Engineering a Scalable Ex Situ Conservation system for Andrographis paniculata via High-Frequency Somatic Embryogenesis and Synthetic Seed Technology

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Abstract

Andrographis paniculata Nees. is a medicinally important species subjected to sustained harvesting pressure, while its natural regeneration is constrained by prolonged seed dormancy, low viability, and poor germination. Although in vitro regeneration has been reported earlier, protocols explicitly addressing conservation and germplasm preservation remain limited. In the present study, a conservation-oriented in vitro system integrating somatic embryogenesis with synthetic seed technology was developed for *A. paniculata*. Somatic embryos were induced from hypocotyl and cotyledon explants of aseptic seedlings cultured on Murashige and Skoog (MS) medium supplemented with defined plant growth regulator combinations. Maximum induction of embryogenic callus and globular embryos was achieved on MS medium containing 2,4-dichlorophenoxyacetic acid (2,4-D; 9.05 µM). Subsequent modulation of auxin levels, incorporation of $AgNO_3$, cytokinins, and elevated sucrose concentrations supported embryo maturation and conversion into plantlets. Mature somatic embryos and in vitro-derived shoot buds were successfully encapsulated using sodium alginate to generate synthetic seeds, which retained regeneration potential following short-term low-temperature storage. The integration of high-frequency somatic embryogenesis with synthetic seed production provides a reproducible framework for ex situ conservation, short-term germplasm storage, and sustainable propagation of *A. paniculata*.

Introduction

Medicinal plants constitute an essential component of global healthcare systems (Theodoridis, S.,et al.,2023;), yet many species face increasing threats due to overexploitation, habitat loss, and limited natural regeneration Volenzo, T., & Odiyo, J. (2020). Conservation of medicinal plant germplasm has therefore emerged as a major challenge (Thirisha Rani, D., et al.,2026), particularly for species that exhibit poor seed viability or prolonged dormancy. Biotechnological approaches that combine efficient regeneration with germplasm preservation are increasingly recognized as valuable tools for addressing these constraints(Arshad, Z., et al.,2025).

Andrographis paniculata Nees. (Acanthaceae) is widely used in traditional and modern medicine owing to the presence of bioactive diterpenoids, particularly andrographolide (Anandalwar and Vasantha, 1989; Malik, A. K., et al.,2025). The species is distributed across South and Southeast Asia, including India, Sri Lanka, Pakistan, and Indonesia (Hooker, 1885). Rising industrial demand for raw plant material has led to intensive harvesting from natural populations, raising concerns regarding sustainability.

Natural propagation of *A. paniculata* is restricted by intrinsic biological limitations, including extended seed dormancy of five to six months, low seed viability, and inconsistent germination Siddiqui, Z. H., & Hakeem, K. R. (Eds.). (2025). These factors significantly reduce natural population recovery and complicate cultivation efforts. Conventional vegetative propagation methods are insufficient to meet conservation and commercial requirements, highlighting the need for alternative propagation strategies that reduce dependence on wild collections.

Somatic embryogenesis represents one of the most effective in vitro regeneration pathways, offering high multiplication rates, genetic uniformity, and the potential for large-scale propagation (Soczyńska, J., et al.,2025). Importantly, somatic embryos can be encapsulated to produce synthetic seeds, enabling storage, transport, and controlled regeneration—features of particular relevance for conservation programs (Das, A., et al.,2026). While somatic embryogenesis in *A. paniculata* has been previously demonstrated (Martin, 2004), earlier studies primarily emphasized regeneration efficiency, with limited focus on storage, handling, and conservation utility.

The present study advances previous work by developing a conservation-oriented protocol that integrates somatic embryogenesis with synthetic seed technology. The objectives were to (i) establish a high-frequency somatic embryogenesis system from different explants, (ii) optimize embryo maturation and conversion, and (iii) evaluate the potential of encapsulated propagules for short-term germplasm conservation of *A. paniculata*.

Materials and Methods

To minimize pressure on natural populations, explants were obtained exclusively from aseptic seedlings raised under controlled conditions. Hypocotyl and cotyledon explants from 30–45-day-old seedlings were selected due to their juvenile physiological state and documented embryogenic competence.

Explants were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of auxins and cytokinins to induce embryogenic callus and somatic embryos. Systematic modulation of growth regulators was employed to promote embryo differentiation, maturation, and conversion.

For conservation-oriented propagation, mature somatic embryos and in vitro-derived shoot buds were encapsulated using sodium alginate via the gel complexation method. Encapsulated propagules were subjected to low-temperature storage to assess their viability and regeneration capacity, thereby evaluating their suitability for short-term ex situ conservation and germplasm exchange.

Results and Discussion

The present study establishes a reproducible and conservation-oriented in vitro regeneration system for *Andrographis paniculata* through high-frequency somatic embryogenesis coupled with synthetic seed technology. Hypocotyl and cotyledon explants derived from aseptic seedlings consistently exhibited embryogenic competence, underscoring their suitability as reliable explant sources for repeated propagation cycles. The protocol emphasizes not only regeneration efficiency but also the downstream utility of somatic embryos for encapsulation and short-term storage, aligning with the applied focus of plant tissue culture-based conservation strategies.

From a conservation standpoint, the ability to generate large numbers of somatic embryos from limited explant material is highly significant, as it reduces the need for continuous harvesting of wild plants. The

superior response observed at 9.05 µM 2,4-D suggests the existence of a narrow auxin threshold required for embryogenic reprogramming in *A. Paniculata* (**Table.1 & Fig. 1**).Concentrations below this level resulted in limited embryogenic competence, whereas higher concentrations favoured excessive callus proliferation with reduced embryo differentiation. This response pattern highlights the importance of precise auxin modulation for maintaining embryogenic potential, a feature critical for reproducible somatic embryogenesis protocols.

The inclusion of $AgNO_3$ significantly enhanced somatic embryo differentiation, likely by mitigating ethylene-mediated inhibitory effects commonly observed under in vitro conditions (You, H., Ling.,et al.,2026; Sharma, N., et al.,2026; Zhang, J., et al.,2026). Similarly, elevated sucrose concentrations supported embryo maturation, possibly by acting as both a carbon source and osmotic regulator during late embryogenic stages. Such coordinated regulation of the in vitro microenvironment is essential for producing morphologically normal and physiologically competent somatic embryos suitable for downstream applications such as encapsulation.

Histological analysis confirmed the development of true somatic embryos exhibiting bipolarity and progressing through characteristic embryogenic stages. The successful conversion of mature embryos into complete plantlets demonstrates the reliability of this regeneration system for producing viable propagules suitable for conservation and reintroduction programs.

Encapsulation and Synthetic Seed Conservation

Encapsulation of somatic embryos and in vitro shoot buds resulted in uniform synthetic seeds capable of withstanding handling and short-term storage. Optimal sodium alginate concentrations ensured mechanical stability without impeding shoot or root emergence. Synthetic seeds retained substantial regeneration potential following storage at 5 ± 2°C, whereas lower temperatures resulted in reduced viability (Table 2 **& Fig. 1**).

The successful encapsulation and subsequent regeneration of somatic embryos and in vitro shoot buds demonstrate the functional integration of regeneration and storage within a single protocol. Importantly, the retention of regeneration capacity following low-temperature storage highlights the practical utility of synthetic seeds for short-term germplasm maintenance, exchange between laboratories, and synchronization of propagation cycles without continuous sub culturing.

Synthetic seed technology has emerged as a strategically significant biotechnological intervention for the ex situ conservation of *A. paniculata*, a medicinally indispensable species (Redlarska, E., et al., 2025; Prabhakornritta, P., et al., 2025; Wang, S., et al., 2025). By facilitating the secure storage, long-distance transport, and germplasm exchange of elite genotypes independent of conventional seed systems, this technology strengthens conservation frameworks while enhancing propagation efficiency (Das, A., et al., 2026). Such an approach is particularly critical for protecting high-value medicinal plant resources,

enabling large-scale restoration and organized cultivation programs, and simultaneously reducing harvesting pressure on wild populations (Sun, J., et al., 2026).

Beyond its recognized therapeutic importance, *A. paniculata* has recently gained attention as an eco-friendly and sustainable green corrosion inhibitor for mild steel, expanding its relevance into environmentally responsible industrial applications (Gapsari, F., et al., 2025). The urgency for its conservation is further amplified by its demonstrated efficacy in managing fatty liver disease associated with modern lifestyle disorders (Vikal, A., Maurya, R., Patel, P., & Kurmi, B. D., 2025). Moreover, its bioactive compounds exhibit promising activity against drug-resistant and H37Rv strains of Mycobacterium tuberculosis, reinforcing its contemporary pharmacological significance (Vilvest, J., et al., 2025). Increasing research interest in *A. paniculata* phytochemicals for apoptosis induction in cancer cells further highlights its expanding therapeutic horizon (Thai, Q. K., et al., 2025).

Importantly, the conservation of *A. paniculata* extends beyond direct medicinal benefits. The species contributes to the sustainable management of soil-borne diseases and plays a role in restoring rhizosphere functionality, thereby supporting ecosystem stability (Xu, X., et al., 2025). These ecological services indirectly promote in vivo conservation by fostering healthier natural habitats for plant communities. Collectively, the escalating pharmaceutical, industrial, and ecological value of *A. paniculata* underscores the immediate need for advanced conservation strategies, with synthetic seed technology representing a forward-looking and resilient solution for ensuring its long-term survival and sustainable utilization.

Unlike earlier regeneration reports in *A. Paniculata*, which primarily focused on plantlet recovery, the present study integrates somatic embryogenesis with synthetic seed technology to address practical challenges in germplasm conservation. The ability to generate large numbers of somatic embryos from limited explant material, followed by encapsulation and short-term storage, reduces the need for continuous donor plant maintenance and repeated subculturing. From a tissue culture perspective, this integrated system offers a scalable and cost-effective approach for ex situ conservation, controlled propagation, and potential reintroduction programs (**Table .3**). Such protocol-level integration enhances the translational value of somatic embryogenesis beyond laboratory-scale regeneration.

Conclusion

The present study establishes a conservation-oriented in vitro regeneration system for *Andrographis paniculata* based on somatic embryogenesis and synthetic seed technology. The protocol enables rapid clonal multiplication, short-term germplasm storage, and efficient regeneration of this medicinally important species. Integration of these biotechnological tools provides a sustainable alternative to wild collection and contributes significantly to the ex situ conservation and long-term utilization of *A. paniculata*. The approach developed here can be extended to other medicinal plants facing similar propagation and conservation challenges.

Declarations

ETHICS DECLARATION/APPROVAL

Not applicable

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work the author(s) used perplexity in order to restructure certain sentences for better articulation. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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DATA AVAILABILITY STATEMENT

The authors confirm that the data will be available on reasonable request

AUTHOR CONTRIBUTIONS STATEMENT

SMM, AJA, P.A, MM: Investigation; DK,TS :Methodology; MP: Prepared all figures; RD: Validation and Visualisation; MM: Wrote the main Manuscript; NK: Conceptualisation Editing and review. "All authors reviewed the manuscript."

References

1. Anandalwar TR, Vasantha L (1989) Necessity of identification, cultivation, collection, preservation and promotion of medicinal plants in Karnataka. My For 25(1):67–73
2. Arshad Z, Shahid S, Hasnain A, Yaseen E, Rahimi M (2025) Functional foods enriched with bioactive compounds: Therapeutic potential and technological innovations. Food Sci Nutr, 13(10), e71024
3. Das A, Seth D, Pramanik S, Thakur RM, Debnath S, Rahimi M (2026) Conservation of Natural Plant Resources for Sustainable Development: An Overview. Biotechnol Plant Conserv, 1–12
4. Gapsari F, Setyarini PH, Anam K, Hadisaputra S, Hidayatullah S, Sulaiman AM, Lai CW (2025) Efficacy of Andrographis paniculata leaf extract as a green corrosion inhibitor for mild steel in concentrated sulfuric acid: Experimental and computational insights. Results Surf Interfaces 18:100361

5. Hooker JD (1885) The flora of British India L. Reeve & Co., London, p 501
6. Malik AK, Redhu A, Mohiuddin I, Philippe SK (2025) Exploring the Pharmacological Evaluation of Indian Medicinal Herbs for Managing Diabetes. Current Analytical Chemistry
7. Martin KP (2004) Plant regeneration protocol of medicinally important *Andrographis paniculata* (Burm. f.) Wallich ex Nees via somatic embryogenesis. *In vitro* cellular and Developmental Biology Plant. 40(2): 204–209
8. Prabhakornritta, P., Waranuch, N., Fuangchan, A., Srikham, K., Boonpattharatthiti,K., Barnig, C., … Dhippayom, T. (2025). Exploring the clinical effects of Andrographis paniculata-derived compounds, its extract, or derivatives for the treatment of COVID-19:a systematic review and meta-analysis. Frontiers in Pharmacology, 16, 1598255.
9. Redlarska E, Ożgo M, Pierzchała M, Kępka-Borkowska K, Chałaśkiewicz K, Pareek CS, Lepczyński A (2025) Impacts of Andrographis paniculata supplementation on health and productivity in monogastric farm animals: A comprehensive review. Anim Nutr 23:381–395
10. Sharma, N., Bakshi, P., Dhotra, B., Sheikh, Z. N., Alharby, H. F., Hakeem, K. R.,… Rahimi, M. (2026). Integrated preharvest salicylic acid maleic hydrazide and postharvest 1 MCP delay softening and preserve strawberry quality during cold storage. Scientific Reports.
11. Siddiqui ZH, Hakeem KR (eds) (2025) Plant Tissue Culture: Current Status and Opportunities in a Changing Environment. CRC
12. Soczyńska, J., Gawełczyk, W., Obrycka, P., Żołyniak, M., Muzyka, A., Majcherczyk, K., … Woźniak, S. (2025). Medical embryology and regenerative medicine: research and applications in clinical practice. Frontiers in Cell and Developmental Biology, 13, 1619036.
13. Sun, J., Zhang, C., Cao, Y., Liang, Y., Huang, J., Zhang, X., … Yin, L. (2026). Germination Requirements of Ottelia cordata (Wall.) Dandy Seeds: Implications for Conservation and Restoration. Aquatic Conservation: Marine and Freshwater Ecosystems, 36(1), e70290.
14. Thai QK, Huynh P, Tran TT, Nguyen BH, Nguyen HTT (2025) Investigating andrographis paniculata compounds for apoptosis induction in cancer. Asian Pac J Cancer Prevention: APJCP 26(7):2657
15. Theodoridis S, Drakou EG, Hickler T, Thines M, Nogues-Bravo D (2023) Evaluating natural medicinal resources and their exposure to global change. Lancet Planet Health 7(2):e155–e163
16. Thirisha Rani D, Priyadharshini S, Devi PB (2026) Conservation of Endemic Plants: Conventional and Biotechnological Approaches. Biotechnology in Plant Conservation. Springer, Cham, pp 25–43
17. Vikal A, Maurya R, Patel P, Kurmi BD (2025) Andrographis paniculata in Fatty Liver Disease: Mechanisms, Nanocarrier Approaches, and Therapeutic Potential. Phytomedicine Plus, 100903
18. Vilvest J, Milton MJ, Yagoo A, Balakrishna K (2025) Evaluation of Andrographolide from Andrographis paniculata against Drug-Resistant and H37Rv strains of Mycobacterium tuberculosis. Folia Microbiol, 1–7
19. Volenzo T, Odiyo J (2020) Integrating endemic medicinal plants into the global value chains: The ecological degradation challenges and opportunities. *Heliyon*, *6*(9)

20. Wang, S., Liang, M., Chen, W., Wan, H., Meng, X., Zhu, X., … Sun, W. (2025). Completing the biosynthesis of the clinically important diterpenoid andrographolide in Andrographis paniculata. Angewandte Chemie, 137(31), e202425303.
21. Xu X, Qin D, Qin X, Gao X, Li C, Liu X, Wu G (2025) Sustainable management of soil-borne disease: integrating fumigation with Andrographis paniculata residues to rebuild rhizosphere function. BMC Microbiol 25(1):460
22. You, H., Ling, L., Hu, H., Li, J., Liu, Y., Chen, J., … Liu, Z. (2026). Mechanistic insights into ethylene-mediated natural aroma compound production in vine tea (Ampelopsis grossedentata) cell suspension culture. Plant Cell, Tissue and Organ Culture (PCTOC), 164(1), 20.
23. Zhang, J., Liu, Z., Farrar, E. J., Li, M., Lu, H., Qu, Z., … Persson, S. (2026). Ethylene modulates cell wall mechanics for root responses to compaction. Nature, 649(8097), 685–692.

Tables

Table 1. Effect of Plant Growth Regulators on Somatic Embryo Induction from Hypocotyl and Cotyledon Explants of *Andrographis paniculata*.

Sl.No	Culture Medium and Plant Growth Regulator Composition (µM)	Mean Number of Somatic Embryos per Explant (± SE)		
		Explant Type		
		Hypocotyls	Cotyledons	Embryos
1	MS+2.4.D.(4.52)	20±0.92[a]	28±1.68[a]	27±1.35[b]
2	MS + 2,4-D (6.78)			26±0.74[b]
3	MS+2.4.D.(9.05)	24±0.50[a]	40±1.65[c]	35±1.02[c]
4	MS + 2,4-D (13.56)			20±1.02[a]
5	MS + TDZ (4.54)		34±1.20[b]	
6	MS + 2,4-D (2.26) + GA_3 (1.44) + $AgNO_3$ (11.76)		42±1.50[c]	
7	MS+2.4.D.(2.26)+$AgNo_3$(5.86)	29±1.22[b]		
8	MS+TDZ.(4.54)+$AgNo_3$(11.76) + 40 gms Sucrose	32±1.77[b]		
9	MS+2.4.D.(1.81) + GA_3(9.21) + ZEA(4.56) + 40gms Sucrose	51±2.65[d]		
10	MS + NAA (5.37) + ZEA (2.28)			27±0.86[b]
11	MS + NAA (2.69) + ZEA (2.28) + GA_3 (1.44) + Glutamine 1368		47±1.93[d]	
12	MS + NAA (2.69) + ZEA (4.56) + 40 gms Sucrose		40±1.46[c]	
13	MS + IBA (9.80) + BAP (8.87) + AS (10.86) + 40 gms Sucrose	43±1.50[c]	51±3.11[d]	
14	MS + IBA (9.80) + GA_3 (2.88) + AS (16.29) + Glutamine (684)		48±2.38[d]	
15	MMS + IBA (2.46) + BAP (8.87) + 40 gms sucrose			42±1.63[d]

Values represent mean ± standard error of three independent experiments.

Table 2. Regeneration Response of Synthetic Seeds After Short-Term Cold Storage: Implications for Ex Situ Germplasm Conservation of *Andrographis paniculata*.

Sl. No.	Duration of Storage 5° ± 2°C	Regeneration Percentage (%)	
		Encapsulated somatic embryos	Encapsulated in vitro shoot buds
1.	1 Week	45.6	48.4
2.	2 Weeks	43.7	47.6
3.	3 Weeks	40.9	44.5
-5° ± 2°C			
4.	1 Week	35.3	37.9
5.	2 Weeks	34.6	36.5
6.	3 Weeks	32.5	34.9

Table 3. Conservation Advantages of Synthetic Seed–Derived Propagules Compared with Conventional Propagation Methods in *Andrographis paniculata*.

Sl. No.	Parameter	Conventional Propagation	Synthetic Seed System
1.	Germplasm storage	Limited	Enabled
2.	Transport	Difficult	Highly feasible
3.	Genetic uniformity	Variable	High
4.	Dependence on wild plants	High	Minimal
5.	Scalability	Moderate	High

Figures

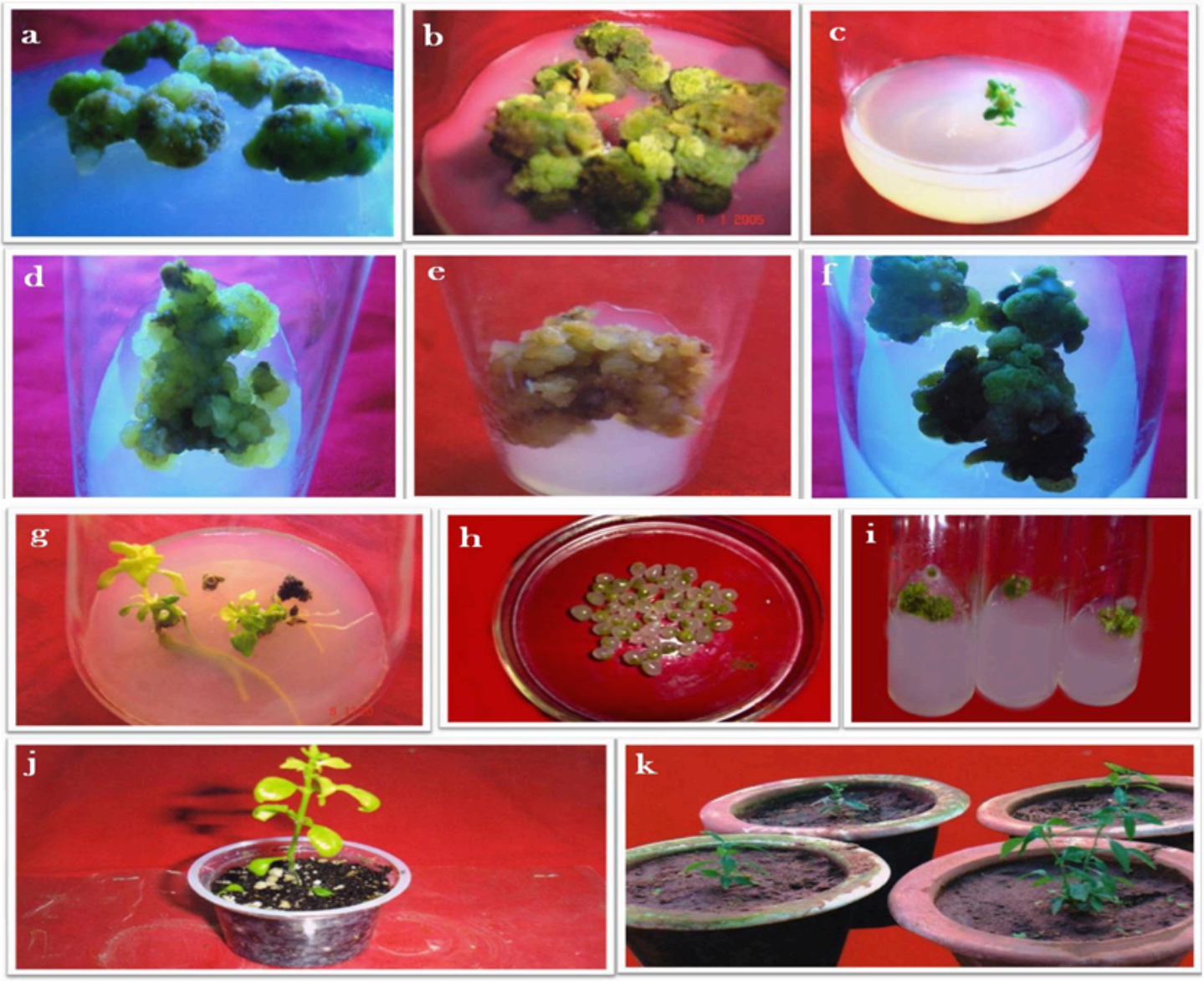

Figure 1

Conservation-oriented regeneration of *Andrographis paniculata* via somatic embryogenesis and synthetic seed technology.

(**A.**) In vitro–raised aseptic seedling serving as the explant source; (**B.**) Excised hypocotyl explant cultured on MS medium; (**C.**) Cotyledon explant exhibiting initial enlargement prior to embryogenic induction; (**D.**) Formation of friable embryogenic callus from cultured explants; (**E.**) Emergence of globular somatic embryos from callus tissue; (**F.**) Heart-stage somatic embryo demonstrating early polarity; (**G.**) Torpedo-stage embryo with a clearly defined embryonic axis; (**H.**) Mature bipolar somatic embryo suitable for germination; (**I.**) Encapsulation of mature embryos in sodium alginate to produce synthetic seeds; (**J.**) Synthetic seed showing shoot emergence following low-temperature storage; (**K.**) Complete plantlet regeneration confirming conversion competence.

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