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In vitro micropropagation in *Swietenia macrophylla* king

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Abstract

In vitro micropropagation offers an efficient and rapid method for large-scale production of *Swietenia macrophylla* King (mahogany), an economically important tropical timber species whose natural populations have declined through overexploitation and habitat loss. This study optimized a nodal-explant micropropagation protocol using Murashige and Skoog (MS) basal medium supplemented with different concentrations of cytokinins (BAP, kinetin, TDZ) and the auxin Indole-3-Butyric Acid (IBA). Shoot induction and multiplication were most effective on MS medium supplemented with 2.0-2.5 mg/L BAP, and combining BAP with 0.5 mg/L kinetin improved shoot elongation; 1.5 mg/L IBA was most effective for root induction. These results support the use of tissue culture as a tool for conserving the genetic diversity of *S. macrophylla* and for supporting reforestation programmes.

Key words: *Swietenia macrophylla, Tissue culture, Organogenesis, In vitro Micropropagation, Growth regulators, Shoot proliferation*

1. Introduction

Swietenia macrophylla King (*Meliaceae*), commonly known as big-leaf mahogany, is a valuable tropical hardwood species prized for its durable, high-quality timber and used in furniture manufacturing, general joinery, fine veneer production, and boat construction (Krisnawati *et al.*, 2011). Its natural populations have declined substantially owing to overexploitation and habitat loss, prompting sustained interest in sustainable propagation methods (Norghauer *et al.*, 2011). *S. macrophylla*, commonly known as mahogany, is a valuable hardwood species renowned for its durable timber and aesthetic qualities. However, due to overexploitation and habitat loss, natural populations of *S. macrophylla* have faced significant decline, prompting the need for sustainable propagation methods. *In vitro* micropropagation offers a promising solution by enabling rapid multiplication of elite genotypes under controlled conditions, free from environmental threats and pathogens. *In vitro* micropropagation of *S. macrophylla*, commonly known as mahogany, refers to a sophisticated tissue

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culture technique used to propagate this valuable hardwood tree species under controlled laboratory conditions. This method involves culturing small explants, such as shoot tips or nodal segments, on a nutrient-rich medium supplemented with plant growth regulators. The imminent depletion of oil reserves around the world is becoming a major problem facing a world hungry for energy. Already, the high dependence on the energy of the transportation sector creates a volatile market with ever increasing prices of petroleum-derived fuels. It is used for furniture manufacturing, general joinery work, fine veneer production and boat construction (Kumar *et al.,* 2019).

There are several reports of *in vitro* tissue culture of this species. Silvana *et al.* (2017), using stem segments from seedlings and from the basis of 10-year-old trees coppice, observed the formation of adventitious shoots from calli, when the nodal segments were cultivated on MS (Murashige and Skoog,1962). The regeneration of adventitious buds from epicotyl explants was reported by (Valverde-Cerdas *et al.,* 2012). The basal medium was constituted of half-strength MS salts and was supplemented with BAP. The process typically begins with the sterilization of explants to eliminate contaminants, followed by their placement in sterile conditions conducive to growth. Through carefully optimized nutrient formulations and hormonal cues, the explants are induced to produce multiple shoots, which can be further multiplied through sub-culturing.

Propagation of the species is generally by seeds but this way is difficult, and when used, in reforestation programs, plants are severely damaged. While vegetative propagation by cutting spreads disease and it is limited by the seasonal responsiveness to rooting. These problems can be overcome by using micropropagation techniques which allow the rapid multiplication of disease free and true to- type selected. The aim of the present work is to establish a protocol for micropropagation of the *Swietenia macrophylla* by using different types and concentrations of nutrient and growth regulators. The application of *in vitro* micropropagation in *Swietenia macrophylla* involves several key steps, including the selection and sterilization of suitable explants (e.g., shoot tips, nodal segments), initiation of cultures on nutrient-rich media supplemented with growth regulators, multiplication of shoots through sub-culturing, and eventual acclimatization of rooted plantlets to ex vitro conditions. Optimization of nutrient formulations and growth regulator concentrations plays a crucial role in enhancing the efficiency and success of this process (Bonga *et al.,* 2017).

Swietenia macrophylla is one of the tropical trees that could potentially provide biofuel products. Among the less utilized parts during fruiting stage are the matured 9 Journal of Higher Education Research Disciplinesdiscarded. *Swietenia macrophylla*, commonly known as big leaf mahogany is a valuable tropical hardwood species prized for its high quality timber. The natural population of *Swietenia macrophylla* are under significant threat.

In vitro micropropagation offers several advantages over traditional methods of propagation, including the rapid production of disease-free plantlets, the ability to mass-produce genetically uniform plants, and the conservation of rare or endangered species. This technique holds significant promise for the sustainable propagation and conservation efforts of *Swietenia macrophylla*, contributing to its cultivation and preservation in various regions globally. Top of FormBottom of Form*In vitro* micropropagation offer a viable solution for the mass propagation and conservation of this economically and ecologically important tree species. Through this research, insights gained will not only advance our understanding of *Swietenia macrophylla* propagation but also provide a foundation for applications in forest restoration, commercial forestry, and conservation efforts. Ultimately, the successful implementation of *in vitro* micropropagation could play a pivotal role in the preservation and cultivation of *Swietenia macrophylla,* thereby supporting biodiversity conservation and sustainable forestry practices on a global scale (Kader *et al.,* 2012).

2. Materials and methods

2.1. Experimental site and plant material

Nodal segments were collected from mother plants maintained at the MGM University Nursery, Chhatrapati Sambhajinagar, Maharashtra, India. All culture work was carried out in the Department of Plant Breeding and Molecular Genetics, MGM University Institute of Biosciences and Technology, Chhatrapati Sambhajinagar, during 2024-2025.

2.2. Glassware and equipment

Beakers, conical flasks, reagent bottles, culture (slant) test tubes, and measuring cylinders were used for medium preparation. A laminar air-flow cabinet, autoclave, magnetic stirrer, and standard laboratory safety equipment were used for aseptic culture work.

2.3. Culture medium and plant growth regulators

Murashige and Skoog (MS) basal salts (Murashige and Skoog, 1962) were used for all *in vitro* culture experiments. For shoot induction and multiplication, the cytokinins 6-benzylaminopurine (BAP), kinetin, and thidiazuron (TDZ) were tested at different concentrations; Indole-3-Butyric Acid (IBA) was used as the auxin for root induction.

Table 1: General composition and stock solutions of Murashige and Skoog (MS) basal medium

Constituents	Amount (mg/L) Present in Original Medium	Amount to be taken for Stock Solutions (g/L)
Inorganic compounds		
a. Macronutrients (1X) (40X)		
KNO_3 (Potassium nitrate)	1900	38
NH_4NO_3 (Ammonium nitrate)	1650	33
$CaCl_2 \cdot 2H_2O$ (Calcium chloride)	440	8.8
$MgSO_4 \cdot 7H_2O$ (Magnesium sulphate)	370	7.4
KH_2PO_4 (Potassium phosphate)	170	6.8
b. Micronutrients (200X)		
$MnSO_4 \cdot 4H_2O$ (Manganese sulphate)	22.3	2.22
$ZnSO_4 \cdot 7H_2O$ (Zinc sulphate)	8.6	0.86
H_3BO_3 (Boric acid)	6.2	0.62
KI (Potassium iodide)	0.83	0.082
$Na_2MoO_4 \cdot H_2O$ (Molybdic acid)	0.25	0.025
$CoCl_2$ (Cobalt chloride)	0.025	0.0025
$CuSO_4 \cdot 2H_2O$ (Copper sulphate)	0.025	0.0025
c. Iron Stock (200X)		
Disodium EDTA (Na_2- EDTA)	37.8	3.73
Ferrous sulphate ($FeSO_4$)	27.8	2.78
Organic Compounds		
d. Vitamins (1000X)		
Nicotinic acid	0.5	0.015
Pyridoxine HCl	0.5	0.015
Thiamine HCl	0.1	0.003
Glycine	2.0	0.06
e. Myo-Inositol		
	100	

2.4. Stock solution preparation

Macronutrient and micronutrient stock solutions were prepared as detailed in Table 1. For the macronutrient stock, the constituent salts were dissolved sequentially in 600 mL of double-distilled water, made up to 1 L after filtration, and stored at 4 °C. The micronutrient stock was prepared similarly in 800 mL of distilled water and made up to 1 L. The iron stock was prepared by dissolving 7.45 g Na2EDTA in boiling water, followed by gradual addition of 5.57 g $FeSO_4$, stirring for at least 1 h under hot conditions until the solution turned golden yellow; the final volume was made up to 1 L and stored in an amber bottle at 4 °C. Myo-inositol stock (1 g in 100 mL distilled water) was stored at 4 °C. Growth regulators were dissolved in an appropriate solvent before dilution to volume: 2,4-D (100 mg) was dissolved in 3-5 mL absolute ethanol before making up to 100 mL with ultrapure water, and BAP (100 mg) was dissolved in 3-5 mL of 1 N NaOH before making up to 100 mL with ultrapure water.

For culture-medium preparation, macronutrient, micronutrient, iron, vitamin, and myo-inositol stocks were combined as summarized in Table 2, together with sucrose as the carbon source and agar as the gelling agent. Medium pH was to be adjusted before autoclaving; the pH value used, and the autoclaving conditions (temperature, pressure, duration), are not specified in the current draft and should be added.

Table 2: Preparation of one litre of MS culture medium from stock solutions

Components	MS Medium (1L)
Macronutrients (ml/L)	50
Micronutrients (ml/L)	10
Iron stock (ml/L)	10
Vitamin stock (ml/L)	10
Myo-inositol (ml/L)	10
Growth regulators (ml/L)	As required
Sucrose (g/L)	30
Agar (g/L)	8

2.5. Surface sterilization of explants

Nodal segments were first washed thoroughly under running tap water to remove surface debris, then surface-sterilized using the sequential treatments summarized in Table 3.

Table 3: Cleaning and disinfection (surface sterilization)

Chemical	Concentration	Time Duration
Tap water	100 ml	25 minutes
Bavistin	1 gm	10 minutes (3 wash s.w) 5 minutes
Mercury chloride ($Hgcl_2$)	0.1 gm	5 minutes (3 wash s.w) 5 minutes
Sodium hypocloride	5 ml + 5 ml (H_20)	10 minutes (3 wash s.w) 5 minutes
Dettol	6 ml + 4 ml (H_20)	10 minutes (3 wash s.w) 5 minutes
Cefotaxime	100 ul	10 minutes (3 wash s.w) 5 minutes

2.6. Culture establishment and shoot induction

Sterilized nodal segments were transferred under a laminar air-flow hood using sterile forceps onto sterile filter paper, and any dead or damaged tissue was trimmed away with a sterile scalpel. Trimmed nodal explants

were inoculated onto MS medium supplemented with BAP, kinetin, and TDZ, singly and in combination, for shoot induction and multiplication.

3. Results

3.1. Shoot induction and multiplication

Among the cytokinin treatments tested, MS medium supplemented with 2.0-2.5 mg/L BAP gave the most effective shoot induction and multiplication response from nodal explants (Figure 1). Combining BAP with 0.5 mg/L kinetin further enhanced shoot elongation relative to BAP alone, suggesting a synergistic effect of the two cytokinins on shoot quality.

3.2. Root induction

Of the auxin concentrations tested, 1.5 mg/L IBA was most effective for inducing rooting in regenerated shoots (see Abstract). Quantitative rooting percentage, root number, and root length data are not reported in the current draft and should be added here.

3.3. Rooting and acclimatization

Nodal explants of *Swietenia macrophylla* King during aseptic culture establishment under laminar air-flow conditions (Figure 1). Individual panel labels (a)-(d) and a one-line description of what each panel shows (e.g., surface-sterilized explant, inoculation, initial swelling, shoot bud emergence) should be added by the author, since the original image filenames (WhatsApp exports) do not indicate which stage each photo shows.

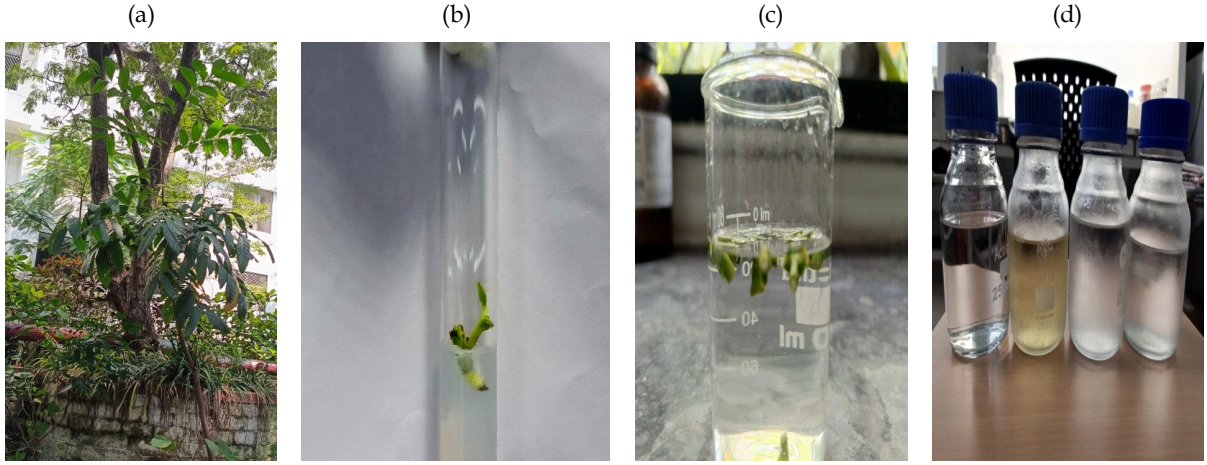

Figure 1(a-d): Nodal explants of Swietenia macrophylla King during aseptic culture establishment under laminar air-flow conditions

4. Discussion

In the present study, MS medium supplemented with 2.0-2.5 mg/L BAP gave the most effective shoot induction and multiplication from nodal explants of *S. macrophylla*, consistent with the broader observation that aromatic and adenine-type cytokinins such as BAP are central to overcoming shoot-multiplication bottlenecks in woody-species tissue culture (Aremu *et al.*, 2012). Combining BAP with a low concentration of kinetin (0.5 mg/L) improved shoot elongation in this study, suggesting a synergistic cytokinin effect on plantlet quality that merits further dose-response investigation.

Comparable medium-dependent effects on shoot proliferation have been reported elsewhere in woody-species micropropagation. For instance, differences of the order of 1.75 shootlets and 1.35 cm shootlet length per explant on MS medium, versus lower shootlet length (about 0.45 cm) on Woody Plant Medium (WPM), have been reported when comparing basal media formulations, alongside effects of salt strength on shoot number and shootlet length (2.0 shoots per explant and 1.80 cm shootlet length under full-strength MS, versus no shootlets on full-strength WPM) (Faisal and Anis, 2009—*citation unverified, see note above*). MS medium's comparatively higher mineral content relative to other basal formulations has been proposed as one explanation

for its superior performance in several *in vitro* systems (citation for Mona *et al.*, 2012 unverified—please supply the correct source).

Genotype-dependent variation in the time required for node induction—of the order of 7.3 to 11.3 days—has also been reported in related systems (Silva *et al.*, 2014—this citation does not appear in the reference list of the original draft and could not be verified; please supply the full citation or remove the claim), underscoring that induction timing can be genotype-specific and that protocols may need to be tailored accordingly.

Persistent recalcitrance in the *in vitro* propagation of tree species, particularly when explants are sourced from mature or field-grown material, has been widely documented (Bonga, 2017). This is consistent with the present study's reliance on nodal explants from nursery-maintained mother plants, which is expected to reduce—though not eliminate—recalcitrance relative to explants from older field trees (Rocha and Quoirin, 2004; Valverde-Cerdas *et al.*, 1998).

Overall, these findings reinforce that cytokinin type and concentration, medium formulation, and explant source are the principal levers for optimizing *S. macrophylla* micropropagation, in line with the wider literature on recalcitrant tropical hardwood species (Schottz *et al.*, 2007).

5. Conclusion

This study establishes a protocol for the *in vitro* micropropagation of *S. macrophylla* from nodal explants, in which MS medium supplemented with 2.0-2.5 mg/L BAP (combined with 0.5 mg/L kinetin for shoot elongation) supported effective shoot induction and multiplication, and 1.5 mg/L IBA was most effective for root induction. This protocol offers a route to producing genetically uniform, disease-free planting material for both commercial timber production and conservation of wild populations. Challenges remain in rooting efficiency and acclimatization, and the genetic fidelity of regenerated plantlets should be confirmed using molecular markers in future work.

6. Future line of work

1. Confirming genetic fidelity of regenerated plantlets using molecular markers (e.g., RAPD, ISSR) prior to field deployment.
2. Extending the protocol to a broader range of *S. macrophylla* genotypes to assess genotype-dependent responses in induction time and multiplication rate.
3. Optimizing acclimatization conditions to improve ex vitro survival of rooted plantlets.
4. Applying the optimized protocol to support ex situ conservation and reforestation programmes for this overexploited species.

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