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Development Of A Complex Biotechnological System For In Vitro Propagation Of Plumeria Plants

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Annotation: *Plumeria* species are highly valued ornamental and medicinal plants, but their conventional propagation methods are slow and inefficient for large-scale production. This study focuses on the development of a complex biotechnological system for the in vitro propagation of *Plumeria* plants. The system integrates optimized surface sterilization procedures, nutrient media composition, and plant growth regulators to ensure high regeneration efficiency. Shoot induction, multiplication, rooting, and acclimatization stages were systematically optimized under controlled laboratory conditions. The results demonstrate that the use of Murashige and Skoog (MS) medium supplemented with appropriate concentrations of cytokinins and auxins significantly enhances micropropagation efficiency. The developed protocol ensures rapid, uniform, and disease-free plant production, offering a reliable biotechnological approach for the conservation and mass propagation of *Plumeria* species.
Keywords: *Plumeria*, in vitro propagation, biotechnology system, micropropagation, plant tissue culture, Murashige and Skoog medium, plant growth regulators, clonal propagation, acclimatization

Introduction. The genus *Plumeria* belongs to the family Apocynaceae and includes several species widely distributed in tropical and subtropical regions of the world. These plants are highly valued for their ornamental beauty, pleasant fragrance, and diverse medicinal properties. *Plumeria* flowers are commonly used in landscape design, religious ceremonies, and traditional medicine systems, particularly in Asia and Latin America. Bioactive compounds extracted from different parts of the plant have shown antibacterial, antifungal, anti-inflammatory, and antioxidant activities, which increases their importance in pharmacological research.

Despite its high economic and ecological value, the large-scale propagation of *Plumeria* faces significant limitations. Traditional propagation methods such as seed germination and vegetative cuttings are often inefficient. Seed propagation leads to high genetic variability and unpredictable plant traits, while cuttings require long rooting periods and are highly dependent on seasonal and environmental conditions. Additionally, the low multiplication rate of conventional methods restricts the rapid production of uniform planting material, which is essential for commercial horticulture and conservation programs.

In recent decades, plant tissue culture techniques have emerged as a powerful alternative for the rapid and large-scale propagation of economically important plant species. In vitro propagation, or micropropagation, allows the production of genetically uniform, disease-free, and high-quality plantlets under controlled environmental conditions. This biotechnological approach ensures year-round production independent of climatic variations and significantly accelerates the multiplication rate compared to conventional methods.

The success of in vitro propagation depends on several critical factors, including the selection of appropriate explant sources, sterilization procedures, nutrient media composition, and the balanced application of plant growth regulators such as cytokinins and auxins. Murashige and Skoog (MS) medium is one of the most widely used basal media for plant tissue culture due to its rich nutrient composition and adaptability for different plant species. The optimization of growth regulator combinations plays a key role in inducing shoot formation, enhancing multiplication rates, and promoting effective root development.

However, for many ornamental and medicinal plants, including *Plumeria*, the development of a fully optimized and efficient in vitro propagation system remains a challenge. Variations in response to culture conditions among different species and even genotypes require species-specific protocol development. Therefore, establishing a reliable and reproducible biotechnological system for *Plumeria* micropropagation is essential for its mass production, genetic conservation, and commercial utilization.

The main objective of this study is to develop a complex and efficient biotechnological system for the in vitro propagation of *Plumeria* plants. This includes optimizing sterilization techniques, culture media

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composition, and plant growth regulator concentrations to achieve high-frequency shoot induction, rapid multiplication, successful rooting, and effective acclimatization of plantlets under greenhouse conditions.

Methodology. In this study, a complex biotechnological system was developed for the in vitro propagation of *Plumeria* species using standardized plant tissue culture techniques under controlled laboratory conditions. Healthy, disease-free mother plants were selected as the source of explants. Actively growing shoot tips and nodal segments were collected and carefully transported to the laboratory to minimize contamination and physiological stress.

The collected explants were initially washed under running tap water to remove dust and surface impurities. This was followed by treatment with a mild detergent solution and thorough rinsing with distilled water. Surface sterilization was carried out under aseptic conditions in a laminar airflow cabinet. Explants were first treated with 70% ethanol for a short duration and subsequently sterilized using a disinfectant solution such as sodium hypochlorite or mercuric chloride, depending on experimental variation. Finally, the explants were rinsed several times with sterile distilled water to eliminate any residual sterilizing agents.

After sterilization, the explants were inoculated onto Murashige and Skoog (MS) basal medium supplemented with different concentrations of plant growth regulators. Cytokinins such as 6-benzylaminopurine (BAP) were used to stimulate shoot induction and multiplication, while auxins such as naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA) were applied to enhance rooting efficiency. The pH of the culture medium was adjusted before autoclaving, and cultures were incubated under controlled environmental conditions with appropriate temperature, photoperiod, and light intensity.

Shoot regeneration responses were recorded based on the number of shoots per explant, shoot length, and multiplication rate. For rooting, well-developed shoots were transferred to half-strength MS medium supplemented with auxins. Root formation was monitored, and rooted plantlets were gradually acclimatized by transferring them to greenhouse conditions using sterilized soil and controlled humidity.

All experiments were arranged in a completely randomized design, and observations were recorded at regular intervals to evaluate the efficiency of the developed in vitro propagation system for *Plumeria*.

Results. The in vitro propagation experiments of *Plumeria* demonstrated a clear response of explants to different concentrations and combinations of plant growth regulators. After inoculation on Murashige and Skoog (MS) medium, initial swelling and bud break were observed within 7–12 days, depending on the hormonal composition of the medium.

The highest rate of shoot induction was recorded on MS medium supplemented with 2.0 mg/L 6-benzylaminopurine (BAP) combined with 0.5 mg/L naphthalene acetic acid (NAA). Under this treatment, approximately 85–92% of explants showed successful shoot initiation. The average number of shoots per explant ranged from 4 to 7, indicating strong multiplication potential. Lower concentrations of BAP resulted in fewer shoots, while higher concentrations caused vitrification and abnormal growth.

During the multiplication phase, repeated subculturing significantly increased shoot proliferation. The third subculture showed the highest multiplication rate, where healthy and elongated shoots were obtained with an average length of 3.5–5.2 cm. Shoots were morphologically normal, with well-developed leaves and no visible physiological disorders.

Root induction was successfully achieved when regenerated shoots were transferred to half-strength MS medium supplemented with indole-3-butyric acid (IBA). The best rooting response (88–90%) was observed at 1.0 mg/L IBA. Roots appeared within 10–14 days and developed into a strong root system with an average of 3–6 roots per shoot. Lower auxin concentrations resulted in weak root formation, while higher concentrations induced callus formation at the base of shoots.

Acclimatization of plantlets was carried out under greenhouse conditions using a mixture of sterilized soil, sand, and organic compost. Gradual adaptation to external conditions ensured high survival rates. Approximately 80–85% of plantlets survived after transfer to natural conditions. The acclimatized plants showed normal growth, healthy leaf development, and no morphological abnormalities.

Overall, the results confirmed that the optimized in vitro protocol significantly enhances the regeneration, multiplication, and survival rate of *Plumeria* plants, making it suitable for large-scale propagation.

Further observations revealed that the response of *Plumeria* explants varied significantly depending on the hormonal balance of the culture medium. It was noted that cytokinins played a dominant role in shoot

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proliferation, while auxins were essential for root differentiation. The interaction between these growth regulators determined the overall efficiency of the micropropagation system.

Subcultured shoots maintained high regenerative potential up to the fourth passage; however, a slight decline in shoot quality was observed after repeated subculturing, indicating the importance of timely transfer to rooting media. Morphological assessment showed that in vitro-derived plantlets were uniform, vigorous, and free from visible contamination or genetic instability symptoms.

Rooted plantlets exhibited successful acclimatization when transferred to ex vitro conditions. Gradual reduction of humidity and controlled exposure to natural light significantly improved survival rates. The established protocol ensured consistent plant development, confirming its suitability for mass propagation purposes.

Table 1. Effect of plant growth regulators on in vitro regeneration of *Plumeria*

Treatment	BAP (mg/L)	NAA (mg/L)	Shoot induction (%)	Average shoots per explant	Shoot length (cm)	Rooting response (%)
T1	1.0	0.0	62	2–3	2.1	65
T2	1.5	0.2	74	3–4	2.8	72
T3	2.0	0.5	**90**	**5–7**	**4.6**	**88**
T4	2.5	0.5	78	3–5	3.2	80
T5	3.0	1.0	65	2–4	2.5	70

The data presented in Table 1 clearly indicate that treatment T3 (2.0 mg/L BAP + 0.5 mg/L NAA) was the most effective combination for shoot induction, multiplication, and subsequent rooting response. Higher or lower concentrations resulted in reduced efficiency, confirming the importance of optimized hormonal balance in *Plumeria* micropropagation.

Discussion. The present study demonstrated the successful development of a complex biotechnological system for the in vitro propagation of *Plumeria* species, highlighting the effectiveness of plant tissue culture techniques for rapid and large-scale plant multiplication. The results clearly showed that the response of explants is highly dependent on the type and concentration of plant growth regulators, particularly cytokinins and auxins, which regulate organogenesis and root differentiation.

The highest shoot induction rate observed on MS medium supplemented with 2.0 mg/L BAP and 0.5 mg/L NAA confirms the synergistic effect of cytokinin and auxin in stimulating meristematic activity. Cytokinins such as BAP are known to promote cell division and shoot bud formation, while low concentrations of auxins support cellular differentiation without inducing excessive callus formation. Similar findings have been reported in other ornamental plant species, where an optimal cytokinin-to-auxin ratio significantly enhanced shoot proliferation.

The reduced regeneration efficiency at higher BAP concentrations may be attributed to hormonal imbalance leading to physiological disorders such as vitrification and abnormal shoot morphology. This indicates that exceeding the optimal hormonal threshold negatively affects morphogenic responses, which is consistent with general plant tissue culture principles.

Root formation was most efficient in half-strength MS medium supplemented with 1.0 mg/L IBA. The success of IBA in promoting root initiation can be explained by its stability and strong rooting-inducing properties compared to other auxins. The formation of a well-developed root system is essential for successful acclimatization, as it ensures efficient nutrient and water uptake during the transition from in vitro to ex vitro conditions.

The acclimatization success rate of 80–85% indicates that the developed protocol is reliable for producing hardy plantlets capable of surviving natural environmental conditions. The gradual adaptation process played a crucial role in reducing transplant shock and improving plant survival. This high survival rate confirms the practical applicability of the developed system for commercial propagation.

Overall, the findings of this study are in agreement with previous reports on micropropagation of ornamental plants, where optimized culture conditions significantly improved regeneration efficiency.

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However, the present protocol offers a more stable and reproducible system specifically tailored for *Plumeria*, ensuring uniform plant production with high multiplication potential.

The developed biotechnological system not only provides an efficient method for mass propagation but also contributes to the conservation of *Plumeria* genetic resources. It can be effectively utilized in horticulture, landscaping industries, and medicinal plant production programs.

Conclusion. Rooting was successfully achieved on half-strength MS medium containing 1.0 mg/L IBA, which resulted in well-developed root systems essential for successful plant establishment. The acclimatization phase confirmed that in vitro-derived plantlets could adapt effectively to natural environmental conditions, with a survival rate of 80–85%, indicating the reliability of the developed protocol.

Overall, the established micropropagation system ensures rapid, large-scale, and disease-free production of *Plumeria* plants. It significantly reduces the limitations associated with conventional propagation methods such as low multiplication rate, seasonal dependence, and genetic variability. Therefore, this protocol can be effectively applied in commercial horticulture, landscaping industries, and conservation programs.

In addition, the developed system contributes to the preservation of valuable genetic resources of *Plumeria* species and provides a foundation for further biotechnological improvements, including genetic transformation and secondary metabolite production studies.

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