"*IN VITRO* PROPAGATION OF BIG-LEAF MAHOGANY (*SWIETENIA MACROPHYLLA* KING) THROUGH TISSUE CULTURE"

By,

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THESIS

Submitted in partial fulfillment of the requirement for the degree

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Declaration

I hereby declare that this thesis entitled **"*In vitro* propagation of big-leaf mahogany (*Swietenia macrophylla* King) through tissue culture"** is a bonafide record 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 to me.

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We, the undersigned members of advisory Committee of Mr. **Girish Sankri,** a candidate for the degree of **Master of Science in Forestry,** agree that this thesis entitled **"*In vitro* propagation of big-leaf mahogany (*Swietenia macrophylla* King) through tissue culture"** may be submitted by Mr. **Girish Sankri** in partial fulfillment of the requirement for the degree.

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Dedicated to My Grandparents and Friends

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ABBREVIATIONS

ABA	Absiscic acid
Avg.	Average
BA/BAP	Benzyl adenine
B5	Gamborg's (1976) medium
cm	centimeter
^0C	Degree Celsius
Fig.	Figure
Hr	hour
et. al.	co-workers
GA_3	Gibberlic acid
g/gm	gram
$HgCl_2$	Mercuric chloride
IAA	Indole 3- acetic acid
IBA	Indole 3-butyric acid
IPA	isopentenyl adenosine
Max.	Maximum
mg	milligram
Min.	Minimum
µm	micro molar
MS	Murashige and Skoog (1962) medium
NAA	α-naphthaleneacetic acid
No.	Numbers
ppm	Parts per million
RH	Relative humidity
TDZ	Thidiazuron
WPM	Woody Plant Medium of Lloyd and McCown (1980)
2-ip	2-isopentenyladenine
2.4-D	2, 4-Dichlorophenoxy acetic acid

INTRODUCTION

INTRODUCTION

Trees as individuals or as principal components of forests that form the green mantle over the earth play multiple roles in nature. Considering the productive, protective, ecological, conservational, educational and recreational roles of forests, the extent of research and developmental activities in forestry are drearily low. Demographic changes, the growing size of population of the world and increasing urbanization have had and will endlessly have a major impact on forest cover and condition, demand for wood and non-wood forest products. While the world's forest area has been progressively decreasing, there has been a continued increase in demand for wood products (Rathore *et al*., 2007).

Swietenia macrophylla King is a member of family Meliaceae, native to Honduras. Mahagony is a large sized, fast growing evergreen tree with a broad round symmetrical crown. It is commonly known as big-leaf mahogany, Brazilian mahogany, Hondurans mahogany and large-leaved mahogany. It is an evergreen tree, which grows up to 30-35 m. It is found in various forest types, grows at elevations from sea level up to 1,400 metres, in areas with an average annual precipitation of 1,600 to 4,000 millimetres and an average temperature of 23 to 28°C. Mahogany prefers rich, deep and well-drained soils. Leaves long, alternate, glabrous, paripinnate. Big-leaf mahogany has a patchy distribution from southern Mexico through Central America and south to Brazil and Bolivia. Bark is grey and smooth when young, turning dark brown, ridged and flaky when old. The fruits of *Swietenia macrophylla* are called "sky fruit", because they seem to hang upwards from the tree. Big-leaf mahogany is one of the most valuable timbers for making furniture in the world due to the decorative and attractive timber with good technical characteristics. It is widely planted in the tropics in reforestation and afforestation programmes. In agroforestry systems it is used for shade and fuel wood.

Among the *Swietenia* genus which consists of five species, *Swietenia macrophylla* and *Swietenia mahagoni* have got good timber value. Wood of big-leaf mahagony is used in making furniture, fixtures, inlay, boat, caskets and many more. Due to its high timber value the tree has been over exploited and the species has become vulnerable. In a vital move for the future protection of this majestic tree, big-leaf mahogany was included on Appendix II of the Convention on International Trade in Endangered Species (CITES) (Blundell, 2004). The tree is also valued for its medicinal uses. The "sky fruit" concentrate is sold as a natural remedy that is said to improve blood circulation and skin. It is also said to have viagra-like qualities regarding erectile dysfunction. Cosmetic products are produced from the oil of the seeds. The infusion of the seeds is used as tonic, painkiller and against typhoid fever It is reported that water extracts of seed have the anti termite properties (Mokabel and Gowda, 2000). A gum is produced from cuts in the bark and it is marketed in both pure form and mixed with other gums. The bark is used for dyeing and tanning leather (World Agroforestry Centre, 2009). The species is facing extinction, due to over exploitation for its valued timber (Wikipedia, 2007).

Production of mahagony wood can be increased only by extensive plantation of this species using superior trees. Economic losses can be reduced by developing varieties resistant to important diseases and pests. Thus owing to high demand in market, large scale production of mahagony is needed which requires good quality propagules in large numbers. This species is mostly propagated through seeds. Development of abnormal seedlings has been reported by Kader and Sethalakshmi, (2001). Sexually reproduced progeny from the seeds of the selected superior trees need not be superior like mother trees and also they will not be uniform among themselves. When seeds are used in reforestation programs, seeds as well as plants are severely damaged by the shoot-borer, *Hypsipyla grandella* Zellar. (Schottz *et al*., 2007; Hauxwell, 2001) which inhibits germination and establishment of plants.

Under such circumstances, vegetative propagation of the selected plus trees will be ideal to pass on all the favourable attributes of this tree to its progeny. In vegetative propagation all the progeny will be uniformly better performers and identical to their selected parents. In conventional methods of vegetative propagation like grafting, budding and layering, plantlets produced will be quiet insufficient to meet the requirements of thousands of planting material required in forestry programmes. This problem can be overcome through micropropagation. This method has advantage over the conventional methods because it facilitates producing large number of plantlets of the superior parents through *in vitro* techniques using a relatively smaller proportion of explants. This technique is of great importance in clonal forestry to overcome the constraints like scarce seed supplies, germination problems and long regeneration time (Leaky, 1987).

Under this background, the present project has been formulated with the objective of standardizing a protocol for micropropagation of big-leaf mahogany (*Swietenia macrophylla* King) through tissue culture.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Discovery of the cell and subsequent propounding of the cell biology has laid the foundation for development of the science of tissue culture. More than two century ago pioneering experiments on wound healing in plants by Duhamel-duMoceau (1756), have demonstrated impulsive callus formation on the decorticated region of elm plants. These studies, according to famous biologist Gautheret (1941), could be considered as preface for the development of plant tissue culture as a science.

In 1900 well-known German Botanist Haberlandt (1902) explicated the concept of *in vitro* cell culture. He was the first to culture isolated, differentiated cells in a nutrient medium containing glucose, peptone and Knoop's salt (Knoop, 1805). The first known plant growth regulator, Indole-3-acetic-acid was identified by Kogl (1934) and later by Thimman (1935). This has made it possible to control growth of plants, tissues and cells. Tissue culture has been commercially applied in production of true-to type plants at rapid rate compared to the conventional methods (Levy, 1981).

Micropropagation of woody species through tissue culture has dawdled far behind, due to the difficulties experienced at primary culture establishment, root induction and partially due to the existence of phenolic compounds in tissues. As quoted by Anand and Bir (1984) slow growing habit of trees and long dormancy pose difficult problems for tissue culturists. They have also noticed that calli of trees are hard to differentiate. Over the past three decades considerable advancement has been achieved on Micropropagation methods of forest trees. Many of the soft wood species have been successfully propagated through tissue culture. Some recent reports available comprise, *Juniperus*

oxycedrus (Gomez and Segura, 1994), and different species of picea (Arnald and Erikson, 1986), *Pinus caribaea* Mor. var. *Hondurensis* (Ifeoma and Eneobong, 2008), *Pinus roxberghii* (Parasharami *et al.*, 2003), *Pinus wallichiana* (Mathur and Nadgauda, 1999, Bastola *et al.*, 2000, Malabadi and Nataraja, 2007), *Prunus serotina* (David *et al.*, 1985), *Quercus euboica* (Epaminondas and Maria, 2007) and *Quercus floribunda* (Purohit *et al.*, 2002) etc.,

The literature pertaining to *Swietenia macrophylla* and other important broad leaved tree species in which micropropagation has been carried out are reviewed here under.

2.1 MICROPROPAGATION

'Micropropagation' that is mass production of selected individuals through *in vitro* techniques – is of great importance in clonal forestry to trounce the constraints like inadequate seed supplies, germination problems, long regeneration time, etc. (Leaky, 1987). It has long been evident that genetic gains can be captured by clonal propagation (Durzan, 1998) and under such circumstances *in vitro* propagation is known to be a promising method in many tree species especially where conventional methods are not economically realistic (Bonga, 1982).

Plant tissue culture is defined as the procedure of cultivating cells, tissues or organs of plants on synthetic media under aseptic conditions. During the last few decades tissue culture has made great progress in the fields of fundamental botany and applied science like, agriculture, horticulture, forestry etc.

2.2 PLANT TISSUE CULTURE: A HISTORIC PERSPECTIVE

The concept of developing whole plant from a single cell came from the cell theory proposed by Schwann (1839). The theory states that each living cell of an organism if provided with proper environment would be capable of independent development. Cell theory gave birth to the concept of totipotency which forms the basic principle behind tissue culture. The principle states that it should be possible to produce an organism from any of its nucleated cells since all information needed to specify an organism is contained in its DNA.

Haberlandt (1902), first time cultured cells on artificial medium. He observed obvious growth of cells on the media. But the cultures lacked cell division. Later, White (1931) found out that the material used by Haberlandt were all mature differentiated cells lacking any meristematic activity. Research during the later period (1940-1950) was mainly concentrated on determining the nutritional requirements for obtaining sustained growth of tissues in cultures. The discovery of plant hormones and vitamins contributed tremendously to the development of tissue culture. The classical demonstration of hormonal control of organogenesis in callus cultures came from Skoog and Miller (1957). They demonstrated that shoot and root initiation could be regulated by a subtle ratio of auxin and cytokinin.

Muir and Hildebrandt (1954) reported the growth of cell cultures in liquid medium for the first time. Later Jones *et al*. (1960) were successful in inducing growth in single isolated cells. The field of protoplast culture was thrown open by Cocking (1960) when he isolated protoplast for the first time. Over the years protoplasts have been isolated from different plant parts like

roots, leaves, coleoptiles, fruit tissues, pollen mother cells etc. Isolation and culture of protoplasts guided to the innovation of somatic hybridization, which later developed as a potent tool for research in somatic cell genetics.

Morel (1960) engaged meristem culture for the first time for clonal propagation. This technique is also used to produce virus free plants (Walkey, 1980). Guha and Maheswari (1964) cultured anthers for the first time to produce haploid cultures. This technique was also used to double the chromosome number of plants developed from anther to produce homozygous diploids (Maheswari and Rangaswami, 1963).

One of the applications of tissue culture in plant breeding is embryo culture. Various hybrid embryos have been successfully cultured and germinated *in vitro* (Maheswari and Rangaswami, 1963). This can facilitate production of distant hybrids. This area is now on limelight since it leads to the isolation of plants which are tolerant to pesticides, alkalinity, pathogens etc. Nickell (1964) was the first to demonstrate that variants could be isolated from sugarcane cultures. Later many investigators worked on embryo culture (Nabors *et al*., 1975; Smith and McComb, 1981). One of the major advantages is that the induction and screening of mutants can be done at cellular level. This technique is used for the conservation of rapidly depleting biodiversity. This has been employed in preserving the tissues of many species like chrysanthemum (Bannier and Steponkus, 1972), datura (Bajaj, 1976), and nicotiana (Maddox *et al*., 1983).

Secondary metabolites of plants which are having industrial and medicinal importance can be extracted from the cell cultures. This technique has been tried successfully in many plants like *Dioscorea deltoidea* (Kaul *et*

al., 1969), *Morinda citrifolia* (Zenk *et al.*, 1975), and *Nicotiana tubacum* (Tabata and Hiraoka, 1976).

Micropropagation through tissue culture in tree species

The big boom in plantation forestry during the past two decades has created an ever increasing demand for quality planting materials. Micropropagation because of its inherent advantage is regarded as the best way to meet this demand. With this view, the technique has been tried in many economically important trees and various other woody species including jatropha. A very brief account of some of the salient works carried out in mahogany and other important species are reviewed below.

Swietenia species

There are several reports on *in vitro* tissue culture of mahogany. Lee and Rao (1988), using stem segments from seedlings and from the basis of 10-year-old trees coppice, observed the formation of adventitious shoots from calli, when the nodal segments were cultivated on MS medium supplemented with 8.87 or 22.2 μM BA. Maruyama and Ishii (1997) reported the use of WPM (Lloyd and McCown, 1980) mineral medium supplemented with 10 μM zeatin for multiplication stage of *Swietenia macrophylla*. Medium B5 (Gamborg *et al*., 1968), in its original composition or modified, supplemented with 0.09 to 4.44 μM BA and/or 0.1 to 4.9 μM IBA, induced polybud formation.

The regeneration of adventitious buds from epicotyl explants was reported by Valverde-cerdas *et al*. (1998). The basal medium was constituted of half-strength MS salts and was supplemented with BA. Venketeswaran *et al*. (1988)

tested MS and WPM culture media containing kinetin (4.65 μM) or BA (17.8; 44.85 and 89.77 μM). The work of Albarran *et al*. (1997) described the development of axillary buds from nodal segments cultured on half-strength MS mineral medium containing 8.87 μM BA and 11.42 μM IAA in only 10% of the explants. Lopes *et al.* (2001) reported the multiplication of the same species on MS medium supplemented with 4.44 μM BA and 0.54 μM NAA, but did not indicate the multiplication rate. This work describes the results of multiplication experiments in which two mineral salt formulations and two cytokinins, isolated or in combinations, were tested for micro propagation of big-leaf mahogany.

Multiplication stage of micro propagation of *Swietenia macrophylla* was developed using juvenile material (Schottz *et. al*., 2007). Seeds were germinated in MS solid culture medium. Shoot formation from seeds occurred during five months. These explants were excised, each containing one axillary bud, and transferred on multiplication media. The media was supplemented with BA (2.5 to 50.0 μM), 2-ip (1.1 to 8.8 μM), combinations of BA (2.5 to 50.0 μM) and 2-iP (2.2 μM). When BA was tested alone, the maximum point of multiplication rate average was obtained on medium containing 23.61 μM, while 2-iP did not induce bud multiplication. The maximum point of multiplication rate average was 5.7 μM, obtained when the MS culture medium was supplemented with 18.51 μM BA and 2.2 μM 2-iP. The nodal segments and apical buds of mahogany (Collado *et al.,* 2006) were cultivated under controlled light and temperature, in MS medium with 50% reduction of nitrates and supplemented with five concentrations of cytokinin (0.1, 0.2, 0.3, 0.5 and 1.0 mg l^{-1} BA). The establishment of vigorous shoots of mahogany was observed at 0.2 mg l^{-1} BA using nodular segments as explants in the initial step of direct propagation. Micropropagation of *Swietenia macrophylla* × *Swietenia mahogani* (hybrid mahogany) was reported by Rodriguez *et al.* (2003) in which best explant quality

and multiplication rates were achieved in media supplemented with BA at 0.50 mg l^{-1}. The use of IBA at 0.50 mg l^{-1} for rooting induction achieved favourable results.

Plantlets were produced by culturing nodal segments in a half-strength MS medium supplemented with different combinations of NAA and BA, specified by the Central Compositional Rotable Statistical Design (CCRSD) method, within a range of 0-3 mg l^{-1} for both hormones. An optimal axillary bud elongation was observed on a medium containing BA 1.94 mg l^{-1} and NAA 0.38 mg l^{-1}. Mahogany plantlets obtained were successfully acclimated. (Tacoronte, *et al.*, 2004). Regeneration of adventitious buds was achieved by Valverde-cerdas (1998) from epicotyl explants from *Swietenia macrophylla.* Explants were cultured on half-strength modified MS basal medium supplemented with BA. *S. macrophylla* responded positively to the presence of BA in the culture medium. In a study conducted by Rocha and Quoirin (2004) explants (leaf and root fragments from *in vitro* cultured plants) were cultured in petri dishes containing modified MS culture medium, with three-quarters of salt concentration, vitamins, 30 g.l^{-1} sucrose and 7 g l^{-1} agar. The growth regulator combinations used were NAA (0.11 µM and 0.54 µM) and one type of cytokinin, kinetin (1.2 µM, 2.3 µM, 4.7 µM and 9.3 µM), BA (2.2 µM, 4.4 µM and 8.8 µM) or 2-iP (2.5 µM). Proembryogenic calluses from mahogany were induced from main and secondary roots, leaves and apical meristems form *in vitro* germinated plantlets (Gonzalez and Pena, 2007).

In vitro regeneration techniques of mahogany (*Swietenia macrophylla*), developed through epicotyl segments from mahogany plantlets germinated in culture medium (Brunetta *et al.*, 2006). The epicotyl segments were inoculated on MS medium supplemented with different combinations of BA and NAA. Results had shown that after 40 days of inoculation callus formation was

observed at the ends of the segments. On media containing 0.50 mg l^{-1} NAA and 0.50 mg l^{-1} BA, 0.25 mg l^{-1} NAA and 1.00 mg l^{-1} BA and 0.50 mg l^{-1} NAA and 1.00 mg l^{-1} BA, 100% of explants showed callus varying the intensity and texture.

Acacia species

Multiple shoots were developed from axillary buds excised from *in vitro* grown seedlings of *Acacia auriculiformis* on Gamborg's (B5) basal medium supplemented with coconut milk and BA 1.0 µM concentration. These shoots, transferred individually to B5 medium, containing IAA 0.1 µM or NAA 1 µM or 0.1 µM produced roots (Mittal *et al*., 1989).

Micropropagation technique for *Acacia catechu* was standardized by Selvan *et al.* (2003). Nodal and terminal explants were obtained from mature trees and plantlets obtained by germinating seed *in vitro*. The explants were cultured in MS medium with 100 mg l^{-1} myo-inositol, 3% sucrose and 0.8% agar. Basal medium was supplemented with cytokinins (BA and kinetin) and auxins (NAA or IAA) alone and in combination. The maximum percentage of shoots were achieved on MS medium with BA (4.0 mg l^{-1}) and NAA (0.5 mg l^{-1}) along with other additives - adenine sulfate (25 mg l^{-1}), ascorbic acid (20 mg l^{-1}) and glutamine (150 mg l^{-1}). Low levels of auxin (NAA 0.5 mg l^{-1}) with BA had a synergistic effect on the per cent shoot proliferation. Rooting was done on one-fourth strength MS medium supplemented with IAA (2.0, 0.5 mg l^{-1}) and 1.5% sucrose. Results have also depicted that the juvenile explants had a higher rate of success than the mature explants. *In vitro* somatic embryogenesis was achieved (Nanda *et al.*, 2003b) from immature zygotic embryos of *Acacia catechu* on MS medium containing BA and 2,4-D. The embryogenic callus line was developed

from non-embryogenic callus on MS basal medium supplemented with 2.2-4.4 µM BA and 4.5 µM 2,4-D.

Kaur and Kant (2000) have described clonal propagation of *Acacia catechu* using shoot apices as explants. Murashige and Skoog medium supplemented with BA at 1.5 mg l^{-1} and kinetin at 1.5 mg l^{-1} has given the maximum number of (12) shoots. *In vitro* shoots were rooted on 1/4 strength MS medium with IAA at 3.0 mg l^{-1} and 1.5% sucrose. Cotyledon explants of *A. catechu* were cultured on MS medium supplemented with 2,4-D (3 mg l^{-1}) and BA (0.5 mg l^{-1}) for callus proliferation (Kaur and Kant, 1999). Shoot bud differentiation and multiple shoot formation were achieved from cell aggregates on liquid MS medium containing BA (3 mg l^{-1}) and NAA (mg l^{-1}). Isolated shoots were rooted in MS medium (1/4 strength) containing IAA (3 mg l^{-1}). Kaur *et al.* (1998b) reported highest number of shoots (eight to ten) using nodal explants from mature elite trees of *A. catechu* on MS medium supplemented with BA (4.0 mg l^{-1}) and NAA (0.5 mg l^{-1}). Addition of adenine sulphate (25.0 mg l^{-1}), ascorbic acid (20.0 mg l^{-1}) and glutamine (150.0 mg l^{-1}) to the medium was found beneficial for maximum shoot bud induction. Excised shoots were rooted on 1/4-strength MS medium with IAA at 3.0 mg l^{-1} and 1.5% sucrose.

Nanda *et al.* (2004) achieved bud sprout from mature nodal explants of 10-years-old tree of *Acacia mangium,* on MS basal medium supplemented with BA 1.0 mg l^{-1}, GA_3 1.0 mg l^{-1} and IAA 0.05 mg l^{-1}. After 13–14 days of culture excised shoots were rooted on half-strength MS basal salts supplemented with 0.5 mg l^{-1} IBA and IAA and 2 % sucrose. Nodal segments from the seedlings of *A. mangium* could give rise to plantlets when cultured on MS media containing 0.5 mg l^{-1} BA (Ahmed, 1990). Xie and Hong (2001) reported somatic embryogenesis and whole plant regeneration in callus cultures derived from

immature zygotic embryos of *A. mangium*. Embryogenic callus was induced on MS medium with combinations of TDZ 1.0-2.0 mg l^{-1}, IAA (0.25-2.0 mg l^{-1}) and a mixture of amino acids.

In a study conducted by Nanda and Rout (2003a) *in vitro* somatic embryogenesis and plant regeneration was achieved in callus cultures from immature zygotic embryos of *Acacia arabica* (*A. nilotica*) on semi-solid MS basal salts supplemented with 8.88 µM BA, 6.78 µM 2,4-D and 3 % sucrose. Maximum number of somatic embryos per callus (72.6) was on medium containing 6.66 µM BA and 6.78 µM 2,4-D. Cotyledonary nodal explants of *Acacia nilotica* differentiated multiple shoots on B5 medium supplemented with BA (1.5 mg l^{-1}). Individual shoots, when transferred to B5 medium containing IAA (2.0 mg l^{-1}) produced healthy roots in 100 per cent cultures (Dewan *et al*., 1992).

A protocol for *in vitro* propagation of *Acacia sinuata* has been developed (Vengadesan *et al.*, 2003) using nodal explants from a mature elite tree. Maximum shoot proliferation (75.2%) was achieved in MS medium supplemented with 8.9 µM BA, 2.5 µM TDZ, and 135.7 µM adenine sulfate. GA_3 at 1.8 µM promoted shoot elongation. *In vitro* regenerated shoots produced prominent roots when transferred to half strength MS medium containing 7.4 µM IBA.

Aegle marmelos

Cotyledonary nodes of *Aegle marmelos* were cultured on MS medium supplemented with BA (0–8.8 µM), kinetin (0–9.4 µM), and IAA (0–1.14 µM) alone and in combinations (Nayak and Behera, 2007). Highest regenerative

response was observed on medium containing 6.6 μM BA+1.14 μM IAA. Cultures on kinetin supplemented medium showed very poor response. Rooting was best in medium supplemented with 14.7 μM IBA. Plantlets were acclimatized and transferred to the field. Rapid clonal multiplication of *Aegle marmelos* was achieved by enhanced axillary bud proliferation on MS nutrient medium (Ajithkumar and Seeni, 1998). Bud break was dependent on cytokinin supply, but the synergistic combination of BA (2.5 mg l^{-1}) and IAA (1.0 mg l^{-1}) induced the formation of shoots. Shoot cuttings were best rooted in half-strength MS medium with IAA (0.5 mg l^{-1}) or IBA (10.0 mg l^{-1}).

Ailanthus triphysa

Nodal explants were used and maximum bud initiation was obtained on MS medium supplemented with 2.0 mg l^{-1} BA and 2.0 mg l^{-1} kinetin. Rooting was obtained on half strength MS medium containing 4.0 mg l^{-1} IAA and 0.4 mg l^{-1} IBA (Natesha, 1999).

Albizzia lebbeck

Micropropagation *Albizzia lebbeck* by tissue culture was achieved (Mamun *et al.*, 2004) using cotyledon, nodal segment of *in vitro* grown seedlings and nodal segments of field grown mature trees. Among different combinations of growth regulators, BA and NAA combination with MS medium was proved best. Highest callus induction was observed in MS medium supplemented with 2 mgl^{-1} BA+0.2 mgl^{-1} NAA. Multiple shoots were produced from the hypocotyl, root, cotyledon and leaflet explants of *A. lebeck*, both directly and indirectly. Rooting was achieved on transfer of the shoots to MS medium containing 2.0 mg l^{-1} IAA (Paramjith *et al.*, 1982).

Albizia guachapele

Regeneration of adventitious buds was achieved (Valverde-cerdas *et al.*, 1998) from hypocotyl explants of *Albizia guachapele* (guayaquil). Explants were cultured on half-strength modified MS basal medium and 5 concentrations of BA were tested. *A. guachapele* responded positively to the presence of BA in the culture medium.

Anacardium occidentale

Hybrid plantlets of cashew were regenerated by *in vitro* culturing of embryos from immature nuts (Aliyu and Awopetu, 2005). Explants were cultured into basal MS agar medium and MS medium supplemented with 1 µM each of NAA, BA and GA_3. It is reported that MS medium supplemented with 1 µM of GA_3 supported germination and growth of embryos.

Anogeissus pendula

Joshi *et al.* (1991) were successful in obtaining multiple shoots from cotyledonary and epicotyledonary nodes. Cotyledonary nodes, which produced 15-20 shoots, were found better than epicotyledonary nodes, which produced 4-5 shoots when cultured on MS medium containing 1.0 mg l^{-1} BA and 0.1 mg l^{-1} IAA. Shoots were rooted on half strength MS medium containing 15 mg l^{-1} of IBA and 0.1 mg l^{-1} of kinetin.

Artocarpus heterophylus

Nodal explants of Singapore jackfruit (*A. heterophyllus*) were cultured in half-strength MS medium supplemented with 1, 2, 4, 6 and 8 mg l^{-1} BA and kinetin (Adiga *et al.,* 2004). Results shown that MS medium supplemented with 2 mg l^{-1} BA has given highest number of shoots, adventitious buds per shoot and number of leaves per shoot. Chavan *et al.* (1996) reported establishment of jack fruit explants on media (both MS and WPM) supplemented with BA 10 mg l^{-1} + NAA 5 mg l^{-1} + GA 5 mg l^{-1}. Jackfruit was micropropagated (Singh and Tiwari, 1996) by culturing nodal explants on modified MS medium containing BA 18.0 mg l^{-1} + IBA 0.2 mg l^{-1}. Highest number of shoots (4-5) was produced after repeated sub culturing on medium supplemented with 2.0 mg l^{-1} BA and IBA at 0.2 mg l^{-1}. These shoots were rooted *in vitro* in half-strength MS medium containing 1.0 mg l^{-1} IBA. Roy *et al.* (1996) reported multiple shoot production from shoot tip and nodal segments of *A. heterophylus* cultured on MS medium supplemented with BA (2.5 mg l^{-1}) and IAA (0.5 mg l^{-1}). Excised shoots were rooted on half strength MS medium containing NAA and IBA each at 1.02 mg l^{-1} concentration.

Azadirachta indica

Root and shoot explants of neem were cultured on MS medium supplemented with different concentrations and combinations of BA (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg l^{-1}) and NAA (0.05 mg l^{-1}) to develop a protocol of regeneration (Shahin-uz-zama *et al.,* 2008). Regenerated shoots were subcultured and rooted on a medium supplemented with IBA and IAA. Leaf explants of neem seedlings were cultured on MS medium supplemented with NAA. Calli from the most explants showed root differentiation (Kota *et al.,*

2006). Azadirachtin was produced by hairy root cultures of neem (Satdive *et al.,* 2005). Hairy roots were cultured on MS and Gamborg's media without growth regulators.

In vitro clonal propopagation of neem was achieved through axillary shoot proliferation (Chaturvedi *et al.,* 2004). Half MS medium was used for establishment of nodal segment cultures. Multiple shoots were observed in 1/2 MS medium containing BA 1 µM + GA_3 0.5 µM and shoot elongation was achieved on full MS medium supplemented with BA 1 µM. Shoots rooted on 1/4 MS medium fortified with IBA 0.5 µM. Evaluation of azadirachtin production in micro propagated plantlets was attempted by Roshni (2003). It was reported that the MS medium is better than WPM. MS medium when supplemented with kinetin showed positive effects in terms of establishment and growth of the culture.

Somatic embryogenesis was obtained in neem (*Azadirachta indica*) using mature seeds, which were cultured on MS medium supplemented with TDZ. TDZ was very effective and induced somatic embryogenesis across a wide range of concentrations (1-50 µM). However, somatic embryogenesis was accompanied by callus formation at concentrations of 20 µM and above. Plants were regenerated from both directly formed somatic embryos and somatic embryos derived from cell suspensions placed on semisolid medium devoid of growth regulators. Regenerated plantlets continued to grow after transfer to greenhouse environment and were similar phenotypically to zygotic seedlings (Murthy and Saxena, 1998).

A micropropagation protocol was developed for *A. indica* using axillary bud as explants. Multiple shoots were obtained on MS medium supplemented

with 0.1 mgl^{-1} BA. Rooting of shoots occurred best on MS medium containing 0.1 mgl^{-1} IBA (Anaz and Vijayakumar, 1996). Organogenesis of germinating seeds of *A. indica* was observed when maintained in MS medium supplemented with various growth substances (Rier and Obasi, 1996). Eeswara *et al.* (1997) reported that shoots of *A. indica* could be induced from leaf explants when cultured on MS medium containing BA 1.0 mg l^{-1}, kinetin 0.8 mg l^{-1} and adenine sulphate 6.0 mgl^{-1}.

Balanites aegyptiaca

Micropropagation procedure for *Balanites aegyptiaca* was developed using axillary bud explants obtained from mature trees. Cultures were established in MS medium supplemented with 2.5 mg l^{-1} BA and 0.1 mg l^{-1} NAA (Ndoye *et al.,* 2003). In the study effects of BA and kinetin on shoot growth and proliferation *in vitro* were investigated. Shoot multiplication was observed on MS media supplemented with 2.5 mg l^{-1} BA. Results revealed that presence of BA or kinetin significantly affect the shoot elongation. Rooting of shoots *in vitro* was achieved on MS medium containing 20 mg l^{-1} of the auxin IBA.

Barringtonia racemosa

Behbahani *et al.* (2007) reported a protocol for *in vitro* propagation of *B. racemosa* using leaf explants. Calli were produced on WPM containing different concentrations of 2,4-D. Woody Plant Medium containing kinetin at 2 mg l^{-1} + IBA 0.2 mg l^{-1} and kinetin 2 mg l^{-1} + NAA 0.4 mg l^{-1} resulted in shoot multiplication. The good rooting percentage and highest number of roots per shoot were achieved on WPM medium supplemented with 0.3 % of active charcoal and 0.8 mg l^{-1} of IBA.

Cabralea canjerana

Multiplication experiments in *Cabralea canjerana* were carried out using nodal segments, excised from *in vitro* germinated plants (Rocha *et al.*, 2007). The explants were inoculated in MS or WPM culture medium, supplemented with BA and/or 2-iP at 2.5 or 5 µM. The nodal segments cultured in the presence of 2.5 µM BA showed the best result, with a multiplication rate of 1.77 per month on MS medium. The rooting of the microcuttings was 87.5 per cent; when they were culture of media supplemented with 5 µM IBA.

Caesalpinia pulcherima

Nodal explants from the trunk sprouts were used for callusing on MS medium containing NAA alone and 2, 4-D in any combination. The greatest rooting was obtained in medium containing IAA and cytokinin (Rohman *et al*., 1996).

Calamus species

Embryo culture of *Calamus latifolius* was achieved in three media namely MS medium and WPM medium supplemented with growth hormones. Seedlings from embryo culture grew well on WPM medium with growth regulators when compared to other medium. When BA supplied alone at 4.54-8.88 µM produced more shoot length than supplied in combination with NAA (Meitram and Sharma, 2006).

In *Calamus tenuis* plantlet regeneration through organogenesis has been reported by Sett *et al.* (2002). Explants produced callus on MS medium

containing 9.05 µM 2,4-D. Shoot differentiation was observed on MS medium supplemented with 22.20 µM BA and 8.04 µM NAA. Regenerated shoots were rooted on MS medium with 0.49 µM IBA.

Capparis deciduas

In *Capparis deciduas* an efficient procedure for direct somatic embryogenesis has been developed by culturing mature zygotic embryos on MS liquid medium with 2,4-D (0.1 mgl^{-1}) and BA (0.5 mgl^{-1}). Explant treated with ABA promoted maturation of somatic embryos and BA (1 mg l^{-1}) promoted germination (Tyagi *et al.*, 2005).

Cedrela odorata

Valverde-cerdas *et al.,* (1998) reported that regeneration of adventitious from hypocotyl explants of *Cedrella odorata*. Explants were cultured on half-strength modified MS basal medium supplemented with 5 concentrations of BA. It is reported that *C. odorata* required media supplementation with both cytokinin (BA) and auxin (NAA) to induce adventitious buds. Results are presented by Rodriguez *et al.* (2003) in a study on the micropropagation of Cedrela using seeds, multiplication rates were achieved in media supplemented with BA at 0.50 mg l^{-1}. The use of IBA at 0.50 mg l^{-1} for root induction achieved favourable results.

Cleistanthus collinus

Quraishi *et al.* (1994) micropropagated *Cleistanthus collinus* using nodal explants. Explants were cultured on MS medium supplemented with BA (2.2

µM). Shoot proliferation was enhanced when the BA concentration was lowered to 1.1µM. Rooting was achieved on half strength MS medium with 22.8 µM IAA.

Crataeva species

Micropropagation of *Crataeva adansonii* through multiple shoot formation from axillary bud cultures was achieved (Sharma *et al.,* 2003). Optimum response in shoot multiplication was recorded on MS medium supplemented with BA at $3mgl^{-1}$ and 13.3 mg l^{-1}, and NAA at 0.05 mgl^{-1} and 0.27 mg l^{-1}. An efficient continuous *in vitro* shoot production in *Crataeva nurvala* was achieved by Walia *et al.* (2007). Nodal explants cultured on MS medium supplemented with 2.22 µM BA produced multiple shoots; elongation of shoots was satisfactorily attained on the same medium.

Dalbergia species

Hypocotyl segments and shoot tips of *in vitro* germinated seedlings of Indian rosewood were excised and cultured on MS medium supplemented with cytokinins and auxin (Rai and Chandra, 1989). Roots were induced when individual shoots were treated first with half strength MS medium supplemented with NAA, IAA and IBA (1 mg 1^{-1} each) and subsequently transferred to hormone-free half-strength MS medium. A procedure for the establishment of a proliferating cell suspension culture of *Dalbergia latifolia* was outlined and plant regeneration was achieved (Pradhan *et al.,* 1998c). *In vitro* propagation of East Indian rosewood through shoot proliferation from cotyledonary nodes was reported by Pradhan *et al.* (1998a).

Kannan (1995) in his study on *D. latifolia*, has reported that WPM with kinetin 1.0 mg $l^{-1}$and IAA at 0.1 mg l^{-1} was best for getting enhanced release of axillary buds. Multiple shoots (3.5 shoots / explant) were induced on MS medium containing 2.0 mg l^{-1} BA. *In vitro* rooting was obtained in half-strength basal medium after giving a pulse treatment with 1000 mg l^{-1} IBA solution to the base of the shoots produced from buds of young trees. Direct shoot and root formation was observed from nodal explants when cultured on WPM supplemented with1.0 or 2.0 mg l^{-1} IAA. Rooting could not be obtained from shoots produced from nodal explants of mature elite rosewood trees. Cotyledon and hypocotyl explants of *D. lanceolaria* were cultured on MS medium supplemented with different growth regulators. Multiple shoots were obtained on MS medium containing 0.5 mg l^{-1} NAA and 2 mg l^{-1} BAP. All shoots were rooted on MS basal medium supplemented with 1 mg l^{-1} IPA and plants were hardened before transplanting to soil (Dwari and Chand, 1996).

Semi-mature zygotic embryos of *Dalbergia sissoo* were employed for plant regeneration (Chand and Singh, 2005). Maximum response for callus formation was 78.3% on MS medium containing 9.04 µM 2,4-D and 1.16 µM Kn. Maximum response (45%) for shoot regeneration was achieved when calli clumps were transferred to MS medium supplemented with 8.88 µM BA and 1.34 µM NAA. *In vitro* rooting was done on 1/2 MS medium containing 1.23 µM IBA. Chand and Singh (2004b) in a study on plant regeneration of *D. sissoo* have employed encapsulated nodal segments as explants. Nodal segments collected were encapsulated in calcium alginate beads. Maximum response (85%) for plantlet production from encapsulated nodal segments was achieved on 1/2-MS medium without plant growth regulators.

Efficient plant regeneration through somatic embryogenesis was achieved from callus cultures derived from semi-mature cotyledon explants of *Dalbergia sissoo* (Singh and Chand, 2003). Callus cultures were produced from cotyledon pieces on MS medium supplemented with 4.52, 9.04, 13.57, and 18.09 µM l^{-1} 2,4-D and 0.46 µM l^{-1} kinetin. Plantlets were produced by transferring somatic embryos on 1/2-MS medium containing 10% sucrose for 15 days prior to transfer on 1/2-MS medium with 2% sucrose. Gill and Gosal (1996) successfully regenerated shoots from nodal segments of coppice shoots of mature trees of *Dalbergia sissoo* on MS medium supplemented with various concentrations and combinations of auxin and cytokinins. They reported that multiple shoot production was highest on MS medium containing BA (1.0 mg l^{-1}) +NAA (1.0 mg l^{-1}) + activated charcoal (0.2%). The regenerated plants developed roots on 1/2 MS medium, and were successfully planted under field conditions.

Emblica officinalis

The *in vitro* multiplication of *Emblica officinalis* plantlets using nodal shoot explants was tried by Maneesh *et al.* (2001). Explants were dipped in 1.0 per cent bavistin (carbendazin) for 60-90 minutes followed by 0.1 per cent $HgCl_2$ treatment for 8 minutes, which significantly reduced explant contamination. Multiple shoot production was obtained in MS medium containing kinetin at 0.2, 0.4, and 0.6 mg l^{-1}. Supplementation with 1.0 mg l^{-1} $GA_3$and 0.4 mg l^{-1} kinetin favoured the internodal elongation of shoots. Adventitious shoot induction was attempted by Ying *et al*. (2002) using tender buds of *Phyllanthus emblica* as explants on MS medium. Experimental results revealed that MS medium supplemented with BA (0.5 mg l^{-1}) and NAA (0.1 mg l^{-1}) was found to be suitable for adventitious bud inducement and the rate of differentiation was more than 90 per cent. Murashige and Skoog's medium with BA (0.1 mg l^{-1}) and

NAA (0.1 mg l^{-1}) was suitable for the growth of shoots. Successful rooting was achieved on half strength MS with NAA (0.25 mg l^{-1}) or with NAA (0.25 mg l^{-1}) + IBA (0.25 mg l^{-1}).

Eucalyptus species

Pattanaik and Vijaykumar (1997) have successfully induced multiple shoots in *Eucalyptus globulus* using nodal segments cultured on MS medium containing IBA (0.5 mg l^{-1}). Rooting was obtained on half strength MS medium containing IAA (0.5 mg l^{-1}) and IBA (0.5 mg l^{-1}). Effect of antibiotics on *in vitro* morphogenesis of *E. grandis* was studied by Picoli *et al.* (2005). Results revealed, carbenicillin and timentin increased the frequency of explant regenerating calluses and decreased necrosis.

Mature zygotic embryos of *Eucalyptus tereticornis* were cultured to obtain somatic embryogenesis and plant regeneration (Prakash and Gurumurthi, 2005b). Murashige and Skoog's and B5 basal media supplemented with different concentrations of NAA and 2,4-D were evaluated for callus induction and different BA concentrations for somatic embryogenesis. Callus induction and somatic embryogenesis was found to be highest on MS medium compared to B5 medium. The embryos were germinated on MS basal medium to produce plantlets. In another study by the same authors it was reported that seedling explants were used for organogenesis and plant regeneration (Prakash and Gurumurthi, 2005a). Prabha *et al*. (2000) cultured nodal explants of *Eucalyptus tereticornis* on MS medium. Cultures established on medium containing BA (1.0 mg l^{-1}) + NAA (0.1 mg l^{-1}) and maximum multiplication of shoots was achieved by transferring to medium containing 1.0 mg l^{-1} BA + 1.0 mg l^{-1} NAA. Best rooting was observed on half strength MS medium with 0.5 mg l^{-1} IBA.

Excocaeria agallocha

Protocol for *in vitro* propagation of *Excocaeria agallocha* was developed by Rao *et al.* (1998). Nodal segments were cultured on MS medium containing BA, zeatin and IBA in concentrations of 13.3 µM, 4.65 µM and 1.23 µM, respectively. Multiple shoot induction was complemented with efficient shoot elongation, and repeated subculture of binodal segments from axillary shoots resulted in more number of (10-12) shoots per explant in 3 months. Rooting was achieved by growing shoots in the new medium with 0.23 µM IBA.

Feronia limonia

Hypocotyl and internodal segments of *Feronia limonia* from *in vitro* grown seedlings were cultured on MS medium supplemented with BA or adenine or kinetin at 0.5 to 5 µM. Maximum response for shoot proliferation occurred on the medium containing 2 µM BA. *In vitro* shoots produced roots when transferred to half strength MS medium supplemented with 1 µM NAA (Hiregoudar *et al.,* 2005).

Gmelina arborea

In *Gmelina arborea* shoots were induced by culturing axillary buds on WPM medium (Thirunavoukkarasu and Debata, 1998) supplemented with different concentrations of BA (0.25 - 0.5 mg l^{-1}). Melendez and Contreras (2000) had attempted clonal micro propagation of *Gmelina arborea* using axillary buds from 1 to 2 year old plants as explants. These buds were cultured on ½ half strength MS medium supplemented with myoinositol (100 mg l^{-1}), thiamine (0.10 mg l^{-1}), nicotinic acid (0.5 mg l^{-1}), pyiridoxin (0.1 mg l^{-1}) and

glycine (2.0 mg l^{-1}). Multiple shoots were obtained after 6 weeks of culture. Rooting was induced on medium containing NAA (0.1 mg l^{-1}).

Hardwickia binata

Somatic embryogenesis of *Hardwickia binata* from semi mature zygotic embryos was reported by (Chand and Singh, 2001). Direct somatic embryogenesis in high frequency was achieved on MS medium supplemented with 2.26 mM 2,4-D.

Hevea brasiliensis

Immature anthers of *Hevea brasiliensis* were cultured on medium containing 4 cytokinins (BA, adenine, zeatin, kinetin and thiabendazole) (Jayasree and Thulaseedharan, 2005). Thidiazuron at 0.25 mg l^{-1} was the most beneficial for embryo germination and plant regeneration followed by benzyladenine and zeatin. Media components were standardized by Das *et al.* (2003) for somatic embryogenesis from anthers. Embryogenic callus was obtained using modified MS medium with BA (1 mg l^{-1}), NAA and 2,4-D (1 mg l^{-1}) each. Multiple shoots from axillary buds of *Hevea brasiliensis* was obtained (Mendanha *et al*., 1998) on MS medium supplemented with kinetin (1.0 mg l^{-1}), 2, 4-D (1.0 mg l^{-1}), sucrose (20.0 g l^{-1}) and agar (4.0 g l^{-1}). Rooting was obtained on MS medium containing NAA (5.0 mg l^{-1}), IBA (3.0 mg l^{-1}), sucrose (50 g l^{-1}) and 4.0 g l^{-1} agar.

Khaya senegalensis

An *in vitro* cloning protocol was developed for *Khaya senegalensis.* *In vitro* shoot multiplication was achieved by culturing explants on medium rich in mineral salts (MS medium) in the presence of BA 2.2 µM + IBA 0.26 µM. The rooting of microshoots was favoured by a less concentrated medium (1/2 MS) and a weak auxin concentration (IBA 5.2 µM) (Danthu *et al.*, 2003).

Nothapodytes foetida

Clonal propagation of *Nothapodytes foetida* (Rai, 2002) was achieved through multiple shoot induction from hypocotyl explants. Murashige and Skoog's medium supplemented with different concentrations and combinations of cytokinins was used in the study for culturing explants. MS medium containing TDZ at 2.2 µM produced multiple shoots. Inhibition of shoot elongation by TDZ was overcome by transferring shoot cultures to medium containing 2.2 µM BA.

Pterocarpus marsupium

A protocol for *in vitro* propagation of *Pterocarpus marsupium* was developed by Husain *et al.* (2007) using cotyledonary explants. The highest shoot regeneration frequency (90%) and maximum number (15.2) of shoots per explant was recorded on MS medium amended with 0.4 µM TDZ. *In vitro* regenerated shoots produced a maximum number (4.4) of roots per shoot on medium containing low concentration of 0.2 µM IBA along with phloroglucinol (3.96 µM). Chand and Singh (2004a) developed a protocol for *in vitro* plant regeneration from cotyledonary nodes of *P. marsupium*. Cultures were

established on MS medium containing 2.22-13.32 µM BA or 2.32-13.93 µM kinetin alone or in combination with 0.26 µM NAA. Highest response of explants (85%) and maximum number of shoots per explant (9.5) were obtained on MS medium supplemented with 4.44 µM BA and 0.26 µM NAA.

Tiwari, *et al.* (2004) has reported *in vitro* propagation of *P. marsupium* using nodal segments as explants. The maximum number of shoot induction was obtained on MS medium containing 3.0 mg l^{-1} BA and 0.5 mg l^{-1} NAA. Maximum Shoot length was achieved in MS medium supplemented with 0.2 mg l^{-1} IBA. Regenerated plants were acclimatized and successfully transferred under field conditions. Tissue culture protocol for *Pterocarpus marsupium* (Santoshkumar, 1993), has been standardized using nodal segments as explants. In this study MS medium was noted to be suitable for primary culture establishment. Woody plant medium supplemented with 2.0 mg l^{-1} kinetin and 0.1 mg l^{-1} IAA was the best for inducing multiple shoots from primary explants. The various growth regulator combinations however failed to induce leaf morphogenesis in shoots.

Pterocarpus santalinus

Cotyledons from *in vitro* germinated seedlings were cultured for *in vitro* shoot regeneration of *Pterocarpus santalinus.* Murashige Skoog medium containing NAA (0.1 mg l^{-1}), BA (1 mg l^{-1}) and kinetin (1 mg l^{-1}) was used for multiple shoot production. *In vitro* shoots rooted on 1/4 strength MS medium with IAA (1 mg l^{-1}) and the fully developed plantlets were successfully established in the soil (Arockiasamy *et al.,* 2000). Proficient protocols were established for *in vitro* shoot multiplication of *P. santalinus* by Anuradha and Pullaiah (1999). The highest shoot bud regeneration was achieved by culturing

mesocotyl explants on B5 medium fortified with 3.0 mg l^{-1} BA and 1.0 mg l^{-1} NAA. Shoots treated with IAA, NAA and IBA (1.0 mg l^{-1}each) prior to transferring them to the rooting medium exhibited better rooting than those with no prior treatment.

Santalum album

Adventitious shoot buds were induced from leaf cultures of sandal on both MS and WPM basal media (Mujib, 2005). Micropropagation in sandal was reported by Radhakrishnan *et al*. (2001) using nodal segments as explants. Callus initiation and organogenesis was highest on MS medium supplemented with BA at 4.0 mg l^{-1}. Rooting was observed in MS medium amended with 3.0 mg l^{-1} IBA. BA at low concentrations (0.44 and 2.22 µM) was effective in this organogenetic process. Direct organogenesis from shoot tips of sandal has been reported by Sanjay *et al*. (1998). Adventitious buds were initiated on MS medium containing various combinations of cytokinins.

Sterculia urens

Hussain *et al.* (2007) described a protocol for rapid and large scale propagation of *Sterculia urens*, by *in vitro* culture of cotyledonary nodes. Out of four different cytokinins (TDZ, 2-iP, zeatin and adenine sulphate) evaluated in supplements to MS medium, TDZ at 2.27 µM was most effective in inducing bud break. Addition of ascorbic acid (0.1%) enhanced frequency of shoot regeneration (93.3%) and number of shoots per explant (19.0). Rooting was best induced (80.0%) on a quarter strength MS medium fortified with IBA (9.80 µM).

Tamarindus indicus

Regeneration of plants through adventitious bud formation from mature zygotic embryo axis of tamarind has been attempted by Mehta *et al.* (2004). Explants consisting of longitudinal section of the embryo axis with attached cotyledon were cultured on MS medium with various combinations and concentrations of NAA, BA and sucrose. Induction of adventitious shoot buds was achieved on the cut surface of the axis when cultured in a medium containing NAA (2.69 μM), BA (44.39 μM) and 4 per cent sucrose. A medium consisting of zeatin (0.91 μM), BA (2.22 μM), calcium pantothenate (0.41 μM) and biotin (0.40 μM) supported differentiation of the buds to form elongated shoots. The shoots developed roots in a half strength MS medium with 2.0 per cent sucrose following a 72-hour treatment with auxin mixture in the dark. On transfer to soil 24 per cent plants survived.

Tectona grandis

Shoot tips of teak were cultured on MS medium containing NaCl, supplemented with IBA + kinetin at varying concentrations (2.5, 5.0, 7.5 and 10.0 mg l^{-1}). Results showed that the highest culture response for callus induction was recorded in the control (Shakila and Rajeswari, 2006). *In vitro* clonal propagation procedure for *T. grandis* was described by Shirin *et al.* (2005). Multiple shoots were induced from nodal segments through axillary bud proliferation. Highest shoot multiplication rate (6.33) was achieved on MS medium supplemented with 10 μM BA and 1 μM NAA. Tiwari *et al.* (2002) reported an improved protocol for micro propagation of teak. Average number of shoots was obtained on MS medium supplemented with 22.2 μM BA and 0.57 μM IAA. *In vitro* raised shoots were dipped in IBA 99.8 μM for two minutes to

obtain rooting. Protocol for micropropagation of teak has been standardized by Sharma (2000).

Terminalia species

Terminal bud and mature nodal explants of *Terminalia arjuna* were cultured to obtain direct shoot regeneration (Thomas *et al.,* 2003). WPM medium with 2 mg l^{-1} BA produced maximum shoots. For rooting, IAA at 1 mg l^{-1} found to be better than IBA and NAA. The rooted plantlets were successfully established in the field. Axillary bud proliferation from nodal segments was employed for micropropagation of *T. bellirica* (Ramesh *et al.,* 2005). Explants were cultured on MS medium with different concentrations of BA (4.4, 8.9, 13.3, 17.8, or 22.2 µM) or kinetin (4.6, 9.3, 14.0, 18.6, or 23.2 µM). The medium containing 13.3 µM BA showed the highest shoot length (1.9 cm) in the primary culture. Rooting of the shoots was achieved under *in vitro* conditions on modified Gamborg's (B5) medium or WPM, both supplemented with 4.9 µM IBA. Regenerated plants were established in the greenhouse.

Deshmukh *et al.* (2005) evaluated effect of plant growth regulators (BA, IBA and NAA) on the establishment, proliferation and root induction in *T. chebula* under *in vitro* condition. Results showed that 1.2 mg l^{-1} BA+0.05 mg l^{-1} NAA was the best combination than BA used either alone or in combination with IBA.

Toona ciliata

Callus formation and plant regeneration in *Toona ciliata* was achieved from *in vitro* propagation (Daquinta *et al.,* 2005). Explants were cultured on MS médium

supplemented with 0-1 mg l^{-1} TDZ. *In vitro* regeneration of plants from nodal single-bud segments of *T. ciliata* trees were obtained by Mroginski *et al.* (2003). Quarter strength MS medium with 3% sucrose, supplemented with 0.1 mg l^{-1} IBA and BA at 0.5 mg l^{-1} was found to be best suited for shoot regeneration. Rooting of regenerated shoots was observed in MS medium with 0.1 mg l^{-1} IBA.

Vateria indica

Organogenesis and embryogenesis in *Vateria indica* were attempted by Divatar (1994). Moderate callusing was obtained from leaf and internodal segments cultured on MS medium and half strength MS medium supplemented with growth regulators like 2-iP, 2,4-D and IBA. However, further response from callus was not observed.

Wrightia tomentosa

Micropropagation protocol for *Wrightia tomentosa* was achieved using shoot segments through forced axillary branching. An average of 4 shoots was produced on MS medium supplemented with 2.0 mg 1^{-1} BA. Rooting was achieved by dipping the lower ends of *in vitro* shoots in 100 mg l^{-1} IBA solution for 15 min followed by implantation on modified MS medium (Purohit *et al.*, 1994).

2.3 CONTROLLING FACTORS IN MICROPROPAGATION

2.3.1 Nutrient medium for *in vitro* cultures

Successful culture establishment has been achieved by standardizing different nutrient combinations. Murashige and Skoog (1962), White's medium

(White, 1963), B5 (Gamborg *et al.* 1968), Linsmaier and Skoog's medium (Linsmaier and Skoog, 1965) medium, Woody Plant medium (WPM) (Llyod and McCown, 1980) and Nitsch medium (1951) are some commonly used media in plant tissue culture (Narayanaswamy (1997) and Gupta (1995). Among them Murashige and Skoog medium (MS) is the most popular one.

A medium specially designed for tree species is the WPM developed by Llyod and McCown (1980). Compared with MS it is low in ammonium nitrate, potassium, and chloride and high in sulphate. After 1980, the most popular media was WPM, especially for woody species. Nodal explants of *Dalbegia sissoo* were cultured on two media namely MS and B5, MS media was found to be suitable (Joshi *et al.*, 2003).

2.3.2 Growth regulators

Growth regulators are organic compounds (other than a nutrient) which in small amounts promote, inhibit or qualitatively modify growth and development (More, 1979). As stated by Krikorian *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. Plant growth regulators (PGR's) include naturally occurring hormones such as IAA, GA_3, BA, ABA etc., and also a number of synthetic chemicals that affect or control growth and development in plants (Minocha, 1987). Bhojwani and Razdan, (1983) reported that, it is generally necessary to add one or more of these PGRs to support good growth of tissues and organs. Addition of growth regulators into the callus regeneration media' modified the genotypic effect on shoot formation frequency, more or less decreasing the genotypic differences and the number of shoots formed (Kallak *et al.*, 1997). Skoog and Miller (1957) proposed the concept of hormonal control.

Their classic experiments on tobacco pith cultures showed that root and bud initiation were conditioned by balance between auxin and cytokinin. High concentration of auxin promoted rooting where as proportionately more cytokinin initiated bud or shoot formation.

Application of cytokinins has been commonly found effective in promoting direct or indirect shoot initiation in *A. Indica* (George and Sherrington, 1984). Kinetin and BA are the most commonly used cytokinins, while 2-ip and zeatin are used less frequently (Thorpe and Patel, 1984). Evans *et al.* (1981) reported that for 75 per cent of the species forming shoots, kinetin/BA was used in the concentration of 0.05 µM and 46 µM.

Razdan, (1993) reported that success in employing these various media in all probability lies in the ratios as well as concentrations of nutrients nearly match the optimum requirement with regard to the growth and differentiation of respective cell or tissue systems.

Cytokinins and adenine derivatives are frequently used for shoot production, proliferation and elongation. In a study Yousef (1997), results revealed cytokinins and adenine derivatives combination with auxin they produce callus. However, according to Bon *et al.* (1998) extents of effects of these growth regulators vary from species to species. This was supported by a study (Singh and Mangia 1998), which reported that multiple shoot formation did not occur without growth regulators in *Acacia tortilis.* In general, it appears that BA is the most effective cytokinin for stimulating axillary shoot proliferation, followed by, decreasing order kinetin and 2-ip (Bhojwani, 1980; Hasegawa, 1980; Lundergan and Janick, 1980; Kitto and Young, 1981). Too high a concentration of auxin may not only inhibit axillary bud branching, but

also induce callus formation, especially when 2,4-D is used (Hasegawa, 1980).

2.3.3 Carbon energy sources

All media require the presence of a carbon source, as the source of energy. The previous studies by Gautheret (1941, 1945), Hilderbrandt and Riker (1949) had documented that a number of carbohydrate could support growth, but over the years through several studies it has become apparent that sucrose is generally the best carbon and energy source (Street, 1969; Thorpe, 1982; Bhojwani and Razdan, 1983). Carbohydrates not only function as a carbon source in metabolism, but they also play an important role in the regulation of the external osmotic potential (Brown and Thorpe, 1980). In an experiment (Von and Eriksson, 1981) it has been revealed that sucrose was the only sugar essential for bud induction in *Pinus contorta*. Despite the widespread use of sucrose, this compound is not always the most effective carbohydrates for shoot initiation. Fructose and glucose were served as the best source for mulberry bud culture (Oka and Ohyama, 1982).

It has been stated that (Dodds and Robert, 1985) the choice and concentration of the sugar to be used depends mainly on the plant tissue to be cultured and the purpose of the explant. In *Alnus crimastogyne* type and concentration of sugar used in the multiplication medium were observed to be critical factors for both multiple shoot induction and bud elongation (Tang *et al.*, 1996).

2.3.4 Vitamins

It is necessary to supplement the medium with required vitamins and amino

acids to achieve the best growth of the tissue. Some consider that thiamine (vitamin B_1) may be only essential vitamin for nearly all plant tissue cultures, where as nicotinic acid (niacin) and pyridoxine (vitamin B6) may stimulate growth (Gamborg *et al.*, 1976). Some other vitamins that have been employed in tissue culture media include p-amino-benzoic acid (PABA; vitamin Bx), ascorbic acid (vitamin C), biotin (vitamin H), cyanocobalamine (vitamin B12), folic acid (vitamin Be), Calcium pantothenate and riboflavin (Huang and Murashige, 1977; Gamborg and Shyluk, 1981).

Linsmaier and Skoog (1965) confirmed that most vitamins are not essential for callus growth in tobacco. Pyridoxine, biotin and nicotinic acid could be deleted nom medium without serious impact on growth. Ascorbic acid which may be employed with other organic acids, is useful as an antioxidant to alleviate tissue browning (Reynold and Murashige, 1979).

2.3.5 Explant size and its position on the mother plant

Rate of shoot multiplication in *Crataeva adansonii* was affected by position of bud on the foliage twig (Sharma *et al.*, 2003). Skirvin (1980) in his study reported that the type of the explant varied with each plant species and the most suitable one should be determined for each species. Hussey (1983) quoted that, as a rule, larger the size of the explant more rapid is the growth rate and greater are the rates of survival. However, larger the explant size more will be the chance of harbouring contaminant microorganisms. In contrast, when eradication of viral infection is one of the culture objectives, small explant should be used. In an experiment shoot tip explants of *Dianthus caryophyllus* less than 2.0 mm, induced roots (Bhan, 1998). If explants measuring 7.5 mm long roots were used, virus could not be removed. So the optimum explants are

2-5 mm in length. Pseudo terminal buds of *Betula uber*, approximate 5 mm long, opened after 4 to 5 days of culturing and produced 3 leaves in a week, whereas 3-4 mm axillary bud took 10-20 days to increase in the same size (Vijayakumar *et al.*, 1990).

Dhawan (1993) in his experiment reported that the position of the explant of the parent plant has an active role in *in vitro* response. It was evident that buds taken from stem part located close to tip when cultured yielded more callus than shoot whereas axillary buds at distant position from apical buds yielded more shoots (Periera *et al.*, 1995).

2.3.6 Age of explant

Explants are the potential source for *in vitro* cultures. Different explants have been reported to give stable cultures. Meristematic tissues generally have a high degree of morphogenic competence than older tissues. However, patentability of juvenile explants from hard-to-root species to multiply in cultures suggests that it should be possible to *in vitro* propagate their elite trees if they can be induced to develop juvenile shoots by suckering, coppicing or hormone treatment. To some degree rejuvenation of shoots also occurs after serial sub cultures (Zimmerman and Broone, 1981). In contrast to this, in some species namely *Betula tatewakiana* (Ide, 1995; Jones *et al.*, 1996), *Fagus sylvatica* (Meier and Reuther, 1994), *Fraxinus excelsa* L. (Hammatt, 1992), *Dalbergia sissoo* (Chauhan *et al.*, 1994), *Tectona grandis* (Gupta *et al.*, 1980; Devi *et al.*, 1994), *Azadirachta indica* (Eeswara *et al.*, 1997) successful *in vitro* clonal propagation has been achieved by taking nodal and terminal bud explants from mature trees.

Suryanarayanan and Pai (1998) have reported that among various explants,*viz.* florets, stem and shoot tips and florets yielded less callus than others in *Coleus forshohlii* . Sharma *et al.,* (2003) reported age of explant has an important role in shoot multiplication.

2.3.7 Season of collecting the explant

The physiological state of the mother plant at the time of explant excision has a definite influence on the response of the buds. The physiological state of plant depends on season. Best results in tissue culture have been achieved from the explants from actively growing shoots at the beginning of the growing season (Anderson, 1980). The nodal explants harvested during March-April and August-September-October was found more suitable for establishment of cultures of *Capparis decidua* (Deora and Shekhawat, 1995). Young (1991) in his study on *Litchi chinensis* experienced that the explant taken after 10 continuous rainy days was completely contaminated and those taken after 15 continuous sunny days had a low contamination rate of 20 per cent. Meier and Reuther (1994) reported that February was the most beneficial month for the collection of explants having dormant buds since infection with endogenous bacteria was still low and *in vitro* growth of the plant material was the highest.

2.3.8 Genotype

Successful application of *in vitro* technology to the production of clone depends upon inducibility of growth and differentiation in tissues of woody plants and the regeneration of true to the type viable plants in selected genotypes. As stated by Ahuja (1983), great differences exist in organogenesis, embryogenesis and regeneration of plantlets among plant species, varieties and

even individuals of the same varieties.

McComb and Bennet (1982) revealed that there was large difference in the capacity of explants from different selections of mature *Eucalyptus marginata* tree to survive in culture. Specific effect of genotype has been reported for *Sequoia sempervirens* (Sul and Korban, 1994). Shekhawat *et al.,* (1993) produced 6-8 shoots per explant by culturing explants from thorn less trees of *Prosopis cineraria* on MS medium containing 0.1 mgl^{-1} IAA, 5.0 mg l^{-1} BA and additives. Kallak *et al.* (1997) observed differences in shoot and root regeneration efficiency of carnation calli depending on genotype. The calli from different cultivars revealed significant differences in growth, colour and structure.

2.3.9 Surface sterilization

Surface of plant parts may harbour a wide range of microbial contaminants which when inoculated to a nutrient medium; contaminate the entire *in vitro* system. To avoid this, the tissue must be thoroughly surface sterilised before planting it on the nutrient medium. To disinfect the plant tissues various surface sterilizing agents have been used at varying durations of time. Kannan and Jasrai (1998) reported that washing of explants in ethanol (90 %) followed by soaking in mercuric chloride 0.1 per cent for one minute gave contamination free cultures in *Vitex negundo.* Srividya *et al.* (1998) reported that treating explants of *Azadirachta indica* in 70 per cent ethanol for 30 seconds followed by treatment with 0.1 per cent mercuric chloride for 8 minutes gave contamination free cultures.

2.3.10 Systemic contaminants

Leifert and Woodward (1998) highlighted that surface sterilization is often inefficient in establishment of aseptic cultures. This problem may be due to the disinfectant being inactive or microorganisms being protected within the plant tissue used as the explant. Thus culture contamination control is extremely difficult and with many contaminants, impossible to control.

Shields *et al.* (1984) and Dodds and Robert (1985) recommended the use of various fungicides and antibiotics in the culture medium to reduce systemic fungal and bacterial contamination respectively. Mallika *et al.* (1992) advocated growing stock plants under controlled conditions with regular sprayings using systemic and contact fungicides in order to avoid problem of contamination to some extent.

Dodds and Roberts (1985) observed that it is preferable to avoid the use of antibiotics for sterilization because they or their degradation product may be metabolized by plant tissue with unpredictable result.

2.3.11 Culture environment

Physical conditions such as pH of the medium, light, temperature and relative humidity, season of culturing etc. have been found to have a profound influence on the rate of growth and extent of differentiation exhibited by the cultured cells. Changes in the physical condition of the medium are brought about by changing the concentration of the gelling agent in the medium. Mahato (1992) reported that, higher pH precipitated nutrients and influenced nutrient and hormone uptake in case of *Dalbergia latifolia* cultures. Bonga (1982) remarked

that pH of the medium is usually set at about 5.0 for liquid cultures and 5.8 for semi solid cultures. In plant cell media the pH is generally adjusted to 5.7-5.8.

In neem the optimum temperature of 28±2° C with a light intensity of 3000 lux was found suitable for the growth of culture (Srividya *et at.,* 1998). Relative humidity is rarely a problem except in climates, where rapid drying occurs. The humidity of air is rarely controlled and when controlled, 70 per cent has been found to be the most frequent setting (Hu and Wang, 1983).

2.3.12 Rooting of *in vitro* produced shoots

In vitro produced shoots can be successfully rooted through *in vitro* methods. A low salt medium is found satisfactory for rooting of shoots in a large number of plant species. Often where shoot multiplication was induced on fullstrength MS medium, the salt concentration may be reduced to half (Garland and Scoltz, 1981; Zimmerman and Broone, 1981) or to a quarter (Skirvin and Chu, 1979) for rooting. It is the endogenous auxin: cytokinin balance that is the key factor in the initiation of the rooting (Thorpe, 1980).

Generally, auxin favours root formation. Among the auxins, NAA has been the most effective one for the induction of rooting (Ancora *et al.,* 1981). Sometimes a combination of auxins may give a better response (Gupta *et al.,* 1980). Drew (1993) reported that longer exposure to IDA reduced rooting percentage in *Azadirachta indica,* as they took longer time to initiate roots and produced excess callus at the base of the explant. Venkateswarlu *et al.* (1998) obtained rooting in *Azadirachta indica* on full strength MS with 3.0 mg rl IAA.

2.3.13 Hardening and planting out

Tissue culture plantlets are tender and their transfer from the artificial environment of the culture vessel to the self dependent green house or similar environment makes their existence and establishment tough. The plantlets formed in the culture are deficient in photosynthetic efficiency and mechanism *to* control water loss. The humidity inside the culture vessel is very high (close to 95%) thus the plants lack the protective cuticle. Success in acclimatization depends upon not only post transfer condition but the pre-transfer culture conditions also (Ziv, 1986).

Joarder *et al.* (1993) transferred rooted plantlets *of A. indica* into the mixture of garden soil and compost (1: 1) and were covered with glass beaker for 7-10 days there after they were transferred to sandy soil. Similar reports were quoted by Venkateswarlu *et al.* (1998) when the plantlets *of A. indica* were transferred *to* soil + vermiculite mix and grown fewer than 90 per cent humidity.

By thorough reviewing the past information available on tissue culture of different wood species it can be concluded that, mercuric chloride can be used as surface sterilant to establish aseptic cultures. MS basal media alone or in combination with cytokinins and auxins has produced profitable results in production of superior plantlets.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present investigation titled "*In vitro* propagation of big-leaf mahogany (*Swietenia macrophylla* King) through tissue culture." was undertaken during the year 2007-2009 in the Plant Tissue Culture Laboratory, College of Forestry, Vellanikkara, Thrissur District, Kerala which is situated at 10^0 32' N latitude and 76^0 10' E longitudes at an altitude of 22.25 m above MSL. The location experiences a humid tropical climate with a mean annual rain fall of 2668.6 mm most of which is received between June to September. The minimum temperature varies from 22.2^0 C (December) to 24.7^0 C (May) and maximum from 28.6^0 C (July) to 36.2^0 C (March). The details of the materials used and the techniques / methodology employed in the experiment during the course of investigation are described in this chapter.

3.1. MATERIALS

3.1.1. Culture Media

For culturing the explants MS medium (Murashige and Skoog, 1962), Woody Plant Medium (WPM) (Lloyd and McCown, 1980) and B5 (Gamborg *et al.,* 1976) medium were used in the present study. The composition of the different media used is presented in Table. 1.

The growth regulators used were auxin (IAA and IBA) and cytokinins (BA and kinetin). Analytical grade chemicals were used for preparing media. Different combinations of growth regulators used in the study are given in Table. 2.

Table. 1 Chemical composition (mg l^{-1}) of various culture media used for in *vitro* propagation of *Swietenia macrophylla*

Components	MS Medium	WPM medium	B5 Medium
$(NH_4)_2S0_4$	-	-	134
$MgS0_{4\ x}\ 7H_2O$	370	370	500
KCl	0.83	-	-
K_2S0_4	-	990	-
$CaCl_{2\ x}\ 2H_2O$	440	96	150
$KN0_3$	1,900	-	3,000
KCI	0.83	-	-
$Ca(NO_3)_{2\ X}\ 4H_2O$	-	556.0	-
NH_4N03	1,650	400.0	-
$NaH_2PO_{4X}H_2O$	170.0	170.0	150
KH_2P0_4	170	-	-
$FeS0_4$ x $7H_2O$	27.8	27.8	27.8
Na_2EDTA	37.3	37.3	37.3
$MnS0_4$ x $4H_2O$	22.3	22.3	10 (1 H2O)
$ZnS0_4$ x $7H_2O$	8.6	8.6	2
$CUS0_4$ x $5H_2O$	0.025	0.025	0.025
$CoC1_2X\ 6H_2O$	0.025	-	0.025
KI	0.83	0.83	0.75
H_3B0_3	6.2	6.2	3
$Na_2M_0O_4$	0.25	0.25	0.25
Sucrose	30000.0	20000.0	20000.0
Myo-Inositol	100.0	100.0	100
Nicotinic Acid	0.5	0.5	1.0
Pyridixine HCl	0.5	0.5	1.0
Thiamine HCl	0.1-1	0.1	10
Glycine	2	2.0	-

Table. 2 Growth regulator combinations used for induction of bud break and shoot development in MS medium

Cytokinetinins mgl^{-1}	Auxins mgl^{-1}	
	IAA	IBA
BA 1		
BA 2		
BA 3		
BA 0.5	0.1	
BA 0.5	0.5	
BA 0.5	1	
BA 1	0.1	
BA 1	0.5	
BA 1	1	
BA 2	0.1	
BA 2	0.5	
BA 2	1	
BA 3	0.1	
BA 3	0.5	
BA 3	1	
BA 0.5		0.1
BA 0.5		0.5
BA 0.5		1
BA 1		0.1
BA 1		0.5
BA 1		1
BA 2		0.1
BA 2		0.5
BA 2		1
BA 3		0.1
BA 3		0.5
BA 3		1

Table. 2 continued

Cytokinetinins mgl^{-1}	Auxins mgl^{-1}	
	IAA	IBA
kinetin 1.0		
kinetin 2.0		
kinetin 3.0		
kinetin 0.5	0.1	
kinetin 0.5	0.5	
kinetin 0.5	1	
kinetin 1.0	0.1	
kinetin 1.0	0.5	
kinetin 1.0	1	
kinetin 2.0	0.1	
kinetin 2.0	0.5	
kinetin 2.0	1	
kinetin 3.0	0.1	
kinetin 3.0	0.5	
kinetin 3.0	1	
kinetin 0.5		0.1
kinetin 0.5		0.5
kinetin 0.5		1
kinetin 1.0		0.1
kinetin 1.0		0.5
kinetin 1.0		1
kinetin 2.0		0.1
kinetin 2.0		0.5
kinetin 2.0		1
kinetin 3.0		0.1
kinetin 3.0		0.5
kinetin 3.0		1

3.1.2 Explants

The explants used in the present experiment are nodal segments containing axillary buds collected from 1-2 years old seedlings of *Swietenia macrophylla* available at the College of Forestry experimental garden.

3.2 METHODS

3.2.1 Preparation of stock solution

For the easiness in media preparation, stock solutions of major and minor nutrients were prepared. Each stock was prepared separately by following standard procedures as given by Gamborg and Shyluk (1981). For this required quantities of the chemicals were weighed accurately. Distilled water was taken in a beaker and the chemicals were added one after the other and dissolved by constant stirring. Care was taken while the preparation of iron stock since it precipitates readily. To avoid this Na_2EDTA and $FeSO_4.7H_2O$ were dissolved in separate beakers with approximately 200 ml distilled water each. Both beakers were placed on hot plates and brought to the point of almost boiling. Then $FeSO_4.7H_2O$ solution was added slowly to Na_2EDTA over a 15 minute period with constant stirring. Then the volume was made up to one litre in a volumetric flask by adding distilled water. The mixture was allowed to cool in room temperature. The stock solutions were labelled indicating the stock number and date of preparation. They are stored in amber coloured bottles under refrigerated condition.

3.2.2 Preparation of the culture media

In a beaker (2000 ml) 300 ml water was taken and then ionositol (100 mg) and

sucrose (30 g) were added and dissolved by constant stirring. Then the quantity of stock solutions required for preparing one litre media was added to this mixture. Sufficient volume of water was added to dissolve the mixture. Different plant growth hormones were added to the basal MS medium. The stock solutions of growth regulators at 10 mg / 100 ml were prepared and stored in refrigerator and aliquots were taken from stock solution for use. These aliquots were added to the medium, before the pH was adjusted. The pH of the media was adjusted to 5.7 (using 1 N NaOH or 1 N HCl) with the help of digital pH meter. To this medium required quantity of (7-8 g l^{-1}) of agar was added.

The solution was then heated on a gas burner or in a microwave oven to dissolve the agar. The boiling media (approximately 15-20 ml per tube) was poured into the well cleaned, oven dried culture tubes of 150 mm X 25 mm size. Then culture tubes were plugged tightly with non-absorbent cotton wool plugs.

3.2.3 Sterilization of culture medium

The media was sterilized using a pressure cooker at pressure of 1.06 kg cm^{-2} for 20 minutes at 121 ^{o}C. After sterilization, the culture tubes were stored in an air-conditioned culture room until further use.

3.2.4 Sterilization of equipment

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

3.2.5 Collection and preparation of explants

Stems of approximately 10 to 20 cm were cut from nursery grown seedlings of *Swietenia macrophylla* using a secature and brought to the laboratory soon to avoid desiccation. The leaves were removed close to the stem leaving small part of the petiole. Stem segments were first washed in tap water. Then they were cut into small segments containing an axillary bud. After this, they were washed thoroughly in a frothing solution of detergent (Teepol 0.01%) to remove all extraneous materials adhered to the segments. The explants were dipped in a mixture of the systemic fungicide, Bavistin (Carbendazim) and the contact fungicide Indofil M- 45 (Mancozeb) each at 0.2 percent for about 1 hr. Then they were taken out, washed under running water and then washed with soap solution. The soap is removed by washing again in tap water.

3.2.6 Surface sterilization of explants

Surface sterilization is done under perfect aseptic condition in a laminar air flow chamber. For surface sterilization explants were immersed in 0.1 per cent mercuric chloride for stipulated period with occasional stirrings by swirling movements. After that, they were washed three times with sterilized distilled water so as to remove the traces of sterilant.

3.2.7 Inoculation of explants

Culturing is done under perfect aseptic condition in a laminar air flow cabinet. For this cotton plug of the test tubes containing the media was opened near a flame and one nodal segment each was transferred to the medium using forceps. The cotton plug was immediately replaced. Then cultures were properly labelled and kept in the

culture room maintained at 25±2^{0}C with a light intensity of 2000 lux for 16 hours light period.

3.2.8 Induction of bud breaks and shoot development

For induction of bud break and shoot development various treatment combinations of growth regulators were tried. For each treatment a minimum of twenty tubes were used and was replicated three times.

3.3 OBSERVATIONS

Each trial was carried out with minimum of 20 tubes replicated three times. Observations were taken for a period of four weeks with an interval of seven days. The data collected are presented on the basis of cultures that remained uncontaminated. The observations which are recorded in each treatment are the following:

Number of cultures uncontaminated

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

Number of cultures showing bud break

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

3 Time taken for bud initiation

Time taken for bud initiation was recorded and expressed in days.

No of explants showing leaf production

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

Time taken for leaf production

It was recorded and expressed in days.

Average number of leaves

Average number of leaves was worked out as mean of the total number of leaves from the number of cultures showing leaf production.

Maximum number of leaves

Maximum number of leaves was expressed as maximum number of leaves produced per explant in a particular treatment.

Number of explants with shoot development

It was expressed as percentage of total number of surviving cultures.

Average number of shoots per culture

The average number of shoots per culture was expressed as mean of the total number of shoots produced in different cultures of a particular treatment.

Average length of shoots per culture

The average length of shoots per culture was expressed in cm as a mean of total length of shoots from the number of cultures showing shoot development.

Maximum shoot length

Maximum shoot length was expressed in cm as the maximum length of shoots produced per explant in a particular treatment.

Number of cultures rooted *in vitro*

Number of culture rooted *in vitro* was counted and expressed as percentage total cultures in a particular treatment.

Number of cultures showing callus production

Number of cultures showing callusing were counted and expressed as percentage of total cultures.

Number of cultures showing browning

Number of cultures showing browning were counted and expressed as percentage of total number of cultures.

3.4 ESTABLISHMENT OF CONTINUOUS CULTURES

An effort was made to produce continuous cultures using *in vitro* shoots of mahogany. Sub culturing of microshoots produced was carried out on various growth regulator combinations, the details are presented in Table 3. After 20 days of main culture, *in vitro* shoots were taken out carefully without any damage and they were cut into segments containing average of one axillary bud and cultured on the media containing different growth regulator combinations.

3.5 STATISTICAL ANALYSIS

The data recorded were transformed wherever necessary and statistically

Table. 3. Growth regulator combinations used for production of continuous cultures of *Swietenia macrophylla* in MS medium

Cytokinins mg l^{-1}		Auxins mg l^{-1}	
BA	Kinetin	IAA	IBA
1.0	-	-	-
2.0	-	-	-
1.0	-	0.1	-
2.0	-	0.1	-
2.0	-	0.5	-
3.0	-	0.5	-
0.5	-	-	1.0
1.0	-	-	0.1
2.0	-		0.1
-	3.0	-	-
-	1.0	0.1	-
-	2.0	0.1	-
-	2.0	0.5	-
-	2.0	-	0.5
-	3.0	-	0.5

analyzed using the statistical package SPSS. The treatment means were compared using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS

4. RESULTS

The results of various experiments on micropropagation of *Swietenia macrophylla* King conducted at the Tissue Culture Laboratory of College of Forestry, Vellanikkara during 2007-2009 are presented in this chapter.

4.1 SURFACE STERILIZATION OF EXPLANTS

The nodal segments containing an axillary bud, which were used as the explants during the present study, were subjected to different surface sterilization treatments to prevent culture contamination. The results obtained in the study are presented in Table. 4. Observation on culture contamination was recorded in weekly interval for three weeks. The results revealed that there was significant difference among the treatments in establishing the aseptic cultures. After one week of culture explants got contaminated; However, the percent contamination was less with the highest being 35.56 per cent when explants were treated with 0.05 per cent mercuric chloride for 10 min. Surface sterilization treatments *viz.* dipping of explants in 0.2 per cent mercuric chloride, pretreatment of explants in fungicidal solution of Carbendazim and Mancozeb (0.2 per cent each) for different duration (1/2 hr and 1 hr) and followed by immersing in 0.1 per cent mercuric chloride for different period (10 min, 15 min) have controlled cent per cent contamination. Cultures treated with 0.2 per cent mercuric chloride for 10 min and 15 min have shown a negligible per cent of contamination.

After two weeks of culturing it is observed that treatments involving dipping in 0.05 per cent mercuric chloride for 10 min and 15 min have completely failed to control the contamination. Explants treated by immersing in 0.2 per cent mercuric chloride for 20 min and pretreatment with 0.2 per cent fungicidal solution and later

Table. 4 Effect of different sterilization treatments on culture establishment of *Swietenia macrophylla* in MS medium

Treatments with duration	% cultures contaminated		
	I Week	II Week	III Week
0.05% $HgCl_2$ - 10 min	35.56^{F} (0..37)	100^{F} (1.57)	100^{E} (1.57)
0.05% $HgCl_2$ – 15 min	33.33^{EF} (0.35)	100^{F} (1.57)	100^{E} (1.57)
0.05% $HgCl_2$ – 20 min	22.09^{CDE} (0.22)	77.16^{E} (0.90)	100^{E} (1.57)
0.10% $HgCl_2$ - 10 min	15.55^{BCD} (0.16)	84.19^{E} (1.01)	100^{E} (1.57)
0.10% $HgCl_2$ – 15 min	17.78^{BCD} (0.18)	32.70^{CD} (0.33)	76.90^{D} (0.88)
0.10% $HgCl_2$ – 20 min	26.67^{DEF} (0.27)	29.09^{BC} (0.30)	56.67^{C} (0.60)
0.20% $HgCl_2$ - 10 min	8.89^{AB} (0.09)	25.54^{ABC} (0.28)	39.63^{B} (0.41)
0.20% $HgCl_2$ – 15 min	2.22^{A} (0.02)	18.10^{ABC} (0.18)	31.82^{AB} (0.32)
0.20% $HgCl_2$ – 20 min	0.0^{A} (0.0)	11.11^{A} (0.11)	35.69^{B} (0.37)
0.2 % fungicidal solution – 30 min + 0.1% $HgCl_2$ - 10 min	0.0^{A} (0.0)	46.67^{D} (0.49)	100^{E} (1.57)
0.2 % fungicidal solution – 30 min + 0.1% $HgCl_2$ - 15 min	0.0^{A} (0.0)	26.67^{ABC} (0.27)	75.56^{D} (0.88)
0.2 % fungicidal solution – 60 min + 0.1% $HgCl_2$ - 10 min	11.11^{ABC} (0.11)	22.71^{ABC} (0.23)	38.89^{B} (0.40)
0.2 % fungicidal solution – 60 min + 0.1% $HgCl_2$ - 15 min	0.0^{A} (0.0)	13.34^{AB} (0.14)	20.33^{A} (0.21)
SEm $\pm$	5.50	6.84	5.76
F	11.18*	44.34*	58.67*

* Significant at 5 %

Figures in parenthesis are arcsine transformed values

Figures with the same superscript do not differ significantly

by dipping in 0.1 per cent mercuric chloride have controlled the contamination to great extent.

After three weeks of culture incubation, among the various sterilization treatments employed, treatment involving pretreatment of explants by dipping for 1 hr in 0.2 per cent fungicidal solution (Bavistin and Indofil M-45) followed by 15 min dip in 0.1 per cent $HgCl_2$ was found effective in controlling the contamination of explants. This treatment was significant over all other treatments. This was followed by dip in 0.2 per cent $HgCl_2$ for 15 min with 31.82 per cent contamination rate.

However, the treatments involving dip in 0.2 per cent $HgCl_2$ for 10 min, dip in 0.2 per cent $HgCl_2$ for 20 min and pretreatment by dipping in 0.2 per cent fungicidal solution followed by dip in 0.1 per cent $HgCl_2$ were found on par with the contamination rate of 36.93 per cent, 35.69 per cent and 38.89 per cent, respectively. Treatments, namely, dip in 0.05 per cent $HgCl_2$ for 10 min, 15 min, 20 min and dip in 0.1 per cent $HgCl_2$ for 10 min were found to be absolutely ineffective in establishing aseptic cultures.

4.2 SEASONAL INFLUENCE ON ESTABLISHING ASEPTIC CULTURES

The seasonal influence on contamination of axillary bud cultures is evident from the Table. 5. During this experiment explants were pretreated with 0.2 per cent fungicidal solution, followed by 15 min dip in 0.1 per cent mercuric chloride and cultured on the medium. The cultures were contaminated at different rate individually by fungi and bacteria. The cultures from explants collected in rainy season (June) completely failed to establish. On the other hand the explants cultured during February to April have shown more than 50 per cent survival. Fungal contamination was identified as the major contaminant in the study, and from the

Table. 5 Seasonal influence on contamination and culture establishment in axillary bud cultures of *Swietenia macrophylla*

Month	Contamination (%)		Survival of cultures (%)	Culture establishment (%)
	F.C	B.C		
January	46.44	3.1	50.46	32.70
February	26.33	5.55	68.12	56.10
March	24.21	2.27	73.52	64.57
April	6.75	NIL	93.25	77.57
May*	-	-	-	-
June	100.0	NIL	NIL	NIL
July	89.18	1.38	9.44	NIL
August	97.45	NIL	2.55	NIL
September	96.45	NIL	3.55	NIL
October	87.81	NIL	12.19	3.6
November	53.51	2.38	44.11	35.50
December	60.55	3.35	31.95	17.21

*Explants were not cultured in that month

F.C. - Fungal contamination

B.C. - Bacterial contamination

results it is clear that explants had bacterial contamination during November to March. The contamination rate varied from 47 per cent to 100 per cent. Culture contamination was found to be highest from June to October.

During the present investigation, season of collection of explants was found to be influencing the culture establishment and growth. The highest per cent of culture establishment (77.57 %) was recorded in April and lowest was recorded in October (3.6 %).

4.3 EFFECT OF DIFFERENT BASAL MEDIA ON CULTURE ESTABLISHMENT AND GROWTH IN AXILLARY BUDS OF MAHOGANY

In the present investigation three basal media, namely, B5, Murashige and Skoog (MS), and Woody Plant Medium (WPM) were used for culture establishment in axillary buds of *S. macrophylla*. The results obtained are presented in Table. 6. There was a significant difference among the three media used with respect to per cent bud initiation, shoot initiation, leaf initiation and callusing. The treatments have also shown difference in the time taken for bud initiation, shoot initiation and leaf initiation.

Among the three media tried MS medium was found significantly different in per cent bud initiation. It has shown highest bud initiation (58.08 %) followed by WPM (39.92 %) and B5 (32.0 %). Explants cultured on MS medium have shown early bud initiation (7.13 days) followed by WPM (9.17days) and B5 (9.85 days). However, the two media *viz.*, WPM and B5 were found to have same effect on per cent bud initiation and time taken for bud initiation. Shoot initiation from established cultures was found to be highest (39.26 %) in MS media with lowest (25.33 %) was in B5 medium. The per cent shoot initiation in WPM (34.25 %) was on par with MS

Table. 6 Effect of different basal media on culture establishment and growth in axillary buds of *Swietenia macrophylla*

Basal media	Bud initiation		Shoot initiation	Shoot length (cm)		Leaf initiation		No. of leaves		Callusing %	Browning %
	% culture	Days	% culture	Avg.	Max.	% culture	Days	Avg.	Max.		
MS	58.08^{A} (0.62)	7.13^{A}	39.26^{A} (0.40)	0.65	1.07	30.74^{A} (0.31)	9.33^{A}	1	1	23.70^{A} (0.24)	35.93^{A} (0.37)
WPM	39.92^{B} (0.41)	9.17^{B}	34.25^{A} (0.35)	0.48	1.63	16.61^{B} (0.17)	11.67^{B}	1.23	1.67	24.51^{A} (0.25)	24.65^{B} (0.25)
B5	32.0^{B} (0.33)	9.85^{B}	25.33^{B} (0.26)	0.52	1.3	14.67^{B} (0.15)	13.33^{C}	1.42	1.67	0.0^{B} (0.0)	0.0^{C} (0.0)
SEm $\pm$	3.65	0.39	2.75	0.07	0.32	3.52	0.67	0.36	0.61	2.48	2.49
F	26.85*	26.85*	13.16*	2.99	1.59	12.41*	18.17*	0.67*	0.80*	62.98*	108.64*

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

medium.

Per cent leaf initiation was also higher in MS (30.74 %) when compared to WPM (16.61 %) and B5 (14.67 %). Early leaf initiation was observed in MS medium (9.33 days) followed by WPM (11.67 days). There was a delayed leaf initiation up to (13.33 days) in cultures established on B5 medium. The media MS and WPM were found on par in per cent callogenesis with 23.70 and 24.51 per cent respectively. There was no callus formation in B5 medium. Though the treatments were non-significant with respect to average shoot length, maximum shoot length, average number of leaves and maximum number of leaves, average shoot length (0.65 cm) was highest in MS medium compared to WPM (0.48 cm) and B5 (0.52 cm). However, maximum shoot length (1.63 cm) and maximum no of leaves (1.67) were highest in WPM in comparison with MS medium showing maximum shoot length of 1.07 cm and maximum of one leaf. Average number of leaves was found to be highest (1.42) in B5 medium when compared with MS (1.0) and WPM (1.23) media.

After a comparative perusal of various growth parameters in all three media, MS medium was found to be superior to B5 and WPM media, for tissue culture in big-leaf mahogany. Different stages of culture establishment in MS and WPM media are shown in plate 1 and 2.

4.4 EFFECT OF PLANT GROWTH REGULATORS ON CULTURE ESTABLISHMENT AND GROWTH

Out of the three basal media used, MS medium was selected for further experiments. This was fortified with different cytokinins and auxins at various concentrations either singly or in combination to evaluate the best growth regulator

Plate. 1. Different stages of cultures establishment and leaf morphogenesis in MS basal medium

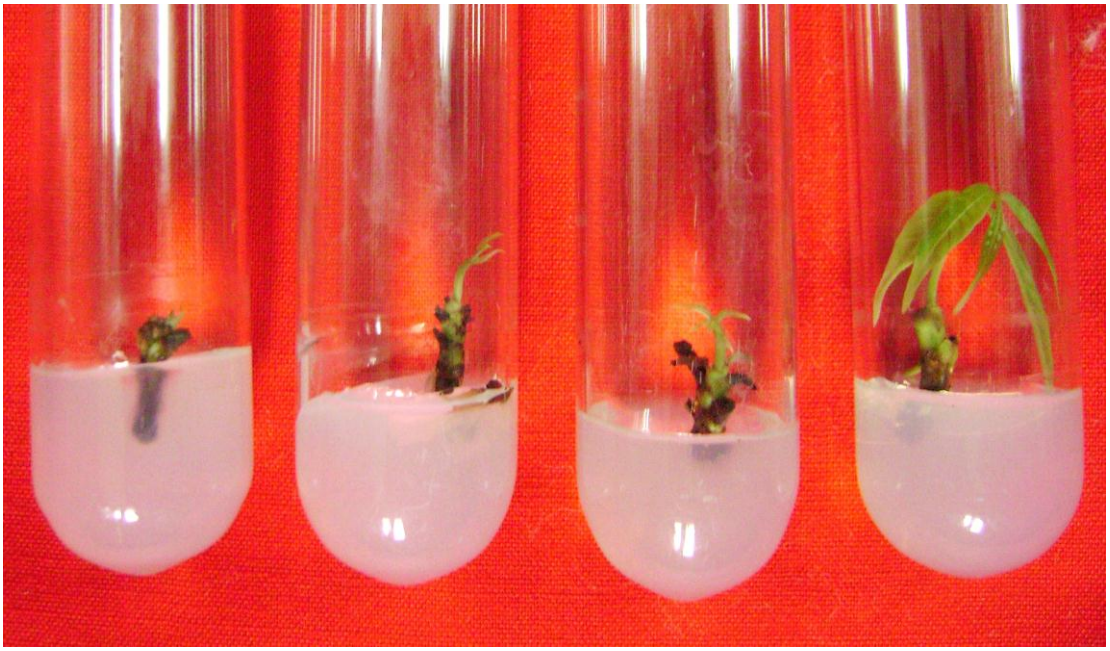

Plate. 2. Different stages of cultures establishment and leaf morphogenesis in WPM basal medium

media combination for maximum culture establishment and growth in *S. macrophylla.*

4.4.1 Effect of BA on culture establishment and growth in MS medium

In this study MS medium was supplemented with three different concentrations of BA (1.0, 2.0 and 3.0 mg l^{-1}). The effect of various concentrations of BA on growth parameters of mahogany are presented in Table. 7 (Figure 2a., 2b., and 2c in page no. 83). Percentage of bud initiation (41.67 %) and cultures with multiple shoots (18.33 %) (Plate. 3) was found to be high in MS medium supplemented with 1.0 mg l^{-1} BA. However, in MS+2.0 mg l^{-1} BA, 28.33 per cent and 16.67 per cent bud initiation and cultures with multiple shoots was recorded respectively. In general per cent bud initiation and leaf initiation were higher, when MS medium was supplemented with lower concentrations of BA.

Time taken for bud initiation in media containing 2.0 mg l^{-1} BA was as low as (10.06 days) followed by MS with 1.0 mg l^{-1} BA (10.63 days) and MS+3.0 mg l^{-1} BA (11.86 days) (Fig. 2b in page no. 83). It is observed that media supplemented with optimum concentration of BA shown early bud initiation. However, all the three combinations were found to be non-significant with respect to percentage bud initiation, shoot initiation and time taken for bud initiation.

Per cent leaf initiation (18.33 %) in media containing 1.0 mg l^{-1} BA was highest followed by MS+2.0 mg l^{-1} BA (10.0 %) and 6.67 % in MS+3.0 mg l^{-1} BA (Fig. 2a). Delayed leaf initiation up to 19.69 days was observed in media with 1.0 mg l^{-1} BA followed by 19.39 days when MS medium was fortified with 2.0 mg l^{-1} (Fig. 2b). With respect to leaves, average number of leaves per explant was more (1.61) in media containing 2.0 mg l^{-1} BA (Fig.2c in page no. 83). However, the effect of

Table. 7 Effect of BA on bud break and shoot development in axillary bud cultures of *Swietenia macrophylla* in MS medium

Concentration of BA ($mg\ l^{-1}$)	Bud initiation		% culture with multiple shoots	Shoot length (cm)		No. of shoots		Leaf initiation		No. of leaves	
	% culture	Days		Avg.	Max.	Avg.	Max.	% culture	Days	Avg.	Max.
1.0	41.67 (0.44)	10.63	18.33 (0.19)	0.55	1.17^{A}	2.14	3.0	18.33 (0.19)	19.69	1.22	1.67
2.0	28.33 (0.29)	10.06	16.67 (0.17)	0.42	0.67^{B}	1.84	2.67	10.0 (0.10)	19.39	1.61	2.0
3.0	21.67 (0.22)	11.86	8.33 (0.08)	0.51	0.73^{B}	1.78	2.33	6.67 (0.07)	16.17	0.78	1.0
SEm $\pm$	10.00	2.24	7.45	0.05	0.05	0.35	0.38	10.45	7.20	0.62	0.86
F	2.07	0.34	1.03	3.87	66.33*	0.63	1.50	0.66	0.76	0.89	0.70

* Significant at 5 %

Figures in parenthesis are arcsine transformed values

Figures with the same superscript do not differ significantly

Plate.3 Multiple shoots in MS + 1.0 mg l^{-1} BA

different concentrations of BA on per cent leaf initiation and number of leaves (average and maximum) was non-significant.

The effect of different treatments on average shoot length was non-significant. The media added with 1.0 mg l^{-1} BA produced average of 0.55 cm lengthy shoots followed by 0.51 cm in MS+3.0 mg l^{-1}. Treatments were found significant on maximum shoot length, with BA at 1.0 mg l^{-1} showing, a maximum of 1.17 cm shoots, media containing 2.0 mg l^{-1} BA (0.67 cm) and 3.0 mg l^{-1} BA (0.73 cm) found on par with each other. MS media supplemented with 1.0 mg l^{-1} BA produced an average number of 2.14 shoots per explants and a maximum of three shoots per explant. Average of 1.84 and 1.78 shoots per explant and a maximum of 2.67 and 2.33 shoots per explants were observed at 2.0 mg l^{-1} and 3.0 mg l^{-1} BA, respectively.

4.4.2 Effect of kinetin on culture establishment and growth in MS media

In the present study kinetin was added at three different levels (1.0, 2.0 and 3.0 mg l^{-1}) to MS media to know its effect on culture establishment and growth. The effect of different levels of kinetin on culture establishment and growth of mahogany cultures is presented in Table. 8. MS media supplemented with kinetin at 2.0 mg l^{-1} and 3.0 mg l^{-1} has shown highest percentage (41.67 %) of bud initiation followed by 35.0 percent at kinetin 1.0 mg l^{-1} (Fig. 3a in page no. 85). It is observed that at all the three levels of kinetin there was no significant difference in bud initiation and multiple shoot induction. However, in media containing 3 mg l^{-1} kinetin, early bud break in 9.58 days was observed (Fig. 3b in page no. 85). It is delayed up to 13.13 days and 10.63 days at 1.0 mg l^{-1} and 2.0 mg l^{-1} kinetin respectively. Kinetin at high concentration of 3 mg l^{-1} gave rise to multiple shoots in 11.67 percent of cultures.

All the three treatments were not differing significantly with respect to shoot

Table. 8 Effect of kinetin on bud break and shoot development in axillary bud cultures of *Swietenia macrophylla* in MS medium

Concentration of kinetin (mg l^{-1})	Bud initiation		% culture with multiple shoots	Shoot length (cm)		No. of shoots		Leaf initiation		No. of leaves	
	% culture	Days		Avg.	Max.	Avg.	Max.	% culture	Days	Avg.	Max.
1.0	35.0 (0.36)	13.13	5.0 (0.05)	0.60^{A}	0.97	1.21	1.67	20.0 (0.20)	16.19	1.3	2.0
2.0	41.67 (0.43)	10.63	6.67 (0.07)	0.50^{AB}	0.97	1.21	2.0	25.0 (0.25)	17.28	1.33	2.0
3.0	41.67 (0.43)	9.58	11.67 (0.12)	0.36^{B}	0.50	1.14	1.67	5.0 (0.05)	11.0	0.67	1.0
SEm $\pm$	9.91	1.23	6.94	0.08	0.27	0.20	0.77	7.82	4.61	0.31	0.54
F	0.32	4.44	0.50	6.78*	2.06	0.07	0.13	3.55	1.06	2.95	4.0

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

length. Kinetin at 1.0 mg l^{-1} produced shoots with average length of 0.6 cm. Longer shoots were produced at 1 mg l^{-1} and 2 mg l^{-1} of kinetin concentrations. Average number of shoots per explants was highest (1.21) in media containing 1.0 mg l^{-1} kinetin. 2.0 mg l^{-1} kinetin, followed by (1.14) in media with 3.0 mg l^{-1} kinetin. MS+2.0 mg l^{-1} kinetin produced a maximum of two shoots per explants, and in media added with 1.0 mg l^{-1} and 3.0 mg l^{-1} kinetin it was low (1.67).

Percentage of leaf initiation was highest (25.0 %) in media supplemented with kinetin at 2.0 mg l^{-1}, followed by 1.0 mg l^{-1}kinetin (20.0 %); lowest (5.0 %) was observed in MS media with 3.0 mg l^{-1} kinetin. Kinetin at higher concentration (3.0 mg l^{-1}) has shown early leaf initiation in 11.0 days. Time for leaf initiation was 16.19 days and 17.28 days in MS media added with kinetin at 1.0 mg l^{-1} and 2.0 mg l^{-1} respectively. Media supplemented with 1.0 mg l^{-1} and 2.0 mg l^{-1} kinetin produced average of 1.3 leaves per explant (Fig. 3c in page no. 85). However, kinetin at all the levels was found non-significant with respect to various growth parameters.

4.4.3 Effect of BA and IAA on culture establishment and growth in MS media

Effect of BA at four different concentrations (0.5, 1.0, 2.0, and3.0 mg l^{-1}) in all possible combinations with IAA at three levels (0.1, 0.5 and 1.0 mg l^{-1}) is presented in Table. 9. There was a significant difference on the percent bud initiation due to different treatments. The percentage of bud initiation was highest (78.33 %) in media containing 2.0 mg l^{-1} BA+1.0 mg l^{-1} IAA, followed by 1.0 mg l^{-1} BA+0.5 mg l^{-1} IAA (75.0 %) and 2.0 mg l^{-1} BA+0.1 mg l^{-1} IAA (73.33 %). These three treatments do not differ significantly among themselves; however they are statistically significant over other combinations tried with respect to bud initiation. Lowest bud initiation (15.0 %) was recorded in MS+0.5mg l^{-1} BA+0.1 mg l^{-1} IAA.

Table. 9 Effect of combination of BA and IAA on bud break and shoot development in axillary bud cultures of *Swietenia macrophylla* in MS medium

Concentration of BA+IAA (mg l^{-1})	Bud initiation		% culture with multiple shoots	Shoot length (cm)		No. of shoots		Leaf initiation		No. of leaves		Callusing %
	% culture	Days		Avg.	Max.	Avg.	Max.	% culture	Days	Avg.	Max.	
0.5+0.1	15.0^{A} (0.15)	9.44^{BC}	1.67^{A} (0.02)	1.08^{DEF}	1.23^{BC}	1.33	1.33^{A}	5.0^{A} (0.05)	13.50	0.83^{A}	1.0^{A}	1.67 (0.02)
0.5+0.5	45.0^{BCD} (0.47)	8.29^{AB}	10.0^{AB} (0.10)	1.11^{DEF}	1.33^{BC}	1.52	2.67^{AB}	36.67^{BCDE} (0.38)	14.82	1.52^{ABC}	2.67^{BC}	1.67 (0.02)
0.5+1.0	26.67^{AB} (0.27)	8.75^{ABC}	3.33^{A} (0.03)	0.97^{DE}	1.17^{B}	1.10	1.33^{A}	16.67^{A} (0.17)	15.42	1.07^{AB}	1.33^{AB}	10.0 (0.10)
1.0+0.1	40.0^{BC} (0.41)	9.77^{BC}	21.67^{ABC} (0.22)	0.51^{A}	0.70^{A}	1.99	3.33^{B}	21.67^{ABC} (0.22)	14.81	1.94^{BC}	2.67^{BC}	3.33 (0.03)
1.0+0.5	75.0^{E} (0.85)	10.16^{BC}	30.0^{BC} (0.31)	0.68^{AB}	1.07^{B}	1.90	2.67^{AB}	53.33^{E} (0.57)	16.73	2.27^{C}	2.67^{BC}	10.0 (0.10)
1.0+1.0	68.33^{DE} (0.76)	11.11^{C}	18.33^{ABC} (0.19)	0.76^{BC}	1.07^{B}	1.65	3.0^{B}	41.67^{CDE} (0.43)	16.44	1.97^{BC}	2.67^{BC}	16.67 (0.17)
2.0+0.1	73.33^{E} (0.87)	7.93^{AB}	33.33^{C} (0.35)	0.91^{CD}	1.3^{BC}	1.80	3.33^{B}	46.47^{DE} (0.50)	16.38	1.64^{ABC}	3.0^{C}	6.67 (0.07)
2.0+0.5	65.0^{CDE} (0.71)	6.91^{A}	28.33^{BC} (0.29)	0.98^{DE}	1.37^{BCD}	2.01	3.0^{B}	43.33^{CDE} (0.45)	16.06	1.31^{AB}	2.0^{ABC}	13.33 (0.13)
2.0+1.0	78.33^{E} (0.92)	7.89^{AB}	21.67^{ABC} (0.22)	1.18^{EF}	1.63^{DE}	1.57	3.0^{B}	41.67^{CDE} (0.43)	15.25	1.08^{AB}	1.33^{AB}	15.0 (0.15)
3.0+0.1	55.0^{CDE} (0.60)	8.50^{AB}	21.67^{ABC} (0.22)	1.26^{F}	1.83^{E}	1.63	3.0^{B}	26.67^{ABCD} (0.27)	17.15	1.13^{AB}	1.33^{AB}	15.0 (0.15)
3.0+0.5	61.67^{CDE} (0.67)	9.64^{BC}	20.0^{ABC} (0.20)	1.56^{G}	1.87^{E}	2.00	3.33^{B}	35.0^{BCDE} (0.36)	16.98	1.61^{ABC}	2.67^{BC}	11.67 (0.12)
3.0+1.0	53.33^{CDE} (0.57)	9.39^{BC}	16.07^{ABC} (0.17)	1.16^{ABC}	1.5^{EF}	1.59	2.67^{AB}	10.0^{A} (0.10)	14.15	1.17^{A}	1.33^{AB}	3.30 (0.03)
SEm ±	10.80	1.02	8.98	0.10	0.13	0.31	0.65	9.65	3.28	0.39	0.58	5.53
F	6.75*	2.58*	2.41*	17.09*	13.04*	1.62	2.26*	5.07*	1.33	2.91*	3.25*	1.79

* Significant at 5 %

Figures in parenthesis are arcsine transformed values

Figures with the same superscript do not differ significantly

The treatments MS+2.0 mg l^{-1} BA+0.5 mg l^{-1} IAA, MS+3.0 mg l^{-1} BA+0.1 mg l^{-1} IAA, MS+3.0 mg l^{-1} BA+0.5 mg l^{-1} IAA and MS+3.0 mg l^{-1} BA+1.0 mg l^{-1} IAA were found on par with each other in per cent bud initiation. Statistically treatments were differed in time taken for bud initiation. An early bud initiation in 6.91 days was observed in media supplemented with 2.0 mg l^{-1} BA+0.5 mg l^{-1} IAA. Time taken for bud initiation was as high as 11.11 days at 1.0 mg l^{-1} BA+1.0 mg l^{-1} IAA.

Significant difference was recorded for different treatments on producing multiple shoots in axillary bud cultures of mahogany. Highest per cent of cultures with multiple shoots (33.33 %) was shown by MS+2.0 mg l^{-1} BA+0.1 mg l^{-1} IAA (Plate. 4) followed by(30.0 %) in 1.0 mg l^{-1} BA+0.5 mg l^{-1} IAA. Lowest per cent was recorded (1.67 %) in MS+0.5 mg l^{-1}BA+0.1 mg l^{-1}IAA. For per cent cultures with multiple shoots, treatments *viz.* MS+1.0 mg l^{-1} BA+0.1 mg l^{-1} IAA, MS+1.0 mg l^{-1} BA+1.0 mg l^{-1} IAA, MS+2.0 mg l^{-1} BA+1.0 mg l^{-1} IAA, MS+3.0 mg l^{-1} BA+0.1 mg l^{-1} IAA, MS+3.0 mg l^{-1} BA+0.5 mg l^{-1} IAA, and MS+3.0 mg l^{-1} BA+1.0 mg l^{-1} IAA were found at par with each other.

Effect of treatment on average length of shoot was significant. Highest shoot length (1.56 cm) was produced in media containing 3.0 mg l^{-1} BA+0.5 mg l^{-1} IAA (Plate. 5) followed by (1.26 cm) in MS+3.0 mg l^{-1} BA+0.1 mg l^{-1} IAA. Lowest shoot length (0.51) was recorded in media added with 1.0 mg l^{-1} BA+0.1 mg l^{-1} IAA.

Maximum number of shoots (2.01) per explant was recorded in MS media containing 2.0 mg l^{-1} BA+ 0.5 mg l^{-1} IAA. Murashige and Skoog media supplemented with 3.0 mg l^{-1} BA+0.5 mg l^{-1} IAA (Plate. 5) and MS+1.0 mg l^{-1}+0.1 mg l^{-1}IAA number of shoots was 2.0 and 1.99 respectively. These three treatments were found significant over all other combinations of BA and IAA tried in the experiment.

Plate. 4. Multiple shoots in MS+2.0 mg l^{-1} BA+0.1 mg l^{-1} IAA

Plate. 5. Multiple shoots and leaf morphogenesis in MS+3.0 mg l^{-1} BA+0.5 mg l^{-1} IAA

Effect of treatments on per cent leaf initiation was found to be significant. Highest per cent of leaf initiation (53.33 %) was observed in cultures established on MS+1.0 mg l^{-1} BA+0.5 mg l^{-1} IAA, which was found superior to other combinations. The lowest per cent leaf initiation (5.0 %) was observed in media supplemented with 0.5 mg l^{-1} BA+0.1 mg l^{-1} IAA. Treatment effect on time taken for leaf initiation was non-significant. However, after 13.50 days leaf initiation was noticed on MS media supplemented with 0.5 mg l^{-1} BA+0.1 mg l^{-1} IAA. While leaf initiation was prolonged up to 17.15 days on media added with 3.0 mg l^{-1} BA+0.1 mg l^{-1} IAA. Statistically significant difference was observed in effect of treatment on average number of leaves per explants. Average number of leaves per explants was found to be highest (2.27 leaves) in MS+1.0 mg l^{-1} BA+0.5 mg l^{-1} IAA. The lowest (0.83 leaves) was recorded on MS+0.5 mg l^{-1} BA+0.1 mg l^{-1} IAA. Callusing was highest (16.67 %) at 1.0 mg l^{-1} BA+1.0 mg l^{-1} IAA; the lowest (1.67 %) was found in MS+0.5 mg l^{-1} BA+0.1 mg l^{-1} IAA and MS+0.5 mg l^{-1} BA+0.5 mg l^{-1} IAA. However, there was no significant difference due to treatment on callusing.

4.4.4 Effect of BA and IBA on culture establishment and growth in MS medium

The data recorded on effect of different combinations of BA (0.5, 1.0, 2.0 and 3.0 mg l^{-1}) and IBA (0.1, 0.5 and 1.0 mg l^{-1}) on culture establishment and growth in MS medium are presented in Table. 10.

Effect of treatment on time taken for bud initiation and percentage of leaf initiation were found to be significant. Highest percentage of bud initiation (70.0%) was observed on media supplemented with 0.5 mg l^{-1}BA+1.0 mg l^{-1} IBA. This was followed by 1.0 mg l^{-1} BA+ 0.5 mg l^{-1} IBA (58.33%), 2.0 mg l^{-1} BA+1.0 mg l^{-1} IBA (56.67 %) and 3.0 mg l^{-1} BA+1.0 mg l^{-1} IBA (60.33 %). Lowest percentage of bud

Table. 10 Effect of combination of BA and IBA on bud break and shoot development in axillary bud cultures of *Swietenia macrophylla* in MS medium

Concentration of BA+IBA (mg l^{-1})	Bud initiation		% culture with multiple shoots	Shoot length (cm)		No. of shoots		Leaf initiation		No. of leaves		Callusing %
	% culture	Days		Avg.	Max.	Avg.	Max.	% culture	Days	Avg.	Max.	
0.5+0.1	25.0 (0.26)	8.03^{CDE}	10.0 (0.10)	1.02	1.17	1.41	2.0	18.33^{ABCD} (0.18)	14.31	1.60	2.33	3.33 (0.03)
0.5+0.5	43.33 (0.45)	9.0^{DEF}	1.67 (0.02)	1.13	1.33	1.08	1.33	30.0^{CDE} (0.31)	15.28	1.22	1.67	1.67 (0.02)
0.5+1.0	70.0 (0.78)	9.55^{F}	5.0 (0.05)	1.03	1.23	1.33	1.67	58.33^{F} (0.62)	15.44	1.47	1.67	1.67 (0.02)
1.0+0.1	51.67 (0.55)	9.25^{EF}	15.0 (0.15)	1.06	1.33	1.56	3.0	15.0^{ABC} (0.15)	14.08	1.28	1.67	0.0 (0.0)
1.0+0.5	58.33 (0.67)	8.09^{CDEF}	11.67 (0.12)	1.01	1.43	1.52	3.33	43.33^{E} (0.45)	16.22	1.27	2.0	0.0 (0.0)
1.0+1.0	51.67 (0.55)	9.08^{DEF}	11.67 (0.12)	1.15	1.50	1.08	3.0	33.33^{DE} (0.34)	16.83	1.28	2.33	0.0 (0.0)
2.0+0.1	41.67 (0.44)	7.69^{BCD}	10.0 (0.10)	1.19	1.45	1.10	1.33	13.33^{AB} (0.13)	13.39	0.73	1.50	0.0 (0.0)
2.0+0.5	48.33 (0.51)	6.86^{ABC}	5.0 (0.05)	1.09	1.37	1.39	2.33	28.33^{BCDE} (0.29)	14.89	1.66	2.33	0.0 (0.0)
2.0+1.0	56.67 (0.61)	6.03^{A}	8.33 (0.08)	1.02	1.23	1.42	2.0	33.33^{DE} (0.34)	15.33	1.67	2.33	5.0 (0.05)
3.0+0.1	34.12 (0.36)	6.64^{ABC}	5.17 (0.05)	1.03	1.23	1.47	2.33	8.69^{A} (0.09)	15.60	1.17	1.33	0.0 (0.0)
3.0+0.5	45.0 (0.48)	7.46^{ABC}	5.0 (0.05)	0.75	0.8	1.34	2.33	31.67^{DE} (0.32)	14.26	1.0	1.0	0.0 (0.0)
3.0+1.0	60.33 (0.65)	6.21^{AB}	3.33 (0.03)	0.72	0.77	1.1	1.67	28.33^{BCDE} (0.28)	13.03	1.34	1.67	0.0 (0.0)
SEm ±	12.12	0.66	5.97	0.24	0.29	0.40	0.77	6.81	2.18	0.29	0.65	1.67
F	2.01	6.95*	0.92	0.79	1.14	0.41	1.25	8.17*	1.73	1.75	1.18	1.99

* Significant at 5 %

Figures in parenthesis are arcsine transformed values

Figures with the same superscript do not differ significantly

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initiation (25.0 %) was observed in media containing 0.5 mg l^{-1} BA+0.1 mg l^{-1} IBA. In media added with 2.0 mg l^{-1} BA+1.0 mg l^{-1} IBA early bud initiation (6.03 days) was recorded. It was delayed up to 9.55 days in MS+05 mg l^{-1} BA+1.0 mg l^{-1} IBA.

Percent culture with multiple shoots was highest (15.0 %) in MS+1.0 mg l^{-1} BA+0.1 mg l^{-1} IBA, followed by (11.67 %) in MS+1.0 mg l^{-1} BA+0.5 mg l^{-1} IBA. The media supplemented with 3.0 mg l^{-1} BA+1.0 mg l^{-1} IBA has shown lowest percent (3.30 %) of cultures with multiple shoots. Average shoot length recorded was highest (1.19 cm) in MS media supplied with 2.0 mg l^{-1}BA+0.1 mg l^{-1} IBA. it was as low as 0.72 cm in media with 3.0 mg l^{-1} BA+1.0 l mg l^{-1} IBA. An average of 1.56 shoots per explants were produced in MS+1.0 mg l^{-1} BA+0.1 mg l^{-1}IBA, it is followed by 1.52 shoots per explants in MS+1.0 mg l^{-1} BA+ 0.5 mg l^{-1} IBA. In media containing 3.0 mg l^{-1} BA+1.0 mg l^{-1} IBA lowest average number of shoots per explants was noted.

Percent leaf initiation in media containing 3.0 mg l^{-1} BA+0.1 mg l^{-1}IBA was found significantly inferior to MS+0.5 mg l^{-1} BA+1.0 mg l^{-1} IBA, 1.0 mg l^{-1} BA+0.5 mg l^{-1} IBA, and 1.0 mg l^{-1} BA+1.0 mg l^{-1} IBA. In former media, the percent leaf initiation was observed as 8.69 %, whereas in latter they were 58.33 %, 43.33 % and 33.33 % respectively. Leaf initiation media combination 3.0 mg l^{-1} BA+1.0 mg l^{-1} IBA was early (13.03 days) in comparison with 16.83 days in 1.0 mg l^{-1} BA+1.0 mg l^{-1} IBA. Average no of leaves per explant produced was highest (1.67 leaves) in 2.0 mg l^{-1} BA+1.0 mg l^{-1} IBA. However, the effect of treatment on time taken for leaf initiation and callus production was found non-significant.

4.4.5 Effect of kinetin and IAA on culture establishment and growth in MS medium

Effect of different combinations of kinetin at four levels namely 0.5, 1.0, 2.0 and 3.0 mg l^{-1} with three different levels of IAA (0.1, 0.5 and 1.0 mg l^{-1}) on growth was evaluated. The result obtained is presented in Table. 11. Percentage of bud initiation was highest (61.67 %) on MS+2.0 mg l^{-1} kinetin+1.0 mg l^{-1} IAA; this was followed by 60.0 percent on 0.5 mg l^{-1} kinetin+0.1 mg l^{-1} IAA. Least bud initiation (33.33 %) was recorded in 1.0 mg l^{-1} kinetin+0.1 mg l^{-1} IAA. Highest percentage of cultures with multiple shoots (11.67 %) was produced on media supplemented with 3.0 mg l^{-1} Kinetin+0.1 mg l^{-1} IAA. Whereas MS media supplied with 2.0 mg l^{-1} kinetin+0.1 mg l^{-1} IAA and 2.0 mg l^{-1} kinetin+0.5 mg l^{-1}IAA failed to produce multiple shoots. Per cent leaf initiation was found to be highest (43.33 %) in MS+2.0 mg l^{-1} kinetin+1.0 mg l^{-1} IAA and a minimum of 23.33 percent leaf initiation was recorded in media added with 1.0 mg l^{-1} kinetin+0.1 mg l^{-1} IAA and 1.0 mg l^{-1} kinetin+0.5 mg l^{-1} IAA. Even though, there was no significant difference on these growth parameters due to treatment.

Treatment effect on time taken for bud initiation and leaf initiation was found to be significant. Bud initiation was early (6.38 days) on MS+2.0 mg l^{-1} kinetin+1.0 mg l^{-1} IAA and it was delayed up to 10.77 days on media supplemented with 1.0 mg l^{-1} kinetin+0.1 mg l^{-1} IAA. Time taken for leaf initiation was 11.08 days in MS media supplemented with+2.0 mg l^{-1} kinetin+1.0 mg l^{-1} IAA. It was delayed (17.97 days) in media containing 2.0 mg l^{-1} kinetin+0.5 mg l^{-1} IAA.

The effect of treatment on average number of shoots, average number of leaves and callus formation was statistically different. A maximum of 1.75 shoots per explant was produced in the media fortified with 1.0 mg l^{-1} kinetin+0.1 mg l^{-1} IAA;

Table. 11 Effect of combination of kinetin and IAA on bud break and shoot development in axillary bud cultures of *Swietenia macrophylla* in MS medium

Concentration of kinetin+IAA (mg l^{-1})	Bud initiation		% culture with multiple shoots	Shoot length (cm)		No. of shoots		Leaf initiation		No. of leaves		Callusing %
	% culture	Days		Avg.	Max.	Avg.	Max.	% culture	Days	Avg.	Max.	
0.5+0.1	60.00 (0.65)	8.61^{BC}	10.0 (0.10)	0.50	0.67	1.19^{AB}	1.67^{AB}	26.67 (0.27)	15.77^{B}	1.12^{AB}	1.67^{ABC}	1.67^{B} (0.002)
0.5+0.5	40.00 (0.42)	8.36^{ABC}	3.33 (0.03)	0.54	0.63	1.11^{AB}	2.0^{AB}	25.0 (0.26)	14.53^{B}	1.33^{AB}	1.33^{AB}	3.33^{B} (0.03)
0.5+1.0	58.33 (0.63)	8.55^{BC}	5.0 (0.05)	0.59	0.67	1.3^{ABC}	1.67^{AB}	28.33 (0.29)	15.79^{B}	1.31^{AB}	2.33^{BC}	11.67^{A} (0.12)
1.0+0.1	33.33 (0.34)	10.77^{D}	8.33 (0.08)	0.74	0.97	1.65^{C}	2.0^{AB}	23.33 (0.24)	15.37^{B}	1.0^{A}	1.0^{A}	0.0^{B} (0.0)
1.0+0.5	43.33 (0.45)	13.05^{E}	8.33 (0.08)	0.73	1.07	1.42^{ABC}	2.67^{B}	23.33 (0.24)	16.87^{B}	1.87^{BC}	2.33^{BC}	0.0^{B} (0.0)
1.0+1.0	46.67 (0.51)	9.50^{BCD}	6.67 (0.07)	0.72	0.93	1.28^{ABC}	2.33^{AB}	35.0 (0.36)	14.73^{B}	1.23^{AB}	1.67^{ABC}	0.0^{B} (0.0)
2.0+0.1	51.67 (0.55)	7.67^{AB}	0.0 (0.0)	0.75	0.9	1.0^{A}	1.0^{A}	28.33 (0.29)	15.13^{B}	1.70^{ABC}	2.0^{ABC}	0.0^{B} (0.0)
2.0+0.5	53.33 (0.57)	7.97^{ABC}	0.0 (0.0)	0.72	0.87	1.0^{A}	1.0^{A}	35.0 (0.36)	17.97^{B}	2.10^{C}	2.67^{C}	0.0^{B} (0.0)
2.0+1.0	61.67 (0.68)	6.38^{A}	5.0 (0.05)	0.68	0.90	1.26^{ABC}	2.33^{AB}	43.33 (0.45)	11.08^{A}	1.0^{A}	1.0^{A}	0.0^{B} (0.0)
3.0+0.1	46.67 (0.49)	8.98^{BCD}	11.67 (0.12)	0.69	0.83	1.49^{BC}	2.67^{B}	26.67 (0.27)	15.87^{B}	1.77^{BC}	2.0^{ABC}	0.0^{B} (0.0)
3.0+0.5	51.67 (0.55)	10.03^{CD}	8.33 (0.08)	0.70	0.77	1.49^{BC}	1.67^{AB}	35.0 (0.36)	14.62^{B}	1.17^{AB}	1.67^{ABC}	0.0^{B} (0.0)
3.0+1.0	55.0 (0.59)	8.13^{ABC}	6.67	0.72	0.77	1.49^{BC}	2.67^{B}	25.0 (0.26)	15.87^{B}	1.77^{BC}	2.0^{ABC}	0.0^{B} (0.0)
SEm ±	12.64	0.89	4.76	0.11	0.15	0.19	0.56	8.02	1.54	0.31	0.49	2.04
F	0.90	7.33*	1.18	1.12	1.52	2.45*	2.24*	1.18	2.30*	2.83*	2.26*	5.54*

* Significant at 5 %

Figures in parenthesis are arcsine transformed values

Figures with the same alphabet do not differ significantly

this was found superior over the all other treatments. Murashige and Skoog medium containing 3.0 mg l^{-1} kinetin+0.1 mg l^{-1} IAA, 3.0 mg l^{-1} kinetin+0.5 mg l^{-1} IAA and 3.0 mg l^{-1} kinetin+1.0 mg l^{-1} IAA has produced 1.49 shoots per explant, and on media supplemented with 1.0 mg l^{-1}kineitn+0.5 mg l^{-1} IAA, 1.0 mg l^{-1} kinetin+1.0 mg l^{-1} IAA, 2.0 mg l^{-1} kinetin+1.0 mg l^{-1} IAA average number of shoots was 1.42, 1.28 and 1.26 respectively. A minimum of one shoot per explant was observed on MS+2.0 mg l^{-1} kinetin+0.1 mg l^{-1} IAA and MS+2.0 mg l^{-1} kinetin+0.5 mg l^{-1} IAA. Average number of leaves per explant was maximum (2.1 leaves) in MS+2.0 mg l^{-1} kinetin+0.5 mg l^{-1} IAA; which was found superior over all other combinations tried with respect to number of leaves. The lowest (1.0 leaves per explant) was recorded on media supplied with 1.0 mg l^{-1} kinetin+0.1 mg l^{-1} IAA and 2.0 mg l^{-1} kinetin+1.0 mg l^{-1} IAA.

4.4.6 Effect of kinetin and IBA of culture establishment and growth in MS medium

The data collected on the effect of different concentrations of kinetin and IBA on growth and culture establishment are presented in Table. 12. Effect of treatment on per cent bud initiation was non-significant. Though, highest percentage of bud initiation (61.67 %) was observed on media supplemented with 0.5 mg l^{-1} kinetin+0.5 mg l^{-1} IBA and 1.0 mg l^{-1} kinetin+0.5 mg l^{-1} IBA. In media containing 0.5 mg l^{-1} kinetin+0.1 mg l^{-1} IBA bud initiation was 18.33 percent, which was found to be lowest of all the combinations tried. The time taken for bud initiation was maximum (10.88 days) in MS media fortified with 2.0 mg l^{-1} kinetin+1.0 mg l^{-1} IBA. Early bud initiation (7.09 days) was observed in MS media containing 3.0 mg l^{-1} kinetin + 0.5 mg l^{-1} IBA.

Table. 12 Effect of combination of kinetin and IBA on bud break and shoot development in axillary bud cultures of *Swietenia macrophylla* in MS medium

Concentration of kinetin+IBA (mg l^{-1})	Bud initiation		% culture with multiple shoots	Shoot length (cm)		No. of shoots		Leaf initiation		No. of leaves	
	% culture	Days		Avg.	Max.	Avg.	Max.	% culture	Days	Avg.	Max.
0.5+0.1	18.33 (0.18)	9.11	0.0 (0.0)	0.0^{A}	0.0^{A}	0.0^{A}	0.0^{A}	0.0^{A}	0.0^{A}	0.0^{A}	0.0^{A}
0.5+0.5	61.67 (0.67)	8.40	1.67 (0.02)	0.52^{B}	0.73^{BC}	1.05^{B}	1.33^{B}	21.67^{B} (0.22)	13.69^{BC}	1.34^{CD}	1.67^{C}
0.5+1.0	43.33 (0.46)	10.82	0.0 (0.0)	0.64^{BC}	0.77^{BC}	1.0^{B}	1.0^{AB}	26.67^{B} (0.27)	14.67^{BC}	1.0^{B}	1.0^{B}
1.0+0.1	31.67 (0.32)	10.46	0.0 (0.0)	0.0^{A}	0.0^{A}	0.0^{A}	0.0^{A}	0.0^{A}	0.0^{A}	0.0^{A}	0.0^{A}
1.0+0.5	61.67 (0.68)	9.06	0.0 (0.0)	0.56^{BC}	0.63^{B}	1.0^{B}	1.0^{AB}	30.0^{B} (0.31)	13.07^{BC}	1.12^{BC}	1.33^{BC}
1.0+1.0	48.33 (0.51)	8.93	1.67 (0.02)	0.63^{BC}	0.70^{BC}	1.22^{B}	1.33^{B}	23.33^{B} (0.27)	14.02^{BC}	1.0^{B}	1.0^{B}
2.0+0.1	38.33 (0.41)	9.72	1.67 (0.02)	0.57^{BC}	0.67^{BC}	1.07^{B}	1.33^{B}	28.33^{B} (0.29)	15.27^{BC}	1.0^{B}	1.0^{B}
2.0+0.5	51.67 (0.55)	9.54	3.33 (0.03)	0.59^{BC}	0.67^{BC}	1.15^{B}	1.67^{B}	30.0^{B} (0.31)	12.49^{B}	1.62^{E}	2.33^{D}
2.0+1.0	55.0 (0.59)	10.88	1.67 (0.02)	0.59^{BC}	0.70^{BC}	1.08^{B}	1.33^{B}	33.33^{B} (0.34)	15.14^{BC}	1.0^{B}	1.0^{B}
3.0+0.1	58.33 (0.64)	7.78	0.0 (0.0)	0.68^{BC}	0.77^{BC}	1.0^{B}	1.0^{AB}	20.0^{B} (0.20)	16.57^{C}	1.0^{B}	1.0^{B}
3.0+0.5	43.33 (0.46)	7.09	3.33 (0.03)	0.78^{C}	0.90^{C}	0.78^{B}	1.33^{B}	21.67^{B} (0.22)	15.03^{BC}	1.0^{B}	1.0^{B}
3.0+1.0	41.67 (0.44)	10.36	5.0 (0.05)	0.75^{BC}	0.8^{BC}	1.2^{B}	1.67^{B}	21.67^{B} (0.22)	15.50^{BC}	1.48^{DE}	2.33^{D}
SEm ±	13.26	1.37	2.64	0.10	0.11	0.21	0.49	7.30	1.52	0.11	0.27
F	1.89	1.52	0.79	12.47*	14.83*	8.34*	2.54*	4.40*	28.80*	39.55*	14.43*

* Significant at 5 %

Figures in parenthesis are arcsine transformed values

Figures with the same alphabet do not differ significantly

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The effect of treatments was significantly different on shoot length and average number of shoots. Average shoot length was found to be highest (0.78 cm) on media containing 3.0 mg l^{-1} kinetin+0.5 mg l^{-1} IBA. Average number of shoots per explant was maximum (1.22 shoots) on media MS+1.0 mg l^{-1} kinetin+1.0 mg l^{-1} IBA. However, cultures established on media having 0.5 mg l^{-1} kinetin+0.1 mg l^{-1} IBA and1.0 mg l^{-1} kinetin+0.1 mg l^{-1} IBA were failed to produce shoots.

Treatments showed significant difference on percentage of leaf initiation. Murashige and Skoog's medium containing 1.0 mg l^{-1} knetin+0.1 mg l^{-1} IBA failed to produce leaves. Treatments other than this were found to be on par with each other. The percent leaf initiation recorded was highest (33.33 %) on MS+2.0 mg l^{-1} kinetin+1.0 mg l^{-1} IBA. The same trend was observed in treatments with respect to number of leaves. The highest number of leaves (1.62) was recorded on media supplemented with 2.0 mg l^{-1} kinetin+0.5 mg l^{-1} IBA.

4.5 ESTABLISHMENT OF CONTINUOUS CULTURES OF *SWIETENIA MACROPHYLLA*

The result obtained is presented in Table. 13. After 20 days of sub-culturing cultures died without any growth. The percentage of death varied from 49 percent to 100 per cent. However, the cultures which were alive did not show further growth, except that they remained green. In some cultures leaf falling was noticed.

Table. 13 Different growth regulators used singly or in combination, to establish continuous cultures of *Swietenia macrophylla* in MS medium

Growth regulators (mg $l^{-1)}$	% culture	
	Dead	Living
BA 1.0	100	0
BA 2.0	83	17
BA 1.0 + IAA 0.1	69	31
BA 2.0 + IAA 0.1	49	51
BA 2.0 + IAA 0.5	73	27
BA 3.0 + IAA 0.5	53	47
BA 0.5 + IBA 1	82	18
BA 1.0 + IBA 0.1	72	28
BA 2.0 + IBA 0.1	85	15
Kin 3	84	16
Kin 1.0 + IAA 0. 1	61	39
Kin 2.0 + IAA 0.1	89	11
Kin 2.0 + IAA 0.5	90	10
Kin 2.0 + IBA 0.5	75	25
Kin 3.0 + IBA 0.5	87	3

DISCUSSION

5. DISCUSSION

Studies to evaluate the effects of basal media and different plant growth regulators on culture establishment and growth in *Swietenia macrophylla* King were taken up in the present investigation. The basic objective of the study was to standardize the protocol for micropropagation of big-leaf mahogany through tissue culture. The information amassed from the present studies will help us to formulate the suitable tissue culture protocol for micropropagation of big-leaf mahogany to produce large quantity of quality planting material to meet the afforestation demand. The salient findings of the present investigation in *S. macrophylla*, undertaken to formulate a protocol for *in vitro* propagation by using axillary buds are discussed hereunder.

5.1 ASEPTIC CULTURES AND CULTURE ESTABLISHMENT

It is reported that, the single most important source of losses in commercial and scientific plant tissue culture laboratories is microbial contamination (Leifert and Woodward, 1998). If the explant employed in a study is not properly disinfected, we will end up with contamination of all cultures. At times, control of contamination is extremely difficult and with many contaminants it is impractical (Leifert and Woodward, 1998). Hence more emphasis will have to be given on early recognition and prevention of contaminant at source itself.

In the present experiment, culture contamination was a serious problem since the explants were collected from seedlings grown in open field. During the study it was observed that variation exists in contamination rate of cultures depending on the time of explant collection. Explants collected during rainy season had shown as high as 100 per cent contamination. When explants were collected during November to

April, cultures exhibited contamination to a lesser extent Table. 5. In this study fungal contamination was found to be the major problem when compared to the bacterial contamination. These results are supporting the findings of Kalkoor (2007) in *Jatropa curcus.* This can be imputed to an amiable and conducive weather conditions which favour the uncontrolled proliferation of microbial inoculum in the field. The fungal contamination was may be due to succulent nature of explants and high amount of microbial inoculum in the field explants.

Variety of preventive measures had been taken to restraint the culture contamination during the present investigation. Among the various measures employed, culture contamination was substantially reduced by pre treating by dipping the explants in a solution of Carbendazim (Bavistin) and Mancozeb (Indofil M-45) each at 0.2 %, for one hour and finally surface sterilised by using 0.1 % mercuric chloride for 15 min Table. 4. Similar results were also presented by Kalkoor (2007) in *Jatropa curcus*.

Use of mercuric chloride as surface sterilant was reported in *S. macrophylla* x *S. mahogani* (Rodriguez *et al.,* 2003), in *Azadirachta indica* by Anaz and Vijayakumar (1996), Srividya *et al.* (1998) and Eeswara *et al.* (1999). However, by looking at the results presented in Table. 4, we can notice that immersing the explants in 0.1 % $HgCl_2$ for 20 min and in 0.2 % for 10 min, 15 min, and 20 min contamination can be controlled. But during the study it is experienced that the explants treated with higher concentrations of $HgCl_2$ have shown charring symptoms which was not observed in treatment involving pretreatment of explants in fungicidal solution and immersing in mercuric chloride (0.1 %) 15 min.

It is always desirable for economic viability of tissue culture techniques to have minimum percentage of contamination. During this study the cultures were

contaminated as late as after two weeks of culturing and also with bud sprout. This is also evident by the results presented by Roshni (2003) in *A. indica* and Kalkoor (2007) in *J. curcus*. This may be because of the presence of dormant spores of micro organisms deep inside the tissues which easily get escaped during surface sterilization and later when availability of favourable conditions favour the growth and hence expression after a long gap.

During the present study it was evident that the season of explant collection had an influence on the cultures establishment and growth. Highest per cent of culture establishment (77.56 %) was recorded during March-April and lowest was in the month of October (Table. 5). These results are in concordance with the findings of Husain and Anis (2009) in *Melia azedarach*, Sanjay *et al.* (2006) in sandal and Sharma *et al.* (2003) in *Crataeva adansonii*. As quoted by Sanjay *et al.* (2006), influence of season on culture establishment may be due to long flowering and seed setting habit of trees. In some studies, Tiwari *et al.* (2002) and Hussain and Anis (2009) have reported that this influence was due to the active growing season of the host plant. In mahogany new flush will be just before the rainy season; hence the explants collected during March-April have shown the higher performance because of the physiological stage of the explants.

5.2 EFFECT OF BASAL MEDIA ON CULTURE ESTABLISHMENT AND GROWTH

MS (Murashige and Skoog, 1962), WPM (Llyod and McCown, 1980) and B5 (Gamborg *et al.,* 1976) medium are most extensively used in forest tree micropropagation. During the present study a comparative study was done to evaluate the effect of three different media *viz.* MS medium, WPM medium and B5 medium on culture establishment and growth of axillary buds of big-leaf mahogany.

In the present investigation, through preliminary screening it was found that MS medium showed significantly higher performance for the growth parameters of cultures. This was followed by WPM (Table.6). Murashige and Skoog's medium was the best suited for culture establishment and growth of axillary buds in mahogany, when compared with other two media (Fig. 1a and 1b). In some of the earlier studies also MS medium was reported as the best basal media for shoot production in *S. macrophylla* (Albarran *et al.*, 1997; Lopes *et al.*, 2001; Rocha and Quoirin, 2004; Gonzalez and Pena 2007).

Considering the above observations, MS was employed for further studies to find out the effect of plant growth regulators on culture establishment of mahogany. The medium was supplemented with various combinations of plant growth regulators.

5.3 EFFECT OF PLANT GROWTH REGULATORS ON CULTURE ESTABLISHMENT AND GROWTH

Plant growth regulators (auxin and cytokinin) or their synthetic counterparts are required either singly or in combination to initiate and continue cell division and elongation. The physiological activity of phytohormones is more when supplied in meager quantities. The concentration and ratio of hormone requirement vary from plant to plant and it has to be standardized for particular plant tissue. Axillary bud is a predetermined organ having meristematic potential to develop into shoot in the absence of apical dominance. Sachs and Thimman (1964) reported that application of cytokinin to the axillary buds helps to overcome the effects of apical dominance and stimulates lateral buds to grow in the presence of terminal buds. Studies conducted by Roshni (2003) and by Chaturvedi *et al*. (2004) have confirmed that growth regulators viz. cytokinin, auxin and gibberlic acid play an important role in

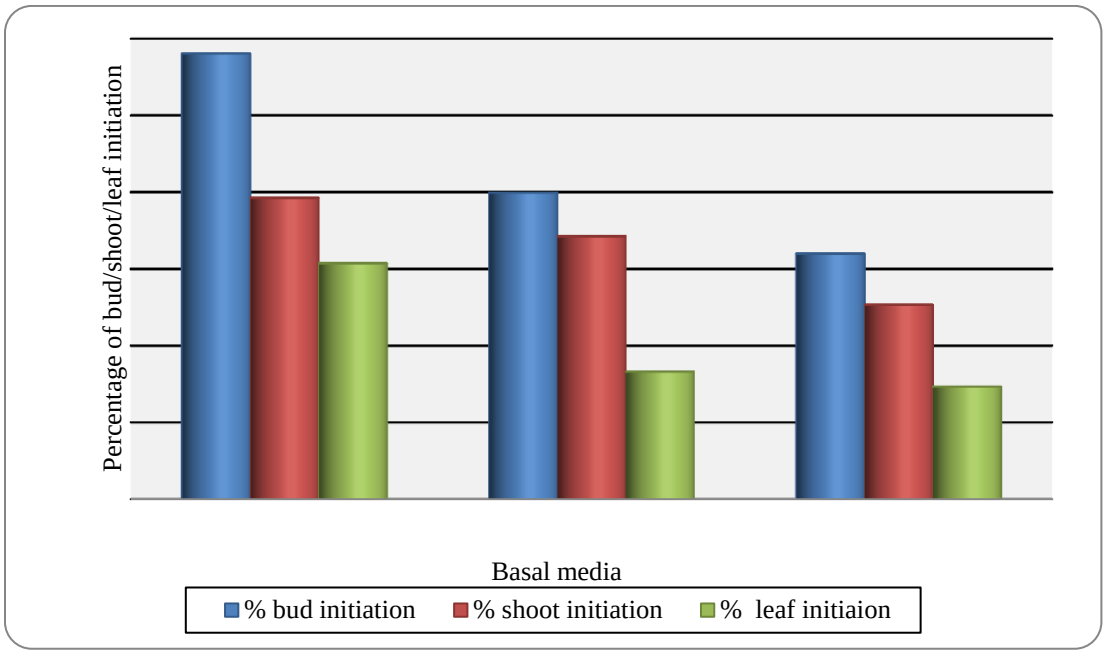

Fig. 1a. Effect of basal media on culture establishment and growth in *S. macrophylla*

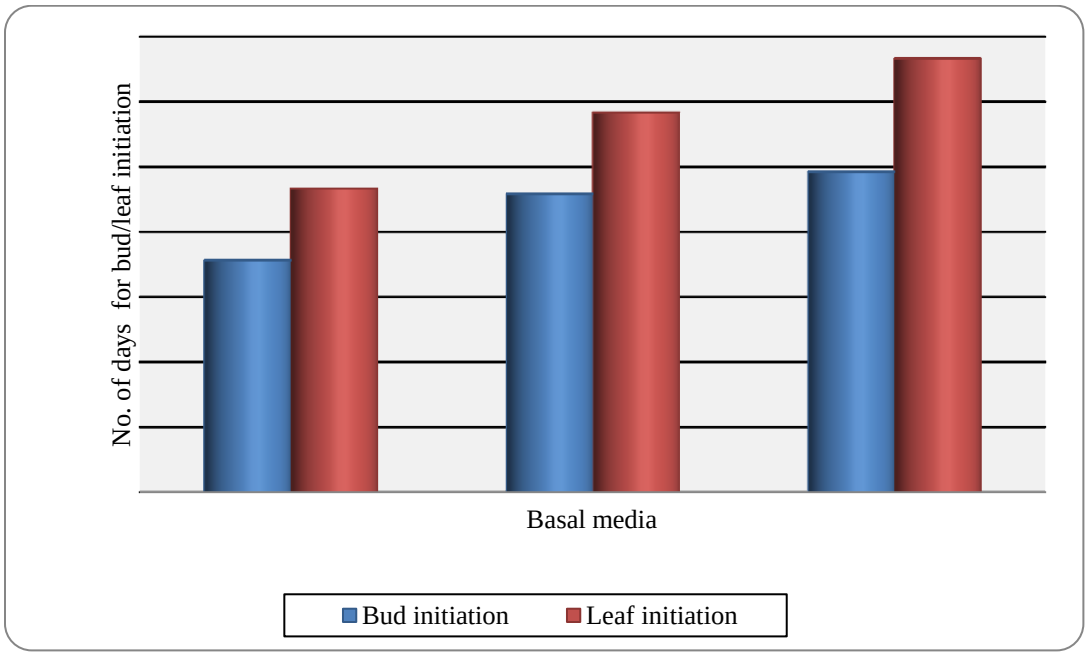

Fig. 1b. Effect of basal media on time taken for bud/leaf initiation in axillary buds of *S. macrophylla*

culture establishment and growth from axillary bud cultures. Synergistic effect of BA in combination with GA_3 has been recorded by Chaturvedi *et al*. (2004).

As stated by Ahmed (1990), in trees species generally BA has been frequently and extensively used to induce better shoot growth and multiplication than other cytokinins. In the present study it was observed that BA at lower concentrations has induced high percentage of bud, shoot and leaf initiation (Fig. 2a). When the concentration of BA was increased in the medium, bud initiation and leaf morphogenesis was hindered and there was delay in leaf formation (Table. 7 and Fig.2b). Similar results have been reported in *Cedrela odorata* by Maruyama *et al*. (1989) and in mahogany by Venketeswaran *et al.* (1988) and Schottz *et al.,* (2007). It is reported that BA in high concentrations causes hyperhydricity in the explants, due to interaction with the agar of culture medium. Excess of cytokinin can lead to other negative effects like the formation of a great number of adventitious buds, which is not desirable for clonal integrity (Grattapaglia and Machado, 1998). In contrast to this kinetin at lower concentrations has inhibited the bud initiation and leaf initiation (Table. 8), but in higher concentrations it has shown to induce bud and leaf initiation (Fig. 3a). Supplementation of BA to MS medium was found to be superior to supplementation with kinetin in terms of shoot production. These results are in supportive of results obtained by Pradhan *et al*. (1998b) in *Dalbergia sissoo* and Nayak and Behera (2007) in *Aegle marmelos.* When the effect of kinetin was compared with BA, it is observed that kinetin yielded better results. Similar reports had been documented by many workers in species like *Azadirachta indica* (Roshni, 2003; Kota, 2006), *Jatropa curcas* (Sujatha and Mukta, 1996), *Syzyzium cumini* (Roy *et al.,* 1996).

In the present investigation addition of cytokinins to the basal MS medium has not given significant performance in comparison with the basal MS medium. The

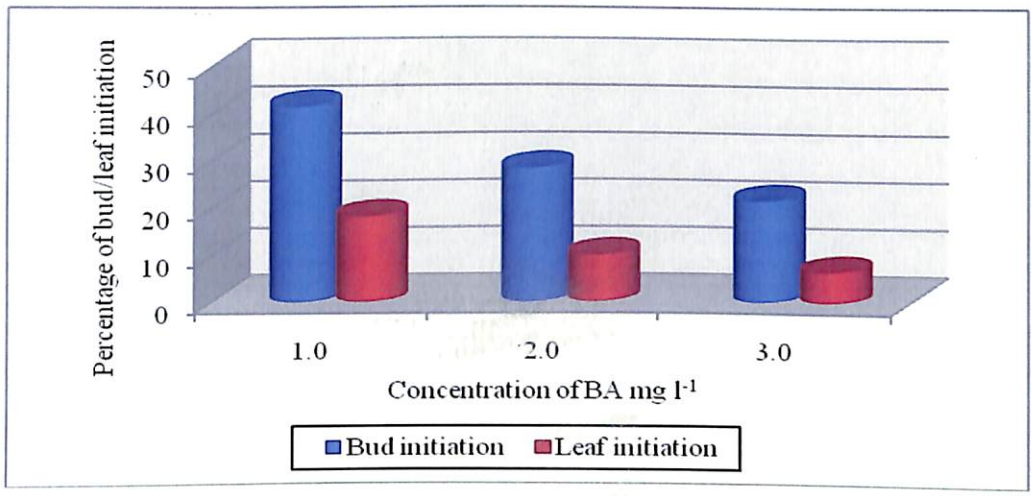

Fig. 2a. Effect of BA on bud/leaf initiation

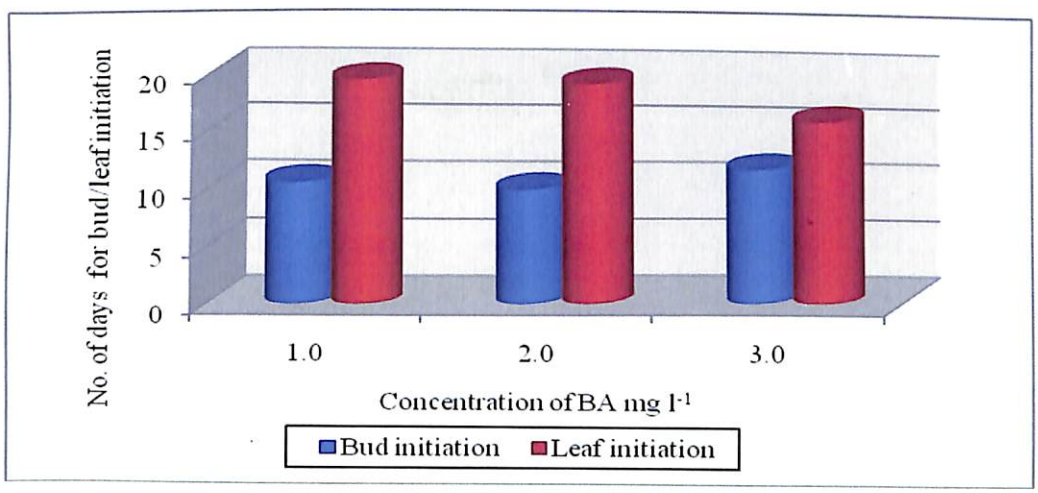

Fig. 2b. Effect of BA on time taken for bud/leaf initiation

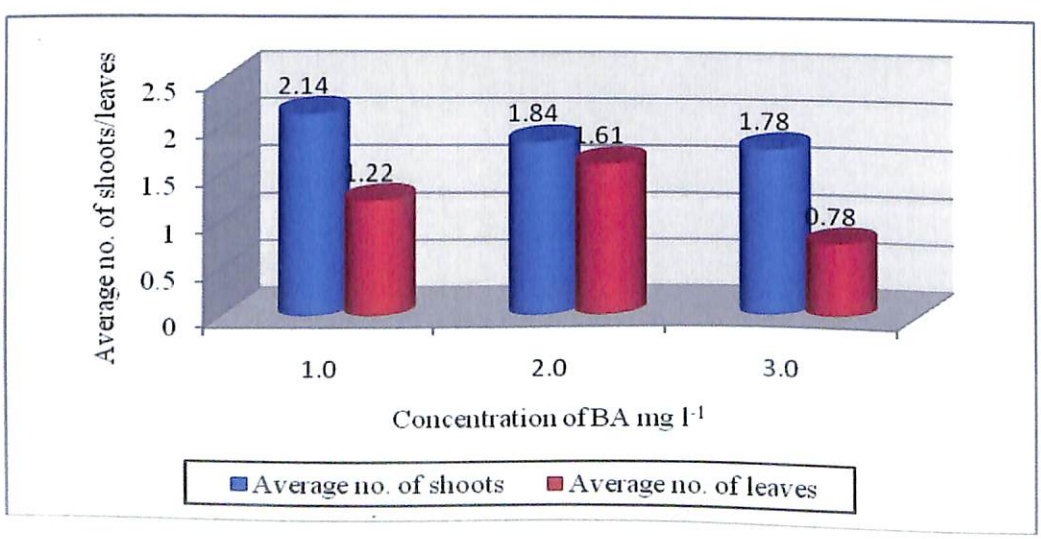

Fig. 2c. Effect of BA on average number of shoots/leaves

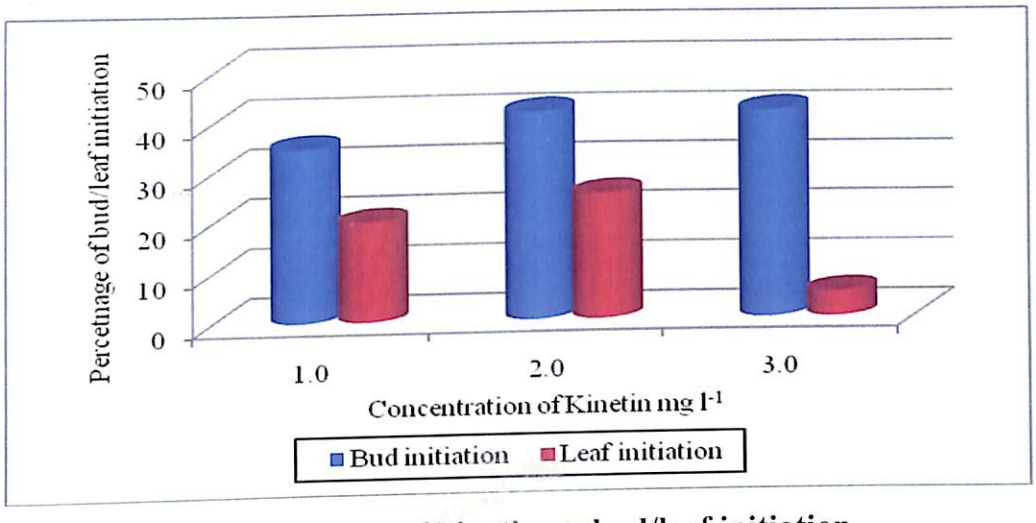

Fig. 3a. Effect of kinetin on bud/leaf initiation

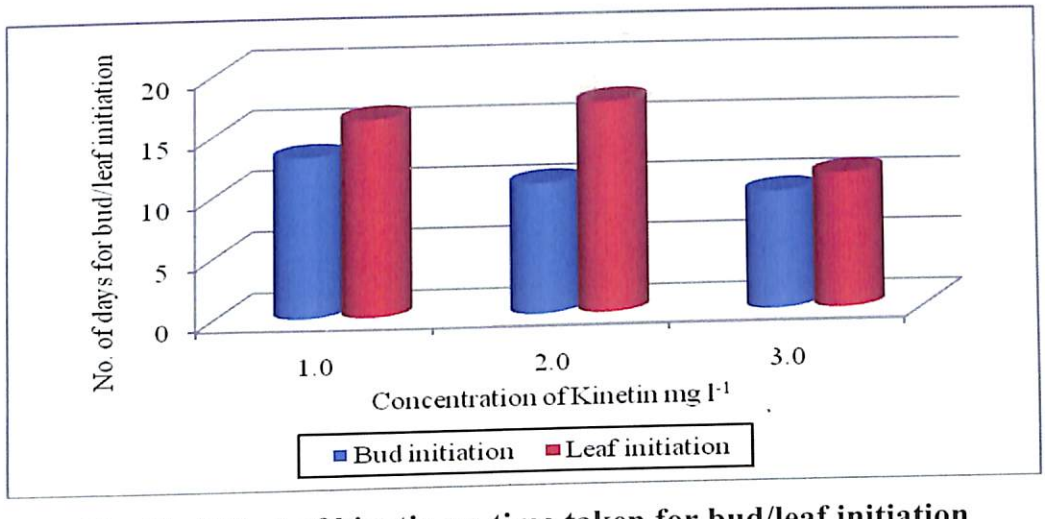

Fig. 3b. Effect of kinetin on time taken for bud/leaf initiation

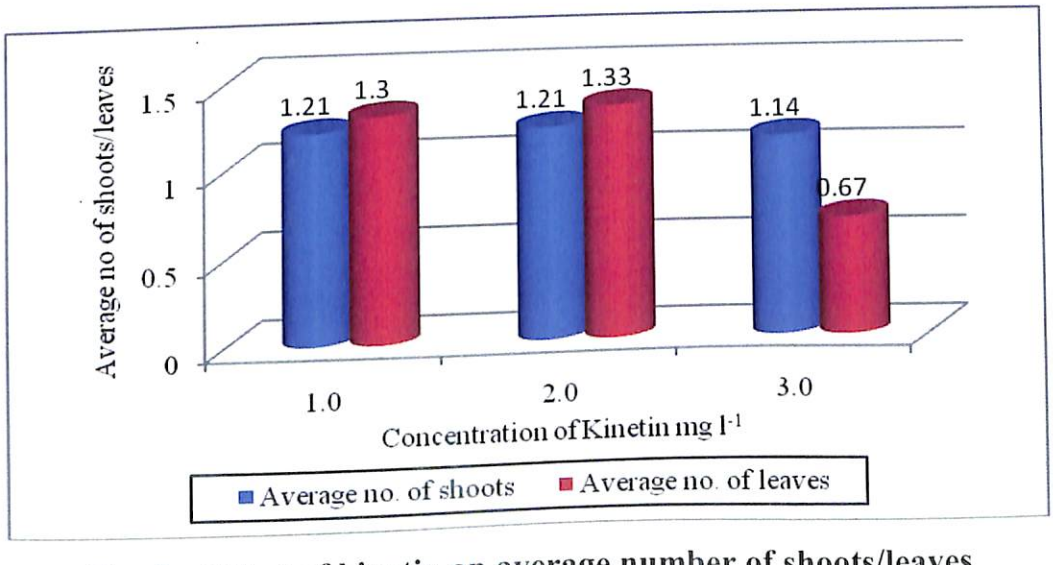

Fig. 3c. Effect of kinetin on average number of shoots/leaves

culture establishment was found to be reducing in the media containing growth regulators. These results are in contrast to the role of plant growth regulators; which may be again due to the season. The explants were cultured on the MS media containing growth regulators during January-February, which is the fruit ripening period in mahogany. Hence, due to this reproductive phase the performance of explants might have been reduced when compared to basal medium. However, the results could not be confirmed.

In plant tissue culture, auxins are extensively used to exploit its potentiality for stimulation of cell division and elongation. The requirement of these growth hormones varies considerably with reference to the types and its concentration. It is believed that, the response of the tissues to various auxins varies depending upon their endogenous levels in the tissues. In general, auxins are supplemented to media singly or in combination with cytokinin. When supplemented with cytokinin, the interaction between two is complex. Kaur *et al.* (1998a) reported production of maximum number of shoot buds in *Acacia senegal* on MS media fortified with auxin and cytokinin. In earlier experiments, inhibitory effect of higher concentration of cytokinin alone in media has been observed. Lundergan and Janick (1980) observed the nullifying the suppressive effect of high cytokinin by auxin. Results in *Syzygium alternifolium* have shown the synergistic effect of cytokinin and auxin combination on shoot production (Khan *et al*., 1997).

In the present investigation, combinations of BA and IAA (1.0 mg l^{-1} BA+0.5 mg l^{-1} IAA and 2.0 mg l^{-1} BA+0.1 mg l^{-1} IAA) have exhibited the synergistic effect in resulting bud and leaf initiation from the axillary buds (Table. 9 and Fig. 4a). Similar results have been reported in bud and leaf initiation of *Azadirachta indica*, MS media supplemented with 0.5 mg l^{-1} BA+1.5 mg l^{-1} IAA and by Roshni (2003). Benzyl adenine at 1.0 mg l^{-1} supplied in combination with IAA (0.5 mg l^{-1}) has

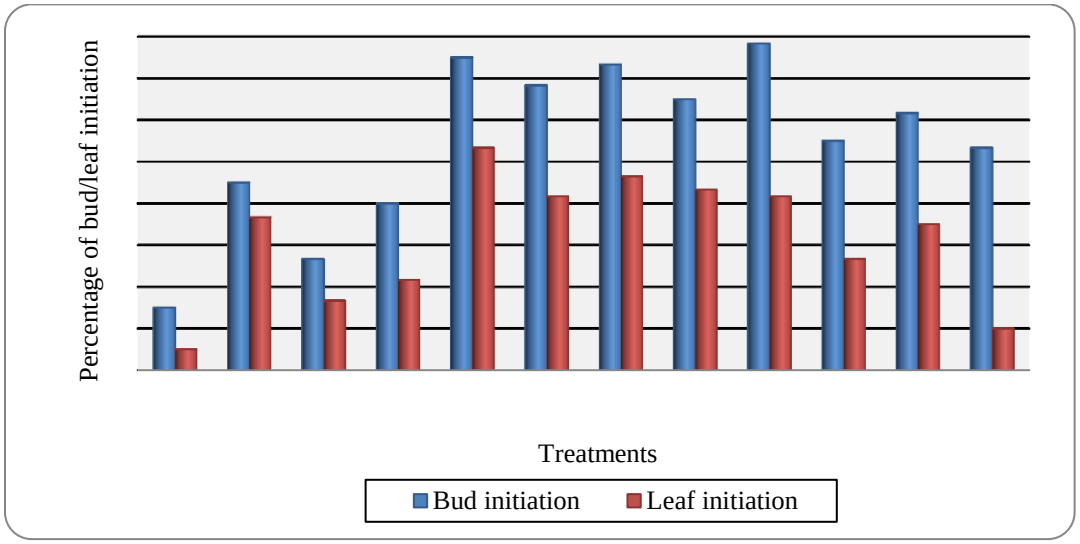

Fig. 4a. Effect of BA and IAA on bud/leaf initiation

1- MS+0.5 mg l^{-1} BA+0.1 mg l^{-1} IAA

2- MS+0.5 mg l^{-1} BA+0.5 mg l^{-1} IAA

3- MS+0.5 mg l^{-1} BA+1.0 mg l^{-1} IAA

4- MS+1.0 mg l^{-1} BA+0.1 mg l^{-1} IAA

5- MS+1.0 mg l^{-1} BA+0.5 mg l^{-1} IAA

6- MS+1.0 mg l^{-1} BA+1.0 mg l^{-1} IAA

7- MS+2.0 mg l^{-1} BA+0.1 mg l^{-1} IAA

8- MS+2.0 mg l^{-1} BA+0.5 mg l^{-1} IAA

9- MS+2.0 mg l^{-1} BA+1.0 mg l^{-1} IAA

10- MS+3.0 mg l^{-1} BA+0.1 mg l^{-1} IAA

11- MS+3.0 mg l^{-1} BA+0.5 mg l^{-1} IAA

12- MS+3.0 mg l^{-1} BA+1.0 mg l^{-1} IAA

produced more number of shoots (Fig. 4b). These results are on par with the findings of studies by Shahin-uz-zaman *et al.* (2008) in shoot initiation of *A. indica* and Kalia *et al.* (2004) in multiple bud induction from *Dalbergia sissoo* nodal explants.

In the present study combination of another auxin, IBA along with BA was found to be non-significant (Table. 10) unlike the IAA in combination with BA, with respect to percentage bud initiation and multiple shoots. These results are on par with the findings of Roshini (2003) in neem. However, BA along with IBA (0.5 mg l^{-1} BA+1.0 mg l^{-1} IBA) has produced higher percentage of leaf initiation (Fig. 5a) compared to the combination of BA with IAA. It is noticed that early bud initiation in MS medium containing 2.0 mg l^{-1} BA+1.0 mg l^{-1} IBA and leaf initiation in media supplemented with 3.0 mg l^{-1} BA+1.0 mg l^{-1} IBA (Fig. 5b). Benzyl adenine when fortified at 1.0 mg l^{-1} with 0.1 mg l^{-1} IBA has produced high per cent (15 %) of cultures with multiple shoots. Number of shoots and leaves were maximum on media containing 2.0 mg l^{-1} BA+0.1 mg l^{-1} IBA and 2.0 mg l^{-1} BA+1.0 mg l^{-1} IBA respectively.

During the present investigation another cytokinin (kinetin) was used in combination with IAA and IBA individually. When kinetin was supplemented along with IAA to the MS media, maximum per cent of bud initiation recorded was 61.67% followed by 60 % in media fortified with 2.0 mg l^{-1} kinetin+1.0 mg l^{-1} IAA and 0.5 mg l^{-1} kinetin+0.1 mg l^{-1} IAA (Table. 11). It was observed that leaf initiation was high when kinetin was supplied in higher concentration along with low concentration of IAA (Fig. 6a). These results are on par with findings by Behbahani *et al.* (2007) in *Barringtonia racemosa*. It is reported that in some studies kinetin at low concentration along with high concentration of IAA has produced maximum per cent of bud initiation and leaf initiation in *A. indica* (Roshni, 2003) and in *Albizzia lebbeck* by Mamun *et al.* (2004). Even though the effect of Kin in combination IAA

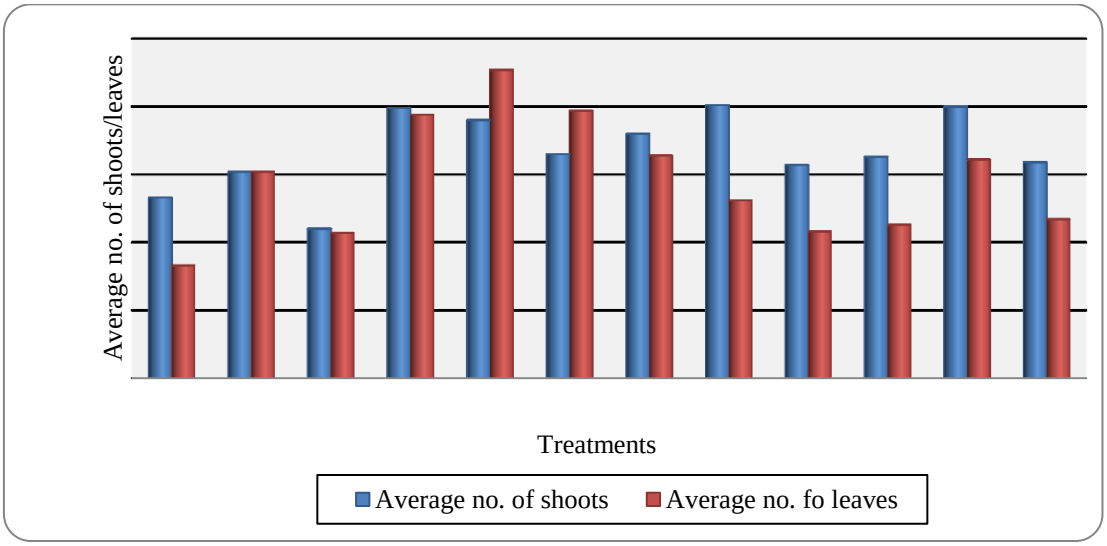

Fig. 4b. Effect of BA and IAA on production of multiple shoots

1- MS+0.5 mg l^{-1} BA+0.1 mg l^{-1} IAA

2- MS+0.5 mg l^{-1} BA+0.5 mg l^{-1} IAA

3- MS+0.5 mg l^{-1} BA+1.0 mg l^{-1} IAA

4- MS+1.0 mg l^{-1} BA+0.1 mg l^{-1} IAA

5- MS+1.0 mg l^{-1} BA+0.5 mg l^{-1} IAA

6- MS+1.0 mg l^{-1} BA+1.0 mg l^{-1} IAA

7- MS+2.0 mg l^{-1} BA+0.1 mg l^{-1} IAA

8- MS+2.0 mg l^{-1} BA+0.5 mg l^{-1} IAA

9- MS+2.0 mg l^{-1} BA+1.0 mg l^{-1} IAA

10- MS+3.0 mg l^{-1} BA+0.1 mg l^{-1} IAA

11- MS+3.0 mg l^{-1} BA+0.5 mg l^{-1} IAA

12- MS+3.0 mg l^{-1} BA+1.0 mg l^{-1} IAA

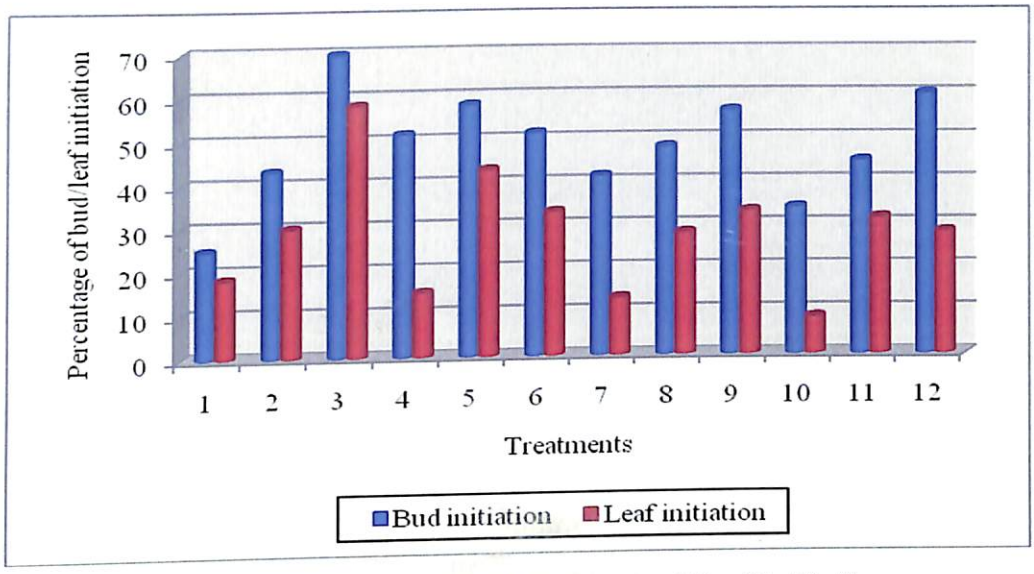

Fig. 5a. Effect of BA and IBA on bud/leaf initiation

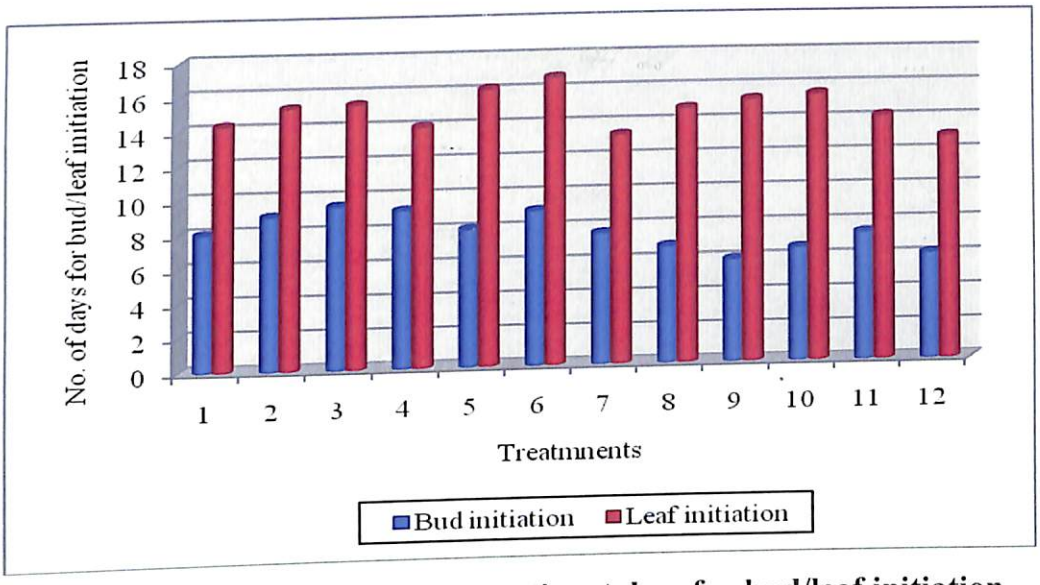

Fig. 5b. Effect of BA and IBA on time taken for bud/leaf initiation

1- MS+0.5 mg l^{-1} BA+0.1 mg l^{-1} IBA

2- MS+0.5 mg l^{-1} BA+0.5 mg l^{-1} IBA

3- MS+0.5 mg l^{-1} BA+1.0 mg l^{-1} IBA

4- MS+1.0 mg l^{-1} BA+0.1 mg l^{-1} IBA

5- MS+1.0 mg l^{-1} BA+0.5 mg l^{-1} IBA

6- MS+1.0 mg l^{-1} BA+1.0 mg l^{-1} IBA

7- MS+2.0 mg l^{-1} BA+0.1 mg l^{-1} IBA

8- MS+2.0 mg l^{-1} BA+0.5 mg l^{-1} IBA

9- MS+2.0 mg l^{-1} BA+1.0 mg l^{-1} IBA

10- MS+3.0 mg l^{-1} BA+0.1 mg l^{-1} IBA

11- MS+3.0 mg l^{-1} BA+0.5 mg l^{-1} IBA

12- MS+3.0 mg l^{-1} BA+1.0 mg l^{-1} IBA

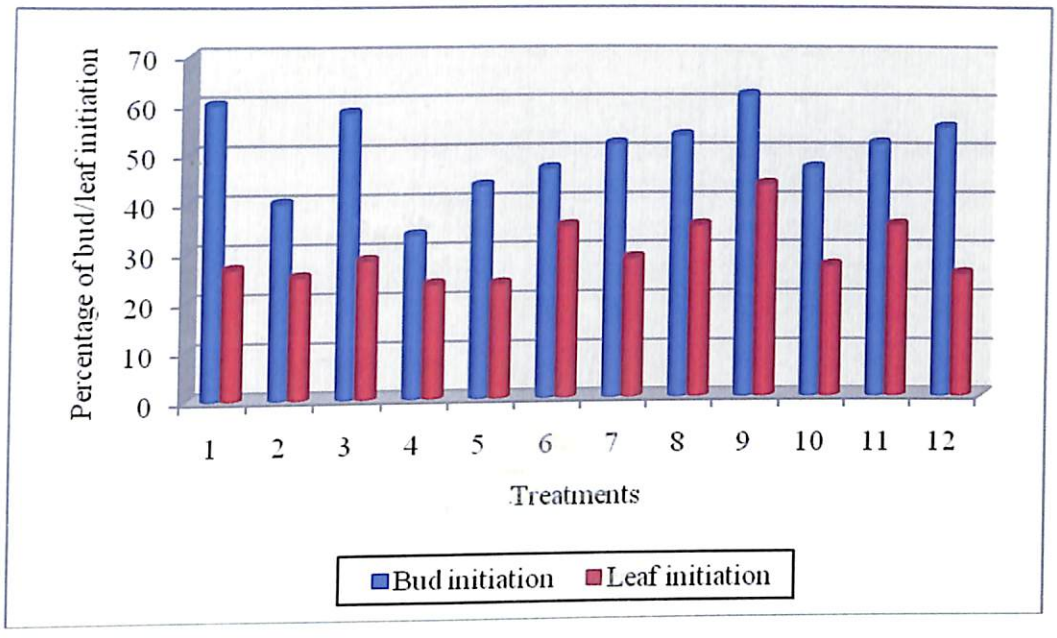

Fig. 6a. Effect of kinetin and IAA on bud/leaf initiation

1- MS+0.5 mg l^{-1} kinetin+0.1 mg l^{-1} IAA
2- MS+0.5 mg l^{-1} kinetin+0.5 mg l^{-1} IAA
3- MS+0.5 mg l^{-1} kinetin+1.0 mg l^{-1} IAA
4- MS+1.0 mg l^{-1} kinetin+0.1 mg l^{-1} IAA
5- MS+1.0 mg l^{-1} kinetin+0.5 mg l^{-1} IAA
6- MS+1.0 mg l^{-1} kinetin+1.0 mg l^{-1} IAA
7- MS+2.0 mg l^{-1} kinetin+0.1 mg l^{-1} IAA
8- MS+2.0 mg l^{-1} kinetin+0.5 mg l^{-1} IAA
9- MS+2.0 mg l^{-1} kinetin+1.0 mg l^{-1} IAA
10- MS+3.0 mg l^{-1} kinetin+0.1 mg l^{-1} IAA
11- MS+3.0 mg l^{-1} kinetin+0.5 mg l^{-1} IAA
12- MS+3.0 mg l^{-1} kinetin+1.0 mg l^{-1} IAA

on bud initiation and leaf initiation.

Kinetin when supplied with IAA has shown synergistic effect on time taken for bud and leaf initiation and per cent cultures with multiple shoots. In the media fortified with 2.0 mg l^{-1} kinetin+1.0 mg l^{-1} IAA bud initiation and leaf initiation was early (6.38 days and 11.08 days, respectively) (Fig. 6b). Per cent culture with multiple shoots was found to be maximum when kinetin at 0.5mg l^{-1} supplied with IAA at 0.1 mg l^{-1}.

In our study kinetin was supplemented to MS medium with IBA to evaluate the effect on culture establishment and growth parameters. Kinetin at 0.5 mg l^{-1} and 1.0 mg l^{-1} supplied each along with 0.5 mg l^{-1} IBA has induced higher per cent of bud initiation from axillary buds of big-leaf mahogany (Fig. 7). Leaf initiation was observed to be maximum on higher concentration of kinetin with lower concentration of IBA (Table. 12). Same trend was noticed in time taken for bud initiation when media was fortified with 3.0 mg l^{-1}kinetin+0.5 mg l^{-1} IBA. During the study more number of shoots per explants was produced when kinetin and IBA were supplied in equal quantities. However, some contrasting results were reported in *A. indica* (Roshni, 2003) and *Barringtonia racemosa* (Behbahani *et al.*, 2007). From the results it is clear that average shoot length and average number of leaves was highest in medium 3.0 mg l^{-1} kinetin+0.1 mg l^{-1} IBA and 2.0 mg l^{-1} kinetin+0.5 mg l^{-1} IBA respectively. However, combination of kinetin along with auxin was not effective as BA in combination with auxin.

5.3.1 Bud and leaf initiation

Some of the media combinations that have given the excellent response in terms of bud break and leaf initiation in axillary bud cultures of mahogany are

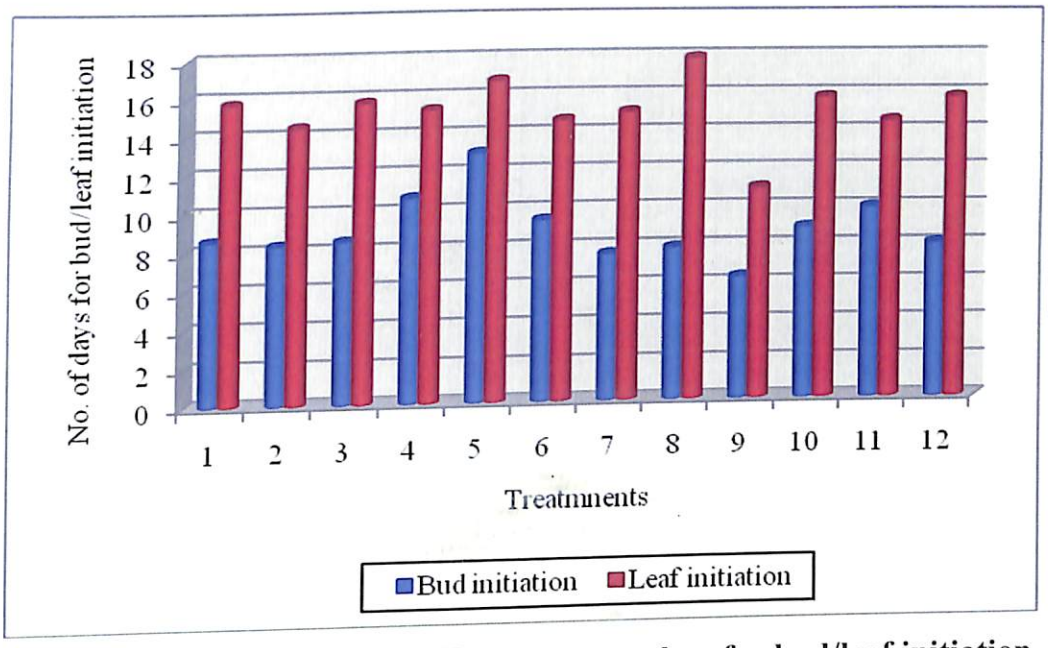

Fig. 6b. Effect of kinetin and IAA on time taken for bud/leaf initiation

1- MS+0.5 mg l^{-1} kinetin+0.1 mg l^{-1} IAA
2- MS+0.5 mg l^{-1} kinetin+0.5 mg l^{-1} IAA
3- MS+0.5 mg l^{-1} kinetin+1.0 mg l^{-1} IAA
4- MS+1.0 mg l^{-1} kinetin+0.1 mg l^{-1} IAA
5- MS+1.0 mg l^{-1} kinetin+0.5 mg l^{-1} IAA
6- MS+1.0 mg l^{-1} kinetin+1.0 mg l^{-1} IAA
7- MS+2.0 mg l^{-1} kinetin+0.1 mg l^{-1} IAA
8- MS+2.0 mg l^{-1} kinetin+0.5 mg l^{-1} IAA
9- MS+2.0 mg l^{-1} kinetin+1.0 mg l^{-1} IAA
10- MS+3.0 mg l^{-1} kinetin+0.1 mg l^{-1} IAA
11- MS+3.0 mg l^{-1} kinetin+0.5 mg l^{-1} IAA
12- MS+3.0 mg l^{-1} kinetin+1.0 mg l^{-1} IAA

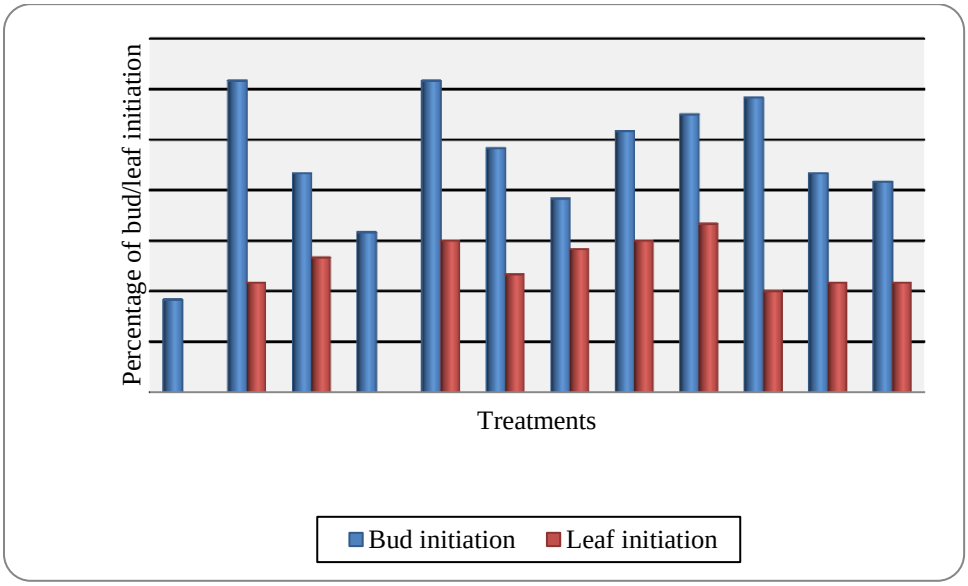

Fig. 7. Effect of kinetin and IBA on bud/leaf initiation

1- MS+0.5 mg l^{-1} kinetin+0.1 mg l^{-1} IBA

2- MS+0.5 mg l^{-1} kinetin+0.5 mg l^{-1} IBA

3- MS+0.5 mg l^{-1} kinetin+1.0 mg l^{-1} IBA

4- MS+1.0 mg l^{-1} kinetin+0.1 mg l^{-1} IBA

5- MS+1.0 mg l^{-1} kinetin+0.5 mg l^{-1} IBA

6- MS+1.0 mg l^{-1} kinetin+1.0 mg l^{-1} IBA

7- MS+2.0 mg l^{-1} kinetin+0.1 mg l^{-1} IBA

8- MS+2.0 mg l^{-1} kinetin+0.5 mg l^{-1} IBA

9- MS+2.0 mg l^{-1} kinetin+1.0 mg l^{-1} IBA

10- MS+3.0 mg l^{-1} kinetin+0.1 mg l^{-1} IBA

11- MS+3.0 mg l^{-1} kinetin+0.5 mg l^{-1} IBA

12- MS+3.0 mg l^{-1} kinetin+1.0 mg l^{-1} IBA

presented in Table. 14 (Fig. 8). It was observed that bud initiation was highest (78.33 %) on MS media supplemented with 2.0 mg l^{-1} BA+1.0 mg l^{-1}IAA, followed by 75 per cent and 73.33 per cent on media containing 1.0 mg l^{-1} BA+0.5 mg l^{-1} IAA and 2.0 mg l^{-1} BA+0.1 mg l^{-1} IAA. The lowest per cent of bud initiation (58.33 %) was recorded on MS+3 mg l^{-1} kinetin+0.1 mg l^{-1} IBA. Leaf initiation from the axillary bud cultures was found to be highest (58.33 %) on MS media fortified with 0.5 mg l^{-1} BA+1 mg l^{-1} IBA and least was in MS+3.0 mg l^{-1} kinetin+0.1 IBA.

5.3.2 Multiple shoot

In commercial and lucrative tissue culture, production of one shoot from one bud is neither reasonable nor enviable. So to have cost effective production of planting material through tissue culture from inadequate and superior source available, it is essential to develop a protocol to obtain multiple shoots. These shoots can be obtained in large quantities simultaneously by sub culturing of single explant on suitable medium.

In mahogany a normal explant (nodal segment) has one bud owing to the alternate leaf arrangement. Thus one shoot from one explant was considered as normal. However, in the present study some of the growth regulator combinations have produced multiple shoots in the primary cultures (Table. 15 and Fig. 9). Highest number of shoots per explant (3.33) was recorded in MS media fortified with relatively high concentration of BA at 3.0 mg l^{-1} along with 0.5 mg l^{-1} IAA, which indicated that there was quantitative interaction between BA and IAA. This kind of quantitative interaction between cytokinin and auxin was also reported in other tissue systems (Bhojwani and Johri, 1970). Multiple shoots were also produced on media supplemented with BA along with IBA and kinetin with IAA and IBA. Similar findings were reported by Vandina *et al.* (1995) in *Moringa ptrerygosperma* and by

Table. 14 Some promising growth regulator combinations which induced bud initiation and leaf initiation in axillary bud cultures of *S. macrophylla* on MS medium

Cytokinins (mg $l^{-1)}$		Auxins (mg $l^{-1)}$		Bud initiation (%)	Leaf initiation (%)
BA	Kinetin	IAA	IBA		
2.0	-	1.0	-	78.33	41.67
1.0	-	0.5	-	75.0	53.33
2.0	-	0.1	-	73.33	46.67
0.5	-	-	1.0	70.0	58.33
-	2.0	1.0	-	61.67	43.33
-	1.0	-	0.5	61.67	30.0
-	0.5	-	0.5	61.67	21.67
	0.5	0.1		60.0	26.67
1.0		0.5		58.33	43.33
	3.0		0.1	58.33	20.0

Table. 15 Some promising growth regulator combinations which induced multiple shoots in axillary bud cultures of *Swietenia macrophylla* on MS medium

Growth regulators (mg l^{-1})			Avg. shoot length (cm)	Avg. no. of shoots	Maximum no. of shoots
BA	IAA	IBA			
3.0	0.5	-	1.56	2.0	3.33
2.0	0.1	-	0.91	1.8	3.33
1.0	0.1	-	0.51	1.99	3.33
1.0		0.5	1.01	1.52	3.33
1.0	1.0	-	0.76	1.65	3.0
2.0	0.5	-	0.98	2.01	3.0
2.0	1.0	-	1.18	1.57	3.0
3.0	0.1	-	1.26	1.63	3.0
1.0	-	0.1	1.06	1.56	3.0
1.0	-	1.0	1.15	1.08	3.0

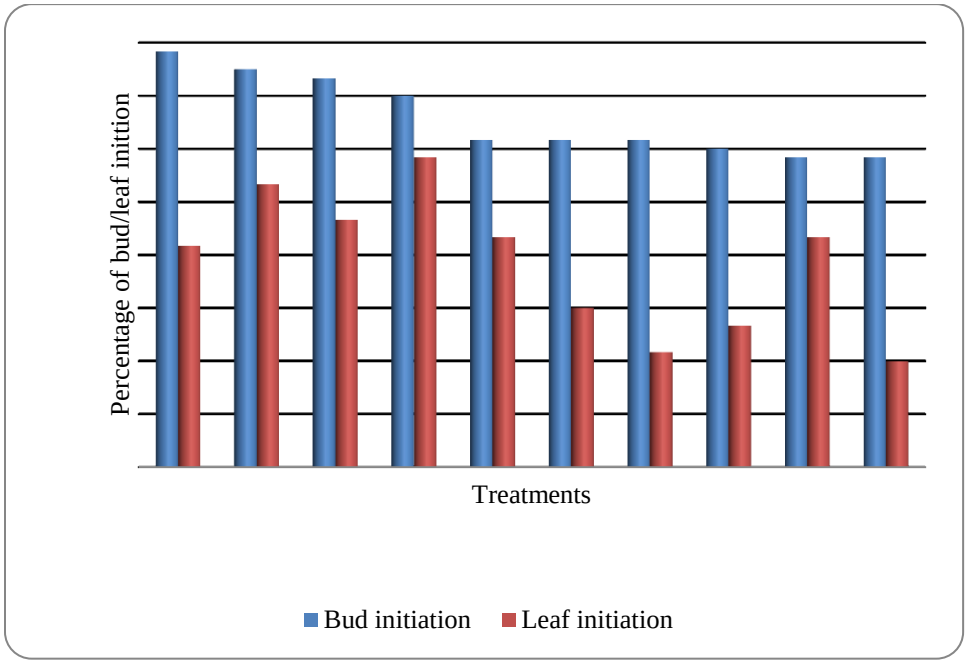

Fig. 8. Some promising growth regulator combinations with respect to bud and leaf initiation

1-MS+2.0 mg l^{-1} BA+1.0 mg l^{-1} IBA

2- MS+1.0 mg l^{-1} BA+0.5 mg l^{-1} IAA

3- MS+2.0 mg l^{-1} BA+0.1 mg l^{-1} IAA

4- MS+0.5 mg l^{-1} BA+1.0 mg l^{-1} IBA

5- MS+2.0 mg l^{-1} kinetin+0.1 mg l^{-1} IAA

6- MS+1.0 mg l^{-1} kinetin+0.5 mg l^{-1} IBA

7- MS+0.5 mg l^{-1} kinetin+0.5 mg l^{-1} IBA

8- MS+0.5 mg l^{-1} kinetin+0.1 mg l^{-1} IAA

9- MS+1.0 mg l^{-1} BA+0.5 mg l^{-1} IAA

10- MS+3.0 mg l^{-1} kinetin+0.1 mg l^{-1} IBA

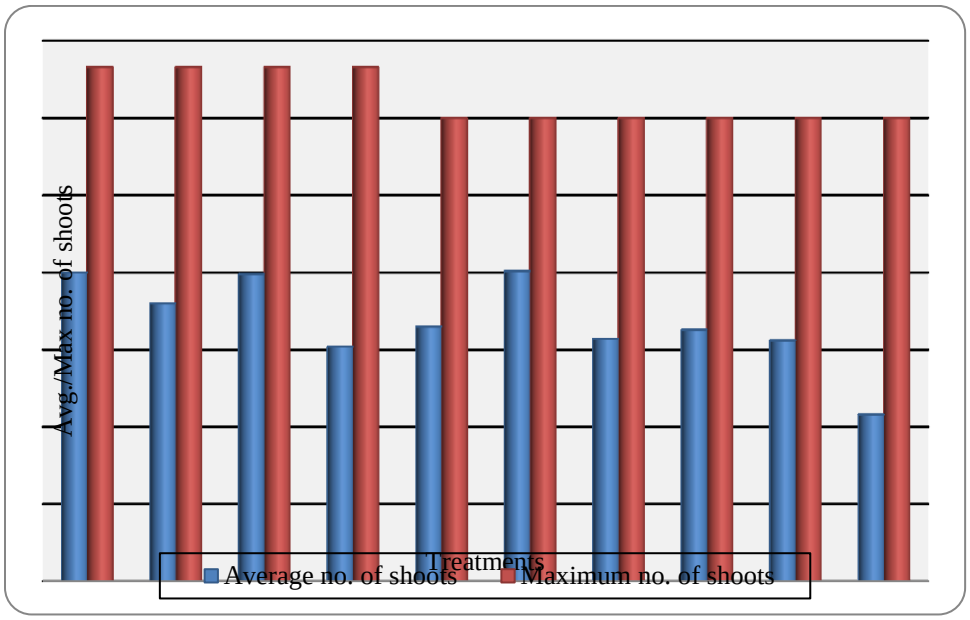

Fig. 9. Some promising growth regulator combination which induced multiple shoots

1- MS+3.0 mg l^{-1} BA+0.5 mg l^{-1} IAA

2- MS+2.0 mg l^{-1} BA+0.1 mg l^{-1} IAA

3- MS+1.0 mg l^{-1} BA+0.1 mg l^{-1} IAA

4- MS+1.0 mg l^{-1} BA+0.5 mg l^{-1} IBA

5- MS+1.0 mg l^{-1} BA+1.0 mg l^{-1} IAA

6- MS+2.0 mg l^{-1} BA+0.5 mg l^{-1} IAA

7- MS+2.0 mg l^{-1} BA+1.0 mg l^{-1} IAA

8- MS+3.0 mg l^{-1} BA+0.1 mg l^{-1} IAA

9- MS+1.0 mg l^{-1} BA+0.1 mg l^{-1} IBA

10- MS+1.0 mg l^{-1} BA+1.0 mg l^{-1} IBA

Roshni (2003) in neem. Induction of multiple shoots has been successfully achieved in many tree species like *Aegle marmelos* (Nayak and Behera, 2007), *Azadirachta indica* (Kota *et al.,* 2006), *Dalbergia sissoo* (Chand and Singh, 2005), *Swietenia macrophylla* (Brunetta *et al.,* 2006; Lopes *et al.,* 2001).

5.4 ESTABLISHMENT OF CONTINUOUS CULTURES

It is very much essential to produce the large number of shoots to produce huge quantity of planting material. More number of shoots can be produced by repeated and subsequent sub culturing. During the study an effort was made to produce continuous cultures by culturing microshoots on various growth regulator combinations, results are presented in Table. 13. A greatest difficulty experienced was abscission of leaves in the subcultures (Plate. 6) and lack of shoot elongation (Plate. 7) in the primary cultures. Many cultures died without showing further proliferation and the culture which is alive remained green for about three to four week after sub culturing, but they did not show any growth. These types of obstacles are reported by *Patri et al.* (1988) in *Pterocarpus santalinus,* Santoshkumar (1993) in *P. marsupium* and in *Jatropha curcas* by Kalkoor (2007). Due to these problems rooting experiments were not carried out. Hence a precise protocol for micropropagation of big-leaf mahogany was could not be developed.

The major constraint for orderly progress of any *in vitro* culture is inadequacy of the reliable quantitative physiological, biochemical and cell biological data related to the species to be cultured. Also, lack of understanding of critical functions of plant growth regulators and other media constituents. This is more so in forest tree species and consequently, plant cell and tissue culture of forest trees is based more on empirical experience than fundamental principles.

Plate. 6. Abscission of leaves and shoots on MS+2.0 mg l^{-1} BA+0.1 mg l^{-1} IAA

Plate. 7. Retarded shoot growth in MS + 3.0 mg l^{-1} BA + 0.5 mg l^{-1} IAA

The study provides preliminary information for framing out a potential programme for micropropagation of *S. macrophylla*. Media combinations which have produce multiple shoots can be used to produce microshoots and these shoots can be used for further rooting experiments.

SUMMARY

6. SUMMARY

A research programme entitled "*In vitro* propagation of big-leaf mahogany (*Swietenia macrophylla* King) through tissue culture" was carried out at the college of Forestry, Vellanikkara during the period 2007 to 2009. The salient features from the study are summarised below:

1.The extent of culture contamination, primarily due to fungus, was found to be very high and more so during rainy season. This may be due to high amount of microbial inoculum in the field explants.

2. A prophylactic spray with a solution (0.2 %) of fungicides (Carbendazim and Moncozeb) was found to reduce culture contamination.

3. A seasonal effect on culture contamination was recorded with the peak contamination in rainy periods.

4. Mercuric chloride at 0.05 per cent, 0.1 per cent and 0.2 per cent was used for surface sterilization for a stipulated period (each concentration for 10 min, 15min and 20 min). These treatments were significantly different from each other. Pretreatment of explants by dipping in a fungicidal mixture of 0.2 percent of each Bavistine (Carbendazim) and Indofil M-45 (Mancozeb) followed by 15 min. surface sterilization with mercuric chloride (0.1 %) was found to be effective to establish aseptic cultures.

5. Considerable differences in culture establishment were evident between basal media compositions with MS basal medium giving the best results when compared to WPM and B5 medium. Highest percent of bud initiation (58.08 %),

shoot initiation (39.26 %) and leaf initiation (30.74 %) was recorded in this medium.

6. Benzyl adenine and kinetin supplied to MS medium has reduced the cultures response with respect to bud initiation and leaf initiation. However, results could not be confirmed; this contrasting result may be due to the season of explant collection.

7. However, benzyl adenine supplied to MS medium induced bud initiation from axillary buds. Highest percent bud initiation (41.67 %), cultures with multiple shoots (18.33 %) and leaf initiation (18.33 %) was observed at BA 1.0 mg l^{-1} in MS medium. Highest number of shoots per explant (2.14) with average shoot length of 0.55 cm was recorded in the same medium.

8. Increasing the concentration of BA has substantially reduced the bud initiation (58.08 % to 21.67 %) and leaf initiation (30.74 % to 6.67 %).

9. Kinetin was found to be effective in per cent bud initiation (41.67 %) and cultures with multiple shoots (11.67 %) when supplied at higher (3.0 %) concentrations. However, leaf initiation (25 %) and average number of leaves per explant (1.33) was found to be maximum on MS medium supplemented with kinetin 2.0 mg l^{-1}.

10. Kinetin at 1.0 mg l^{-1} has produced more number of shoots per explant (1.21) with an average length of 0.6 cm.

11. Benzyl adenine was found to be more effective for bud initiation and leaf initiation compared to kinetin when both are supplied alone to the MS basal medium.

12. Synergistic effect of BA and IAA has been noticed on media containing 2.0 mg l^{-1} BA + 0.1 mg l^{-1} IAA and 2.0 mg l^{-1} BA + 1.0 mg l^{-1} IAA in per cent bud initiation, leaf initiation and culture with multiple shoots.

13. Murashige and Skoog media supplemented with 2.0 mg l^{-1} BA and 1.0 mg l^{-1} IAA has produced highest percent (78.33 %) of bud initiation, where as media containing 2.0 mg l^{-1} BA and 0.1 mg l^{-1} IAA has produced highest cultures with multiple shoots (33.33 %) and leaf initiation (46.47 %).

14. Benzyl adenine and IBA combination when fortified to the MS medium was found to be non-significant over culture establishment and growth. However, bud initiation (70 %) and leaf initiation (58.33 %) was recorded in MS+0.5 mg l^{-1} BA+1.0 mg l^{-1} IBA.

15. The combination of BA with IAA and IBA was found to be better in bud and leaf initiation when compared to control (basal MS medium).

16. Kinetin in combination with IAA was found to be non significant on bud initiation and leaf initiation. However, the effect was found to be significant on time taken for bud initiation, leaf initiation, average number of shoots and average number of shoots.

17. Kinetin at 2.0 mg l^{-1} in combination with 1.0 mg l^{-1} IAA has shown early bud initiation (6.38 days) and leaf initiation (11.08 days) when compared with other combinations. Average number of shoots (1.65) and average number of leaves (1.87) was maximum on media containing 1.0 mg l^{-1} kinetin+0.1 mg l^{-1} IAA and 1.0 mg l^{-1} kinetin +0.5 mg l^{-1} IAA, respectively.

18. Murashige and Skoog media supplemented with kinetin along with IBA was not effective in bud initiation. Maximum leaf initiation was found to be on media

containing 2.0 mg l^{-1} kinetin + 1.0 mg l^{-1} IBA. This combination was found significant in production of multiple shoots.

19. Callusing was observed on MS basal medium, WPM basal and MS medium supplemented with BA + IAA, BA + IBA and kinetin + IAA. Highest percent of callusing (24.51 %) was observed on WPM basal medium.

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Note: * Originals not seen

"*IN VITRO* PROPAGATION OF BIG-LEAF MAHOGANY (*SWIETENIA MACROPHYLLA* KING) THROUGH TISSUE CULTURE"

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THESIS

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ABSTRACT

The study entitled "*In vitro* propagation of big-leaf mahogany (*Swietenia macrophylla* King) through tissue culture" was carried out at the Tissue Culture Laboratory of College of Forestry, Vellanikkara during the period 2007-2009. The objective of the programme was to standardize a protocol for *in vitro* micropropagation of big-leaf mahogany (*Swietenia macrophylla* King).

Fungal contamination was found to be prominent in rainy season and was a major problem in establishing the aseptic cultures of mahogany through axillary bud break. Explants collected during November to April were shown less percentage of contamination. To establish contamination free cultures, explants were pretreated with a fungicidal solution of Bavistine (Carbendazim) and Indofil M-45 (Moncozeb) each at 0.2 percent for 1 hr and followed by surface sterilization with 0.1 percent mercuric chloride for 15 minute. During the present investigation, season of explant collection has also influenced the culture establishment and growth.

Out of the three media employed, *viz.* MS, WPM and B5, MS medium was found to be suitable for culture establishment and growth. Murashige and Skoog media was used for further studies, fortified with different plant growth regulators (PGR) viz. cytokinin (BA and Kinetin) and auxin (IAA and IBA). Cytokinins at different concentrations (0.5, 1.0, 2.0 and 3.0 mg l^{-1}) were supplied individually or in combinations with auxins at various concentrations (0.1, 0.5 and 1.0 mg l^{-1}).

Kinetin supplemented alone to MS at higher concentrations was found to be enhancing bud initiation when compared to its counterpart BA which was effective, when supplied alone at lower concentrations. For multiple shoot production supplementation of BA to MS medium was more effective than kinetin.

Benzyl adenine fortified in combination with IAA was effective for culture establishment and growth from axillary buds of mahogany, over other combinations such as BA + IBA, kinetin + IAA and kinetin + IBA. Murashige and Skoog media supplemented with 2.0 mg l^{-1} BA+0.1 mg l^{-1} IAA was found to be best suited for bud initiation, shoot proliferation and leaf initiation. However, maximum number of shoots (3.33) was recorded on MS media supplemented with 1.0 mg l^{-1} BA along with 0.5 mg l^{-1} IBA.

Subsequent production of multiple shoots from microshoots of primary cultures through sub culturing failed due to shoot elongation and abscission of leaves in sub cultures. Due to such obstacles rooting experiments were not carried out and hence, a precise and profitable protocol for *in vitro* micropropagation of mahogany could not be developed in the present study.