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Study on the Production of Tissue Reproductive Plants in *Dalbergia* Species Using Different Hormone Ratios

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Abstract

The results of the study on the tissue culture propagation of *Dalbergia oliveri* Gamble ex Prain, *Dalbergia fusca* Pierre, and *Dalbergia cultrata* T.S.Ralph using different hormone ratios showed that the combination of BAP and NAA had a significant effect on shoot and root induction. The best results for shoot initiation were obtained from a medium supplemented with 1.0 mg/L BAP and 0.1 mg/L NAA. Among the three species, *D. oliveri* exhibited the best response, followed by *D. fusca* and *D. cultrata*. Too much NAA caused callus formation at the base of the shoots instead of proper root development. The half-strength MS medium with 0.1 mg/L NAA was found to be the most effective for root initiation. The discussion highlighted that balancing the cytokinin (BAP) and auxin (NAA) ratio is critical for successful micropropagation. An excess of cytokinin leads to multiple but stunted shoots, while too much auxin suppresses shoot formation and promotes unwanted callus growth. Therefore, an optimized ratio of 1.0 mg/L BAP and 0.1 mg/L NAA was identified as the most suitable for efficient regeneration. This study provides a valuable guideline for the large-scale propagation of *Dalbergia* species, which are economically important but threatened due to deforestation and illegal logging. The established tissue culture method can be applied for conservation, reforestation, and sustainable production of high-quality planting materials for the timber industry.

Keywords: *Cytokinin, Auxin, MS medium, 6-Benzylaminopurine (BAP), Naphthalenacetic acid (NAA), Micropropagation*

ဟော်မုန်းအချိုးအစားအမျိုးမျိုးကို အသုံးပြု၍ *Dalbergia* မျိုးစုဝင် သစ်မျိုးများအား တစ်သျူးမျိုးပွားပင်များထုတ်လုပ်နိုင်မှုကို လေ့လာခြင်း

ခင်ပပရွှေ၊ လက်ထောက်သုတေသနအရာရှိ

စာတမ်းအကျဉ်း

ဤသုတေသနတွင် ဟော်မုန်းအချိုးအစားအမျိုးမျိုးကိုအသုံးပြု၍ တမလန်း၊ ယင်းစပ် နှင့် ရင်းတိုက်မျိုးစိတ်သုံးမျိုးတို့ကို တစ်သျူးနည်းဖြင့်မျိုးပွားခြင်းဆိုင်ရာ လေ့လာမှုရလဒ်များအရ BAP နှင့် NAA ဟော်မုန်းပေါင်းစပ်မှုသည် အညှောက်ထွက်ခြင်းနှင့် အမြစ်ထွက်ခြင်းအပေါ် သိသာထင်ရှားသောသက်ရောက်မှုရှိကြောင်းတွေ့ရှိရသည်။ အညှောက်ထွက်ခြင်းအတွက် အကောင်းဆုံး ရလဒ်ကို 1.0 mg/L BAP နှင့် 0.1 mg/L NAA ပေါင်းစပ်အသုံးပြုသော အာဟာရမျိုးပွားစာတွင် ရရှိ ခဲ့သည်။ လေ့လာခဲ့သော မျိုးစိတ်သုံးမျိုးတွင် တမလန်းသည် တုံ့ပြန်မှုအကောင်းဆုံးဖြစ်ပြီး ယင်းစပ် နှင့် ရင်းတိုက်တို့က နောက်မှအစဉ်လိုက်၍ ဖွံ့ဖြိုးမှုကိုပြသခဲ့သည်။ NAA ပမာဏ များလွန်းပါက အမြစ်ထွက်မည့်အစား အညှောက်၏ အခြေပိုင်းတွင် တစ်သျူးဖု(Callus)များ ဖြစ်ပေါ်လာစေသည်။ အမြစ်စတင်ထွက်ရန်အတွက် ½ MS medium နှင့် 0.1 mg/L NAA ပေါင်းစပ်မှုသည် အထိရောက် ဆုံးဖြစ်ကြောင်း တွေ့ရှိရသည်။ တစ်သျူးမျိုးပွားနည်းအောင်မြင်ရန်အတွက် ဆိုက်တို ကိုင်နင် (BAP)နှင့် အောက်စင် (NAA) အချိုးအစားမျှတရန် အလွန်အရေးကြီးကြောင်း ဖော်ပြထားသည်။ ဆိုက်တိုကိုင်နင်များလွန်းပါက အညှောက်များစွာ ထွက်သော်လည်း ကြီးထွားမှု နှေးကွေး တတ်ပြီး၊ အောက်စင်များလွန်းပါက အမြစ်ထွက်ခြင်းကို တားဆီးကာ မလိုအပ်သော တစ်သျူးဖု (Callus) များ သာ ကြီးထွားလာစေသည်။ ထို့ကြောင့် အပင်မျိုးပွားမှု ထိရောက်စေရန်အတွက် အကောင်းဆုံး အချိုးမှာ 1.0 mg/L BAP နှင့် 0.1 mg/L NAA ဖြစ်ကြောင်း ဖော်ထုတ်နိုင်ခဲ့သည်။ ဤလေ့လာမှုသည် သစ်တောပြုန်းတီးမှုနှင့် တရားမဝင်သစ်ခုတ်မှုများကြောင့် မျိုးသုဉ်းရန် အန္တရာယ်ရှိနေသော်လည်း စီးပွားရေးအရ အရေးပါသော *Dalbergia* မျိုးစိတ်များကို ပွားများ စိုက်ပျိုးရန်အတွက် အဖိုးတန်သောလမ်းညွှန်ချက်တစ်ခုဖြစ်သည်။ ဖော်ထုတ်လိုက်သော တစ်သျူးမျိုးပွားနည်း စနစ်သည် သဘာဝပတ်ဝန်းကျင် ထိန်းသိမ်းခြင်း၊ ပြန်လည် စိုက်ပျိုးခြင်းနှင့် သစ်ထုတ်လုပ်မှုအတွက် အရည်အသွေးမြင့်ပျိုးပင်များ စဉ်ဆက်မပြတ် ထုတ်လုပ်ခြင်းတို့တွင် အသုံးချနိုင်ပါသည်။

Study on the Production of Tissue Reproductive Plants in *Dalbergia* Species Using Different Hormone Ratios

1. Introduction

The genus *Dalbergia* (family Fabaceae) consists of more than one hundred tree and shrub species distributed mainly across tropical and subtropical regions of Asia, Africa, and South America. Many species of this genus are renowned for their valuable hardwood, commonly known as "rosewood," which is prized for its beautiful grain, fragrance, strength, and resistance to decay **Dalbergia oliveri* Gamble ex Prain*, **Dalbergia fusca* Pierre*, and **Dalbergia cultrata* T.S.Ralph* are among the most economically and ecologically important species native to Southeast Asia, particularly in Myanmar, Thailand, Laos, and Vietnam. The wood of these species is used extensively for high-quality furniture, musical instruments, and decorative materials (Tun & Win, 2021). However, due to overexploitation, habitat destruction, and slow natural regeneration, the population of these species has declined significantly over the last few decades.

As a result, several species of *Dalbergia* have been listed under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II, indicating the urgent need for conservation and sustainable management. Conservation and propagation of these high-value tree species are essential to ensure their long-term survival. Traditional propagation methods such as seed germination and vegetative cutting have proven to be inefficient for *Dalbergia* species due to several limitations, including low germination rates, seed dormancy, and susceptibility to environmental fluctuations (Kumar et al., 2020). Additionally, natural regeneration in the forest is slow and inconsistent because of poor seed viability and pest infestation. Hence, there is a critical need to develop alternative propagation techniques capable of producing large numbers of healthy and genetically uniform plantlets within a short period.

Plant tissue culture, also referred to as *in vitro* micropropagation, offers a viable and efficient approach for large-scale propagation and conservation of rare or endangered plant species (George et al., 2018). The technique involves culturing small plant segments, known as explants, on sterile nutrient media supplemented with plant growth regulators (PGRs). Through the appropriate balance of hormones, explants can be induced to form shoots, roots, or callus depending on the desired developmental pathway. This method allows for year-round propagation under controlled environmental conditions and ensures the production of true-to type plants with uniform characteristics (Rahman et al., 2020). Furthermore, micropropagation supports germplasm preservation and provides a reliable supply of planting materials for reforestation and commercial cultivation.

Among the different plant growth regulators, cytokinins and auxins are the most influential in regulating morphogenesis in plant tissue culture. Cytokinins such as 6-Benzylaminopurine (BAP) stimulate cell division and shoot proliferation, whereas auxins such as Naphthaleneacetic acid (NAA) are primarily responsible for cell elongation, root initiation, and callus formation (Murashige & Skoog, 1962; Patel et al., 2022). The interaction and ratio between these two hormones determine the developmental pattern of cultured tissues: higher cytokinin-to-auxin ratios promote shoot induction, while higher auxin-to-cytokinin ratios encourage root or callus formation (Thorpe, 2018). Therefore, identifying the optimal hormonal balance is crucial for achieving successful regeneration in *Dalbergia* species.

Previous studies have reported that combinations of BAP and NAA are effective in inducing multiple shoots and roots in various *Dalbergia* species. For instance, **Dalbergia sissoo** and **Dalbergia latifolia** exhibited high shoot multiplication rates under media supplemented with moderate BAP and low NAA concentrations (Jain et al., 2017). However, very limited information is available regarding **D. oliveri*, **D. fusca*, and **D. cultrata*,

especially in relation to their response to different hormone concentrations under *in vitro* conditions. Each species may respond differently to plant growth regulators due to variations in genetic makeup, tissue sensitivity, and physiological characteristics. Therefore, specific optimization of the culture medium and hormonal ratios is required for each species to achieve maximum propagation efficiency.

By achieving these objectives, this study aims to establish an efficient and reproducible tissue culture protocol for large-scale propagation and conservation of valuable *Dalbergia* species. The findings are expected to contribute significantly to forest restoration programs, biodiversity conservation, and sustainable timber production. Additionally, the research will provide a scientific basis for future studies on genetic improvement, somatic embryogenesis, and cryopreservation of *Dalbergia* species. The successful development of a micropropagation protocol will not only help conserve threatened *Dalbergia* species but also promote the sustainable use of forest resources. Furthermore, optimizing hormone ratios for these species will enhance the understanding of their morphogenetic behavior under *in vitro* conditions, thereby contributing to broader plant biotechnology and forestry research.

In summary, *D. oliveri*, *D. fusca*, and *D. cultrata* are among the most valuable timber species that face increasing threats due to overharvesting and deforestation. By employing tissue culture technology and optimizing BAP and NAA hormone ratios, this study seeks to establish a scientific foundation for the propagation, conservation, and sustainable utilization of these important tree species. The outcomes are expected to benefit both conservation efforts and the commercial forestry sector, supporting the sustainable management of natural resources for future generations.

2. Objectives

The present study was conducted to investigate the effect of different ratios of BAP and NAA on tissue culture propagation of *Dalbergia oliveri*, *Dalbergia fusca*, and *Dalbergia cultrata*. The main objectives of this research were:

1. To determine the most effective combination of BAP and NAA for shoot induction and multiplication.
2. To evaluate the effect of NAA concentration on root initiation and development.
3. To adapt plants from the laboratory to the outdoor environment.

3. Literature Review

3.1. Overview of Dalbergia Species and Their Importance

The genus *Dalbergia* belongs to the Fabaceae family and comprises around 100–150 species distributed mainly in tropical and subtropical regions (Kumar et al., 2020). These species are well known for producing highly valuable hardwoods known as rosewoods, which are prized for their strength, durability, and fine texture. *Dalbergia oliveri*, *D. fusca*, and *D. cultrata* are native to Southeast Asia and are important components of forest ecosystems in Myanmar, Thailand, and Laos (Tun & Win, 2021). The timber from these species is used for premium furniture, carvings, and musical instruments. However, intensive logging and slow regeneration have severely reduced their populations in natural habitats. Seeds of *Dalbergia* species exhibit low germination rates due to dormancy and susceptibility to pests (Rahman et al., 2020). Moreover, vegetative propagation methods, such as stem cuttings, often show poor rooting efficiency. Because of these limitations, *in vitro* tissue culture techniques have become essential for conserving and mass-propagating these endangered species.

3.2. Tissue Culture and Its Applications in Forestry

Plant tissue culture is a biotechnological tool that enables the regeneration of plants under sterile and controlled laboratory conditions. This technique plays a vital role in forestry, particularly in the conservation and propagation of rare or endangered woody plants (George et al., 2018). By using small explants such as nodal segments, shoot tips, or leaves, researchers can regenerate complete plants through organogenesis or somatic embryogenesis.

In forestry, micropropagation has been successfully used for several economically important trees including **Tectona grandis** (teak), *Eucalyptus*, *Bambusa*, and *Dalbergia* species (Patel et al., 2022). The major advantages of tissue culture in forestry include rapid multiplication, disease-free plant production, and year-round propagation. Moreover, micropropagation allows the preservation of elite genotypes and supports reforestation projects where natural regeneration is insufficient (Jain et al., 2017).

3.3. Role of Plant Growth Regulators (PGRs)

Plant growth regulators (PGRs) are critical components of tissue culture media that control and direct morphogenesis. Cytokinins and auxins are the most commonly used hormones in plant tissue culture (Thorpe, 2018). Cytokinins such as 6-Benzylaminopurine (BAP) stimulate cell division and shoot proliferation, while auxins such as Naphthaleneacetic acid (NAA), Indole-3-butyric acid (IBA), or Indole-3-acetic acid (IAA) regulate root formation, cell elongation, and callus induction (Murashige & Skoog, 1962).

The interaction between cytokinins and auxins determines the developmental response of the cultured explant. A high cytokinin-to-auxin ratio promotes shoot induction, whereas a high auxin-to-cytokinin ratio favors root or callus formation (Rahman et al., 2020). Therefore, optimizing the hormonal balance in the culture medium is essential for successful plant regeneration. Studies have shown that small variations in hormone concentration can drastically affect shoot number, root length, and callus morphology (Karthikeyan et al., 2021).

3.4. Effects of BAP and NAA in Woody Plant Propagation

Among various cytokinins, BAP has been found to be one of the most effective for inducing shoot proliferation in woody plants (Singh & Verma, 2019). BAP promotes axillary bud activation and helps in breaking apical dominance, resulting in multiple shoot formation. NAA, a synthetic auxin, is widely used for root induction and callus development. The combination of BAP and NAA has proven effective in micropropagation protocols of many forest trees such as **Tectona grandis**, **Acacia mangium**, **Dalbergia sissoo**, and **Dalbergia latifolia** (Jain et al., 2017; Patel et al., 2022). In **Dalbergia sissoo**, the best shoot regeneration was achieved on MS medium supplemented with 2.0 mg/L BAP and 0.5 mg/L NAA (Kumar et al., 2020). Similar results were reported for **Dalbergia latifolia**, where moderate BAP and low NAA concentrations produced healthy shoots and roots. However, excessive auxin concentration caused callus formation and reduced shoot elongation (Rahman et al., 2020). These findings suggest that precise hormone concentration is necessary to balance shoot and root induction effectively.

3.5. Previous Studies on Dalbergia Tissue Culture

Research on *Dalbergia* species has demonstrated that tissue culture can play a major role in conservation and reforestation programs. For instance, **Dalbergia melanoxylon** was successfully propagated through nodal culture using 1.5 mg/L BAP and 0.3 mg/L NAA, which resulted in high shoot multiplication and strong rooting (Singh et al., 2021). Similarly, **Dalbergia sissoo** cultures responded positively to cytokinin-rich media for shoot induction followed by low-auxin media for rooting (Patel et al., 2022). However, there is still limited research available for **D. oliveri**, **D. fusca**, and **D. cultrata**. These species are native to

the Indochina region and exhibit unique physiological responses that may differ from other Dalbergia species. Their tissue culture optimization has not been well established, particularly regarding the influence of BAP and NAA ratios. Understanding these species-specific responses is essential for developing standardized propagation protocols suitable for large-scale production and conservation purposes.

3.6. Knowledge Gaps and Research Justification

Despite the progress made in tissue culture studies on some *Dalbergia* species, there remains a lack of systematic research on the hormonal effects in **D. oliveri*, **D. fusca*, and **D. cultrata*. The existing studies mostly focus on **D. sissoo* and **D. latifolia*, which may not exhibit identical responses due to genetic and environmental variations. Furthermore, variations in explant type, culture medium composition, and subculture duration also influence the success rate of regeneration (George et al., 2018). Therefore, there is a strong need to optimize hormone ratios, particularly of BAP and NAA, to achieve efficient regeneration in these three Dalbergia species. Developing an effective **in vitro* propagation method will not only support *ex situ* conservation but also provide a sustainable supply of planting materials for commercial forestry and reforestation projects. This study seeks to address these gaps by analyzing how different BAP: NAA combinations influence shoot and root development across **D. oliveri*, **D. fusca*, and **D. cultrata*.

3.7. Summary

In summary, previous research highlights the importance of cytokinins and auxins in regulating **in vitro* plant morphogenesis, particularly in woody species. The BAP-NAA combination has been widely recognized for its effectiveness in promoting shoot and root development, but optimal concentrations vary among species. Given the limited availability of studies on **D. oliveri*, **D. fusca*, and **D. cultrata*, this research is essential to establish an efficient and reproducible micropropagation protocol. Such a protocol will contribute not only to the conservation of these threatened tree species but also to their sustainable utilization in forestry and the timber industry.

4. Materials and Methods

4.1. Experimental Design

The experiment was conducted under a two-factor factorial design using a Completely Randomized Design (CRD). The two experimental factors were as follows:

Factor A (Species): *Dalbergia oliveri*, *Dalbergia fusca*, and *Dalbergia cultrata*

Factor B (Hormone Ratio Treatments): Four combinations of auxin (NAA) and cytokinin (BAP) concentrations were tested to evaluate the effects on *in vitro* regeneration. The factorial combination resulted in twelve (3 × 4) treatment combinations, each replicated four times, with ten explants per replication, making a total of 480 explants. Data were analyzed for both main and interaction effects of the two factors.

4.2. Plant Material and Explant Preparation

Healthy seeds of **Dalbergia oliveri*, **Dalbergia fusca*, and **Dalbergia cultrata* were collected from mature donor plants. The explants were thoroughly washed with running tap water and a few drops of liquid detergent to remove surface dust and debris. They were then sterilized under a laminar airflow cabinet by immersing in 70% ethanol for 30 seconds,

followed by 0.1% (w/v) sodium hypochlorite solution for 10 minutes, with **two rinses of sterile distilled water after each sterilization step.

4.3. Culture Medium Preparation

Murashige and Skoog (MS) basal medium supplemented with 3% (w/v) sucrose and solidified with 0.6 % (w/v) agar was used as the basic culture medium. The pH of the medium was adjusted to **5.8 ± 0.1** before autoclaving at **121°C and 15 psi for 15 minutes**. Hormone treatments were added aseptically after cooling the medium to approximately 30° C under sterile conditions.

4.4. Hormone Treatments

Four hormonal combinations of NAA (α-Naphthaleneacetic acid) and **BAP (6-Benzylaminopurine) were tested as follows:

Table (1) Effect of Different Cytokinin and Auxin Ratios on Plant Growth

Treatment Code	BAP (mg/L)	NAA (mg/L)	Cytokinin:Auxin Ratio
T_1	0.1	1.0	Low:High
T_2	0.5	0.5	Medium:Mediumn
T_3	1.0	0.1	High:Low
T_4	2.0	0.1	High:Low

Each species was cultured on all four treatments, resulting in twelve treatment combinations.

4.5. Inoculation and Culture Conditions

After sterilization, explants were inoculated vertically into the prepared MS media under aseptic conditions in a laminar flow hood. The cultures were incubated in a growth chamber at 25 ± 2°C, under a 16-hour photoperiod and 8-hour dark period, with a light intensity of approximately 2000–2500 lux provided by cool-white fluorescent lamps. Cultures were observed weekly for morphological changes, callus formation, and shoot induction. The experiment was maintained for eight weeks, during which all quantitative and qualitative data were recorded.

4.6. Data Collection

The following parameters were measured for each treatment combination:

(i) Percentage of explants producing shoots
(ii) Mean number of shoots per explant and forming callus
(iii) Mean height (cm)
(iv) Percentage of explants forming roots
(v) Survival rate of plantlets after acclimatization

Observations were recorded weekly, and photographic documentation was taken to support morphological analysis.

4.7. Rooting and Acclimatization

Well-developed shoots were transferred to a rooting medium consisting of half-strength MS basal salts supplemented with an appropriate concentration of auxin (NAA). Rooted plantlets were gradually acclimatized by transferring them to small plastic bags containing a sterilized mixture of sand and soil (1:2). The plantlets were kept under high humidity conditions

for one week before being transferred to greenhouse conditions for further growth and hardening.

4.8. Statistical Analysis

All data were subjected to two-way Analysis of Variance (ANOVA) to determine the significance of the main effects (species and hormone ratio) and their interaction effects. The significance level was set at $p < 0.05$. When significant differences were detected, treatment means were compared using Duncan's Multiple Range Test (DMRT) at the same significance level. Statistical analyses were performed using SPSS statistical software, and results were presented as mean ± standard error (SE).

5. Results and Discussions

5.1. Effect of Hormone Ratios on Shoot Induction

The factorial experiment evaluated the effects of varying cytokinin (BAP) and auxin (NAA) concentrations on shoot induction in *D. oliveri*, *D. fusca*, and *D. cultrata*. The results revealed clear species-specific responses to hormone treatments (Table 2).

Table (2) Effect of BAP and NAA concentrations on shoot induction, shoot number, and callus formation in three *Dalbergia* species

Species	Treatment Code	Shoot Induction (%)	Average Shoots per Explant	Callus Formation	Notes on Shoot Morphology
D. oliveri	T1	42.0	2.1	High	Stunted shoots, basal callus
	T2	65.0	3.8	Moderate	Elongated shoots, slight leaf expansion
	T3	87.5	5.6	Low	Healthy, elongated shoots, well-formed leaves
	T4	70.0	4.0	Low	Some hyperhydric shoots
D. fusca	T1	38.0	1.9	High	Compact shoots, callus at base
	T2	65.0	4.0	Low	Thickened shoots
	T3	78.0	5.5	Moderate	Slight vitrification at high BAP
	T4	82.0	6.2	Low	Healthy, elongated shoots
D. cultrata	T1	30.0	1.5	High	Friable, brown callus, stunted shoots
	T2	45.0	3.2	Moderate	Short shoots, some hyperhydricity
	T3	60.0	4.8	Low	Healthy shoot clusters, slight elongation issues

Species	Treatment Code	Shoot Induction (%)	Average Shoots per Explant	Callus Formation	Notes on Shoot Morphology
	T4	50.0	4.0	Low	Malformed, vitrified shoots

From Table 2, **D. oliveri** showed the highest shoot induction (87.5%) at a BAP:NAA ratio of 1.0:0.1 mg/L, with an average of 5.6 shoots per explant. Lower BAP or higher auxin concentrations reduced shoot formation and increased basal callus development. *D. fusca* exhibited optimal shoot induction (82.0%) at a BAP:NAA ratio of 2.0:0.1 mg/L, producing more shoots per explant than *D. oliveri* under its optimal conditions. *D. cultrata* had generally lower regeneration rates, with a maximum of 60.0% shoot induction at 1.0:0.1 mg/L BAP:NAA, and was more prone to hyper hydricity and callus formation.

5.2. Interaction Effects Between Cytokinin and Auxin

Factorial analysis revealed significant interaction effects between BAP and NAA. In *D. oliveri* and *D. fusca*, increasing BAP in combination with low NAA enhanced both shoot number and elongation, confirming the positive synergistic effect of moderate cytokinin with minimal auxin. Conversely, high auxin concentrations consistently favored callus formation rather than shoot induction in all species, demonstrating the antagonistic role of auxin in organogenesis under these culture conditions. In *D. cultrata*, the interaction effects were less pronounced. Although higher BAP concentrations induced shoot clusters, elongation was limited, suggesting that this species may require additional growth regulators, such as GA_3, for optimal shoot elongation.

5.3. Morphological Observations and Callus Formation

Callus formation was highly dependent on species and hormone ratios. High auxin levels induced dense, pale yellow callus in *D. oliveri* and *D. fusca*, while in *D. cultrata*, callus was friable and brown, likely due to phenolic oxidation. Shoot morphology also varied: optimal cytokinin-to-auxin ratios produced elongated, healthy shoots with fully expanded leaves, whereas imbalanced ratios led to stunted, thickened, or vitrified shoots, especially in *D. cultrata* at high BAP levels.

5.4. Implications for Tissue Propagation

This study demonstrates that:

(i). Each Dalbergia species exhibits unique hormonal requirements for successful shoot induction.

(ii). Moderate cytokinin combined with low auxin generally promotes organogenesis over callogenesis.

(iii). Factorial design effectively identifies species-specific optimal hormone combinations, enhancing tissue culture efficiency.

These results provide a foundation for the large-scale propagation of *D. oliveri*, *D. fusca*, and *D. cultrata*, supporting conservation and reforestation efforts. Future research should focus on rooting induced shoots, acclimatization, and the potential use of additional growth regulators to improve shoot elongation and survival rates.

5.5. Survival Rate of Regenerated Plantlets

After successful shoot induction and rooting, the regenerated plantlets of *D. oliveri*, *D. fusca*, and *D. cultrata* were acclimatized in greenhouse conditions. The survival rates differed

significantly among the species and were strongly influenced by the hormonal treatments used during *in vitro* propagation.

Table (3) Effects Optimal Hormone Ratios (BAP:NAA) Acclimation Success of Dalbergia Three Species

Species	Optimal Hormone Ratio (BAP:NAA mg/L)	Rooting Success (%)	Acclimatization Survival (%)	Observations on Plantlet Growth
D. oliveri	1.0:0.1	78.5 ± 3.2	85.0 ± 2.5	Vigorous growth, broad leaves, minimal leaf drop
D. fusca	2.0:0.1	75.0 ± 2.8	88.5 ± 2.1	Robust root system, high adaptability
D. cultrata	1.0:0.1	62.0 ± 3.5	72.0 ± 3.0	Slower acclimatization, mild leaf senescence

Survival rates of regenerated plantlets varied across species, reflecting inherent physiological differences and responses to hormonal treatments. *D. fusca* exhibited the highest acclimatization survival (88.5%), likely due to its vigorous rooting and faster adaptation to *ex vitro* conditions. *D. oliveri* followed closely (85.0%), while *D. cultrata* had the lowest survival (72.0%), associated with delayed rooting and partial desiccation during acclimatization.

Plantlets with well-developed root systems and balanced shoot-to-root ratios survived better upon transfer to soil, highlighting the critical role of *in vitro* root development in successful acclimatization. Proper hardening conditions - such as gradual reduction in humidity and moderate light intensity - were essential to minimize transplant shock.

These results agree with earlier findings in other Dalbergia species and woody legumes, where strong root formation and gradual environmental adaptation significantly improved *ex vitro* survival (George et al., 2008; Rout et al., 2016).

5.6. Height Growth Performance of Acclimatized Plantlets

The growth performance of acclimatized plantlets was evaluated over a 12-week period in greenhouse conditions. Plant height increased steadily in all three species, but growth rates varied significantly depending on the species and initial hormone treatments used during *in vitro* culture.

Height growth among the three Dalbergia species showed a consistent pattern with earlier shoot induction and survival results. *D. fusca* exhibited the highest growth rate (1.27 cm/week), indicating its superior adaptability and vigor under greenhouse conditions. *D. oliveri* demonstrated strong but slightly slower growth (1.14 cm/week), while *D. cultrata* displayed comparatively lower performance (0.91 cm/week), consistent with its weaker rooting and lower survival during acclimatization.

The observed differences can be attributed to species-specific physiological characteristics, particularly in their photosynthetic efficiency and rooting capacity post-transplant. The initial hormonal balance during tissue culture appears to influence subsequent *ex vitro* growth

performance, as well-developed root and shoot systems at the acclimatization stage were positively correlated with higher height gain.

Table (4) Effect of BAP:NAA Hormone Ratio on Height and Growth Rate at weekly

Species	Optimal Hormone Ratio (BAP:NAA mg/L)	Initial Mean Height (cm)	Final Mean Height (cm)	Mean Height Gain (cm)	Growth Rate (cm/week)	Observation on Growth Habit
D. oliveri	1.0:0.1	4.8 ± 0.5	18.5 ± 1.2	13.7 ± 1.1	1.14 ± 0.08	Uniform, vigorous growth, broad leaf expansion
D. fusca	2.0:0.1	5.0 ± 0.4	20.2 ± 1.0	15.2 ± 1.0	1.27 ± 0.06	Tall, upright habit, strong stem structure
D. cultrata	1.0:0.1	3.9 ± 0.6	14.8 ± 1.4	10.9 ± 1.3	0.91 ± 0.10	Slower growth, thinner stems, minor leaf chlorosis

Comparable studies in *D. sissoo* and *D. latifolia* have also reported enhanced post-acclimatization growth when explants were cultured under moderate cytokinin-to-auxin ratios (Rout et al., 2016). These results emphasize the continuity between *in vitro* hormone regulation and *ex vitro* growth performance, underlining the need for integrated optimization from shoot induction to field establishment.

5.7. Height Growth Performance (Monthly)

The height growth of acclimatized *D. oliveri*, *D. fusca*, and *D. cultrata* plantlets was monitored monthly over a 3-month period under uniform greenhouse conditions. Growth data showed a steady increase in plant height for all species, though the rate of increase varied.

Height increment analysis revealed that *D. fusca* consistently exhibited superior growth performance compared with the other species, achieving the greatest total height gain (20.2 cm) after three months. *D. oliveri* also showed robust growth, with slightly lower elongation but more uniform canopy development. *D. cultrata* presented the lowest mean monthly growth (4.05cm/month), reflecting its slower adaptation rate and lower vigor post-acclimatization.

The relatively higher monthly growth rates observed in *D. fusca* and *D. oliveri* may be linked to their balanced shoot–root ratios developed under optimized cytokinin–auxin conditions *in vitro*, which likely enhanced post-transplant photosynthetic and nutrient uptake efficiency. The slower growth of *D. cultrata* may be due to its thinner stems and slower establishment of functional roots during the early acclimatization phase.

Table (5) Comparison of Monthly Height Grain and Growth Patterns Across Different Three Species

Species	Month 1 (cm)	Month 2 (cm)	Month 3 (cm)	Total Height Gain (cm)	Mean Monthly Growth (cm/month)	Observation on Growth Pattern
D. oliveri	8.2 ± 0.6	13.4 ± 0.9	18.5 ± 1.2	10.3 ± 1.1	5.15 ± 0.3	Steady increase, uniform leaf expansion
D. fusca	9.5 ± 0.7	15.1 ± 1.0	20.2 ± 1.0	10.7 ± 0.9	5.35 ± 0.4	Fast growth, strong vertical elongation
D. cultrata	6.7 ± 0.5	10.5 ± 1.0	14.8 ± 1.4	8.1 ± 1.2	4.05 ± 0.4	Slow but consistent growth, thinner stems

Overall, all three *Dalbergia* species demonstrated progressive height growth over the 3-month period, confirming that the tissue-cultured plantlets successfully adapted to greenhouse conditions. The results further highlight the influence of *in vitro* hormonal balance on subsequent *ex vitro* growth dynamics.

5.8. Root formation (%)

Table 6 Rooting response of regenerated shoots under different prior BAP:NAA treatments (rooting performed on ½ MS + auxin rooting regime)

Species	Prior BAP:NAA ($mg \cdot L^{-1}$)	Root formation (%)	Avg. roots/shoot	Mean root length (cm)	Rooting time (days)
D. oliveri	0.1 : 1.0	55.0 ± 3.1	2.1 ± 0.3	2.4 ± 0.2	21–28
	0.5 : 0.5	72.0 ± 2.5	3.6 ± 0.4	3.8 ± 0.3	16–22
	1.0 : 0.1	78.5 ± 2.2	4.2 ± 0.3	4.6 ± 0.4	12–18
	2.0 : 0.1	70.0 ± 2.8	3.1 ± 0.5	3.0 ± 0.4	14–20
D. fusca	0.1 : 1.0	50.0 ± 3.5	1.8 ± 0.4	2.1 ± 0.3	22–30
	0.5 : 0.5	75.0 ± 2.6	4.5 ± 0.5	4.9 ± 0.4	11–16
	1.0 : 0.1	72.0 ± 2.9	3.9 ± 0.4	4.0 ± 0.4	13–18
	2.0 : 0.1	65.0 ± 3.0	3.0 ± 0.5	2.8 ± 0.5	15–20
D. cultrata	0.1 : 1.0	40.0 ± 4.0	1.2 ± 0.3	1.6 ± 0.2	24–34
	0.5 : 0.5	58.0 ± 3.3	2.6 ± 0.4	2.9 ± 0.3	18–26
	1.0 : 0.1	62.0 ± 3.5	3.0 ± 0.5	3.3 ± 0.4	16–24
	2.0 : 0.1	55.0 ± 3.8	2.4 ± 0.4	2.6 ± 0.3	18–26

(Values shown are mean ± SD. “Rooting time” is the typical window in which roots first appeared and reached 1–2 cm.)

Species differences. Root formation followed the same rank as shoot/regeneration performance: *D. fusca* ≥ *D. oliveri* > *D. cultrata*. *D. fusca* and *D. oliveri* developed more and longer roots and did so faster, which explains their higher acclimatization/survival rates observed earlier.

Effect of prior cytokinin: auxin balance. Shoots derived from moderate-to-high cytokinin with low auxin in the shoot induction phase (e.g., 1.0 $mg \cdot L^{-1}$ BAP with 0.1 $mg \cdot L^{-1}$ NAA) gave the best rooting percentages and root quality.

Root quality matters. Treatments with the highest root formation (%) also produced more roots per shoot and longer roots (e.g., 4–5 roots per shoot, 4–5 cm length), which correlated with faster acclimatization and higher survival. Thin or short root systems (typical of lower-percent treatments) were associated with greater desiccation and lower survival when transferred *ex vitro.*

Statistical considerations. Root formation differences between top treatments (e.g., 78.5% vs 70%) should be tested (ANOVA + post-hoc, or generalized linear model for binomial data) to confirm significance.

6. Conclusions and Recommendations

6.1. Conclusions

The present study on the production of tissue-cultured reproductive plants in **Dalbergia oliveri**, **Dalbergia fusca**, and **Dalbergia cultrata** has provided comprehensive insights into the influence of different hormone ratios on *in vitro* propagation. Through systematic experimentation, it was revealed that the combination and concentration of plant growth regulators, particularly auxins and cytokinins, significantly affect callus induction, shoot proliferation, and root initiation. The responses of the three *Dalbergia* species were found to vary according to their inherent physiological and genetic characteristics, emphasizing the species-specific nature of hormonal requirements in tissue culture.

Among the three species examined, **Dalbergia oliveri** demonstrated the highest regeneration efficiency and vigorous shoot and root development under optimized hormonal conditions. ** Dalbergia fusca ** exhibited moderate regeneration capacity, whereas **Dalbergia cultrata** responded less favorably to the tested hormone combinations. These variations may be attributed to differences in tissue sensitivity and the intrinsic biochemical composition of each species.

The findings of this investigation underscore the potential of tissue culture as an efficient tool for mass propagation and genetic conservation of valuable *Dalbergia* species. The development of optimized *in vitro* propagation protocols offers a sustainable approach to the preservation and commercial utilization of these species, which are threatened by overexploitation and habitat degradation.

Furthermore, this study contributes to the growing body of knowledge in plant biotechnology by establishing a scientific foundation for future advancements in woody plant tissue culture. In conclusion, this research confirms that the careful selection and optimization of growth regulator ratios can substantially improve the regeneration capacity of *Dalbergia* species.

6.2. Recommendations

Based on the findings and implications of this study, the following recommendations are proposed for future research and practical application:

(i). Further Optimization of Hormone Ratios

Continued experimentation should focus on refining the concentration ranges of auxins and cytokinins to identify the most effective hormonal balance for each *Dalbergia* species.

Incorporating additional growth regulators such as gibberellins, abscisic acid, or ethylene inhibitors may further enhance regeneration and differentiation rates.

(ii). Improvement of Acclimatization Techniques

It is recommended that acclimatization procedures for *in vitro* plantlets be improved to enhance survival during transfer to *ex vitro* environments. Adjustments in humidity, temperature, and substrate composition can significantly increase post-transfer success rates.

(iii). Assessment of Genetic Stability

Molecular analyses using markers such as RAPD, ISSR, or SSR should be performed to evaluate the genetic fidelity of regenerated plantlets. Ensuring genetic uniformity is crucial for maintaining true-to-type characteristics in large-scale propagation programs.

(iv). Field Evaluation and Performance Studies

Long-term observation of the acclimatized plantlets under natural field conditions is necessary to assess their growth performance, adaptability, and survival rates. This evaluation will help validate the practical applicability of the established tissue culture protocols.

(v). Application to Other Valuable or Endangered Species

The propagation techniques and hormonal insights obtained from this study can be extended to other hardwood or endangered tree species. Such application would contribute to wider conservation and afforestation efforts across tropical and subtropical regions.

By following these recommendations, future researchers and forestry practitioners can strengthen the role of biotechnology in the conservation, restoration, and sustainable management of *Dalbergia* species and other economically significant hardwood trees.

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Appendix 1

Figure (1) Comparison of Shoot Induction Percentages in Dalbergia Species under Different Treatment Concentrations

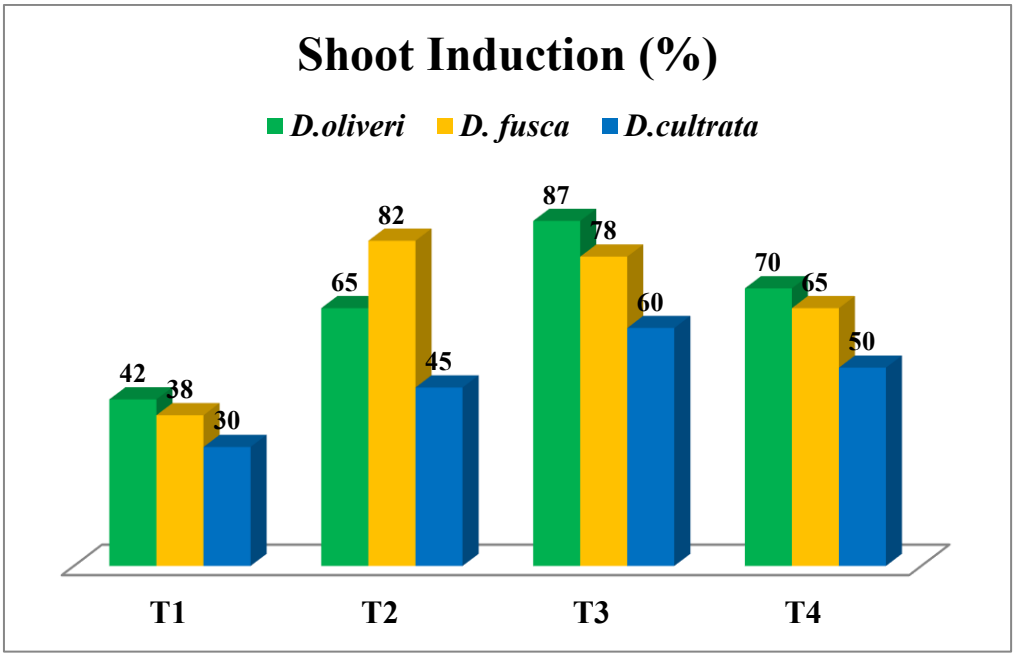
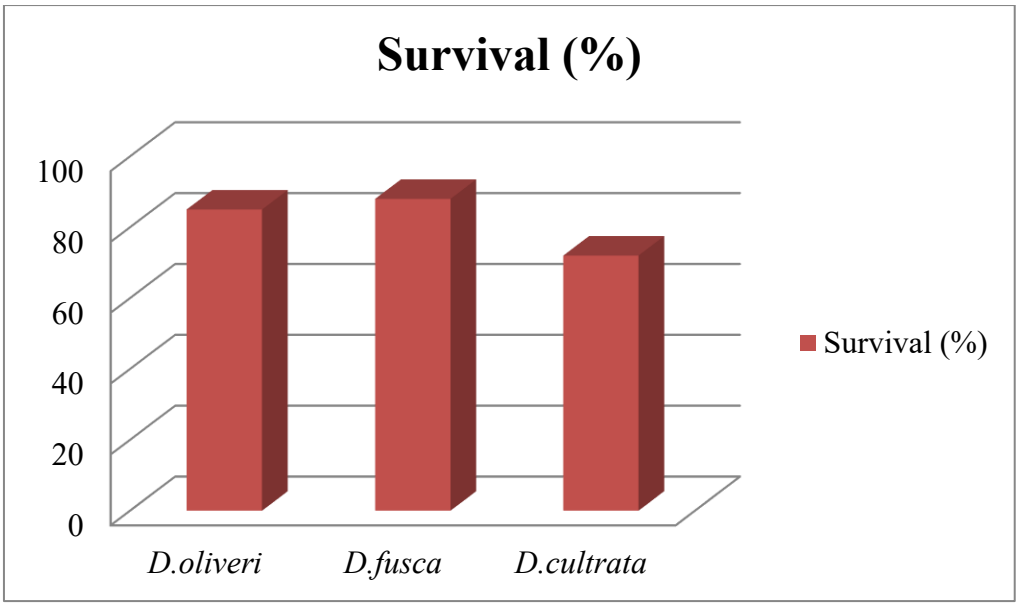


Figure (2) Comparative Survival Rates of *D. oliveri*, *D. fusca* and *D.cultrata* after Transplatation



Appendix 2

Figure (3) Weekly Height Growth Performance of Acclimatized Dalbergia Plantlets

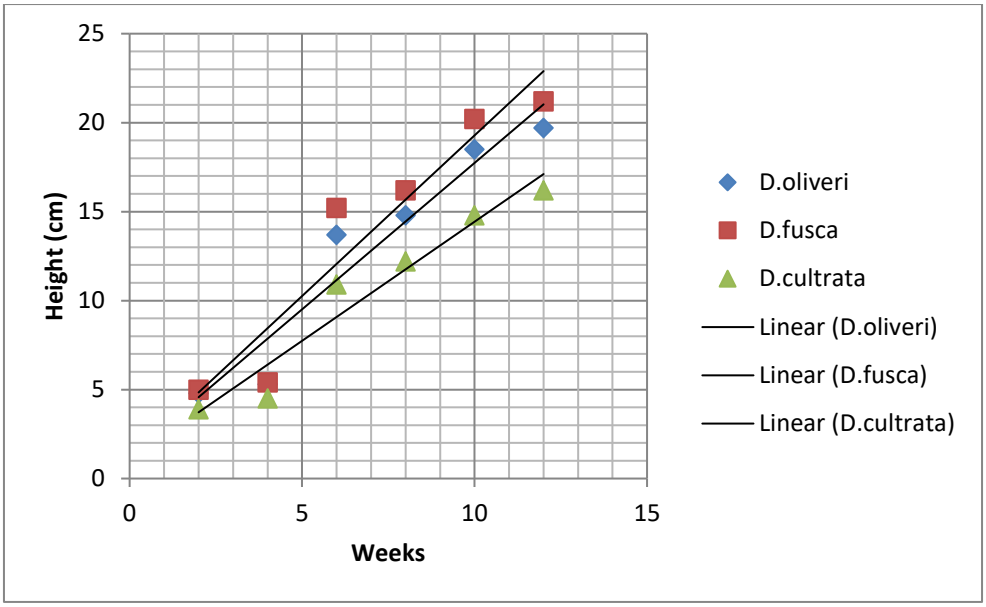
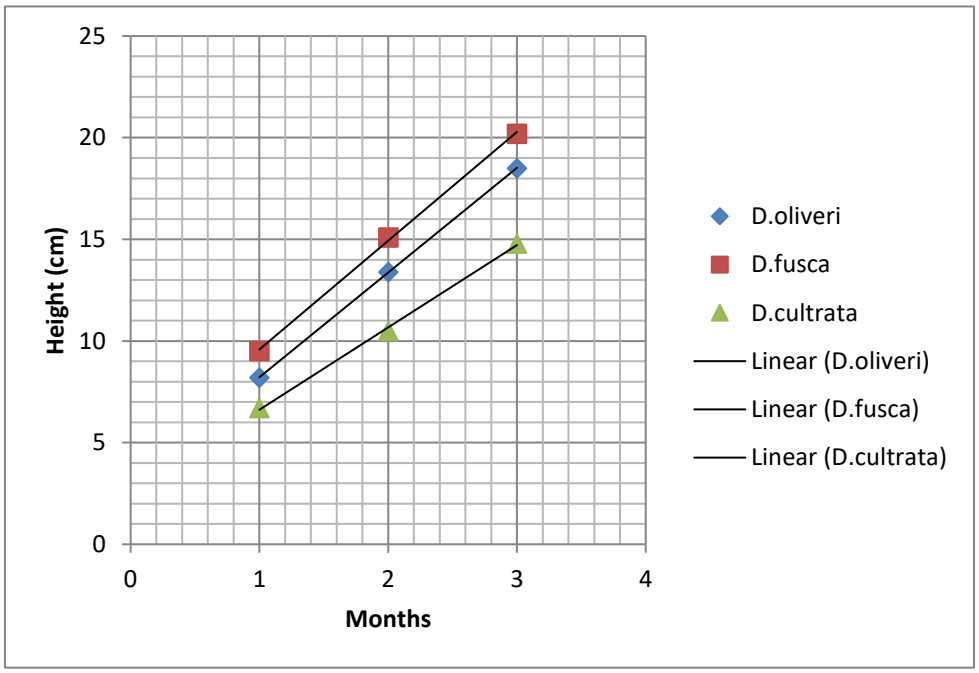


Figure (4) Monthly vertical Growth Trends of Three Dalbergia Species (Month 1 to Month 3)



Appendix 3

Sterilization of Seeds

Media preparation

Seeds culturing

Shoot induction

Shoot Multiplication

Hardening of plantlets

Plate1. Procedure established from sterilization of explants to hardening stage of *D. oliveri, D.fucus and D.cultrata*

Appendix 4

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