In Vitro PROPAGATION OF MALABAR WHITE PINE (*Vateria indica* L.) THROUGH TISSUE CULTURE

By

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THESIS

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requirement for the degree of

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College of Forestry
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1994

Dedicated to
my
Loving and Devoted
Uncle

DECLARATION

I hereby declare that this thesis entitled "**In vitro propagation of Malabar white pine (*Vateria indica* L.) through tissue culture**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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We, the undersigned members of the Advisory Committee of **Sri. Ashok B. Divatar**, a candidate for the degree of Master of Science in Forestry with major in Tree Physiology and Breeding, agree that the thesis entitled **"In vitro propagation of Malabar white pine (Vateria indica L.) through tissue culture"** may be submitted by Sri. Ashok B. Divatar, in partial fulfilment of the requirement for the degree.

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LIST OF ABBREVIATIONS

ABA	-	Abscisic acid
$AgNO_3$	-	Silver nitrate
BAP	-	Benzyl amino purine/Benzyl adenine
cv.	-	cultivar
CCC/Cycocel	-	Chloro Choline Chloride
2,4-D	-	2,4-Dichlorophenoxy acetic acid
Fe EDTA	-	Ferric salt of ethelene diamine tetra acetic acid
GA_3	-	Gibberellic acid
IAA	-	Indole-3-acetic acid
IBA	-	Indole-3-butyric acid
Kin	-	Kinetin
µM	-	Micromole
MS	-	Murashige and Skoog (1962) medium
NAA	-	Naphthyl acetic acid
ppm	-	parts per million
v/v	-	volume in volume
w/v	-	weight in volume
WPM	-	Lloyd and McCown's (1980) Woody Plant Medium

Introduction

INTRODUCTION

Malabar White pine (Vateria indica Linn.), popularly known as 'Vellapine' in the vernacular, is a large handsome evergreen tree belonging to the family Dipterocarpaceae. This is found in South Western India from North Kanara in Karnataka to Tirunelveli in Tamil Nadu through entire Kerala, upto 760 m altitude (Troup, 1921). It is most plentiful in all the important evergreen forests. In Kerala it is found in almost all the divisions. It is also extensively planted as an avenue tree outside the forests in the above areas (Troup, 1921).

The white pine is well known in South India, along the West Coast, as a useful wood for tea chests, packing cases, coffins, plankings, interior fittings of buildings, floorings ceilings etc. Large quantities of this timber used to be shipped to Bombay from Malabar and sold as "Malabar White Pine". It is also suitable for bobbins and shuttles in the textile industry. Its usability for production of durable plywood as well as match splints has been established. It is extensively used in Kerala for the above purposes (Rama Rao, 1914). Dried kernels of white pine yield, by solvent extraction, a fat called "Piney tallow". It is often used in

candle making, soap manufacture and as a medium for chronic rheumatism (Krishnamurthy, 1993). The tree also yields piney-gum resin called White dammar, Indian copal or dhupa which under the influence of gentle heat combines with wax and oil and forms an excellent resinuous ointment which is a good substitute for resin. Fine shavings of this is administered internally to check diarrhoea.

The seeds of Vateria indica loses its viability comparatively quickly and because of this recalcitrant nature they cannot be stored for long periods (Huang and Villanueva, 1993). The seeds, however, have good germinative capacity. The seedlings stand a considerable amount of shade but are sensitive to drought and frost. Top canopy shade and lateral growing space help the establishment of large number of seedlings as wildlings. However, for raising nursery the recalcitrant nature of the seeds pause a serious problem. Most often wildlings collected from the forests are used for secondary nursery/field planting. In the above procedure the survival and establishment of seedlings is less than 25 per cent. Under these circumstances, enough quantity of planting materials is a limiting factor for large scale plantation programmes of this species.

Ashton (1981) proposed genetic improvement and the development of methods for producing clonal stock as

priorities for future dipterocarp research. However, ccnventional forestry procedures are difficult to be implemented in most of the dipterocarps including Vateria indica.

Micropropagation is attracting considerable attention for obtaining large number of genetically pure elite populations of forest tree species using in vitro methods. This technique is one of the possibilities of overcoming the problems associated with growing Vateria indica in large scale. The possibility of producing adequate quantity of seedlings of known superior genotypes with uniform quality can be assured through this method of propagation. In addition, standardization of this technique offers possibilities of genetic manipulations of the species through protoplast manipulation, callus culture, embryo culture etc. Such techniques offer possibilities for assisting in tree improvement programmes.

There have, to date, been few reports of tissue culture of dipterocarps. Smiths and Struycken (1983) induced callus and roots on leaf explants of Shorea curtisii whereas nodal explants of S. obtusa sprouted axillary shoots, but complete plantlets were not obtained. Scott et al. (1987) produced complete plantlets of Shorea roxburghii G.

In this study attempt was made to propagate *V. indica* from dormant terminal as well as axillary buds under *in vitro* conditions with the objective of standardizing methods for rapid multiplication as well as helping in long term tree improvement programmes.

Review of Literature

REVIEW OF LITERATURE

Development of the science of tissue culture is historically linked to the discovery of the cell and subsequent propounding of the cell theory. More than 234 years ago, Henri-Louis Dohamel du Monceau's (1756) pioneering experiments on wound healing in plants demonstrated spontaneous callus formation on the decorticated region of elm plants. His studies, according to noted biologist Gautheret (1985), could be considered a 'foreword' for the discovery of plant tissue culture.

Later in the early 1900 German Botanist Haberlandt (1902) developed the concept of *in vitro* cell culture. He was the first to culture isolated, fully differentiated cells in a nutrient medium containing glucose, peptone, and Knop's salt solution.

The greatest success using this technique has been achieved in herbaceous horticultural species. This success is partially due to the weak apical dominance and strong root regenerating capacities of many herbaceous plants. Examples of successful herbaceous ornamental species propagated through *in vitro* axillary bud proliferation include *Anigozanthos*, *Anthurium*, *Cephalotus*, *Chrysanthemum*, *Dianthus*, *Fuchsia*,

Gerbera, Gladiolus, Gloxinia, Hosta, phlox; the herbaceous vegetables include Allium, Arachis, Asparagus, Beta, Brassica, Phaseolus, and Fragaria; and the agronomic and other herbaceous genera like Capsella, Glycine, Vigna, Zea.

Compared to herbaceous crops, the micropropagation of woody species has lagged far behind. The greatest difficulty is experienced at primary culture establishment, root induction and partially due to the existence of polyphenolic compounds in the tissue.

Considerable progress has been made over the last two decades on micropropagation methods for forest trees. The successful examples of axillary bud proliferation include species in the following genera; Araucaria, Cryptomeria, Eucalyptus, Populus, Tectona and Thuja. Recent success in tree tissue culture is the controlled flowering of in vitro propagated bamboo (Nadgauda et al., 1990). The work done in tissue culture propagation of broad leaved tree species in recent years is reviewed below.

2.1 Clonal propagation

Sinha and Mallick (1993) reported that multiple shoot formation from segments of in vitro seedlings of Albizzia falcataria was seen in MS media supplemented with BA (4.4-8.9 μM).

Callus mediated shoot bud formation and rooting of shoots in Dalbergia latifolia, have been reported (Rao, 1986; Sita et al., 1986). Successful induction of multiple shoots on excised hypocotyl segments and shoot tips of in vitro germinated seedlings of Indian rosewood on MS supplemented with cytokinins and auxins has been achieved by Rai and Chandra (1989). Elite Indian rose wood trees of 60-80 years age could be micropropagated through axillary bud culture in MS supplemented with 1.0 ppm BA and 0.05 ppm NAA or 0.5 ppm IAA (Swamy et al., 1992). Protocol for clonal propagation of mature rose wood through axillary bud culture has been standardised (Mahato, 1992). Indian rosewood could be regenerated from callus tissue of cambial origin (Kumar et al., 1991). Successful plantlet regeneration of Indian rose wood from leaf disc cultured on MS (3/4 reduced major elements) supplemented with 5.0 ppm BA and 0.5 ppm NAA has been achieved by Sita and Swamy (1993).

Callus of Sesbania bispinosa could be obtained from both cotyledon and mature leaf explants in MS containing BA at 0.5 ppm and 2,4-D at 2.0 ppm. Callus could be later regenerated into shoot in MS medium supplemented with 2.0 ppm BA and 15 per cent (v/v) coconut milk (Sinha and Mallick, 1991). Sesbania grandiflora could be micropropagated by using hypocotyl and cotyledon segments (Shankar and Ram, 1990).

Yadav et al. (1990) reported that multiple shoot formation can be obtained from shoot tip explants of Syzygium cumini seedlings, cultured on MS medium supplemented with 0.23-8.9 μM of BA alone or in combination with auxins (IAA, IBA or NAA) at the range of 0.12-1.0 μM.

Sita et al. (1980) reported callus induction followed by differentiation in Santalum album using endosperm tissues from seeds of green fruits. The explants were cultured on MS amended with 2,4-D (1.0-2.0 ppm), Kinetin (0.1-0.2 ppm), BA (0.5-2.0 ppm) and NAA (1.0 ppm).

Shoot growth and rooting of Faidherbia (Acacia) albida was obtained on MS basal medium supplemented with BAP at 10^{-7}M and NAA at 10^{-7}M but rooting was most successful on medium supplemented with 10^{-7}M NAA alone (Ruredzo and Hanson, 1993). Induction of shoot buds in Acacia nilotica under in vitro conditions, in MS media incorporated with IAA (0.1-10.0 ppm) was achieved by Mathuri and Chandra (1983). Multiple shoot formation from excised axillary bud explants of the same species on MS containing 50 per cent (v/v) coconut water and 1 μM of BA was reported by Mittal et al. (1989). In Acacia nilotica, BA (1.5 ppm) supplemented to B_5 medium supported maximum multiple shoot differentiation from cultured cotyledonary nodal explant and in the medium amended with

2.0 ppm IAA, 100 per cent of shoots developed roots (Dewan et al., 1992). Acacia mangium nodal segments of seedling origin could give rise to plantlets when cultured on MS containing 0.5 ppm BA (Ahmad, 1990). The capacity for the shoot morphogenesis in internodal segments of Acacia holosericea and A. saligna were demonstrated by Jones et al. (1990) through indirect organogenesis. In the same study A. divenosa, A. holosericea, A. salicina, A. sclerosperma and A. saligna showed enhanced release of axillary buds in MS medium.

Multiple shoot formation was induced from excised seedling segments of Anogeissus pendula through indirect organogenesis. Cotyledon segments were noticed to have a higher organogenetic potential than epicotyl segments, when cultured in MS added with 1.0 ppm BA and 0.1 ppm IAA (Joshi et al., 1991).

Mascarenhas et al. (1982) observed that terminal buds from 10-20 year old Havea brasiliensis when cultured on MS medium along with 0.5 ppm kinetin, 2.0 ppm BA, 2000 ppm caseinhydrolysate, 0.1 ppm calcium panthothenate and 0.1 ppm biotin induced a maximum of three shoots per explant.

Excised leaf discs of neem (Azadirachta indica) cultured on Wood and Braun's medium supplemented with Kinetin

and BAP produced adventitious shoot buds (Ramesh and Padhya, 1990). Each isolated bud grown on medium containing gibberellic acid (GA_3) developed into a healthy shoot.

Kumar (1992) reported successful micropropagation of 15-18 year old Bauhinia purpurea trees through callus culture. Stem segments cultured in MS with 10 μM 2,4-D developed callus, which regenerated into plantlets with 2-3 leaves in the same basal medium supplemented with 5.0 μM kinetin. Mathur and Mukunthakumar (1992) reported in vitro propagation protocols established for two leguminous trees Bauhinia variegata and Parkinsonia aculeata from explants taken from mature trees.

Grellier et al. (1984) has stated that multiplication of Betula pendulosa and Betula pubescens was achieved on MS medium containing low levels of cytokinins. Pseudoterminal buds of B. uber when placed in medium containing 0.6 and 0.05 ppm of BA and IAA respectively opened in four to five days and produced upto three leaves in one week (Vijayakumar et al., 1990). The plantlets regenerated have been field planted.

In vitro propagation of tamarind has been reported from nearly all parts of seedlings when cultured in MS containing cytokinins and auxins (Mascarenhas et al., 1987). Shoot tip cultures in tamarind on MS with 0.5-5.0 ppm BA was

effective for shoot induction (Kopp and Nataraja, 1990). High frequency plant regeneration from cotyledons of the same species in MS supplemented with 5.0 µM of BA was reported by Jaiwal and Gulati (1991).

The successful induction of multiple shoot formation from terminal buds of 100 years old teak trees was achieved by Gupta et al. (1980). Over 500 plants could be produced from a single bud of a selected elite tree in MS containing Kinetin (0.15 ppm) and BA (0.15 ppm).

Axillary bud from Caesalpinia pulcherrima cultured on MS medium containing NAA and BA/kinetin exhibited shooting and the greatest number of roots were produced on medium containing IAA and cytokinin (Rohman et al., 1993).

Wickremesinhe and Arteca (1993) reported the establishment of callus cultures from stem explants of Cephalotaxus harringtonia cultured on MS medium supplemented with 4.5 µM 2,4-D and 0.05 µM kinetin. Transfer of callus on to a hormone free medium was givingrise to both shoots and roots.

Aboel-nil (1987) reported that in vitro propagation method has been found successful in Casuarina species, namely C. glauca, C. cunninghamiana and C. equisetifolia. Callus was induced from juvenile and mature stem segment on MS medium

supplemented with 0.5 μM each of 2-ip and NAA. Buds were regenerated from callus tissue and stem segment explants on MS medium containing BA at 2.2-11.0 μM combined with IAA at 0.5 μM.

Organogenesis from shoot callus of Pterocarpus indicus in MS medium after irradiating the calli (10-60 Kr) was reported by Callinawan and Halos (1981). Patri et al. (1988) attempted culturing Pterocarpus santalinus and observed that shoots could be obtained through callus culture in 1/4 MS supplemented with 3.0 ppm BA and 40 ppm adenine. Production of plantlets of the same species by induction of multiple shoots from shoot tip cultures established from germinating seedling has been reported by Sita et al. (1992). Multiple shoot formation from axillary buds of Pterocarpus marsupium was obtained on WPM medium supplemented with 2.0 ppm kinetin and 0.1 ppm of IAA (Santhoshkumar, 1993).

Shoot tip explants of Ficus lacor saplings could be induced to form shoots at a rate of 20-30 in MS containing 1.0 ppm BA, 1.0 ppm kinetin and 1000 ppm casein hydrolysate (Amatya and Rajbhandary, 1990).

Plantlets have been obtained from embryoderived cotyledonary nodes of common ash (Fraxinus excelsior) as reported by Hammatt and Ridout (1992). The addition of

thidiazuron (TDZ) to MS salts and vitamins, instead of BAP increased both the culture weight and the proportion of common ash embryo hypocotyl explants that produced adventitious shoots and resulting shoots were rooted in half strength WPM with 1.0 ppm IBA (Tabrett and Hammatt, 1992).

Nadgir et al. (1984) obtained multiple shoot from nodal segments of mature tree of Dendrocalamus strictus, Bambusa vulgaris and B. arundinaceae. Explants cultured on MS medium containing BA (0.02 ppm) and coconut water (5%, v/v) produced shoots. Dendrocalamus strictus embryos started callusing in B_5 medium having 2,4-D at 10.30 µM. The callus on subculture gaverise to somatic embryos and germinated in the same medium (Rao and Rao, 1988). Multiple shoots were produced from axillary buds of 54 species from 15 genera of bamboo, cultured on MS medium containing 22.0 µM BA. Rooting occurred in media containing 2.7-5.4 µM NAA (Prutpongse and Gavinlertvatana, 1992).

Differentiation could be obtained from hypocotyl segments of Prosopis cineraria cultured in MS ammended with 4.5 ppm kinetin and 0.25 ppm IAA/NAA (Goyal and Arya, 1981). Shoot formation from nodal explants of elite tree of Prosopis cineraria, on MS medium containing NOA and NAA at 3.0 ppm each was achieved by Kackar et al. (1991). Nandwani and Ramawat (1991) observed multiple shoot formation from nodal explants

of Prosopis juliflora on MS medium supplemented with cytokinin and auxin. Shoots could be rooted on MS medium containing NAA or IBA. Callus mediated regeneration of Prosopis tamarugo on MS medium containing 5.0 ppm BA was achieved by Nandwani and Ramawat (1992).

In vitro shoot multiplication of Prosopis glandulosa through indirect organogenesis could be obtained in a variety of combinations involving different media and growth regulators (Jang et al., 1988). Shoot cultures of Prosopis deltoides have been successfully regenerated from internodal stem explants (Colleman and Ernst, 1990). Shoot formation from root segments of Prosopis alba x P. grandidenta cultured in WPM containing zeatin was reported by Son and Hall (1990).

Best response from nodal explants of Melia azedarach was obtained on MS medium containing 0.5 ppm kinetin and 0.5 ppm BA (Dhingra et al., 1991).

Rapid clonal multiplication of mulberry plant could be obtained by culturing axillary buds in MS with 2.0 ppm BA (Tewary and Subba Rao, 1990).

Manzanera and Pardos (1990) obtained shoot proliferation from apical buds and nodal stem segments of Cork Oak (Quercus suber L.) cultured on Sommer's or Heller's medium

supplemented with 0.1 ppm BA and rooting of shoots were observed with IBA added to the above medium.

Ashok (1985) reported success in in vitro propagation of Eucalyptus citriodora. Activated charcoal increases shoot development. Kumar and Ayyappan (1987) used juvenile tissues of in vitro germinated seedlings of E. camaldulensis and produced multiple shoot on MS supplemented with BA, adenine sulphate and sodium dihydrogen phosphate. Multiple shoot formation from nodal explants of E. tereticornis and E. globulus cultured on MS medium containing kinetin (0.2 ppm), BA (0.3 ppm), Calcium panthothenate (0.1 ppm) and biotin (0.1 ppm) has been reported (Gupta and Mascarenhas, 1987 and Das and Mitra, 1990).

Successful in vitro multiplication for clonal propagation of Leucaena leucocephala through axillary bud culture was reported by Datta and Datta (1985) and Goyal et al. (1985).

Kim and Lee (1988) reported that axillary buds of Ziziphus cv. Geumsung showed best shoot and growth when 500 ppm activated charcoal was added to half strength MS with 0.5 ppm BA.

2.2 Factors affecting success of *in vitro* propagation

2.2.1 Culture medium

Most tree tissue cultures are carried out on basal media similar to those used for herbaceous crops. The most commonly used medium is that of Murashige and Skoog (1962) and various modifications thereof. A medium specifically designed for tree species is the "Woody plant medium" of Lloyd and McCown (Lloyd and McCown, 1980). Compared with the Murashige and Skoog medium, it is low in ammonium, nitrate, potassium, chloride and high in sulphate. Several fruit trees have a high manganese requirement (Quoirin and Lepoivre, 1977). The nutrient needs of cultures may vary seasonally. The nitrate requirement of buds of 2-year-old *Pseudotsuga menziesii* seedlings *in vitro* decreased from November to April (Evers, 1981).

2.2.1.1 Basal media

A number of basic media have been developed by various workers to suit particular requirements of a cultured tissue. A standard or a basal medium consists of a balanced mixture of macronutrients and micronutrient elements, vitamins, a carbon source, organic growth factors, a source of reduced nitrogen supply and plant hormones.

Addition of deproteinized coconut milk, tomato juice, water melon juice, orange juice and other plant extracts have also been tried. Composition of different media has been developed by different workers and named after them such as, Gautheret (1942), Hildebrandt et al. (1946), Nitsch (1951), Heller (1953), Reinert and White (1956), Murashige and Skoog (1962), White (1963), Gamborg et al. (1968), Schenk and Hildebrandt (1972) etc.

The Murashige and Skoog (MS) (1962) or Linsmaier and Skoog (LS) (1965) salt composition is used widely, particularly if the desired objective is plant regeneration. The B_5 medium, or its various derivatives, have been valuable for cell and protoplast culture (Gamborg et al., 1968; Kao, 1977). The medium designated N_6 was developed for cereal antherculture and is used with success in other types of cereal tissue culture (Chu, 1978). The E_1 medium supports rapid growth of cells for embryogenesis and for the culture of protoplasts (Gamborg et al., 1983). In the special class of antherculture, the medium devised by Nitsch and Nitsch (1969) is used frequently. Widely used medium, especially for woody plants is WPM (Lloyd and McCown, 1980).

2.2.1.2 Growth regulators

There are four broad classes of growth hormones which

are of known importance in tissue culture. They are the auxins, cytokinins, gibberellins and abscisic acid.

A common feature of auxin is the property of inducing cell division. The compounds include 2,4-dichlorophenoxy-acetic acid (2,4-D), Indole-3-Acetic Acid (IAA) and Naphthalene Acetic Acid (NAA). Other effective compounds are 2,4-5-trichlorophenoxyacetic acid (2,4,5-T) and 4-amino-3,5,6,-trichloropicolinic acid (Picloram). The auxins also stimulate root initiation.

Cytokinins are adenine derivatives which have an important role in shoot induction. The most frequently used cytokinins are 6-Furfurylaminopurine (Kinetin), N-6-Benzyladenine (BA) 6-(4-Hydroxy-3-Methyl-but-2-enylamino) purne (Zeatin) and iso-pentenyl adenine (IPA). The gibberellins are normally used in plant regeneration after the formation of primordia has occurred (Gamborg, 1984). Ethylene is also an important growth hormone. The compound is produced by cultured cells, but its role in cell and organs in culture is not known (Gamborg, 1984).

A small quantity of cytokinin may be synthesized by shoots grown in vitro (Koda and Okazawa, 1980), roots being the principal site of cytokinin biosynthesis. BA is most effective for meristem, shoot tip, and bud cultures, followed

by kinetin, cytokinin 2-ip has been used less frequently (Nair et al., 1979). It has been suggested that exogenous auxin is not essential but is beneficial for the growth of stage I cultures (Dale, 1975). When GA is supplemented, its function is primarily for bud elongation (Schnabdrauch and Sink, 1979).

In general, it appears that BA is the most effective cytokinin for stimulating axillary shoot proliferation, followed by, in decreasing order kinetin and 2-ip (Bhojwani, 1980a; Hasegawa, 1980; Lundergan and Janick, 1980; Kitto and Young, 1981). A quite different order of cytokinin effectiveness may exist in certain species, such as Rhododendron (Anderson, 1975) and mountain laurel (Lloyd and McCown, 1980) in Ericaceae. One of the possible roles of auxin in stage II (Multiplication of the propagule) medium is to nullify the suppressive effect of high cytokinin concentrations on axillary shoot elongation, and restore normal shoot growth (Lundergan and Janick, 1980). 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). Wochok and Sluis (1980) observed that a topical treatment of Atriplex shoot explants with GA was effective not only in stimulating shoot elongation, but also in enhancing shoot multiplication beyond that of the most effective auxin-cytokinin combination.

Sankhla et al. (1993) reported that gibberellin biosynthesis inhibitors increased shoot formation in Albizzia julibrissin, conversely, GA_3 decreased shoot formation, indicating that modification of gibberellin status can have a strong impact on the number of shoots formed.

2.2.1.3 Other organic compounds

Culture media are often supplemented with a variety of organic extracts which have constituents of an undefined nature. These include protein (casein) hydrolysates, coconut milk, yeast and malt extracts, ground banana, orange juice and tomato juice etc. to promote the growth of certain calli and organs. In tissue culture the success achieved with use of coconut milk (5 to 20 per cent) and protein (casein) hydrolysates (0.05 to 1.0 per cent) has been significant. Similarly, potato extract has been found as a suitable medium for anther culture (Razdan, 1993). Pollard et al. (1965) reported that coconut water contains myo-inositol which lead to the inclusion of this chemical in many culture media. Certain other organic compounds like adenine and its more soluble form adenine sulphate are often added to the culture media. These can enhance growth and organ formation due to their cytokinin like capacity (Skoog and Tsui, 1948). Amino acids are routinely added to the media as they provide an immediately available source of nitrogen and their up take can

be much more rapid than that of inorganic nitrogen in the same medium (Simkins et al., 1970). Polyphenolic compounds like phloroglucinol in the medium has been found to have a beneficial effect on organogenesis and growth (Hunter, 1979; Mallika et al., 1992). Hawker et al. (1973) stated that replacement of casein with coconut milk could double growth rate of callus. Many researchers have warned against the indiscriminate use of these compounds, as it will lead to little control over the experiment (Gamborg and Shyluk, 1981).

The addition of activated charcoal to culture media is reported to stimulate growth and differentiation paradoxically its effect has also been proved inhibitory. Incorporation of this chemical into nutrient medium stimulates androgenesis in some systems (Anagnostakis, 1974; Bajaj et al., 1977), presumably by removing the growth inhibitors from the medium (Keller and Stringam, 1978; Kohlenbach and Wernicke, 1978; Weatherhead et al., 1979). Addition of activated charcoal may also prevent browning of tissues (Tisserat, 1979). Harmful effects of activated charcoal include binding of plant growth regulators and other metabolites has been reported by Weatherhead et al., 1979.

2.2.2 Root induction

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 full strength MS medium, the salt concentration was reduced to half (Garland and Stoltz, 1981; Zimmerman and Broome, 1981) or a quarter (Skirvin and Chu, 1979) for rooting. Abundant rooting was observed when salt concentration of medium was reduced to one-half or one third (Lane, 1979).

Riboflavin is reported to improve the quality of root system in Eucalyptus ficifolia. In the presence of IBA and under diffuse light it induced the formation of long roots which grew into medium and bore very short laterals. In contrast, the roots developed in the absence of riboflavin were short, bore laterals and grew near the surface of the medium (Gorst and De Fossard, 1980).

Generally, auxin favours root formation. Among the auxins, NAA has been the most effective one for induction of rooting (Ancora et al., 1981). The concentration of auxin required is often critical to provide sufficient stimulus to initiate roots while preventing the excessive formation of callus (Yeoman, 1986). Sometimes a combination of auxins may give a better response (Gupta et al., 1980). The root elongation phase has been found to be very sensitive to auxin concentration. High concentration of auxin inhibited root elongation (Thimman, 1977). Rooting of the shoots in vitro

and *in vivo* has been reported by the simple, highly efficient and more economical methodology by Yeoman (1986), Schwarz *et al*. (1988) and Vijayakumar *et al*. (1990). This is achieved by using vermiculite, sand and peat singly or in combination in tubes or pots as the rooting medium and maintaining it in high humid conditions.

Jones (1983) reported that phloroglucinol promoted rooting in a number of rosaceous fruit trees but the whole subject of the effectiveness of phloroglucinol in rooting has been a subject of controversy.

2.2.3 Acclimatisation and planting out

Acclimatisation is critical to any micropropagation scheme since shoots and plantlets produced *in vitro* must be readapted to the less humid environmental conditions outside of the culture vessels. Acclimatization done concurrently with rooting saves time and reduced cost (Zimmerman, 1986). Physical, chemical and biological properties of the planting medium are important for plant establishment (Kyte and Briggs, 1979). Thorough washing of the plantlet to remove traces of nutrient sticking to it is essential for eliminating the problems of fungal infection (Anderson, 1975).

The large internal air spaces that are present result in excess water loss from the leaves of micropropagated

plants. Stomatal density is also greater in leaves of *in vitro* plantlets than in leaves of acclimatised or green house grown plantlets of sweet gum (Wetzstein and Sommer, 1983).

If one surveys the tissue culture literature, it becomes apparent that a large proportion of it deals with clonal propagation. The ability to regenerate plant by micropropagation is an essential step in almost all aspects of tissue culture research and application. The economic potential of the aseptic culture of forest trees is still at the down of exploitation. However, significant advances have been made during the past decade in regenerating a few hardwood tree species. Needless to say that more work and workers are needed in this field.

Materials and Methods

MATERIALS AND METHODS

The present investigations on *in vitro* propagation of Malabar white pine (*Vateria indica* Linn.) through tissue culture were carried out at the College of Forestry, Vellanikkara during the period 1992-94. Details regarding the experimental materials and the methods adopted for the study are described below.

3.1 MATERIALS

3.1.1 Explant

Nodal and internodal segments of about one cm size and leaf segments (1 cm x 1 cm) collected from 3 to 15 month old seedlings were used as the explants.

The source seedlings were raised in beds. Three month old seedlings were transplanted to polybags (40 cm x 15 cm) containing potting mixture (1:1:1 sand:soil:Farm Yard Manure). These seedlings were maintained in glass house.

3.1.2 Media

The basic media used for the study include Murashige and Skoog medium (Murashige and Skoog, 1962), ½MS and Woody Plant medium (Lloyd and Mc Cown, 1980). The chemical

composition of the media are given in Table 1. The basic media were supplemented with plant growth regulators and other additives in the different experiments.

The chemicals used for preparing the media were of analytical grade from, Sisco Research Laboratories (SRL), Merck, Sigma and British Drug House (BDH).

3.2 METHODS

3.2.1 Collection and preparation of explant

Since the seedlings selected for collecting the explants were growing in the glass house chances of harboring microbial populations were quite high. To control the rate of contamination in cultures, the seedlings were sprayed with a mixture of a systemic fungicide 'Bavistin' 50 per cent WP (Carbendazim) and the contact fungicide 'Indofil M-45' (Mancozeb) 0.3 per cent once in 10 to 15 days. The frequency of spray was increased to once in alternate days during rainy season (May to August).

Stem segments of approximately 25 to 30 cm with 10 to 12 nodes were excised from the seedlings using a sharp blade and brought to the laboratory as quickly as possible. The leaves were then removed leaving 0.5 cm of the rachis. Stem segments were washed in running tap water using a detergent to

Table 1. Chemical composition of various culture media used for *in vitro* propagation in *Vateria indica*

Compound	Amount (mg/l)	
	Murashige and Skoog (MS)	Woody Plant Medium (WPM)
Inorganic		
Ammonium nitrate	1650.0	400.0
Boric acid	6.2	6.2
Calcium chloride - 2 hydrate	440.0	96.0
Calcium nitrate - 4 hydrate	-	556.0
Cobalt chloride - 6 hydrate	0.025	-
Copper sulphate - 5 hydrate	0.025	0.025
Ferrous sulphate - 7 hydrate	27.8	27.8
Manganese sulphate - 1 hydrate	22.3	22.3
Magnesium sulphate - 7 hydrate	370.0	370.0
Na_2 EDTA - 2 hydrate	37.3	37.3
Potassium chloride	0.83	-
Potassium nitrate	1900.0	-
Potassium sulphate	-	990.0
Sodium dihydrogen phosphate	170.0	170.0
Sodium molybdate - 2 hydrate	0.25	0.25
Zinc sulphate - 7 hydrate	8.6	8.6

Contd.

Table 1 (Contd.)

Compound	Amount (mg/l)	
	Murashige and Skoog (MS)	Woody Plant Medium (WPM)
Organic		
Inositol	100.0	100.0
Nicotinic acid	0.5	0.5
Thiamine HCl	0.1	0.1
Pyridoxine HCl	0.5	0.5
Glycine	2.0	2.0
Others		
Sucrose (in per cent w/v)	3.0	2.0
Agar (in per cent w/v)	0.7	0.7
pH	5.8	5.6

½MS denotes ½ the amounts of the inorganic constituents per litre

remove traces of fungicide and dust adhering to it. After drying the segments in blotting paper, they were swabbed with a cotton dipped in 70 per cent (v/v) alcohol. Further sterilisation procedures were carried out under perfect aseptic conditions in a 'Klenzaids' laminar air flow cabinet. The stem segments were then cut into nodal segments of size 1.5 cm (approx.) and subjected to surface sterilisation using different chemicals described in Table 2. For all the treatments the explants were immersed in the given chemical for stipulated period with occasional stirring. The explants after surface sterilisation were removed from the chemical and rinsed thrice in sterilised distilled water to remove traces of sterilant sticking to the surface. The cut ends of the explants were then further trimmed to give a final size of approx. one cm to the explant. In similar way, internodal and leaf segments were collected and prepared. Another experiment was conducted to estimate the optimum size of the explants. Explants with lengths <0.5 cm, 0.5-1.5 cm and >1.5 cm were used for culturing the study.

3.2.2 Preparation of culture media

Standard procedures (Gamborg and Shyluk, 1981) were followed for the preparation of the media. Stock solutions of major and minor nutrients were prepared by dissolving the required quantity of the chemicals in distilled water and were

Table 2. Chemicals used, their concentration and duration of treatment for surface sterilisation of explants of *Vateria indica*

Sterilant	Concentration	Duration (minutes)
Mercuric chloride	0.1%	1
		2
		3
		4
		5
		6
		8
		9
		10
		15
	0.05%	5
		6
		8
		10
	0.01%	5
		6
		8
		10
Chlorine water	3.5 mg l^{-1}	5
		8
Bavistin + Indofil + $HgCl_2$	(0.1%+0.1%) + 0.1%	10+5
		10+8
		10+10

stored in amber coloured bottles under refrigerated conditions. The stock solution of nutrients were prepared fresh every four weeks and that of vitamins, aminoacids and growth regulators every week.

Specific quantities of the stock solutions of the chemicals and growth regulators were pipetted out into a 1000 ml beaker. Sucrose and inositol were added fresh and dissolved. The volume was then made upto about 1000 ml by adding distilled water. The pH of the solution was checked with a pH meter and adjusted to the range 5.6 to 5.8 using 1 N NaOH or 1 N HCl. Agar was then added to the medium and the final volume made upto 1000 ml.

The solution was then boiled for melting the agar by keeping in a microwave oven. Twenty ml each of the melted media was poured hot to the oven dried culture tubes (150 x 25 mm), which were previously washed and rinsed in distilled water. The containers with the medium were then tightly plugged with absorbent cotton wool plugs. The media were autoclaved for 15-20 min at 15 psi pressure and 121°C temperature (Dodds and Roberts, 1985). After sterilisation, the culture vessels were immediately transferred to the culture room.

3.2.3 Inoculation and culturing of the explants

All the inoculation operations were carried out under perfect aseptic conditions in a laminar air flow cabinet.

To inoculate the explants to the culture medium, the cotton wool plug of the culture vessel was removed and the vessel neck was first flamed over a gas burner kept in the chamber. The sterile explants were quickly transferred into the medium using sterile forceps. The neck of the culture vessels was once again flamed and the cotton wool plug replaced.

The culture vessels were then transferred to the culture racks where they were incubated at a temperature of 27 ± 2°C. Artificial illumination was provided using cool white fluorescent lamps. The light intensity was maintained at 2000 lux. Photo period was fixed as 16 h per day.

3.2.4 Shoot induction

3.2.4.1 Standardisation of medium supplement

Studies were conducted to evaluate the effect of various plant growth regulators on bud break, shoot induction, multiple shoot production and growth from axillary buds. For this study separate experiments were conducted using WPM, MS and ½MS as basal medium. A total of 240 combinations of

different levels of cytokinins and auxins were tried in this experiment. Details regarding the various plant growth regulators tried and concentrations used are furnished in Table 3. Apart from growth regulators, various organic and inorganic additives were evaluated for their ability to produce shoots from axillary bud explants of Vateria indica. A total of 70 treatment combinations were tried with different media additives in ½MS and WPM. Details regarding the organic supplements other than growth regulators tried are presented in Table 4.

3.2.4.2 Standardisation of physical condition

As physical condition of the culture plays a profound influence on the shoot induction and growth from axillary explants, a study on the influence of these conditions was carried out. An experiment was conducted where varying levels of agar (0%, 0.6%, 0.7%, 0.8%) were incorporated and their influence studied. Study on the influence of the pH of the medium on culture establishment was done by adjusting pH of the medium to 5.0, 5.5 and 6.0. Another trial was conducted to study the influence of light on shoot induction and growth of Vateria indica axillary bud explants. Treatments tried included culture incubation in the dark, in white fluorescent light at 500 lux, 1000 lux, 2000 lux, as well as red light and sunlight.

Table 3. Treatments of plant growth regulators tried for culture establishment bud break from axillary buds and callus induction from internode and leaf segments of Vateria indica

Basal medium	Treatment
MS	Kinetin (1.0, 2.0, 3.0 ppm), BA (1.0, 2.0, 3.0 ppm) and 2-ip (1.0, 2.0, 3.0 ppm)
MS	3x3 combinations of Kinetin (1.0, 2.0, 3.0 ppm) and IAA (0.1, 0.5, 1.0 ppm)
MS	3x3 combinations of Kinetin (1.0, 2.0, 3.0 ppm) and NAA (0.1, 0.5, 1.0 ppm)
MS	3x3 combinations of Kinetin (1.0, 2.0, 3.0 ppm) and 2,4-D (0.1, 0.5, 1.0 ppm)
MS	3x3 combinations of BA (1.0, 2.0, 3.0 ppm) and IAA (0.1, 0.5, 1.0 ppm)
MS	3x3 combinations of BA (1.0, 2.0, 3.0 ppm) and NAA (0.1, 0.5, 1.0 ppm)
MS	3x3 combinations of BA (1.0, 2.0, 3.0 ppm) and 2,4-D (0.1, 0.5, 1.0 ppm)
MS	3x3 combinations of 2-ip (1.0, 2.0, 3.0 ppm) and IAA (0.1, 0.5, 1.0 ppm)
MS	3x3 combinations of 2-ip (1.0, 2.0, 3.0 ppm) and NAA (0.1, 0.5, 1.0 ppm)
MS	3x3 combinations of 2-ip (1.0, 2.0, 3.0 ppm) and 2,4-D (0.1, 0.5, 1.0 ppm)
½MS	3x3 combinations of Kinetin (1.0, 2.0, 3.0 ppm) and IBA (0.1, 0.5, 1.0 ppm)
½MS	3x3 combinations of BA (1.0, 2.0, 3.0 ppm) and IBA (0.1, 0.5, 1.0 ppm)
½MS	3x3 combinations of 2-ip (1.0, 2.0, 3.0 ppm) and IBA (0.1, 0.5, 1.0 ppm)

Contd.

Table 3 (Contd.)

Basal medium	Treatment
½MS	Gibberellic acid (1.0, 2.0, 3.0 ppm) in combination with 2 ppm 2-ip and 0.1 ppm IBA
½MS	ABA (1.0, 2.0, 3.0 ppm) in combination with 2 ppm 2-ip and 0.1 ppm IBA
WPM	Kinetin (1.0, 2.0, 3.0 ppm), BA (1.0, 2.0, 30 ppm) and 2-ip (1.0, 2.0, 3.0 ppm)
WPM	3x3 combinations of Kinetin (1.0, 2.0, 3.0 ppm) and IAA (0.1, 0.5, 1.0 ppm)
WPM	3x3 combinations of Kinetin (1.0, 2.0, 3.0 ppm) and IBA (0.1, 0.5, 1.0 ppm)
WPM	3x3 combinations of Kinetin (1.0, 2.0, 3.0 ppm) and NAA (0.1, 0.5, 1.0 ppm)
WPM	3x3 combinations of Kinetin (1.0, 2.0, 3.0 ppm) and 2,4-D (0.1, 0.5, 1.0 ppm)
WPM	3x3 combinations of BA (1.0, 2.0, 3.0 ppm) and IAA (0.1, 0.5, 1.0 ppm)
WPM	3x3 combinations of BA (1.0, 2.0, 3.0 ppm) and IBA (0.1, 0.5, 1.0 ppm)
WPM	3x3 combinations of BA (1.0, 2.0, 3.0 ppm) and NAA (0.1, 0.5, 1.0 ppm)
WPM	3x3 combinations of BA (1.0, 2.0, 3.0 ppm) and 2,4-D (0.1, 0.5, 1.0 ppm)
WPM	3x3 combinations of 2-ip (1.0, 2.0, 3.0 ppm) and IAA (0.1, 0.5, 1.0 ppm)
WPM	3x3 combinations of 2-ip (1.0, 2.0, 3.0 ppm) and IBA (0.1, 0.5, 1.0 ppm)
WPM	3x3 combinations of 2-ip (1.0, 2.0, 3.0 ppm) and NAA (0.1, 0.5, 1.0 ppm)
WPM	3x3 combinations of 2-ip (1.0, 2.0, 3.0 ppm) and 2,4-D (0.1, 0.5, 1.0 ppm)

Table 4. Treatments of media additives tried for culture establishment and bud break from axillary buds and callus induction from internode and leaf segments of *Vateria indica*

Medium	Treatment
½MS	Casein hydrolysate (200, 300, 500 ppm) in combination with 2.0 ppm 2-ip and 0.1 ppm IBA
½MS	Cycocel (0.1, 0.5, 0.75, 1.0, 2.0, 3.0 ppm) in combination with 2.0 ppm 2-ip and 0.1 ppm IBA
½MS	Activated charcoal (1,2,3,4 per cent w/v) in combination with 2.0 ppm 2-ip and 0.1 ppm IBA
½MS	Adenine sulphate (10,20,30,40,60 ppm) in combination with 2.0 ppm 2-ip and 0.1 ppm IBA
½MS	Silver nitrate (5,10,15,20 ppm) in combination with 2.0 ppm 2-ip and 0.1 ppm IBA
½MS	Copper sulphate (5,10,15,20 ppm) in combination with 2.0 ppm 2-ip and 0.1 ppm IBA
½MS	Coconut water (10,20,30 per cent v/v) in combination with 2.0 ppm 2-ip and 0.1 ppm IBA
½MS	Cobalt chloride (1,2,3 ppm) in combination with 2.0 ppm 2-ip and 0.1 ppm IBA
½MS	Gibberellic acid (1,2,3 ppm) in combination with 2.0 ppm 2-ip and 0.1 ppm IBA
½MS	ABA (1,2,3 ppm) in combination with 2.0 ppm 2-ip and 0.1 ppm IBA
WPM	Casein hydrolysate (200, 300, 500 ppm) in combination with 2.0 ppm 2-ip and 0.1 ppm IBA
WPM	Cycocel (0.1, 0.5, 0.75, 1.0, 2.0, 3.0 ppm) in combination with 2.0 ppm 2-ip and 0.1 ppm IBA

Contd.

Table 4 (Contd.)

Medium	Treatment
WPM	Activated charcoal (1,2,3,4 per cent w/v) in combination with 2.0 ppm 2-ip and 0.1 ppm IBA
WPM	Adenine sulphate (10,20,30,40,60 ppm) in combination with 2.0 ppm 2-ip and 0.1 ppm IBA
WPM	Silver nitrate (5,10,15,20 ppm) in combination with 2.0 ppm 2-ip and 0.1 ppm IBA
WPM	Copper sulphate (5,10,15,20 ppm) in combination with 2.0 ppm 2-ip and 0.1 ppm IBA
WPM	Coconut water (10,20,30 per cent v/v) in combination with 2.0 ppm 2-ip and 0.1 ppm IBA
WPM	Cobalt chloride (1,2,3 ppm) in combination with 2.0 ppm 2-ip and 0.1 ppm IBA

3.2.5 Indirect organogenesis/embryogenesis

3.2.5.1 Callus induction and multiplication

Explants such as leaf segments and internodal segments of seedlings were used for callus induction. Different media combinations tried for callus induction and later proliferation, are furnished in Tables 3 and 4. The relative performance of different explants for callus induction and proliferation was observed.

Observations were recorded for callus induction, growth rate and morphology. Callus index (CI) was worked out as below

$$CI = P \times G$$

where P is the percentage of callus initiation and

G is the growth score

Scoring was made based on the spread of the calli and a maximum score of four was given for those that have occupied the whole surface of the media within four weeks culture period in culture tubes.

3.2.6 Observations

Each trial was conducted with a minimum of fourteen tubes. The observations were recorded between third and

fourth weeks after culture inoculation. All the data were calculated based on cultures that remain without contamination after the required period of incubation.

The following observations were recorded from various experiments.

a. Number of cultures uncontaminated: Number of cultures free from contamination were expressed as a percentage of total number of cultures.

b. Number of explant showing bud break: Number of cultures showing bud break/sprout were expressed as a percentage of total number of surviving cultures.

c. Number of explants that produced leaves: Number of cultures that produced leaves were expressed as a percentage of total surviving cultures.

d. Number of cultures that showed callusing at nodal base: The number of cultures that had callusing at base were expressed as a percentage of total surviving cultures.

e. Number of cultures that showed leaf callusing: The number of leaf cultures that showed callusing were expressed as a percentage of total surviving cultures.

f. Number of cultures that showed stem/internodal callusing: The number of stem cultures that showed callusing were expressed as a percentage of total surviving cultures.

g. Callus index: The percentage of callus initiation, multiplied by the growth score.

Results

RESULTS

The results of various experiments on the standardization of *in vitro* propagation technique in *Vateria indica*, conducted during 1992-94 at the Plant Tissue Culture Laboratory of the Department of Tree physiology and Breeding, College of Forestry, Vellanikkara, Trichur, are presented in this chapter.

4.1 Culture establishment and bud break

4.1.1 Explant choice

The effect of explant size on culture establishment is presented in Table 5. Nodal explants of 0.5 to 1.5 cm were found to be better for culture establishment where 21.4 per cent of the cultures showed release of axillary buds. Bigger explants of size more than 1.5 cm had lower number of cultures showing bud sprout (14.3 per cent). More chance of contamination was noticed in these cultures. Only 7.1 per cent of the cultures from smaller explants of less than 0.5 cm showed bud break.

4.1.2 Surface sterilisation of explants

The results of the trial for surface sterilisation of the explants are presented in Table 6. Among the various

Table 5. Effect of size of explants in axillary bud cultures of Vateria indica on initiating shoot bud release

Explant size	% cultures showing response
< 0.5 cm	7.1
0.5 cm - 1.5 cm	21.4
> 1.5 cm	14.3

Culture medium ½MS + 2.0 ppm 2-ip + 0.1 ppm IBA

surface sterilants tried, mercuric chloride 0.1 per cent was found to be most effective in controlling contamination of explants. An initial wiping of explants with 70 per cent alcohol, followed by immersion in 0.1 per cent mercuric chloride for a period of 5 minutes resulted in the less rate of contamination in nodal explants whereas a period of 4 minutes was found sufficient for surface sterilisation of leaf segments. The percentage of survival was 89.3 and 75.0, respectively, for the stem and leaf segments. Chlorine water alone tried was found to be totally ineffective for controlling contamination. In different levels of fungicidal dip tried, the rate of survival of explants was found to be poor.

Data on polyphenol exudation from tree explants is presented in Table 7. In case of tender nodal explant tissue, polyphenol exudation was negligible with bud sprout in 21.7 per cent of the cultures, whereas the mature nodal explant tissue exhibited good amount of phenol excretion in some of trial cultures. Bud break was recorded in 7.1 per cent of these cultures.

4.1.3 Seasonal variation in the rate of culture establishment

The seasonal influence on culture establishment of nodal explant is evident from the results presented in

Table 6. Effect of various surface sterilants on culture establishment of Vateria indica

Treatments	Duration of treatment (minutes)	Percentage of survival without contamination after 15 days		Percentage of establishment 21 days after inoculation	
		Nodal segment	Leaf segment	Nodal segment	Leaf segment
$HgCl_2$ (0.1%)	1		64.3		42.8
	2		66.6		50.0
	3		70.8		62.5
	4		75.0		66.6
	5	89.3		71.4	
	6	85.7		60.7	
	8	71.4		53.0	
	9	64.2		47.7	
	10	57.1		42.4	
$HgCl_2$ (0.05%)	5	78.6		67.8	
$HgCl_2$ (0.01%)	5	75.0		47.8	
Chlorine water	5	5.0		5.0	
	8	5.0		5.0	
Fungicidal (0.1% Bavistin + 0.1% Indofil) dip + $HgCl_2$ (0.1%)	10+5	17.4		Nil	
	10+8	15.0		Nil	
	10+10	14.3		Nil	

Table 7. Phenol exudation and percentage of bud sprout in Vateria indica using different explant sources

	Culture of axillary and terminal buds from	
	Tender nodal tissue	Mature nodal tissue
Exudation of phenolics	+	++
Percentage of induced buds	21.7%	7.1%

\+ Negligible amount

++ Good amount

Table 8. The rate of fungal contamination varied greatly with respect to the season of collection of explants. The cultures expressed better survival during February to May and the infection was as high as 30 to 100 per cent during other months of the year. Fungal infection was identified as the main source of contamination during culture establishment. The causal organism was identified as Colletotrichum sp. based on its morphology. Extent of culture establishment also was influenced by season. It was maximum during months of February, March, April and May (74.5, 96.4 and 62.5 per cent, respectively).

4.1.4 Standardisation of basal medium for culture establishment

The data on the establishment of different explants in various basal media tried are presented in Table 9. Medium MS with half the concentration of inorganic salts was identified as the best basal medium for nodal explants, internodal segments and leaf segments with an establishment rate of 79.1 per cent, 31.9 per cent and 28.6 per cent, respectively.

4.1.5 Effect of plant growth regulators

In order to find out the effect of plant growth regulators on bud break and shoot elongation from axillary buds of V. indica, MS, ½MS as well as WPM media were

Table 8. Seasonal influence on contamination and culture establishment in axillary bud cultures of Vateria indica

Month	Contamination (%)		Survival of cultures (%)	Culture establishment (%)
	F.C.	B.C.		
January	30.3	3.7	66.0	41.0
February	18.2	Nil	81.8	74.5
March	Nil	Nil	100.0	96.4
April	1.8	Nil	98.2	94.6
May	23.2	Nil	76.8	62.5
June	100.0	Nil	Nil	Nil
July	96.9	Nil	3.1	Nil
August	97.7	Nil	2.3	Nil
September	98.2	Nil	1.8	Nil
October	96.4	Nil	3.6	Nil
November	28.2	3.9	67.9	50.0
December	50.0	3.6	46.4	25.0

F.C. - Fungal contamination

B.C. - Bacterial contamination

Table 9. Culture establishment from various explants of *Vateria indica* in different basal media

Media	Culture establishment after three weeks culture period (%)		
	Nodal segment	Internodal segment	Leaf segment
MS	54.8	19.6	19.0
½MS	79.1	31.9	28.6
WPM	31.8	8.3	12.5

supplemented with different levels of cytokinins like kinetin, BA and 2-ip independently and in combination with auxins, namely, IAA, IBA, NAA and 2,4-D. Effect of different levels of ABA as well as GA were also studied. The results obtained are presented below.

4.1.5.1 Effect of kinetin and auxins

Observations on the effect of different concentrations of kinetin and auxins supplemented to MS medium are presented in table 10.

Axillary buds cultured in MS medium containing 1.0, 2.0 and 3.0 ppm kinetin in absence of any auxin exhibited bud break in all the combinations. Maximum bud break of 28.6 per cent was obtained with 2.0 ppm kinetin. But none of the above levels produced leaves.

Kinetin in combination with IAA, could not induce bud break or shoot growth in any of the levels tried. However, maximum culture establishment of 21.4 per cent was recorded when kinetin and IAA were added to the basal medium at concentrations of 1.0 ppm each.

Along with 1.0 ppm kinetin in MS media, NAA at 0.1 ppm produced bud break in 21.4 per cent of the cultures and 7.1 per cent cultures produced leaves. Bud break was reduced to

Table 10. Effect of kinetin with auxin combinations on culture establishment and bud break in nodal segments of Vateria indica in MS media

Concentration of kinetin (ppm)	Auxin	Concentration (ppm)	Cultures established (%)	Established cultures sprouted (%) (Bud break)
1.0			85.7	14.3
2.0			85.7	28.6
3.0			85.7	14.3
1.0	IAA	0.1	14.3	Nil
		0.5	14.3	Nil
		1.0	21.4	Nil
2.0		0.1	14.3	Nil
		0.5	14.3	Nil
		1.0	Nil	Nil
3.0		0.1	Nil	Nil
		0.5	14.3	Nil
		1.0	14.3	Nil
1.0	NAA	0.1	35.7	21.4
		0.5	57.1	7.1
		1.0	50.0	7.1
2.0		0.1	57.1	7.1
		0.5	35.7	Nil
		1.0	21.4	7.1
3.0		0.1	28.7	21.7
		0.5	35.7	14.3
		1.0	35.7	Nil
1.0	2,4-D	0.1	92.9	7.1
		0.5	92.9	Nil
		1.0	100.0	Nil
2.0		0.1	100.0	7.1
		0.5	100.0	Nil
		1.0	100.0	Nil
3.0		0.1	92.9	Nil
		0.5	100.0	Nil
		1.0	100.0	Nil

7.1 per cent when NAA concentration was increased to either 0.5 ppm or 1.0 ppm maintaining the kinetin level at 1.0 ppm. Among these two levels, kinetin at 1.0 ppm and 0.5 ppm of NAA produced leaves in 7.0 per cent of the cultures. Maximum culture establishment of 57.1 per cent in kinetin and NAA combination was observed in 1.0 ppm kinetin with 0.5 ppm NAA and 2.0 ppm kinetin with 0.1 ppm NAA. Two ppm of kinetin in combination with 0.1 and 1.0 ppm NAA resulted in bud break in 7.1 per cent each. Whereas no response was obtained at 0.5 ppm NAA with the above level of kinetin. Along with 0.1 or 0.5 ppm NAA, kinetin at 3.0 ppm level produced bud break in 21.7 per cent and 14.3 per cent, respectively. But bud break was absent when NAA concentration was increased to 1.0 ppm maintaining kinetin level at 3.0 ppm.

Among the nine combinations of kinetin and 2,4-D tried in MS, bud break was observed only in the combinations involving kinetin at 1.0 ppm with 0.1 ppm 2,4-D and kinetin at 2.0 ppm with 0.1 ppm 2,4-D. These combinations resulted in 7.1 per cent of the cultures producing bud break and in all responded cultures, callus was produced at cut end of the explants. In remaining combinations eventhough good culture establishment was recorded bud break was not observed.

Kinetin in combination with IBA in half MS media resulted in bud sprout (Table 11). Bud break was observed in

Table 11. Effect of kinetin with auxin combinations on culture establishment and bud break in nodal segments of Vateria indica in half MS media

Concentration of kinetin (ppm)	Auxin	Concentration (ppm)	Cultures established (%)	Established cultures sprouted (%) (Bud break)
1.0	IBA	0.1	85.7	7.1
		0.5	50.0	14.3
		1.0	42.8	Nil
2.0		0.1	71.4	7.1
		0.5	78.4	14.3
		1.0	64.2	Nil
3.0		0.1	93.3	6.6
		0.5	57.1	Nil
		1.0	78.6	7.0

7.1 per cent of the cultures when kinetin and IBA were added to the basal medium at concentrations of 1.0 ppm and 0.1 ppm, respectively. Higher concentration of auxin (0.5 ppm) with same level of kinetin resulted in marginal increase in number of cultures showing bud sprout (14.3 per cent) (Fig.2a). However, IBA at 1.0 ppm at this level of kinetin (1.0 ppm) could not induce bud break. Both the combinations in which bud break was observed, the cultures showed callusing at cut end of the explants. Kinetin at 2.0 ppm with IBA 0.1 ppm and 0.5 ppm produced 7.1 per cent and 14.3 per cent bud break, respectively (Fig.2b). Higher concentration of IBA (1.0 ppm) at this level of kinetin could not induce bud break. Callusing at the proximal end of the explant was also noticed in the sprouted cultures. Auxin IBA at concentrations of 0.1 ppm and 1.0 ppm with 3.0 ppm of kinetin in half MS medium resulted in 6.6 per cent and 7.0 per cent bud break, respectively. Other combination of IBA at 0.5 ppm with 3.0 ppm kinetin failed to induce bud break.

Data obtained from the study of the influence of kinetin alone or in combination with auxins in WPM media are presented in Table 12.

Among the different levels of kinetin (1.0, 2.0, 3.0 ppm) tried independently, only kinetin at 1.0 ppm produced bud break (7.1 per cent). Of the different combinations of

Table 12. Effect of kinetin with auxin combinations on culture establishment and bud break in nodal segments of Vateria indica in WPM media

Concentration of kinetin (ppm)	Auxin	Concentration (ppm)	Cultures established (%)	Established cultures sprouted (%) (Bud break)
1.0			35.7	7.1
2.0			35.7	Nil
3.0			42.7	Nil
1.0	IAA	0.1	Nil	Nil
		0.5	Nil	Nil
		1.0	7.1	Nil
2.0		0.1	7.1	7.1
		0.5	7.1	Nil
		1.0	Nil	Nil
3.0		0.1	Nil	Nil
		0.5	7.1	Nil
		1.0	Nil	Nil
1.0	IBA	0.1	7.0	Nil
		0.5	7.0	Nil
		1.0	Nil	Nil
2.0		0.1,0.5,1.0	Nil	Nil
3.0		0.1	Nil	Nil
		0.5	7.0	Nil
		1.0	Nil	Nil
1.0	NAA	0.1	Nil	Nil
		0.5	42.9	21.5
		1.0	7.1	Nil

Contd.

Table 12 (Contd.)

Concentration of kinetin (ppm)	Auxin	Concentration (ppm)	Cultures established (%)	Established cultures sprouted (%) (Bud break)
2.0		0.1	28.6	21.0
		0.5	7.1	Nil
		1.0	14.3	Nil
3.0		0.1	Nil	Nil
		0.5	7.0	7.0
		1.0	21.4	14.3
1.0	2,4-D	0.1	42.9	Nil
		0.5	50.0	Nil
		1.0	50.0	Nil
2.0		0.1	57.1	Nil
		0.5	64.3	Nil
		1.0	50.0	Nil
3.0		0.1	57.1	7.0
		0.5	57.1	Nil
		1.0	42.9	Nil

kinetin at 1.0 ppm with three levels of IAA tried, only IAA at 1.0 ppm produced bud break (7.1 per cent). Kinetin at 2.0 ppm along with IAA at 0.1 ppm also resulted in 7.1 per cent bud break. However, higher dose of kinetin (3.0 ppm) in combination with IAA at 0.1, 0.5 and 1.0 ppm levels, could not induce bud break. Kinetin at 1.0 ppm with 0.1 or 0.5 ppm IBA could induce culture establishment in 7.0 per cent of the cultures in both combinations. Kinetin at 2.0 ppm in combination with any of the three levels of IBA tried failed to induce bud sprout. With higher dose of kinetin at 3.0 ppm supplemented with 0.5 ppm IBA 7.0 per cent of cultures exhibited establishment. Kinetin at 1.0 ppm when supplemented with three levels of NAA (0.1 0.5, 1.0 ppm) only NAA at 0.5 ppm could induce bud break. Combination of NAA at 0.1 ppm with 2.0 ppm kinetin produced bud sprout in 21.0 per cent of the cultures. Higher concentration of NAA (0.5 or 1.0 ppm) could not produce sprout. Kinetin at 3.0 ppm with 0.1 ppm NAA could not have any effect on bud break, however, NAA at 0.5 or 1.0 ppm with same level of kinetin induced bud break in 7.0 per cent and 14.3 per cent of the cultures, respectively.

Among the nine combinations of kinetin and 2,4-D tried in WPM only the combination involving kinetin at 3.0 ppm with 0.1 ppm 2,4-D, produced bud sprout (7.0 per cent).

4.1.5.2 Effect of BA and auxins

The cytokinin BA alone at the concentration of 1.0 ppm and 2.0 ppm induced bud break in MS in 7.1 per cent and 14.3 per cent of cultures, respectively (Table 13). However, higher concentration of BA (3.0 ppm) failed to induce bud sprout.

Among the nine combinations of BA and IAA tried, only BA at 1.0 ppm with 0.1 ppm IAA and 3.0 ppm BA with 1.0 ppm IAA induced bud break. The remaining combinations were ineffective in inducing bud sprout.

In all levels of BA and NAA tried good culture establishment was observed. Auxin NAA at a concentration of 0.1 ppm or 0.5 ppm with 1.0 ppm kinetin resulted in 14.3 per cent and 14.2 per cent of the cultures exhibiting bud sprout, but NAA at 1.0 ppm with same level of BA could not induce bud sprout. BA at 2.0 ppm with NAA at 0.1, 0.5 and 1.0 ppm produced sprouts in 21.4, 14.3 and 7.1 per cent, respectively. Higher concentration of BA (3.0 ppm) with NAA 0.1 ppm failed to produce bud release. The same level of BA supplemented with NAA at the concentrations of 0.5 or 1.0 ppm could induce sprouts in 7.1 per cent of the cultures in both combinations.

Auxin 2,4-D was not found effective for bud release, however, good culture establishment was achieved. None of the

Table 13. Effect of BA with auxin combinations on culture establishment and bud break in nodal segments of *Vateria indica* in MS media

Concentration of BA (ppm)	Auxin	Concentration (ppm)	Cultures established (%)	Established cultures sprouted (%) (Bud break)
1.0			21.4	7.1
2.0			28.6	14.3
3.0			21.4	Nil
1.0	IAA	0.1	21.4	7.1
		0.5	14.2	Nil
		1.0	21.4	Nil
2.0		0.1	28.6	Nil
		0.5	28.6	Nil
		1.0	42.9	Nil
3.0		0.1	14.2	Nil
		0.5	14.2	Nil
		1.0	35.7	7.1
1.0	NAA	0.1	64.3	14.3
		0.5	35.7	14.2
		1.0	21.4	Nil
2.0		0.1	64.3	21.4
		0.5	28.6	14.3
		1.0	64.2	7.1
3.0		0.1	42.9	Nil
		0.5	57.1	7.1
		1.0	50.0	7.1
1.0	2,4-D	0.1	42.9	Nil
		0.5	35.7	Nil
		1.0	64.3	Nil
2.0		0.1	50.0	Nil
		0.5	42.8	Nil
		1.0	42.8	Nil
3.0		0.1	57.1	Nil
		0.5	57.1	Nil
		1.0	64.2	Nil

nine treatments of 2,4-D with BA in MS medium induced bud sprout.

All the combinations of BA and IBA tried in half MS medium were not effective except BA at 1.0 ppm with 1.0 ppm of IBA. This combination of growth regulators also induced callus at the cut ends of the explants (Table 14). With all the remaining combinations good culture establishment ranging from 71 to 91 per cent was observed. However, they failed to produce sprout.

Data on the effect of BA in combination with different levels of auxin in WPM media on culture establishment and bud sprout are presented in Table 15. Different levels of BA alone in WPM were not effective for bud release, however, culture establishment was achieved to the maximum of 28.6 per cent of cultures. All combinations of auxin IAA with BA could not produce sprout and culture establishment was found to be very less. None of the combinations of BA with IBA in WPM induced bud sprout except BA at 2.0 ppm with IBA 1.0 ppm which induced bud release in 7.1 per cent of the cultures.

None of the combinations of auxin 2,4-D with BA could produce bud break, eventhough good culture establishment was noticed.

Table 14. Effect of BA with auxin combinations on culture establishment and bud break in nodal segments of Vateria indica in half MS media

Concentration of BA (ppm)	Auxin	Concentration (ppm)	Cultures established (%)	Established cultures sprouted (%) (Bud break)
1.0	IBA	0.1	78.6	Nil
		0.5	92.9	Nil
		1.0	78.5	7.1
2.0		0.1	71.4	Nil
		0.5	92.8	Nil
		1.0	100.0	Nil
3.0		0.1	86.6	Nil
		0.5	71.4	Nil
		1.0	78.6	Nil

Table 15. Effect of BA with auxin combinations on culture establishment and bud break in nodal segments of Vateria indica in WPM media

Concentration of BA (ppm)	Auxin	Concentration (ppm)	Cultures established (%)	Established cultures sprouted (%) (Bud break)
1.0			28.6	Nil
2.0			14.3	Nil
3.0			14.3	Nil
1.0	IAA	0.1	7.1	Nil
		0.5	21.4	Nil
		1.0	Nil	Nil
2.0		0.1,0.5,1.0	Nil	Nil
3.0		0.1,0.5,1.0	Nil	Nil
1.0	IBA	0.1,0.5,1.0	Nil	Nil
2.0		0.1	Nil	Nil
		0.5	Nil	Nil
		1.0	7.1	7.1
3.0		0.1	Nil	Nil
		0.5	21.4	Nil
		1.0	42.9	Nil
1.0	2,4-D	0.1	57.1	Nil
		0.5	35.7	Nil
		1.0	50.0	Nil
2.0		0.1	57.1	Nil
		0.5	57.1	Nil
		1.0	42.8	Nil
3.0		0.1	28.6	Nil
		0.5	35.7	Nil
		1.0	57.1	Nil

4.1.5.3 Effect of 2-ip and auxins

Data on the effect of 2-ip in combinations with different levels of auxin in medium MS on bud break are presented in Table 16.

Addition of the cytokinin 2-ip in MS at a concentration of 1.0, 2.0 and 3.0 ppm resulted in 21.4, 7.1 and 14.3 per cent of the culture producing sprouts, respectively.

The auxin IAA at a concentration of 0.1 ppm or 0.5 ppm with 2-ip at 1.0 ppm in MS media resulted in bud sprout in 14.3 per cent of the cultures in both combinations. Higher concentration of IAA (1.0 ppm) along with the above concentration of 2-ip could not induce bud release. Addition of 2-ip at a concentration of 2.0 ppm along with IAA (0.1, 0.5, 1.0 ppm) in MS, were not effective in producing bud sprouts, even with better culture establishment. Higher concentration of 2-ip (3.0 ppm) along with 0.1 ppm IAA could induce 78.6 per cent culture establishment without bud release. Increase in IAA (0.5 or 1.0 ppm) with same concentration of 2-ip induced bud break in 7.1 per cent and 13.3 per cent of cultures, respectively.

A combination of NAA (0.1 ppm) along with 2-ip at 1.0 ppm induced bud break in 14.3 per cent of the cultures. Cut

Table 16. Effect of 2-ip with auxin combinations on culture establishment and bud break in nodal segments of *Vateria indica* in MS media

Concentration of 2-ip (ppm)	Auxin	Concentration (ppm)	Cultures established (%)	Established cultures sprouted (%) (Bud break)
1.0			35.7	21.4
2.0			57.1	7.1
3.0			42.9	14.3
1.0	IAA	0.1	71.4	14.3
		0.5	28.6	14.3
		1.0	35.7	Nil
2.0		0.1	78.6	Nil
		0.5	71.4	Nil
		1.0	78.6	Nil
3.0		0.1	78.6	Nil
		0.5	64.3	7.1
		1.0	73.3	13.3
1.0	NAA	0.1	50.0	14.3
		0.5	64.3	21.4
		1.0	50.0	7.0
2.0		0.1	57.1	14.3
		0.5	64.3	Nil
		1.0	Nil	Nil
3.0		0.1	14.3	7.0
		0.5	57.1	Nil
		1.0	Nil	Nil
1.0	2,4-D	0.1	100.0	Nil
		0.5	100.0	Nil
		1.0	92.8	Nil
2.0		0.1	100.0	Nil
		0.5	100.0	Nil
		1.0	100.0	Nil
3.0		0.1	92.8	Nil
		0.5	100.0	Nil
		1.0	78.6	Nil

end of the explants produced callus in 7.0 per cent of the cultures. The auxin NAA at 0.5 ppm along with the above concentration of 2-ip had effect on bud break in 21.4 per cent of the cultures. Among the established cultures, 7.1 per cent of cultures produced proximal end callus. Further increase in NAA concentration to 1.0 ppm produced bud break in 7.0 per cent of the cultures. The cytokinin 2-ip at 3.0 ppm with 0.1 ppm NAA resulted in 7.0 per cent of cultures producing sprouts. Higher concentration of NAA (0.5 or 1.0 ppm) failed to induce bud break.

None of the combinations of 2-ip with 2,4-D in MS induced bud break, but maximum culture establishment of 78.6 to 100 per cent was noticed in these combinations.

The cytokinin 2-ip at 1.0 ppm in combination with any of the three levels of IBA tried in half MS media failed to induce bud break, but had culture establishment of 64.3 to 85.7 per cent (Table 17). Combination of 2-ip with 0.1 ppm of IBA in half MS produced bud break in 7.0 per cent of cultures with 85.7 per cent culture establishment. Leaf production could be obtained in 7.0 per cent of the cultures. Higher concentration of IBA (0.5 ppm) along with same level of 2-ip was not effective in producing bud sprout, however, culture establishment was observed in 92.9 per cent of the cultures.

Table 17. Effect of 2-ip with auxin combinations on culture establishment and bud break in nodal segments of Vateria indica in half MS media

Concentration of 2-ip (ppm)	Auxin	Concentration (ppm)	Cultures established (%)	Established cultures sprouted (%) (Bud break)
1.0	IBA	0.1	64.3	Nil
		0.5	78.5	Nil
		1.0	85.7	Nil
2.0		0.1	85.7	7.0
		0.5	92.9	Nil
		1.0	92.9	7.1
3.0		0.1	85.7	Nil
		0.5	85.7	Nil
		1.0	92.8	Nil

The auxin IBA at 1.0 ppm with above level of 2-ip induced 7.1 per cent bud sprout in cultures. Higher concentration of 2-ip (3.0 ppm) in combination with any of the three levels of IBA tried failed to induce bud break, even with better culture establishment.

Data obtained from the study of the influence of 2-ip alone or in combination with auxins in WPM are presented in Table 18. Among all levels of 2-ip (1.0, 2.0, 3.0 ppm) tried in this media, only 2-ip at 1.0 ppm could produce bud sprout (7.1 per cent).

None of the combinations of 2-ip and IAA tried in WPM produced bud break. Even culture establishment was also very less.

All the nine combinations of 2-ip and IBA tried in WPM exhibited culture establishment, but none of the combinations induced bud release.

None of the combinations of 2-ip with NAA tried in WPM induced bud release.

Among all levels of 2-ip and 2,4-D tried, only the combination of 2-ip at 2.0 ppm with 0.5 ppm 2,4-D induced bud break (7.0 per cent). Remaining treatments could not produce any response.

Table 18. Effect of 2-ip with auxin combinations on culture establishment and bud break in nodal segments of *Vateria indica* in WPM media

Concentration of 2-ip (ppm)	Auxin	Concentration (ppm)	Cultures established (%)	Established cultures sprouted (%) (Bud break)
1.0			21.4	7.1
2.0			7.1	Nil
3.0			7.1	Nil
1.0	IAA	0.1,0.5,1.0	Nil	Nil
2.0		0.1	Nil	Nil
		0.5	7.1	Nil
		1.0	Nil	Nil
3.0		0.1	7.1	Nil
		0.5	Nil	Nil
		1.0	Nil	Nil
1.0	IBA	0.1	14.3	Nil
		0.5	57.1	Nil
		1.0	42.9	Nil
2.0		0.1	50.0	Nil
		0.5	21.4	Nil
		1.0	21.4	Nil
3.0		0.1	42.8	Nil
		0.5	50.0	Nil
		1.0	28.6	Nil
1.0	NAA	0.1	57.1	Nil
		0.5	14.3	Nil
		1.0	Nil	Nil
2.0		0.1,0.5,1.0	Nil	Nil
3.0		0.1,0.5,1.0	Nil	Nil

Contd.

Table 18 (Contd.)

Concentration of 2-ip (ppm)	Auxin	Concentration (ppm)	Cultures established (%)	Established cultures sprouted (%) (Bud break)
1.0	2,4-D	0.1	35.7	Nil
		0.5	57.1	Nil
		1.0	64.3	Nil
2.0		0.1	35.7	Nil
		0.5	71.4	7.0
		1.0	35.7	Nil
3.0		0.1	21.4	Nil
		0.5	21.4	Nil
		1.0	28.6	Nil

4.1.6 Effect of other media supplements

Data on the trials conducted to evaluate the effect of media supplements other than plant growth regulators in half MS and WPM are given in Table 19 and 20, respectively.

4.1.6.1 Casein hydrolysate

Effect of casein hydrolysate in culture establishment and bud induction was studied in both half MS and WPM media (Table 19 and 20). None of the combinations in both media induced bud release. Culture establishment also was noticed to be very poor.

4.1.6.2 cycocel

Cycocel (2 Chloro ethyl trimethyl ammonium chloride) was tried at six different levels in half MS supplemented with 2.0 ppm 2-ip and 0.1 ppm IBA. Data obtained is presented in Table 19. At the lowest level of the chemical tried (0.1 ppm) 14.2 per cent of the cultures produced sprout. A concentration of 0.75 ppm of CCC in the same basal formulation increased the number of cultures producing bud sprouts to 21.4 per cent but further increase of CCC (2.0, 3.0 ppm) resulted in 7.1 per cent of the cultures producing bud break in both combinations. In above all combinations culture establishment was poor.

Table 19. Effect of media supplements on the culture establishment from primary nodal segments of Vateria indica in half MS media containing 2.0 ppm 2-ip and 0.1 ppm IBA

Treatments	Concentration	Cultures established (%)	Established cultures sprouted (%) (Bud break)
Casein hydrolysate	200 ppm	Nil	Nil
	300 ppm	Nil	Nil
	500 ppm	6.6	Nil
Cycocel	0.10 ppm	21.4	14.2
	0.50 ppm	21.4	7.1
	0.75 ppm	35.7	21.4
	1.00 ppm	28.6	Nil
	2.00 ppm	28.6	7.1
	3.00 ppm	50.0	7.1
Adenine sulphate	10 ppm	78.6	Nil
	20 ppm	42.9	Nil
	30 ppm	57.1	Nil
	40 ppm	78.5	Nil
	50 ppm	78.5	7.0
Silver nitrate	5 ppm	14.3	Nil
	10 ppm	28.6	7.0
	15 ppm	14.3	Nil
	20 ppm	21.4	7.0
Cobalt chloride	1 ppm	85.7	7.0
	2 ppm	71.4	Nil
	3 ppm	71.4	Nil
Coconut water	10% (v/v)	21.4	7.0
	20% (v/v)	14.3	7.0
	30% (v/v)	21.4	Nil
Gibberellic acid	1 ppm	7.1	7.1
	2,3 ppm	Nil	Nil

All levels of cycocel added in WPM supplemented with 2.0 ppm 2-ip and 0.1 ppm IBA exhibited culture establishment (Table 20). Cycocel at 0.1 ppm and 1.0 ppm incorporated to WPM resulted in sprout in 14.2 per cent and 7.1 per cent of the cultures, respectively.

4.1.6.3 Adenine sulphate

All levels of adenine sulphate (10, 20, 30, 40 and 50 ppm) added in half MS supplemented with 2.0 ppm 2-ip and 0.1 ppm IBA resulted in average culture establishment (Table 19). However, bud break was observed only at 50 ppm of adenine sulphate. This combination resulted in 7.0 per cent of cultures producing sprout.

4.1.6.4 Silver nitrate

The effect of silver nitrate as a media supplement was studied in half MS containing 2.0 ppm 2-ip and 0.1 ppm IBA. Data on observations are presented in Table 19. Silver nitrate at 10 ppm or 20 ppm added to this media resulted in 7.0 per cent of cultures producing bud sprout in both combinations. Silver nitrate at 5 ppm or 15 ppm could not induce bud break. In all combinations culture establishment was very poor.

Table 20. Effect of media supplements on axillary bud culture of Vateria indica in WPM containing 2.0 ppm 2-ip and 0.1 ppm IBA

Treatments	Concentration	Cultures established (%)	Established cultures sprouted (%) (Bud break)
Casein hydrolysate	200 ppm	7.1	Nil
	300 ppm	Nil	Nil
	500 ppm	7.1	Nil
Cycocel	0.10 ppm	21.4	14.2
	0.50 ppm	14.3	Nil
	0.75 ppm	14.3	Nil
	1.00 ppm	7.1	7.1
	2.00 ppm	14.3	Nil
	3.00 ppm	7.1	Nil
Silver nitrate	5 ppm	7.1	Nil
	10 ppm	7.1	Nil
	15 ppm	14.3	7.1
	20 ppm	50.0	40.0
Coconut water	10% (v/v)	14.3	3.6
	20% (v/v)	21.4	7.0
	30% (v/v)	7.1	7.1

Silver nitrate incorporated at levels of 15 ppm and 20 ppm in WPM media (Table 20) could induce bud break in 7.1 per cent and 40 per cent of the cultures, respectively. Poor culture establishment without further response was observed when silver nitrate was added at 5 ppm or 10 ppm to WPM media.

4.1.6.5 Cobalt chloride

Influence of cobalt chloride on bud release of white pine was studied in half MS supplemented with 2.0 ppm 2-ip and 0.1 ppm IBA (Table 19). With all three levels of cobalt chloride (1.0, 2.0, 3.0 ppm) good culture establishment was noticed, however, bud break (7.0 per cent) was induced at 1.0 ppm cobalt chloride only(Fig.2a).

4.1.6.6 Coconut water

Effect of coconut water on bud break was studied in half MS supplemented with 2.0 ppm 2-ip and 0.1 ppm IBA. Data generated are presented in Table 19. When 10% (v/v) or 20% (v/v) of coconut water was incorporated in the medium, 7.0 per cent of the cultures produced bud break. Coconut water at 30% (v/v) could not induce bud sprout. Culture establishment was poor in all the level of coconut water.

Effect of adding coconut water in WPM supplemented with 2.0 ppm 2-ip and 0.1 ppm IBA is shown in Table 20. In

three levels of coconut water (10% (v/v), 20% (v/v), 30% (v/v)) culture establishment was poor, however, bud sprout in 3.6 per cent, 7.0 per cent and 7.1 per cent of the cultures could be obtained, respectively.

4.1.6.7 Gibberellic acid

The influence of GA_3 for enhanced release of axillary buds in V. indica was studied in half MS medium supplemented with 2.0 ppm 2-ip and 0.1 ppm IBA. When 1.0 ppm of GA_3 was incorporated in the basal formulation only 7.1 per cent of the cultures exhibited culture establishment and bud sprout (Table 19).

4.2 Shoot growth and leaf morphogenesis

Percentage of bud break in the various media composition tried varied from 7.00 to 40.00 per cent. However, in most of these cultures further growth of the sprouted buds were not noticed. Only in four media combinations shoot elongation and leaf morphogenesis was achieved. The data is presented in Table 21. Medium MS supplemented with 1.0 ppm kinetin and 0.1 ppm NAA or MS supplemented with 1.0 ppm 2-ip registered the maximum bud break. In both these combinations an average of one shoot measuring about 0.5 cm was produced. The number of leaves per shoot was only one.

Table 21. Growth performance of axillary bud cultures in various media identified for bud break in *Vateria indica*

Treatments	Days taken for bud break	Bud break (%)	No. of shoots produced	Mean shoot length after 1 month culture period (cm)	No. of leaves produced	No. of leaves retained after 2 month culture period	% of cultures with leaf expansion	Culture showing callusing tendency (%)
MS + 1.0 ppm Kinetin + 0.1 ppm NAA	24	21.4	1.0	0.6	1.0	Nil	Nil	7.0
MS + 1.0 ppm Kinetin + 0.5 ppm NAA	25	7.1	1.0	0.8	1.0	Nil	Nil	Nil
MS + 1.0 ppm 2-ip	23	21.4	1.0	0.5	1.0	Nil	Nil	7.0
½MS + 2.0 ppm 2-ip + 0.1 ppm IBA	18	7.1	1.0	1.0	2.0	2.0	7.1	Nil

Axillary buds cultured in half MS supplemented with 2-ip 2.0 ppm and IBA 0.1 ppm exhibited better growth response. The average shoot length was 1.0 cm with two expanded leaves, two months after culture (Fig.3b). However, the rate of bud break was lower than that in the medium with 2-ip alone or with kinetin 1.0 ppm along with NAA 1.0 ppm. The number of days taken for bud break also was minimum (18) with the above media composition. Except this combination, none of the cultures retained leaves after two months culture period. The growth was arrested after subculturing the shoots in the same medium. Sustained growth of the shoot was not achieved in any of the combinations identified for this purpose.

4.3 Indirect organogenesis/embryogenesis

4.3.1 Callus induction and proliferation

4.3.1.1 Effect of plant growth regulators

The effect of various growth regulator combinations for inducing calli in leaf explant is presented below.

Among the various growth regulator combinations tried in MS basal media for leaf explants (Table 22) kinetin at levels of 1.0, 2.0 and 3.0 ppm in combination with 2,4-D at 0.1, 0.5 and 1.0 ppm showed the response for callusing in 4.1 to 35.7 per cent of the cultures. The callus induced in these

Table 22. Effect of different growth regulator combinations on callus induction and later proliferation in leaf explants of Vateria indica in MS media

Treatments combinations (ppm)	Days taken for callus induction (mean)	% callusing	Callus index	Callus morphology
Kin 1.0 + NAA 0.1	29	14.1	14.1	Green compact
Kin 1.0 + NAA 0.5	31	7.1	7.1	"
Kin 1.0 + 2,4-D 0.1	28	14.3	28.6	Yellow compact
Kin 1.0 + 2,4-D 0.5	27	14.3	24.3	"
Kin 1.0 + 2,4-D 1.0	24	7.1	9.23	"
Kin 2.0 + 2,4-D 0.1	29	21.4	49.2	"
Kin 2.0 + 2,4-D 0.5	25	28.6	45.7	"
Kin 2.0 + 2,4-D 1.0	30	35.7	82.1	"
Kin 3.0 + 2,4-D 0.1	24	7.1	7.1	"
Kin 3.0 + 2,4-D 0.5	26	35.7	58.1	"
Kin 3.0 + 2,4-D 1.0	35	4.1	4.1	"
BA 1.0 + 2,4-D 0.1	25	14.3	28.6	Dark brown compact
BA 2.0 + 2,4-D 0.5	27	21.4	44.9	"
BA 2.0 + 2,4-D 1.0	30	14.3	25.7	"
BA 3.0 + 2,4-D 1.0	24	7.1	14.2	"
2-ip 1.0 + 2,4-D 0.1	28	35.7	85.7	Pale yellow friable
2-ip 1.0 + 2,4-D 0.5	28	21.4	42.8	"
2-ip 1.0 + 2,4-D 1.0	26	42.9	85.8	"
2-ip 2.0 + 2,4-D 0.1	29	21.4	42.8	"
2-ip 2.0 + 2,4-D 0.5	26	28.6	47.1	"
2-ip 2.0 + 2,4-D 1.0	27	21.4	27.8	"
2-ip 3.0 + 2,4-D 0.1	28	50.0	80.0	"
2-ip 3.0 + 2,4-D 0.5	24	13.0	26.0	"
2-ip 3.0 + 2,4-D 1.0	25	50.0	100.0	"
2-ip 1.0 + NAA 0.1	28	7.1	7.1	Green compact
2-ip 1.0 + NAA 0.5	25	7.1	7.1	"
2-ip 2.0 + NAA 1.0	29	14.3	14.3	"
2-ip 3.0 + NAA 0.5	29	7.1	7.1	"
2-ip 1.0 + IAA 0.1	32	14.3	28.6	Pale green compact
2-ip 3.0 + IAA 0.5	30	14.3	28.6	"
2-ip 3.0 + IAA 1.0	33	14.3	7.1	"

combinations was yellow and compact, whereas NAA at 0.1 or 0.5 ppm with kinetin at 1.0 ppm induced green, compact callus in 14.1 per cent and 7.1 per cent of the cultures. Supplementing IBA at 0.1, 0.5 and 1.0 ppm along with kinetin (1.0, 2.0 and 3.0 ppm) in half MS media (Table 23) showed callus induction in 7.1 to 42.9 per cent of the cultures. The calli were pale yellow and friable.

At three levels of BA (1.0, 2.0, 3.0 ppm) in combination with 2,4-D at 0.1, 0.5 and 1.0 ppm in MS medium (Table 22) dark brown and compact calli were induced in 7.1 to 21.4 per cent of the cultures. The above same levels of BA supplemented with IBA at 0.1, 0.5 and 1.0 ppm in half MS medium (Table 23) could induce pale yellow and compact callus, at low level of BA (1.0 ppm) in 7.1 to 42.9 per cent of the cultures. At high levels of BA (2.0, 3.0 ppm) the callus morphology was pale yellow and friable.

Combinations of 2-ip at 1.0, 2.0 and 3.0 ppm along with 2,4-D at 0.1, 0.5 and 1.0 ppm in MS medium could induce pale yellow and friable callus in 8.3 to 50.0 per cent of the cultures. NAA at 0.1 ppm or 0.5 ppm along with same level of 2-ip in MS medium induced callusing in 7.1 to 14.3 per cent of the cultures, showing green compact appearance of the callus. The auxin IAA at 0.1, 0.5 and 1.0 ppm with 2-ip at 1.0 and

Table 23. Effect of different growth regulator combinations on callus induction and later proliferation in leaf explants of Vateria indica in half MS media

Treatments combinations (ppm)	Days taken for callus induction (mean)	% callusing	Callus index	Callus morphology
Kin 1.0 + IBA 0.1	31	28.6	60.0	Pale yellow friable
Kin 1.0 + IBA 0.5	35	7.1	7.1	"
Kin 1.0 + IBA 1.0	32	14.3	18.6	"
Kin 2.0 + IBA 0.1	36	14.3	28.6	"
Kin 2.0 + IBA 0.5	30	21.4	51.1	"
Kin 2.0 + IBA 1.0	34	14.3	28.6	"
Kin 3.0 + IBA 0.1	31	21.4	42.8	"
Kin 3.0 + IBA 0.5	33	42.9	68.6	"
Kin 3.0 + IBA 1.0	33	14.3	28.6	"
BA 1.0 + IBA 0.1	29	42.9	77.2	"
BA 1.0 + IBA 0.5	28	28.6	57.2	Pale yellow compact
BA 1.0 + IBA 1.0	28	7.1	7.1	"
BA 2.0 + IBA 0.1	29	28.6	37.2	"
BA 2.0 + IBA 0.5	24	35.7	35.7	Pale yellow friable
BA 2.0 + IBA 1.0	26	7.1	7.1	"
BA 3.0 + IBA 0.1	27	14.3	28.3	"
BA 3.0 + IBA 0.5	29	7.1	7.1	"
BA 3.0 + IBA 1.0	25	42.9	58.9	"
2-ip 1.0 + IBA 0.1	28	35.7	71.4	"
2-ip 0.5 + IBA 0.5	30	35.7	35.7	"
2-ip 1.0 + IBA 1.0	30	50.0	50.0	"
2-ip 2.0 + IBA 0.1	29	64.3	64.3	"
2-ip 2.0 + IBA 0.5	31	35.7	71.4	"
2-ip 2.0 + IBA 1.0	31	50.0	100.0	"
2-ip 3.0 + IBA 0.1	29	61.5	61.5	"
2-ip 3.0 + IBA 0.5	36	4.1	4.1	"
2-ip 3.0 + IBA 1.0	29	42.9	85.8	"

3.0 ppm induced callusing in 14.3 per cent of cultures showing pale green and compact morphology (Table 22).

All combinations of auxin IBA with BA at 1.0, 2.0 and 3.0 ppm levels in half MS media (Table 23) induced callusing in 7.1 to 42.9 per cent of the cultures with pale yellow compact habit.

The maximum (100.0) callus index for leaf explant was recorded for the combinations of 2-ip at 3.0 ppm along with auxin 2,4-D at 1.0 ppm in MS media, whereas low callus index (4.1) was recorded with combinations of kinetin at 3.0 ppm with 1.0 ppm of 2,4-D in MS media and 3.0 ppm 2-ip with auxin IBA at 0.5 ppm in half MS medium.

Kinetin at concentration of 1.0 ppm or 3.0 ppm with 2,4-D at 0.1 or 0.5 ppm in WPM media (Table 24) resulted in callus induction in 7.0 to 14.3 per cent of the leaf cultures with yellow and compact callus. Addition of BA at 1.0 ppm along with 2,4-D at 0.1, 0.5 and 1.0 ppm in WPM media could obtain callusing in 7.0 to 14.3 per cent of cultures with dark brown and compact callus. In all combinations of 2-ip and 2,4-D tried in WPM media (Table 24) callusing was obtained in 7.1 to 21.4 per cent of the cultures with pale yellow and friable callus. Auxin IBA at 1.0 ppm with 2-ip at 1.0 ppm in WPM was not found effective in callus induction.

Table 24. Effect of different growth regulator combinations on callus induction and later proliferation in leaf explants of Vateria indica in WPM media

Treatments combinations (ppm)	Days taken for callus induction (mean)	% callusing	Callus index	Callus morphology
Kin 1.0 + 2,4-D 0.1	32	14.3	28.6	Yellow compact
Kin 3.0 + 2,4-D 0.1	30	14.3	28.6	"
Kin 3.0 + 2,4-D 0.5	31	7.1	14.2	"
BA 1.0 + 2,4-D 0.1	34	7.1	14.2	Dark brown compact
BA 1.0 + 2,4-D 0.5	33	14.3	28.6	"
BA 1.0 + 2,4-D 1.0	35	7.1	7.1	"
2-ip 1.0 + 2,4-D 0.1	24	21.4	63.9	Pale yellow friable
2-ip 2.0 + 2,4-D 0.1	26	21.4	42.8	"
2-ip 2.0 + 2,4-D 0.5	28	14.3	28.6	"
2-ip 3.0 + 2,4-D 0.1	25	7.1	14.2	"
2-ip 3.0 + 2,4-D 0.5	27	14.3	28.6	"
2-ip 1.0 + IBA 1.0	34	7.1	7.1	"

The maximum callus index for leaf explants (63.9) was observed in the combination of 2-ip at 1.0 ppm with 2,4-D at 0.1 ppm in WPM media and low callus index (7.1) was obtained with BA with 1.0 ppm along with 2,4-D at 1.0 ppm in WPM and 2-ip at 1.0 ppm with IBA at 1.0 ppm in WPM medium.

The response of internodal segments to various growth regulator combinations tried for callusing is presented below.

Among the various growth regulator combinations tried in MS basal media (Table 25) for internodal explants, kinetin at the levels of 1.0, 2.0 and 3.0 ppm in combination with 2,4-D at 0.1, 0.5 and 1.0 ppm showed callusing in 7.1 to 35.7 per cent of cultures. The morphology of induced callus was yellow and compact. Whereas NAA at 0.1 or 0.5 ppm with kinetin at 1.0 ppm induced callus in 7.0 per cent of the cultures, showing pale green compact appearance, IBA at 0.1, 0.5 and 1.0 ppm along with the above levels of kinetin in half MS media tried, could induce callusing in 7.1 to 42.9 per cent of the cultures showing pale yellow and friable character (Table 26).

Three levels of BA (1.0, 2.0, 3.0 ppm) in combination with 2,4-D at 0.1, 0.5 and 1.0 ppm in MS medium (Table 25) induced dark brown and compact callus in 7.1 to 21.4 per cent of cultures. The auxin IBA at concentrations of 0.1, 0.5 and

Table 25. Effect of different growth regulator combinations on callus induction and later proliferation in internodal explants of Vateria indica in MS media

Treatments combinations (ppm)	Days taken for callus induction (mean)	% callusing	Callus index	Callus morphology
Kin 1.0 + NAA 0.1	24	7.1	7.1	Pale green compact
Kin 1.0 + NAA 0.5	10	7.1	7.1	"
Kin 1.0 + 2,4-D 0.1	35	7.1	14.2	Yellow compact
Kin 1.0 + 2,4-D 0.5	33	28.6	78.6	"
Kin 1.0 + 2,4-D 1.0	35	7.1	14.2	"
Kin 2.0 + 2,4-D 0.1	17	14.3	28.6	"
Kin 2.0 + 2,4-D 0.5	18	14.3	21.5	"
Kin 2.0 + 2,4-D 1.0	18	21.4	35.5	"
Kin 3.0 + 2,4-D 0.1	17	35.7	49.9	"
Kin 3.0 + 2,4-D 0.5	18	35.7	78.6	"
Kin 3.0 + 2,4-D 1.0	21	21.4	70.6	Pale yellow friable
BA 1.0 + 2,4-D 0.1	41	7.1	7.1	Dark brown compact
BA 2.0 + 2,4-D 0.1	43	7.1	14.2	
BA 2.0 + 2,4-D 0.5	41	7.1	14.2	"
BA 3.0 + 2,4-D 1.0	46	21.4	42.8	"
2-ip 1.0 + NAA 0.1	23	14.3	14.3	Green compact
2-ip 1.0 + NAA 0.5	19	7.1	7.1	"
2-ip 2.0 + NAA 1.0	11	7.1	7.1	"
2-ip 3.0 + NAA 0.5	11	7.1	7.1	"
2-ip 1.0 + IAA 0.1	30	7.1	7.1	Pale green compact
2-ip 3.0 + IAA 0.5	32	7.1	7.1	"
2-ip 3.0 + IAA 1.0	33	14.3	14.3	"
2-ip 1.0 + 2,4-D 0.1	25	42.9	85.8	Pale yellow friable
2-ip 1.0 + 2,4-D 0.5	28	35.7	71.4	"
2-ip 1.0 + 2,4-D 1.0	35	21.4	35.5	"
2-ip 2.0 + 2,4-D 0.1	31	21.4	49.2	"
2-ip 2.0 + 2,4-D 0.5	37	50.0	110.0	"
2-ip 2.0 + 2,4-D 1.0	39	28.6	42.9	"
2-ip 3.0 + 2,4-D 0.1	23	50.0	120.0	"
2-ip 3.0 + 2,4-D 0.5	26	35.7	71.4	"
2-ip 3.0 + 2,4-D 1.0	25	35.7	71.4	"

1.0 ppm with the same levels of BA in half MS medium could induce callusing in 7.0 to 42.9 per cent of cultures showing pale yellow and compact morphology (Table 26).

Combinations of 2-ip at 1.0, 2.0 and 3.0 ppm along with 2,4-D at 0.1m 0.5 and 1.0 ppm in MS medium resulted in callusing in 21.4 to 50.0 per cent of the cultures with pale yellow and friable callus (Table 25) (Fig.4b). NAA at 0.1 or 0.5 ppm along with same levels of 2-ip in MS medium could induce callusing in 7.1 to 14.3 per cent of the cultures. Induced callus was green and compact in nature. Cultures were showing callus induction in 7.1 to 14.3 per cent in the combination of auxin IAA (0.1, 0.5, 1.0 ppm) with 2-ip at 1.0 and 3.0 ppm in MS.

All combinations of auxin IBA with 2-ip at the above levels in half MS media could result in callusing of internodal explants in 35.7 to 64.3 per cent of the cultures, with pale yellow and friable callus (Table 26).

The maximum callus index (120.0) was recorded for the internodal explants in the combinations of 2-ip at 3.0 ppm along with auxin 2,4-D at 0.1 ppm in MS media (Table 25), whereas low callus index (7.1) was recorded with combinations of 2-ip at 1.0, 2.0 and 3.0 ppm with auxin, NAA at 0.1 or 0.5 ppm and 2-ip (1.0 or 3.0 ppm) with IAA (0.1 or 0.5 ppm).

Table 26. Effect of different growth regulator combinations on callus induction and later proliferation in internodal explants of *Vateria indica* in half MS media

Treatments combinations (ppm)	Days taken for callus induction (mean)	% callusing	Callus index	Callus morphology
Kin 1.0 + IBA 0.1	33	14.3	14.3	Pale yellow friable
Kin 1.0 + IBA 0.5	33	14.3	14.3	"
Kin 1.0 + IBA 1.0	44	14.3	14.3	"
Kin 2.0 + IBA 0.1	43	28.6	28.6	"
Kin 2.0 + IBA 0.5	34	7.1	7.1	"
Kin 2.0 + IBA 1.0	40	21.4	28.5	"
Kin 3.0 + IBA 0.1	37	14.3	14.3	"
Kin 3.0 + IBA 0.5	40	21.4	27.8	"
Kin 3.0 + IBA 1.0	37	42.9	42.9	"
BA 1.0 + IBA 0.1	40	28.6	28.6	"
BA 1.0 + IBA 0.5	29	28.6	28.6	Pale yellow compact
BA 1.0 + IBA 1.0	27	42.9	68.6	"
BA 2.0 + IBA 0.1	28	7.0	7.0	"
BA 2.0 + IBA 0.5	28	42.9	42.9	"
BA 2.0 + IBA 1.0	24	35.7	42.8	"
BA 3.0 + IBA 0.1	21	14.3	14.3	"
BA 3.0 + IBA 0.5	29	7.1	7.1	"
BA 3.0 + IBA 1.0	29	35.7	57.1	"
2-ip 1.0 + IBA 0.1	26	[illegible]	50.0	Pale yellow friable
2-ip 1.0 + IBA 0.5	35	35.7	35.7	"
2-ip 1.0 + IBA 1.0	29	50.0	[illegible].0	"
2-ip 2.0 + IBA 0.1	25	35.7	64.3	"
2-ip 2.0 + IBA 0.5	26	64.3	90.0	"
2-ip 2.0 + IBA 1.0	27	42.9	55.8	"
2-ip 3.0 + IBA 0.1	26	35.7	57.1	"
2-ip 3.0 + IBA 0.5	18	61.5	98.4	"
2-ip 3.0 + IBA 1.0	25	64.3	70.7	"

Combinations of kinetin at 1.0 ppm with auxin NAA at 0.1 or 0.5 ppm, or 2,4-D at 0.1 ppm added to MS media resulted in low (7.1) callus index (Table 25).

The combinations of kinetin (1.0, 3.0 ppm) with 2,4-D (0.1 or 0.5 ppm) in WPM media resulted in the induction of yellow compact callus in 7.1 to 14.3 per cent of the cultures. Addition of BA at 1.0 ppm with auxin 2,4-D at 0.1, 0.5 and 1.0 ppm in WPM media could induce callusing in 7.1 to 14.3 per cent of cultures showing brown compact appearance. Combinations of 2-ip (1.0, 2.0, 3.0 ppm) with auxin (0.1 or 0.5 ppm) added to WPM resulted in callusing of internodal explants in 7.1 per cent of the cultures. The calli had pale yellow friable morphology. Whereas 2-ip and IBA at 1.0 ppm each in WPM could induce pale yellow friable callus in 7.1 per cent of cultures (Table 27).

The maximum (14.3) callus index for internodal explants was recorded in the combinations of kinetin at 1.0 ppm with 2,4-D at 0.5 ppm added to WPM media and also with the same medium supplemented with BA 1.0 ppm and 0.1 ppm 2,4-D. Whereas the low (7.1) callus index was recorded in combinations of 2-ip (1.0, 2.0, 3.0 ppm) with 2.4-D (0.1 or 0.5 ppm) added to WPM media (Table 27).

Table 27. Effect of different growth regulator combinations on callus induction and later proliferation in internodal explants of *Vateria indica* in WPM media

Treatments combinations (ppm)	Days taken for callus induction (mean)	% callusing	Callus index	Callus morphology
Kin 1.0 + 2,4-D 0.5	30	14.3	14.3	Yellow compact
Kin 3.0 + 2,4-D 0.1	17	7.1	7.1	"
Kin 3.0 + 2,4-D 1.0	30	7.1	7.1	"
BA 1.0 + 2,4-D 0.1	17	14.3	14.3	Brown compact
BA 1.0 + 2,4-D 0.5	30	7.1	7.1	"
BA 1.0 + 2,4-D 1.0	30	7.1	7.1	"
2-ip 1.0 + 2,4-D 0.1	30	7.1	7.1	Pale yellow friable
2-ip 2.0 + 2,4-D 0.1	17	7.1	7.1	"
2-ip 2.0 + 2,4-D 0.5	30	7.1	7.1	"
2-ip 3.0 + 2,4-D 0.1	17	7.1	7.1	"
2-ip 3.0 + 2,4-D 0.5	30	7.1	7.1	"
2-ip 1.0 + IBA 1.0	38	7.1	7.1	"

4.3.1.2 Effect of other media supplements

The effect of other supplements like adenine sulphate, coconut water and casein hydrolysate on callus induction and growth is presented in Table 28.

The effect of adenine sulphate as a media supplement was studied in half MS and WPM media containing 2.0 ppm 2-ip and 0.1 ppm IBA. All the levels of adenine sulphate (10, 20, 50 ppm) added to the above media did not influence much the callus induction in Vateria leaves and internodal segments. The callus index recorded was also low in all the combinations of adenine sulphate.

The callus induction obtained in the combination of coconut water 30 per cent (v/v) added to the half MS media formulation was low. Callus index also was low in both the internodal and leaf segments. Callus induction was, however, earlier in internodal segments than leaf segments in this trial.

Influence of casein hydrolysate on callus induction was studied in half MS media supplemented with 2.0 ppm 2-ip and 0.1 ppm IBA. Casein hydrolysate at 100 ppm added to this media resulted in 21.4 per cent and 14.3 per cent callus induction in leaf and internodal segments, respectively. Whereas casein hydrolysate at 300 ppm supplemented to the same

Table 28. Effect of media supplements on callus induction and later proliferation of Vateria indica in media containing 2.0 ppm 2-ip and 0.1 ppm IBA

Media	Treatments		Days taken for callus induction (mean)	% callusing	Callus index	Callus morphology
½MS	Adenine sulphate 10 ppm	L	32	14.3	14.3	Pale yellow friable
		I	38	14.3	14.3	"
	Adenine sulphate 20 ppm	L	30	7.1	7.1	Pale yellow friable
		I	30	14.3	14.3	"
	Adenine sulphate 50 ppm	L	30	7.1	7.1	Pale yellow friable
		I	30	14.3	14.3	"
	Coconut water 30% (v/v)	L	29	7.1	7.1	Pale brown compact
		I	13	7.1	7.1	Pale yellow friable
	Casein hydrolysate 100 ppm	L	29	21.4	21.4	Pale yellow friable
		I	30	14.3	14.3	"
	Casein hydrolysate 200 ppm	L	29	7.1	14.2	Pale yellow friable
		I	30	14.3	14.3	"
	Casein hydrolysate 300 ppm	L	28	14.3	14.3	Pale yellow friable
		I	30	21.4	21.4	"
WPM	Adenine sulphate 50 ppm	L	36	14.3	14.3	Pale yellow friable
		I	43	7.1	7.1	"

L = Leaf segment I = Internodal segment

Table 29. Response of *Vateria indica* stem callus subcultured from different combinations of callus induction media to MS medium with GA_3 (1.0 ppm) + 2-ip (1.0 ppm) + 2,4-D (0.5 ppm) + 0.2% sucrose

Callus induction media	Intensity of callus proliferation	% of cultures showing proliferation	Callus morphology
MS + 1.0 ppm 2-ip + 0.5 ppm 2,4-D	CCC	50.0	Creamy friable
MS + 2.0 ppm 2-ip + 0.1 ppm 2,4-D	C	Nil	Dark brown compact
MS + 2.0 ppm 2-ip + 0.5 ppm 2,4-D	C	Nil	Dark brown compact
MS + 3.0 ppm 2-ip + 0.5 ppm 2,4-D	CCC	50.0	Creamy friable

C - No callus growth

CCC - Some callus growth units observed

media could induce callusing in 14.3 per cent and 21.4 per cent in leaf and internodal segments, respectively.

The maximum (21.4) callus index was recorded for leaf segments in combination of casein hydrolysate 100 ppm added to half MS containing 2.0 ppm 2-ip and 0.1 ppm IBA. In internodal segments the maximum (21.4) callus index could be obtained in the combination of casein hydrolysate 300 ppm supplemented to half MS containing 2.0 ppm 2-ip and 0.1 ppm IBA.

The adenine sulphate levels (10, 20, 50 ppm) in both WPM and half MS media were showing low (7.1) callus index in both leaf and internodal segments.

4.3.2 Organogenesis/embryogenesis

The callus induced in different media combinations were cultured upto third subculture by different growth regulator combination (Table 29). The callus induced from the combinations of MS supplemented with 1.0 ppm 2-ip and 0.5 ppm 2,4-D or MS supplemented with 3.0 ppm 2-ip and 0.5 ppm 2,4-D showed response with respect to callus proliferation of creamy friable morphology. None of the combinations tried gave favourable response to organogenesis or embryoid formation.

Discussion

DISCUSSION

Veteria indica, is one of the well known species for commercial plywood and construction timber of medium quality in South India. This tree is known for its medicinal property also. Propagation of V. indica, however, is rather difficult due to its recalcitrant seeds (Huang and Villanueva, 1993). This conventional forestry procedures are difficult to be implemented for mass propagation of this species.

Attempts were made for the first time to standardise the in vitro propagation technique of Vateria indica in the Plant Tissue Culture Laboratory, Department of Tree Physiology and Breeding, College of Forestry. The results obtained during the study are discussed in the light of available literature and presented below.

5.1 Explant choice and surface sterilization

It is recognised that success in plantlet formation under in vitro condition is dependent to a large extent on the careful selection of the explant (Murashige, 1974; Sommer and Caldas, 1981). Considerable difference in organogenic capability often exists among different parts of the plant (Bonga, 1982). For clonal propagation, axillary and terminal buds have been routinely exployed (Bonga, 1981). In the

present study, nodal segments were used as the explant from 3 to 15 month old seedlings, to exploit the potentiality of axillary buds to grow and give rise to shoots under ideal culture conditions. The optimum explant size has been found to be 0.5 to 1.5 cm. Reduction in size of explant usually ended up in poor survival, probably due to the high cut surface: volume ratio. It has been reported by Hussey (1983) that with larger explants the growth and survival is rapid. However, observations in V. indica has shown that microbial contamination is more when larger explants were used for culture.

All tissue cultures are likely to end up contaminated if the inoculum or explant used is not obtained from properly disinfected plant material. Surfaces of the explant parts carry a wide range of microbial contaminants. Contamination rates are often higher if tissues from field grown specimens are used rather than from material raised in growth chamber or green house. Doublin (1984) observed that the percentage of infection was more than 90 for field explants regardless of the procedure used for their sterilization. The results were similar in our studies with Vateria. Irrespective of the explant sterilization protocol adopted, the culture contamination was nearly 100 per cent when field explants were used. Prophylactic sprayings given to the mother plants on

alternate days with a combination of a systemic fungicide Bavistin-50 per cent WP (Carbendazim) and contact fungicide Indofil M-45 (Mancozeb) both at 0.3 per cent could control contamination to considerable extent. Reduction in culture contamination by prophylactic spraying of mother plant has been reported earlier by different authors (Legrand and Mississo, 1986; Mahato, 1992; Santhoshkumar, 1993).

Mercuric chloride has been reported to be a very effective sterilant to remove the surface contamination of explants (George and Sherrington, 1984; Sita et al., 1986; Mahato, 1992). Of the different treatments tried with mercuric chloride during this investigation, dipping the explants in the sterilant at 0.1 per cent for five minutes followed by three rinses in sterile distilled water was found to be ideal for nodal segments. For leaf segments four minutes treatment was found to be effective. While lower treatment level results in higher rate of contamination, increase in treatment time resulted in high rate of mortality of explants.

Fungicidal dip in a combination of fungicides Bavistin-50 per cent WP (Carbendazim) and Indofil M-45 (Mancozeb) both at 0.1 per cent for ten minutes followed by mercuric chloride (0.1 per cent) for ten minutes given to the explants had no influence in reducing the rate of

contamination rather, the rate of explant survival was found to be poor. This is probably due to the fungicidal toxicity on the explant. Similar results of fungicide toxicity have been reported by Brown et al. (1982) and Shields et al. (1984). Dodds and Roberts (1985) suggested to avoid the use of fungicides for sterilisation since they or their degradation products may be metabolised by plant tissues with unpredictable results.

Exudation of polyphenols from explants which pauses a serious problem in most of the tree tissue culture programme was not a serious handicap in V. indica. However, it has been noticed that mature nodal tissues tended to show slightly higher amount of polyphenol exudation in comparison to the tender nodal tissues extracted from new flush. At higher concentrations the polyphenol can get oxidised and such compounds are reported to be highly toxic which may inhibit the enzyme activity (Mayer and Harel, 1979; Hu and Wang, 1983). The factors like age of the stock plant and location on the stem from which the explants are removed have been reported to greatly affect the establishment of tissues in vitro (Compton and Preece, 1988).

Predominant influence of seasonal variation on microbial interference in cultures of V. indica was noticed. The peak contamination occurring during June to October can be

directly related to the high ambient relative humidity prevailing during this season, which favours an increased amount of microbial inoculum in the environment. This is further supported by the relatively low contamination rates during the dry seasons of February to May. Such a season dependent culture contamination has been reported earlier by other authors also (Hu and Wang, 1983). Seasonal influence of physiological state of plant and its effect on culture establishment has also been reported (Borrod, 1971; Seabrook et al., 1976).

5.2 Culture establishment and bud break

The most extensively used culture media for micropropagation in trees are MS (Murashige and Skoog, 1962) and WPM (Lloyd and McCown, 1980). Reducing salt concentration to half its normal strength of MS has also been reported to be effective in certain tree species like Shorea roxburghii (Scott et al., 1990), Hopea odorata (Scott et al., 1990), Ziziphus cv. (Kim and Lee, 1988) and Leucaena leucocephala (Datta and Datta, 1984). Considering these different combinations of growth regulators as well as growth supplements in MS, half MS and WPM have been tried to identify a suitable culture media for axillary bud cultures of Vateria indica.

5.2.1 Effect of plant growth regulators

The axillary buds of Vateria indica, are normally dormant due to strong apical dominance. An axillary bud is a predetermined organ with great potential to give rise to a shoot in the absence of apical dominance. The basic phenomenon involved in the induction of bud break and subsequent plantlet production in vitro is reported to be the action of plant hormones. Though little is known how a hormone evoke a particular response (Thorpe and Meier, 1973), the favourable effects on bud bursting and shoot production by cytokinins had been demonstrated by Murashige (1974).

In the present study, out of 310 growth regulator combinations tried in different media, bud break was observed in only 73 combinations. Of the 73 combinations, ten have been identified as the better ones for inducing bud break in axillary buds of V. indica. These ten combinations are given below:

MS + Kin 2.0 ppm

MS + Kin 1.0 ppm + NAA 0.1 ppm

MS + Kin 1.0 ppm + NAA 0.5 ppm

MS + Kin 3.0 ppm + NAA 0.1 ppm

MS + BA 2.0 ppm + NAA 0.1 ppm

MS + 2-ip 1.0 ppm

MS + 2-ip 1.0 ppm + NAA 0.5 ppm

½MS + 2-ip 2.0 ppm + IBA 0.1 ppm

WPM + Kin 1.0 ppm + NAA 0.5 ppm

WPM + Kin 2.0 ppm + NAA 0.1 ppm

Kinetin is a common natural cytokinin widely used in plant tissue culture works (Sita et al., 1980; Mascarenhas et al., 1982). In the present study kinetin alone at 2.00 ppm level added to the media MS induced bud break. Combinations of kinetin with certain levels of auxins were also found to be favourable for organogenesis in V. indica. In both MS and WPM media, NAA was the best auxin along with kinetin than IAA, IBA and 2,4-D in inducing bud break. Other auxins had very less effect in inducing bud sprout. Concentration of NAA at 0.1 and 0.5 ppm in combination with kinetin exhibited a remarkable effect on bud break in both the media. The favourable effect of kinetin on bud break has been reported in other tree species like Eucalyptus tereticornis and E. globulus (Gupta and Mascarenhas, 1987) and Pterocarpus marsupium (Santhoshkumar, 1993).

Among the various cytokinins used in plant tissue culture, BA is the cheapest and one of the most effective (Aboel-nil, 1987; Rai and Chandra, 1989). In the present investigation, however, BA was found to be less effective in

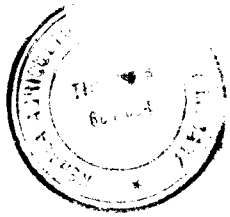
inducing bud break in V. indica. The cytokinin BA (2.0 ppm) along with auxin NAA (0.1 ppm) incorporated in MS media alone induced bud break. Cytokinin BA alone supplemented to the medium did not exhibit any favourable influence on the axillary buds of Vateria. In comparison, BA has lesser effect than kinetin. In Dalbergia latifolia, it was observed that presence of auxins inhibited BA's capacity for inducing morphogenesis (Mahato, 1992). However, in present study, it was observed that auxin NAA enhanced the effect of BA in inducing bud break from axillary bud cultures. Bud break and proliferation was noticed in MS medium supplement with 2.0 ppm BA and 0.1 ppm NAA. The favourable effect of BA in bud break has also been reported earlier in various tree species like Betula uber (Vijayakumar et al., 1990), Acacia albida (Ruredzo and Hanson, 1993) and Caesalpinia pulcherrima (Rohman et al., 1993).

The naturally occurring cytokinin, 2-ip has been reported to be more effective than kinetin or BA in a number of species like Rhododendron (Anderson, 1975), Kalmia latifolia (Lloyd and McCown, 1980) and Allium sativum (Bhojwani, 1980b). In the present study, when 2-ip was used alone or in combination with auxins NAA or IBA in MS and half strength MS media bud break could be obtained. Among these two auxins NAA has better effect than IBA in combination with

2-ip in V. indica. There is earlier report of 2-ip being not very effective in inducing bud break in hybrid Willow (Bhojwani, 1980a). From the present study, 2-ip could be rated better than BA and next to kinetin with regard to its potential for inducing bud break in Malabar white pine.

The action of 2-ip might be stronger than kinetin or BA with other auxin combinations in half MS. The combination of higher concentration of 2-ip and relatively low IBA favoured bud break and later growth in V. indica. The growth regulator combinations of kinetin or BA could not induce shoot formation though it gave a better culture establishment.

Many aspects of cellular differentiation and organogenesis from tissues have been found to be controlled by an interaction between cytokinin and auxin concentrations (Skoog and Miller, 1957). A higher cytokinin:auxin ratio promotes shoot formation while the reverse induces rhizogenesis. The interaction of cytokinin and auxin, however, is more complex. Auxin added to the medium has already been reported to nullify the suppressive effect of high cytokinin content on axillary shoot growth (Lundergan and Janick, 1980). In the present study auxin NAA has been found to be the best auxin to be supplemented with cytokinins for inducing bud break in Vateria indica in both MS and WPM media. The synergistic effect of NAA along with cytokinins in bud



break has also been reported earlier in various tree species like Santalum album (Sita et al., 1980) and Syzygium cumini (Yadav et al., 1990).

Among these combinations identified for bud break, half MS media having 2-ip (2.0 ppm) and IBA (0.1 ppm) alone produced good shoots with normal leaves. Cytokinin kinetin or BA alone or in combination with auxin did not favour healthy shoot formation eventhough bud break was noticed in some of these combination.

The maximum bud break recorded for Vateria indica, in the present study is only 21.4 per cent in the best media identified. Hence, the media combinations tried in the present study is presumed to be not sufficient to eliminate the limiting factors for the in vitro culture of Vateria indica.

5.2.2 Effect of other media supplements

Plants synthesise vitamins as well as amino acids endogenously and these are used as catalysts in various metabolic processes. When plant cells and tissues are grown in vitro, some essential vitamins are synthesised but only in sub-optimal quantities. Hence it is necessary to incorporate the medium with required organic and inorganic supplements to achieve the best growth of the tissue.

Incorporation of casein hydrolysate, a complex mixture of amino acids, has been attempted by several workers. Enhancement in multiple shoot production by adding casein hydrolysate has been reported by Mascarenhas et al. (1982) in Hevea brasiliensis and Mahato (1992) in Dalbergia latifolia. Murashige and Skoog (1962) observed that casein hydrolysate allowed vigorous organ development when supplemented with a broad range of kinetin and IAA in MS media. However, in the present study addition of casein hydrolysate in both half strength MS and WPM media along with cytokinin and auxins gave no response in V. indica. Similar observations of no favourable effect of casein hydrolysate have been reported by George and Sherrington (1984) in several plant species both in monocots and dicots. It had also no beneficial effect on shoot formation and growth in Pterocarpus marsupium (Santhoshkumar, 1993).

Cycocel is a choline derivative containing substituted chlorine. Addition of CCC to half strength MS medium containing 2-ip (2.0 ppm) and IBA (0.1 ppm) had a profound influence on bud break in Vateria. The effect of CCC, however, was not so pronounced when this chemical was supplemented in WPM with the above growth regulators. Influence of cycocel on leaf morphogenesis and leaf anatomy including palliside cell length, spongy parenchyma layer

thickness and mesophyll air space has been reported by Gausman (1986). This compound is a well known inhibitor of GA synthesis, but not its action. Usually CCC treated plants have shorter internodes and thicker and green leaves (Davis and Curry, 1991). In addition to these, it may prolong the leaf retention by delaying leaf senescence (Davis and Curry, 1991) which would be a factor that influenced axillary bud sprout in *V. indica*. In our study CCC, however, could not induce leaf expansion and retention in the cultures. It had effect on leaf production in *Pterocarpus marsupium* which negatively influenced bud break from axillary buds of this species (Santhoshkumar, 1993).

Adenine, a nitrogenous base of DNA, when added to the culture medium has been found to have beneficial effects in *Pterocarpus santalinus* (Patri *et al.*, 1988) and *Dalbergia latifolia* (Mahato, 1992) etc. Adenine and its more soluble form adenine sulphate when added to culture media enhance growth and organ formation, probably due to their cytokinin like activity (Skoog and Tsui, 1948). *Vateria indica*, cultures, however, did not show any positive response to adenine supplements. Adenine sulphate when supplemented to half strength MS media containing auxins and cytokinins did not affect the frequency of bud break. However, good culture establishment was observed. The inhibitory role of adenine

sulphate in shoot formation has reported earlier by Jarret et al. (1980). In Pterocarpus marsupium, reduction in number of shoots and shoot length has been noticed, when adenine sulphate was supplemented (Santhoshkumar, 1993).

Silver nitrate is a growth supplement which is reported to have positive as well as negative influence to in vitro cultures. During the present study, effect of this compound on Vateria bud culture was assessed by adding it to both half MS and WPM. In both media silver nitrate have been found to have beneficial effect on bud break. This influence was more pronounced when axillary buds were cultured in WPM supplemented with silver nitrate (20.0 ppm) along with 2-ip and IBA. Reports of enhanced shoot morphogenesis and growth in a number of species with addition of silver nitrate in the culture medium are available (Vasil et al., 1979; Songstad et al., 1980). The positive effect of silver nitrate is probably due to its action on ethylene. Silver ion is a potent inhibitor of ethylene action and it prevents the accumulation of ethylene in culture vessels (Beyer, 1976). Although silver ions inhibits the action of ethylene, it does not inhibit ethylene biosynthesis (Liberman, 1979). At higher concentrations, however, the silver compounds are found to be toxic to the plants. This has been evidenced by observations of Pious et al. (1993) who reported the deleterious effect of

silver nitrate in in vitro cultures of Pennisetum americaum. In Pterocarpus marsupium also the number of responding cultures were not influenced by silver nitrate supplements in the media (Santhoshkumar, 1993).

Ethylene is known to have inhibitory action in in vitro cultures. Cobalt ions inhibit ethylene production by blocking the conversion of 1-amino cyclopropane-1-Carbolic acid to ethylene (Yang and Hoffman, 1984). In order to exploit the anti-ethylene characteristic of cobalt ions, cobalt chloride was supplemented in half strength MS medium. However, no favourable influence was noticed in the cultures of V. indica. Cobalt chloride added to the media is found to inhibit the shoot regeneration slightly at lower strength and completely at higher strength in Pterocarpus marsupium (Santhoshkumar, 1993).

Addition of coconut water in medium has been reported to enhance both shoot growth and multiple shoot formation in a number of tree species (Mittal et al., 1989; Rai and Chandra, 1989). The favourable effects of coconut water in the promotion of growth and differentiation of excised tissues and organ have been attributed to the presence of cytokinins and gibberellin like substances in it (Straus and Rodney, 1960). It contains a number of cell division factors and free amino acids (Shantz and Steward, 1952). Coconut water contain

myo inositol which lead to the inclusion of this chemical in many media (Pollard et al., 1965). Addition of this compound in half strength MS and WPM media, however, did not give any favourable response to the axillary buds of V. indica. Lack of beneficial effect of coconut water on shoot formation and leaf production has been noticed in Dalbergia latifolia (Mahato, 1992). This material is also known to induce callusing in different crop species (Hawker et al., 1973).

Gibberellic acid tried at various combinations did not yield any favourable result for the growth of cultures. Though GA_3 is known to enhance cell division and growth, its inhibitory effects in combination with auxins and cytokinins concentration which would otherwise promote morphogenesis were reported by Thorpe and Meier (1973). It is presumed that the enhancement of endogenous auxin level in the explant in the presence of GA_3 might have suppressed the bud break. Inhibitory effect of GA_3 in cultures has been reported by Heide (1974) in Begonia, Mahato (1992) in Dalbergia latifolia and Santhoshkumar (1993) in Pterocarpus marsupium.

Eventhough a wide range of media compositions involving different basal composition, growth regulators and additives were tried, none was proved to be fully efficient. A media composition with perfect repeatability could not be identified during the present investigation. Another hurdle

in formulating a viable protocol in Vateria indica was the loss of morphogenetic potential of the buds, with time. Eventhough some media formulations could be identified with a good percentage of bud break, further morphogenesis of shoots leading to the production of leaves and continued growth could be noticed only in few cases. Further growth of the shoots were not very satisfactory and therefore rooting and as well as other aspects of propagation could not be carried out. No clearcut interpretation could be made to explain the behaviour of the explant under culture. The present study being the first of its kind in Vateria indica, no earlier reports could be made use of to provide a plausible explanation to the recalcitrant behaviour of the species to micropropagation. Response to tissue culture exhibited by certain other members of Dipterocarps like Hopea purviflora and Shorea obtusa are also not much different from the result obtained in V. indica, during this present investigation. Based on similar observations made in certain other tree species like Pterocarpus santalinus, Pterocarpus marsupium, Fraxinus excelsior etc. Patri et al. (1988) remarked that such observations could be due to the endogenous levels of some unknown factors, probably auxins and cytokinins inherited with the explant.

5.3 Indirect organogenesis

Indirect or callus mediated organogenesis is an alternative method for micropropagation. It is the fastest method of shoot multiplication and been suggested as a potential method of cloning plant species (Murashige, 1974). The most of serious drawback in the use of callus culture is the possible genetic instability of the cells. However, callus mediated organogenesis as a method of clonal propagation has been reported in a number of tree species. Successful micropropagation technique following this route have been reported in several species including Sesbania bispinosa (Sinha and Mallick, 1991), Bauhinia Purpurea (Kumar, 1992) and Cephalotaxus harringtonia (Wickremesinhe and Arteca, 1993) in addition to some of the soft wood species. Considering the potentiality of indirect organogenesis as a method of micropropagation callus induction, proliferation and organogenesis have been tried in V. indica during the present investigation.

5.3.1 Callus induction and proliferation

Different explants of Vateria indica like leaf segments and internodal segments were tried for inducing calli. Different basal media were used and cultures were found to be established in all basal media tried, with varying

frequencies. Half strength MS promoted better culture establishment after three weeks culture period for leaf and internodal segments.

Different growth regulators as well as organic compounds were supplemented to the basal media MS, half MS and WPM, to arrive at the most appropriate media composition for maximum callus growth.

Among the various growth regulator combinations tried in the MS basal media for leaf explants, 2-ip at level of 1.0 to 3.0 ppm with 2,4-D at 0.1 to 1.0 ppm or 2-ip at 1.0 to 3.0 ppm with IBA at 0.1 to 1.0 ppm in half strength MS gave the best results for callus induction with an establishment percentage of 21.4 to 64.1 and callus index of 35.7 to 100.0. Both leaf and stem explants responded similarly for callus induction and proliferation. Callus induction rate and its morphology varied according to the growth regulators used. Use of kinetin instead of 2-ip with 2,4-D induced yellow compact callus. Similarly use of BA at lower concentration (1.0 ppm) instead of 2-ip with IBA induced pale yellow compact callus with low callus index. Use of NAA along with kinetin or 2-ip induced green compact, pale green compact callus. The callus proliferation rate was more with 2-ip than kinetin or BA levels. Use of 2,4-D for callus induction was reported earlier in several tree crops (Kumar, 1992; Wickremesinhe

and Arteca, 1993). Use of NAA for callus induction was reported by Nandwani and Ramawat (1992) in Prosopis tamarugo and Sita and Swamy (1993) in Dalbergia latifolia.

Organic supplements like adenine sulphate, coconut water and casein hydrolysate were incorporated in the media to observe the response of calli. Adenine sulphate tried at various levels in half strength MS and WPM media did not yield any favourable result for callus induction and proliferation in Vateria indica. Favourable effect of coconut water on callus induction and growth was reported by Sita and Swamy (1993) and Mahato (1992) in Dalbergia latifolia and Hawker et al. (1973) in grape berries. Contrary to this result, in Vateria indica, coconut water did not give any favourable result on callus induction and growth when added to half strength MS media. The cytokinin like compounds present in the coconut water might have caused this type of response.

Incorporation of casein hydrolysate, a complex mixture of amino acids had favourable effect on Dalbergia latifolia (ManjuAnand and Bir, 1984). In the present study, casein hydrolysate when incorporated in half strength MS medium supplemented with plant growth regulators did not enhance callus induction in Vateria indica. Inhibitory effect of casein hydrolysate has been observed in Dalbergia latifolia (Mahato, 1992).

5.3.2 Indirect organogenesis/embryogenesis

The callus induced on stem segments of Vateria indica were subcultured to different media combinations, so as to induce embryoids. None of the treatments tried gave favourable response on callus differentiation. Most of the media combinations tried were unfavourable for embryogenesis but some callus growth units were observed in MS medium.

Somatic embryogenesis was attempted in a number of tree species, and successful reports include on Santalum album (Sita et al., 1979) and Sesbania bispinosa (Sinha and Mallick, 1991).

Response of Vateria indica, through indirect organogenesis to obtain normal plantlet was found not encouraging. All the normal procedures for callus induction and proliferation were attempted for this crop. However, callus induction and proliferation achieved by the growth regulator combinations was poor. Few levels of growth regulators were attempted at various stages of callus growth to induce embryogenesis but with no favourable response. Eventhough moderate callus proliferation was achieved but the treatment combinations tried in the present study were not sufficient to identify a suitable media that could support profuse callus growth, leading to organogenesis or embryoid formation and germination.

Summary

SUMMARY

The present investigation was carried out during the period 1992-94 in the Tissue Culture Laboratory of the Department of Tree Physiology and Breeding, College of Forestry, Vellanikkara with the objective of standardising the technique of micropropagation of Vateria indica. The salient findings of the investigation are presented below.

1. Nodal segments of size 0.5 to 1.5 cm were found to be the best explants for initiating axillary bud break in culture.

2. Of the various surface sterilants tried, mercuric chloride at 0.1 per cent level for 5 minutes was found effective for nodal segments in establishing aseptic cultures of Vateria indica. A treatment time of 4 minutes in mercuric chloride at 0.1 per cent was found to be sufficient for leaf explants.

3. Prophylactic spraying of the source plants with the contact fungicide Indofil M-45 (Mancozeb) and systemic fungicide Bavistin (Carbendazem) was found to reduce culture contamination.

4. Fungicidal dip of nodal explants did not reduce the contamination rate.

5. Culture establishment of nodal explant from different sources of Vateria indica did not face the problem of polyphenol interference.

6. Seasonal variation was observed for the microbial interference and the period from February to May was identified as the best season for establishing the cultures.

7. Among the different basal media tried half strength MS was identified suitable for establishing cultures of nodal and leaf segments.

8. Half strength MS basal medium with 2.0 ppm 2-ip and 0.1 ppm IBA gave bud break and leaf production from nodal segments.

9. Addition of gibberellic acid did not have any favourable effect on bud break and shoot elongation.

10. Casein hydrolysate had no beneficial effect in axillary bud culture of Vateria indica.

11. Cycocel added to the media enhanced the bud break to certain extent.

12. Adenine sulphate incorporated to half MS had failed to induce bud break.

13. Silver nitrate added to the culture media had more beneficial effect on bud break of Vateria indica in WPM than in half strength MS media.

14. Cobalt chloride used in Vateria indica had no favourable effect on bud break in half strength MS media.

15. Incorporation of coconut water had no beneficial effect on either bud break or shoot formation in half MS and WPM media.

16. MS basal medium with 2-ip at 1.0 to 3.0 ppm and 2,4-D at 0.1 to 1.0 ppm or 2-ip at 1.0 to 3.0 ppm with IBA at 0.1 to 1.0 ppm in half MS were identified as the best basal media for callus induction in Vateria indica.

17. Callus morphology and proliferation rate varied with the growth regulator combinations.

18. No favourable response was obtained with addition of adenine sulphate, coconut water and casein hydrolysate in callus induction and proliferation.

19. The callus maintained in MS basal medium supplemented with GA_3 + 2-ip + 2,4-D + 2 per cent sucrose did not respond to embryogenesis but callus growth units were observed.

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* Originals not seen

Plates

Plate 1a Explant with axillary bud in culture

Plate 1b Explant with terminal bud in culture

Plate 2a Nodal segment of Vateria showing axillary bud break in half MS medium supplemented with kinetin 1.0 ppm and IBA 0.5 ppm

Plate 2b Nodal segment showing axillary bud break in half MS medium supplemented with kinetin 2.0 ppm and IBA 0.5 ppm

Plate 2c Nodal segment showing axillary bud break in half MS medium supplemented with cobalt chloride 1.0 ppm, 2-ip 2.0 ppm and IBA 0.1 ppm

Plate 3a Shoot elongation and leaf morphogenesis in MS medium supplemented with kinetin 1.0 ppm and NAA 0.5 ppm

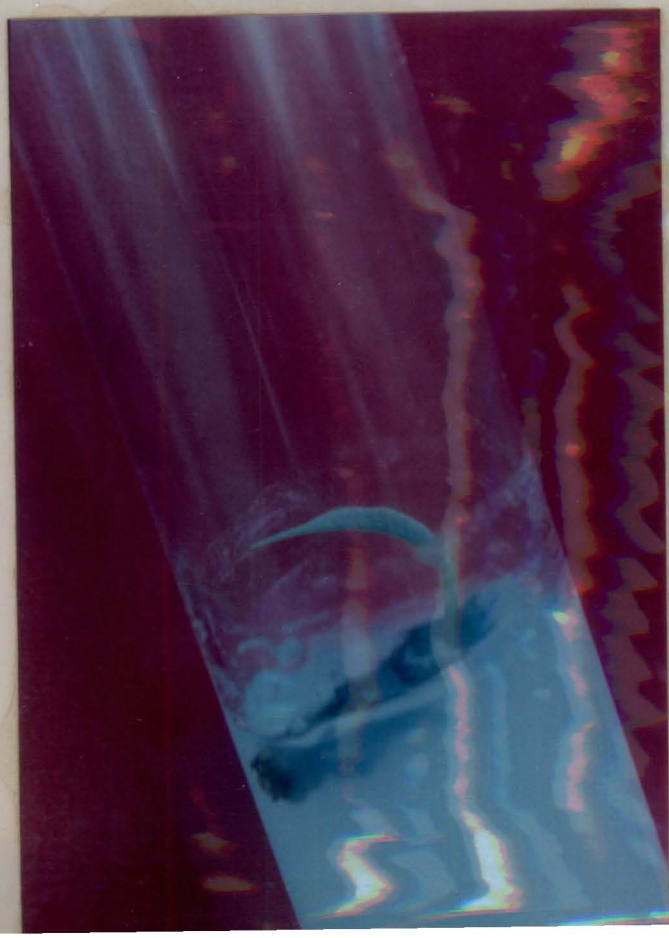


Plate 3b Shoot elongation and leaf morphogenesis in half strength MS medium supplemented with 2-ip 2.0 ppm and IBA 0.1 ppm

Plate 3c Leaf morphogenesis and expansion in half strength MS medium supplemented with 2-ip 2.0 ppm and IBA 0.1 ppm

Plate 4a Yellow compact callus induced in MS medium supplemented with kinetin 3.0 ppm and 2,4-D 1.0 ppm

Plate 4b Pale yellow friable callus induced in MS medium supplemented with 2-ip 1.0 ppm and 2,4-D 0.5 ppm

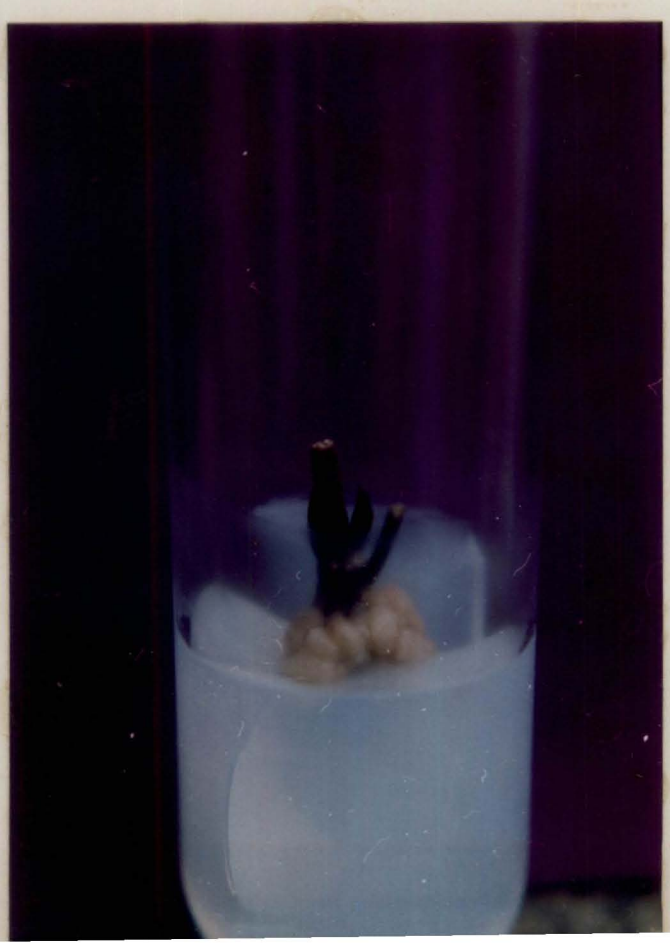
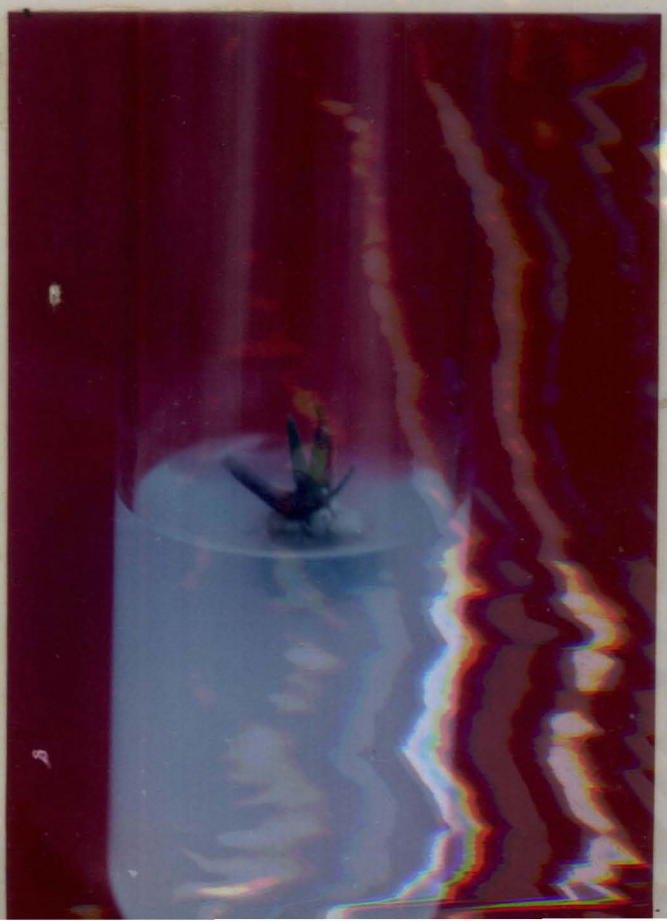


Plate 4c Pale yellow friable callus induced in MS medium supplemented with 2-ip 3.0 ppm and 2,4-D 0.5 ppm

Plate 4d Pale yellow compact callus induced in half strength MS medium supplemented with 2-ip 3.0 ppm and IBA 0.5 ppm



In Vitro PROPAGATION OF MALABAR WHITE PINE (*Vateria indica* L.) THROUGH TISSUE CULTURE

By

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ABSTRACT OF A THESIS

Submitted in partial fulfilment of the
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ABSTRACT

The present investigation was carried out at the Department of Tree Physiology and Breeding, College of Forestry, Vellanikkara, during 1992-94, to standardise the *in vitro* technique for multiplying *Vateria indica* which is commonly known as Malabar white pine. This being the first attempt of micropropagation in this tree species, the methodology was to be standardised from the initial stage itself. Nodal and internodal segments as well as leaf segments collected from seedling maintained in the College of Forestry were used as explants.

Different routes like enhanced release of axillary buds, organogenesis and embryogenesis were attempted for this species. It was found that nodal segments of size 0.5 to 1.5 cm was ideal as the explants. Prophylactic spray of seedlings with a mixture of Bavistin and Indofil M-45 combined with surface sterilization of explants with 0.1 per cent mercuric chloride for 5 minutes for nodal explants and 4 minutes for leaf explants, could control culture contamination to the greatest extent. Seasonal variation was observed for the fungal interference and the period from February to May was identified as the best season for establishing the cultures of *Vateria indica*. Half strength MS medium was noted to be

suitable for primary culture establishment for both nodal and leaf segments. Out of the various growth regulator combinations tried for bud break and shoot elongation in Vateria, 2-ip and IBA could support bud break and shoot production. Among the various media additives tried, CCC had less effect on bud break in half MS medium and silver nitrate had moderate effect on bud break in WPM medium. Casein hydrolysate, adenine sulphate, cobalt chloride and coconut water were the other additives, tried without having any beneficial effect on bud culture of Malabar white pine.

Moderate callusing could be induced from leaf and internodal segments on MS and half strength MS media supplemented with growth regulators 2-ip + 2,4-D (in MS media) and 2-ip + IBA (in half strength MS) with a callus index ranging from 35.7 to 100.0. The calli did not respond to organogenesis but growth units of callus were obtained. The morphology and growth rate varied according to the growth regulator combinations tried.

The results of the present study being first of its kind in Vateria indica would have significance to disentangle the in vitro response of this species for micropropagation. Since this species is recalcitrant in nature, much more regulated efforts are to be made for standardising the protocol for micropropagation of Vateria indica.