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Role of Tissue Culture in Conservation of Rare Plant Species

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Dr. Syed Feroze Ahamed
Asst. professor
Anjuman-E-Islam's
Nehru Arts Science and Commerce College, Ganthikeri, Hubli, Karnataka-580020
Orcid id https://orcid.org/0009-0005-7200-9631

Abstract— The accelerating loss of plant biodiversity due to habitat destruction, climate change, overexploitation, and invasive species has placed a growing number of rare and endangered plant taxa at risk of extinction. Conventional in situ conservation, while ecologically preferable, cannot always keep pace with the rate of habitat loss or protect species with fragmented, dwindling populations. Plant tissue culture has therefore become an essential complementary strategy for ex situ conservation, offering the means to multiply scarce genetic material, establish disease-free clonal stocks, and store germplasm for extended periods through slow-growth storage and cryopreservation. This paper reviews the role of tissue culture techniques, including micropropagation, in vitro slow-growth storage, and cryopreservation, in safeguarding rare plant species. It examines documented case studies, evaluates the genetic stability of regenerated material, and discusses the practical challenges that limit broader application of these methods. The review concludes that while tissue culture cannot replace habitat protection, it provides a scientifically robust safety net that, when integrated into comprehensive conservation programs, significantly improves the prospects of survival for the world's most vulnerable plant species.

Keywords: Plant tissue culture, In vitro propagation, Micropropagation, Rare and endangered species, Germplasm conservation, Cryopreservation, Somatic embryogenesis, Ex situ conservation

INTRODUCTION

Plant biodiversity underpins ecosystem stability, agricultural resilience, and the continued discovery of compounds with pharmaceutical, industrial, and ecological value. Yet a substantial proportion of the world's flora is currently threatened, with international assessments repeatedly identifying habitat fragmentation, overharvesting, pollution, and climate change as the principal drivers of decline. For many rare species, natural regeneration is slow, seed production is unreliable, or seeds themselves are recalcitrant, meaning they cannot tolerate the drying and freezing conditions used in conventional seed banking. These biological constraints make many rare and endangered plants poor candidates for standard ex situ conservation through seed storage alone, necessitating alternative approaches capable of preserving living genetic material directly.

Plant tissue culture, the aseptic cultivation of plant cells, tissues, or organs on nutrient media under controlled conditions, offers a practical answer to this problem. Because tissue culture requires only small amounts of starting material, often a single shoot tip or a few cells, it is particularly well suited to species whose wild populations are too small or too sensitive to disturb through large-scale seed or cutting collection. Beyond simple multiplication, tissue culture provides pathways for medium-term storage through slow growth techniques and, more significantly, for long-term storage through cryopreservation in liquid nitrogen, a method capable of halting biological ageing almost indefinitely. This paper reviews the scientific literature on the application of these techniques to rare and endangered plant conservation, discusses documented successes and persistent challenges, and considers the broader role of tissue culture within integrated conservation strategy.

LITERATURE REVIEW

Foundational work in plant tissue culture rests on the standardized nutrient medium developed by Murashige and Skoog, whose formulation for rapid growth and bioassay in tobacco tissue culture remains the base medium adapted for the

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overwhelming majority of conservation-oriented micropropagation protocols across plant families.

Reviewing the application of these methods specifically to threatened flora, Oseni, Pande, and Nailwal examined plant tissue culture as a technique for the propagation and conservation of endangered plant species, concluding that micropropagation enables the rapid multiplication of rare genotypes from very limited starting material while also allowing the elimination of systemic pathogens through meristem-tip culture, a factor that often determines whether reintroduction efforts succeed. Their review also identified cryopreservation as the most significant long-term conservation technique available, noting its capacity to secure germplasm indefinitely once an effective protocol has been established for a given species.

The theoretical and technical basis of cryopreservation has been extensively developed by Engelmann, whose review on the use of biotechnologies for the conservation of plant biodiversity described how storage of plant material in liquid nitrogen at minus one hundred ninety-six degrees Celsius arrests nearly all metabolic and physical processes, allowing tissues to be preserved for extremely long periods without genetic drift associated with repeated subculturing. In an earlier review focused specifically on cryopreservation progress and prospects, Engelmann traced the development of vitrification and encapsulation-based cryoprotection methods and noted that despite considerable progress, protocols had at that time been established for only a limited number of species, underscoring the species-specific nature of successful cryopreservation.

Practical guidance for applying these techniques was consolidated by Villalobos and Engelmann in a widely referenced practical guide to the cryopreservation of plant germplasm, which set out standardized approaches including vitrification, encapsulation-dehydration, and droplet-vitrification, methods that have since been adapted by researchers working with rare and threatened taxa around the world.

A clear demonstration of these principles applied to an actual endangered species was provided by Wilkinson, Wetten, Prychid, and Fay, who examined the suitability of cryopreservation for the long-term storage of the rare ornamental species *Cosmos atrosanguineus* using shoot tip encapsulation and dehydration in alginate strips. Their study achieved survival rates of up to one hundred percent and shoot regeneration rates of up to thirty-five percent, and genetic fingerprinting using amplified fragment length polymorphism analysis found no detectable genetic variation between material before cryopreservation and after twelve months of storage in liquid nitrogen, providing strong evidence that cryopreservation can maintain genetic fidelity in conserved rare plant material.

Similarly rigorous evidence comes from Sharma and colleagues, who investigated the cryopreservation and genetic stability of regenerants of the critically endangered medicinal plant *Dioscorea deltoidea* for the purpose of cryobanking germplasm, confirming through molecular marker analysis that regenerated plantlets retained genetic uniformity comparable to the source material, supporting the reliability of cryopreservation as a conservation tool for critically endangered medicinal species.

The broader ex situ conservation value of in vitro slow-growth storage combined with cryopreservation was reinforced by Panis, Nagel, and Van den Houwe, who reviewed the challenges and prospects for conserving crop genetic resources across field genebanks, in vitro collections, and liquid nitrogen storage, concluding that a combined strategy using multiple conservation methods offers the most resilient protection against the loss of genetic resources, a conclusion directly applicable to rare and endangered wild species as well as cultivated crop germplasm.

Finally, at the level of technique optimization, Ozaslan, Can, and Aytekin demonstrated that the choice of explant source significantly affects the success of in vitro propagation in *Paulownia tomentosa*, a finding with direct relevance to conservation programs, since the availability of high-quality explant material from rare species in the wild is often severely limited, making explant selection a critical determinant of overall protocol success. Complementary work by Ebrahimi, Mokhtari, and Amirian on somatic embryogenesis in the endangered medicinal plant *Kelussia odoratissima* further illustrated how alternative regeneration pathways, beyond simple shoot proliferation, can be developed for species that respond poorly to standard micropropagation, expanding the toolkit available for difficult-to-propagate rare taxa.

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Taken together, this literature demonstrates a coherent scientific progression: from the standardization of basic culture media, through species-specific propagation and cryopreservation protocol development, to molecular confirmation of genetic stability in conserved material, establishing tissue culture as a methodologically sound and empirically validated tool for rare plant conservation.

In Vitro Conservation Strategies

Conservation through tissue culture is generally organized into three complementary time frames, each suited to different conservation goals and resource constraints.

Conservation Strategy	Storage Duration	Underlying Principle	Typical Application
Active/base collections (routine culture)	Weeks to months	Continuous subculturing on standard growth media	Ongoing propagation and distribution of plant material
Slow growth storage	Months to a few years	Reduced temperature, osmotic stress, or growth regulator manipulation to slow metabolism	Medium-term backup requiring less frequent subculturing
Cryopreservation	Theoretically unlimited	Storage at minus one hundred ninety-six degrees Celsius in liquid nitrogen, halting metabolic activity	Long-term genetic security for critically endangered or exceptional species

Slow growth storage is typically achieved by lowering incubation temperature, reducing light intensity, adding osmotic agents such as mannitol or sorbitol to the culture medium, or using growth retardants, all of which extend the interval between subcultures and reduce the labor and contamination risk associated with frequent handling. Cryopreservation, by contrast, requires more technically demanding protocols, most commonly vitrification, encapsulation-dehydration, or droplet-vitrification, each involving controlled dehydration and cryoprotection of tissues prior to immersion in liquid nitrogen, followed by careful rewarming and recovery procedures upon retrieval.

Techniques Employed in Conservation Tissue Culture

Technique	Procedure	Conservation Relevance
Meristem/shoot tip culture	Excision and culture of the actively dividing apical meristem	Produces pathogen-free clonal material from minimal wild-collected tissue
Micropropagation	Multiplication of shoots through repeated subculture on cytokinin-rich media	Rapidly increases numbers of a rare genotype for reintroduction
Somatic embryogenesis	Induction of embryo-like structures from somatic cells	Provides an alternative regeneration route for species recalcitrant to shoot proliferation
In vitro slow growth storage	Reduced temperature and/or osmotic stress applied to established cultures	Extends subculture intervals, reducing genetic drift risk and labor cost
Vitrification	Treatment of tissues with concentrated cryoprotectant solution before liquid nitrogen immersion	Prevents ice crystal formation, widely used for shoot tips and embryos
Encapsulation-dehydration	Encapsulation of shoot tips or embryos in alginate beads followed by dehydration	Effective for species with delicate or complex tissue architecture, as demonstrated for *Cosmos atrosanguineus*
Droplet-vitrification	Small droplets of cryoprotectant containing the explant are placed on foil strips and plunged into liquid nitrogen	Enables rapid cooling and warming rates, improving survival in recalcitrant species

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Case Studies in Rare and Endangered Species Conservation

The practical value of these techniques is best illustrated through documented case studies. In the case of *Cosmos atrosanguineus*, a rare ornamental species with very limited surviving genetic diversity, Wilkinson and colleagues demonstrated that encapsulation-dehydration of shoot tips achieved survival rates of up to one hundred percent following cryopreservation, with molecular analysis confirming no detectable genetic change after a full year of storage in liquid nitrogen, providing one of the clearest published demonstrations that cryopreservation can secure a rare species' genetic identity over time.

For *Dioscorea deltoidea*, a critically endangered medicinal plant valued for its steroidal saponin content, Sharma and colleagues established a cryopreservation protocol and confirmed the genetic stability of regenerated plantlets using molecular markers, an outcome of particular importance given the dual conservation and pharmaceutical significance of the species.

For *Kelussia odoratissima*, an endangered medicinal plant endemic to parts of Iran with historically poor propagation success from seed, Ebrahimi and colleagues developed a highly efficient somatic embryogenesis protocol from callus tissue, demonstrating that alternative in vitro regeneration pathways can rescue species for which conventional propagation and even standard micropropagation prove inadequate.

Species	Conservation Status Context	Technique Applied	Reported Outcome
Cosmos atrosanguineus	Rare, narrow genetic base	Cryopreservation via encapsulation-dehydration	Up to 100% survival, up to 35% shoot regeneration, no detectable genetic change after 12 months
Dioscorea deltoidea	Critically endangered medicinal species	Cryopreservation with molecular stability assessment	Confirmed genetic stability of cryopreserved regenerants
Kelussia odoratissima	Endangered, poor natural regeneration	Somatic embryogenesis from callus	High rate of embryo induction, establishing a reliable propagation pathway
Paulownia tomentosa	Explant-source dependent propagation study	Micropropagation with explant source comparison	Explant origin significantly affected shoot proliferation and rooting success

Genetic Stability and Risks Associated with In Vitro Conservation

A central concern in using tissue culture for conservation is whether regenerated plants remain genetically true to the original wild material. Repeated subculturing, prolonged callus phases, and the stresses associated with cryopreservation can, in principle, introduce somaclonal variation, unintended genetic or epigenetic changes that alter the phenotype or genotype of regenerated plants. This risk is especially significant in a conservation context, since the goal is to preserve the existing genetic diversity of a threatened population rather than to generate novel variation.

Encouragingly, the case studies reviewed here provide direct molecular evidence that well-optimized protocols can minimize this risk. Both the *Cosmos atrosanguineus* study using amplified fragment length polymorphism analysis and the *Dioscorea deltoidea* study using molecular marker assessment found no significant genetic divergence between source material and cryopreserved regenerants. This suggests that when protocols are carefully matched to species-specific requirements, particularly with respect to cryoprotectant concentration, dehydration duration, and cooling and warming rates, cryopreservation can achieve a level of genetic fidelity that makes it a trustworthy tool for long-term conservation

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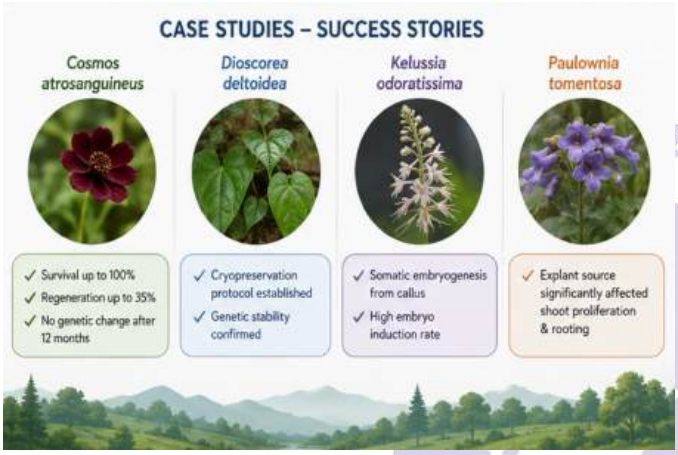
rather than a source of unwanted variability. Nonetheless, routine genetic stability testing using molecular markers is recommended as standard practice for any conservation tissue culture program, since the degree of stability can vary considerably between species and even between protocols applied to the same species.



Challenges and Limitations

Despite its demonstrated value, the application of tissue culture to rare plant conservation faces several practical constraints. Protocol development is highly species specific, and in many cases genus specific or even population specific, meaning that a technique successful for one rare species cannot simply be transferred to another without extensive optimization, a process that can take years of research for particularly recalcitrant taxa. Contamination remains a persistent operational challenge, particularly when working with field-collected material from wild populations that may carry endophytic microorganisms not always eliminated by standard surface sterilization. The technical infrastructure required for cryopreservation, including reliable liquid nitrogen supply, controlled-rate freezing equipment, and trained personnel, is costly and often unavailable in the very regions where much of the world's rare plant diversity is concentrated, creating a geographic mismatch between conservation need and technical capacity. Additionally, for many critically endangered species, the amount of material available for research is itself extremely limited, restricting the scope for the trial-and-error experimentation that protocol development typically requires. Finally, even successful in vitro conservation does not by itself guarantee species survival, since the conserved material must eventually be regenerated, acclimatized, and in many cases reintroduced into suitable habitat, each step carrying its own risk of failure.

Future Directions and Sustainability

Advancing the role of tissue culture in rare plant conservation will require continued investment in several directions. Development of more generalized cryopreservation protocols, informed by comparative studies across related taxa, could reduce the time needed to establish species-specific methods. Expansion of regional cryobanking infrastructure, particularly in biodiversity-rich developing countries, would help close the current gap between where rare species occur and where the technical capacity to conserve them exists. Greater integration of molecular monitoring as a routine component of conservation tissue culture would help ensure that genetic fidelity is maintained across generations of stored material. Importantly, tissue culture conservation should continue to be positioned as a complement to, rather than a substitute for, in situ habitat protection, since ex situ collections alone cannot preserve the ecological relationships, pollinator interactions, and evolutionary processes that sustain species in their natural environments. When combined with habitat restoration and community-based conservation initiatives, however, tissue culture and cryopreservation provide a scientifically robust safety net that can prevent irreversible genetic loss while longer-term ecological recovery efforts proceed.

Conclusion

Plant tissue culture has become a scientifically validated and increasingly indispensable tool in the conservation of rare and endangered plant species. Through micropropagation, somatic embryogenesis, slow growth storage, and cryopreservation, researchers can multiply scarce genetic material from minimal starting tissue, eliminate pathogens that threaten weakened wild populations, and secure genetic resources for extended, potentially indefinite, periods of time. The literature reviewed here, spanning foundational medium development, cryopreservation methodology, and species-specific case studies with molecular confirmation of genetic stability, demonstrates that these techniques can preserve rare plant genotypes reliably when protocols are carefully developed and validated. Challenges relating to species-specific optimization, infrastructure cost, and limited source material remain significant, but continued research and expanded regional capacity can help address these constraints. Ultimately, tissue culture and cryopreservation should be understood as a critical component within a broader, integrated conservation strategy, one that combines ex situ genetic security with sustained efforts to protect and restore the natural habitats upon which the long-term survival of rare plant species ultimately depends.

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