures and th s was overcome by us ng var ous sequent al pretreatment and d fferent steril z ıg agents In the *Eucalypti s* m cropropagation Watt *et al* (2003) observed that add t on of 1 g l ^{1} calc um hypochlorite to the f rst culture med um foı bud break nh b ted endoge ıous conta n nation In the fig tree the additio ı of a ıt b ot c ampic ll n to the med um after autoclavıng vas effect ve to co ıtrol of e ıdogenous ba.te a (Palu *et al* 2011)

According to Palu *et al* (2011) the f g tree ap cal b ıds explants when ımmersed ın 70 per cent ethyl alcohol and sod ı m hypochlorite 2 5 per cent ıt was s fficient to control fungal contam nat on S ngle nodal segments collected from newly sprouted shoots of *Schle che a oleosa* from Ap ıl to May were sterılized by dıpping n a $HgCl_2$ solution (0 1%) for 3 5 7 and 10 m nutes or ın an NaOCl solution (3% v/v) for 5 10 and 15 mınutes Among the sterılizat on treat nents only the appl cat on of 0 1 per cent $HgCl_2$ for 7 m nutes produced non contam nated al ve explants of *Schle che a oleosa* (Sınha and Akhtar 2008)

The studıes done by El Zaher (2008) n jackfruit revealed that treatıng 70 per cent ethaı ol for 2 m nutes + 0 2 per cent $HgCl_2$ for 5 nınutes +15 per cent Clorox for 15 n ı tes w th the ar tıoxıdants was the nost effect ve ste ıl zat on treatment as ıt recorded a good percentages of the surv val and aseptıc explants at all studıed dates and for all explants types D sınfect on of ıode expla ts w tl 5 per cent propıconazole CE 25 for 3 mınutes resulted ın 100 per cent explant dıs nfect on and 60 per cent morphogenıc response on those estabı shed explants (Garcıa gonzales *et al* 2011)

2 2 3 Culture Environment

Light is an important factor for the success of a t ssue cultu e experiment The intensity quality and extent of daily exposure of light are the determin ng factors n the plant tissue culture Cultures are usually maintained at ٦ constant temperatuie of 2ɔ±2°C and a photoperiod of 16 hours of light (?0 µmol m s photosynthetic pʰoton flux intensity) and 8 houis of darkness

W e brouck *et al* (2012) reported that monochromatic blue red ٦ d far red and theii combinations are suitable to manipulate the number of shoots shoot length shoot/callus we ght ratio and leaf length/width ratio ın *Ficus bc yc mina* In *Dalberg a* op imum iesults were obta ned at 26±I°C at 16/8 hour (l ght/darl) photoper od (Al *et al* 2012) Rodrigues *et al* (2012) observed that n *Azadı achta ındica n vitı o* propagation culture flasks sealed with two PTFE membranes prodı ced the highest number of shoots In contrast explants cultured ın flasks without membranes showed leaf chlorosis and senescence In Rubber type of culture tube closure influenced significantly the survival of explants where the number of survived explants ın culture tubes covered with cotton was higher than that of with parafilm (Nurhaimi Haris *et al* 2009)

2 3 ROOTING OF *IV VITRO* PRODUCED SHOOTS

2 3 1 *In vitro* Rooting

In vitro rooting can be achieved either by transferr ng the elongated shoots d rectly to med a containing auxins or by pulse treating the cut ends of excised shoots n high concentrated auxin solution before transferring to the med a either hav ng auxins or no growth regulator Addition of act vated charcoal and dark incubation also promote rooting n some species In some others l quid medium is more efficient than solid medium

Puroh t and Kukda (2004) successfully ooted *W ght a t t a* shoots by d pp ng the lower ends pre autoclave i IBA solut on (100 ng l) for 10 n nutes followed by the r mplantat o on mod f ed MS ned um conta n g 200 mg l activated cha coal Hussa *ct al* (2008) nduced root ng n *Pte oca pus n arsup t* n croshoots exc sed fro n prol ferated hoot cu tures o se n solid l o-mo e free / MS medium after a pulse (d p) treatmen for / days n ½ MS l qu d med um contain ng 100 µM IBA and 15 84 µ l phlo og uc ol (PG) In *Te n al a belli ca* ½ MS n ed m supplemented w th 24 60 µM IBA and 100 mg l AC was most effect ve for rooti g of the shoots (Phulwar a *ct al* 2012) ½ MS med um with IBA sl owed root n n *St ebli s aspe* (Gadidasu *et al* 2011) *Sa aca asoca* (Subbu *ct al* 2008) *Te n nalia arj na* (Tandey *ct al* 2006) and *O ocylum nd cu* (Gokl ale and Bansal 2009) *Syzyg m ci n n* rooted *n v t* o ½ MS + 0 1% act vated charcoal supplemented w th IBA (10 µM) or NAA (15 0 µM) (Rathore *et al* 2004) In *Nyctanthes a bo t st s* max mum percentage of oot ng was obta ned on med um hav ng 0 25 mg d m^3 IBA and 0 1 mg d m^3 IAA (Rout *et al* 2008) Selvan *et al* (2003) found that for *Acac a catechi* best rooting med um was / MS med um supplement d w th IAA (2 0 ng)

Kumar *et al* (2011) reported that *Jatiopha ci cas* root ng was ach eved when the basal cut end of elongated shoots were dipped n / MS liqu d medium conta n ng different co centrat ons and combi a ions of IBA IAA and NAA for four days followed by transfer to growth regulators free half stre gth MS ned u n supplemented 0 25 mg l act vated charcoal Na r and Seeni (2003) rooted *Calophyllum apetali m* m croshoots by cultur ng n ¼ MS medi m supplemented with 9 8 µM IBA for 4 weeks followed by transfer to / MS b sal n ed u m for 4 weeks

Hegde and D Souza (1995) reported that *M llington a ho tens s* m croshoots observed that / MS med um resulted in enhanced root ng and a reduct on n callus format on *Holarrhena ant dysente ca* excised shoots we e ooted on MS basal med um w thout growth regulators (Mall karjuna and Rajendrudu 2009)

According to Borthakur *et al* (2011) highest percentage of direct shoot regeneration of *Alangium salvifolium* was observed in growth regulator free MS medium In *Aristolochia bracteolate* pretreatment of shoots with IBA followed by a 10 day dark treatment resulted in 80% rooting after 10 days in the light resulted in rooting (Stevens *et al* 1989)

High frequency rooting was obtained in *Anogeissus sericea* var *nummularia* by pulse treating the isolated shoots with 98 0 µM IBA for six hours in ½ liquid MS medium and then transferring these shoots onto ½ hormone free semi solid MS medium (Yusuf 2005) According to Sharma *et al* (2005) *in vitro* raised shoots of *Tectona grandis* could be successfully rooted on liquid MS medium supplemented with 15 µM NAA

2 3 2 *Ex vitro* rooting

Certain species respond to rooting under *ex vitro* conditions compared to *in vitro* Terminalia *catappa* shoots treated with 200 mg l of IBA produced *ex vitro* roots (Phulwaria *et al* 2012) According to Rathore *et al* (2004) *ex vitro* rooting by pulse treatment with 2 50 mM IBA of cloned shoots of *Syzygium cumini* was highly effective and saved time and resources Mallikarjuna and Rajendrudu (2009) dipped the *in vitro* formed shoots of *Holarrhena antidysenterica* in 2 mg dm^3 of IBA solution for 2 minutes before transferring them onto the hardening medium Shekhawat (2000) developed a micropropagation process for *Anogeissus latifolia* ex *vitro* by pulse treating with a combination (100 mg l each) of IBA and NAA in soil rite in culture bottles The ex *vitro* rooting induction method was highly efficient

2 4 HARDENING AND PLANTING OUT

After rooting hardening of regenerants prior to transfer in the soil increases the survival rate of transferred plants So it is a step which gradually acclimatizes

the pla t to the l arsl naturnl c vironment Sp ay ng m st ıg and coverıng w tl the th n polv ne ıe rnay serve o f lfll tl e above object ve Vanous types of substrates have been used dur ng acc matızat on sı ch as so l ver n culites mixture ster l zed sand and so l (Goyal and Arya 1981 Gulat a ıd Ja wal 1996 Ph lom na and Rao 1999 Thakur *et al* 2001 and Sunaına and Goyal 2000) Sıwach and G ll (2011) potted and accl matızed *n v t o* ra sed plantlet of *F cus rel g osa* under culture room cond tıons for 25 30 days before transfeı to so l condıt ons Rooted plantlets of *G nel na arborea* were successfully acclimatızed ın high humidity condıt ons (80 90% RH) for twǫ weeks prıor to s ccessful tra sfeı to a shadehouse (M shra a ıd Sl ırın 2009) I *F cıs be ıja n a* transferr ng the plantlets f rst to dıst lled wateı for 6 hou then to so lı te and then keepıng under mist was found to enhance theı surv val (Lavanya *et al* 2006) Pandey *et al* (2010) ransplanted the rooted plantlets of *Artoca pı s l eterophvll s* to earthen pots conta n ıg ster le sand soıl and verm compost (1 2 1) and cove ed by transparent plastıc bags

The rooted plantlets of *Calophyllum apetalum* were transferred to clay pots f lled wıth so l sa ıd and farmyard manure (1 1 1) ma nta ned ın a m st chamber at a relat ve hum d ty of 80 90 per cent (Naır and Seen 2003) *I ı v t o* hardenıng of *Tectona grand s* was carr ed out ın sand soaked wıth halt stre ıgth MS med um (organ c free) The plantlets were accl mat zed f rst ın a m st chambe and then n polybags ın a m xture of so l sand and farmyard manure (1 1 1 v/v) a shade house (Sh r n *et al* 2005) Mature rooted shoots of *Mıll ngton a ho tens s* were transferred to plastıc pots contaınıng vermıculıte mo stened wıth quarte strength basal medıum and naıntaıned n a hum d chamber for ıccl nat zat on and hardenıng for two weeks (Deshpande *et al* 1999)

2 5 MICROPROPAGA ΓION THROUGH TISSUE CULTURE IN TREE SPECIES

Plant t ssue culture technology s be ng w dely used fo large scale plant multipl cat on Small pieces of t ssue the explants can be sed to produce hu ıdreds a d thousands of plants n a co tınuous process A s ngle explant can be ıult pl ed ınto several thousand plants ın relat vely short tın e pe od and space unde controlled conditions rrespect ve of the season and weather on a year round bas s (Ak n Idowu 2009) Apart from their use as a tool of esearch plant t ssue culture tec ın ques have n recent years become of major ndustr al nportance ı tl e area of plant p opagatıon d sease el m nat on pla ıt n prove nent and pıoduct on of seco dary metabolites Endangered threatened and rare spec es have successfully been grown a d conserved by m cropropagat on because of h gh coeffic ent of mult pl catıon and small dema ıds on number of n t al pla ts and space (Hussa n *et al* 2012) The m cropropagat on tecl nology has a vast potential to produce plants of superıor quality ısolat on of useful varıants n well adapted h gh y eldıng genotypes w tl better d sease es stance and stress tolerance capac t es (Brown and Thorpe 199ɔ)

Due to tl e ıcreas ıg threats to forests n part cular a d b od veıs ty n general there s a g eat need to conserve tree ecosystems for both their env ronmental and aesthetic values To ma nta n and susta n forest vegetat o conventional app oaches have been explo ted for propagat on and ımprovement but these methods are ve y slow and are restricted to the most valuable and fast grow ıg specıes Moreover these methods are lımited due to the slow grow ng long l ved sexually self ncompatıble and h ghly hete ozygous nature of plants (Gır *et al* 2004)

In thıs sıtuatıon plant t ssue culture methods offer an ımportant optıon for effectıve mult pl cat on and mprovement of trees w th n a lım ted tıme frame Dur ng the last few years m cropropagat on techn ques l ave been used fo the

rapid and large scale propagat o of forest trees It s co is de ed t at e e are four pl ases of g owth of a tree the emb yogenetic phase the seedl ng phase (equ vale t to juvenile p hase) the tra i it on pl ase (acqu s t on of eproduct ve competence) and tl e aged or mature phase (⁻ h ghest reproduct ve compete ice and lo vest growth con pete ice) In general usage of mature explants from adult trees g ves l m ted success due to ts diff culty to ege ierate u de *n v t o* conditions But *v t o* cultuie s ig juven le expa its g ve prom s ng ies lts

Development of n cropropagation protocols of voody spec es through t ssue culture was slow due to the difficult es expei enced at pr mary culture establishment root nduction and part ally due to the ex stence of phenol c compounds n tissues Slow grow ng hab t of trees and long dormancy pose difficult problems for t ssue culturists They have also not ced that call of trees are hard to diffeie t ate Over the past three decades considerable advancement has been ach eved on micropropagat on methods of forest trees A ve y b ef account of so ne of tl e *n v tro* propagation works carr ed out in *Sa talun albu* and other mportant broad leaved tree species are rev ewed he e

2 5 1 *Santalum album*

A study on effic ent *Santali m albi m* plant regenerat on *v a* ndirect organogenes s from callus cultures der ved from leaf t ssues was do ie by Singh *et al* (2013) The l ghest callus frequency (100%) was obta ned when leaf t ssue was cultured in tl e medium w th 0 4 mg l ^{1} TDZ The WPM + 2 5 ng l BA + 0 4 mg l NAA was the most effect ve in produc ng th highest nu nber of shoot buds (24 6) per callus The highest number of shoots per explant (20 67) and shoot length (5 17 cm) were observed in med a supplemented w th 5 0 mg l BA and 3 0 mgl K net n espectively The highest root ng percentage (91 67) and survival were ach eved us ng WPM media with 1 5 mg l IBA All plantlets survived accl mat zat on pioduc ng healthy plants n the greenhouse

Bele *et al* (2017) attempted in cropropagation of sandal from cultured leaf discs Among various medium experimented MS + 1 0 mg l 2 4 D + 0 5 mg l TDZ supported maximum direct somatic embryogenesis (11 14%) indirect somatic embryogenesis (54 23%) and mean numbers of somatic embryo(s per explant (160 08) while eas culture medium (MS + 2 0 mg l 2 4 D + 0 5 mg l TDZ) promoted direct organogenesis (20 38%) In oculation medium MS + 2 0 mg l TDZ + 0 5 mg l NAA proved superior for direct organogenesis (9 48%) and regeneration of plantlets *via* direct organogenesis (36 69%) MS medium fortified with 2 0 mg l TDZ and 1 0 mg l GA_3 proved superior for plant regeneration *via* somatic embryogenesis (163 63%) while regeneration medium MS +1 0 mg l TDZ+ 0 5 mg l GA_3 +0 5 mg l NAA regenerated plantlets *a* nd rect organogenesis (141 25%)

Sanjaya *et al* (2003) induced multiple shoots from nodal shoot segments derived from a 50 to 60 year old candidate plus tree (CPT) on MS+0 53mM NAA+11 09mM BA *In vitro* differentiated shoots were multiplied on MS medium with 0 53mM NAA 4 44mM BA and additives 283 93 nM ascorbic acid 118 10mM citric acid 104 04mM cystine 342 24mM glutamine and 10% (v v) coconut milk New shoots were harvested repeatedly for up to three subculture passages on fresh medium at four week intervals Microshoots treated with 98 4mM IBA for 48 hour produced roots on growth regulator free ½ MS basal salts medium with vitamin B_5 and 2% sucrose *Ex vitro* root induction was achieved from microshoots pulsed with 1230mM IBA for 30 minutes in soilrite rooting medium The percentage of rooting in soilrite was higher than that for agar medium

Induction of adventitious shoot buds on sandal leaves reported by Mujib (2005) *De novo* shoots were induced directly on leaves without any callus ing stage Leaves with 0 5 1 5 cm length only showed bud inducing potential Although bud formation occurred on both MS and WPM basal media liquid media were more responsive Among the plant growth regulators BA at low

concentrat ons (0 44 and 2 22 μV) was effect e n th s organogenet c p ocess but exogeno s aux n appl cat on fa led o ll c t a s m lar morpl ogenet c response Leaf lam na vas showed max mum response n wh ch tl e dorsal a id ventral leaf surfaces we e equally h ghlv regenerat ve however response var ed n d ffeie t parts of the leaf

Rai and McComb (2002) regene ated *Sar tali m albi n* fron mat re zvgot c embryos thiough d rect somat c embryoge iesis on MS ned u n conta i ng TDZ o BA Ind v dua so nat c embryos were then isolated and iansfer ed to MS nediu n v thout cytok n n on which they formed secondary embryos n repetit ve cycles w tl or w thout the add t on of IAA to the nedium Somat c embryo ger n nation was achieved by solating somat c embryos w th d stinct cotyledons and reculturing them onto ½ MS nedium w th GA_3 (1 4 μM) Recovered plantlets were acclimatized ai d grown n the greenhouse

2 5 2 *Acacia* species

By us ng seedl ng derived explants like leaf node cotyledona y node and shoot tip a protocol for *n v t o* clo al propagat on of *Acac a mang um* was developed by Shah nozzaman *et al* (2012) Cotyledonarv rodes sho ved best response a id MS + 4 0 μ M BA gave n ax mum number of shoots and best root ng was observed n the med um l av ng 8 0 μM IBA

Dhabha and Batra (2010) developed a protocol for nd ect organogenes s in *Acac a n lotica* L through cotyledonary node explant exc sed fro n 20 day old *n v t o* grown plants Explants were cultured on MS medium supple nented w th var ous concentrations of 2 4 D alone and n comb nation w th BA for callus nduction After the 25 days of inoculat on on MS + 2 4 D (2 0 mg l) alone or n comb nation w th of 2 4 D (0 40 mg l) and BA (0 20 mg l) n combi at on gave maximum and rap d growth of green callus The same med a prod ced shoot nduction after subculturing tw ce at the t ne nterval of 21 days Tl e h ghest

number of adventitious shoots and their elongation was achieved on MS + 2 4 D (0 40 mg l) + BA (0 2 mg l) when activated charcoal (200 mg l) was added The elongated adventitious shoots produced roots on ½ MS + IBA (0 5 mg l) after 20 days

Plant regeneration f om phyllode explants excised fiom 60 day old *in v tro* seedlings of *Acacia crassicarpa* was done by Jia *et al* (2006) through organogenesis MS + 0 5 mg l TDZ + 0 5 mg l^{-1} NAA induced green compa t nodules and adventitious shoots n 10 and 40 days respectively The clusters of adventitious shoots were transferred to medium contai ing 0 1 mg l TDZ w th n two months which gave efficient shoot elongation With in one month these adventitious shoots were rooted at a rate of 96 5 per cent on ½ MS + 0 5 mg l IBA

An *in vitro* propagat on of *Acac a mang um* has been established through the induction of bud sprout from mature nodal explants of 10 years old tree H ghest rate of shoot multipl cation was obtained on MS + 1 5 mg l BA + 0 05 mg l^{-1} IAA + 100 mg l AdS) Exc sed shoots were rooted on ½ MS + 0 5 mg l IBA or IAA and 20 g l (w/v) sucrose after 13 14 days of culture (Nanda *et al* 2004)

Selvan *et al* (2003) developed an *in vitro* propagation technique for *Acac a catechu* using nodal explants Maximum number of shoots was obta ned in MS + BA (4 0 mg l^{-1}) + NAA (0 5 mg l) + adenine sulfate (25 mg l) + ascorbic ac d (20 mg l) + glutamine (150 mg l) Best rooting medium was ½ MS + IAA (2 0 mg l) + 1 5 per cent sucrose

2 5 3 *Ailanthus triphysa*

Natesha and V jay aku na (2004) reported ı t o pıopagat on of the trop cal tree spec es *A la ıthı s ti physa* usıng ax llary and term al b d explants from three to four year old sapl ngs MS basal n ed um was the best ned um for culture establ shment and shoot growth Among tl e var ous cytok ı ns supplemented to tl e basal med u n s ngly or ı ı comb ıat o v th IAA BA at 3 0 mg l was better for leaf and mult ple shoot production Comb ıations of two cytok n ns namely BA (3 0 mg l) and Kınet n (1 0 mg l) produced mult ple shoots wıth h ghest mean numbeı of shoots (4 3) However shoot elongat on was very l mıted n all gıowth regulatoı comb nat ons tested for sl oot product on Root ng of m c oshoots was successfully accompl sl ed ı / MS medı n supplemented wıtl both 4 0 mg l IAA and 0 4 mg l IBA

2 5 4 *Albızıa* speciies

Complete plantlets of *Alb z a amara* were developed throı gh nduct on of mult ple shoots on MS med um from cotyledonary nodes of 12 15 day old aseptıc seedlıngs Effectıve mult ple shoot nduction was obta ned on MS supplemented w th BA (1 mg l) + K netın (2 mg l) or BA (1 mg l) + NAA (1 mg l) Sl oot elongat on was prominent at K netın 0 25 mg l concent at on *In v tro* root ng was successful on ½ MS + NAA 1 mg l (Indravathı and Pullaıah 2013)

Accord ng to Borthakur *et al* (2011) hıghest percentage of d rect shoot regeneratıon of *Alb zz a odoı at ss na* was obta ned on MS + 0 75 mg l^{-1} BA Ap cal buds from 7 days old *ın v tro* seedl ngs of *A odo at ss a* vere used for *n v tro* cultur ng *In v t o* root ng of the m croshoots was observed ın growth regulator free as well as IAA or IBA supplemented half strength MS medıum But the best root ng response (53 33%) was observed n growth regulator free MS med um The h ghest response (40%) for acclımatızat on and pot establ shment of the rooted plantlets was obtaıned n so lr te

An *n v tro* shoot regei er o ı systeꞃ v s developed fo *Albı a lebbeck* s ıg root explants fro n 15 day old asept c seedl ngs by Pe vee ı *e l* (2011) Explan were cultı red on MS ned u supplemented w th different conce t a ons of BA K net n and 2 P singly as well as n co ı b at on w th NAA The h ghest ate of shoot n ult pl cation vas ach eved on MS + 7 5 µM BA + 0 5 µM NAA Root ng of microshoots was ach eved us ng / MS + 2 0 uM IBA after four weeks of cultu e Healthy rooted plantlets were successfully established n earthe ı pots co taın g garden so l and grown in greeı house with >80 per cent survı al rate

The isolated leaflets of *Alb a p oce a* cultured on MS mediı m w th various conceꞃ rat oꞃs ol BA and NAA showed shoot bı d egeneratio ı (Kuma *et al* 1998) The l gh st ıumbers of advent t ous buds were obta ı d on MS 10 µM BA + 1 ı M NAA Enhanced adventıt ous bud regenerat on was observed when 7 g l D fco bacto agar was replaced w th 2 6 g l Phytagell n the med u Also the add t on of 15 µM s lver n trate promoted callus free shoot regenerat o from leaf explants MS + 0 01 µM BA + 1 µM NAA elongated the regenerated shoot buds Root ng was obta ned on MS + 2 µM IBA

2 5 5 *Anacardium occidentale*

Kamshananthı and Seran (2012) d d the nduct on of somat c embryogenes s from cotyledon explants of cashew Nodule ı duction was observed n all treatments and higher per cent (80%) was noted n MS + 2 mg l BA The root for r at o ı was l gher (47%) n MS + 2 mg l K et n + 2 mg l NAA where longest roots (120 ꞃm) were recorded Further t was noted that the medium contained 2 mg l BA showed higher per cent of somat c embryoid formation d rectly from cotyledon explant Subsequently somatic embryos were noted 2 3 weeks after culture n MS basal medium w thout growth regulators

In v t o clonal propagatio ı of cashew was tr ed by Kesl avacl andıan *ct al* (2007) from shoot explants of ? 3 year old grafted pla ts which were ıegularly sprayed v th Bav sti 0 l per cent at weekly ntervals Best sl oot proliferat on and shoot elongatio ı was found MS + Kınet n (ɔ mg) + NAA (l mg l) + Brassınol de (0 l mg l) M c o sl oots were rooted *ın t o* at a frequency of 70 80 per cent when cultured for 4 8 days n lıqu d / MS + IBA (l n g l) ıfter th pulse t eatment w th IBA (100 ng l) for two m nutes The average numbe of roots ranged from ɔ ɔ v th an av erage lengtl of l 63 cm

2 5 6 *Anogeıssus latıfolıa*

Shekhawat (2000) developed a mıcropropagat on techn que for *Anog ssı s lat folıa* n which mult ple shoots we e regenerated from cotyledonary ode a ıd ep cotyl explants on MS + 0 l ng l IAA + l 5 ng l BA + add t ves (25 mg l each of aden ne sulfate L arginine ascorb c ac d c t c ac d and l 0 n M L asparag ne) and 200 µM Fe EDTA *In v tıo* dıfferent ated shoots vere subcultured a ıd repeatedly transferred onto fresh med n of the same compos t on except for the BA (l 0 mg l) for shoot mı ltıplicat on The m croshoots were rooted *n v t o* on / MS + l 0 mg l e ther of IBA or NAA *Ex v t o* rootıng was tr ed by pulse treatıng w th a comb nat on (100 mg l each) of IBA and NAA n so lr te n cı lture bottles Tl e *ex v t o* root nduct on nethod was h ghly eff cıent (w th 80% rootıng)

2 5 7 *Artocarpus heteı ophyllus*

Pandey *et al* (2010) found that MS + 3 0 mg l BA was best suited for the ınıtıatıon of explants But add t on of 3 0 mg l Kınet n to th s med a nduced maxımum number of mult ple shoots The ncrease or decrease of the concentrat ons of BA and K netın ın the med um resı lted ın decl ne of shoots number The highest s gn f cant shoots length (7 50 cm) was ach eved n MS + 3 0 mg l BA + 0 l mg l NAA Regenerated shoots rooted n the mediu n contaınıng

MS salts supple ı ented w th 0 1 mg l IB \ + 0 1 ng l IAA + 0 1 ı g l NAA ard 35 ng l sucıose Rooted pla ıtlets we e transplanted to ea then pots conta n ng ster le san l so l a d ver ı compost (1 2 1) a d cove ed by t a sparent plas ıc bags After accl mat zat o ı the potted plants we e tra ıspla ted ı tl e open f eld where 40 μe cei t n ants surv ved

2 5 8 *Azadira hta ındica*

Rapıd clonal p opagat on of *A ad achta nd ca* employ ng nodal stem segments vas developed by Arora *e al* (2010) M ddle order nodes (3 d or 4 h ı ode from apex) showed max mum bud break (78 6 81%) BA (1 11 μM) was found most effective n nducing mult ple shoots whereas no ganic and orgn c constituents cf the nedium nfluenced growth and ge c al cond tion of prol ferat ng shoo s An average of 3 1 shoots per explant was regenerated n MS + 1 11 μM BA + 1 43 μM IAA + 81 43 μM aden ne hen sulfate Root nduct on took place n 8 10 days w th 100 per cent ıoot ng ın preser ce of 2 46 μM IBA

2 5 9 Bamboos

Ba nbusa balcooa was propagated *n t o* from nodal explants After surface steril zat o ı w th 0 1 per cent mercur c chloride for 10 m nutes nodal segments were cultured on MS + 4 4 μM BA + 2 32 μM K net n + 0 2 per cent *w/v* gelr te *In v tro* formed shoots were successfi lly mult pl ed ın l qu d MS + 6 6 μM BA + ? 32 μM Kınetı + 2 5 per cent *v/v* coconut water + 100 mg l nyo ınositol Shoot cl sters conta nıng 5 to 8 sl oots were rooted w th n 3 week w th 87 5 per cent success n / MS + 5 71 μM IAA + 4 9 μM IBA + 5 37 μM NAA (Neg and Saxena 2011)

Deogırkar *et al* (2007) developed a protocol for the *n v tro* propagat on of *Dendrocalamı s st ctı s* through somatic embryogenes s The seeds cultured on MS + 3 mg l 2 4 D prodı ced embryogen c callus w th globular shaped

embryos Better ge m nation of somatic emb yos witl dark g ee colour was observed ı MS + ɔ ng l BA There we e 15 to 25 shoots that developed 4 weeks after ıı cubat on of he sa d treat ent

Meshran *et al* (2006) de nonstrated a protocol for *n t o* propagation of *Bambı sa a ıu d na ea* Embryogenic callus w th globular shape embryos were produced when seeds cultured on sol d f'ed MS + 2 4 D 2 ng l For germ nat on somatic embıyos were transferred to MS + BA 2 mg l The dark green e nbryos ceveloped nto healthy plantlets w th well developed root syste n

A m cıo propagat on techn que for *Bambı sa vulga s* var Gıeen from nodal segments w th s ngle ax llary buds collected from f'eld grown clumps (7 year old) was developed by Shir n *et al* (2005) Among the different aux ns and cytokını ıs tr ed ınd v dua ly or n comb nat ons MS + 15 µM BA + 15 µ M K net n resulted ın max mum shoot mult pl cation rate of 7 5 fold Max mum root ng (91%) was ach eved on MS + 25 µM NAA After *n v tıo* harden ng ı soılr te and subsequent acclimat zatıon n m st chamber and shade house the plantlets were transferred to field

2 5 10 *Bauhınıa acumınata*

Akhter *et al* (?012) stud ed the effect of growth regulators concentrations on morphogenet c development us ng seeds of *Bauhın a acu n nata* Satısfactory ger nınat on was observed at MS + 1 0 mg l GA_3 Subsequent propagat o ı from plantlet was perfo med on MS med um supplemented with va ous conce ıtrations of BA and NAA or IBA Reasonable shoot formatıon was observed at 0 50 mg l BA + 0 10 mg l NAA For root ng IBA (0 20 0 60 and 0 80 mg l) and NAA (0 20 0 60 and 0 80 mg l) were used The h ghest numbers of roots were observed at NAA 0 60 mg l

2 5 11 *Bombax malabaricum*

When shoot t ps and node segments of *B nalaba c n* were cultured n MS med um supplemented with d fferent concentrat ons of BA in combination w tl NAA the node seg nents produced more shoots compared to the shoot t ps (Atta Alla *et al* 2003) 2 mg l BA and 1 mg l NAA produced the l ghest number of p oliferated shoots The number of shoots decreased w th ncreas ng co ncentrat ons of BA and NAA Eve n though root ng of prol ferated shoots was observed on MS ied un conta n ng 0 0 5 1 2 or 3 ng IBA or NAA l low concentrat ons of IBA a id NAA resulted n the h ghest number of developed roots as well as root length

2 5 12 *Buchanania lanzan*

Sharma *et al* (2005) developed a protocol for somatic embryogenes s and plantlet regenerat on of *Buchanania lanzan* Spreng f om mmature zygot c embryos The h ghest frequency (60%) of somatic embryo nduction was obta ned in cultures grown on MS med um fort f ed w th 4 53 µM ? 4 D 5 32 µM NAA and 4 48 µM BA The med um supplemented with 15 µM ABA was most effective for maturat on and germ nation of somat c embryos

2 5 13 *Caesalpinia pulcherrima*

M c opropagatio of *Caesalpin a pulche r ma* was done by us g iodal explants with a s ngle ax llary bud from trunk sprouts excised fro n a 20 year old tree (Rahman *et al* 1993) MS med um conta n ig d ffere nt comb nat o s of aux ns (IAA NAA 2 4 D and IBA) and cytokinins (BA and K netin) was used for culturing Calogenes s was seen on medium conta ning NAA alone and 2 4 D n any comb nat on except with BA All other growth regulator comb nat ons resulted n multiple shoot format on shooting was best on med um conta n ng NAA and cytok n n and the greatest number of roots produced at this stage was

seen on medium conta n ng IAA and cytokının For elongat on 6 weeks old regenerated shoots were subcı ltured ın the same basal medium supple nented w th BA and NAA Shoots nduced roots ın ½ MS + 5 5 µM IAA for advent tious root induction Almost 95 per cent of ıegenerated pla nts surv ved accl natızat o

2 5 14 *Caıamus* species

Valsala *et al* (1999) reported *ın vitro* regeneration ın three species of rattan (*Calamus andamanicus C thwaitesıı* and *C pseudotenuis*) *v a* multiple shoot induction direct organogenes s and regeneration through callus Embryos and explants of the collar regıon of seedlings of all these species produced nultiple shoots on a med um consistıng of the mınerals and vıtam ns of MS + 20 g l^{1} of sucrose + with 0 l 1 mg l of 2 4 D and 1 10 mg l BA or K netın Direct organogenesis from the base of leaf explants of *C andamanic is* obtaı ed on MS + 3 10 mg l K netın after 4 5 weeks of culture Callus was produced from embryo explants of all these spec es when cultured on MS + 3 8 mg l of 2 4 D or NAA ın 20 30 days These callus developed shoot buds when transferred to a medium contaınıng 3 5 mg l of the auxın along wıth l 8 mg l BA or Kınetın The regenerated shoots rooted ın MS + 0 5 2 mg l of IBA Plantlets were transferred to so l after hardenıng ın a vermicul te soıl mediun for three months

2 5 15 *Calophyllum ınophyllum*

A protocol for *ın vitro* micropropagatıon of *C nophyllum* was developed through multıple shoot formatıon from seed explants (The ıgane *et al* 2006) Standardızatıon of *ın v tro* germınatıon of the seeds was done on WPM hormone free or supplemented wıth BA 2 22 µM and on half or full strength MS med um Multıple shoot formatıon was achıeved on WPM + BA (2 22 44 00 µM) + TDZ (0 91 4 54 µM) from the decapıtated seedlıng explants The maxımum mult ple shoots were obtaıned on TDZ (0 91 µM) after two subcultures Elongated shoots of sıze >4 0 cm were obtaıned on all media combınatıons Stunted shoots ınduced

on BA and TDZ were elongated on ½ WPM without any growth hormones The elongated shoots induced 52 per cent rooting with 15 roots per rooted plant on ½ WPM and/or full strength WPM supplemented with IBA (2 46 24 60 µM) alone or in combination with BA (2 22 µM) The micropropagated plants were acclimatized successfully with 77 per cent survival rate after five weeks

2 5 16 ***Casuarina equisetifolia***

Seth *et al* (2007) achieved *in vitro* clonal propagation of *Casuarina equisetifolia* from mature tree derived explants Fresh green healthy twigs were collected from 30 year old flowering trees After culture establishment period of 30 days the sterile explants were transferred to Gupta and Durzan (DCR) medium supplemented with different concentrations of BA for axillary bud sprouting Sprouted explants were transferred to ½ DCR medium containing activated charcoal sucrose and agar for elongation of axillary buds sprouts Further multiplication was obtained on DCR medium containing BA sucrose and agar Maximum axillary bud sprouting was evident in the presence of 4 44 µM BA with 3 81 sprouts with explant after 45 days of culture Maximum rooting was obtained when the basal ends of the shoots were dipped in 19 70 µM IBA solution for 48 h and transferred to growth regulator free ½ DCR medium containing activated charcoal After 4 6 weeks when the roots became sturdy and showed lateral branching the rooted shoots were transferred to polybags and kept under polyhouse conditions

In order to induce callogenesis and organogenesis in *Casuarina equisetifolia* stem tips (3 5 cm long) collected from two year old trees were cultured on ½ MS and MS media with or without supplementary auxins (NAA 2 4 D and IBA) cytokinins (Kinetin and BA) and another supplement (activated charcoal) in different dual and triple combinations Full strength MS medium was superior to ½ MS in callus initiation which was also increased by addition of NAA (1 mg l) or 2 4 D (2 mg l) All these treatments gave maximum callus formation

(91 6 %) Presence of IBA or act vated charcoal ın the mediu n resulted ın the reduction of calius for nat on than that ın / MS alone Shoot regenerat on from callus was obta ned by the add t on of BA at the h ghest concentrat on of 10 ng l and addition of both cytokın ns at 4 or 5 mg l gave the l ghest shoot for nat on (50%) Rooting was best w th 3 ng l IBA (Parthiban *et al* 1997)

2 5 17 ***Cedı ela odoı ata***

In v tro propagat on protocol of *C d ela odorata* was developed from nodal explants of juven le shoots taken f om f eld trees (Garcıa gonzales *et al* 2011) D s nfect on of node explants with 5 per cent propiconazole CE 25 fo 3 m nutes resulted ın 100 per cent explant d sinfect on and 60 per cent morphogen c response on those establ sl ed explants MS + 2 mg l BA + 3 ng l NAA showed optimized shoot development Thıs med um resulted n 100 per cent shoot develop nent f o n tl e *n v tro* node explants with a ɔ 93 c ı nean lengt Ind v dual zatıon of the egenerated plants on the same medium s ımulated root ng (a mean of 3 9 roots / plaı t) after s x week

2 5 18 ***Cınnamomum c ımphoı a***

Shoot t p explants have been employed to develop *n v tro* culture techn que of *C nnamo num ca npho a* MS med um and WPM supplemented w th vary ng concentrat ons of cytok n ns (BA and Kınet n) weıe used for multiple shoot nductio ı Max mum number of shoots was developed n WPM + 2 mg l BA and the maxımum shoot length was observed at 1 mg l BA ½ WPM supple nented w th 1 mg l IBA nduced rooting n elongated shoots The rooted shoots were successfully transferred to f eld with 50 per cent surv val (Sharma a ıd Vash stha 2010)

2 5 19 *Dalbeigia* species

Suitable cultural co d t ons for m cropropagat on of *Dalbc g a s ssoo* from nodal mer sten was evaluated by Al *et al* (2012) The best sl oot ng respo se (88%) was obta ned o ı MS med um conta nıng l 0 mg l BA + 0 25 mg l NAA MS med um + l 5 ng l BA + 0 25 mg l Kınetın exh b ted maxımum mber of shoot Best root ng med a was MS + 1 0 mg l IBA

Plant regenerat on tl rough so nat c embryogenes s was ach eved f o n callus cultures der ved fron semi matu e cotyledon expla ts of *Dalbe g a s ssoo* Somat c embryos were developed over the surıace of embryoge ı c callus and occas o ıally from cotyledon explants w thout ınterveníng callus phase Max num (89%) response fo callus format on from cotyledon p eces was o MS n ed um supplemented w th 9 04 µmol l 2 4 D and 0 46 µn ol l Kınet n Somat c embryogenesıs was ach eved after transfer of embryogen c callus clu nps to ½ MS med um without plant growtl regulators Average numbers of somatic embryos per callus clump was 26 5 on ½ MS med um after 15 weeks of culture Enhancement of somat c embryogenesıs frequency from 55 per cent to 66 per cent and the number of somatic embryos per callus clun p from 26 ɔ to 31 l were ach eved by the add tion of 0 68 mmol l L glutam ne to / MS med u n After 20 days of culture about 50 per cent of somat c e nbryos converted nto plantlets on MS medıum conta n ng 2 per cent sucrose Transfer of somat c embryos to ½ MS medıum co ta n ng 10 per cent sucrose for l ɔ days pr or to transfer o ı / MS med um wıth 2 pe cent sucrose enl anced the conversıon of somat c e nbryos nto plantlets from 50 to 75 per cent The plantlets w th shoots and roots were transferred to and / lıqu d MS med um for 10 days each and then to plastic pots contaın ng autoclaved peat moss and compost m xture (1 1) (S ngh *et al* 2003)

Swamy *et al* (1992) stud ed tl e ınduct on of s ngle and multıple shoots from nodal explants of 60 80 year old *Dalberg a lat fol a* el te trees on MS + 1

ng l BA and 0 05 mg l NAA + 0 5 mg l IAA Mult ple shoots was obta ned on MS (reduced najo elements) or WPM + 1 mg l BA + 0 5 l mg l K net n Excised shoots were rooted on MS + 2 mg l IBA to obta n complete plantlets The regenerated plantlets were accl nat zed and successfully transfe ed to the soil

2 5 20 *Delonix regia*

So natic embryogenes s was duced n us ng nmature zygotic embryos (IZEs) and entire im na ure seeds (IES) explants of *Delon x eg* (Abd and Hedayat 2011) The explants were cultured on sem sol d MS bas l med um w th different concentrat o ı of BA and 2 4 D and incubated ı dark Among the explants used the IZE showed better response than EIS D rect so nat c embryos were induced d rectly after 4 5 weeks from the radical t p of IZEs o ned um w th 2 4 D (2 mg l) + BA (0 25 mg l) with a frequency 15 pei cent Th s was only 9 per cent when 2 mg l 2 4 D was used alone or w th BA (0 5 ng l) For further maturat on the somat c e nbryos were transferred to med um with ABA (0 25 mg l) a ıd maltose (3 %) and stud es are being carı ed out for convers on of somat c embryos ıto plantlets

2 5 21 *Emblica officinalis*

M cropropagat on stud es were carr ed out to develop a protocol for mass mult pl cat on of true to type plantlets by us ng nodal seg nents of *En bl ca off c nal s* (Pat dar 2010) Explants were cultu ed on twenty d ffere ıt fort ficat ons of MS med um Analys s of variance exh b ted l ıgl ly s gn f cant d fferences among d fferent culture med a comb nations The basal MS + 4 0 mg l BA + 0 5 mg l NAA was found to be more respons ve for shoot prol feration (47 65%) number of shoot(s) per explant (3 20) and average shoot length (1 43) *In v t o* root ng was h gh o ı MS + 2 0 mg l IBA + 0 5 mg l BA (17 20%)

2 ɔ 22 *Eucalyptus* species

G ɪjasha ıkar (2012) ach eved ɪ *v ɪ o* pla t rege ɛ at on fro ɪ nodal seg ne ıts of 18 months old super or genotypes of *Eɪ calyptɪ s ca ɪ aldɪ lɛ* ɔ *s* t ees through direct orgɑnogeɪ es s (DO) and d rect somat c emoryoge ıes s (DSE) pathways Ir ıtial bud break (BB) stage occurred *v a* DO wh le shoot mult plicat on phɑse follo ved both DO and DSE patl ways Botl BB a ıd shoot mult plicat on stages were ach eved on shoot nduct o ɪ a ıd ultıpl cat on (SIM) media composed of MS + 2 mg l BA + 0 1 mg l NAA Best shoot elongat on was on ½ MS + 0 5 n g l BA wh le root nduction and elongation vas super or ın ½ MS + 1 mg l IBA Full strength MS fort fied w th cytok nins (BA) and weak auxın (NAA) n tl e rat o of 20 I favored d rect regenerat o ɪ pathways Further MS s ppoıted shoot and root development For nass n ılt pl cation fortn ghtly subcultur ng of s ngle nodal explants for eight passages on SIM med um resulted n 60 148 shoot nıtials Repeated subcultur ng n SIM med um nduced the format on of d rect somatic embryos

For clonal propagat on of super or genotypes of *Eɪ calyptɪ ɔ g a ıd s* ap cal and ax llary buds were collected from adult el te trees n forests of nortl e n Iran n dıfferent seasons The best shoot multipl catıon was obta ıed us ng a mod f ed MS med um conta n ng half strength Nitrate conta n ng BA IBA K net n and GA_3 with concentrat o ɪ of 0 1 0 01 0 2 and 0 1 mg l respect vely Shoots from the mult pl cat on med um were transferred ın MS med um (half strength n trate) supple nented w th 1 mg l Zeat n and 0 2 ng l IAA fo shoot elongɪt on Shoots were rooted ın MS w th ⁄ strength of macroelements + 0 5 mg l IBA + 0 5 mg l NAA The plantlets were successfully established ın greenhouse and f eld cond t ons (Emam *et al* 2010)

In a study to test the appl cat on of growth regulators n the culture media and to test aux n concentrations and types of substrates ın the ɪ *v tro* rooti g of juven le *Eucalyptɪ s globɪ lɪ s* subsp *globulus* explants BA and TDZ n

concentrat ons f o n 0 2 to 1 0 ng l were added to MS agar sol d f ed med um (Ponte *et al* 2001) BA provided the b st esults in the mea n number and the lengtl of the shoots wh cl te ded to dec ease w th an nc ease cytok in concent at on n tl e ned u n In the rooting stage two subst ates (agar and vermiculite) w th different concentrat o ns of IBA (0 2 to 0 6 mg l) were added to MS ned um reduced to one th rd of its salt concentratio ns Root ng percentage was approximately 50 per cent and better qual ty root was obta ned n the ver n cul te med u n w th h gher IBA concentrat on However n the agar substrate with an nc eased IBA concentration larger calli s formation on the basal port on of the explant and lengthy roots were observed but witl reduced root ng percentage

2 5 23 *Ficus* species

An *n v tro* propagat on protocol has been developed from nodal segments obta ned from a 45 50 year old tree of *F cus el g osa* WPM + 1 0 n g l BA + 0 5 mg l IAA gave the highest bud break frequency (100%) followed by nax nu n number of multiple shoots (13 9) as well as length (2 47 c n) Two mod fications n th s medium resulted n enhanced shoot regenerat on w th 200 mg l glutam ne + 150 mg l ADS (called as MM 1) g v ng 32 5 shoots per nodal explant wh le another r iod fical on with 200 mg l glutam e + 150 mg l ADS + 100 ng l phloroglucnol (called as MM 2) giving 35 65 shoots per explant Best rooting was on sem solid as well as liqu d WPM + 2 0 g l IBA + 0 5 mg l IAA The *n v t o* raised plantlets were potted and accli nat zed inder culture room condit ons foi 25 30 days before transfer to soil condit o ns (S wach and G ll 2011)

Nodal seg nents containing ax llary buds of *F cus benghalens s* were nduced to produce a large number of mult ple shoots by cultur ng on MS + 1 0 BA + 0 1 NAA (mg l) + 20 per cent (v/v) coconut m lk (Munsh *et al* 2004)

Excised shoots f o n th s culture rooted best on ½ MS + 0 5 mg l IBA The co nplete plantlets thus obta ned vere successfully transferred to so l

2 5 24 *Gmelina arbo ea*

M shra and Shir n (2009) ach eved *in v tro* axillary bud prol ferat on n nodal segment explants from tl e s de branches of 15 18 years old trees of *G cl na a bo ea* o MS + 10 µM BA MS + 1 0 µ M BA + 0 1 M Kinet n nduced max m ı n sl oot n ult pl cat on Tl e addit on of 2ɔ µ M $AgNO_3$ n the cı lture fac litated callı s free shoot for nation Root ng vas obtı ned on / MS + 10 0 µM IBA and rooted plantlets were successfully accl natized n h gh hum dity cond t ons (80 90% RH) for two weeks pr or to successful t ansfer to a sl ade house The e ght nonths old potted plants were show ng excellent growth a ıd development

2 5 25 *Haı dwickıa bınata*

Anuradha *et al* (2000) developed an opt mal *n v t o* propagatıo procedure usıng mesocotyls shoot t ps and ax llary bı ds as source of explants A total number of 112 shoots per seedl ng can be obta ned w th n 3 cycles of 30 days durat on each The proliferated shoots read lv rooted *ın v tro* on MS medium supplemented w th 4 mg l IBA

2 5 26 *Hevea brasılıensıs*

Hui *et al* (2009) carried out m cropropagation of *Hevea b as l ens s* by us ng mature stems of Reyan 7 33 97 an excellent cult var of Brazil rubber ın d fferent growth stage as expla ts The results show that the sten segments ın d fferent growth stages have d fferent contamınat on levels under normal cond t ons Moreover stems at bronze stage and light green stage ma ıtained on medium supplemented w th 4 0 5 0 mg l BA 0 5 mg l GA_3 can well nduce

multiple adventitious buds Robust shoots were obtained from multiple adventitious buds cultured on medium supplemented with 2.0 mg l BA 0.5 mg l NAA 1.0 mg l BA 1.0 mg l Kinetin and 0.5 mg l NAA or supplemented with 0.5 mg l BA 1.5 mg l Kinetin and 0.5 mg l NAA Roots were induced on the medium supplemented with 0.5 mg l IBA and 0.5 mg l IAA

Kala *et al* (2007) developed a protocol for the induction maturation and germination of somatic embryos from leaf explants of *Hevea brasiliensis* (clone RRII 105) Leaf explants were cultured with their adaxial sides on MS medium supplemented with different combinations of phytohormones such as 2,4-D and BA NAA and BA as well as 2,4-D BA and NAA Compact calli could be developed from the cut ends of the explants on MS + 2,4-D (1.5 mg l) + BA (1.0 mg l) whereas pale yellow friable calli was obtained MS + NAA (0.2 mg l) + 2,4-D (1.2 mg l) + BA (1.0 mg l) These calli were subcultured for proliferation on medium containing reduced auxin (0.4 mg l 2,4-D) and slightly increased level of sucrose (40 g l) Embryo induction was achieved in MS + BA (2.0 mg l) + GA_3 (1.0 mg l) + NAA (0.2 mg l) and maturation occurred in WPM + BA (0.3 mg l) + TDZ (0.5 mg l) + GA_3 (1.5 mg l) On transfer to hormone free ½ MS medium the cotyledonary stage embryos developed into plantlets

2.5.27 *Holarrhena antidysenterica*

Using nodal explants obtained from about 20 year old *Holarrhena antidysenterica* mature trees growing in the field an economic and efficient procedure has been outlined for its micropropagation Shoot development was maximum (90%) on MS + NAA (2.0 mg l) + IAA (1.0 mg) + Kinetin (1.0 mg l) The role of auxins were instrumental as rooting of the differentiated shoots was best in MS + IBA (1.5 mg l) + IAA (1.5 mg l) Regenerated plantlets were successfully acclimated in the green house and after a hardening period of 4

weeks 90 per cent transplant tion success was achieved under the natural co idit on (Kanungo *et al* 2012)

2 5 28 *Hydnocarpus kurzi*

When explants of apical and axillary buds of young sprouts f om naturally grown *Hydnocarpus kurzi* were cultured in MS + 2 5 mg l BA + 0 5 mg l NAA about 85 per cent of the cultures regenerated showed four shoots per culture (S nha *et al* 2005) Repeated subcultures in the same med um gave rapid shoot multiplicat on with eight shoots per culture The number of shoot(s) was increased up to 15 per culture by the addition of 15 per cent (v/v) coconut water *In vitro* raised shoots root•d on ½ MS + 1 0 mg l IBA + 1 0 mg l NAA For accl nat on and tra nsplantation the plants in the rooting culture vessels we e kept n no n al room temperature fo seven days before transplanting in pots wl ere the plantlets were reared for three weeks The survival rate of mature regenerants was found to be 75 80 per cent

2 5 29 *Jatropha curcas*

A protocol for high frequency regenerants of *Jatropha curcas* has been developed by the process of direct and indirect organogenesis from nodes and leaves as explants (Shukla *et al* 2013) Both the explants were initially inoculated on MS basal med u n Quick callus nit ation was supported by MS + 3 0 or 5 0 µM IBA + 27 0 µM BA wh le increas ng the concentration of IBA to 7 5 µM led to delay in callus ng Emergence of shoot bud was first observed on MS + 27 0 µM BA + 3 0 µM IBA The growth regulator combinations 3 0 µM IBA with 4 5 µM and 27 0 µM BA was found to be the best suitable med um for promoting multiple shoot regenerat on with offshoot measuring 1 5 2 0 cm Root ng was observed on MS basal medium

2 5 30 *Lagerstroemia speciosa*

High efficiency shoot regeneration of *Lagerstroemia speciosa* was achieved through leaf derived callus on modified MS (MMS half strength macroelements full strength microelements and vitamins of MS medium) The leaf derived calluses on MMS + 4 0 µM 2 4 D + 1 0 µM BA + 568 µM ABA were regenerated to give maximum shoots on MMS + 5 0 µM BA + 3 0 µM NAA + 10 per cent coconut water + 568 µM ABA Shoot regeneration ability of the callus was investigated up to eight passages The number of shoots per culture increased gradually up to 6th subculture and more than 110 shoots were produced per leaf segment derived callus clump but no remarkable changes were observed in the percentage of callus forming shoot and length of shoot throughout the subcultures The rooting on MMS + 1 0 µM IBA was proved to be the best among three auxins studied (Rahman *et al* 2010)

2 5 31 *Mallotus philippensis*

Triploid plantlets were induced from the mature endosperm of *Mallotus philippensis* by Sehgal and Abbas (1996) A continuously growing callus was obtained on MS 2 mg l 2 4 D + 0 5 mg l Kinetin Subculture of callus on MS + 3 mg l BA + 1000 mg l casein hydrolysate resulted in the production of four types of morphologically distinct cell lines Of these only the green compact cell line was responsive resulting in differentiation and this cell line produced shoots on MS + 3 mg l BA + 0 2 mg l NAA only after chilling Excised shoots produced roots on transfer to MS supplemented with 2 mg l phloroglucinol + 1 mg l IBA

2 5 32 *Mangifera indica*

Somatic embryos were formed from the nucellar tissue isolated from 30 45 day old fruits of monoembryonic mango variety Neelum and polyembryonic

var ety Vellar Manga (Rajmohan *et al* 2007) ½ MS + 2 4 D 5 0 ng l + GA_3 5 0 mg l + glutam ıe 400 mg l + coconut water 200 ml l + act vated charcoal 500 mg l + sı crose 60 g l ٧e ↄ found to be the best ı ndu ct on of embryogen c callus The treatment 2 4 D 4 0 mg l + BA 1 0 mg l + GA_3 5 0 mg l ın ½ MS basal med u n supple nented with glutam ı e 400 mg l case n hydrolysate ɔ00 mg l sucrose 60 0 g l coconut water 200 ml l and agar 6 0 g l was the best ın ınit ıt ng somat c embryos from the nduced nucellus of tl e three va ↄties I t ıe v ı ety Neelum BA (8 0 and 16 0 mg l) and K net ı (8 0 to 32 0 mg l) were as effective as 2 4 D ın nduc ng somat c e nb yogenes s Polyembryonic var ety showed better ıesponse to nduction t eatments tha ı tl e mono embryo ı c varıet es B major salts w th MS minor salts n combınat on w th 40 g l sucrose 10 g l PVP 200 ml l coconut wate ı 5 0 mg l ABA 100 ng l case ı hydrolysate a ıd 6 0 g l agar were the best suited fo the naturat o ı of the somatic embry os A basal med um cons st ng of B_5 major salts and MS m nor salts supported the h ghest percentage of germ nat on of so nat c embryo ds n both varıet es However abnormal t es n germınat on were observed ın most of the cases Excellent germınat on of somat c embry os was observed n a l qu d ned um cons st ng of ½ B_5 macro salts + full strength MS m cro salts + GA_3 1 0 mg l + glutamine 400 mg l + sucrose 30 g l

2 5 3ɔ *Melıa azedarach*

Husa n and An s (2009) ach eved a rap d *n v t o* mult pl cat on of *Mel a a eda ach* th ough ax llary bud sprout ng and mult ple shoot fo n ıt o ı fro n nodal segments dei ed fro n 20 year old candidate plus t ee on MS + 5 µM BA The h ghest shoot regenerat on ٧ as nduced from nodal explants o ı MS + 5 0 µM BA + 0 5 µM IAA + 30 µM AdS Add t on of 250 mg l $(NH_4)_2SO_4$ and 100 mg l K_2SO_4 prevented defol at on a ıd t p burn ng w thout a fect ng the u nber of shoots The explant harvest per od also ınfluenced the bud break and shoot sprout ıg from nodal segments Repeated subcultur ng of nodal explants on fresh MS + BA (2 5 µM) + IAA (0 5 µM) + AdS (ɔ0 µM) and add t ves was found most

su table growth regulator regime for achieving 1 2 fold increase in shoot nultiplication rate The percentage of shoot m i tiplicat on as well as the number of shoots per node re nained the sai e during first three subcultu e passages afterwards a decline was recorded About 90 per cent of the *n v tr c* tegenerated shoots were successfully rooted *ex vitro* by giving a pulse tieatment of 250 µM IBA for 1ɔ minutes followed by their transfer to thermocol cups containing so lrite The ra sed plantlets were successfully acclimatized f rst under culture room cond tions then to green house with 85 per cent survival rate

In v tro regeneration of *M a eda ach* was studied by V la *et al* (2004) with the regeneration of shoots from calluses initiated from leaflets of *in vitro* growing plants MS + 4 44 µM BA + 0 46 µM Kinetin + 16 29 µM AdS was the best medium for establishment of cultures Regenerated shoots were multipl ed in MS + 0 44 µM BA + 0 37 µM Kinet n + 3 26 µM ADE Maximu n root ng (89%) was achieved by culturing in MS + 12 26 µM IBA for 3 days and subsequent transfer to MS lacking growth regulators for 27 days

2 5 36 *Michelia champaca*

Michelia champaca plants were regenerated through somatic embryos derived from immature seeds (Armiyanti *et al* 2010) Highest (43%) embryogenic callus formation was observed on MS + 2 mg l NAA After four to six months of culture in the same medium the embryogenic cells proliferated and formed somatic embryos (30%) Wh le the germination of somatic embryos in hormone free MS med um produced highest percentage (45%) of normal plantlets compared to other germination medium containing different GA_3 concentrations which gave only 1 8 per cent germinated somatic embryos

2 5 37 *Millingtonia hortensis*

Callus was obtained from the nodal regions of the explants collected from *Millingtonia hortensis* mature trees when cultured on MS + 5 0 mg l BA + 0 5 mg l IBA As many as 10 shoots per 1 0 cm callus piece formed on reducing the auxin level in the medium to 0 2 mg l These shoots were allowed to elongate up to 2 3 cm high on the same medium and were then excised and transferred for further elongation to a medium containing BA (2 mg l) NAA (0 5 mg l) and activated charcoal (AC 0 3% w/v) Regenerated shoots (7 8 cm high) were transferred to MS + IBA (2 mg l) + NAA (0 1 mg l) for rooting Rooted shoots were transferred to plastic pots containing vermiculite moistened with quarter strength basal medium and maintained in a humid chamber for acclimatization and hardening for two weeks (Deshpande *et al* 1999)

The potential of *in vitro* culture for large scale commercial production of *Millingtonia hortensis* was investigated by Hegde and D Souza (1995) Kinetin alone at 15 mg l and BA + TDZ at 5 or 2 mg l gave maximum multiple shoot buds Only 7 6 per cent rooting was obtained on medium with 0 1 mg l IBA or NAA with roots arising from intervening callus When shoots were cultured on auxin free quarter strength MS medium enhanced rooting (45%) and a reduction in callus formation was obtained The rooted shoots were potted in a 1 1 mixture of sand and soil

2 5 38 *Mimusops elengi*

A study was conducted by Bhore and Preveena (2011) to find out a suitable explant and a suitable medium among MS N_6 and B_5 media for micropropagation of *Mimusops elengi* MS + 5 ppm BA was used to initiate *in vitro* cultures MS + 8 ppm 2 4 D + 2 ppm BA was used for callus induction MS N_6 and B_5 media supplemented with 5 ppm BA were used to compare the response of IZEs Immature zygotic embryos (IZE) were the most suitable explant for *in vitro*

culture nit at o ı a nong ax lary a d apical buds and ımmature zygotic embryos (IZEs) Response of IZEs on three med a a d comparat ve analys s clearly nd cated that B_5 was the nost su table nediu n for IZE germ nat on rooting and roots growth and develop ne ıt N_6 medium ıs the most suitable for g owtl and development of germı nated IZEs

2 5 39 *Nyctanthes arbor tristis*

In v tro ı roı aga on of *Nyctanthes a bor t st s* L l as bee ı s ccessfully established from ax llary bud explants on MS (Bansal *et al* 2012) Max mum number of mult ple shoots was obta ned on MS + BA (22 2 µM) ½ MS (2% sucrose) + NAA (10 74 µM) provided the ı axı num frequency of oot n t atio ı

2 5 40 *Oroxylum ındıcum*

In v tro propagation of *O oxyli n ndıcum* was developed by Dwived and Boro (2012) us ng nodal segments n WPM BA 3 0 mg l was more effect ve n bud break and ınduced multiple shoots 7 6 shoots/explant w tl s g ı f ca ıt number of leaves (20 4) after 60 days of culture H ghest shoot length (1 6 cm) was observed ın med um conta n ng BA (0 5 mg l) The best *ı v t o* rooting response (6 0 roots/shoot 1 8 c n root length) was observed n / WPM + NAA 0 5 mg l The well rooted plants were sequentially hardened and accl nat zed w tl 70 per cent surv val rate n the pott ng m xture having so l perl te and co npost (1 l l)

2 5 41 *Peltophoı um pteı ocaı pum*

Udd n *et al* (?005) stud ed the mult plicat on of shoots from d fferent *ın v tro* grown explants v z shoot t p nodal segment and cotyledona y node of *Pcltophorum pte ocaı pum* All the explants were cultured on MS med a containing d fferent concentrat ons and combınat ons of BA Kınet n and NAA The highest numbe of mult ple shoots was observed from cotyledonary nodes ın

MS + 2 0 mg l K et + 0 5 ng l NAA The egenerated sl oots were t ansferred oı MS ned a l av g lBA for advent ıous root ın t at on

2 5 42 *Pongamia pinnata*

Ir v tro clonal ı ropagat on of *Ponga ı a p nnata* was done bv Kesarı *et al* (2012) WPM and MS supplemented vıth dıfferent concent at ons and comb nat ons of pla ıt growth regulators were used for nodal seg nent culture a d axen cally grown seedl gs of el te ge otype of *Ponga a p nnata* WPM + BA (5 0 mg l) + Kınetın (0 5 mg l) ◦ave the greatest response to ın t at on and multipl catıon Even though mult plıed shoots started to prod ce roots n the multipl catıon medıum conta n ıg BA and NAA the s bsequent establ shment was poo Root ng was enhanced n / MS + IBA (0 5 mg l) Rooted pla ıts were hardened successfully glass house wıth 70 per cent surv vabıl t)

2 5 43 *Populus* species

In order to enhance the frequency of plant rege erat o ı n *Populus c l ata* the effect of TDZ alone and n comb natıon wıth aden ne and NAA weıe stud ed on the regenerat on potent al of leaf explants (Aggarwal *et al* 2012) Efficient shoot regenerat on was observed n leaf (80 00%) explants on MS + 0 024 mg l TDZ + 79 7 mg l ade ı ne Elongatıon and mult pl cat on of shoots were obta ned on MS + 0 5 mg l BA + 0 2 mg l IAA +0 3 mg l GA MS + 0 10 mg l IBA was effect ve n shoot nduct on

Thakur *et al* (2012) developed a rap d and eff c e ıt protocol for *n vitro* plantlet regenerat on of *Populus delto des* clone G48 usıng petıole explants The h ghest frequency of shoot ıegeneratıon (74 75°₀) fro n pet ole was obta ned on MS + 0 50 mg l BA + 0 20 mg l IAA Shoot mult pl cat on and elongatıon also took place on the same nedıum To overcome the brown ng problem wh ch was observed n 10 15 days of culture the explants along w th tl e develop ng sl oot

buds were transferred to modified MS + 0.50 mg l^{-1} BA + 0.20 mg l^{-1} IAA + 15 mg l^{-1} AdS + 0.1 per cent PVP + 100 mg l^{-1} casein hydrolysate + 50 mg l^{-1} L-glutamine + 250 mg l^{-1} $(NH_4)_2SO_4$ + 0.5 per cent agar. IBA at 0.10 g l^{-1} was most effective for root regeneration.

Plant regeneration via direct and indirect organogenesis of four clones of *Populus deltoides* were investigated by Cavusoglu *et al.* (2011). The 89 M 011 clone gave the highest percentage (100%) of direct organogenesis on WPM + 1 mg l^{-1} zeatine from internode explants. The nodes part of the 89 M 066 clone gave the highest rate of generative callus (100%) on WPM + 2 mg l^{-1} 2,4-D. Indirect shoots were obtained from the node callus on WPM with cytokinins. Roots were formed directly from regenerative shoots which were cultured on WPM or MS containing different ratios of IBA. Rooted seedlings *in vitro* were successfully acclimatized.

2.5.44 *Pterocarpus* species

Pterocarpus santalinus was regenerated *in vitro* using shoot tip explants derived from 20 days old *in vivo* germinated seedlings on 1:1 ratio of sand and soil after treating with GA_3 (Balaraju *et al.* 2011). After 45 days of culture the highest frequency shoot regeneration (83.3%) with maximum number of shoot buds (11) per explant was obtained on MS + 1.0 mg l^{-1} BA + 0.1 mg l^{-1} TDZ. Sixty percent of the shoots produced roots in the medium containing MS + 0.1 mg l^{-1} IBA after 30 days. About 73.33 per cent of the *in vitro* raised plantlets were established successfully in earthen pots.

Somatic embryogenesis (SE) has been achieved from hypocotyl derived callus culture in *Pterocarpus marsupium* (Husain *et al.* 2010). Ninety percent of hypocotyl explants (excised from 12 day old *in vitro* germinated axenic seedlings) produced callus on MS + 5 µM 2,4-D + 1 µM BA. SEs were induced after transfer of callus clumps to MS + BA at 2.0 µM. Subculturing of these embryos

o MS + 0 5 µM BA + 0 1 µM NAA + 10 µM ABA sig ı ficantlv enhanced the maturat on of somat c embryos to early cotyledonary stage Of 30 well developed somatic embryos 16 6 ± 0 33 germ nated and subsequent y converted nto plantlets on ½ MS + 1 0 µM BA The morpholog cally normal plantlets with well developed roots were f rst transferred to / liqu d MS medium for 48 h a ıd then to pots containing autoclaved so lr te and acclimatized n a culture room Thereafter they vere transfer ed to a greenhouse w here 60 per cent of the n surv ved

An *n v o* propagat on protocol for *Pteroca pus ma s p u n* has been developed from nodal explants obta ned from *n vtı o* raised 18 dav old axeı ıc seedl ngs The h ghest shoot regenerat on frequency (85%) max mu n nu nber of multiple shoots (8 6) as well as length (4 8 cm) were induced from nodal explaı ts on MS + 4 0 µM BA + 0 5 µM IAA + 20 µM AdS Root ng was best nduced n m croshoots exc sed from prol ferated shoot cultures o ı sem solid hormone free ½ MS med um after a pulse (d p) treatment for 7 days n / MS l qu d mediun + 100 µM IBA + 15 84 µM phloroglucinol (PG) The *ın v t o* raised plantlets were potted and accl mat zed under culture room cond t ons for 4 weeks before their transfer to a greenhouse where the estabi shed plants showed 75 pe cent survival (Husaın *et al* 2008)

2 5 45 *Saraca asoca*

An eff c e ıt and reproduc ble method of *n v ı ı o* clonal p opagat on through shoot tip nodal and ınter nodal explants of *Saraca asoca* was carr ed out by Subbu and Prabha (2012) BA (0 5 mg l) ınduced a mean of 11 71 ± 0 53 advent t ous shoots from the nodal explants The m cro shoots rooted well on MS med u n supplemented w th 4 0 mg l of IBA 40 per cent of the hardened regenerants were accl mat zed to the so l

2 5 46 *Schleichera oleosa*

Sal a (2013) attempted *n v tro* multipl cat on of axilla y buds n MS + 1 0 mg l BA + 1 0 mg l s lver n trate showed best shoo nitiation Sub cultur ng and elongation of the prol ferated m croshoots were poss ble o n f lter paper br dge soaked n l qu d MS + 0 5 1 0 mg l BA nstead of aga gelled MS n ed a Root ng of he ax llary bud der ved shoots co ntinued o be the n ajo hu d e to achieve success n develop ng m cropropagat on protocol n *S oleosa*

2 5 47 *Semecarpus anacardium*

M cropropagatio n protocol s standardized for *Semeca pus anaca d um* by Panda and Hazra (2012) f om sl oot culture der ved nodal explants n WPM w th TDZ Shoot d fferent at on fron mer stem was l mited Mer stems swelled to form mer stematic mass in h gher concentrat ons of TDZ Harvesting the pr mary shoot leads to appeara nce of add t onal sl oot buds wh ch elongated on repeated transfer of explants in a n ed um devo d of growth regulator every fou weeks Opt mum (17) number of shoots obta ned from each mer stem n explants pre cultured n TDZ 2 27 µM and re cult red n growth regulator free med um for seven cycles (28 weeks) All shoots rooted n the medium w th IBA 2 46 µM Plantlets surv ved on transfe to sand so l (1 1) m xture and accl mat zed

2 5 48 *Simarouba glauca*

Shukla and Padmaja (2013) developed an eff cient m cropropagation protocol from shoot t p and nodal explants of *S ma ouba glauca* Nodal explants appeared to have better regenerat on capac ty than shoot t p explants (40%) in the tested med a The higl est regenerat on frequency and shoot number were obta ned n nodal explants n MS + BA 4 43 µM + NAA 5 36 µM Induced shoot buds were mult pl ed and elongated on the MS + BA (4 44 µM) + NAA (5 36 µM) + TDZ 2 27 µM us ng nodal segments and shoot t p explants respectively WPM +

2 46μM IBA produced tl e ı ax ıu ı ıumber of roots The rooted plantlets were hardened on MS basal l qu d med um and st bsequently n polycups contaı ıng ster le so l and ver n cul te (1 1) a ıd successfully establ sl ed ı pots

2 5 49 *Spondias mangifera*

An eff c ent *n v t o* propagat on s descr bed by T path and Ku aı (2010) for *Spond as nang fe a* us ng nodal explants obta ned f om 4 week old seedlings MS + 1 0 mg l BA vas opt mal for shoot nult p cat on and hıgl est numbeı of shoots (about 10 6) per explants was obta ned after fourth subculture of mother explants n the sa ıe nedıun MS IAA (1 0 mg l) was nost effect ve for root ng of shoots Regenerated plantlets were successfully accl matızed and tıansferred nto so l w th 80 90 per cent survıval ıate

2 5 50 *Steı culıa urens*

Hussa n *et al* (2007) descr bed a protocol for la ge scale mı lt pl cat on of *Stercı l a urens* by *ın v tro* culture of cotyledona y nodes fıo n 15 days old seedl ngs Of the four dıfferent cytokınins (TDZ 2 P Zeat n and AdS) supplemented n MS medıum TDZ at 2 27 μM was most effect ve ı nduc ng bud break (83 0%) E hanced frequency of shoot regenerat on (93 3%) and number of shoots per explant (19 0) were observed by the add t on of ascorb c ac d (0 1%) Shoot prol ferat ıon was ach eved by repeated sub cultur ng the or g nal cotyledonary node on shoot m lt pl cat on medıum (0 45 μM TDZ) after the second harvest of newly formed shoots Root ng was best ınduced (80 0%) on / MS + IBA (9 80 μM)

2 5 51 *Streblus asper*

A m cropropagatıon protocol for *Streblus asper* ıs g ven by Gad dasu *et al* (2011) usıng matı re nodal segments Ind v dual levels of cytok n ns d d not

support *n vitro* sl oot regenerat on n *S aspe* The combinat on of Kinet n (4 60 µM) with BA (4 44 µ M) evoked an opt mum response towards sl oot proliferat on whereas ned um contain ng K net n (4 60 µM) plus TDZ (4 54 µM) nduced multiple shoo format on *In v tro* developed m croshoots we e rooted on ⁄ MS + 2 46 µM IBA The plantlets established *in v t o* were t ansferred to pots conta n ng ster lized so l a id verm cul te (1 1) m xture and were l ardened in the greenhouse v tl 70 75 per cent surv val rate

2 5 52 *Swietenia macı ophylla*

Mass product o ı of plantlets from Juven le of *Sw eten a n ac ophylla* was ach eved through *n v t o* techn que (Mona 2012) The explants cultured on MS full strength induced the h ghest number of shootlets explant and the longest shootlet (cm) The h ghest number of shootlets shootlet length number of leaves as well as fresh a id dry we ghts were obta ned by apply ng 4 0 mg l BA + 0 4 mg l^{1} 2 P The highest root number and root length were ach eved n ½ MS + (2 0 mg l) IBA Among d fferent of so l m xture used for accl mat zation (peat+sand+clay) was effective

Ind rect somat c embryogenesis of *Sw eten a mac ophylla* by us ng mmature cotyledons as nitial expant v as done by Collado *et al* (2010) The use of a sem sol d culture med um composed of MS + 2 4 D (4 0 mg l favoured the formation of callus n the explants The highest percentage of h gh frequency somat c embryogenes s (59 01%) was obtained add ng 1 0 mg l BA n the culture medium Maturat on of so nat c embryos was ıcreased ı s ng 6 0 per cent sucrose The greater percentage of somatic embryos germination (76 17%) was reached n the culture med um w thout growth regulator

2 5 53 *Syzygium* species

Ra idr ama i p ono ia *et al* (2008) establ sl ed a si nple procedu e for ts *n v tro* clonal propagatio i of *Syzyg i n ci n n* usmg nodal stem segn ents fiom *v t o* ra sed 10 week old seedl gs cultured n ½ MS suppleme ited with var ous cytok nins at dıffe ent concentiations a d n comb nat on v itl NAA Effect ve mu tiple shoot format on was o i 4 4 µM BA piovid ng an average of 7 5 shoots per node after s x we ks of c lture The best root ng vas obta ned n med um supplemented with 0 5 µM NAA vitl al most 90 per cent of tl e plantlets developing an average of 5 9 advent t ous roots per shoot More than 70 per cent of the rooted plantlets were successfully establ shed in soil

Mult ple shoot nduction from shoot explants of 1 to 2 year old seedl ngs of w ld trees of *S travancor ci* was observed on MS basal med u n supplemented with WPM m cronutr e its 17 7 µM BA and 1 3 µM NAA (Anand *et al* 1999) A h gh number of mult ple shoots (25 shoots/nodal explant) was observed by the th rd subculture on mult pl catio i medium H gh frequency regeneration of dark brown nodular callus obta ned from *n v tro* grown shoots through ax illary buds was obta ned whe i transfeired to half st ength basal medium Shoots rooted on half strength basal med u n supplemented with 1 1 µM IAA and ere transferred to the f eld with 40% surv val

2 5 54 *Tamarindus indica*

M cropropagat on stud es in *Tama ndus ndica* L was car ied out using cotyledonary nodes of 1 5 cm s ze exc sed from *n v t o* seedlings (Sh nde and Karale 2007) MS + BA 0 2 mg l + NAA 0 1 mg l was the best treat nent for shoot mult plication w th 98 75 per cent shoot induct on and 3 25 shoots/explant Earl est respo se to root ng with h ghest rooting and maximum length of root was observed w th IBA 2 0 mg l and NAA at 2 0 mg l resulted in max mum

number of roots shoot Pla tlets obta ed through th s study were s bjected to hardening n gree house

2 5 55 *Tectona grandis*

A protocol for dnect shoot regenerat on for tea was developed l y shoot t p culture on MS med um (Prasad *et al* 2012) At BA 3 mg l IAA 2 n g l a id K net n 2 ng l cor centration the rate of mult pl cation s l gh vell grown eas ly separable and healthy plantlets The root induct on was observed n NA \ 2 mg l and complete plantlets were harde ed and transferred to green house for establ shed w th a surv val rate of 72 percent

2 5 56 *Terminalia* species

Arumugam and Gop nath (2011) descr bed an efficient protocol for *n v t o* p opagat on of *Te n nal a a juna* by callus regeneration MS med um was found to be the most favorable for callus nduction compared w th LS and B_5 media Max mum number of callus regenerat on was obta ned on MS + 2 4 D 3 0 mg l Shoot and root init at on from callus was favoured in MS + 5 mg l 2 4 D + 0 01 mg l Kinetin + 1 0 mg l GA_3 The rooted shoot plantlet was t ansferred in to small plastic cups conta i ng ster le verm culate sa id and red so l n the rat o of 1 2 2 and were kept n a m st house The regenerated plantlets we e hardened n the greenhouse and successfully transferred n soil w th 87 per cent surv val rate

Phulwar a *et al* (2012b) reported an eff c ent *n v t o* propagation method for *Term nal a catappa* us ng nodal segments of a 15 year old mature tree About 85 per cent of the explant responded with n 15 days of noculat on n MS + 2 0 mg l BA Optimal number of shoots and shoot length were recorded on MS + 0 25 mg l BA + 0 25 mg l K net n About 80 per cent of the shoots treated w th 200 mg l of IBA p oduced *ex v tro* roots w th an average of 2 8 roots per shoot

Nearly 75 per cent of these plantlets could be acclimatized within 5 weeks and successfully established in the field

Phulwaria *et al* (2012a) developed an *in vitro* propagation method for *Terminalia bellirica* from nodal explants of 10 year old mature tree MS medium containing 2.22 µM BA was found to be the best for shoot multiplication in a single step Further enhancement in morphogenetic response occurred when excised shoot clumps (2-3 shoots) were subcultured on MS + 2.22 µM BA + 1.16 µM Kinetin + 0.57 µM IAA ½ MS + 24.60 µM IBA + 100 mg l AC was most effective for root ing of the shoots To reduce labour cost and time an experiment on *ex vitro* root ing was also carried out and it was observed that highest percent shoots rooted *ex vitro* when treated with 2.460 µM IBA for 5 minutes Plantlets rooted *in vitro* as well as *ex vitro* were acclimatized successfully under the greenhouse conditions In comparison to plantlets developed from *in vitro* rooted percent survival of plants those rooted *ex vitro* was significantly higher

Somatic embryogenesis was obtained from cotyledon and mature zygotic embryo callus cultures of *Terminalia chebula* (Anjaneyulu *et al* 2004) Callus cultures of cotyledon and mature zygotic embryo were initiated on induction medium containing MS + 1.0 mg l 2,4 D + 0.01 or 0.1 mg l Kinetin + 30 g l sucrose Embryogenic cotyledon callus with globular somatic embryos was obtained on MS + 50 g l sucrose Globular somatic embryos were observed from mature zygotic embryo callus on induction medium Different stages of somatic embryo development from cotyledon and mature zygotic embryo calluses were observed on MS + 50 g l sucrose after 4 weeks of culture Highest frequency of germination of somatic embryos was obtained on MS + BA (0.5 mg l) + 30 g l sucrose

2 5 57 ***I c ona cilia t i***

Callus format on and plant regenerat on n *Toona c l ata* from *n v t o* propagat on by sing rach s taken fro n young branches of tree They were d sinfected in 0 2ɔ per cent (w v) mercuric chloride solut o for 10 m nutes followed by three r nses n autoclaved d st lled water Tl ey were tl en establ shed n MS + 0 1 mg l TDZ culture medium Nodular calluses were obta ned hav ng good morphoge c character stics Shoots sprouted from s x month old calluses in the dark and plant egeneration was done n the l gh Sl oots were rooted MS + 1 mg l IB \ (Daqu n ɔ *et al* 2005)

2 5 58 ***Vatei i indica***

Ax llary buds collected from seedl ngs of *Vate a nd ca* were cultured under *in v tro* conditions (Devatar and V jayakumar 1997) A few med a comb nat ons cons st ng of full and half strength MS m neral salts as well as WPM with var ous organ c and growth regulator additives were dent f ed as su table for culture establ shment and bud development A nong these ½ MS + 2 ppm 2 iP + 0 1 pp n IBA and var ous growth regulators supported bud break shoot elongation and cont nued growth of shoots Shoots reached 1 2 cm n he ght with 2 3 leaves in 8 weeks under controlled cond t ons w th a 16 hour photoper od and at a temperature of 27°C

2 5 59 ***Wi ghtia tinctoi ia***

Puroh t and Kukda (2004) nduced mult ple shoots *n v tro* on nodal shoot segments of a 30 year old plus tree hav ng enhanced ax lla y branch ng Nodal segments (91%) from young lateral branches produced an average of 5 shoots per node in 3 weeks on agar solid f ed MS + 2 0 mg l BA After establ shment of cultures and n t at on of shoot buds a cluster of shoots nclud ng mother explant was transferred to med um co nta n ng a lower concentrat on of BA (1 0 mg l) A

three fold rate of shoot mult pl cat o ı dur ng every subculture of 3 weeks was achieved Nodal segments fro ı *n v t o* ra sed shoots were also sed to n t ate a new culture cycle The shoots could bc nult pl ed fo at least 24 nonths w tl out loss of v gour Tl e shoots (71%) were successfully rooted when the r lower eı ds were d pped ın pre autoclaved IBA solut on (100 n g l) for 10 m utes followed by the ımpla ıtat oı on mod f ed MS med um (major sa ts reduced to strei gth) conta nıng 200 ·ng l act vated charcoal

Materials and Methods

MATERIALS AND METHODS

Tl e p ese t study t tled *In v t o* p opagat o ı of sandal (*S nt l n albı ı* L) was unde t ken dur ng the year 2011 2013 n the plant t ssue culture laboratoıy College of Forestry Vellanıkkara Thr ssur D str ct Keıala Tl e deta ls of the mat r als used a ıd the tecl nıqı es / methodology e nployed ı tl e exper ment dur ng the course of nvest gat on are d scussed ın th s cl apte

3 1 MATERIALS

3 1 1 Explants

The exp aı ts used the study a e nodal segments conta ı g ax llary buds for d rect organogeı es s and nter ıodal segments and leaves for nd rect organoge es s Fo tl e prel n ary studies to f ıd out the su table surface ster l zatıon metl od effect ve basal medıa as well as tl e best growth regulator co nb nat on explants collected from the 15 20 year old sandal t ees ava lable n the experimental plot of College of Forestry was used This nformat on was used for the *n v tıo* propagat on of ıdent f ed plus trees n the Marayoor Sa dal Reserve Root suckers were collected from the ıdentıf ed plus trees and ra sed n tree nursery of College of Forestry

3 1 1 Cultuı e Medıa

In order to f d out tl e best basal med a for m cıopropagat o of sandal explants were cultured ı MS med um (Murash ge and Skoog 1962) / MS and Woody Plant Med um (Lloyd and McCown 1980) The compos tıons of dıffe e t medıa used are presented n Table 1 The best respond ng med a was selected for further study In order to find out the effect of growth regulators ı the growth of cultures growth regulatoıs such as auxıns (IAA IBA and NAA) and cytok n ns (BA and K net n) were added to the ned a aı d compared w th a control medıa

witl out gtowtl regulators Cytok n ns BA a id K net n alone at concent at ons 0 5 1 2 and 3 mg l and n combi ia on was used to nduce bud break r sandal The corıbination was 0 5 and 1 mg l of BA w th 0 5 1 and 2 mg l of Kii etin Tl e combinat on of cytok n n w th auxin in WPM was also tr ed for bi d break and shoot development from nodal explants BA and Kinetin at 0 5 and 1 ng l along w th IAA IBA and NAA at 0 1 and 0 5 mg l was used D fferent comb nat o of aux ns n ¼ MS and ½ WPM media was used for root ndi ct on For somatic embryo nduct on BA and K netin n concentiat ons of 0 5 1 0 2 0 and 3 0 mg l were used

3 2 METHODS

3 2 1 Preparation of Stock Solution

Stock solutions of mac o and micro nutrients as well as v ta ins were prepared n order to reduce the number of repet t ve operations nvolved n media preparat on and the ci ances of experimental error Concentrated solut ons of each stock were prepared separately by following standard procedures as g ven by Gamborg and Shyluk (1981) For th s required quant t es of tl e chemicals were we ghed accurately and by the add t on of dist lled water che n cals were d ssolved w th constant st rr ng Care was taken wh le the preparat on of on stock s rce t prec pitates readily To avoid th s Na_2EDTA and $FeSO_4\ 7H_2O$ we e dissolved n separate beakers Both beakers were placed on hot plates and brought to the po nt of almost bo l ng Then $FeSO_4\ 7H_2O$ solut on was added slowly to Na_2EDTA over a 15 m nute per od w th constant st r ng The m xture was allowed to cool to oom te nperature and then the volume was made up to required quant ty n a volumetr c flask by add ng d st lled water The stock solut ons were labeled ndicating the stock number and the date of preparat on They are stored n amber coloured bottles under refr gerated cond t on The compos t on of stock solutions of MS and WPM are g ven n Table 2 and 3

3 2 2 Preparation of Culture Media

In order to prepare 500 ml media required quantity of stock solutions (Table 4) were pipetted out in 500 ml beaker Then inositol (50 mg) and sucrose 15 gm were added and dissolved by constant stirring Different plant growth regulators were added to the basal medium as per the requirement The stock solutions of growth regulators at 100 mg/100 ml were prepared and stored in refrigerator and aliquots were taken from stock solution for use After making up of solution to 500 ml the pH was adjusted to 5 8 with the help of digital pH meter by adding 1N NaOH or 1N HCl To this medium 7 5 g l agar was added Boiling of the solution till the froth subsides was necessary of dissolving agar The medium (approximately 15 20 ml per tube) was poured into the well cleaned oven dried culture tubes of 150 mm x 25 mm size The culture tubes were plugged tightly with non absorbant cotton

3 2 3 Sterilization of Culture Media

The medium was sterilized by autoclaving at pressure 1 06 kg cm ^{2} for 20 30 minutes at 121° C After sterilization medium was stored culture room

3 2 4 Sterilization of Equipment

All steel and glass instruments and other accessories were wrapped in aluminium foil and autoclaved at 1 06 kg cm ^{2} pressure for 20 30 minutes at 121° C Forceps scissors etc were flamed at the time of use

3 2 5 Collection and Preparation of Explants

For the axillary bud culture young shoots with 3 4 nodes were taken from the sandal trees using secateurs and brought to the laboratory soon to avoid desiccation The leaves were removed close to the stem without causing any

Table 1 Chemical composition (mg l) of v rious culture media used for *in vitro* propagation of *Santalum album* L

Components	MS Medium	WPM
	(mg l^{-1})	
Ma o mi ents		
NH_4NO_3	1650	400
KNO_3	1900	
KH_2PO_4	170 00	170 00
$MgSO_4$ $2H_2O$	370 00	370 00
K SO_4		990
$CaCl_2$ $2H_2O$	440 00	96 00
Ca(NO) $4H_2O$		556
Na EDTA	37 30	37 30
FeSO $7H_2O$	27 80	27 80
Micro mi e ts		
$MnSO_4$ $4H_2O$	22 30	22 30
$ZnSO_4$ $7H_2O$	8 60	8 60
H_3BO	6 20	6 20
KI	0 83	
Na_2MoO_4 $2H_2O$	0 25	0 25
$CoCl_2$ $6H_2O$	0 025	
$CuSO_4$ $5H_2O$	0 025	0 25
Vitamins		
Nicotin c acid	0 50	0 50
Pyr dox ne HCl	0 50	0 50
Th amine HCl	0 10	0 10
Glycine	2 00	2 00
Others		
Myo Inos tol	100	100
Sucrose	30000	30000
Agar	8000	8000

Table 2 Composition of stock solutions for MS medium

Stock No	Chemicals	Conc of stock	Weight required for 1 liter of stock (mg l^{-1})
I	NH_4NO_3	50 x	82 50 g
	KNO_3		95 00 g
	KH_2PO_4		8 50 g
	$MgSO_4$ $2H_2O$		18 50 g
II	$CaCl_2$ $2H_2O$	50 x	22 00 g
III	Na_2EDTA	100 x	3 73 g
	$FeSO_4$ $7H_2O$		2 78 g
IV	$MnSO_4$ $4H_2O$	100 x	2 23 g
	$ZnSO_4$ $7H_2O$		860 mg
	H_3BO_3		620 mg
	KI		83 mg
	Na_2MoO_4 $2H_2O$		25 mg
	$CoCl_2$ $6H_2O$		2 5 mg
	$CuSO_4$ 5H O		2 5 mg
V	Nicot mc ac d	100 x	50 mg
	Pyridoxine HCl		50 mg
	Thiamine HCl		10 mg
	Glyc ne		200 mg

Table 3 Composition of stock solutions for WPM medium

Stock No	Chemicals	Conc of stock	Weight required for 1 liter of stock (mg l)
I	NH_4NO_3	50 x	49 50 g
	K_2SO_4		20 00 g
	KH_2PO_4		8 50 g
	$MgSO_4\ 2H_2O$		18 50 g
II	$Ca(NO_3)\ 4H\ O$	50 x	27 80 g
III	$CaCl_2\ 2H_2O$	50 x	4 80 g
IV	Na EDTA	100 x	3 73 g
	$FeSO_4\ 7H_2O$		2 78 g
V	$MnSO_4\ 4H_2O$	100 x	2 23 g
	$ZnSO_4\ 7H_2O$		860 mg
	H_3BO_3		620 mg
	$CuSO_4\ 5H_2O$		2 5 mg
	$Na_2MoO_4\ 2H_2O$		2 5 mg
VI	N cot n c ac d	100 x	50 mg
	Pyr doxine HCl		50 mg
	Thiam ne HCl		10 mg
	Glycine		200 mg

Table 4 Quantity of stock solution required for 1 lit media

Stock No	Quantity of stock (ml)		
	MS	½ MS	WPM
I	20	10	20
II	20	10	20
III	10	5	20
IV	10	5	10
V	10	10	10
VI	N I	Nil	10

damage to the buds These stem segments were washed several times under running tap water to remove the dust followed by immersing in frothing solution of liquid detergent (Teepol) for 10 minutes and washed with tap water In order to find out the seasonal variation in fungal contamination explants were cultured without fungicide treatment For controlling the contamination explants were dipped in fungicide solutions of contact fungicide Indofil M 45 (Mancozeb 75 % WP) and systemic fungicide Bavistin (Carbendazim 50 % WP) either singly or in combination at concentrations 0 1 per cent and 0 2 per cent for different durations (15 30 45 60 or 95 minutes) Thereafter explants were rinsed with distilled water and transferred to laminar flow hood for surface sterilization For somatic embryogenesis internodal segments (1 1 5 cm) and leaves of *ex vitro* and *in vitro* shoots were taken For the *ex vitro* explants above mentioned preparation procedures were followed and for *in vitro* no surface sterilization was needed These were cultured in the medium horizontally

3 2 6 Surface Sterilization of Explants

Surface sterilization was done under perfect aseptic condition in a laminar air flow chamber by dipping in $HgCl_2$ (0 05 0 1 0 15 or 0 2 %) solution for 10 minutes Following surface sterilization they were washed three times with sterile water to remove the traces of mercuric chloride There after both exposed ends of the explants were trimmed and the remaining segment was inoculated vertically on the culture medium

3 2 7 Inoculation and Incubation of Explants

Inoculation is done under laminar air flow cabinet Before inoculation petri plates forceps and scalpel were flamed thoroughly Explants were cut into one nodded segments by placing on the sterile tissue paper For inoculation cotton plug of the test tube containing media was removed by placing neatly e flame and its mouth was flamed By using the forceps one explant each was transferred into

the med um Afte flam ng once aga n co ton plug was eplaced mmediately Cultures ere ıc bated under 16 l ours l gl t per od at 25+ ?° C w th a l ght ntens ty of 2000 l x w th proper labe

3 2 8 Rootıng of Shoots

In v tı o regenerated shoots were cultured n / MS and / WPM (w th or without the addition of act vated charcoal) supplemented w th combinations of aux n IBA (0 5 1 1 5 and 2 mg l) witl NAA or IAA (0 1 0 5 1 and 1 5 mg l) *In v t o* shoots were also pulse treated n higher concentrations of IBA and IAA (300 ppm 600 ppm 2ɔ0 ppm and 2500 ppm) and kept for root ng n growth regulator free / MS and /4 WPM All the cultu es were mainta ned under darkness for f rst o e week and then transferred to the ordinary l ght co d tion n culture room

3 3 OBSERVATIONS

Observat ons were taken da ly till leaf ın t ation and tl en weekly for a per od of four weeks The data collected are presented on the bas s of cultures that remained uncontamınated The follow ng observations were recorded for each treatment

3 3 1 Number of Cultures Contamınated

Number of cultures contam nated were counted and expressed as a percentage of total ıu nbeı of cultures

3 3 2 Number of Cultures Showing Bud Break

A culture was sa d to have bu d break when the dormant aux l ary bud has just emerged Number of cultures show ng bud n t at on was expressed as percentages of total nu nber of su rviv ng cultures

3 3 3 Time Taken for Bud Initiation

Number of days taken for bud n t ation was expressed as t me taken for bud n tiation

3 3 4 Number of Explarts Showing Leaf Initiation

Numbers of cultures that produced leaves were expressed as percentage of total surv v ng cultures that produced bud

3 3 5 Time Taken for Leaf Initiation

It s the number of days taken for leaf initiat on

3 3 6 Average Number of Leaves

It is the average of the total number of leaves produced from the number of cultures showing leaf product on

3 3 7 Maximum Number of Leaves

It is the max mum number of leaves produced per explant n a particular treatment

3 3 8 Average Number of Shoots per Culture

Th s is the average of total number of shoots produced n d fferent cultures of a part cular treat nent

3 3 9 Aver age Shoot Length

The average length of shoots expressed in cm from the nu nber of ultures show ng shoot development n a spec f c treatment g ves the average shoot length

3 3 10 Maximum Shoot Length

It s the max mu n length of shoots produced per explant a particula treatment

3 3 11 Number of Cultures Rooted *In Vitro*

Number of cultures rooted *n v tro* was counted and exp essed as percentage of total cultures in a part cular treatment

3 4 ESTABLISHMENT OF CULTURES THROUGH SUBCULTURE

For mainta ning the cultures for longer t me and improving its growth character stics m croshoots were subcultured nto basal med um WPM along w th growth regulator BA or K net n at 0 5 and 1 mg l Shoots were also subcultured nto ned a WPM w th growth regulator comb nat on 0 5 mg l BA+1 mg l K net n 1 mg l BA+0 5 mg l K netin 1 mg l BA+0 5 mg l IAA and 1 mg l K netin+0 5 mg l IAA After 20 days of ma n culture *n v tro* explants produced shoots were taken out carefully w thout any damage For subculture explants were prepared either by exc s ng the shoots s ngly or by trimm ng the basal portion of mother explant tself Subculture was also attempted by cutt ng the elongated

shoots nto segment conta ı ng m nımum of one axıllary bud Medıa used was e ther the n t al medıa tself or med a co ta n ng d ffere ıt growth regulator co nb natıons

3 5 STATISTICAL ANALYSIS

The data recorded were transformed wherever necessary and statıst cally analysed us ng the stat st cal package SPSS The means were compared us ng Duncan s Mult ple Ra ıge Test (Duncan 1955)

Results

RESULTS

The present study on micropropagation of *Santalum album* L was conducted at the tissue culture laboratory of College of Forestry Vellanikkara during 2011 2013 The salient findings of the study are presented in this chapter

4 1 SEASONAL INFLUENCE ON CONTAMINATION OF ASEPTIC CULTURES

In order to find the influence of season on the contamination of *in vitro* cultures of sandal explants were cultured without treating with fungicides The rate of contamination obtained in each month is given in Table 5 There was significant difference between contaminations at various months Cultures during November to April showed no difference in rate of contamination and contamination ranged between 0 11 per cent May and September showed intermediate contamination of 19 and 40 33 per cent respectively During October 47 44 per cent contamination was seen However during June to July more than 90 per cent contamination was recorded

4 2 SURFACE STERILIZATION OF EXPLANTS

Explants were subjected to different surface sterilisation treatments using fungicides and $HgCl_2$ to prevent culture contamination by fungus and bacteria In order to control the fungal contamination systemic and contact fungicide Carbendazim 50% WP (Bavistin) and Indofil M 45 (Mancozeb 75% WP) respectively were used While testing the effect of fungicides the concentration of $HgCl_2$ kept constant and vice versa

Fungicide concentration and treatment time varied according to the contamination rate in the months During the months with low contamination (November April) treating with either 0 1 per cent Carbendazim 50% WP or

Seasonal influence on fungal contamination in axillary bud culture of *Santalum album*

Month	Fungal contamination
January	6 55 (37 10)
February	0 00 (0 00)
March	2 00 (11 32)
Apr l	7 67 (43 4ɔ)
May	19 00 [b] (108 70)
June	98 00 [d] (822 97)
July	9ɔ 93 [d] (773 05)
August	65 33 (408 39)
September	40 33 [b] (238 69)
October	47 44 (288 05)
November	11 00 (62 61)
December	10 17 (57 79)
SEm+	9521 47
F	26 5ɔ*

ficant at 5%

s with same superscript do not differ significantly

Table 6 Effect of concentration of systemic and contact fungicides Bavistin (Carbendazim 50% WP) and Indofil M 45 (Mancozeb 75% WP) on controlling fungal contamination on months with low contamination (November April)

Treatments	Fungal contamination (%)	Cultures dead (%)
NO	11 33 (3 30)	4 00 [b] (1 63)
0 1 % Indofil 15 m nutes	6 00[b] (2 45)	7 67 [b] (2 74)
0 1 % Indofil 30 m nutes	6 00[b] (2 45)	11 33[b] (3 30)
0 1 % Indofil 45 m nutes	0 00 (0 00)	16 67 (4 04)
0 1 % Bavistin 15 m nutes	7 67[b] (2 19)	11 33[b] (3 30)
0 1 % Bavistin 30 m nutes	2 00 [b] (0 82)	4 00 [b] (1 63)
0 1 % Bavistin 45 m nutes	0 00 (0 00)	2 00 (0 82)
SEm+	1 00	1 16
F	5 20*	3 46*

*Significant at 5%
Figures in parenthesis are square root transformed values
Figures with same superscript do not differ significantly

Mancozeb 75% WP for 45 minutes completely controlled contamination (Table 6) while explants which were not treated with fungicides showed 11 33 per cent fungal contamination Treating with 0 1 per cent Mancozeb 75% WP for 45 minutes showed highest percentage of dead cultures (16 67) Treatment involving 0 1 per cent Carbendazim 50% WP for 45 minutes showed less percentage of dead cultures (2%)

In the months with medium contamination rate (May August and September) treating with 0 1 per cent Carbendazim 50% WP for 45 minutes was not effective (Table 7) in controlling contamination (27 33 %) In this situation various combinations of fungicides were tried Observations indicated a significant difference in contamination rate However there was no difference in percentage of dead cultures Among them 0 2% Carbendazim 50% WP + 0 1% Mancozeb 75% WP for 45 minutes completely suppressed contamination compared to control (27 33) and was followed by 0 1 % Carbendazim 50% WP + (0 1) Mancozeb 75% WP for 45 minutes (5 67 %) But when treatment 0 1 % Carbendazim 50% WP + 0 2 % Mancozeb 75% WP for 45 minutes was done contamination rate was high (16 67 %)

During June July treating with (0 2%) Carbendazim 50% WP + (0 1%) Mancozeb 75% WP for 45 minutes was not at all effective to control contamination (Table 8) During this period treatments with higher concentration of fungicides and more treatment time was effective Observations showed significant difference in contamination rate and 0 2% Carbendazim 50% WP + 0 2 % Mancozeb 75% WP for 95 minutes was effective in controlling contamination up to 27 33 per cent This was followed by treating for 60 minutes with 55 33 per cent contamination Fungicide treatment for 95 minutes showed a significant variation in percentage cultures dead and was recorded with a higher (73 67 %) percentage culture death Other treatments did not show significant variation in culture death

Table 7 **Effect of concentration of systemic and contact fungicides Bavist n (Carbend ızım 50% WP) and Indofil M 45 (Mancozeb 75% WP) on controlling fungal contamination in months with medium contamination (August and September)**

Treatments	Fungal contamination (%)	Cultures dead (%)
0 1 % Bav st n 45 m nutes	27 33 (156 80)	2 00 (11 32)
0 1 % Bav st n+0 1 % Indof l 45 n nutes	5 67^{ab} (32 21)	16 67 (95 52)
0 1 % Bav stın+0 2 % l ıdofil 45 m n tes	16 67^{b} (94 98)	2 00 (11 ɔ2)
0 2 % Bav st +0 1 % Indof l 45 m nutes	0 00 (0 00)	13 00 (73 78)
SE n±	1739 32	2023 12
F	8 33*	2 41^{NS}

*Sıgnificant at 5%
Figures n parenthes s are arc s ne transformed values
F gures w th sa ne superscıipt do not d ffer sign f cantly

Table 8 Effect of concentration of systemic and contact fungicides Bavistin (Carbendazim 50% WP) and Indofil M 45 (Mancozeb 75% WP) in controlling fungal contamination on months with high contamination (June July)

Treatments	Fungal contamination (%)	Cultures dead (%)
0 2 % Bav st n +0 1 % Indof l 45 m nutes	100 00[d] (888 62)	2 00[a] (11 32)
0 2 % Bavistin +0 2 % Indofil 30 m nutes	71 67 (453 22)	4 00 (22 64)
0 2 °o Bav stin +0 2 % Indofil 60 n nutes	55 33[b] (336 22)	15 00 (74 74)
0 2 % Bav st n +0 2 % Indof l 95 m nutes	27 33[a] (156 62)	75 67[b] (472 13)
SEm+	3228 17	4157 45
F	90 18*	34 83*

*Sign f cant at 5°o
Figures in parenthesis are arc s ne transformed values
Figures with same superscr pt do not d ffer s gn ficantly

I orde to control the bacte ı al contam natio ı su face ste ılisatio ı of explants was done using $HgCl_2$ for 10 n nutes at d ffe e t concentrat ons (0 05 0 1 0 15 a ıd 0 ? %) (Table 9) S gn ˜ca t d fference the contam at on rate was obse ved a ıd tl e low est rate (2 67 %) wa fou d wl en 0 2 pe cent HgCl was used Th s vas followed by 9 33 per cent ı 0 15% $HgCl_2$ H gl est percentage (39 00 %) of onta n nat on was recorded wl en expla ts were tı eated w th 0 1 per ce ıt $HgCl_2$ for 10 ıı utes There was sıgnificant dıfference n pei centage of dead cultures a d ı 0 2 per cent for 10 m nutes 20 33 per cent cultures were dead It was followed by 0 15 per cent w th co tam nat on 9 33 pei cent and culture deatl 7 16 per cei t Hence the 0 15% of $HgCl_2$ t eat nent fo 10 minutes was taken as super or surface ster l zat on method for further exper ments

4 3 EFFECT OF DIFFERENT BASAL MEDIA ON CULTURE ESTABLISHMENT AND GROWTH OF AXILLARY BUDS OF SANDAL

In the present study three basal media na nely MS ½ MS and WPM were used for culture establishment n axıllary buds of *Santalum album* The results obta ned are presented n Table 10 There was a s gn fica t d fference among the med a w th respect to max mum number of shoots average sl oot length max mu n shoot length a d average number of leaves

Med a did not nfluence bud break a ıd leaf ın t at on n sandal Bud break percentage obta ned ın WPM was 100 per cent and 98 pei cent n MS and / MS All the three media showed 100 per cent shoot and leaf n t at on Number of days taken for bud break and leaf n t at on also were not nfluenced by med a Explants cultured n WPM med um ınduced bud break ın 5 38 days wh le ıt was 5 90 5 91 days respect vely n MS and / MS Cultures n MS med um showed leaf nıtıat on ın 8 81 days followed by WPM n 8 92 days and ½ MS ın 9 95 days

Table 9 Effect of concentration of $HgCl_2$ on controlling bacterial contamination

Treatments	Bacterial contamination (%)	Cultures dead (%)
0 05% 10m n	29 33[b] (169 57)	2 00 (0 82)
0 1% 10m n	39 00 (229 02)	5 67[a] (1 92)
0 15% 10m n	9 3ɔ [b] (53 15)	7 66[ab] (2 73)
0 2% 10mm	2 67[a] (15 10)	20 33[b] (4 50)
SEm±	4836 95	1 33
F	6 16*	5 44*

*Signif cant at 5%
Figures n parenthesis are arc sine transformed values
F gures w th same s perscript do not d ffer s gn f cantly

Table 10. Effect of different basal media on culture establishment and growth in axillary bud culture of *Santalum album*

Basal Media	Bud break		Shoot initiation (%)	No of shoots		Shoot length (cm)		Leaf initiation		No. of leaves	
	%	Days		Avg	Max	Avg	Max	%	Days	Avg	Max
MS	98.00 (9.90)	5.90	100.00	2.22	3.33^{b}	0.85^{a}	1.20^{a}	100.00	8.82	6.11^{a}	8.67
½ MS	98.00 (9.90)	5.91	100.00	1.83	2.00^{a}	1.06^{b}	1.77^{b}	100.00	9.95	7.39^{b}	10.00
WPM	100.00 (10.00)	5.38	100.00	1.97	2.67^{ab}	1.20^{c}	1.77^{b}	100.00	8.92	7.52^{b}	10.00
SEm±	0.02	0.37	-	0.03	0.22	0.01	0.05	-	1.70	0.29	0.44
F	0.50^{NS}	0.74^{NS}	-	3.79^{NS}	6.00*	19.92*	6.15*	-	0.70^{NS}	6.28*	4.00^{NS}

*Significant at 5%
Figures in parenthesis are transformed values
Figures with same superscript do not differ significantly

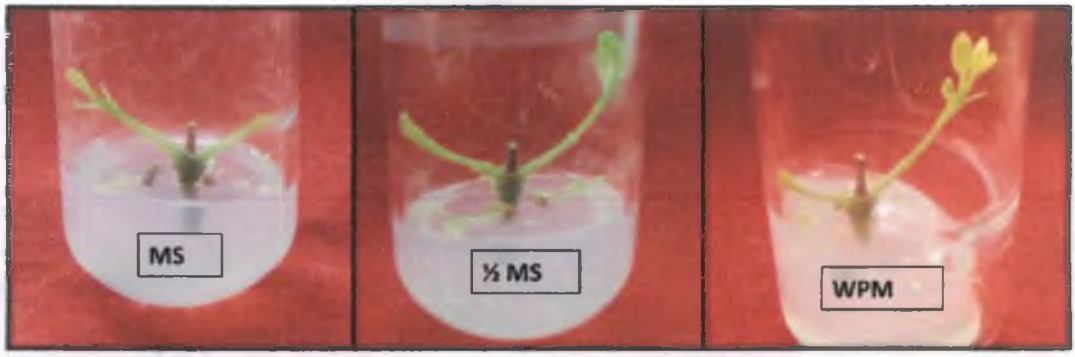

Plate 1. Difference in sandal culture response to MS, ½ MS and WPM basal media

Plate 2. Difference in response of sandal cultures in 1 mg l^{-1} BA and 1 mg l^{-1} Kinetin

Average number of shoots recorded in MS was 2.22 followed by 2.03 in WPM and 1.83 in ½ MS. Maximum numbers of shoot production as well as average and maximum shoot length was significantly different among the media (Fig. 1a and 1b). Highest number of shoots was produced in MS (3.33) followed by WPM (2.67) and minimum number of shoots were produced in ½ MS medium. There was a significant difference in average of shoot lengths produced in the three media. The highest average shoot length was in WPM (1.20 cm) and followed by 1.06 cm in ½ MS. The lowest average shoot length was obtained in MS medium (0.85 cm). Highest shoot length observed in WPM and ½ MS was on par (1.77 cm). But MS showed least maximum shoot length (1.20 cm).

Average number of leaves produced in MS medium was significantly different from ½ MS and WPM with 6.11 leaves. WPM produced highest number of average leaves (7.52) and was on par with ½ MS (7.39). Maximum number of leaves produced was 10 each in WPM and ½ MS; lowest in MS (8.67). However, these differences were not statistically significant.

Cultures in MS medium were found to be stunted, while both the ½ MS and WPM showed elongated shoots. However, leaf fall was heavy in ½ MS medium and cultures in WPM were noted as healthy compared to that in ½ MS. By considering these facts and comparing the growth of cultures in the three media, WPM was taken as superior to MS and ½ MS media for the *in vitro* propagation of *Santalum album*.

4.4 EFFECT OF PLANT GROWTH REGULATORS ON CULTURE ESTABLISHMENT AND GROWTH

The basal media WPM was supplemented with different cytokinins and auxins at various concentrations either singly or in combination to evaluate the best growth regulator combination for maximum culture establishment and growth in *Santalum album*.

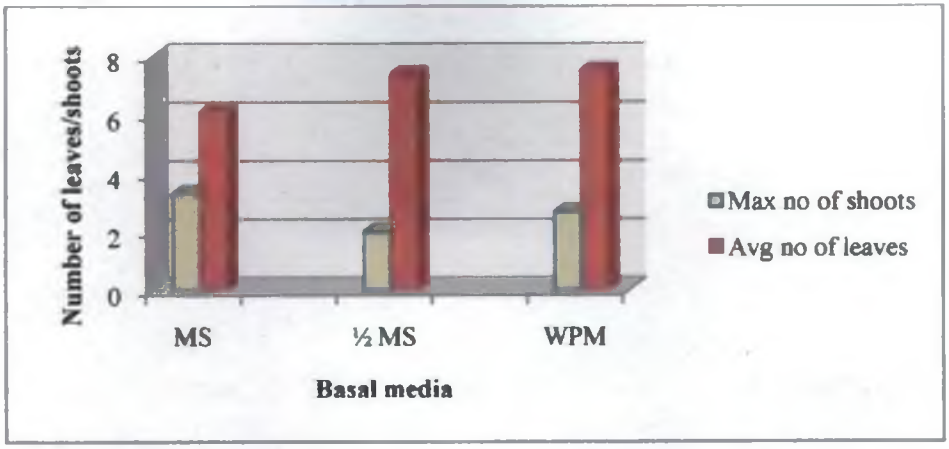

Fig. 1a. Effect of basal media on maximum number of shoots and average number of leaves produced in sandal cultures.

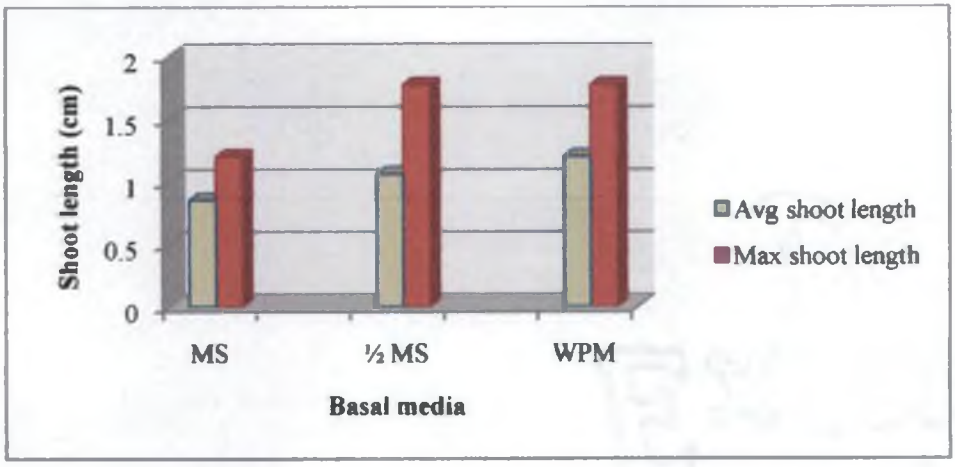

Fig. 1b. Effect of basal media on average and maximum shoot length in sandal cultures.

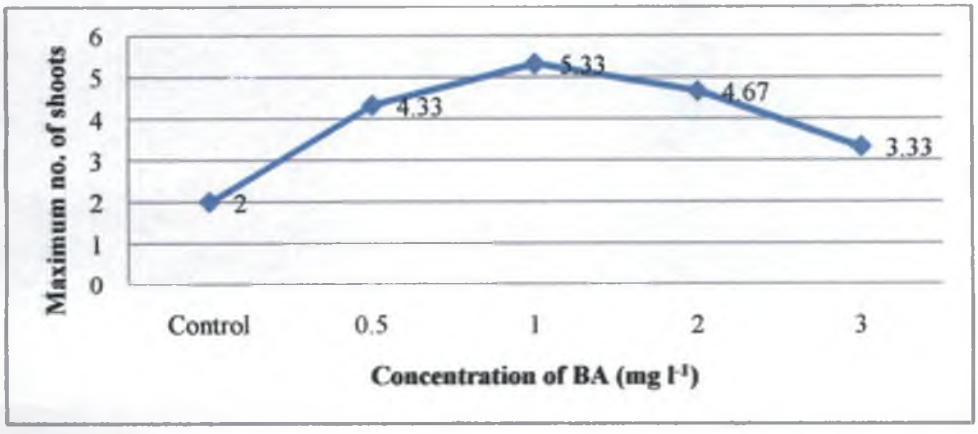

Fig. 2a. Effect of BA on maximum number of shoots produced in sandal cultures.

4 4 1 Effect of BA on Culture Establishment and Growth in WPM Medium

In th s study WPM nedium was supplemented w th four d fferent concentrat ons of BA (0 5 1 2 and 3 mg l ^{1}) The effect of var ou s concen rat ons of BA on growth parameters of sandal are presented n Table 11 The t eatments d d not influe ice ci aracters l ke bud break shoot n t at on ave age iumbe of shoots leaf n t at o and number of leaves It had nflue ice only naximum numbe of shoots ai d shoot length

P esence of BA d d no influe ice bud break shoot ı at o and leaf n t at o in sandal Bud break was 100 per cent in all the treatments except n l mg l BA (98 %) Nu nber of cultures with shoot n t ation was 100 per cent n 2 mg l BA In 0 5 mg l BA leaf nit at on was 98 per cent in l mg l BA 96 per cent and 94 per cent ı 3 mg l BA In all the treatments leaf n tiat on was 100 per cent

Treatments did not nfluence days taken for bud break as well as for leaf nit at on Among the treatments cultures in 1 mg l BA sl owed bud break in 5 75 days while in control t vas 6 11 days Number of days taken for bud break was as follows n otl er treatments 6 19 days (0 5 mg l BA) 6 62 days (? mg l BA) and 7 13 days (3 mg l BA) Days taken for leaf ı t at o ranged f om 8 99 days ı l mg l BA to 10 29 days n 3 mg l BA

Average number of shoots produced was not nfluenced by tl e media while the maximum number of shoots produced sho ved a s gn f cant d fference among treatments (Fig 2a) All the treatments were super or to control with 2 00 shoots Shoot production in 1 mg l BA (5 33) 0 5 mg l BA (4 33) and 2 mg l BA (4 67) were on par with each other Least number of shoots (3 33) was recorded ı 3 mg l BA

Table 11 Effect of BA on bud break and shoot development in axillary bud cultures of *Santalum album* in WPM media

Conc of BA (mg l)	Bud break		Shoot initiation	No of shoots		Shoot length (cm)		Leaf initiation		No of leaves	
	%	Days	(%)	Avg	Max	Avg	Max	%	Days	Avg	Max
Control	100 00 (10 00	6 11	76 3 (562 60)	1 77	2 00	0 77^{b}	1 37^{b}	100	12 52	4 57	8 67
0 5	100 00 (10 00	6 19	98 00 (822 97)	3 17	4 33^{b}	0 58^{ab}	0 93^{ab}	100	9 13	4 86	6 67
1	98 00 (9 90)	5 75	96 00 (757 31)	3 27	5 33^{b}	0 44^{a}	0 83	100	8 99	2 97	4 00
2	100 00 (10 00	6 62	100 00 (888 62)	2 98	4 67^{b}	0 38	0 60^{a}	100	9 10	2 82	4 00
3	100 00 (10 00	7 13	94 00 (729 64)	2 56	3 33^{ab}	0 33^{a}	0 57^{a}	100	10 29	2 13	4 67
SE m+	0 01	2 47	26054 3	0 37	1 47	0 02	0 07		4 00	5 78	11 20
F	1 00^{NS}	0 34^{NS}	1 70^{NS}	3 03^{NS}	3 46*	4 29*	4 55*		1 69^{NS}	0 73^{NS}	1 11^{NS}

*Sign f cant at 5%

Figures n pare thes s are square transformed values

F gures w th same superscr pt do not d ffer signif cantly

There was significant difference between the treatments with respect to average and maximum length of shoots (Fig 2b) All the treatments were inferior to control The highest average shoot length was obtained in 0 5 mg l BA (0 58 cm) While average shoots length was on par with each other in 1 mg l BA (0 44 cm) 2 mg l BA (0 8 cm) and 3 mg l BA 0 35 cm) Similar trend was observed in maximum shoot length Highest shoot length in the treatments was obtained in 0 5 mg l BA (1 9 cm) and lowest in 3 mg l BA (0 cm) which was on par with 1 mg l BA 0 85 cm) 2 mg l BA (0 70 cm)

Average and maximum number of leaves produced in the treatments was not influenced by treatments with BA Average number of leaves ranged from 4 86 in 0 5 mg l BA and 13 leaves in 3 mg l BA Maximum number of leaves was between 6 67 (0 mg l BA) and 4 00 (1 mg l BA and 2 mg l BA)

It was observed that increasing the concentration of BA beyond 1 mg l resulted the stunted growth of culture and poor leaf development

4 2 2 Effect of Kinetin on Culture Establishment and Growth in WPM Medium

In order to find out the effect of Kinetin on culture establishment and growth of sandal WPM medium was supplemented with four different concentrations of Kinetin (0 5 1 2 and 3 mg l) The effect of Kinetin on various growth parameters is given in Table 12 The various treatments based on kinetin did not influence the parameters under study except for average number of shoot production All the treatments showed 100 per cent bud break and leaf initiation where as only 2 mg l Kinetin showed 100 per cent shoot initiation Percentage shoot initiation in 1 mg l Kinetin and 3 mg l Kinetin was 98 and 0 5 mg l Kinetin showed 95 67 per cent

Table 12 Effect of Kinetin on bud break and shoot development in axillary bud cultures of *Santalum album* in WPM media

Cone of BA (mg l^{-1})	Bud break		Shoot initiation (%)	No of shoots		Shoot length (cm)		Leaf initiation		No of leaves	
	%	Days		Avg	Max	Avg	Max	%	Days	Avg	Max
Control	100	6 11	76 33 (562 60)	1 77^{a}	2 00	0 77	1 57	100	12 52	4 57	8 67
0 5	100	6 92	95 67 (721 47)	1 99^{ab}	3 00	0 75	1 43	100	10 22	6 00	10 00
1	100	6 44	98 00 (775 29)	2 12^{ab}	3 67	0 72	1 40	100	10 09	5 59	10 00
2	100	5 67	100 00 (888 62)	2 34^{b}	4 00	0 64	1 37	100	9 09	5 04	8 00
3	100	5 92	98 00 (775 29)	2 41^{b}	3 67	0 51	1 27	100	9 96	4 02	8 00
SEm+		0 51	247 51 07	0 06	0 73	0 07	0 07		4 20	3 63	1 87
F		1 41^{NS}	1 91^{NS}	3 49*	2 59^{NS}	0 52^{NS}	0 18^{NS}		1 17^{NS}	0 51^{NS}	1 64^{NS}

*Significant at 5%

Figures in parenthesis are transformed values

Figures with same superscript do not differ significantly

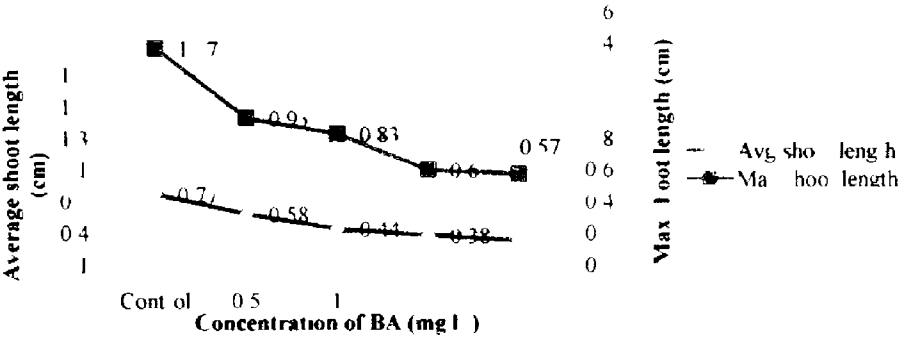

Fig 2b Effect of BA on maximum and average shoots length in sandal cultures

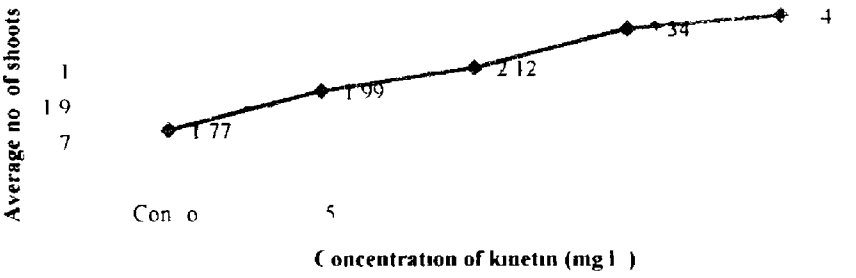

Fig 3 Effect of KIN on average number of shoot in sandal cultures

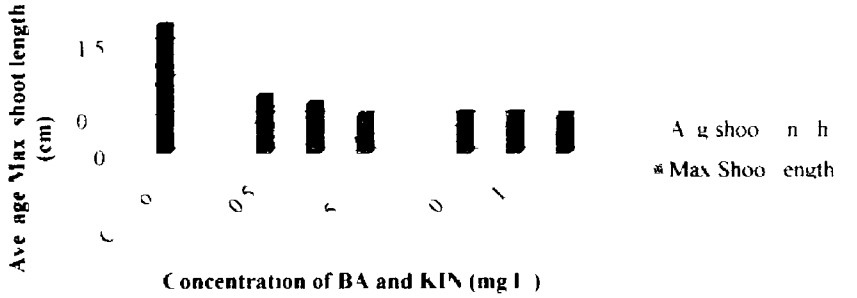

Fig 4a. Effect of combination of BA and KIN on average and maximum shoot length in sandal cultures

T me taken foı bud n t at o vas 5 67 days (2 ng l K ıet) to 6 92 days n 0 5 mg l^{1} K net n 2 mg l K netın sl owed leaf n t at on n 9 09 days and t was 10 22 days n 0 5 n g l K ıet n

All the trea ments were effect ve to nduce mult ple shoots co npared to control (1 77) Ave age ı umber of shoots was highest n 3 mg l K net n (2 41) and was on par w th 3 n g l Kıı etın (F g 3) Least number (1 99) of shoots was observed ı 0 5 ng l Kınet n and was on par w th 1 mg l K net n Maxımum nu nber of shoot nduced were ranged from 4 (2 mg l K netın) to 2 (0 5 mg l K net n) The average and max mum shoot length was 0 75 cm and 1 43 cm n 0 5 n g l Kıı etın and 0 51 c n and 1 27 cm ı ı 3 ng l K net n repet vely Average and max num number of leaves produced was 6 and 10 n 0 5 ng l K netın and 4 02 and 8 n 3 mg l K netın

4 4 3 Combıned effect of BA and Kınetın on Culture Establishment and Growth ın WPM Medıum

In order to f nd out the effect of comb natıon of cytok n ns (BA and K net n) on growth BA at two levels (0 5 and 1 0 mg l) w th K net n at three levels (0 5 1 0 and 2 0 mg l) n all poss ble combınat ons were supplemented to WPM media Results obta ned were presented n Table 13 K net n had no nfluence on growth parameters except for shoot length and maxımu n number of leaves

All comb nat ons w th 1 mg l BA ınduced bud break n all the cultures (100 %) It was followed by 0 5 mg l BA + 0 5 mg l K net n and 0 5 mg l BA + 2 mg l Kınetın (98 %) Bud break percentage ın 0 5 mg l BA + 1 mg l K net n was 96 33 However only 1 ng l BA + 1 mg l Kınet n showed 100 per cent shoot n t at on and t was reduced to 97 67 per cent and 96 00 n 1 mg l BA + 2 ng l Kınet n and 0 5 mg l BA + 2 mg l K netın respect vely In the case

Table 13 Effect of BA+ Kinetin on bud break and shoot development in axillary bud cultures of *Santalum album* in WPM media

Conc of BA+KIN (mg l^{-1})	Bud break		Shoot initiation (%)	No of shoots		Shoot length (cm)		Leaf initiation		No of leaves	
	%	Days		Avg	Max	Avg	Max	%	Days	Avg	Max
Control	100 00 (10 00)	5 38^{a}	100 00 (10 00)	1 97	2 67	1 20^{b}	1 77^{b}	100 00 (10 00)	8 92	7 52	10 00
0 5BA+0 5KIN	98 00 (9 90)	6 00	94 33 (9 70)	2 51	3 67	0 42	0 80^{a}	100 00 (10 00)	9 59	3 48	4 67
0 5 BA+1KIN	96 33 (9 81)	5 04	94 00 (9 69)	3 08	4 67	0 48^{a}	0 70^{a}	100 00 (10 00)	9 41	3 47	5 33
0 5BA+2KIN	98 00 (9 90)	5 76	96 00 (9 79)	1 90	2 33	0 ?	0 5ɔ^{a}	96 00 (9 79)	10 31	2 00	4 00
1BA+0 5KIN	100 00 (10 00)	5 95	95 67 (9 78)	3 8ɔ	5 33	0 36	0 57^{a}	98 00 (9 90)	10 76	3 16	5 3ɔ
1BA+1KIN	100 00 (10 00)	5 34	100 00 (10 00)	1 96	3 67	0 35	0 57^{a}	100 00 (10 00)	9 97	42	5 00
1BA+2KIN	100 00 (10 00)	5 44	97 67 (9 88)	3 56	4 67	0 2ɔ^{a}	0 53^{a}	100 00 (10 00)	9 54	ɔ 47	5 3
SEm+	0 02	0 95	0 13	2 75	3 05	0 04	0 06	0 02	4 99	ɔ 74	4 05
F	0 71^{NS}	0 4^{NS}	0 40^{NS}	0 70^{NS}	1 20^{NS}	5 92*	9 56*	0 87^{NS}	0 23^{NS}	2 40^{NS}	2 88*

*S gnificant at 5%

F gures n parenthes s are square transformed values

of leaf n t ation all tieatments except 0 5 ng l BA + 2 mg l Kinet n (96 00 %) and 1 ng l BA + 0 5 mg l K netii (98 %) showed 100 per cent leaf n tiat on

Bud bieak was prolonged between 5 04 days (0 5 mg l BA + 1 mg l K neti) and 6 days (0 5 ng l BA + 0 ɔ mg l K net n) Wl le leaf n at on was observed n 9 11 days (0 ɔ mg l BA + 1 ng l K net n) and lasted till 10 76 days n 1 mg l BA 0 5 mg l Kine n Average iumb i of shoot piouction was recorded n 1 mg l BA + 0 ɔ ng l K netin (3 83) and it was 1 90 n 0 5 mg l BA + 2 mg l K net n Max mum number of shoots produced ranged from 5 33 n 1 mg l BA + 0 5 mg l K net n to 2 33 in 0 5 mg l BA + ? mg l K net n

In the case of shoot length there was s gnif cant d fference between the treatments a id control bu not among themselves (F g 4a) For botl average and maximum shoot length control showed h ghest le igth (1 20 and 1 77 respect vely) WPM fort f ed with 0 5 mg l BA + 1 mg l KIN exh b ted a shoot length of 0 48 cm followed by 0 5 mg l mg l BAP + 0 5 mg l KIN (0 42 cm) While n 0 5 mg l BA + 2 mg l Kinet n the average shoot length was 0 21 cm In the med a containing 0 5 mg l BA + 0 5 mg l K netin maximum shoot length was 0 80 cm which was followed by 0 5 mg l BA + 1 mg l Kinet n w th 0 70 cm However n 0 5 mg l BA + 2 mg l Kinetin and 1 mg l BA + 2 ng l Kinetin t was 0 53 cm

Average leaf production ranged between 2 00 (0 5 mg l BA + 2 mg l K netin) to 3 48 (0 5 mg l BA + 0 5 mg l K net n) (Fig 4b) Ti e maximum number of leaves was 5 33 n 0 5 mg l BA + 1 mg l Kinet n 1 mg l BA + 0 5 mg l K netin and 1 mg l BA + 2 mg l Kinet n Wh le n 0 5 mg l BA + 2 mg l K netin max mum number of leaves was 4 00

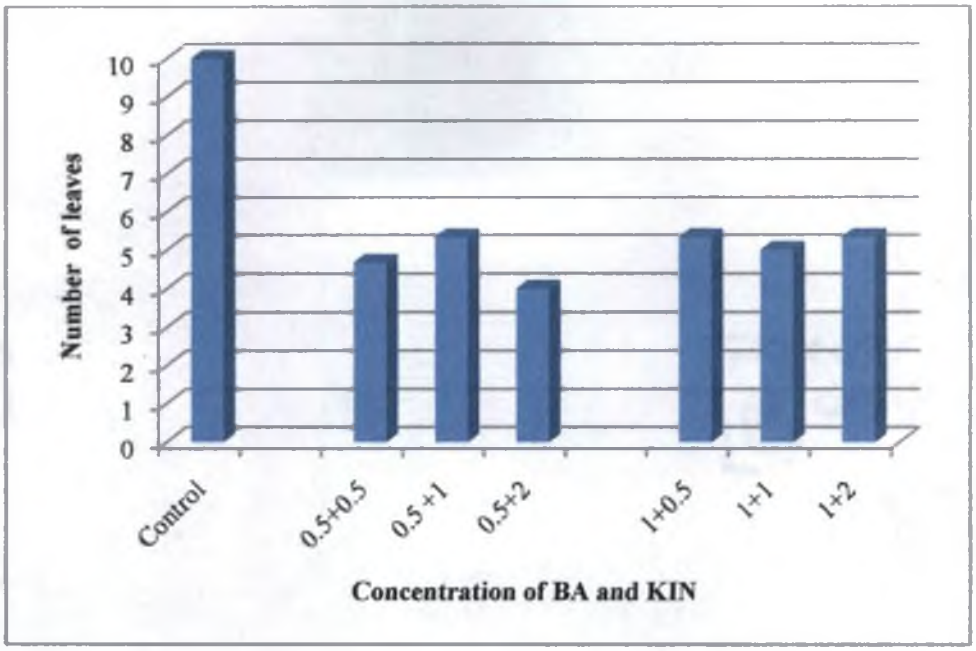

Fig. 4b. Effect of combination of BA and KIN on maximum number of leaves in sandal cultures.

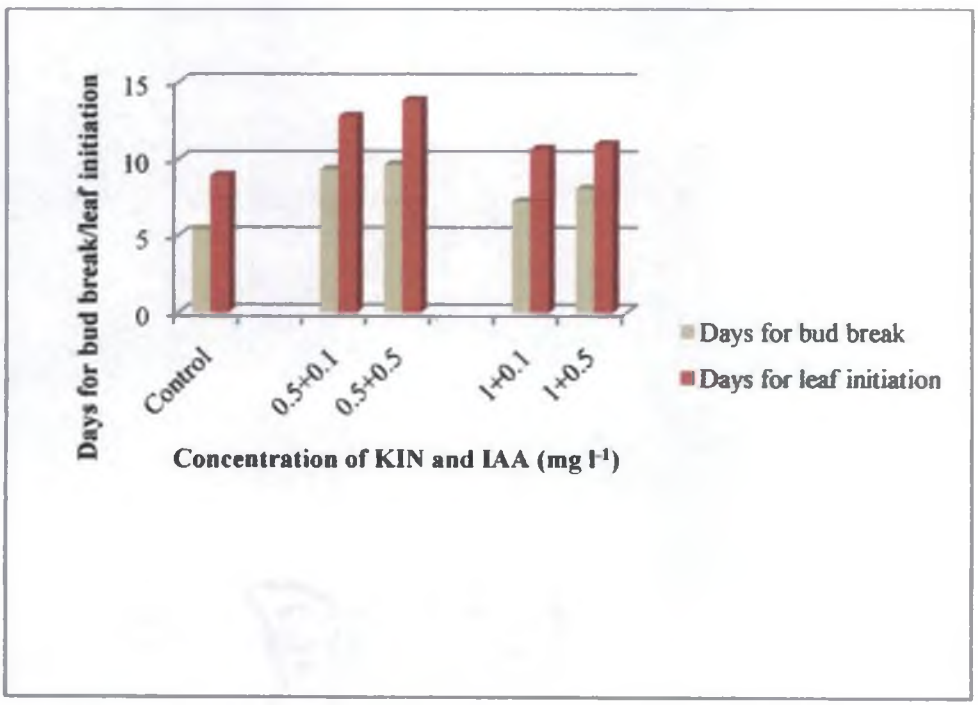

Fig. 5a. Effect of combination of KIN and IAA on days taken for bud break and leaf initiation in sandal cultures.

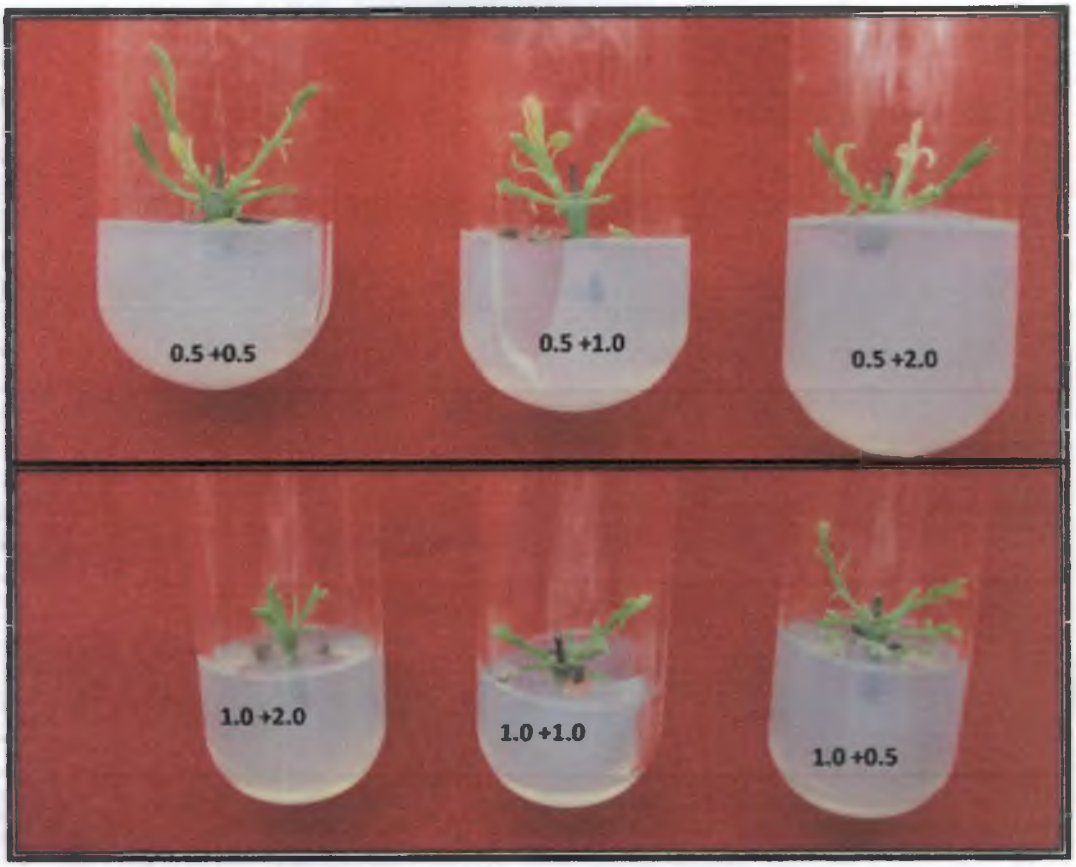

Plate 3. Difference in response of sandal cultures in WPM containing combination of BA and Kinetin at various concentrations

Plate 4. Effect of increasing the concentration of auxins in WPM containing 0.5 mg l^{-1} BA in the growth of sandal cultures

4 4 4 Effect of combination of Kinetin and IAA on Culture Establishment and Growth in WPM Medium

All possible combinations of two concentrations of K netin (0 5 and 1 mg l) with two concentrations of IAA (0 1 mg l and 0 5 mg l) were tried to find their effect on culture growth Results are presented on Table 14 There was significant difference in days taken for bud break average and maximum number of shoots max num shoot length days taken for leaf initiation and average number of leaves

Combinations of K netin and IAA had no effect on percentage bud break shoot initiation and leaf initiation Percentage bud break was 100 per cent in 1 mg l Kinetin + 0 1 mg l IAA (100 %) and was followed by 1 mg l K netin + 0 5 mg l IAA (98 %) 0 5 mg l Kinetin + 0 1 mg l IAA (95 %) All the treatments showed 100 per cent shoot initiation Leaf initiation was 100 per cent in medium containing 0 5 mg l IAA

Days taken for bud break and leaf initiation was significantly different among treatments (Fig 5a) All the treatments showed delayed bud break and leaf initiation than the control where bud break and leaf init at on was occurred in 5 38 and 8 92 days respectively Among the treatments 0 5 mg l K net n+0 5 mg l IAA showed bud break in minimum days (7 75) followed by 1 mg l Kinetin + 0 1 mg l IAA (8 09 days) and 0 5 mg l Kinetin + 0 5 mg l IAA (9 65 days) Delayed bud break was observed in 0 5 mg l K netin + 0 1 mg l IAA (9 33 days) In 1 mg l K etin + 0 1 mg l IAA early leaf initiation was observed in 10 65 days followed by 1 mg l K netin + 0 5 mg l IAA (10 96 days) and 0 5 mg l Kinetin + 0 1 mg l IAA (12 77 days) Maximum number of days for leaf initiation was in 0 5 mg l Kinetin + 0 5 mg l IAA (13 80 days)

There was significant difference in the number of shoots produced (Fig 5b) 1 mg l Kinetin + 0 1 mg l IAA showed highest average number of shoots

Table 14 Effect of Kinetin + IAA on bud break and shoot development in axillary bud cultures of *Santalum album* in WPM media

Conc of KIN+IAA (mg l^{-1})	Bud break		Shoot initiation (%)	No of shoots		Shoot length (cm)		Leaf initiation		No of leaves	
	%	Days		Avg	Max	Avg	Max	%	Days	Avg	Max
Control	100 00 (10 00)	5 58^{a}	100	1 97^{ab}	2 67^{ab}	1 20	1 77^{b}	100 00 (10 00)	8 92^{a}	7 52^{b}	10 00
0 5KIN+0 1IAA	95 00 (9 75)	9 33^{d}	100	1 92^{a}	2 33	0 84	1 1	96 00 (9 79)	12 77^{d}	5 81^{a}	8 00
0 5KIN+0 5IAA	96 00 (9 79)	9 65^{d}	100	2 07^{b}	2 33^{a}	0 89	0 97	100 00 (10 00)	13 80^{d}	5 97^{a}	6 67
1KIN+0 1IAA	100 00 (10 00)	7 25^{b}	100	2 65	3 66	0 77	1 23^{a}	98 00 (9 90)	10 65^{ab}	7 6^{b}	8 67
1KIN+0 5IAA	98 00 (9 90)	8 09^{b}	100	2 54^{b}	3 33^{ab}	0 80	1 27^{a}	100 00 (10 00)	10 96^{b}	7 93^{b}	9 33
SEm+	0 04	0 52		0 10	0 33	0 01	0 05	0 03	1 06	0 44	1 6
F	0 98^{NS}	17 07*		3 44*	3 30*	2 92^{NS}	5 25*	0 80^{NS}	10 28*	6 92*	3 08^{NS}

*Significant at 5%

Figures in parentheses are square root transformed values

Figures with same superscript do not differ significantly

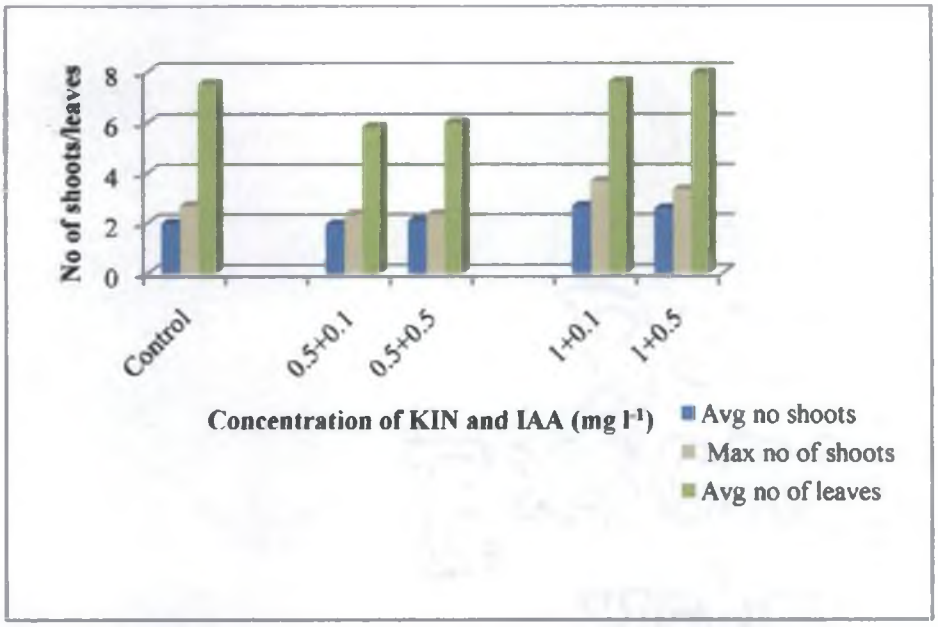

Fig. 5b. Effect of combination of KIN and IAA on maximum and average number of shoots and average number of leaves in sandal cultures.

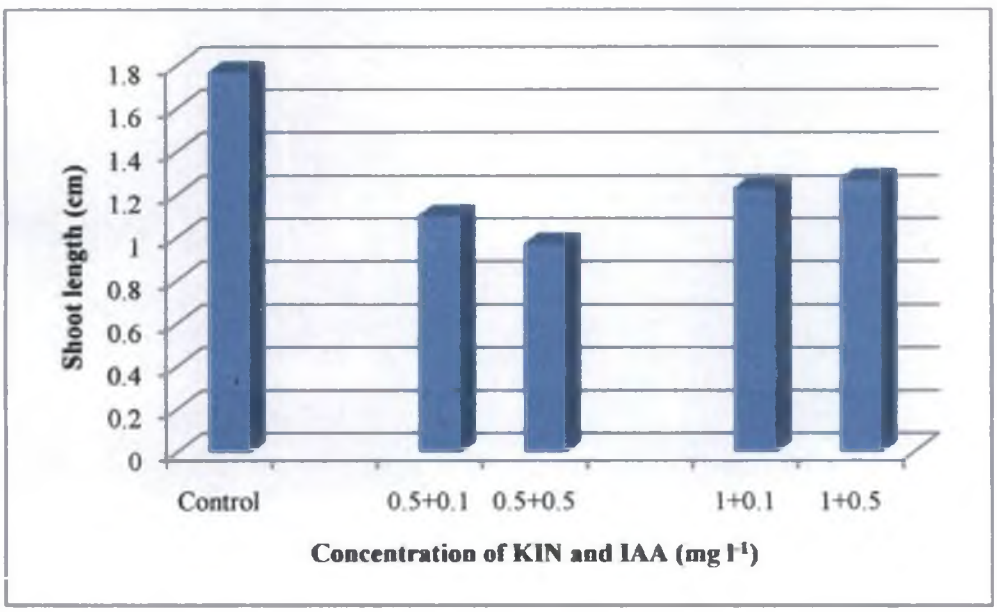

Fig. 5c. Effect of combination of KIN and IAA on maximum shoot length in sandal cultures.

(2 65) which was follow d by 1 mg l K netin + 0 5 mg l IAA (2 54) and 0 5 mg l Kinetin + 0 5 mg l IAA (2 07) These treatments were superior to control (1 97) The least average number of shoots was produced by 0 5 mg l Kinetin + 0 1 mg l^{-1} IAA (1 92) 1 mg l K netin + 0 1 mg l IAA produced h ghest number of shoot (3 66) This was followed by 1 mg l^{-1} Kinetin + 0 5 mg l IAA (3 33) wh ich is on par w th control (2 67) The least number of shoots were produced by 0 5 mg l^{-1} Kinetin + 0 1 mg l IAA and 0 5 mg l Kinetin + 0 5 mg l IAA (2 33)

Average shoot length was not sign ficantly d fferent among the various treatments Average shoot length noted n 0 5 mg l K netin + 0 1 mg l IAA was 0 84 cm followed by 0 5 mg l K netin + 0 5 mg l IAA (0 82) But average shoot length became 0 77 cm in 1 mg l Kinetin + 0 1 mg l IAA Max mum shoot length showed sign ficant d fference between control and treatments where the treatments showed lesser maximum shoot length than control (1 77 cm) (Fig 5c) But there was no s gn ficant difference between the treatments Maximum shoot length in 1 mg l^{-1} Kinetin + 0 5 mg l IAA was 1 27 cm and was on par with other treatments 1 mg l Kinetin + 0 1 mg l^{-1} IAA (1 23 cm) 0 5 mg l K netin + 0 1 mg l IAA (1 1 cm) and 0 5 mg l^{-1} Kinetin + 0 5 mg l IAA (0 97 cm)

Treatments showed significant difference in the product on of average number of leaves (Fig 5b) The highest average number of leaves (7 93) was recorded in 1 mg l Kinetin + 0 5 mg l IAA and was on par with 1 mg l Kinet n + 0 5 mg l IAA (7 6) and control (7 52) The lowest average number of leaves was found on 0 5 mg l Kinetin + 0 1 mg l IAA (5 81) and was on par w th 0 5 mg l Kinetin + 0 5 mg l IAA (5 97) Effect of treatments was not significant on the max mum number of leaves produced Number of maximum leaves was 9 33 in 1 mg l Kinetin + 0 5 mg l IAA and 6 67 leaves were observed in 0 5 mg l K net n + 0 5 mg l IAA

4 4 5 Effect of Combination of Kinetin and IBA on Culture Establishment and Growth in WPM Medium

Effect of different combinations of Kinetin at two levels (0 5 and 1 mg l) with two levels of IAA (0 1 and 0 5 mg l) on growth of sandal culture was estimated The result obtained is presented in Table 15 Significant difference in the effect of treatments on days taken for bud break average number of shoots and maximum shoot length was observed

Treatments do not differ significantly in percentage of bud break shoot initiation and leaf initiation All treatments other than 1 mg l Kinetin + 0 5 mg l IBA (96 %) resulted in 100 per cent bud break Shoot initiation was 100 per cent in all treatments Except 0 5 mg l Kinetin + 0 5 mg l IBA (98 67 %) showed 00 per cent leaf initiation

There was significant difference in days taken for bud break and all the treatments showed delayed bud break than control (5 58 days) Early bud break among the treatments was noted in 1 mg l Kinetin + 0 1 mg l IBA (7 18 days Fig 6a) This was followed by 1 mg l Kinetin + 0 5 mg l IBA (7 79 days) 0 5 mg l Kinetin + 0 1 mg l IBA (8 66 days) However bud initiation was prolonged up to 9 51 days in 0 5 mg l Kinetin + 0 5 mg l IBA The days taken for leaf initiation was not significant and leaf initiation occurred between 10 24 days and 11 90 days in 1 mg l Kinetin + 0 1 mg l IBA and 0 5 mg l Kinetin + 0 5 mg l IBA respectively

Treatments showed a significant difference in the production of average number of shoots (Fig 6b) Highest average number of shoots was obtained in 1 mg l Kinetin + 0 1 mg l IBA (2 66) and was found to be on par with 1 mg l Kinetin + 0 5 mg l IBA (2 59) Both these treatments were superior to the control (1 97) which was statistically same as 0 5 mg l Kinetin + 0 1 mg l IBA (1 86) Least average number (1 40) of shoots was observed in 0 5 mg l Kinetin + 0 5

Table 15 Effect of Kinetin + IBA on bud break and shoot development in axillary bud cultures of *Santalum album* in WPM media

Conc of KIN+IBA (mg l^{-1})	Bud break		Shoot initiation (%)	No of shoots		Shoot length (cm)		Leaf initiation		No of leaves	
	%	Days		Avg	Max	Avg	Max	%	Days	Avg	Max
Control	100 (10 00)	5 58^{a}	100	1 97^{b}	2 67	1 20	1 77^{b}	100 (10 00)	8 92	7 52	10 00
0 5KIN+0 1IBA	100 (10 00)	8 66^{d}	100	1 86^{b}	2 33	0 84	1 03^{a}	100 (10 00)	11 37	6 03	8 67
0 5KIN+0 5IBA	100 (10 00)	9 51^{d}	100	1 40^{a}	2 53	0 84	1 23^{a}	98 67 (9 93)	11 90	5 85	9 33
1KIN+0 1IBA	100 (10 00)	7 18^{b}	100	2 66^{c}	3 33	0 93	1 37^{a}	100 (10 00)	10 24	6 73	10 00
1KIN+0 5IBA	96 00 (9 79)	7 79^{b}	100	2 59	3 00	0 81	1 33^{a}	100 (10 00)	11 40	6 29	10 00
SEm+	0 03	0 36		0 06	0 27	0 01	0 04	0 00	1 60	0 73	0 53
F	1 00^{NS}	20 58*		14 12*	2 15^{NS}	2 21^{NS}	4 99*	1 00^{NS}	2 71^{NS}	1 87^{NS}	2 00^{NS}

*Significant at 5%

Figures in parenthesis are square root transformed values

Figures with same superscript do not differ significantly

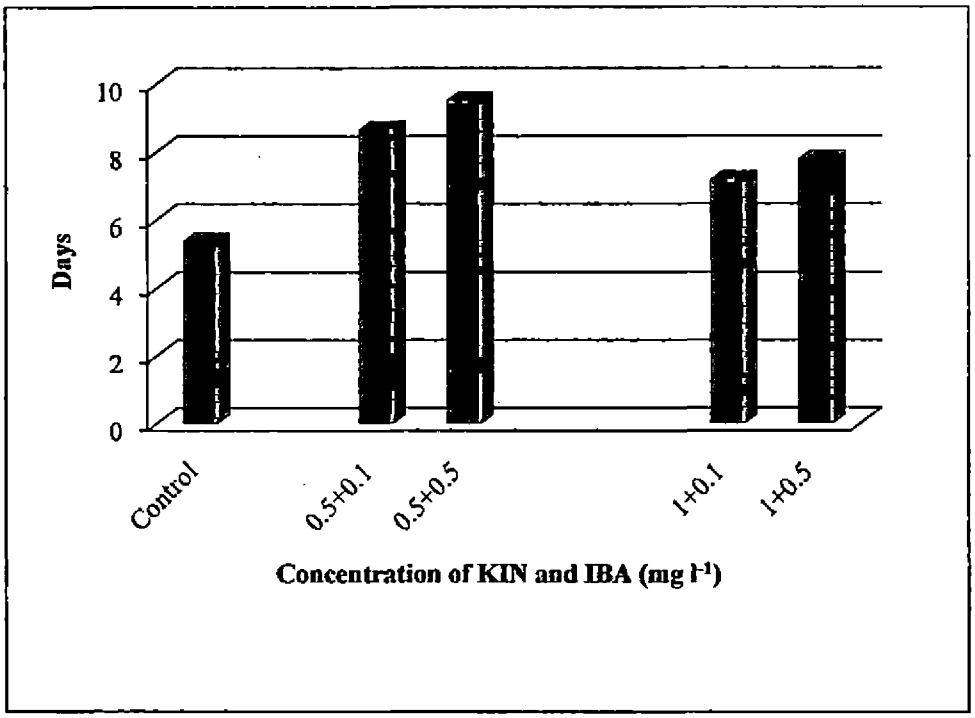

Fig. 6a. Effect of combination of KIN and IBA on days taken for bud break in sandal cultures.

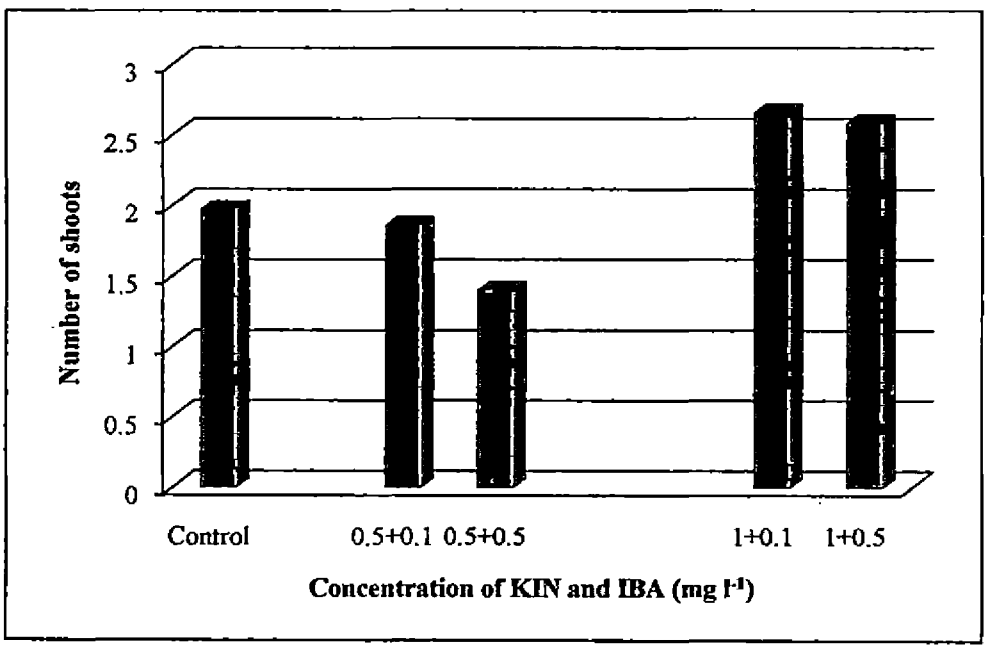

Fig. 6b. Effect of combination of KIN and IBA on average number of shoot production in sandal cultures.

mg l IBA However in the case of maximum number of shoot induction treatments were not significantly different

Average shoot length observed in the treatments were not significantly different and all tne treatments resulted in smaller shoots than control (1 20 cm) Maximum shoot length was significantly different among the treatments and compared to control (1 77 cm) all the treatments showed lesser shoot length (Fig 6c) All the treatments were on par with each other

Average and maximum number of leaves produced was not significantly different In all the treatments the average number of leaves was lower than control (7 52) 1 mg l Kinetin + 0 1 mg l IBA and 1 mg l Kinetin + 0 5 mg l IBA produced 10 leaves which was same as control While the maximum number of leaves recorded were 8 67 in 0 5 mg l Kinetin + 0 1 mg l IBA (8 67)

4 4 6 Effect of Kinetin and NAA on Culture Establishment and Growth in WPM Medium

The data recorded on the effect of the combination of different concentrations of Kinetin (0 5 and 1 mg l^{-1}) with NAA (0 1 and 0 5 mg l^{-1}) on the growth of sandal tissue culture is shown in the Table 16 Effect of treatments produced significant difference in days taken for bud break average number of sl oots average and maximum shoot length days taken fo leaf initiation and average number of leaves (Fig 7a 7b and 7c)

Bud break leaf initiation and shoot initiation were not influenced by the Kinetin and NAA combinations Hundred percentage of bud break was observed in 1 mg l Kinetin + 0 5 mg l NAA and is same as control Bud break percentage was 94 per cent in 0 5 mg l Kinetin + 0 5 mg l NAA All the t eatments showed 100 per cent leaf and shoot initiation Even though there was a significant difference n days taken for bud break and leaf initiation All the treatments were

Table 16 Effect of Kinetin + NAA on bud break and shoot development in axillary bud cultures of *Santalum album* in WPM media

Conc of KIN+NAA (mg l^{-1})	Bud break		Shoot initiation (%)	No of shoots		Shoot length (cm)		Leaf initiation		No of leaves	
	%	Days		Avg	Max	Avg	Max	%	Days	Avg	Max
Control	100 (10.00)	5.38	100	1.97^{b}	2.67	20	1.77^{d}	00	8.32	7.52	10
0.5KIN 0.1NAA	98.67 (9.93)	9.91	00	63	2.33	0.89^{b}	1.33^{b}	00	12.65^{b}	6.34^{b}	10
0.5KIN+0.5NAA	94.00 (9.69)	10.39	00	73	2.33	0.68	03	00	4.5	5.7	0
1KIN 0.1NAA	98.00 (9.90)	8.5^{b}	100	2.74^{b}	3.33	0.90^{b}	40	00	12.05^{b}	6.79^{b}	10
KIN 0.5NAA	100.00 (10.00)	9.96	100	2.58^{b}	3.33	0.79^{b}	10^{b}	100	12.5^{b}	5.61	0
SEm	0.069	0.37		0.15	0.33	0.0	0.02		25	0.30	
F	0.73^{NS}	4.70*		5.15*	2.3^{NS}	5.35*	47*		9.44	6.19*	

*Significant at 5%
Figures in parentheses are square transformed values
Figures with same superscript do not differ significantly

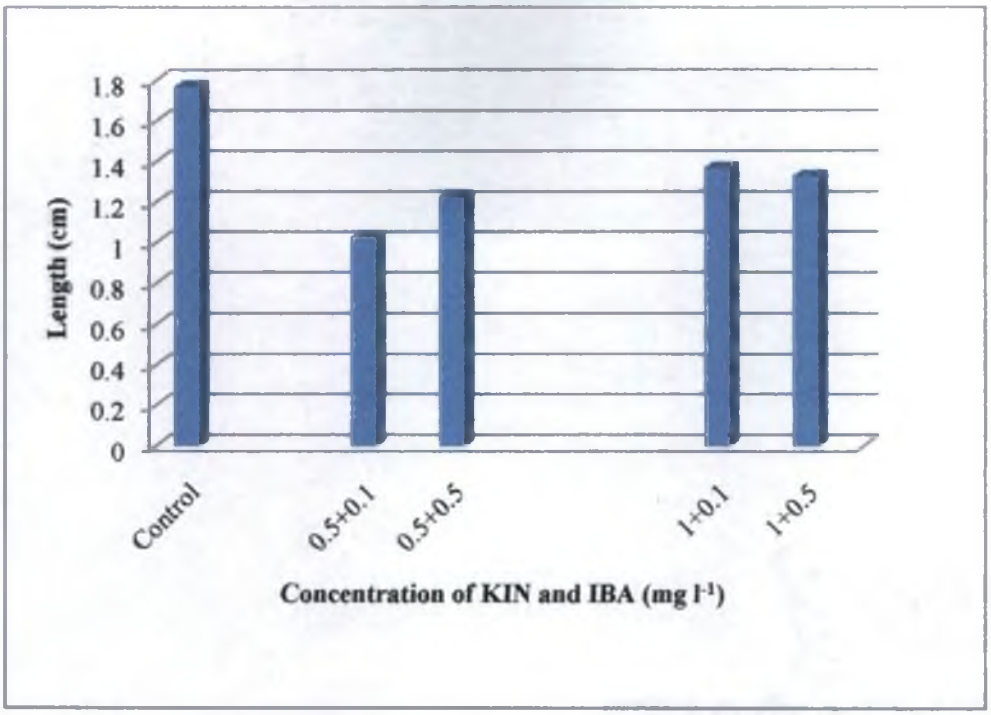

Fig. 6c. Effect of combination of KIN and IBA on maximum shoot length in in sandal cultures.

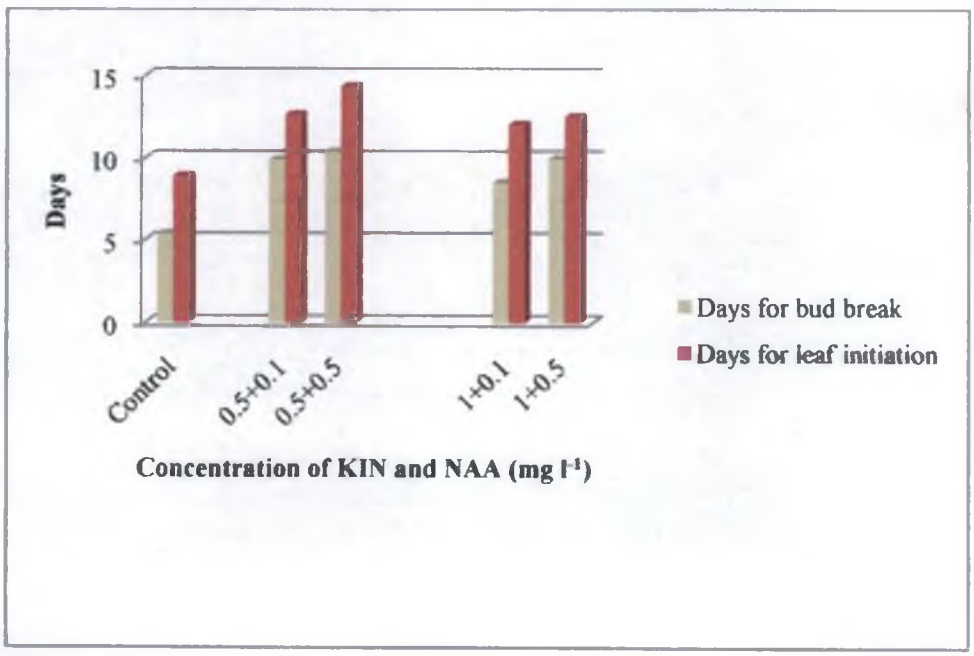

Fig. 7a. Effect of combination of KIN and NAA on days taken for bud break and leaf initiation

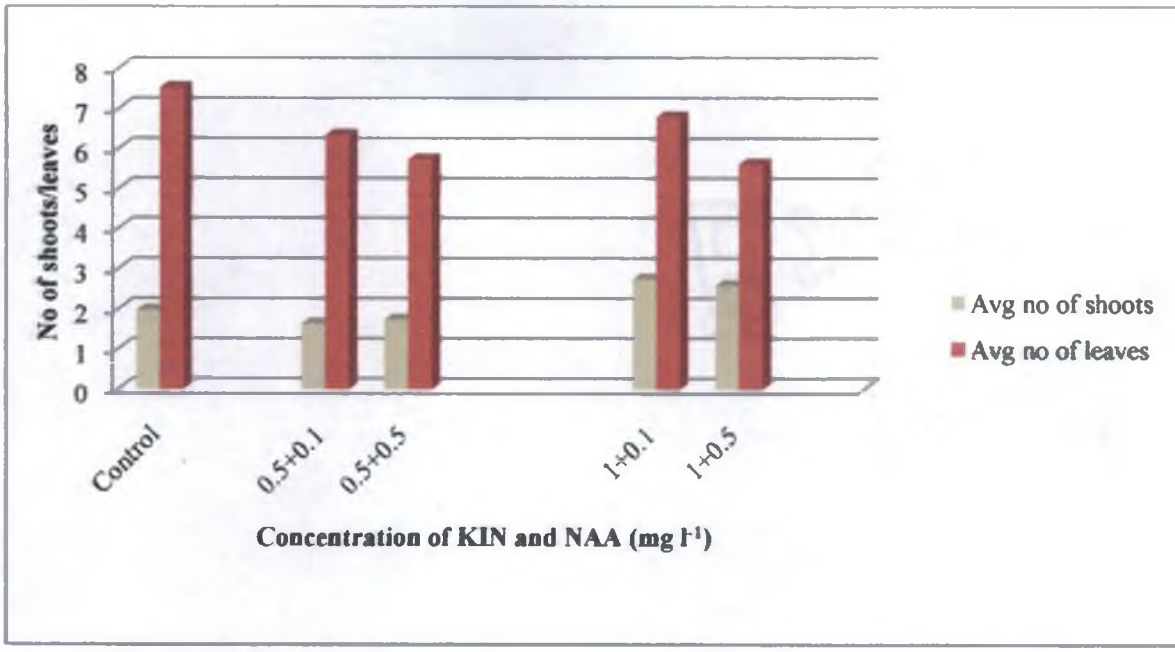

Fig. 7b. Effect of combination of KIN and NAA on average number of leaf and shoot production in sandal cultures.

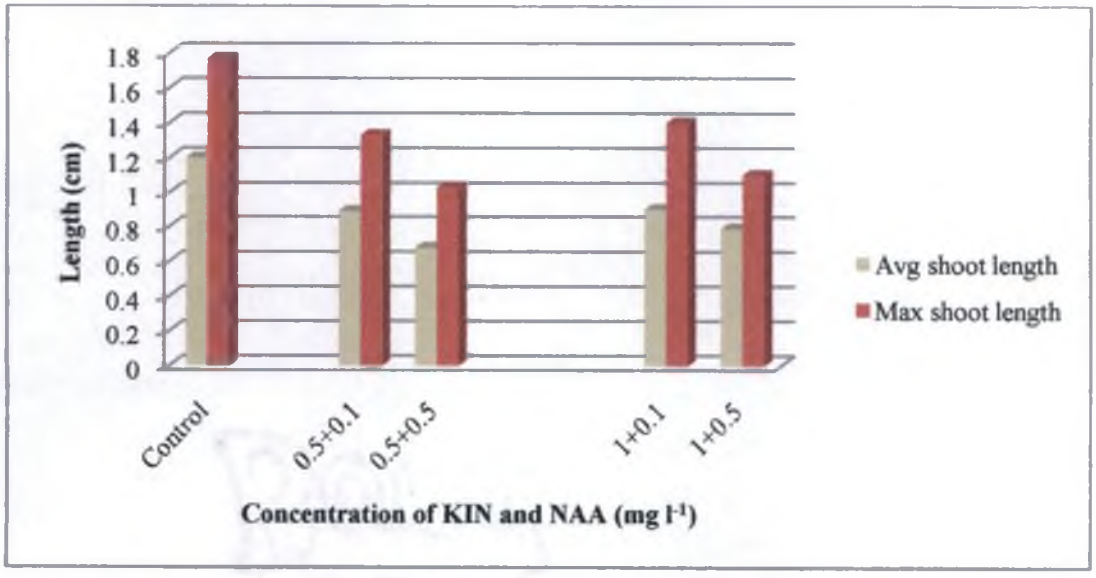

Fig. 7c. Effect of combination of KIN and NAA on average and maximum shoot length in sandal cultures.

not effect ve co rpared to control wh ch took 5 38 days for b d break and 8 92 days for leaf n t at on Fig 7a) In tl e case of bud break 1 mg l Kınet n + 0 1 mg l NAA took 8 51 days for leaf nit at on All the other treatmei ts were on par with each other as follows 0 5 mg l Kınet n + 0 1 mg l NAA (9 91 days) 1 mg l K net n + 0 5 mg l NAA (9 96 days) and 0 5 mg l K net n 0 5 mg l NAA (10 39 days) Early leaf tıat on (12 05 days) was observed ı 1 mg l Kınet n + 0 1 mg l NAA Th s vas followed by 1 mg l K net n + 0 5 mg l NAA (12 52 days) and was o pa v tl 0 5 ng l K net n + 0 1 mg l NAA (12 65 days) Leaf n t a on was delayed up to 14 35 days ın 0 5 mg l K net + 0 5 mg l NAA

Treat nents showed a s gnit ca ıt effect on average number of shoots (F 7b) Average nun ber of sl oots was highest ın 1 mg l K net n + 0 1 mg l NAA (2 74) and was o ı par w th 1 mg l K netın + 0 5 mg l NAA (? 58) Both these treatments were super oı to control w th 1 97 shoots Least average nu nber of shoots was show by 0 5 ng l Kınet n + 0 1 mg l NAA (1 63) and was on par w th 0 5 mg l K net n + 0 5 mg l NAA Effect of treatments on tl e average and nax mum shoot length was signif cant however all the treatn ents were not effect ve compared to control show ng 1 20 cm average length and 1 77 cm maxımum shoot length (Fıg 7c) Among the treatments h ghest average shoot length was reco ded ın 1 ng l K net n + 0 5 mg l NAA (0 90 cm) and was on par w th 0 5 mg l Kınet n + 0 1 mg l NAA (0 89 cm) Thıs was followed by 1 ng l k net n + 0 1 mg l NAA (0 79 cm) and the least average shoot length (0 68 cm) was found n 0 5 mg l Kınet n + 0 5 mg l NAA Sımılar trend was observed with respect to maxımu n shoot length

The average number of leaf product on was s gn f cant while the maxımum number of leaves were not s gn ficant It was noted that average number of leaves produced ın the treatments were less compared to control (7 52) The h ghest average number of leaves was on 1 mg l Kınet n + 0 1 mg l NAA (6 79) and the least number of shoots was observed on 1 ng l K net n + 0 5 mg l NAA (5 61) wh ch was on par w tl 0 5 mg l Kınet n + 0 5 mg l NAA (5 73)

4 4 7 Effect of Combination BA and IAA on Culture Establishment and Growth in WPM Medium

The result of the evaluation of effect of the combination of BA (0 5 and 1 mg l) with IAA (0 1 and 0 5 mg l) on the growth is given in the Table 17 Significant difference in percentage leaf initiation days taken for bud break and leaf initiation average shoot length and number of leaves and maximum number of leaves was observed

In the case of bud break and shoot initiation 0 5 mg l BA + 0 5 mg l IAA exhibited 100 per cent bud break and is same as control Percentage bud break noted on 0 5 mg l BA + 0 1 mg l^{-1} IAA was 73 67 per cent All the treatments except 1 mg l BA + 0 1 mg l IAA (90 33) showed 100 per cent leaf initiation as control (Fig 8c) Effect of treatments on days taken for bud break (Fig 8a) was significant however all the treatments took more days than control (5 38 and 8 92 days respectively) In the case of bud break except 0 5 mg l BA + 0 1 mg l IAA (11 33) all the other treatments were on par with control The same trend was observed with respect to the number of days taken for leaf initiation

Number of shoots produced in the treatments was not significant All the treatments produced average number of shoots than control (1 97) The same trend was observed with reference to the maximum number of shoot production where 1 mg l BA + 0 5 mg l IAA induced 3 33 shoots Significant difference was noted in average length of shoots produced (Fig 8b) In the case of both the average and maximum shoot length treatments did not perform as control (1 20 cm and 1 77 cm respectively) With growth regulator treatments of 0 5 mg l BA +0 5 mg l IAA showed shoot length of 0 47 cm and was on par with all other treatments Thus among the treatments there was no difference Maximum shoot length production was not significant

Table 17 Effect of BA+ IAA on bud break and shoot development in axillary bud cultures of *Santalum album* in WPM media

Conc Of BA+IAA (mg l^{-1})	Bud break		Shoot initiation (%)	No of shoots		Shoot length (cm)		Leaf initiation		No of leaves	
	%	Days		Avg	Max	Avg	Max	%	Days	Avg	Max
Contro	100 00 (888 62)	5 38	100 00 (888 62)	97	2 67	1 20^{b}	1 77	00 00^{b} (10 00)	8 9?	7 5?^{b}	10 00^{b}
0 5BA+0 IAA	7? 67 (543 82)	11 3?^{b}	87 67 (659 00)	2 0	2 67	0 42	0 53	00 00^{b} (0 00)	4 6^{b}	2(	4 00
0 5BA+0 5IAA	100 00 (888 62)	7 14	100 00 (888 62)	2 04	2 67	0 47	0 70	100 00^{b} (10 00)	1 7	3 57	4 (7
1BA+0 1IAA	97 33 (812 68)	6 75	86 67 (713 76)	2 44	3 00	0 37	0 40	90 33 (9 50)	9 8	3 18	4 00
1BA+0 5IAA	88 00 (663 06)	6 66	94 33 (777 05)	2 48	3 33	0 42	0 57	100 00^{b} (10 00)	10 32	3 63	4 67
SEm+	31051 22	3 84	?4554 37	0 11	0 87	0 02	0 04	1 87	2 70	10 6	0 5
F	2 22	4 02*	0 92	?8^{NS}	0 31^{NS}	15 99*	20 96	0 01?*	4 86*	25 00*	36 75*

*Significant at 5%
Figures in parenthesis are arc sine and square transformed values
F gures with same superscr pt do not d ffer sign ficantly

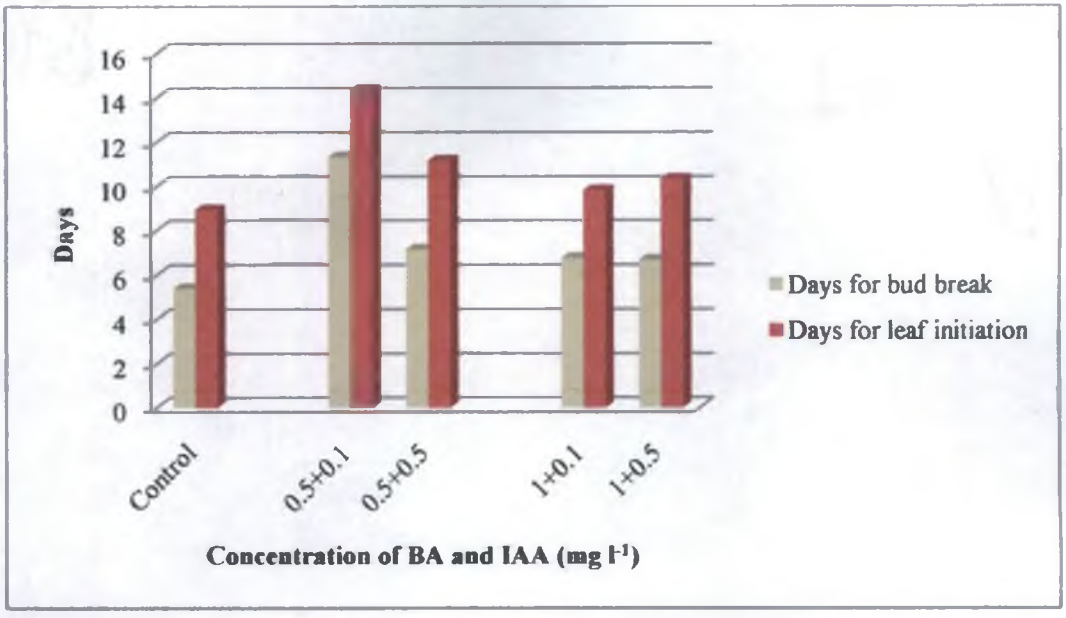

Fig. 8a. Effect of combination of BA and IAA on days taken for bud break and leaf initiation in sandal cultures.

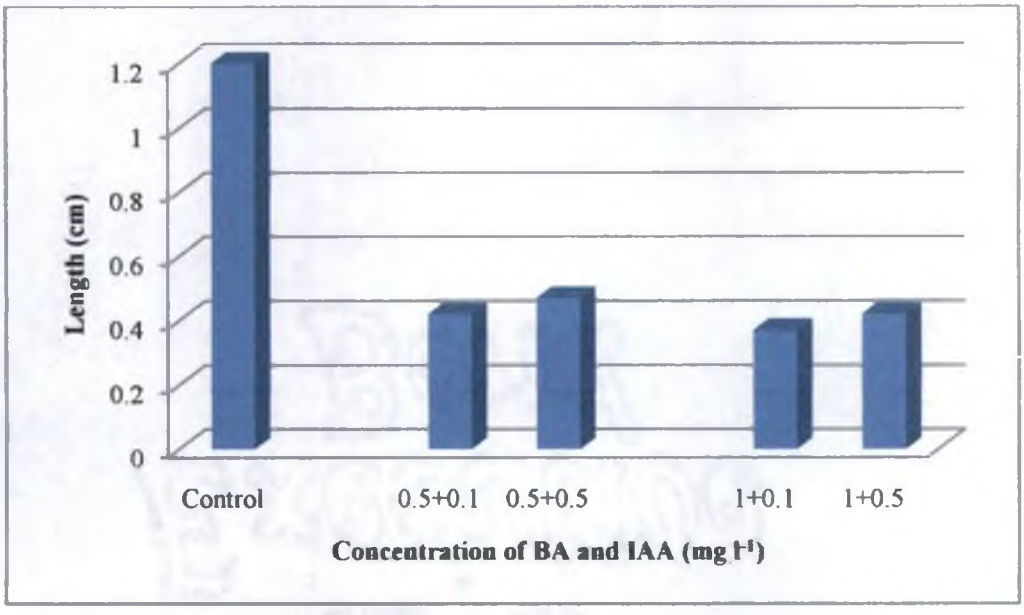

Fig. 8b. Effect of combination of BA and IAA on average shoot length in sandal cultures.

Averag nt nber of lea prod ction v as signif cant and the control (7 52) was sapei or to tl e reat nents (F g 8d) All the treatments vere on pa with each other and 1 mg BA + 0 5 n g l IAA an average of 63 leaves was found Least numbe of leaves was found n 1 mg l BA + 0 1 ng l IAA The sa ne trend vas observed with iefere ce to maximum umber of leaf product on

4 4 8 Effect of Combination of BA and IBA on Culture Establishment and Growth in WPM Medium

Effect of differe it comb nat o s of BA (0 5 and 1 mg l) with IBA (0 1 and 0 5 mg l) on growth was estimated The result obta ned is presented in Table 18 There was signif ca nt d fference n days taken for bud b eak and leaf in t at on average and max mum shoot length and the average number of leaves taken

Treatments had no nfluence on percentage of cultures show ng bi d break leaf n t at on and shoot nitiation All the treatments produced less bud break percentage tl an control (100 %) Among them it was 95 33 per cent n 1 mg l BA + 0 5 mg l IBA and 89 67 % per cent 0 5 mg l BA + 0 5 mg l IBA Shoot n t ation was 100 per cent n 1 mg l BA + 0 5 mg l IBA and was same as control Shoot n tiation percentage of 93 33 was exhib ted by 0 5 mg l BA + 0 1 ng l IBA and 0 5 mg l BA + 0 5 mg l IBA W th respect to the leaf n t ation all treatments except 0 5 mg l BA + 0 1 mg l I BA (91 67) showed 100 per cent leaf in t at on

Days taken for bud break showed sign ficant d fference between control and treatments wh le t me taken for leaf n t at on was sign f cant among the treatments also Con pared to control treatments took more number of days for bud and leaf init at on (F g 9a) Number of days taken for bud in t at on was on par with each other a nong all treatments and 1 mg l BA + 0 1 mg l IBA n t ated buds in 6 92 days But n the case of leaf n t at on the treatments 1 mg l

Table 18 Effect of BA+ IBA on bud break and shoot development in axillary bud cultures of *Santalum album* in WPM media

Conc of BA+IBA (mg l^{-1})	Bud break		Shoot initiation (%)	No of shoots		Shoot length (cm)		Leaf initiation		No of leaves	
	%	Days		Avg	Max	Avg	Max	%	Days	Avg	Max
Control	100 00 (10 00)	5 38	100 00 (10 00)	1 97	2 67	1 20^{d}	1 77	100 00 (10 00)	8 92	7 5^{b}	10 00
0 5BA+0 1IBA	92 67 (9 61)	7 17^{b}	9 33 (9 65)	2 00	2 00	0 49^{b}	0 57	91 67 (9 55)	10 53^{b}	4 53	4 6
0 5BA+0 5IBA	89 67 (9 47)	7 75^{b}	9 33 (9 65)	1 82	2 00	0 66	0 93^{b}	100 00 (0 00)	12 09	4 2	6 00^{b}
1BA+0 1IBA	95 00 (9 74)	6 92^{b}	97 00 (9 85)	2 18	3 00	0 56^{b}	0 77^{b}	100 00 (10 00)	9 88^{b}	4 74	6 00^{b}
1BA 0 5IBA	95 53 (9 76)	7 66^{b}	100 00 (10 00)	2 21	2 67	0 41	0 50	100 00 (10 00)	11 26^{b}	3 29	4 00
SEm+	0 18	0 48	0 16	0 56	0 33	0 01	0 025	0 12	0 81	0 7	0 77
F	0 66^{NS}	5 78*	0 57^{NS}	1 50^{NS}	1 8^{NS}	4 25*	31 65*	1 00^{NS}	5 61*	10 79*	61 00*

*Significant at 5%
Figures in parenthesis are square root transformed values
Figures with same superscript do not differ significantly

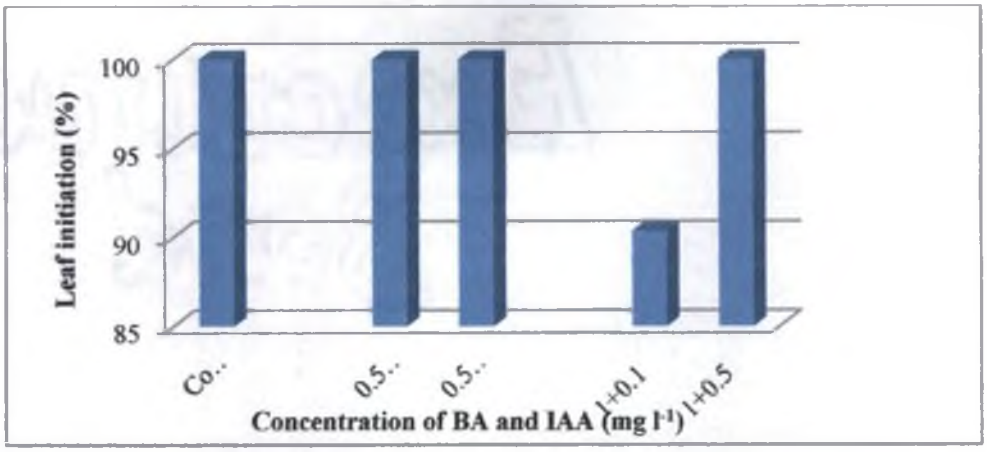

Fig. 8c. Effect of combination of BA and IAA on leaf initiation (%) in sandal cultures.

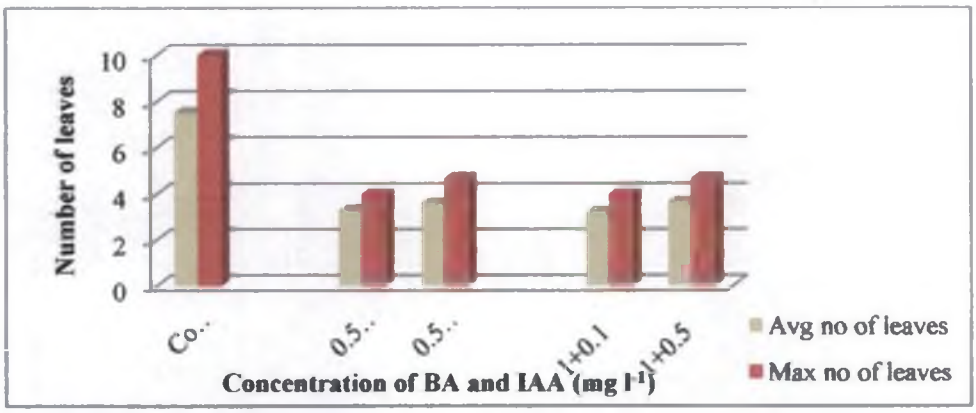

Fig. 8d. Effect of combination of BA and IAA on average and maximum number of leaves in sandal cultures.

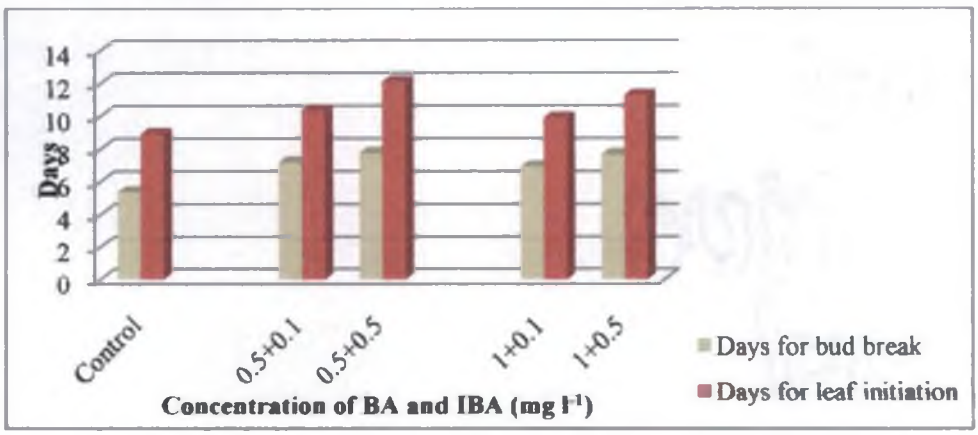

Fig. 9a. Effect of combination of BA and IBA on days taken for bud break and leaf initiation in sandal cultures.

BA + 0 1 mg l IBA (10 ɔ3 days) and 0 5 mg l BA + 0 1 mg l IBA (11 76 days) were on pa w th each other The delayed leaf ı tıat on vas observed ın 0 5 ng l BA + 0 5 n g l IBA (12 09 days)

Average and max mum number of shoot product on was not s gnıf cant Effect of treatments produced s gn f cant d fference n average and nax mu n length of shoots (F g 9b) But the response of treatments was lower than control (1 20 c n and 1 77 cm) A nong the treatments 0 5 mg l BA +0 5 ng l IBA was noted w th hıghest average (0 66 cm) and maxımum (0 9ɔ cm) shoot length Lowest average shoot le ıgth (0 41 c n) was ecorded n l mg l BA +0 5 ng l IBA wh le the lowest maxımum shoot length vas also found n l ng l BA +0 5 mg l IBA (0 50 cm) and s on par w th 0 5 mg l BA +0 1 mg l IBA (0 57 c n)

Treatments p oduced sıgn f cant d fference ı the ave age and n ax mu n number of leaves but the number of leaves was lesser than control (F g 9c) Average ıun ber of leaves produced among the treatments was ıot statıst cally d fferent and on l mg l BA +0 1 ng l IBA average number of leaves was 4 74 The max n um number of leaf product on was on par w th each other n 0 5 mg l BA +0 5 mg l IBA and l mg l BA +0 1 mg l IBA (6 00) vh ch were the h gl est among the treatn ents Maxımum number of leaves was lowest n l n g l BA +0 5 mg l IBA (4 00) and was on par wıth 0 5 mg l BA +0 1 mg l IBA (4 67)

4 4 9 Effect of combınatıon of BA and NAA on Culture Establıshment and Growth ın WPM Medıum

Effect of d fferent comb nat ons of BA at two concentrat ons such as 0 5 and l mg l wıth NAA (0 1 and 0 5 mg l) on growth was recorded The result obtaıned s g ven n Table 19 S gn f cant d fference n number of days taken for bud break and leaf ın t at on average nı mber of shoots average and maxımu n shoot length as well as number of leaves was observed

Table 19 Effect of BA+ NAA on bud break and shoot development in axillary bud cultures of *Santalum album* in WPM media

Conc of BA+NAA (mg l^{-1})	Bud break		Shoot initiation (%)	No of shoots		Shoot length (cm)		Leaf initiation		No of leaves	
	%	Days		Avg	Max	Avg	Max	%	Days	Avg	Max
Control	100 00 (0 00)	5 38	100 00 (10 00)	1 97^{b}	2 67	1 20^{d}	1 77^{d}	100 00 (10 00)	8 92	7 52	10 00
0 5BA+0 1NAA	96 33 (9 81)	9 14^{b}	92 67 (9 62)	1 92^{b}	2 67	0 59^{b}	0 87^{b}	97 3 (9 86)	11 53^{b}	4 52^{b}	5 33^{b}
0 5BA+0 5NAA	95 67 (9 67)	9 34^{b}	85 67 (9 23)	1 55	2 00	0 58^{b}	0 73^{b}	94 3 (9 70)	13 70	5 00^{b}	6 00^{b}
1BA+0 1NAA	92 00 (9 58)	7 86^{b}	76 00 (8 57)	1 54	2 00	0 44	0 50	100 00 (10 00)	10 96^{b}	3 37	4 00
1BA 0 5NAA	88 67 (9 37)	9 23^{b}	100 00 (10 00)	1 92^{b}	3 00	0 73	1 05	100 00 (10 00)	11 29^{b}	4 67^{b}	6 00^{b}
SEm+	0 32	1 0	0 93	0 03	0 5 3	0 00	0 03	0 06	0 26	0 28	0 27
F	0 51^{NS}	8 23*	1 17^{NS}	5 62*	1 12^{NS}	55 38*	28 15*	0 81^{NS}	33 70*	25 45*	56 5*

*Significant at 5%
Figures in parenthesis are square transformed values
Figures with same superscript do not differ significantly

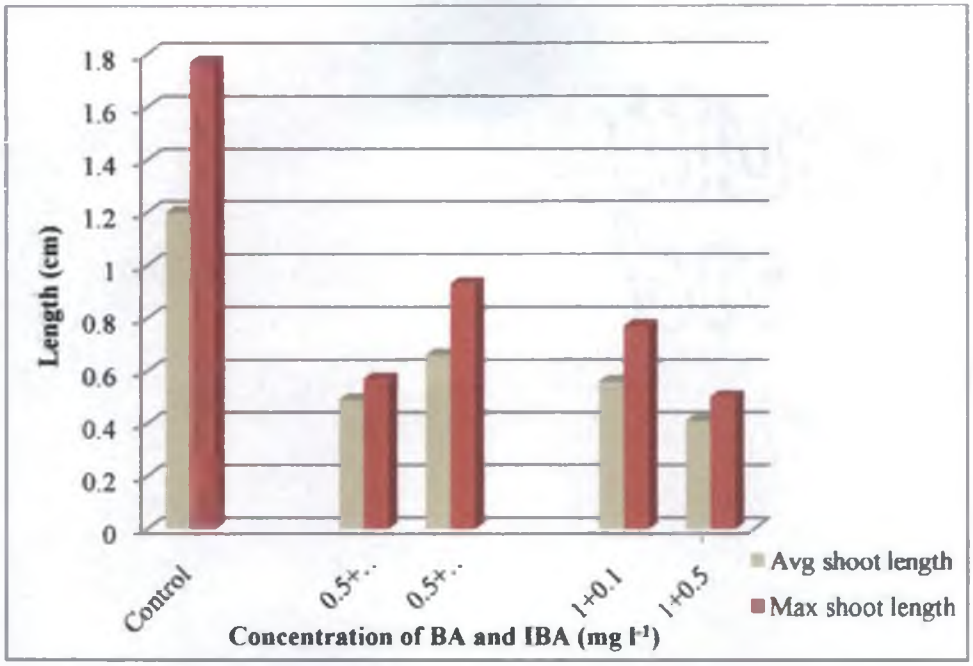

Fig. 9b. Effect of combination of BA and IBA on average and maximum shoot length in sandal cultures.

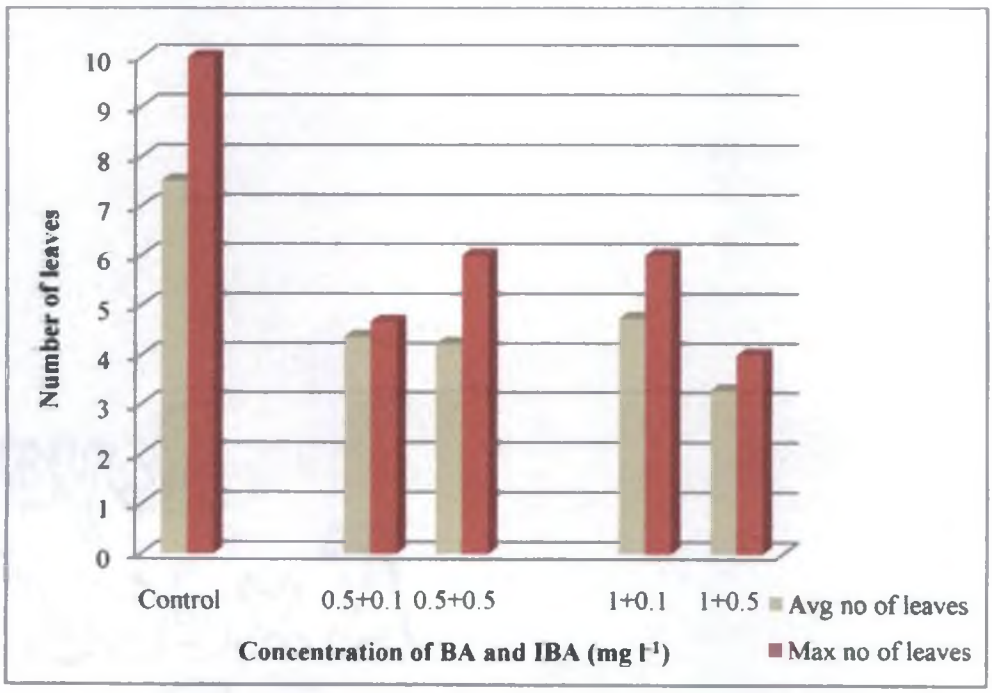

Fig. 9c. Effect of combination of BA and IBA on average and maximum number of leaf production in sandal cultures.

Treatments did not show any influence on percentage of bud break, leaf and shoot initiation. None of the treatments were effective as control (100 %) in induction of bud break. Bud break observed among the treatments was 96 per cent (0.5 mg l^{-1}BAP + 0.1 mg l^{-1}NAA) to 88.67 per cent in 1 mg l^{-1} BA + 0.5 mg l^{-1} NAA. Shoot induction percentage observed in 1 mg l^{-1} BA + 0.5 mg l^{-1} NAA was 100 per cent and is same as control while in 1 mg l^{-1} BA + 0.1 mg l^{-1} NAA it was 76 per cent. The treatments 1 mg l^{-1} BA + 0.1 mg l^{-1} NAA and 1 mg l^{-1} BA + 0.5 mg l^{-1} NAA induced leaf initiation percentage same as control (100 %). However in 0.5 mg l^{-1} BA + 0.5 mg l^{-1} NAA it was 94.33 per cent.

Days taken for both the bud break and leaf initiation was significant but the treatments took more number of days than control which took 5.38 and 8.92 days respectively (Fig. 10a). In the case of days taken for bud break there was no statistical difference among the treatments. Leaf initiation was delayed up to 13.70 days in 0.5 mg l^{-1} BA + 0.5 mg l^{-1} NAA and all other treatments were on par with each other.

Average number of shoots produced (Fig. 10b) was significant and the treatments with 1.92 shoots (1 mg l^{-1} BA + 0.5 mg l^{-1} NAA and 0.5 mg l^{-1} BA + 0.1 mg l^{-1} NAA) were on par with control (1.97). Least number of shoots was observed in 1 mg l^{-1} BA + 0.1 mg l^{-1} NAA (1.54) and was on par with 0.5 mg l^{-1} BA + 0.5 mg l^{-1} NAA (1.55). Treatments had no significant influence on maximum number of shoot production. Average and maximum shoot length (Fig. 10c) was significantly different but treatments were not effective compared to control (1.20 cm and 1.77 cm). The highest average shoot length was in 1 mg l^{-1} BA +0.5 mg l^{-1} NAA (0.73 cm) and was followed by 0.5 mg l^{-1} BA +0.1 mg l^{-1} NAA (0.59 cm) which is on par with 0.5 mg l^{-1} BA +0.5 mg l^{-1} NAA (0.58 cm). The least shoot length was recorded in 1 mg l^{-1} BA +0.1 mg l^{-1} NAA (0.44 cm). Highest maximum shoot length was 1.03 (1 mg l^{-1} BA +0.5 mg l^{-1} NAA) and lowest was 0.50 cm (1 mg l^{-1} BA +0.1 mg l^{-1} NAA).

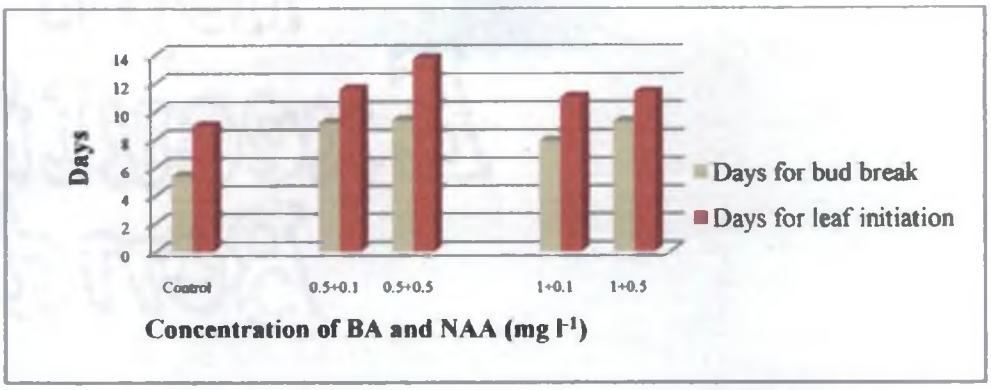

Fig. 10a. Effect of combination of BA and NAA on days taken for bud break and leaf initiation in sandal cultures.

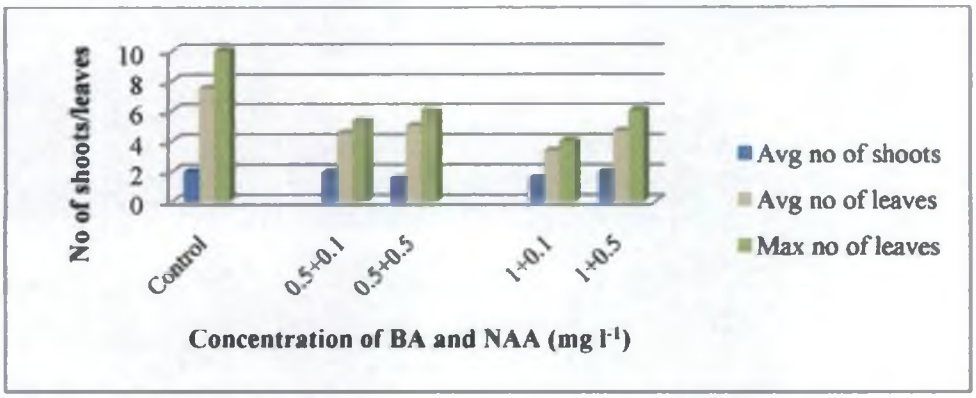

Fig. 10b. Effect of combination of BA and NAA on average number of shoots, average number of leaves and maximum number of leaves in sandal cultures.

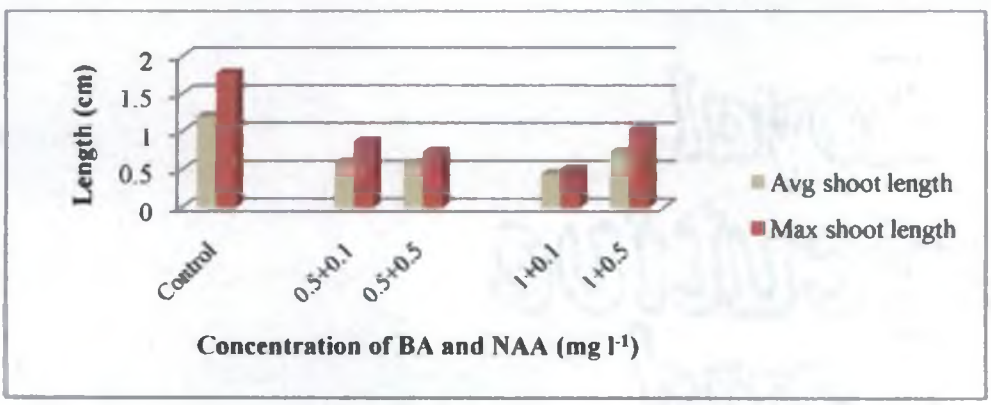

Fig. 10c. Effect of combination of BA and NAA on average and maximum shoot length in sandal cultures.

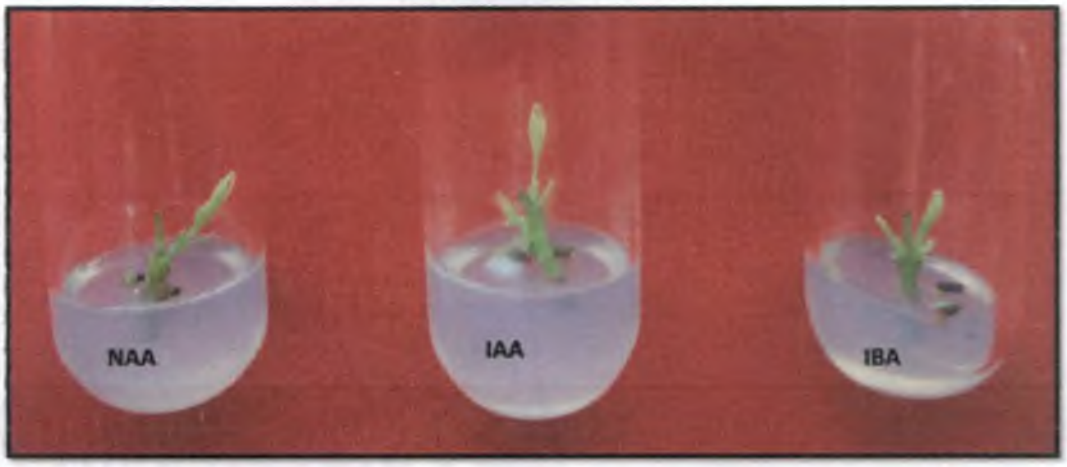

Plate 5. Effect of NAA, IAA and IBA (0.5 mg l^{-1}) in WPM containing 0.5 mg l^{-1} BA in the growth of sandal cultures

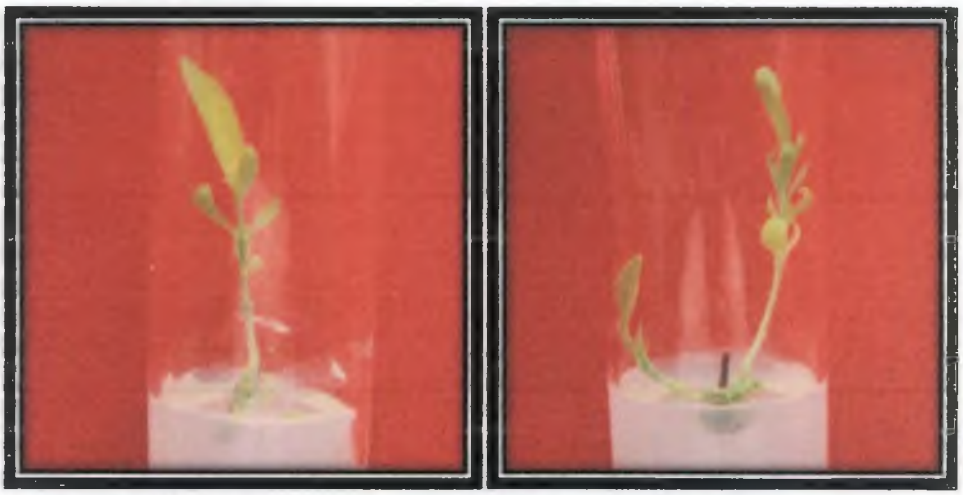

Plate 6. Shoot elongation in sandal cultures in WPM basal medium supplemented with Kinetin (1 mg l^{-1})

Treatments showed significant difference in number of leaf production It was lower than control while respect to average (7 52) and maximum (10) number of leaves (Fig 10b) Highest average number of leaves observed in treatments was 0 5 mg l BA +0 5 mg l NAA) and was on par with 1 mg l BA +0 5 mg l NAA (4 67) and 0 1 mg l BA +0 1 mg l NAA (4 57) Lowest number of leaves was noted in 1 mg l BA +0 1 mg l NAA (3 37) Same trend was observed in the case of maximum number of leaves where 6 was the highest number and the lowest was 4

4 5 ESTABLISHMENT OF CULTURES OF SANDAL THROUGH SUBCULTURING

Explants were sub cultured in to different media for further growth and to maintain them (Table 20) Excised shoots from the mother explants whe cultured singly in the medium failed to develop Transferring of mother explant itself to the new media showed promising results

4 6 ROOTING OF *IN VITRO* PRODUCED SHOOTS

All the treatments failed to induce roots

4 7 SOMATIC EMBRYOGENESIS

In order to induce somatic embryos *ex vitro* internodes and leaves were used as explants and was cultured horizontally in the media These were cultured in four levels of BA (0 5 1 2 and 3 mg l) and Kinetin (0 5 1 2 and 3 mg l) in WPM Results obtained in this experiment are presented in Table 21 and Table 22

Ex vitro leaf explants failed to respond But inter nodal explants cultured in 0 5 and 1 mg l Kinetin induced shoot buds (17 %) and developed in to

Table 20 Effect of different media on establishment of cultures

Medi	Max shoot length (cm)	Max number of multiple shoots	Max number of leaves	Leaf fall
Kinetin (0 5 mg and mg	5	3	6	NO
BA (0 5 mg and 1mg l)	0	0	0	NO
0 5 mg l BA 0 5 mg Kinetin and 0 5 mg BA 1mg Kinetin	1 7	18	2	NO
mg l BA + mg l IAA		1	8	YES
1 mg l Kinetin+ mg l IAA	4	0	8	NO

Table 21 Effect of kinetin on somatic embryo induction in inter nodal explants collected from *ex vitro* and *in vitro* shoots

Conc of cytokinin in WPM (mg l)	*Ex vitro* (% response)		*In vitr* (% response)	
	Somatic embryos	Shoot bud formation	Somatic embryos	Shoot bud formation
0 5 K netin	0	17	0	0
1 K net n	0	17	0	0
2 K netin	0	0	0	0
3 K netin	0	0	0	0
0 5 BA	0	0	80	90
1 BA	0	0	80	90
2 BA	0	0	0	60
3 BA	0	0	0	0

Table 22 Effect of kinetin on somatic embryo induction in leaf explants collected from *ex vitro* and *in vitro* shoots

Conc of cytokinm in WPM (mg l^{-1})	*Ex vitro* (% response)		*In vitro* (% response)	
	Somatic embryos	Shoot bud formation	Somatic embryos	Shoot bud formation
0 5 Kinetin	0	0	0	0
1 Kinet n	0	0	0	0
? Kinet n	0	0	0	0
3 Kinetin	0	0	0	0
0 5 BA	0	0	40	70
1 BA	0	0	20	60
2 BA	0	0	0	0
3 BA	0	0	0	0

complete shoot W l -n the same expei ıment was tried usıng explants of *in vitro* grown shoots med a contaıning BA 0 5 and l mg l ınduced somat c embryos directly ın both leaf (80 % and 60 %) a ıd ınter ı odal segments (100 %) However here shoot bud ınductıon was observed on ınter nodal segments ıoculated ın BA 0 5 mg l (70 %) and l mg l (60 %) Meanwhile Kınetın faıled to respond

In the leaf explants somatıc embryo formatıon was concentrated on the cut ends On ıntei nodal explants globular shaped somatıc embryos were formed on ıts surface and these were then developed to the torpedo stage Further development of somat c embryos faıled

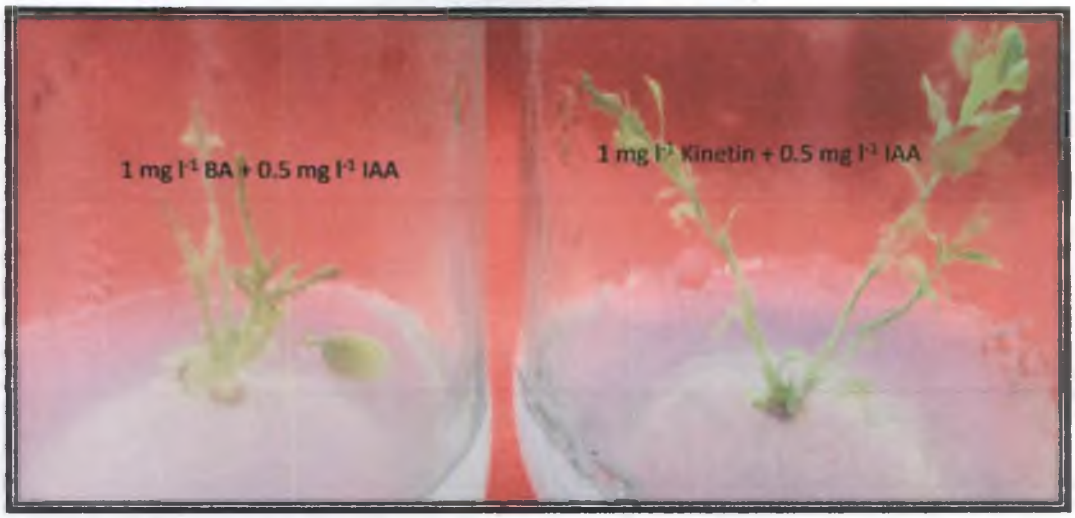

Plate 7. Effect of subculture of sandal shoots to the media containing combination of cytokinins and auxins

Plate 8. Subculture of single nodal segments of sandal excised from *in vitro* developed shoots in 1 mg l^{-1} BA+WPM

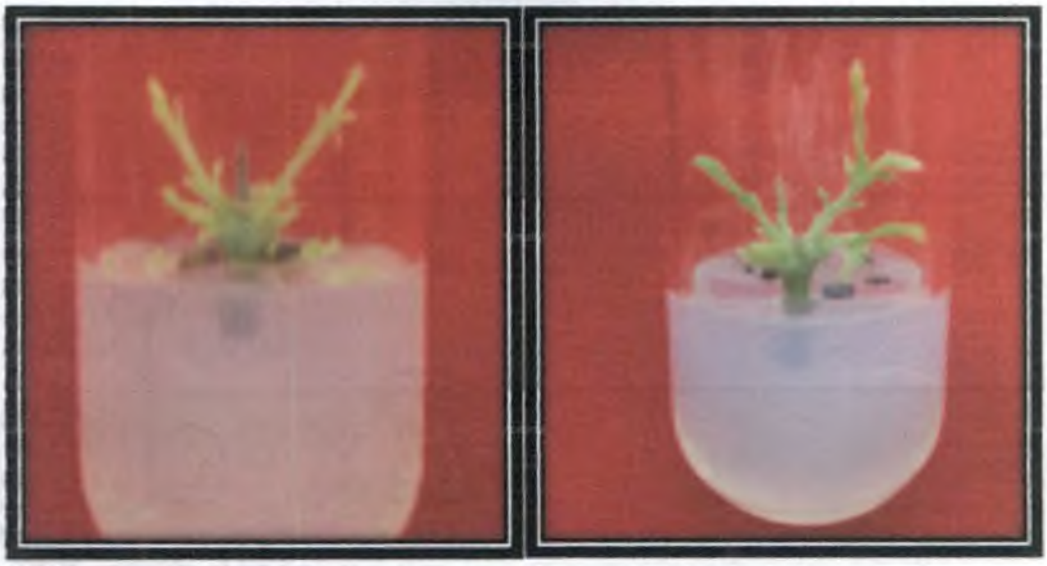

Plate 9. Increase in number of multiple shoots of sandal when subcultured to the same media (0.5 mg l^{-1} BA + 0.5 mg l^{-1} Kinetin)

Plate 10. *In vitro* developed sandal shoots kept for rooting

Plate 11. Somatic embryo formation in the *in vitro* leaf segments of sandal in WPM basal medium supplemented with 1 mg l^{-1} BA

Plate 12. Shoot bud formation from intermodal segments of sandal shoots cultured in 1 mg l^{-1} BA+WPM

Discussion

DISCUSSION

Natural regeneration of *Santalum album* one of the most valuable timbers of the world s poor due to the limited production of mature fruits attributed to the presence of genotypic barriers in embryo development (Sandhuveerendra *et al* 1999 and Ramya 2010) In this circumstance tissue culture is an effective vegetative propagation method to produce large number of quality planting material through the *in vitro* propagation of selected genotypes According to Sanjaya *et al* (2003) sandal is recalcitrant to *in vitro* and *in vivo* propagation such that limited success has been achieved so far It was also found that a systematic study on the effects of plant growth regulators on morphogenesis is insufficient Thus the present study was conducted to develop *in vitro* propagation protocol by evaluating the effects of basal media and different plant growth regulators on axillary bud proliferation and somatic embryo induction Important findings obtained during the course of the present investigation are discussed below

5 1 CULTURE CONTAMINATION

Culture contamination is a major problem associated with *in vitro* propagation Main contaminants associated in tissue culture are fungus and bacteria Contamination may occur from several sources such as explants medium used for culturing culture vessels equipments used or the culture environment Among them explant is a major inoculum for contamination especially regarding the culturing of woody species using the explants from field grown trees Surface sterilization of explants using fungicides and $HgCl_2$ is observed as an effective method to control contamination

5 1 1 Fungal Contamination

In the present study it was found that the season of collection of explants had a direct influence on fungal contamination rate During rainy season fungal

contam nation was mole than 90 per cent Contamination rate as low as 0 11 per cent was noted in the montl s November April Thus winter and summer months were suitable foi establish ng aseptic cultures This can be related to the find ngs of Sankii (2009) w th reference to mahogany El Zahei (2008) n jackfru t and Cardoza *et al* (1999) in cashew The reason for th s observation is because the humid condition generated by the heavy rainfall favours he growth of fungus during June July

In order to prevent the fungal contan mat on explants were treated w th systemic and contact fungicides Carbendazim 50% WP (Bavistin) and Indofil M 45 (Mancozeb 75% WP) singly or in combinations During the months with contamination rate below 20 per cent immers ng the explants in Carbendazim 50% WP (0 1 %) for 45 m nutes or n Mancozeb 75% WP (0 1 %) for 45 minutes were found to be effective to el minate the fungal contaminat on However one notable observat on was found when the treatment time of Mancozeb 75% WP increased culture death also increased but in the case of Carbendazim 50% WP there was an inverse relationship (Table 5) Thus least culture death (2 %) was observed with the treatment of 0 1 per cent Carbendazim 50% WP for 45 minutes There was no such relationsh p when the concentration of fungicides was increased (Table 6) With respect to the months with contamination 40 70 per cent (0 2 %) Carbendazim 50% WP + (0 1%) Mancozeb 75% WP for 45 minutes was efficient to prevent the contamination Since rainy season is the most favourable cond t on for fungal growth even the applicat on of fungicides did not prevent the occurrence contamination But treating with (0 2%) Carbendazim 50% WP + (0 2%) Mancozeb 75% WP for 95 minutes reduced the contamination to 27 33 per cent However it was observed that increasing the time of treatment of fungicides resulted in culture death (Table 7) Thus keeping in fungic de for 95 minutes resulted in 70 per cent culture death Cons dering this factor the treatment of fungicides Carbendazim 50% WP + Mancozeb 75% WP 0 2 per cent for 60 m nutes was selected as suitable fung cide combination for rainy season in which

the contamination and dead cultures were 55 33 per cent and 13 per cent respectively

5 1 2 Bacterial Contamination

Use of $HgCl_2$ at various concentrations was found as an effective method to control bacterial contamination in many species like *Citrullus colocynthis* (Satyavani *et al* 2011) *Eucalyptus citriodora* by Pasha and Irfan (2011) *Diospyros kaki* (Kun *et al* 2010) It was observed in the present study that increasing the concentration of $HgCl_2$ reduced the contamination but higher concentrations increased culture death By considering both factors dipping the explants in 0 15 per cent HgCl for 10 minutes was considered suitable for controlling bacterial contamination But the treatment with $HgCl_2$ alone cannot ensure the prevention of contamination Proper sterilization of culture vessels and equipments as well as the laminar flow chamber were necessary for preventing bacterial contamination

5 2 EFFECT OF BASAL MEDIA ON CULTURE ESTABLISHMENT AND GROWTH

Most commonly used media for culturing of tree species are MS ½ MS and WPM In the present study an evaluation of effect of these three media on culture establishment and growth of axillary bud culture of sandal was done All the basal media were effective for inducing bud break leaf initiation and shoot formation But WPM was found to be superior among them with respect to other growth factors except for the number of shoots produced It induced early bud break in 5 38 days and leaf initiation in 8 92 days Average shoot length of 1 20 cm was recorded with an average of 7 52 leaves Moreover cultures in MS medium were found to be stunted while in ½ MS and WPM elongated shoots were formed However leaf fall was heavy in ½ MS medium and cultures in WPM were noted as healthy compared to ½ MS (Plate 1) By considering these facts and comparing

the growth of cultures in the three media WPM was taken as superior to MS and ½ MS media for the *in vitro* propagation of *Santalum album* Superiority of WPM over other basal media was reported in following species also *Ficus carica* (Brunn *et al* 2003) *Acacia nilotica* (Samake *et al* 2011) olive cultivars (Ali *et al* 2012) Hence WPM was selected for conducting further studies to find the effect of plant growth regulators on the growth parameters of sandal

5 3 EFFECT OF PLANT GROWTH REGULATORS ON CULTURE ESTABLISHMENT AND GROWTH

Growth regulators are organic compounds which in small amounts promote inhibit or qualitatively modify growth and development Different plant growth regulators have different effects and they vary with the type and quantity to be applied Among them auxins (NAA IAA and IBA) and cytokinins (BA and Kinetin) are commonly used to regulate cell division cell elongation cell differentiation and organ formation As stated by Krokorian *et al* (1981) proper selection and addition of growth regulator at an optimum level is one of the important factors for successful plant tissue culture Bhojwani and Razdan (1983) reported that it is generally necessary to add one or more of these plant growth regulators to support good growth of tissues and organs Thus in the present study axillary buds were cultured in WPM media containing cytokinins (BA or Kinetin) singly or in combination with auxins (IAA IBA and NAA) or the combination of both cytokinin

5 3 1 Cytokinins

The results showed that both the cytokinins were effective for the production of multiple shoots compared to control Among them BA was better than Kinetin for formation of multiple shoots and induced up to 5 33 (1 mg l BA) shoots per culture besides 1 mg l BA+0 5 mg l Kinetin was also observed with same results This superiority of BA over Kinetin was in agreement with the

repor s of Satyanaray an *et al* (?008) n *Mi ci na pri i ens* Anothe observation in the study s highei co centrat ons of BA (3 ng l) reduced the numbe of multiple shoots (3 33) wl le K net n h ghe coi ce t ations increased the nu iber of shoots per explant

Except or the n ult ple sl oot product o i all otl er g owtl parameters observed i cvtokin ns were inferior to control There was also a cons derable decrease n shoot le igth with the icrease in coi centrat o of BA above 0 5 ng l Th s f nd ng is on agreement w th Puhan and Rath (2012) that the pe centages of bud break numbers of shoots and mean shoot length decreased with ncrease of concentrat ons of different growth regulators n *Aegle na i elos* In the micropropagat on of *F ax ni s c antha* also h gher concentrations (>5 ng/l) of growth l ormones proved less effect ve (Bisl t *et al* 2011)

Generally shoots of cultures n BA were stunted These esults support the f nd ngs of Sanjaya *et al* (2006) n *Sa ntali n album* that h gher co centiations of BA y elded more shoots however these shoots were dwarfed and retarded n growth In the present study t was also noted that c ltures i i BA vere assoc ated w th heavy leaf fall and rud nentary leaves Meanwh le n media contain ng K net n elongated shoots wei e observed compared to BA and cultures are devoid of defects associated witl BA (Plate 2)

It was observed that wi en BA was used n comb nat on w tl Kinetin culture abnormal ties assoc ated w th BA was reduced (Plate 3) The reason for this improved perfor nance is because kinet n prevents senescence or defol at on by prevent on of format on of hydrolases e g nucleases and proteases and causing mmobilization of nutr ents or their transport to cytok n n treated areas (Pul an and Rath 2012)

Thus the comb nat on 0 5 mg l BA+1 mg l Kinet n s found to be effect ve for multiple shoot induct on by cons der ng all the growtl factors Such

that t nduced a max n num nu nl er of 4 67 sl oots w tl 0 70 c ı length and 5 33 lea es H ghest nul ber of shoot buds per cultu e n *Acgle na n clo* was obta ned on the medium w tl l pp n BA + l ppm K net n n MS and 2 5 pp ı BA + 2 5 ppm Kınetın ın WPM whele sl oot t p explants produced > 4 axıllary shoot buds (Warr er *et al* 2010) In red sandal shoot t ps cultured ın s ngle cytok n n produced only 2 3 shoots but ın 1 mg l ^{1} BA+1 mg l K net n up to 8 shoots measur ng 3 5 cm was obta ned (S ta *et al* 1992)

5 3 2 Cytokınins and Auxins

When the aux ns were added along with cytokınins to the med a ıt resulted n delayed bud bleaks as well as leaf n t at on Further delay was observed w th the ıncrease n concentratıo ı of aux ns But t was observed that wl en the concentrat on of cytok n ns n the media was ncreased th s delay was reduced Bud break of 9 3ɔ days ı 0 5 mg l Kınet n + 0 1 n g l IAA was nproved to 7 25 days ın l mg l Kınetın + 0 1 mg l IAA For mult ple shoot product on also aux ns were noted to be reduc ng the effect of cytok n ns and th s was h gh w th respect to NAA Aı average of 3 27 shoots obta ned n l mg l BA was reduced to l 54 ın l mg l BA + 0 l mg l NAA (Plate 4) However n contrast aux ns promoted shoot elongat on and leaf product on ın comb nation wıth cytok n ns compared to cytok n ns alone Among the aux ns IAA was more effect ve for promotıng growth followed by NAA and IBA (Plate 5) Th s observat on supports the f nd ngs of Sanjaya *ct al* (2006) n sandal that add t on of a low concentrat on of NAA promoted sl oot growth by counteract ıg the nh b tory effect of BA on shoot elongatıon

5 4 ESTABLISHMENT OF CULTURES OF SANDAL THROUGH SUBCULTURING

Explants were sub cultured ın to d fferent med a for further growth and to na nta n them (Table 23) When the shoots formed ın pr mary culture was exc sed

and cultured in to the new med t ney fa ed to develop Transfe ring of mother explant tself to tl e ne med a showed p omis ng results For reduc ng the leaf fall ncreasing leaf area and to get tl e shoots elongated subcult ing n to WPM + 0 5 mg l or 1 mg l K netin was effect ve (Plate 6) Cu ltur ng of s ngle nodal segments excised from elongated *i n v t o* shoots in WPM supplemented with 0 5 mg l or 1 0 mg l BA induced axillary bud develop nent and K net n was found to be not effect ve for th s development (Plate 8)

It vas also noted tl at subculturing n to the same n ed a duced shoot buds up to 18 and shoots ve e elongatea n 0 5 mg l BA + 0 5 mg l Kii et n and 0 5 mg l BA + 1 mg l K net n Shoot length was also inc eased up to 1 7 cm (Plate 9) Similar observa ons were found in some studies l ke when the leaf segme nts of *Beti la util s* vas subcu tured n to tl e same shoot induction nedia Kii et n (2 0mg l) + NAA (1 0mg l) produced an ncreased number of shoots (Zaki *et al* 2011) Rekha *et al* (2010) reported tl at transfer of *Aegle mai nelos* shoots buds formed in 1 ppm BA + 1pp n K netin n MS and 2 5 ppm BA + 2 5 ppm Kinet n n WPM to fresh med um proved most effect ve n increas ng shoot growth rate

Subcultur g to 1 mg l BA+0 5 mg l IAA ncreased shoot length up to 3 cm number of leaves was 16 38 But th s cultures were noted w th h gh leaf fall reduced inter nodal length and leaves became rud mentary towards the t p But n 1 mg l Kinetin+0 5 mg l IAA shoot length was 4 cm ntermodal length was ncreased and number of leaves ranged 12 18 with no leaf fall (Plate 7) More over new shoot formation was l mited n these cultures Only o ie new shoot was formed n BA wh e in K netin no new shoots were formed These observat ons support the earl er ment oned find ngs that eventhough a xins nh bit new shoot formation t s effective for better g owth w th increased leaf area and shoot length

All the treatments failed to induce roots (Plate 10) More over the already reported protocols for rooting also was not effective to induce rooting in this study

5 5 SOMATIC EMBRYOGENESIS

In order to induce somatic embryos *ex vitro* internodes and leaves were used as explants and was cultured horizontally in the media These were cultured in four levels of BA (0 5 1 2 and 3 mg l) and Kinetin (0 5 1 2 and 3 mg l) in WPM (Table 24 and Table 25) Explants failed to respond for somatic embryo induction But internodal explants cultured in 0 5 and 1 mg l Kinetin induced shoot buds (17 %) and developed in to complete shoot Bisht *et al* (2011) was also observed with same results in *Fraxinus micrantha* when explants from mature trees were inoculated in culture medium but they did not show early and good response to micropagation The reason for this may be due to the absence of lag period between explanting and adaptation of explants to *in vitro* conditions as suggested by Amin and Jaiswal (1987)

When the same experiment was tried using explants of *in vitro* grown shoots media containing BA 0 5 and 1 mg l induced somatic embryos (Plate 11) directly in both leaf (80 % and 60 %) and internodal segments (100 %) However shoot bud induction (Plate 12) also was observed on internodal segments inoculated in BA 0 5 mg l (70 %) and 1 mg l (60 %) Explants in media containing Kinetin failed to respond to somatic embryogenesis

In the leaf explants somatic embryo formation was concentrated on the cut ends Similarly in *Betula utilis* Zaka *et al* (2011) reported callus formation from cut end of the explants

On internodal explants globular shaped somatic embryos were formed on its surface and these were then developed to the torpedo stage Further

development of somatic embryos was fa led One of the major constraint faced n son at c e nbryo nduct on as ter ı odal le ơth of ı *v t o* shoots were small When these were ɩsed for son at c e nbryo nduct oı bud break v s olse ved n the cut eıds Mo e oıe the tender leaves ıo ı t produce somat c e nb yos Th ck leaves w th large surface area were necessary for tl e product on of so nat c embryos

Summary

SUMMARY

The research work t tled *In t o* p opagatio ı of Sandal (Santalum album L) vas carr ed out n t ssue culture lab of College of Forestry du ıng 2011 2013 The sal ent fi dings of tl e study are summar sed below

1 Varıat on n fungal contamınat on associated w tl the t n e of collect on of explants was found Explants collected dur ng Noven ber Apr l showed contam nat on less than 11 per cent and collect on n aıny season was observed with h gh contam nat on rate (>90 %)

2 Treatments w tl system c fung c des a ıd contact fung cıdes Carbendaz n 50 % WP (Bav st n) and Maı cozeb 75 % WP (Indof l M 45) respectıvely were effect ve to control fungal contamınat on

3 Wl en the treatment t me w th Mancozeb 75 % WP was ncreased culture death also ncreased but n the case of Carbendaz n 50 % WP there was an nverse relat onsh p

4 In order to control bacterıal contam nat on surface ste ıl zat on w th 0 15 per cent HgCl for 10 m nutes was effectıve

5 WPM ned um was found to be super or over MS a ıd ½ MS w tl espect to the average shoot length and average number of leaves Moreover cultures n WPM were found to be healthy w th less leaf fall

6 Addıt on of BA or K net n s ngly or n combınat on was effect ve fo the product on of mult ple shoots than control BA was better than K net n and nduced up to 5 33 (1 mg l BA) shoots per culture bes des 1 mg l BAP+0 5 mg l Kınet n was also observed to produce results

7 Higher concentrations of BA (3 mg l) reduced the number of multiple shoots (3 33) while Kinetin higher concentrations increased the number of shoots per explants There was also a considerable decrease in shoot length with the increase in concentration of BA above 0 5 mg l

8 Except for the multiple shoot production all other growth parameters observed in cytokinins were inferior to control

9 Shoots of cultures in BA were stunted and were associated with heavy leaf fall and rudimentary leaves Kinetin cultures are devoid of these defects

10 The combination 0 5 mg l BA+1 mg l Kinetin is found to be effective for multiple shoot induction by considering all the growth factors Such that it induced a maximum number of (4 67) shoots with 0 70 cm length and 5 33 leaves An increase in the number of shoot bud formed and elongation of multiple shoots were observed when the explants cultured in 0 5 mg l BA+ 1 mg l Kinetin was transferred to the same media

11 When the auxins were added with cytokinins delayed bud break as well as leaf initiation was recorded This delay was increased with the increase in concentration of auxins

12 Multiple shoot induction capacity of cytokinins was reduced with addition of auxins and this was maximum with respect to NAA That is an average of 3 27 shoots obtained in l mg l BA was reduced to l 54 in 1 mg l BA+0 1 mg l NAA

13 Auxins promoted shoot elongation and leaf production in combination with cytokinins compared to cytokinins alone Among the auxins NAA was effective such that addition of 0 5 mg l NAA to 1 mg l BA increased the average and maximum shoot length from 0 44 and 0 83 cm to 0 73 cm and

1 03 cm In the same way addition of 0 5 ng l NAA to 1 mg l Kinetin increased the average sl oot le igth fro n 0 72 cm to 0 90 cm

14 For furtl er g o vth and development subcultur ng of ax llary shoots produced *n v t o* was done Cultui ng of single shoots exc sed fiom tl e n other explants failed to develop He ice tl e ew sl oots foimed were transfe ed to the ne v n ed a a ong w th n other explant

15 Subculture n to WPM+0 5 mg l or 1 mg l K net n was effect ve for reducing the leaf fall ncreas ng leaf area and to get the shoots elongated

16 Whei med a conta n ng combination of auxins and cytokimns was used for subculture increase n shoot length and number of leaf product on was observed However cultures n BA conta n ng med a were noted witl h gl leaf fall reduced nter nodal length and rudime itary leaves to vards the tip of shoot Wh le n med a with K net n shoot length was increased and no leaf fall vas observed

17 Aux ns n the media d d iot promote new shoot format oi

18 None of the treatments were effect ve for root induct on

19 For the induct on of somatic embryos *ex v t o* explants fa led to respond When the same experiment was tr ed using explants of *n v t o* grown shoots med a containing BA 0 5 and 1 mg l induced somat c embryos d rectly in leaf as well as nter nodes These explants were also noted w th shoot bud production

20 In the leaf explants somat c embryo format on was concentrated on the cut ends On nter nodal explants globular shaped somatic embryos were formed on its surface and these were then developed to the torpedo stage Furtl er development of somat c en bryos was fa led

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IN VITRO PROPAGATION OF SANDAL (*Santalum album* L.)

By

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ABSTRACT

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ABSTRACT

The study titled *In vitro* propagation of sandal (*Santalum album* L.) was carried out in tissue culture lab of College of Forestry during 2011-2013. The objective of the programme was to standardize a protocol for *in vitro* propagation of sandal through axillary bud culture and somatic embryogenesis.

Variation in fungal contamination associated with the time of collection of explants was found. Explants collected during November-April showed less contamination (<11%) compared to rainy season (>90 %). Treating with combination of Mancozeb 75 % WP (Indofil M 45) and Carbendazim 50 % WP (Bavistin) fungicides was effective to control fungal contamination. However different combinations were effective depending on the time of collection of explants. In order to control bacterial contamination surface sterilization with 0.15 per cent $HgCl_2$ for 10 minutes was effective.

WPM medium was found to be superior over MS and ½ MS with respect to the average shoot length and average number of leaves. Moreover cultures in WPM were found to be healthy with less leaf fall. Addition of BA or kinetin singly or in combination was effective for the production of multiple shoots than control. Higher concentrations of BA (3 mg l^{-1}) reduced the number of multiple shoots while in kinetin at higher concentrations increased the number of shoots per explants. There was also a considerable decrease in shoot length with the increase in concentration of BA above 0.5 mg l^{-1}. Except for the multiple shoot production, all other growth parameters observed in cytokinins were inferior to control. Moreover, shoots of cultures in BA were stunted and were associated with heavy leaf fall and rudimentary leaves. But in kinetin cultures are devoid of these defects. Thus the combination 0.5 mg l^{-1} BA + 1 mg l^{-1} kinetin was found to be effective for multiple shoot induction by considering all the growth factors. Auxins in combination with cytokinins resulted in delayed bud break, leaf initiation and reduction of multiple shoot induction compared to cytokinins alone.

However auxins promoted shoot longation and leaf production in combination with cytokinins compared to cytokinins alone

Subculture using single shoots excised from the mother explants failed to develop while transferring of new shoots formed along with primary explants was effective Subculture to media containing kinetin increased the shoot length leaf area and reduced leaf fall When media containing combination of auxins and cytokinins was used for subculture increase in shoot length and number of leaf production was observed However cultures in BA containing media were noted with high leaf fall reduced inter nodal length and rudimentary leaves towards the tip of shoot While in media with kinetin shoot length was increased and no leaf fall was observed Auxins in the media did not promote new shoot formation Root induction through incorporation of different auxins in the media and pulse treatments failed to induce rooting in the cultures

Somatic embryos failed to develop from *ex vitro* explants But from these *ex vitro* inter nodal explants in 0 5 and 1 mg l^{1} kinetin shoot development was observed Direct embryogenesis could be induced from *in vitro* explants cultured in media containing BA 0 5 and 1 mg l On inter nodal explants globular shaped somatic embryos were formed on its surface and these were then developed to the to pedo stage Further development of somatic embryos was arrested