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# Study on effect of various hormones in *in-vitro* culture of teak (*Tectona grandis* Linn.f.)

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## Table of Contents


| | | Page |
|---|---|---|
| | Abstract | i |
| | စာတမ်းအကျဉ်း | ii |
| 1. | Introduction | 1 |
| 2. | Objectives | 2 |
| 3. | Literature Review | 2 |
| 4. | Materials and Methods | 4 |
| 5. | Results and Discussions | 6 |
| 6. | Conclusion | 16 |
| 7. | Acknowledgement | 17 |
| 8. | References | 18 |
| | Appendix | 20 |

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## Abstract

This paper presents the effect of various hormones in *in-vitro* culture of teak (*Tectona grandis* Linn.f.). Micropropagation of selected six clones derived from plus trees teak plantations of Compartment No.72, Ngalaik Reserved Forest, Moe-Swe Research Station, Ottrathiri Township, Nay Pyi Taw was carried out successfully. Shoot cultures were induced to form by culture of shoot tips of epicormic shoots that sprouted from branch cuttings maintained in the mist chamber. Surface sterilized were inoculated on a modified MS, WPM and B5 media. The best medium was with MS. Multiple shoot formation was obtained in first subculture with Kinetin (Kin) in test tubes. The good performance of shooting was found at hot season. Multiple shoot formation was obtained in second subculture onwards on the same media with 0.001 mg/l BAP and 0.001 mg/L Kin. Shoot cultures were maintained through subculture carried out at 4-6 week intervals. *In-vitro* rooting stage, microshoots treated with MS media with BAP (0.001) mg/l and NAA (0.001) mg/l and BAP (0.001) mg + NAA (0.001) mg with half (MS) media. Furthermore, at Fertistart (10ml/lit) for 10 minutes, basal callusing was observed and root formation took place after 4 weeks of transfer. The best result was recorded at BAP (0.001) mg + NAA (0.001) mg with full (MS) media as it gave the maximum number of roots per shoot and root length.

**Keywords:** MS media, Micropropagation, Kinetin (Kin), 6-Benzyle Amino Purine (BAP), Naphthalene Acetic Acid (NAA)

# ကျွန်းတစ်သျှူးမွေးမြူရာတွင် ဟော်မုန်းအမျိုးမျိုးတို့၏ အကျိုးသက်ရောက်မှုကို စမ်းသပ်လေ့လာခြင်း

ဒေါ်ခင်ပပရွှေ (လက်ထောက်သုတေသနအရာရှိ)
ဒေါက်တာသန့်ရှင်း (လက်ထောက်ညွှန်ကြားရေးမှူး)

## စာတမ်းအကျဉ်း

ဤသုတေသနစာတမ်းသည် ကျွန်းတစ်သျှူးမွေးမြူခြင်းနည်းဖြင့် စမ်းသပ်ဆောင်ရွက်ရာတွင် ဟော်မုန်းအမျိုးမျိုးတို့၏အကျိုးသက်ရောက်မှုကို စမ်းသပ်လေ့လာထားခြင်း ဖြစ်ပါသည်။ မိုးစွေ၊ ငလိုက်ကြိုးဝိုင်း၊ အကွက်အမှတ် (၇၂)၊ ဥတ္တရသီရိမြို့နယ်၊ နေပြည်တော်မှ ကျွန်းမျိုးသန့်ကလုန်း(ဖ)မျိုးတို့ကိုရွေးချယ်၍စမ်းသပ်ဆောင်ရွက်ခဲ့ပါသည်။

စတင်မွေးမြူခြင်း အဆင့်တွင် ရေငွေ့ထိန်းအိမ်ခန်းအတွင်းမွေးမြူထားသော အညွှန့်ထိပ်ပိုင်းများဖြင့် စမ်းသပ်ဆောင်ရွက်ခဲ့ပါသည်။ ပိုးသန့်စင်ထားပြီးသော အညွှန့်ထိပ်ပိုင်းများကို အခြေခံမျိုးပွားစာ MS, WPM နှင့် $B_5$ သုံးမျိုးတို့တွင် ခါတ်ခွဲခန်းအတွင်း၌ စတင်စမ်းသပ်မွေးမြူခဲ့ရာ MS မျိုးပွားစာတွင် ရှင်သန်မှုအကောင်းဆုံးဖြစ်ပါသည်။ ရွေးချယ်ခဲ့သောကလုန်းများအား ပထမအကြိမ် ပွားများ မွေးမြူခြင်းအဆင့်တွင် MS မျိုးပွားစာနှင့်အတူ kinetin (0.001)mg/lတွင် စမ်းသပ်ခဲ့ရာ နွေဥတု တွင်ရှင်သန်မှုအကောင်းဆုံးဖြစ်ပါသည်။ ပထမအကြိမ်ပွားများမွေးမြူခြင်းအဆင့်ကို စမ်းသပ်ပြီး နောက် သီတင်းပါတ် (၄–၆)အကြာတွင် ဒုတိယအကြိမ်ပွားများမွေးမြူခဲ့ရာ MS မျိုးပွားစာနှင့် အတူ BAP(0.001)mg/l + kinetin (0.001) mg/l ပေါင်းစပ်မှုဟော်မုန်းတို့တွင် စမ်းသပ်ဆောင်ရွက်ခဲ့ပါသည်။ အမြစ်ထွက်မွေးမြူခြင်းအဆင့်တွင် MS+ BAP (0.001) mg/l + NAA (0.001) mg/l နှင့် ½MS+ BAP (0.001) mg/l + NAA(0.001)mg/l မျိုးပွားစာတို့ဖြင့် ခါတ်ခွဲခန်း အတွင်း စမ်းသပ်ဆောင်ရွက်ခဲ့ပါသည်။ အခြေပိုင်းတွင်ကဲလ်လပ်ထွက်ရှိခဲ့သော အပင်ငယ်များကို ခါတ်ခွဲခန်းပြင်ပတွင် အမြစ်ထွက်ဟော်မုန်း (Ferti–Start) ၌ (၁၀)မိနစ်စိမ်၍ စမ်းသပ်ခဲ့ရာ လေးပါတ်အကြာတွင် အမြစ်ထွက်ရှိခဲ့ပါသည်။ အမြစ်ထွက်အားအကောင်းဆုံးမှာ MS+ BAP (0.001) mg/l+ NAA (0.001) mg/l ဖြစ်ကြောင်း တွေ့ရှိရပါသည်။

# Study on effect of various hormones in *in-vitro* culture of teak (*Tectona grandis* Linn.f.)

## 1. Introduction

Teak (*Tectona grandis*) has the reputation as one of the best timber species in the world because of the durability and appearance of its wood. The timber is suitable for a wide range of uses and there is a high worldwide demand. Teak is indigenous to South East Asia but today grown in several countries of Africa, Central and South America. Because of the problems associated with the conventional tree breeding and genetic improvement of trees in general, clonal forestry with selected clones of superior performance has been widely adopted as a short term measure for improving the quality of plantations (Kaosa-ard *et al.*, 1987; Libby and Ahuja, 1993). Mascarenhas *et al.* (1987), Monteuuis (1999), Palanisamy *et al*. (2003) have highlighted the importance of the using clonal planting stock in teak.

Tissue culture techniques have several advantages over traditional methods of propagation especially when the number of propagules to be raised from a limited stock is large. Not only are the multiplication rates much higher than vegetative methods, but also the plantlets produced are compact, uniform and free of pests and diseases. Year around production is also feasible. Forest tree are usually propagated by seeds, seedlings and stumps. Vegetative propagation is a very useful tool which has been practiced in replicating clonal material. A modern technique of vegetative propagation by grafting, budding and tissue culture also being used for the establishment of clonal orchard. Progress in teak tissue cultures provides tremendous opportunities for promoting mass reproduction of improved material.

Using the tissue culture technology developed, it will be possible to successfully introduce and mass produce any selected Plus-trees, regardless of ortet age. Tissue-cultured plantlets are obviously the only means currently available for successfully exporting teak clones to overseas destinations. Proper coordination in dispatch and arrival timing has resulted in successful deliveries worldwide.

The influencing factors on achievement of tissue culture include explants, sterilization, culture medium, plant growth regulators and cultural conditions. Explants exhibit different capacities for *in vitro* regeneration depending on their location on the donor plant. For example, survival and growth of terminal bud explants are typically greater than lateral bud. The lateral bud explants from bottom to top of single plant part may differently respond *in vitro* regeneration. Additionally, explants exhibit greater dependence on culture medium supplementing with exogenous plant growth regulators for maximum shoot survival and development (Shabde and Murashige, 1977).

The type and concentration of plant growth regulators supplementing into nutrient media are one of the most influential factors affecting the efficiency of *in vitro* regeneration (Smith 2000). Exogenous application of cytokinin namely BAP, kinetin, Zeatin promote the shoot formation in many plants. Among them BAP and Kinetin are commonly used for shoot induction and multiplication in woody plant. The mineral nutrient medium used in previous research is MS (Murashige and Skoog's) medium with sucrose (3% w/v) and agar (0.8%). The response of explants to plant growth regulator, culture medium and growth conditions

vary with different species and even different genotype within same species (George and Sherrington, 1984).

## 2. Objectives

This research was conducted to fulfill the following objectives:

1) To determine effect of plant growth regulator in *in vitro* culture of teak.

2) To determine the seasonal variation influencing of the development of shoots and roots from cutting.

## 3. Literature Reviews

Relevant literatures were cited to support the results of this study.

### 3.1 Background history of teak *in vitro* propagation

Due to the importance role teak plays in the economy of Myanmar 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. 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. Among the teak forests in several regions of Myanmar, a few superior trees have been identified which are 50-80 years old and have wood of high quality.

### 3.2. Affecting factors on *in vitro* regeneration

The success of *in vitro* regeneration is influenced by many factors. These factors include temperature, light, nutrient and plant growth regulator containing in culture media and cultured part of plant.

#### 3.2.1. Temperature

Temperature is one of affecting factors on growth and development of plants as well as behavior of propagules during storage and germination. In most of *in vitro* culture mean culture temperature are generally higher than those which would be experienced by plants of

same species *in vivo*. The average culture temperature employed in a large sample of experimental reports is 25˚C (George and Sherrington, 1984).

### 3.2.2. Light intensity and photoperiod

Light influence on growth and morphogenesis *in vitro* and fluorescent tube is almost universally used for artificial lighting in most micropropagation (George and Sherrington, 1984). Light requirement involves a combination of several components like intensity, quality and duration. An optimum combination of these is required for certain phytomorphogenic events. According to Murashige (1974) the optimum duration of light exposure or photoperiod is sixteen hours for a wide range of plants. In previous works of micro propagation of *Tectona grandis* all cultures were maintained under a 16/8 hour light / dark photoperiod with different light intensity.

### 3.2.3. Culture medium

The success of *in vitro* propagation is greatly influenced by the nature of culture medium. The components of *in vitro* culture medium include mineral nutrient, vitamins, a carbon source, unidentified supplements, plant growth regulators and solidifying agent. Table sugar or sucrose is nearly always added in culture media as carbon source. Agar is exclusively used as the solidifying agent for preparation of solid or semi - solid media. Unidentified supplements were sometimes used to obtain improved result. These include fruits juices, banana, potato, yeast extracts and coconut milk. Unidentified supplements are popular addition to media for orchid culture while they were not used in bamboo *in vitro* culture. The pH of media is highly important since it influences the uptake of various components of media as well as regulating a wide range of biochemical reaction occurring in plant *in vitro* culture. The optimal pH varies with cultured species and type of culture (George and Sherrington, 1984). For *in vitro* culture of *Tectonia grantis* pH of media was adjusted to 5.8 prior to autoclaving at 121˚C for 20 minutes (Aguihotri *et al.*, 2009 and Kaur *et al.*, 2011).

### 3.2.4. Mineral nutrient

Without mineral nutrient, a plant cannot live in both *in vitro* and *in vivo* (Pierik, 1987). Cultured plant tissues require in relatively large amount and such elements are termed macronutrients or major nutrient or essential nutrients. These elements are required more than 1000 ppm and are nitrogen, phosphorus, potassium, calcium, magnesium and sulfur. Some elements are required in very small amount by plant tissues and termed micronutrient or minor nutrient or trace nutrient. These elements are required less than 1000 ppm and include iron, manganese, zinc, boron, copper, molybdenum and chlorine. In order to avoid inaccuracies and inconsistencies in molecular formulae of the inorganic components the media with accurate concentration and chemical formulation including Murashige and Skoog (MS, 1962) were published by George and Sherrington, 1984.

### 3.2.5. Vitamins

Vitamins have catalytic functions in enzyme system and are required only in trace amounts. The most frequently used vitamins for plant *in vitro* cultures are thiamine, pyridoxine (vitamin $B_6$) and nicotinic acid (George and Sherrington, 1984).

### 3.2.6. Plant growth regulators

Plant growth regulators are organic compound which influence the growth and development of plant tissues. These compounds are usually active at very low concentration and known as plant hormone. The growth hormones are generally grouped as auxins, cytokines, and gibberellins, ascorbic acid, growth inhibitor ethelene. In *in vitro* culture of higher plants auxin and cytokinin are very significant. Auxins are used for induction of callus from explant, embryogenesis and root formation. Low concentration promotes root formation whereas high concentrations fails root formation and promote callus formation. The naturally occurring auxin is IAA and synthetic auxins are IBA, NAA and 2,4 D. Cytokinins promote cell division in plant tissue and stimulate bud proliferation but inhibit rooting. Kinetin, BAP, 2iP, and PBA are common used cytokinins. In high concentration, they induce adventitious shoot formation (Pierik, 1987).

## 4. Materials and Methods

There were three experiments in this study.

Experiment I. Effect of basal media on shoot induction

Experiment II. The response of different concentration levels of Detergent and Running tap water treatment on phenolic exudation of teak clones

Experiment III. The effect of seasonal variation in different clones

### 4.1. Plant materials and explants source

Shoot tips for initiation of cultures were obtained either from dormant buds collected from branches of plus trees of teak from Compartment No. 72, Ngalaik Reserved Forest, Moe-Swe Research Station, Ottrathiri Township, Nay Pyi Taw or from epicormic shoots induced on branch cuttings maintained in soil filled bags placed under Mist Chamber, Forest Research Institute, Yezin. All the explants were maintained at 25-32º C (Temp) and 90-100 % (RH) in it.

Axillary buds and shoots tips of the epicormic shoots developing after a few weeks of placing the branch cuttings were collected and used as the explants to initiate cultures. The teak clones used for this study were 17/1, 9/1, 9/2, T21, 17/4 and 20/1.

### 4.2. Sterilization of explants

Axillary shoot explants and epicormic shoots were collected into vessels containing tap water. Shoots were thoroughly washed in running tap water for 30 min and 60 min to remove adherent particles, immersed in 0.5 and 1.0% (w/v) solution of Bavistin, a fungicide for 30 min, then treated with 5% (v/v) Tween 20 solution for 20 min and rinsed thrice with sterile distilled water followed by surface sterilization in 0.1% (w/v) mercuric chloride

solution under sterile conditions for 10 min for axillary shoots and 5 min for epicormic shoots and then rinsed thrice with sterile distilled water. The disinfected shoots (1.5 cm) were inoculated onto shoot induction medium.

### 4.3. Culture media

Murashige and Skoog (MS 1962) medium was used as primary basal medium for *in vitro* studies. Other media like WPM (Lloyd and McCown, 1980) and $B_5$ also tested for comparison of shoot induction and proliferation. Stock solutions were made and stored for many of the chemicals for convenience and accuracy and also ease of storage. All chemicals used in this study were of analytical grade purchased and water was used for preparation of all media was double glass distilled. Stock solutions of the mineral salts and vitamins in distilled water were stored in the refrigerator or freezer respectively. After addition of sucrose, necessary additions of growth regulators and other additives were made from stock solutions stored below 5$^{o}$C. The pH of the media was adjusted to 5.7 with 1 N NaOH or HCl. Agar was added at 0.8 % w/v and melted in a microwave oven before dispensing into the culture vessels. Aliquots of 20 ml were dispensed into each test tube. Media were dispersed into borosilicate test tubes of the size 150 mm x 25 mm or 100 mm x 25 mm (20 ml media /tube) or 600 ml glass bottles with PP lids (30 ml/bottle). Culture media were autoclaved at 1.5 kg-1 cm-2 at 12l˚C for 20 min.

### 4.4. Culture conditions

All cultures were maintained in the culture room where a temperature of 25° C ± 2 °C was maintained through air-conditioning. A photoperiod of 8 hrs light and 16 hrs darkness was provided to the cultures. Cultures were normally maintained for 4-6 weeks of growth before subculture onto fresh media. Depending on the growth response, the culture period was sometimes increased up to 8 weeks.

### 4.5. Stages in culture

#### 4.5.1. Initiation:

Shoot tips were surface sterilized according to the sterilization methods. After treatment to minimize browning due to phenolic exudation and oxidation. Explants were inoculated in test tubes on a solid initiation medium consisting of the basal media supplemented with Agar 0.8 % w/v and sucrose 3 %. The PH was adjusted to 5.7 before autoclaving. The cultures were incubated for 4 week in 25±2ºC under 80-90 % relative humidity. After four weeks the cultures were observed and the successful initiated contamination free cultures were taken for further shoot multiplication.

#### 4.5.2. Multiplication:

Sprouted shoots from shoot tip explants were subcultured on to fresh media and when multiple shoot formation was observed after two- subcultures, shoots were split into clusters of 3-4 shoots for transfer to fresh media. Combination of Cytokinin (BAP and Kinetin) were tested for shoot multiplication.

**4.5.3. Rooting:**

After successful shoot multiplication, the cultures with well morphological characteristics having healthy shoots and good inter nodal gap were selected for root induction. For induction of roots, the shoots of length 3-4 cm were taken and dissected and inoculated in BAP (0.001) mg/l + NAA (0.001) mg/l. After inoculation the cultures were kept for 16 hour photoperiod and 25- 27º C temperatures. Successful rooting percentages were recorded after 30 days.

**4.5.4. Hardening:**

The base of the shoot was dipped in Fertistart for 10 minutes and planted in a 1: 1 mixture of vermiculite and sand in trays. Rooted plantlets after 8 weeks in the mist chamber were transferred to soil in polybags and placed in shade for a week before shifting to the nursery. After successful root induction the plants were transferred to greenhouse for acclimatization. The cultures were kept in greenhouse under relative humidity of 90% with mist chamber.

**4.6. Statistical Analysis**

The experiment was conducted using Completely Randomized Block Design (CRB) with 20 explants for each clone. Collected data has been processed and accumulated by using Microsoft Office Excel 2003.The recorded data has been statistically analyzed by using statistical software. Analysis of Variance (ANOVA) was done by using Statistix 8.0. Mean values were separated by using Least Significant Differences (LSD) test as 0.05 level.

**5. Results and Discussions**

**5.1. Experiment I. Effect of basal media on shoot induction**

**5.1.1. Difference between dormant and epicormic shoots as explants:**

Plus trees, the original source of the explants used to initiate clones are located in remote forest plantations and being very large trees with no lower branches does not permit easily access to the canopy. It is therefore expensive and damaging to the tree to collect explants each time cultures have to be initiated. It is more convenient to collect branch cutting and use the epicormic shoots as source of explants.

**Table 1.a** Dormant shoots in MS (Supplements: BAP and Kinetin)

| **Cytokinin (µM)** | | **% Response** | **Mean no. of shoots/explant** | **Mean shoot length (cm)** |
|---|---|---|---|---|
| **BAP** | **KIN** | | | |
| 0.5 | - | 75 | 3.00 | 1.80 |
| 1.0 | - | 90 | 7.74 | 1.96 |
| 2.0 | - | 80 | 6.00 | 1.46 |
| 2.5 | - | 60 | 0.16 | 0.80 |
| - | 0.5 | 65 | 0.50 | 1.00 |

| | | | | |
|---|---|---|---|---|
| - | 1.0 | 80 | 5.00 | 1.76 |
| - | 2.0 | 70 | 1.16 | 1.26 |
| - | 2.5 | 50 | 0.10 | 0.40 |

**Table 1.b** Epicormic shoots in MS media (Supplements: BAP and Kinetin)

| Cytokinin (µM) | | % Response | Mean no. of shoots/explant | Mean Shoot length (cm) |
|---|---|---|---|---|
| **BAP** | **KIN** | | | |
| 0.5 | - | 50 | 2.20 | 3.20 |
| 1.0 | - | 65 | 3.80 | 4.10 |
| 2.0 | - | 60 | 2.06 | 3.56 |
| 2.5 | - | 55 | 1.10 | 3.00 |
| - | 0.5 | 45 | 0.56 | 2.33 |
| - | 1.0 | 50 | 1.90 | 2.76 |
| - | 2.0 | 35 | 0.13 | 2.50 |
| - | 2.5 | 40 | 1.06 | 2.26 |

Dormant shoots while difficult to collect in large numbers from the plus trees gave better results in initiation of cultures (Table 1a and b). Up to 90 % of shoots sprouted compared to only 65% of the epicormic shoots. Fungal contaimination was a serious problem in the case of latter suggesting that prophylactic treatments should be used in the protocol. The mean number of shoots obtained from the different explants is also significantly different with more than double the shoots resulting from the dormant shoots. For practical reasons the use of epicormic shoots for culture is much more preferred for routine micropropagation since it is envisaged to set up a clonal hedge garden to serve a source of explants of the plus trees.

### 5.1.2. Effect of different basal media on shoot induction and proliferation from epicormic shoots

The MS Medium was found to give significantly higher results in the mean number of shoots produced per explants as well in the shoot length produced.

**Table 2**. Effect of basal media on shoot induction

| Media | % Response | Mean no. of shoots/explant | Mean shoot length (cm) |
|---|---|---|---|
| MS | 85 | 13.00 | 5.80 |
| WPM | 75 | 5.00 | 4.20 |
| B5 | 53 | 1.30 | 2.10 |

### 5.2. Experiment II. The response of different concentration levels of Detergent and Running tap water treatment on phenolic exudation of teak clones.

#### 5.2.1. Initiation

On the initiation medium consisting of MS basal media supplemented with Kinetin (0.001mg/l) and sucrose 3 %, shoot tip explants sprouted to give rise to shoots. Severe browning of the media immediately below many of the explants (Figure 1) occurred within a few hours in spite of the antioxidant treatment and was countered by shifting the position of explants in the same test tube or fresh media if needed. Rinsing of the explants immediately after collection was found to reduce the intensity of phenolic exudation. When that was not possible the plant meterial was brought to the lab and placed in running tap water for 30 min and an hour before further processing and surface sterilization.

**Table 3.a** Effect of Detergent treatment of phenolic exudation

| Plot no. | Clones | Detergent (ml / lit) | No. of bottle | (%) of bottle with phenolic exudation |
|---|---|---|---|---|
| A | 17/1 | 0.5 | 10 | 80 |
| | | 1.0 | 10 | 60 |
| | 9/1 | 0.5 | 10 | 60 |
| | | 1.0 | 10 | 30 |
| | 9/2 | 0.5 | 10 | 90 |
| | | 1.0 | 10 | 70 |
| B | T21 | 0.5 | 10 | 40 |
| | | 1.0 | 10 | 20 |
| | 17/4 | 0.5 | 10 | 80 |
| | | 1.0 | 10 | 60 |
| | 20/1 | 0.5 | 10 | 30 |
| | | 1.0 | 10 | 10 |

**Table 3.b** Effect of Running tap water treatment on phenolic exudation of teak clones

| Plot no. | Clones | (Duration in min) | No. of bottle | (%) of bottle with phenolic exudation |
|---|---|---|---|---|
| A | 17/1 | 30 | 10 | 40 |
| | | 60 | 10 | 20 |
| | 9/1 | 30 | 10 | 60 |
| | | 60 | 10 | 40 |
| | 9/2 | 30 | 10 | 50 |

| | | | | |
|---|---|---|---|---|
| | | 60 | 10 | 20 |
| B | T21 | 30 | 10 | 30 |
| | | 60 | 10 | 10 |
| | 17/4 | 30 | 10 | 40 |
| | | 60 | 10 | 10 |
| | 20/1 | 30 | 10 | 60 |
| | | 60 | 10 | 20 |

### 5.3. Experiment III. The Effect of Seasonal Variation in Different clones

### 5.3.1. Effect of two-noded explants for subculture of shoots

The normal practice adopted so far was to subculture a cluster of several (3-8 nos) shoots into fresh media. When single shoots were available they were excised into single two noded cuttings and subcultrued to ascertain the feasibility of adopting it routinely. 56 to 80 % of explants gave response in the different clones and as high as 3.0 shoots per explants produced (Table 4).

**Table 4.** Effect of two noded explants for Mean no. of shoots/explants at Subculture (1) (Media: MS + Kin (0.001mg/l)

| Plot no. | **Clones** | **% Response** | **Mean no. of shoots/explants** | | |
|---|---|---|---|---|---|
| | | | Hot | Rainy | Cold |
| A | 17/1 | 66 | 3.00 | 3.00 | 3.00 |
| | 9/1 | 80 | 2.00 | 2.00 | 2.00 |
| | 9/2 | 75 | 2.00 | 1.00 | 2.00 |
| B | T21 | 60 | 3.00 | 2.00 | 2.00 |
| | 17/4 | 56 | 1.00 | 1.00 | 1.00 |
| | 20/1 | 78 | 2.00 | 2.00 | 2.00 |
| Pr>F | | | $0.05^{ns}$ | $0.05^{ns}$ | $0.11^{ns}$ |
| CV (%) | | | 14.21 | 31.40 | 27.22 |

The average number of shoots for all clones was 23 in the hot season, 12 in the rainy season and 17 in the cold season (Table 5.a, b, c). ANOVA revealed that there were no significant differences in mean number of shoots among clones ($p<0.05$). The maximan mean shoot length was 3.6 (cm) while the minimum mean shoot length was 1.2 (cm) (Table 6)

**Table 5.** ANOVA for shoot formation of subculture (1) at three seasons
**Table 5.a** ANOVA for shoot formation at hot season

| Source | *DF* | *SS* | *MS* | *F* | *P* |
|---|---|---|---|---|---|
| V001 | 2 | 204.3333 | 102.167 | 9.43 | 0.0508 |
| Error | 3 | 32.500 | 10.833 | | |
| Total | 5 | 236.833 | | | |

*DF* Degree of Freedom, *SS* Sum of Squares, *MS* Mean Sum of Squares, *F* F test, *P* Non significant level: p > .05000

Grand Mean 23.167 CV 14.21

**Table 5.b** ANOVA for shoot formation at rainy season

| Source | *DF* | *SS* | *MS* | *F* | *P* |
|---|---|---|---|---|---|
| V001 | 2 | 252.333 | 126.167 | 8.41 | 0.0589 |
| Error | 3 | 45.000 | 15.000 | | |
| Total | 5 | 297.333 | | | |

*DF* Degree of Freedom, *SS* Sum of Squares, *MS* Mean Sum of Squares, *F* F test, *P* Non significant level: p > .05000

Grand Mean 12.333 CV 31.40

**Table 5.c** ANOVA for shoot formation at cold season

| Source | *DF* | *SS* | *MS* | *F* | *P* |
|---|---|---|---|---|---|
| V001 | 2 | 201.000 | 100.500 | 4.98 | 0.1113 |
| Error | 3 | 60.500 | 20.167 | | |
| Total | 5 | 261.500 | | | |

*DF* Degree of Freedom, *SS* Sum of Squares, *MS* Mean Sum of Squares, *F* F test, *P* Non significant level: p > .05000

Grand Mean 16.500 CV 27.2

**Table 6.** Effect of two noded explants for mean shoot length (cm) at Subculture (1)
(Media: MS + Kin (0.001mg/l)

| Plot no. | Clones | Mean shoot length (cm) | | |
|---|---|---|---|---|
| | | Hot | Rainy | Cold |
| A | 17/1 | 3.60 | 3.60 | 3.60 |
| | 9/1 | 2.40 | 2.40 | 2.40 |
| | 9/2 | 2.40 | 1.20 | 2.40 |
| B | T21 | 3.60 | 2.40 | 2.40 |
| | 17/4 | 1.20 | 1.20 | 1.20 |
| | 20/1 | 2.40 | 2.40 | 2.40 |

### 5.3.2. Multiplication (Subculture 2)

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 0.001 mg/l BAP and 0.001 mg/l Kinetin. Elongation and growth of healthy shoots (Figure 3) was obtained on lower levels of cytokinins. The best results were obtained on media with both BAP and Kinetin where the shoots had bigger leaves. The shoot cultures developed a mass of callus at the base during the passage. In some of the clones, size of the callus growth threatened to disrupt the growth of shoots and had to be removed and the shoots transferred to fresh media.

Sprouted shoots from shoot tip explants were subcultured on to fresh media and were maintained through subcultures. Growth was initially slow for the first two months during which subcultures to fresh media was carried out every three weeks. When multiple shoot formation was observed after subcultures, the shoots were split into clusters of 3-4 shoots for transfer to fresh media. Single shoots as well as bases of shoots were also transferred, but compared to explants consisting of shoot clusters their growth was slower.

**Table 7.** Effect of 2-4 noded explants for Mean no. of shoots/explants at Subculture (2)
(Media: MS + BA (0.001mg/l) + Kin (0.001mg/l)

| Plot no. | **Clones** | **Mean no. of shoots/explants** | | |
|---|---|---|---|---|
| | | Hot | Rainy | Cold |
| A | 17/1 | 5.00 | 5.00 | 5.00 |
| | 9/1 | 2.50 | 2.50 | 2.50 |
| | 9/2 | 2.50 | 2.50 | 2.50 |
| B | T21 | 4.00 | 4.00 | 4.00 |
| | 17/4 | 2.00 | 2.00 | 2.00 |
| | 20/1 | 3.00 | 3.00 | 3.00 |
| Pr >F | | $0.07^{ns}$ | $0.10^{ns}$ | $0.17^{ns}$ |
| CV | | 15.18 | 52.48 | 35.33 |

The average number of shoots for all clones was 70 in the hot season, 29 in the rainy season and 49 in the cold season (Table 8). ANOVA revealed that there were no significant differences in number of shoots among clones ($p>0.05$). Likewise, the maximum mean shoot length was 8.0(cm) while the minimum mean shoot length was 1.2(cm) (Table 9). Likewise, the maximum percentage of survival of all clones was 93 % at hot season but the minimum percentage was 11 at rainy season. (Table 10)

**Table 8.** ANOVA for shoot formation of subculture (2) in MS medium at three seasons
**Table 8.a** ANOVA for shoot formation at hot season

| Source | *DF* | *SS* | *MS* | *F* | *P* |
|---|---|---|---|---|---|
| V001 | 2 | 1692.33 | 846.167 | 7.46 | 0.0686 |
| Error | 3 | 340.50 | 113.500 | | |
| Total | 5 | 2032.83 | | | |

*DF* Degree of Freedom, *SS* Sum of Squares, *MS* Mean Sum of Squares, *F* F test, *P* Non significant level: p > .05000
Grand Mean 70.167 CV 15.18

**Table 8.b** ANOVA for shoot formation at rainy season

| Source | *DF* | *SS* | *MS* | *F* | *P* |
|---|---|---|---|---|---|
| V001 | 2 | 2552.33 | 1276.17 | 5.38 | 0.1017 |
| Error | 3 | 711.00 | 237.00 | | |
| Total | 5 | 3263.33 | | | |

*DF* Degree of Freedom, *SS* Sum of Squares, *MS* Mean Sum of Squares, *F* F test, *P* Non significant level: p > .05000
Grand Mean 29.333 CV 52.48

**Table 8.c** ANOVA for shoot formation at cold season

| Source | *DF* | *SS* | *MS* | *F* | *P* |
|---|---|---|---|---|---|
| V001 | 2 | 2058.33 | 1029.17 | 3.48 | 0.1653 |
| Error | 3 | 887.00 | 295.67 | | |
| Total | 5 | 2945.33 | | | |

*DF* Degree of Freedom, *SS* Sum of Squares, *MS* Mean Sum of Squares, *F* F test, *P* Non significant level: p > .05000
Grand Mean 48.667 CV 35.33

**Table 9.** Effect of 2-4 noded explants for Mean shoot length (cm) at Subculture (2) (Media: MS + BA (0.001mg/l) + Kin (0.001mg/l)

| Plot no. | **Clones** | **Mean shoot length (cm)** | | |
|---|---|---|---|---|
| | | Hot | Rainy | Cold |
| A | 17/1 | 8.00 | 6.40 | 8.00 |
| | 9/1 | 4.00 | 4.80 | 4.00 |
| | 9/2 | 4.00 | 3.20 | 4.00 |
| B | T21 | 6.40 | 4.80 | 6.40 |
| | 17/4 | 3.20 | 1.60 | 3.20 |
| | 20/1 | 4.80 | 1.80 | 4.80 |

**Table 10.** Survival percentage of selected clones at three seasons

| Plot no. | **Clones** | **% Response** | | |
|---|---|---|---|---|
| | | Hot | Rainy | Cold |
| A | 17/1 | 93 | 40 | 65 |
| | 9/1 | 67 | 14 | 23 |
| | 9/2 | 39 | 18 | 23 |
| B | T21 | 92 | 77 | 84 |
| | 17/4 | 65 | 16 | 56 |
| | 20/1 | 65 | 11 | 41 |

**Figure 1.** Phenolic exudation and Callus from shoot tip explants without treatment (left) and control of browning after running water treatment (right)

**Figure 2**. Multiple shoots of Teak

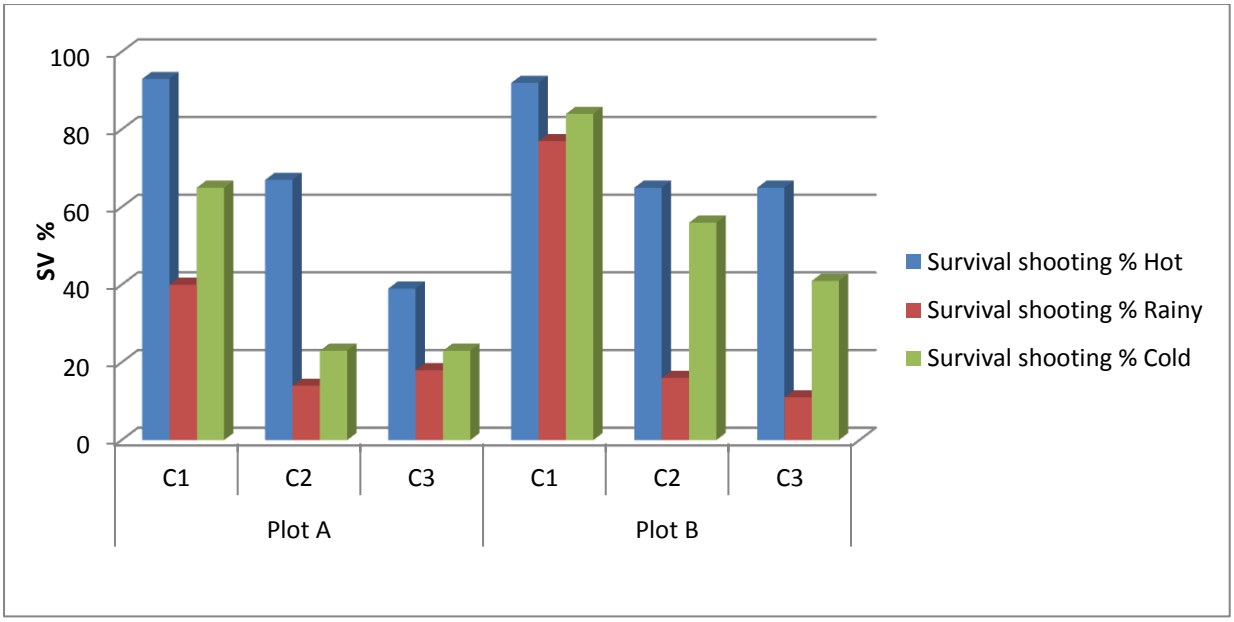


**Figure 3.** Seasonal variation of survival % among the selected clones at multiplication stage.

**5.3.3. *In vitro & Ex vitro* rooting**

After multiple shoot formation had proceeded for two subcultures and sufficient shoots were available for each clone, rooting of the microshoots were attempted. *In vitro* rooting was very successful and in most attempts all the shoots rooted within eight weeks (Figure 4).

The treatment given at BAP (0.001) mg + NAA (0.001) mg with half (MS) media, the roots were very thin and weak. But, the roots produced at the treatment of BAP (0.001) mg + NAA (0.001) mg with full (MS) media, were healthy and strong. It gave the many number of roots per shoot and root length after 8 weeks of transplantation for each clone.

Furthermore, at Fertistart (10ml/lit), basal callusing was observed and many root formation took place after 4 weeks of transfer. The best results were recorded at BAP (0.001) mg + NAA (0.001) mg with full (MS) media as it gave the maximum number of roots per shoot and root length after 8 weeks of transplantation for each clone (Table 11).

Surendran & Seethalakihimi, (1987) indicated that, all the three factors, i.e,growth regulating substance, their concentration and seasonal effect had considerable effect on induction of rooting in cuttings. A treatment of 100 ppm NAA planted in April gave best result for ***T. grandis.*** In this study, teak planted in May i.e., hot season, produced the best result of rooting, and the survival percentage was also high. This demonstrated similar outcome of result mentioned by Surendran & Seethalakihimi.

White (1947), studied the effect of seasonal variation on the rooting of branch cuttings in some bamboo species. His results showed that the average rooting percentage was at a maximum in December (15.8%), 10.5 % in June, 3.3 % in September. March and April was found to be the best for the preparation of branch cutting of Teak (Doo & Thida Mundt, 1989

| Plot no. | **Clones** | **% Response** | | |
|---|---|---|---|---|
| | | MS(BAP(0.001)mg+ NAA(0.001)mg | ½(BAP(0.001)mg+ NAA(0.001)mg | 10ml/l Fertistart |
| A | 17/1 | 93 | 40 | 65 |
| | 9/1 | 67 | 14 | 23 |
| | 9/2 | 39 | 23 | 18 |
| B | T21 | 92 | 77 | 84 |
| | 17/4 | 63 | 16 | 40 |
| | 20/1 | 65 | 11 | 30 |

**5.3.4. Hardening:**

Rooted plantlets for 8 weeks in the mist chamber were transferred to soil in polybags and placed in shade for a week before shifting to the nursery. Newer leaves of the plantlets increased in size as the plantlets established but for the first few months had not attained the stiff and leathery nature of rooted teak stem cutting and were more like teak seedlings. In the three seasonal treatments, rooted plantlets were observed that 40 - 90% in the rainy season, 20 - 70% in the cold season and, 10 - 60% in the hot season among selective clones (Figure.6).

**Figure 4.** Microshoot with root induction after one month

**Figure 5.** Plantlets of teak

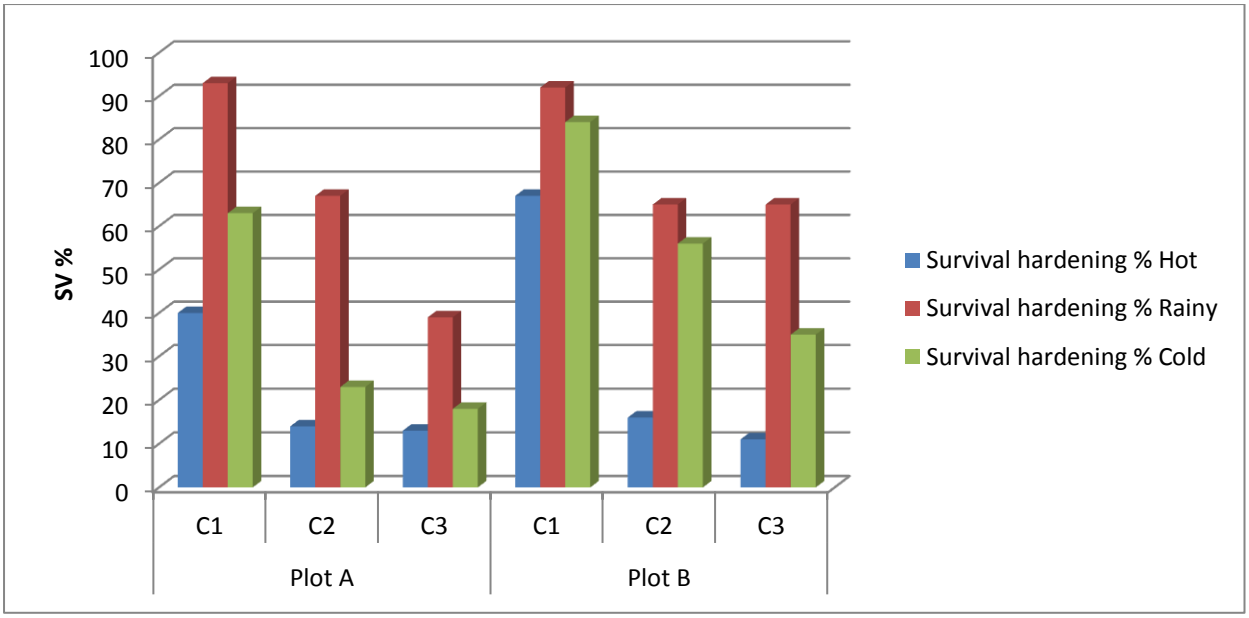


**Figure 6.** Seasonal variation of survival % among the selected clones at hardening stage

## 6. Conclusion

(1) The use of epicormic shoots for culture is much more preferred for routine micropropagation. The MS Medium was found to give significantly higher results in the mean number of shoots produced per explant as well in the shoot length produced. 85% of explants gave response in the different clones and as high as 5.80 cm shoots per explant produced.

(2) Effect of running tap water treatment on phenolic exudation of teak clones gave best results. The plant meterials were placed in running tap water for 30 min and an hour before further processing and surface sterilization. One hour sterilization produced less phenol than 30 min sterilization.

(3) The seasonal effect of shooting was observed that, the maximum percentage in hot season (March to May) and minimum percentage in rainy season (June to September).

(4) The treatment given at BAP (0.001) mg + NAA (0.001) mg with helf (MS) media, the roots were very thin and weak. But, the roots produced at the treatment of BAP (0.001) mg + NAA (0.001) mg with full (MS) media, were healthy and strong.

(5) Furthermore, at Ferti-start (20ml/lit), basal callusing was observed and many root formation took place after 4 weeks of transfer. However, the best results were recorded the maximum number of roots per shoot and root length at the treatment of BAP (0.001) mg + NAA (0.001) mg with full (MS) media after 8 weeks of transplantation for each clone.

## 7. Acknowledgements

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## Appendix

Appendix 1.

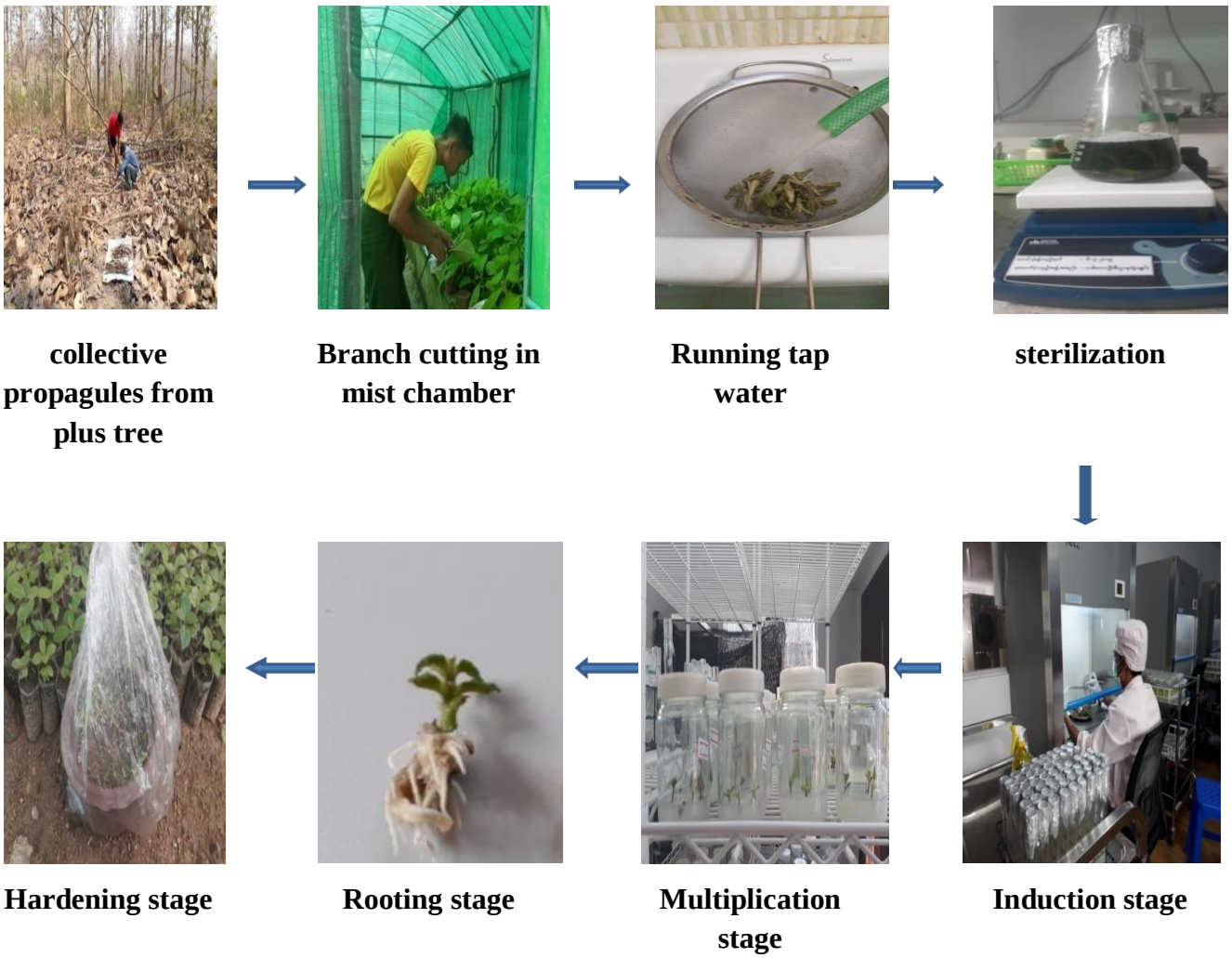


**Plate 1.** Procedure established from collective propagules to hardening stage of *Tactona grandis* (lin.f.)

**Appendix 2.** Composition of full and half strength of Murashige and Skoog's -1962 (MS) medium.

| **Components** | **Concentration in medium ( gm.L$^{-1}$)** | |
|---|---|---|
| | **FMS** | **HMS** |
| $NH_4NO_3$ | 1.65 | 0.825 |
| $KNO_3$ | 1.90 | 0.95 |
| $CaC1_2.2H_2O$ | 0.44 | 0.22 |
| $MgSO_4.7H_2O$ | 0.37 | 0.185 |
| $KH_2PO_4$ | 0.17 | 0.085 |
| $FeSO_4.7H_2O$ | 27.8 | 13.9 |
| NA2 EDTA | 37.3 | 18.65 |
| $H_3BO_3$ | 6.2 | 6.2 |
| $MnSO_4.4H_2O$ | 22.3 | 22.3 |
| $ZnSO_4.4H_2O$ | 8.6 | 8.6 |
| KI | 0.83 | 0.83 |
| $Na_2MoO_4.2H_2O$ | 0.25 | 0.25 |
| $CuSO_4.5H_2O$ | 0.025 | 0.025 |
| $CoCl_2.6H_2O$ | 0.025 | 0.025 |
| Nicotinic acid | 0.5 | 0.5 |
| Pyridoxin.HCl | 0.5 | 0.5 |
| Thiamine.HCl | 0.1 | 0.1 |
| Glycine | 2 | 2 |
| Myoinositol | 0.1 | 0.1 |