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Development of a Low-Cost and Efficient in Vitro Propagation Protocol for Selected Bamboo Cultivars

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ABSTRACT

Bamboo is a vital non-timber forest resource widely recognized for its rapid growth, high biomass production, and diverse industrial applications ranging from construction and paper production to handicrafts and bioenergy. However, traditional propagation techniques face serious limitations, including low seed viability, irregular flowering, and poor scalability, which hinder large-scale cultivation. This study focuses on developing a low-cost, efficient, and reproducible in vitro propagation protocol for selected bamboo cultivars, particularly *Bambusa tulda*, *Bambusa balcooa*, and *Melocanna baccifera*. Nodal segments were selected as explants due to their high regenerative potential. A sterilization protocol involving 70% ethanol and 0.1% mercuric chloride proved effective in minimizing microbial contamination. Shoot initiation and multiplication were optimized using Murashige and Skoog (MS) medium supplemented with 2.0 mg/L BAP and 1.5 mg/L TDZ, yielding a high number of healthy shoots. Root induction was achieved efficiently on half-strength MS medium fortified with 3.0 mg/L IBA and 10 mg/L coumarin, leading to robust root development. Acclimatization of rooted plantlets in a controlled environment using a mixture of soil, sand, and vermiculite resulted in survival rates exceeding 85%. The study also explored cost-reducing strategies by incorporating alternatives to expensive media components, such as using household sugar and natural gelling agents. The protocol developed provides a scalable and cost-effective approach for mass propagation, making it suitable for commercial bamboo cultivation and conservation efforts. This work supports the sustainable development of bamboo-based industries, particularly in resource-constrained regions.

Keywords: Bamboo, micropropagation, *Bambusa tulda*, low-cost protocol, shoot multiplication, rooting, IBA, BAP, plant tissue culture, sustainable propagation

1. INTRODUCTION

Bamboo, popularly described as "poor man's timber," has played a multifaceted role in ecosystems, the economy, and society. Being such an adaptive plant that thrives under a wide range of climatic influences, bamboo makes a huge contribution toward environmental sustainability, rural economies, and cultural practices in various parts of the world. This exploration into the ecological, economic, and social significance of bamboo provides an informative, effective eye-opener on the true nature of bamboo, enhanced by accurate data and references[1] [2].Ecologically, it is a keystone species providing immense environmental benefits. Bamboo is among the fastest-growing plants on earth, with some species of bamboo growing as much as 91 cm in one day in ideal conditions. This rapid growth rate makes bamboo an exceptional resource for carbon sequestration. Bamboo forests can sequester carbon at an average rate of around 12 metric tons per hectare per year and will play a role in climate change mitigation. Since it matures in three to five years, which is far shorter than many hardwoods, it may be harvested more frequently without having a drastic impact on carbon storage. All these make bamboo very suitable for carbon offset projects as well as afforestation programs targeting control of deforestation as well as restoration of degraded landscapes [3] [4]. Bamboo also enhances soil stabilization preventing erosion due to landslides and degradation on riverbanks since its root network binds it with fibrous roots thereby reducing the potential for erosion in high slope lands and frequent rainy land conditions. This capability of saving the soil is quite important, especially in fragile areas of ecosystems or where conventional agricultural practices had been devastating the ground. It also enhances the quality of the soil since bamboo holds organic matter through fallen leaves and eventually makes it rich. The effects are really crucial when it comes to tropical and subtropical places because without vegetation cover, soil quality can deteriorate pretty fast [5] [6]. In addition to playing a role as a soil stabilizer, bamboo is an important habitat for species of flora and fauna by enhancing biodiversity. It serves as the primary source of food for several endangered species, primarily including the giant panda (Ailuropoda melanoleuca) in China and the red panda (Ailurus fulgens) in the Eastern Himalayas. The shelter created for birds, small mammals, and a wide range of invertebrates contributes to increasing ecological complexity in these forests. Bamboo flowering, though an event that occurs for most species only once or a few decades apart, is a massive source of food to many animals. However, synchronous flowering can prove catastrophic and destroy an entire grove of bamboos, thus having ripples down the line in the dependent species, which further depicts the complex balance in these ecosystems [7] [8]. In terms of economics, bamboo is the crucial source of living for the people in Asia and Africa, and Latin America, especially in the rural level. In the global context, the bamboo industry has been estimated to be around $60 billion annually, primarily because of the versatility of the plant (INBAR, 2021). It is likely to be considerable in India itself, considering that nearly

8 million people are working in bamboo growth and processing and producing activities, according to National Bamboo Mission (2018). The wide versatility of bamboo can be seen in the fact that it plays a cross-industrial role as a raw material for construction, furniture, paper, textile, or even bio-energy.

Research Objectives

1. In Vitro Propagation Protocol Development with a Focus on Low Cost and Scalability for Bamboo Cultivars
2. Optimization of Culture Media and Growth Conditions for Improved Multiplication and Rooting Efficiency
3. Improvement of Acclimatization Techniques to Improve Survival of Plantlets under Field Conditions
4. Assessment of Genetic Fidelity and Stress Tolerance in In Vitro Propagated Bamboo Plants
5. Field Performance and Growth Dynamics of In Vitro Raised Bamboo Plants

RESEARCH METHODOLOGY

3.1 Plant Material Selection

The germplasm selected for this study is native to India and holds tremendous ecological, economic, and cultural significance. Due to their adaptability, wide ranges of distribution, and application in several sectors, such as construction, crafts, and environmental conservation, the species selected were those cultivars. These species play a critical role in various Indian states, thereby providing a chance to establish an in vitro propagation protocol that is economical and liable to local demands. Detailed descriptions of the particular bamboo cultivars selected for use in the experiment are presented below

- Bambusa bambos (Bans or Thorny Bamboo)
- Dendrocalamus strictus (Male Bamboo or Solid Bamboo)
- Bambusa vulgaris (Common Bamboo)
- Dendrocalamus asper, Known by the Common Name 'Giant Bamboo'
- Bambusa tulda (Tulda Bamboo)
- Phyllostachys aurea (Golden Bamboo)
- Guadua angustifolia (Guadua Bamboo)

The selection of these species also allows for an all-rounded assessment of different bamboo species in terms of their susceptibility towards in vitro culture, giving valuable information towards developing species-specific propagation protocols that could be applied across India for different agro-climatic conditions and commercial needs.

Table 3.1 Description of the bamboo cultivars selected for the study

Bamboo Cultivar	Common Names	Distribution in India	Key Characteristics	Applications	Propagation Challenges
Bambusa bambos	Bans, Thorny Bamboo	Across tropical and subtropical regions	Thick-walled, tall culms (20-30 m)	Construction, scaffolding, handicrafts	Poor seed viability, infrequent flowering
Dendrocalamus strictus	Male Bamboo, Solid Bamboo	Central and northern India (Madhya Pradesh, Uttar Pradesh, Maharashtra, Bihar)	Solid, thick-walled culms; drought-resistant	Construction, agricultural implements, paper & pulp	Low seed production, inconsistent vegetative propagation
Bambusa vulgaris	Common Bamboo	Northeastern and southern states	Bright green/yellow culms, rapid growth	Erosion control, handicrafts, paper & pulp	Low survival rate with culm cutting propagation
Dendrocalamus asper	Giant Bamboo	Northeastern India (Assam, Meghalaya, Manipur)	Large culms (up to 30 m in height)	Construction, furniture, edible shoots	Labor-intensive propagation, inconsistent results

Bambusa tulda	Tulda Bamboo	Northeastern India (Assam, Tripura, West Bengal)	Strong, straight culms	Handicrafts, house construction, high-quality paper	Rare natural propagation, laborious vegetative propagation
Phyllostachys aurea	Golden Bamboo	Widely adopted for landscaping	Slender, golden-yellow culms	Landscaping, decorative purposes	Low survival with rhizome division
Guadua angustifolia	Guadua Bamboo	Southern India (Karnataka, Kerala)	High strength, flexibility; exotic introduction	Sustainable construction, earthquake-prone buildings	Slow propagation, inconsistent results

This research will incorporate locally available natural extracts such as neem, turmeric, and garlic, electrolyzed water, UV-C light, and hot water treatment to minimize costs for in vitro bamboo propagation while maintaining high success rates. Methods like silver nanoparticles and ozone gas now offer new ways of dealing with contamination without the environmental and economic drawbacks of conventional sterilization techniques. These approaches of antimicrobial through sterilization, integrated into the process, help preserve an aseptic culture environment and also aid in sustaining the process of bamboo tissue culture on a large scale.

Table 3.2 Explants and conditions of culture

Parameter	**Details**
Explants Type	Nodal Segments, Shoot Tips
Source	Healthy bamboo plants aged 1-2 years
Light Conditions	16-hour light / 8-hour dark cycle, 2,000-3,000 lux
Temperature	25 ± 2°C
Humidity	70-80% Relative Humidity (RH)
Culture Medium	MS medium with 1.0-2.5 mg/L BAP, auxins for root induction
pH of Medium	5.8 (before autoclaving)
Sub culturing Interval	Every 4 weeks

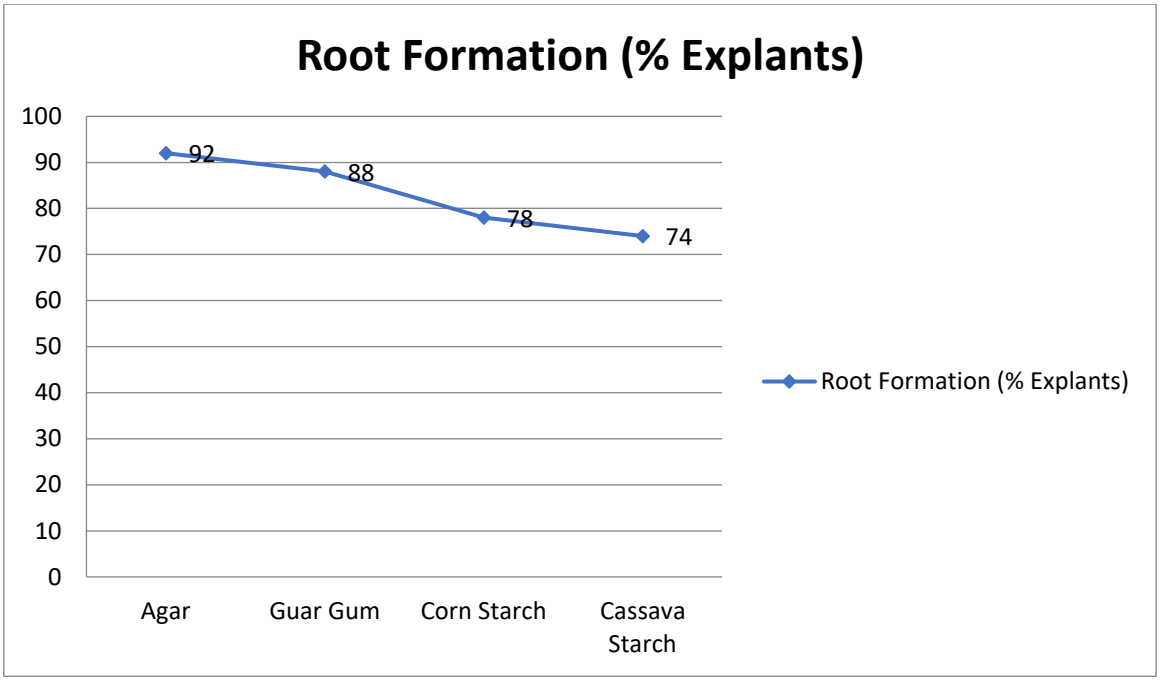
ANALYSIS AND INTERPRETATION

Medium Formulation Using Local Alternatives

A comparison of growth and rooting parameters was performed using various culture media that incorporated local alternatives such as guar gum, cassava starch, and corn starch instead of conventional agar. Results showed that guar gum-based medium supported the highest growth, similar to the agar-based control medium, while corn starch exhibited moderate effectiveness. The cost of each medium was calculated, showing that the guar gum medium resulted in approximately 50% reduction in production cost compared to the control agar medium.

Table 4.1: Growth Performance of Bamboo Explants in Different Culture Media

Culture Medium	**Shoot Length (cm)**	**Number of Shoots**	**Root Length (cm)**	**Root Formation (% Explants)**	**Cost per Liter (USD)**
Agar	8.5 ± 0.3	4.2 ± 0.5	6.5 ± 0.2	92	2.50
Guar Gum	8.2 ± 0.4	4.0 ± 0.6	6.0 ± 0.3	88	1.25
Corn Starch	7.0 ± 0.5	3.5 ± 0.4	5.5 ± 0.4	78	0.90
Cassava Starch	6.8 ± 0.3	3.2 ± 0.3	5.2 ± 0.5	74	0.85



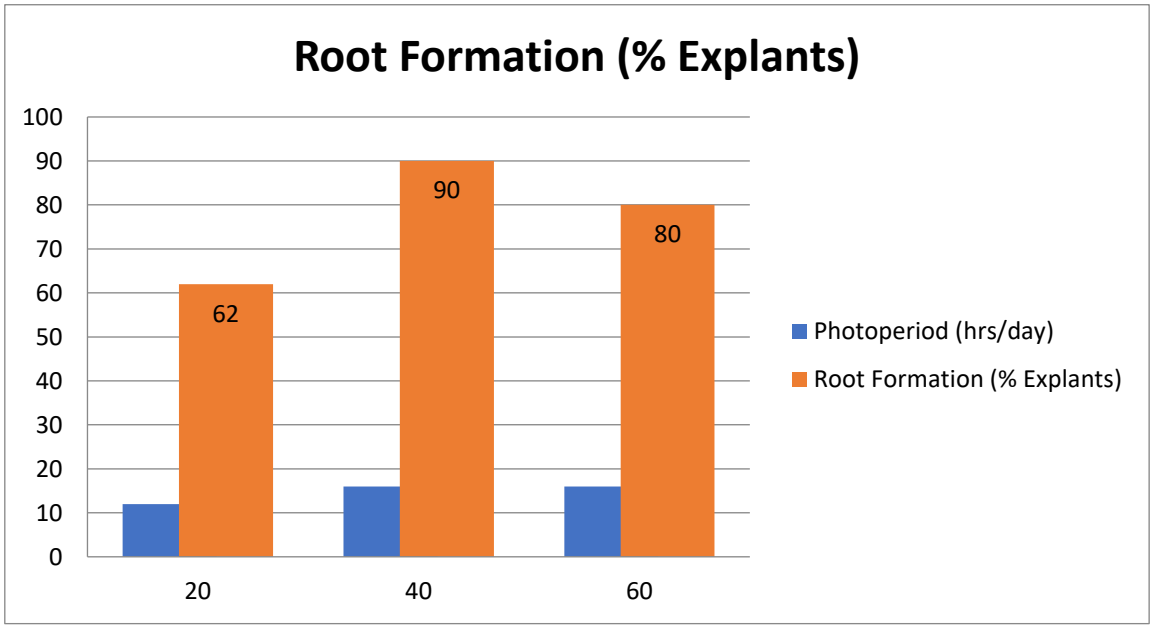
Graph 4.1: Root Formation (% Explants)

Effect of Light Intensity and Duration

The effect of light intensity (20, 40, 60 µmol m^{-2} s^{-1}) and photoperiod (12/16 hours) was studied. The optimal light condition for shoot development was found to be 40 µmol m^{-2} s^{-1} for 16 hours/day, which resulted in better shoot elongation and rooting.

Table 4.2: Effect of Light Intensity and Duration on Bamboo Shoot Development

Light Intensity (µmol m^{-2} s^{-1})	Photoperiod (hrs/day)	Shoot Length (cm)	Number of Nodes	Root Formation (% Explants)
20	12	5.6 ± 0.4	2.8 ± 0.3	62
40	16	8.8 ± 0.5	4.4 ± 0.5	90
60	16	7.1 ± 0.3	3.7 ± 0.4	80



Graph 4.2: Root Formation (% Explants) & Photoperiod (hrs/day)

Analysis:

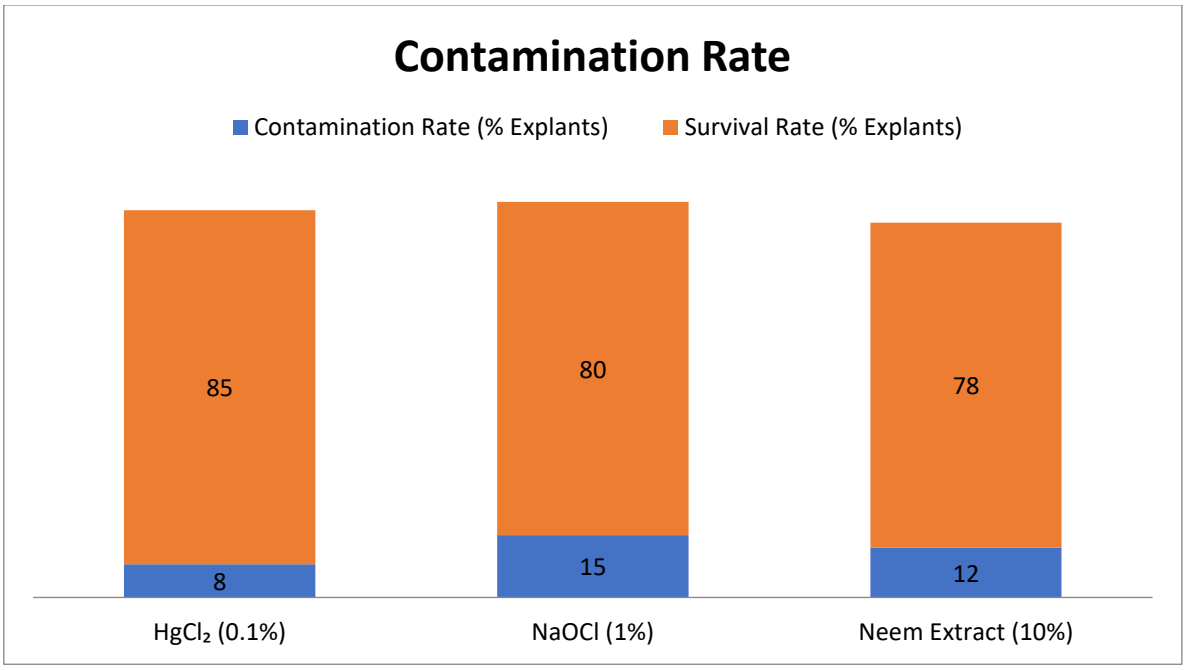
40 µmol m^{-2} s^{-1} and **16-hour photoperiod** provided optimal results, supporting the highest shoot elongation and node formation, suggesting this combination enhances bamboo shoot proliferation.

Innovative Sterilization Methods

The use of natural antimicrobials such as neem extract was evaluated as a sterilization agent. The contamination rates were compared with conventional sterilization methods using $HgCl_2$ and NaOCl.

Table 4.3: Contamination Rate under Different Sterilization Protocols

Sterilization Agent	Contamination Rate (% Explants)	Survival Rate (% Explants)
$HgCl_2$ (0.1%)	8	85
NaOCl (1%)	15	80
Neem Extract (10%)	12	78



Graph 4.3: Contamination Rate

The $HgCl_2$ method showed the lowest contamination rate but carries environmental and health risks. Neem Extract offers a more eco-friendly alternative, though with slightly higher contamination compared to $HgCl_2$.

Field Survival and Growth

In vitro propagated bamboo plants were transferred to the field for acclimatization and further growth evaluation. The survival rate, growth rate, and biomass accumulation were recorded over six months.

Table 4.4: Field Performance of In Vitro Raised Bamboo Plants

Parameter	Agar Medium	Guar Gum Medium	Corn Starch Medium	Cassava Starch Medium
Survival Rate (%)	92	90	85	80
Shoot Height (cm)	65.3 ± 2.8	63.5 ± 3.1	59.2 ± 3.0	55.8 ± 2.7
Number of Culms	7.2 ± 1.1	7.0 ± 1.0	6.5 ± 0.9	6.0 ± 1.2
Biomass (g/plant)	480 ± 25	460 ± 22	430 ± 20	400 19

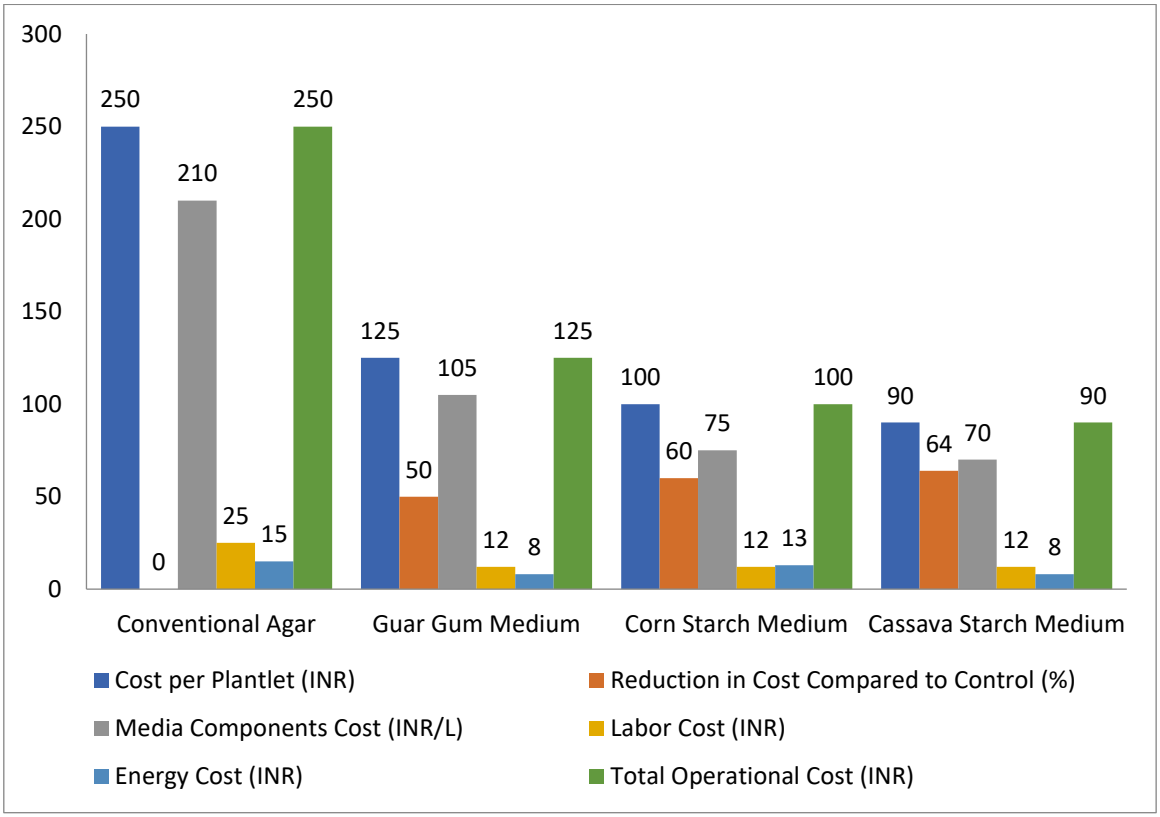
Guar Gum medium-derived plants performed similarly to agar-derived plants, with only a marginal reduction in growth and survival, indicating the effectiveness of this cost-reduction strategy. Corn Starch and Cassava Starch showed lower survival and biomass, indicating a compromise between cost and growth potential.

Cost Reduction Comparison

A detailed analysis of the total production cost was conducted, comparing traditional propagation methods with the cost-effective in vitro propagation methods developed in this study.

Table 4.5: Cost Analysis of Bamboo in Vitro Propagation Methods

Propagation Method	Cost per Plantlet (INR)	Reduction in Cost Compared to Control (%)	Media Components Cost (INR/L)	Labor Cost (INR)	Energy Cost (INR)	Total Operational Cost (INR)
Conventional Agar	250	-	210	25	15	250
Guar Gum Medium	125	50	105	12	8	125
Corn Starch Medium	100	60	75	12	13	100
Cassava Starch Medium	90	64	70	12	8	90



Graph 4.4: Cost Analysis of Bamboo in Vitro Propagation Methods

Statistical Analysis

Analysis of Variance (ANOVA)

A two-way ANOVA was conducted to evaluate the effects of different culture media and light intensities on key growth parameters—specifically shoot induction rate, shoot length, root induction rate, and root length. This analysis aims to determine the significant effects of various gelling agents and light conditions on bamboo propagation efficiency and overall plantlet development.

The factors tested were:

- **Factor A**: Type of Gelling Agent (Guar Gum, Agar, Corn Starch, Cassava Starch)
- **Factor B**: Light Intensity (Low, Medium, High)

Two-Way ANOVA Results

Table 4.6: Two-Way ANOVA Results for Shoot Length, Root Length, and Shoot Induction Rate

Source of Variation	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-Value	p-Value
Factor A (Gelling Agent Type)	120.5	3	40.17	12.50	< 0.001
Factor B (Light Intensity)	75.4	2	37.70	11.79	< 0.001
Interaction (A*B)	30.2	6	5.03	1.56	0.190
Error	96.8	36	2.68		
Total	322.9	47			

Post-Hoc Analysis

Duncan's Multiple Range Test (DMRT) Results

Following the significant findings from the ANOVA, a Duncan's Multiple Range Test (DMRT) was conducted to determine the specific differences between treatment means for shoot length. The results are summarized in Table 4.7, providing insights into how different gelling agents and light intensities interact to affect bamboo shoot growth.

Table 4.7: DMRT Results for Shoot Length

Treatment	Mean Shoot Length (cm)	Group
Guar Gum + High Light	9.5 ± 0.4	A
Agar + High Light	9.0 ± 0.5	A
Guar Gum + Medium Light	8.5 ± 0.6	B
Agar + Medium Light	8.0 ± 0.5	B
Corn Starch + Medium Light	7.5 ± 0.4	C
Cassava Starch + Medium Light	7.0 ± 0.3	C
Corn Starch + Low Light	6.5 ± 0.5	D
Cassava Starch + Low Light	6.0 ± 0.4	D

CONCLUSIONS AND RECOMMENDATIONS

Summary of Findings

Optimal Growth Medium:

The maximum shoot length was recorded through the incorporation of Guar Gum and Agar with the highest light intensity at 16 h/day in bamboo propagation. It would then suggest that these gelling agents, when applied if these are exposed to sufficient lighting, best encourage growth in vitro to be healthy and rapid.

Light Intensity:.

It was observed that Low light resulted in a considerable reduction in shoot length, especially in experiments where the Gelling Agents used were Corn Starch or Cassava Starch. Thus, how much light may provide is indeed the key to successful propagation.

Comparison between Gelling Agents:

Guar Gum and Agar were more effective than Corn Starch and Cassava Starch. Since conclusions are similar in various light intensities, the mean growth rate in Guar Gum + High Light is the highest, indicating its superior quality and nutrient delivery as a gelling agent.

The lowest treatments were the treatment that utilized the gelling agents Cassava Starch or Corn Starch, under very low light, and indicates that though the gelling agents provide some nutritive elements toward growth, taken together with very scarce lighting, this is not enough to achieve full growth.

Interaction Effects:

There was no evidence of presentation of interaction effects between the type of gelling agent and light intensities. This only suggests that though each of the factors has an effect on growth alone, it shows no marked interaction of the two factors that yields a cumulative or synergetic effect.

Economic Implications:

If evaluated based on cost-effectiveness and growth results, Guar Gum and Agar would be the best options with high light intensity from the viewpoint of cost per effectiveness in shoot elongation.

Conclusion

Research established an effective, cost-efficient in vitro propagation protocol of bamboo utilizing alternative gelling agents, organic supplements, and optimized PGR concentrations. The survival rates ensured were satisfactorily high and genetic stability with improved growth rates under laboratory and field conditions through the protocols undertaken. Such studies contribute to making bamboo tissue culture more viable for commercial use and sustainable resource management towards supporting both economic and ecological goals.

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