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# Develop Teak Tissue Culture Methodology

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# ကျွန်းတစ်ရှူးမွေးမြူရေးစနစ်ဖော်ထုတ်ခြင်း

စောစီဒူး

သစ်တောသုတေသနဗဟိုမာန်

## စာတမ်းအကျဉ်းချုပ်

ကျွန်းမျိုးကောင်းမျိုးသန့်ဖော်ထုတ်ရာ၌ အသုံးချနိုင်မည့် ကျွန်းတစ်ရှူး မွေးမြူရေးစနစ်ကို လေ့လာထားခြင်း ဖြစ်ပါသည်။ ကျွန်းဖူး၌ ပါဝင်သော အစိတ်အပိုင်းများကို ဓါတ်နှင့်ဟိုမုန်းအမျိုးမျိုး ပါဝင်သော ဓါတ်စာများတွင် ကျွန်းပျိုးပင်များရရှိစေရန် နည်းအမျိုးမျိုးဖြင့် စမ်းသပ်ထားသော စာတမ်း ဖြစ်ပါသည်။

# Develop Teak Tissue Culture Methodology

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## Abstract

The paper dealt with the unique teak tissue culture methodology necessary to produce seedlings for the development of seed orchard.

Various cultural methods were tested with different culture media. Different plant parts from the teak bud were tested for the production of teak seedlings.

## Contents

## 1. Objective

(1) To develop unique tissue culture methodology necessary to produce seedlings of the chosen species.
(2) Develop the production methodology necessary to mass produce seedlings for planting in seed orchards around Burma.

## 2. Background

Due to the importance role teak plays in the economy of Burma there are great pressures to over-cut the Teak forests. In many instances the natural forest is not regenerating successfully necessitating planting to re-establish the Teak forest. Rather than plant these site with trees or poor genotypic constitution (as is currently done as seed for plantation projects are randomly selected with no regard to important and genetically controlled features of the mother tree), seed from superior trees (good form, straight grain, proper heartwood color, etc.) should be utilised. Seed orchards grown from selected mother trees will provide such planting stock.

Unfortunately vegetative reproduction of superior teak mother trees cannot always be used to establish seed orchards. Cuttings are hard to root and when do, generally form a fibrous rather then the tap rooted system produced by plants grown from seed. Such trees may prove not to be wind firm. Bud grafting and cleft grafting of superior scion material on randomly chosen stock trees, while currently considered an acceptable method of seed orchard establishment, has been shown in certain situation to result in cambial decay at the graft juncture. This sometimes does not manifest itself until the seed are over 20 years old. Seedling produced by tissue culture will not have these defects.

Other forest tree species of Burma, while not of as great and economic important as teak (as a generators of foreign exchange), none the less are commercially important. Many poorly stocked or denuded site are currently being planted to these species. Again it would be desirable to plant these sites using seedling from seed of genetically superior mother trees. As with teak, there are again problems entailed with the vegetative reproduction of "superior" mother trees and thus tissue culture tree production is initiated.

Among the teak forests in several regions of Burma, a few superior trees have been identified which are 50-80 years old and have wood of high quality. Suitable methods are not available for the propagation of such trees by cuttings etc. to give plant with a healthy root system with a tap root.

There has been reports on differentiation and organogenesis, mainly of Conifers (1-8) and the propagation of forest trees (9-16) but very little work has been done on the propagation of mature teak trees by tissue culture.

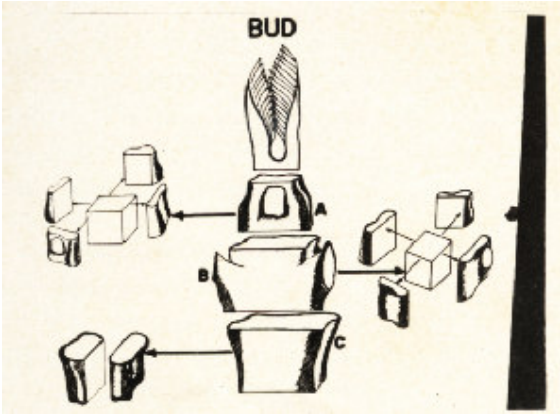


Fig .1

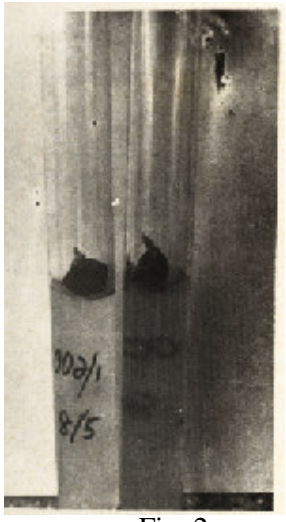

Fig .2

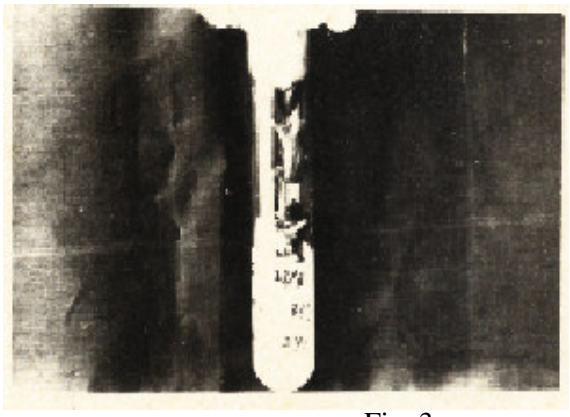

Fig .3

Fig 1. Explants used in teak tissue culture. A = Apical bud. B = Pith region. C = Cambium region.

Fig 2. Initiation of shoot after the explant was cultured in MS-1 media. (15 days old)

Fig 3. Development of shoots after the explant was transferred from MS-1 media to MS-A (added with auxins and kinetin)

## 3. Materials and Methods

Auxiliary and terminal buds for tissue culture propagation were collected from both young and mature trees.

The buds were thoroughly washed in a running water for 15 minutes and excised into three parts the apical bud, the pith and the Cambium (diagram 1.) The materials obtained were then sterilized with 1% Hgel for 2 minutes, rinse in a sterilized distilled water and again sterilized in a 5% Ca-hypoclorite for 10 minutes. Tween wetting agent was used before sterilization.

The material were then rinse in sterilized distilled water and culture in 25 mm x 150 mm test tube containing different media.

A septic conditions was observed throughout the operation.

## Culture Media

The following culture media were used in the course of the experiment. Murashige and Skoog basic media, (MS-1) Murashige and Skoog high salt media, (MS-2) Murashige and Skoog multiplication media A and B (MSA and MSB).

Gamborg's B-5 supplement with 0.5 mg/L IAA, IBA and 2 mg/L of Kinetin. (G).
White's media. (W)
Abacea starting media. ($A_1$ )
Anderson media. ($A_2$ )

The $p^H$ of all the media was adjusted to 5.8. Either solid medium (20 ml containing 0.8% agar in 25 mm x 150 mm test tube) or liquid medium (10 ml in 100 ml conical flask) were used for shoot initiation.

Liquid medium (20 ml in test tube with filter bridge) was used for the induction of rooting. The cultures were kept in a room temperature (28 °C - 30 °C) for 12h in light (1000 lux) and 12h in dark.

All chemicals used were of analytical grades from Sigma and Difco Co.

A 0.5% Polyclar AT (an insoluble Polyvinylpyrrodone, PVP) was also used in the removal of phenolic substances in the tissue.

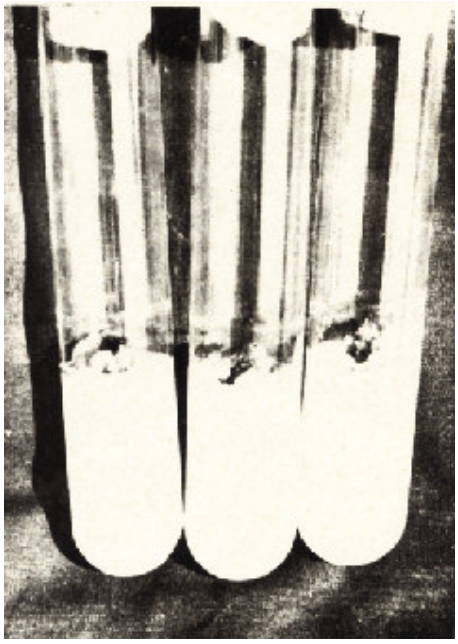

Fig 4. 10 days old callus formed from the explant ( Pith region)

Fig. 5. Development of callus in MS-A media (added with auxins and kinetin) 20 days old. The callus can be multiplied indefinitely in a fresh media.

## 4. Results

Cultural media such as MS-1, MS-2, G,W, $A_1$ and $A_2$ were used as starting medium. Of all the medium MS-1, G, were better than the rest of the media. At least 70% -80% of the tissue proliferated within 5-7 days. After 14 days in the media, tissue obtained from the apical bud produced young shoots. Callus formation was formed with the tissue from the pith and cambium region. The shoots in MS-1 and (G) media did not grow, although they were kept for (30) days in room temperature under light. The shoots (2 cm long) have to be transferred to MS-A and MS-B media for further growth after 14 days in MS-1 and G media.

The shoots than grow to about 6-8 cm with 6-7 nodal section after 14 days.

## Rooting

This was the most difficult section of the experiment. Different concentration of auxins such as IBA (Indole-Butyric-Acid), IAA (Indole-Acetic_Acid), and IPA (Indole-Pupionic-Acid) were added to MS-A media for rooting.

However there was still a negative result. When the shoots were out to about (1) cm in length with (1) nodal section and transferred to MS-1 liquid media added with IBA (0.2 mg/L) and IAA (0.1 mg/L) and also supported by filter bridge, the root developed within 6-7 days. After 5 days the shoot and root were transferred to MS-2 media for further growth.

When tissue from one year old and fifteen year old teak were cultured MS-1 media, younger had a vigorous proliferation compared to older tissue.

Seasonal effect was also found to be significant in the collection of tissue materials for culturing. The most suitable time for collection of tissue materials was found to be in between February and April.

Temperature and light also were paramount important in the development of tissue materials. A 25 °C with a 16 hr. light gave better result in most of the tests.

Fig.6. Development of shoot from apical bud region in MS-A media 30 days old. Development of root is not observed in this picture. The root developed only after nodal section is placed in MS -1 liquid media supported by filter bridge.

## Callus Proliferation

Callus formation was obtained from all the tissue parts Viz. apical bud, pith region, and cambium region. However, the callus did not produce either shoots or roots, when treat with different concentration of auxins and Kinetin.

Although the callus from MS-1 was transferred to MS-A media with auxins and Kinetin and agitate on a shaker for 7 days, there was no changes in the growth of the tissue.

## 5. Discussion

Teak tissue culture has never been done in this country. This is the first attempt to mass produce seedlings of superior quality having the same genotype of parent characteristics. As the chromosome number of root tips of the conventional raised seedlings and of plantlets obtained from the tissue were the same; i.e. 36 (1); there was no danger to argue about the variation of the plantlet.

The methods describe above were not a complete report of the experiment, as more work still remained to accomplish in the near future. The report confined to a break-through of teak tissue culture methodology for mass production in the future.

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