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# MICROPROPAGATION OF TEAK, ROSEWOOD AND SANDAL

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## CONTENTS

# *ABSTRACT*

*Micropropagation of three important tree species viz. teak, rosewood and sandal was attempted In teak, shoot tip and axillary bud collected fiom seedlings and from mature trees were used to initiate shoot cultures. High levels of contamination presumably from endogenous bacteria and fungi, was observed in tissues collected fiom mature trees during the period from May to November. This is attributed to the high humidity during these months. The best period for collection of buds was January to April. Exudation of phenolic compounds and browning of media was also high in tissues of mature trees.*

*In teak, sprouting of shoot tip and axillary bud was better in seedling, than in mature trees. Shoots obtained from mature trees were however thicker and with larger leaves. The effect of cytokinins on shoot multiplication was tested in mature tree cultures and higher levels of benzyl aminopurine and kinetin were found to favour shoot multiplication whereas at lower levels shoot elongated. Excised shoots could also be rooted by transfer to a medium containing 1 mg/l of indole butyric acid for 42 hrs., followed by transfer to a hormone free liquid medium. Plantlets of teak were transferred to a mixture of soil and vermiculite (1 :l) and hardened in a green house under intermittent mist. 60% survival was obtained when plants were shifted to soil after 10 weeks.*

*In rosewood axillary bud explants taken from mature trees of* ***D. latifolia*** *and* ***D. sissoides*** *gave high rates of contamination when collected during the period from June to November. Axillary buds sprouted to form shoots but failed to elongate. No multiple shoot formation was obtained on cytokinin containing media*

*Leaf explants cultured on media containing auxins and cytokinins gave rise to profuse callusing. Callus could be maintained by subculture, but no morphogenesis was obtained on a wide range of media*

*Shoot tips, nodes and internodes of mature sandal trees were cultured for induction of multiple shoot and callus. High rates of contamination was obtained as in teak and rosewood. but were mostly caused by bacteria Axillary buds sprouted to form shoots and few multiple shoots but failed to elongate further. No plantlets could be regenerated*

*Internode and leaves were cultured on auxin containing medium to induce callus. Callus formation occurred on cut edges of tissues but was slow growing. Callus could not be maintained for longer periods and did not show morphogenesis.*

# 1. INTRODUCTION

India has a rich diversity of forest tree species that have been well known for their utility as well as aesthetic value. Our indigenous species like teak, rosewood and sandalwood are amongst the most valuable Umbers in the world. These trees once abundant in the forests throughout the country have been depleted in recent times to meet the increasing demand. Plantations of these species have been established to ensure that future requirements for timber are met but it is becoming evident that a tremendous increase in production will be required. Since a huge increase in the area under plantations is undesirable due to environmental concerns, the other alternative is to enhance the productivity of plantations by tree improvement and other means. Compared to the short term measures like silvicultural management, nutrient inputs and pest and disease management, genetic improvement of trees will have the greatest potential in the long term sustainable increase in productivity.

## 1.1. Tree Improvement

Conventional tree improvement strategies aim at selecting and utilising the variability existing in natural populations or in plantations of different species. Genetic variability is found in various degrees throughout the natural range of the tree species. In a broad sense the variation between various locations can be referred to as provenance variation and usually the first step in any tree improvement programme in a species with a wide distribution would be to select the best provenance to suit the requirements. This is followed by selection of the best trees with the provenance in natural stands or plantations. Primary selection is based on the phenotypic characters of mature trees. Plus trees thus selected are put through a cycle of progeny testing followed by mass multiplication of the trees found to perform best.

## 1.2. Application of clonal propagation in tree improvement

Clonal propagation harnesses the total genetic makeup of the mother plants and makes it available in the next generation. Therefore the utilization of proven clones for operational planting is a rapid means of improving the productivity of forest plantations. The draw back is that mature trees are often not very amenable to conventional clonal propagation techniques like rooting of stem cuttings. The alternative to such methods is to use *in vitro* techniques for clonal propagation. Commonly used *in vitro* techniques for plant regeneration are referred to as micropropagation.

## 1.3. Status of micropropagation of tree species

The application of *in vitro* techniques are considerably more difficult in tree species than in herbaceous plants. However over the last two decades the number of tree species that have been micropropagated has been increasing rapidly. In India micropropagation of forest trees has been relatively successful (Muralidharan and Mascarenhas, 1989). The important timber species of the subcontinent have been studied in culture and *in vitro* plantlet regeneration obtained through the different pathways of morphogenesis.

## 1.4 Species selected for the study

### 1.4.1 Teak

Not only is teak a durable and strong timber but it also has good workability and is termite resistant. Teak timber has been in use in the form of poles or sawnwood for shipbuilding, railway sleepers, furniture and structural work.

Teak has a natural distribution over a large area in South and South-East Asia which shows a variation in soil and climate. In India teak grows in areas with rainfall ranging from below 900 mm to more than 2500 mm. Genetic differences between teak of different provenances has been tested and established through an international network of provenance trials (Keiding *et al*.. 19861.

### 1.4.2 Rosewood

*Dalbergia latifolia* and *D. sissoides* have timber that is practically indistinguishable and are treated in commerce as rosewood. Rosewood is a prized timber because of the deep purple colour of the heartwood. It is used for furniture, cabinet making and carving. *D. latifolia* is distributed widely throughout the deciduous forests of India. The trees are very slow growing and tree form is generally poor. Plus tree selections have been made in many states and plantations established but rosewood has not received a high priority because of its slow growth. The development of clonal propagation methods including micropropagation is expected to contribute to the tree improvement.

### 1.4.3 Sandal wood

Sandalwood is one of the most valuable of all timbers. The heartwood contains sandalwood oil which is valuable in perfumery. The wood is used for carving and has medicinal uses too. Sandal is found mainly in the Southern states of Karnataka. Kerala and Tamilnadu in the drier regions.

The sandal tree is a root parasite growing on several different trees and shrubs. A severe and devastating phyllody disease caused by MLOs called the spike disease is affecting the sandal population in all the three States. No effective preventive or curative measures have been found for the disease. A few individual trees which appear to be free of the disease are found remaining in the affected regions: Testing of these trees for resistance and rapid clonal propagation of the resistant individuals appears to be a strategy with potential for control of the spike disease. Conventional vegetative propagation is not successful in sandal wood although not much research appears to have been done on this aspect.

## 1.5. Status of Micropropagation in teak, rosewood and sandal

### 1.5.1. Teak

Pioneering work on micropropagation of the three tree species were carried out at different laboratories in India. The results obtained in teak in particular is important because it was probably the first such report of regeneration of plantlets using explants from mature trees. Gupta *et al.* (1980) used nodal explants from 100 year old trees and obtained a multiplication of 3-4 shoots per culture. Mascarenhas *et al.* (1987) later modified the procedure and obtained better results.

Monteuuis (1994) was also successful in micropropagation of mature teak trees using a modified procedure. Tiwari and Pandey (1995) also reported successful micropropagation from mature trees. However, the paper does not give details of the procedure or even mention the difference from the work of Gupta *et al.* Micropropagation of teak is being carried out by a few commercial biotechnology units in the country and in Thailand. It is not clear whether the cultures were derived from juvenile plant parts or mature trees of proven superiority.

In this study, attempts have been made to micropropagate teak from mature trees/grafted clones growing in Peechi, Palapilly and Nilambur. Material taken from stumps of seedlings showing good growth characteristics were also used for micropropagation. Trials have shown that such stumps perform better than unselected ones as planting material.

### 1.5.2. Rosewood

Tissue culture of rosewood has been carried out in three laboratories. Lakshmi Sita and Raghava Swamy (1993);Rao (1986)and Rai and Jagadish Chandra (1989) have reported micropropagation of mature rosewood trees. Rao and Lakshmi Sita (1994)have also reported somatic embryogenesis from immature seeds of *D. latifolia*.

In the present study attempts have been made to develop methods of micropropagation using leaf and nodal explants of mature trees of *D. latifolia* and *D. sissoides*. The methods and media used by earlier workers were also tested.

### 1.5.3. Sandalwood

Micropropagation of sandal through somatic embryogenesis has been reported (Lakshmi Sita *et al*.. 1979).Although very successful techniques for somatic embryogenesis has been reported it is surprising that the more common method of micropropagation involving preexisting meristems ie. shoot tip and axillary bud culture has not been reported to be successful.

In our study an attempt was made to culture shoot tips and axillary buds of mature sandal wood trees to induce multiple shoot formation. The advantage of such a method is that no callus formation is involved and consequently the risk of genetic variability in regenerants is minimised. Attempt has been made to regenerate mature trees through somatic embryogenesis in callus cultures using the methods reported in literature.

# 2. MATERIALS AND METHODS

## 2.1. Plant material

The plant materials for culture were collected from mature trees of *Tectona grandis, Dalbergia latifolia, D. sissoides* and *Santalum album* growing in the campus of KFRI. Peechi. One year old stumps of teak, procured from the Silviculture Division of KFRI, were also used as source of explants. Buds of teak were also collected from the various clones assembled at the clonal seed orchard at Nilambur.

Buds and other plant parts were collected from all the three species from the middle part of the crown. Only new vegetative growth were used. Twigs were kept moist in polythene bags until use (upto 24 hrs.).

## 2.2. Pretreatment of explants

Since exudation of phenolic compounds and browning of explants were observed in teak and rosewood, explants were pretreated with running water, activated charcoal (AC). polyvinyl pyrolidone (PVP) or citric acid. Explants were kept after cutting to proper size in running water for 1 hr. or for 30 min.. in solutions of 0.25% (w/v) AC or 0.5% PVP or 150 mg/l citric acid after which surface sterilization was carried out.

## 2.3. Surface sterilization

Explants were prepared for culture by removal of dead tissue and cleaning by tap water containing a few drops of detergent (Extran. Merck) followed by 1-2 washes with distilled water to remove the detergent. This was followed by surface sterilization.

Mercuric chloride solution prepared in double distilled water was used for surface sterilization of all the plants used in this study. The concentrations and duration of the $HgCl_2$ treatment varied with the type of tissue used. For shoot tips and nodal explants of teak, rosewood and sandal wood, 0.1-1.0% of $HgCl_2$ (w/v) was used for 5-10 minutes. For tender stem of sandal 0.05% and for thicker stems and leaves 0.1% of $HgCl_2$ was used for 5 minutes.

Pretreatment or culture in the presence of antibiotics and antimycotics was also tested to control contamination in explants. Bavistin. a systemic

fungicide was used as a 0.2% (w/v) solution for 1 hr. soaking treatment or as an additive to the media. Streptomycin and Gentamycin were added to the media at a concentration of 100mg/l after filter sterilization through a 0.22 μ membrane (Millipore)to autoclaved media just before gelling.

## 2.4. Glassware/Culture containers

Borosilicate glassware was used in this study for preparation of culture media and for use as culture vessels. Test tubes used were of the size 150 mm x 25 mm or 100 mm x 25 mm.

Soda glass bottles (600 ml) with polypropylene lids were also used for some of the experiments and has been indicated in the appropriate place. Test tubes were plugged with adsorbent cotton plugs. All empty glassware and water for surface sterilization of explants were autoclaved (1.5 kg $cm^{-2}$, 121°C) for 20 min.

## 2.5. Labware and surgicals

Stainless steel forceps and surgical scalpels were used for the excision of plant tissue during establishment and subcultures. Pads of unglazed paper were autoclaved and used for carrying out the dissections. These were frequently replaced to minimise cross contamination. 70% rectified spirit was used to swab working surfaces and hands before all sterile operations and for flaming surgical implements in between different batches of cultures.

## 2.6. Culture media

The composition of basal media used in this study is given in Table 1. The mineral salts and vitainins according to Murashige and Skoog (1962) were used as the basal medium. Sucrose was added at 2% (w/v) as the carbon source in all the media. All chemicals used in this study were of analytical grade. The medium was prepared using stock solutions of the mined salts and vitamins (stored frozen) in distilled water. After addition of sucrose, necessary additions of growth regulators and other additives were made from stock solutions stored below 5°C and the pH adjusted to 5.7 with 1 N NaOH or HC1.

For preparation of solid medium, agar (Hi-Media.Bombay) was added at 0.5% w/v and melted in a microwave oven before dispensing into the culture vessels. Aliquots of 20 ml were dispensed into each test tube. Culture media were autoclaved at 1.5$kg^{-1}$ $cm^{-2}$ at 12l°C for 15 min. Table 2. gives the composition of different media used in this study.

**Table 1. Composition of Basal Medium (MS*)**

| Sl. No. | Chemical | Concentration in mg/l |
|---|---|---|
| 1. | $KNO_3$ | 1900.000 |
| 2. | $NH_4NO_3$ | 1650.000 |
| 3. | $CaCl_2$. 2 $H_2O$ | 440.000 |
| 4. | $MgSO_4$. 7 $H_2O$ | 370.000 |
| 5. | $KH_2PO_4$ | 170.000 |
| 6. | $MnSO_4$. 4 $H_2O$ | 22.300 |
| 7. | $ZnSO_4$. 7 $H_2O$ | 8.600 |
| 8. | $H_3BO_3$ | 6.200 |
| 9. | KI | 0.830 |
| 10. | $CuSO_4$.5 $H_2O$ | 0.025 |
| 11. | $Na_2MoO_4$.2 $H_2O$ | 0.250 |
| 12. | $CoCl_2$.6 $H_2O$ | 0.025 |
| 13. | $FeSO_4$. 7 $H_2O$ | 27.800 |
| 14. | $Na_2EDTA$.2 $H_2O$ | 37.300 |
| 15. | Myo inositol | 100.000 |
| 16. | Thiamine HCl | 0.100 |
| 17. | Nicotinic acid | 0.500 |
| 18. | Pyridoxine HCl | 0.500 |

*Mineral salts and vitamins of Murashige and Skoog's Medium (1962).

**Table 2. Composition of media used in this study**

Basal Medium : MS + Sucrose 2% (w/v)

| Media | Composition |
|---|---|
| | Concentration of hormones |
| Cytokinin Series | |
| BAP | 1. 2. 4, 8. 16 and 32 µM |
| Kin | 1, 2, 4, 8, 16 and 32 µM |
| BAP+Kin | 0.5. 1. 2. 4. 8 and 16 µM each |
| Auxin Series | |
| 2.4-D | 0.5. 1, 2. 4 µM |
| NAA | 0.5, 1, 2, 4 µM |
| Rooting Media | |
| IBA | 0.5, 1. 2 µM |

*Agar (0.5%w/v) added to obtain solid media wherever needed.

## 2.7. Culture incubation conditions

Cultures were incubated in a culture room where the temperature was maintained at 25±2°c) and the relative humidity between 60 and 80%. The cultures were illuminated with cool daylight fluorescent tubes and the photosynthetically active radiation (PAR] at the level of the cultures was 18 μ E $m^{-2}$ $s^{-1}$. A photoperiod of 8 hr light and 16 hr darkness was provided to the cultures. Cultures were normally maintained for 4 weeks of growth before subculture onto fresh media. Depending on the growth response, the culture period was sometimes increased upto 8 weeks. Cultures were usually evaluated after three passages on a particular media. The mean of 10 cultures was recorded wherever necessary.

## 2.8. Transfer of plants to soil

Plantlets of teak were shifted from the root inducing medium after 15 days to soil after washing the roots with water to remove the traces of medium. A mixture of soil and vermiculite (2:1) in poly bags was used as the transplanting medium. Plantlets were kept in a green house under intermittent mist and hardened for about 10 weeks after which they were shifted to soil in bigger polybags.

# 3. RESULTS AND DISCUSSION

## 3.1. Contamination of initial cultures

High rates of fungal and bacterial contamination in the initial cultures of all the three species was obtained. The contamination was dependent on the season of collection and was particularly high during the months from June to September. This coincided with the rainy season and the period of high relative humidity in the area from where the specimens were collected. The contaminants proliferated particularly on the cut edges of the explants or when tissues expanded or when abscission of bracts and leaves took place. The contamination is hence suspected to be mostly endogenous in origin since the contaminant was not directly in contact with media. In teak and rosewood fungal contamination was predominant, whereas in sandal, bacterial contamination appeared to be dominant.

With the use of anti-microbials for pretreatment of the explants or included in the culture medium some reduction in number of contaminated explants was obtained but there was also an increase in the time taken for contamination to manifest itself in the culture. During months of heavy rainfall. and specially when explants were collected within 2-4 days of rainfall almost all of the explants were lost by contamination in all the three species. Application of Bavistin was effective for teak and rosewood in control of fungus and gentamycin-streptomycin for control of bacteria in sandal.

## 3.2. Browning of explants and media

In explants of teak and rosewood leaching of phenoltc compounds into the medium commenced within a few hours of inoculation. After 24 hrs. browning of the media was severe in mature teak explants and to a lesser degree in teak seedling and rosewood explants.

All pretreatments to reduce the effect of phenolic compounds were effective to varying degrees. Washing of excised explants in running tap water for 1 hr. and treatment of explants with activated charcoal (AC) was most effective in both teak and rosewood.

Some reduction of the inhibitory effect of browning was obtained by shifting the position of explants on the media every 24 hrs, three times. Gupta *et al.* (1980) treated teak buds for 45 minutes with 0.7% Polyclar AT, a high molecular weight insoluble PVP, suspended in 2% sucrose to control the problem of browning.

## 3.3. Shoot multiplication

3.3.1. Teak

Buds collected during May to November gave 100% fungal contamination even with sterilisation for 10 min with 1% $HgC1_2$. Higher concentration or time resulted in rapid killing of the explants. Contamination rates were minimum in buds taken in March and April (45%). Monteuuis (1994) did not give details of the extend of contamination observed in teak taken from Solomon Islands but the surface sterilisation was done with a rather high concentration of $HgC1_2$ (2%w/v) and for a longer period of time than is usual (30 min.). This could have been due to the presence of higher levels of contamination in the tissues used. Such high doses of $HgC1_2$ were, however. found to be damaging to the tissues in the present experiment.

In 60% of buds collected from seedlings (stumps) sprouting was obtained in two weeks. Multiple shoot formation occurred in explants transferred to fresh medium after 4 weeks. Upto 4 shoots were formed on every nodal explant (Fig. 1).Shoots were cut to 1-2 node cuttings for transfer to fresh medium for subculture.

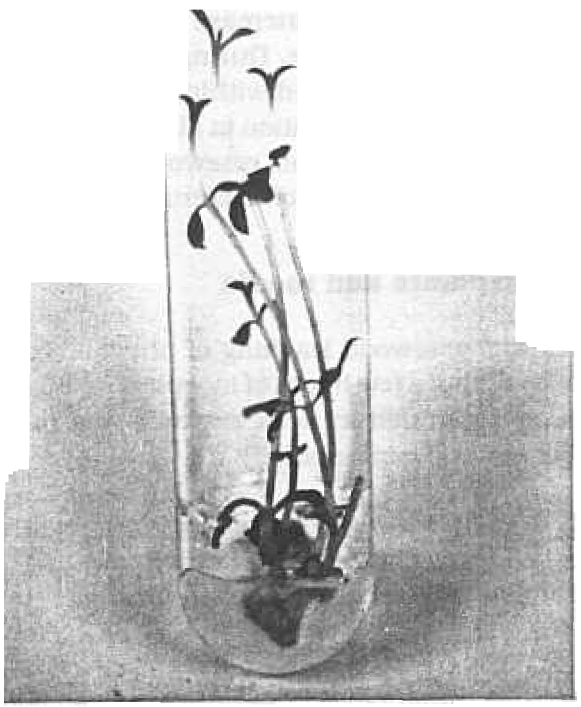

Fig. 1 Shoot cultures of teak derived from seedling nodes

Sprouting of shoot tips and axillary buds (Fig. 2) of mature trees were obtained in only 6% of the buds after 4 weeks of culture. Shoot continued to grow when explants were shifted to fresh medium after 6 weeks. The shoots derived from mature teak had larger leaves and thicker stems when compared to those obtained from seedling material. Multiple shoot formation occured in the second subculture when the shoot was cut into single node cuttings and put on fresh medium. Only 2-4 multiple shoots per node was formed in every passage.

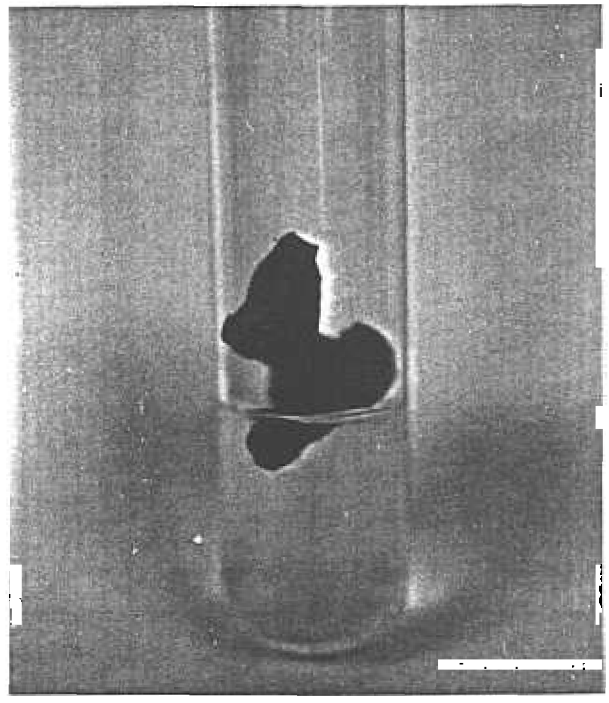

Fig.2. Sprouted shoot tip of mature teak

Shoot cultures derived from buds of mature teak were subcultured on the cytokinin series to test the effect of BAP and Kin, singly and in combinations, on shoot multiplication rates. After three passages the best shoot multiplication was obtained on 16 and 32 µ MBAP and on 8 µ M BAP + 8 µ M Kin (Table 3.1). Elongation and growth of healthy shoots (Fig. 3) was obtained on lower levels of cytokinins. The best results were obtained on 4 µ M BAP, 2 µ M Kin and 0.5µ M and 2 µ M each of BAP + Kin. On media containing both BAP and Kin the plants were healthier and with bigger leaves. Shoots derived from mature trees formed callus at the base of the shoots on all the media but mainly in the higher concentrations. Shoot formation was however always from axillary buds and no callus regeneration was observed.

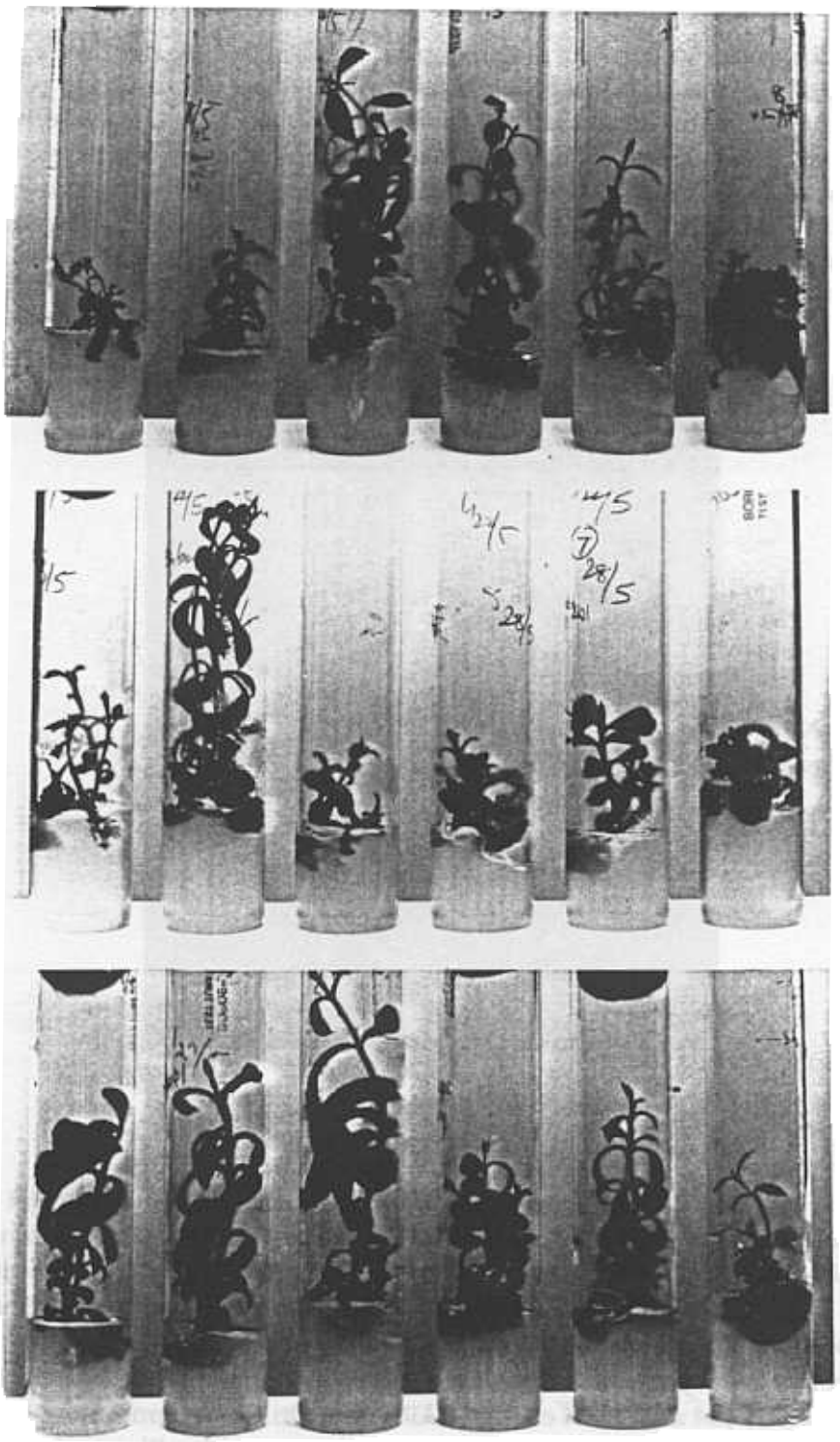

Fig. 3. Effect of cytokinins on shoot multiplication in teak
(Top to bottom - BAP, Kin. and BAP + Kin.)
(Left to right - 1, 2, 4, 8, 16, 32 µM)

**Table 3.1. Effect of cytokinins on shoot multiplication in teak**

| Cytokinin | Conc. (mM) | Multiplication | Elongation | Vigour* |
|---|---|---|---|---|
| BAP | 1 | | - | - |
| | 2 | + | ++ | + |
| | 4 | +++ | +++ | +++ |
| | 8 | +++ | +++ | +++ |
| | 16 | ++++ | ++ | ++ |
| | 32 | ++++ | | |
| Kin | 1 | + | ++ | ++ |
| | 2 | ++ | ++++ | +++ |
| | 4 | ++ | +++ | + |
| | 8 | ++ | ++ | + |
| | 16 | ++ | ++ | + |
| | 32 | +++ | | |
| BAP + Kin | 0.5 each | ++ | ++++ | ++++ |
| | 1 | ++ | ++++ | ++++ |
| | 2 | +++ | ++++ | |
| | 4 | +++ | +++ | ++ |
| | 8 | ++++ | ++ | ++ |
| | 16 | +++ | ++ | + |

- No multiples/tissues dead
+ 1 shoot: Shoots shorter than 15 mm
++ 1-2 multiple shoots:shoots 15-30 mm in length
+++ 3-4 shoots; shoot 30-50 mm
++++ More than 4 multiples: shoots greater than 50 mm
* Scored by visual assessment. Scores are relative.
++++ being a healthy erect shoot with expanded leaves.

To test the effect of shoot tips and nodes as explants for subculture, single shoot tips and a cluster of 2-3 shoot bases were cultured in 600 ml glass bottles. Multiplication was higher in nodes than in single shoots (Fig. 4). The persistence of apical dominance in the presence of cytokinins could be the reason for the poor shoot multiplication from shoot tips as compared to the shoot bases where the tip were excised.

Rooting of shoots was obtained occasionally in the multiplication medium itself. This was mostly found in vigorously growing dominant shoots with larger leaves. Other shoots formed roots in one week, when treated for 24 hrs with 1 mg/l of IBA and shifted to a liquid medium.

Of the plantlets transferred to a sterilised soil-vermiculite mixture (1:1)and plced in a greenhouse, 60% survived after 10 weeks and established well when shifted to soil in polybags (Fig.5).

Fig.4 Effect of explant type on shoot multiplication. (Left - shoots from shoot tips; Right - shoots from nodal explants).

Fig. 5. Plantlet of teak derived from mature tree, 3 months after transfer to soil

3.3.2. Rosewood

High rates of contamination (greater than 80%) was observed in shoot tips and nodes collected in all months from June to February. Survival of buds without contamination could be obtained only in the dry season. Use of 0.2% (w/v) Bavistin in the medium was effective in reducing the contamination to less than 60% and in delaying the appearance of the contamination.

Of the surviving shoot tips and axillary buds sprouting was observed in about 20% of explants in two weeks on cytokinin containing medium. The concentration and type of cytokinin did not appear to have an effect on the initial sprouting response. Shoot tips responded better than axillary buds in developing a sprout. Shoots elongated to about 8-10 mm. in 4 weeks on different concentrations of cytokinins but further development was not obtained. The shoots had only bract-like structures and these often fell off before 4 weeks. The unelongated shoots remained live for a further subculture of 4 weeks after which it turned brown. No multiple shoots were formed in any of the cultures.

Leaf explants cultured on media containing 2,4-D or NAA or 2.4-D and NAA gave rise to callus on all the concentrations. Coconut milk was not essential to obtain callus growth. The callus which developed at the cut edges of the leaf explants was brownish white in colour and soft and friable in texture. The media below the callus often turned brown in many of the cultures. The use of PVP was ineffective in reducing browning in callus cultures. Since the browning did not appear to be inhibitory to callus growth, other antioxidants were not tested. When subcultured to fresh medium the callus could be maintained in a healthy growing state. The maximum callus formation was obtained on 2 µ M 2.4-D.

Calli transferred to the regeneration media containing BAP and NAA and shifted to light developed green and hard nodules. No evidence of organogenesis was however observed. All the different media reported to induce organogenesis failed to give results in this study.

3.3.3. Sandalwood

Nodal and shoot tip explants showed abscission of leaves and petioles after about a week in culture on all the different media. The axillary buds in the nodal explants were swollen and bracts covering them partly open or abscised at this stage. Some amount of soft translucent-white callus developed on the abscission zone. After three-four weeks of culture the buds had elongated in about 20% of the nodal explants cultured on cytokinin containing media (Fig. 6). Multiple shoot formation (4 shoot intials) was observed on media containing BAP (0.5µ M)and Kin (0.5 µ M). Explants also showed callusing

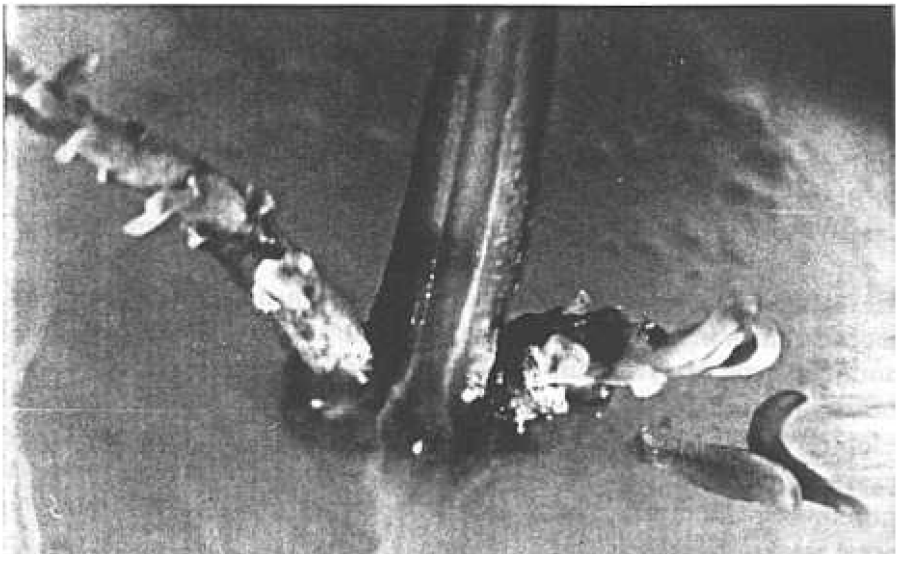

Fig. 6. Nodal explant of mature sandal with axillary bud sprouts

on this medium. Shoot tip explants did not show any sprouting response although they remained green and live at this stage.

Internode explants cultured on auxin containing media showed rupture of the epidermis and formation of callus after one week of culture. The callus grew very slowly and turned light brown in colour. Callus formation was best on 2.4-D containing medium (Fig. 7). Subculture of explants with callus was done after 4 weeks but further callus growth was not obtained. On media containing auxins combined with cytokinins the callus growth was better initially but on subculture no growth could be obtained. No sign of morphogenesis was observed in any of the cultures even after 3 subcultures.

In the present study the material used was collected from Marayur whereas in the work done by other workers the material is presumed to have been always collected from Karnataka. The influence of genotypic differences on response of plant tissue in culture has been recorded in several species. The phenotypic difference in trees from different provenances of sandal wood is well known. The failure to obtain results using the media reported by earlier workers can thus be attributed to the genotypic differences. The efficiency of the various *in vitro* responses like percentage of explants that gave callus, the number of cultures that were embryogenic etc. are not clearly reported in the available literature.

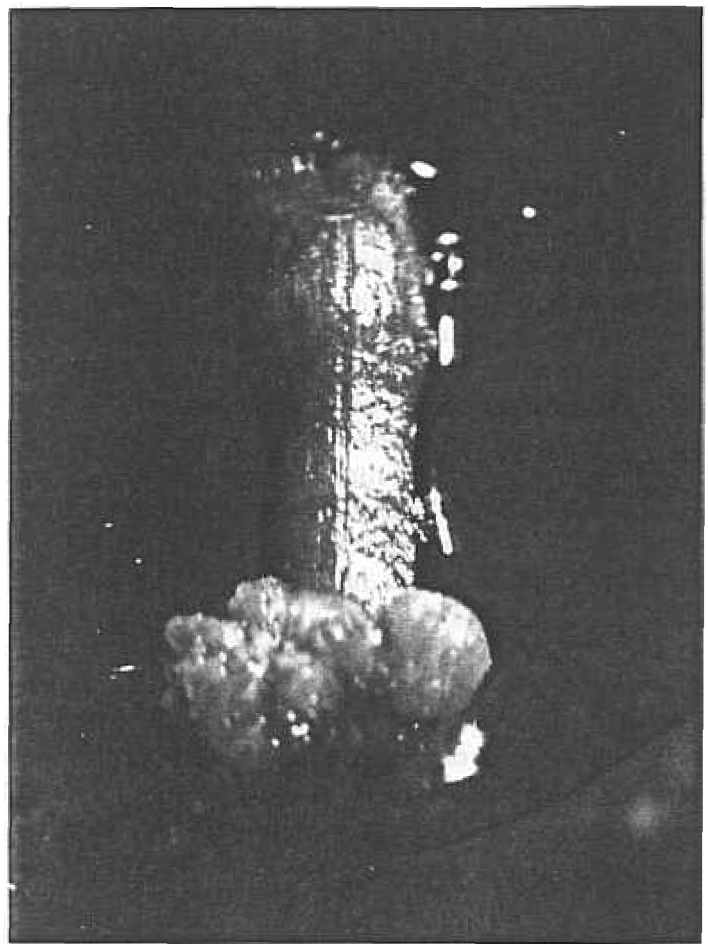

Fig. 7. Internode of sandal with callus formation on cut ends

The induction of multiple shoot formation through culture of nodes or shoot tips has not been described in sandal wood by any of the groups working on sandal wood even though the method would have been the most preferred means of clonal propagation. It is to be concluded that no success in regenerating plants was obtained as in the present study. Perhaps a complete reevaluation of the composition of the basal medium is required to obtain a better response.

# 4. CONCLUSIONS

Methods for micropropagation of teak, rosewood and sandal have been reported by researchers in India. Particularly in rosewood and sandal different groups have been successful in regeneration of plantlets through organogenesis or somatic embryogenesis. However,in the present studies the results have not been satisfactory in any of the three species. One of probable reasons, as already discussed (Section3.3.3). is the difference in provenances of the trees of all the three species used in the experiments. All the work by other groups were with trees from the drier plains whereas in Kerala the trees were from a high rainfall area. The high relative humidity during a large part of the year was responsible for the higher incidence of contamination in initial cultures. It appears that the practical solution for the problem in Kerala is to restrict initiation of cultures to the January-June period.

In rosewood, since callus cultures did not respond to the media reported in literature, a wider range of parameters needs to be tested to find the appropriate media for the provenance. The initial response in multiple shoot formation from axillary buds of sandal wood needs to be optimized and further development of shoots and plantlet formation obtained. Media for induction of somatic embryogenesis from mature tissues also needs to be standardized for Kerala.

The study highlights the importance of standardising all stages of micropropagation of tree species including methods of obtaining sterile material for initiating cultures from field grown trees. As observed in several other plant species there is a high influence of genotype on the in *vitro* responses. The high genetic heterogeneity of forest trees which are essentially still wild populations will pose problems of repeatability since micropropagation protocols are usually developed based on studies done with a few genotypes.

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