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APPLICATION OF PLANT TISSUE CULTURE IN GENETIC CONSERVATION AND BREEDING PROGRAMS

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ABSTRACT

Plant tissue culture has emerged as a pivotal tool in genetic conservation and breeding, enabling the preservation of rare germplasm, rapid propagation of elite lines, and facilitation of controlled genetic modifications. This review highlights the multifaceted applications of plant tissue culture techniques—including micropropagation, somatic embryogenesis, in vitro conservation, and embryo rescue—in both ex situ conservation and crop improvement programs. In genetic conservation, tissue culture ensures the long-term preservation of endangered, wild, and medicinal plant species through techniques such as slow-growth storage and cryopreservation. In breeding programs, tissue culture accelerates the development of uniform, disease-free planting material and supports advanced biotechnological approaches like somaclonal variation, doubled haploid production, and gene transformation.- The integration of plant tissue culture with molecular markers and genetic engineering has expanded its role from simple multiplication to sophisticated breeding strategies, enabling targeted trait improvement and genetic diversity conservation. Despite notable progress, challenges remain in genetic stability, cost, and scalability for field-level application. This review provides a consolidated understanding of current applications, technical advancements, and future prospects of plant tissue culture in genetic resource management and breeding innovation.

Keywords: Plant tissue culture, Genetic conservation, Breeding programs, Micropropagation, Cryopreservation, Embryo rescue, Somaclonal variation

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1. INTRODUCTION

Genetic diversity is fundamental to the sustainability of ecosystems, resilience of crops, and long-term food security. It provides the raw material for plant breeding programs and allows species to adapt to environmental stresses, pests, and diseases. However, increasing anthropogenic pressures such as habitat destruction, climate change, and industrial agriculture have led to the erosion of this diversity, necessitating urgent conservation efforts. In this context, plant breeding emerges as a pivotal strategy, not only for enhancing yield and quality but also for securing global food systems and ecological stability. A critical tool aiding these efforts is plant tissue culture, which offers precise, efficient, and scalable methods for conserving genetic resources and accelerating crop improvement. This review explores the multifaceted applications of plant tissue culture in genetic conservation and breeding programs, highlighting its significance in safeguarding biodiversity and supporting sustainable agricultural practices.

1.1 Scope of tissue culture in breeding and conservation

Plant tissue culture has emerged as a transformative tool in modern plant breeding and genetic conservation programs. Its scope extends from micropropagation to advanced biotechnological interventions, offering both scalability and precision. In breeding, tissue culture facilitates the rapid multiplication of elite genotypes through techniques like somatic embryogenesis and shoot tip culture, thereby accelerating the release of improved cultivars. It enables clonal propagation of hybrids and genetically uniform plants, ensuring trait stability across generations. Moreover, tissue culture provides a platform for haploid and doubled haploid production via anther or microspore culture, significantly reducing the breeding cycle duration and aiding in homozygous line development.

In genetic conservation, tissue culture is vital for preserving endangered, rare, or threatened species ex situ. Cryopreservation of embryonic axes, shoot tips, and somatic embryos ensures long-term conservation of germplasm without genetic drift. Slow-growth storage techniques further support medium-term preservation of plant material under controlled in vitro conditions. This method is especially valuable for species with recalcitrant seeds or vegetatively propagated crops. Additionally, in vitro conservation aids in safeguarding genetic diversity during biotic or abiotic stresses in natural habitats.

Tissue culture also complements genetic transformation and molecular breeding. Regeneration systems are essential for the integration of transgenes, gene editing (e.g., CRISPR/Cas9), and somaclonal variation exploitation, all of which broaden the genetic base and introduce novel traits like stress resistance or quality enhancement. As a result, plant tissue culture stands as a cornerstone of integrated breeding and conservation strategies, promoting sustainable agriculture and biodiversity preservation.

2. OVERVIEW OF PLANT TISSUE CULTURE TECHNIQUES

2.1 Micropropagation

Akin-Idowu et al. (2009) emphasized that micropropagation provides a powerful

technique for the rapid multiplication of elite clones, particularly in crops with slow or poor propagation rates. The authors noted that clonal propagation via in vitro methods ensures genetic uniformity, which is essential in preserving the desirable agronomic traits of high-performing cultivars. This approach has been especially useful in fruit trees and ornamental plants, where maintaining true-to-type characteristics is crucial for commercial viability.

Chaudhury et al. (2010) discussed the application of micropropagation in vegetatively propagated crops like banana, sugarcane, and cassava, where conventional propagation is either too slow or results in the transmission of diseases. They highlighted that tissue culture not only speeds up the multiplication rate but also helps in producing pathogen-free planting material through meristem culture. The authors stressed the importance of this technique for the commercial production of seedless crops, which cannot be propagated through sexual means.

Gamborg and Phillips (2013) elaborated on the advantages of micropropagation in preserving elite genotypes through somatic embryogenesis and shoot culture, pointing out its role in maintaining genetic fidelity across successive plantlets. They reported successful outcomes in elite grapevine and potato clones, where micropropagation ensured consistency in yield and quality. The review also pointed out that this approach can bridge the gap between breeding breakthroughs and large-scale field deployment.

Ray and Bhattacharya (2008) explored the large-scale micropropagation of seedless citrus varieties, which are typically propagated through budding or grafting. They documented the successful establishment of shoot tip and nodal explant cultures that allowed rapid multiplication while maintaining varietal integrity. The authors also argued that micropropagation plays a key role in germplasm exchange and international movement of plant material, as it allows production of disease-free clones in quarantine-compliant formats.

2.2 Somatic Embryogenesis

Bapat and Rao (1988) emphasized the fundamental role of somatic embryogenesis in plant regeneration, noting its capacity to produce whole plants from differentiated somatic cells in various species. They highlighted the importance of this process in propagating genetically uniform plants and stressed its potential for mass multiplication in woody perennials and ornamental crops. Their research laid the foundation for future developments by establishing that somatic embryos can function analogously to zygotic embryos, thus enabling the regeneration of complete plants under controlled in vitro conditions.

Guan et al. (2016) explored the use of somatic embryogenesis for synthetic seed technology, focusing particularly on crops with high economic value. They found that somatic embryos encapsulated in artificial coatings could be stored, transported, and germinated similarly to true seeds, offering an alternative propagation method for elite genotypes and hybrid lines. Their study demonstrated successful regeneration rates from synthetic seeds, emphasizing the utility

of somatic embryogenesis in large-scale plant production, especially where conventional seed production is challenging.

Jiménez (2005) provided a comprehensive overview of the molecular mechanisms governing somatic embryogenesis, discussing key signaling pathways, gene expression patterns, and the influence of plant growth regulators. He outlined how auxins like 2,4-D initiate cellular dedifferentiation, which is critical for embryogenic callus formation. Jiménez's work clarified the stages of embryo development and suggested that somatic embryogenesis is not only a tool for regeneration but also a model for studying plant developmental biology at the cellular and molecular levels.

Rai et al. (2021) investigated the practical applications of somatic embryogenesis in synthetic seed development for conservation and commercial purposes. Their research focused on endangered medicinal plants, revealing that somatic embryogenesis facilitates germplasm preservation by enabling long-term storage of encapsulated embryos. They reported high conversion rates of synthetic seeds into viable plantlets, especially when embedded with nutrients and growth regulators, thus validating the efficiency of this technique in propagation and conservation strategies.

2.3 Callus and Suspension Culture

Abo El-Nil (2020) emphasized the importance of callus and suspension cultures as foundational tools for the mass propagation of undifferentiated plant cells, particularly in species with limited conventional propagation methods. He highlighted how callus cultures derived from explants under controlled hormonal conditions provide the basis for large-scale cell proliferation, which can then be transitioned into liquid suspension cultures for continuous growth in bioreactors. This system not only facilitates uniform cell production but also serves as a flexible platform for metabolic engineering.

Dwivedi et al. (2021) reviewed the application of suspension cultures in producing valuable secondary metabolites, particularly in medicinal plants. They observed that suspension cultures enable high-yield and consistent production of compounds such as alkaloids, flavonoids, and terpenoids, which are often difficult to extract from wild populations. Moreover, they discussed how elicitation and optimization of culture conditions—such as nutrient media, pH, and light—significantly enhance metabolite accumulation in these cultures, making them attractive for commercial pharmaceutical production.

Karami and Deljou (2022) investigated the role of callus cultures in plant genetic transformation, describing them as critical targets for Agrobacterium-mediated gene transfer and biolistic techniques. Their findings revealed that undifferentiated callus cells are highly amenable to genetic manipulation, which allows for the introduction of traits like pest resistance or abiotic stress tolerance. The study also illustrated the callus' ability to regenerate whole plants from single transformed cells, making it an efficient tool in crop improvement programs.

Shinde and Patil (2023) focused on the scalability of suspension cultures in biotechnological applications. They noted

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that with the advancement of bioreactor technologies, it is now feasible to cultivate large volumes of plant cells under sterile and optimized conditions. These cultures not only support the bulk synthesis of secondary metabolites but also act as a model system for studying cell signaling, stress response, and metabolic pathways at the cellular level. Their review further stressed the synergy between suspension culture and synthetic biology in producing novel compounds.

3. TISSUE CULTURE IN GENETIC CONSERVATION

3.1 In Vitro Conservation (Slow-Growth Storage)

In vitro conservation using slow-growth storage is a widely adopted strategy to preserve plant genetic resources, particularly for vegetatively propagated plants and endangered species with recalcitrant seeds or no seed production. This method involves maintaining plant tissues—such as shoot tips, nodal segments, or somatic embryos—under reduced metabolic activity to delay subculturing intervals, thus minimizing labor, cost, and contamination risks.

To achieve slow growth, the in vitro culture conditions are strategically altered. One key approach is modification of the nutrient medium, where reduced concentrations of macronutrients (e.g., nitrogen and phosphate) and carbohydrate sources (e.g., sucrose) are used. Additionally, osmotic agents like mannitol or sorbitol can be added to induce osmotic stress and inhibit cellular division. Growth retardants such as abscisic acid (ABA) or paclobutrazol are also used to slow down shoot elongation and cell proliferation.

Another effective technique includes environmental manipulation—such as reducing the temperature (to 4–15°C), light intensity, or photoperiod—to create conditions where metabolic activity is naturally reduced. These treatments extend the storage life of cultures from a few months to several years without the need for frequent subculturing.

This method is particularly useful for conserving endangered plant species, especially those with limited natural populations, habitat destruction, or poor seed viability. Successful examples include wild relatives of crops, orchids, banana, potato, cassava, and rare medicinal plants, which have been conserved using slow-growth protocols with high recovery rates upon regeneration.

Table: Key Parameters for In Vitro Slow-Growth Storage in Selected Plant Species

Plant Species	Explants Used	Medium Modification	Temperature (°C)	Growth Retardant/ Agent	Storage Duration	Regeneration Rate
Musa spp. (Banana)	Shoot tips	MS medium with 1% sucrose, 1% mannitol	15	ABA (0.5 mg/L)	9 months	90%
Dioscorea spp.	Nodal	Half-strength MS, 2%	12	None	12 months	85%

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(Yam)	segments	sorbitol				
Orchis spp. (Orchid)	Protocorms	MS with 3% sucrose, low nitrogen	10	Paclobutrazol (0.2 mg/L)	8 months	75%
Solanum tuberosum (Potato)	Shoot tips	MS with 1.5% sucrose, reduced NO_3^-	4	None	24 months	95%
Azadirachta indica (Neem)	Shoot tips	MS + 2% mannitol + 0.2 mg/L ABA	15	ABA	10 months	80%

Note: MS = Murashige and Skoog medium; ABA = Abscisic Acid; storage durations and regeneration rates may vary with genotype and exact conditions.

3.2 Cryopreservation

Cryopreservation is the process of storing biological samples—such as shoot tips, somatic embryos, pollen, or seeds—at ultra-low temperatures, typically in liquid nitrogen at -196°C. Under such conditions, all cellular metabolic activities, including degradation, are effectively halted, allowing plant material to be stored indefinitely without genetic alteration or loss of viability. This technique is especially valuable for the conservation of plant species that produce recalcitrant seeds (which cannot be dried and stored conventionally) or those that are clonally propagated.

In practice, cryopreservation involves several steps: pre-culture of the explants on media to promote tolerance to dehydration, treatment with cryoprotectants (e.g., DMSO, glycerol, or sucrose), followed by controlled cooling and immersion in liquid nitrogen. Upon retrieval, rapid thawing and recovery on suitable media allow the regeneration of complete plants. Techniques such as vitrification (formation of glass-like structures to avoid ice crystallization), encapsulation-dehydration, and droplet vitrification have enhanced the success rate of cryopreservation for a wide range of plant species.

Cryopreservation is not only important for biodiversity conservation but also plays a crucial role in safeguarding elite germplasm for future breeding programs. Institutions such as gene banks and botanical repositories rely on it for secure, space-efficient, and long-term storage of valuable plant material. Additionally, cryopreserved tissues maintain their genetic fidelity, making them suitable for true-to-type regeneration.

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Table: Components and Techniques in Cryopreservation of Plant Material

Component	Description	Examples
Target Material	Plant tissues used for cryopreservation	Shoot tips, somatic embryos, seeds, pollen
Cryoprotectants	Substances used to protect cells from ice damage during freezing	DMSO, Glycerol, Sucrose, Ethylene glycol
Cooling Techniques	Methods used to reduce temperature without causing ice formation	Slow freezing, Vitrification, Droplet vitrification
Storage Temperature	The temperature at which materials are stored in liquid nitrogen	-196°C
Thawing Method	Rapid warming to prevent recrystallization	37–45°C water bath for 1–2 minutes
Recovery Media	Nutrient media used to regenerate plants after thawing	MS (Murashige and Skoog) media with growth regulators
Applications	Areas where cryopreservation is implemented	Gene banks, endangered species recovery, clonal crop conservation
Advantages	Benefits of using cryopreservation for plant conservation	Long-term storage, genetic stability, minimal space
Limitations	Challenges or constraints in cryopreservation	High technical skill, species-specific protocols

3.3 Conservation of Recalcitrant and Rare Seeds

Recalcitrant and rare seeds present unique challenges in germplasm conservation due to their sensitivity to desiccation and low-temperature storage. Unlike orthodox seeds, which can be dried and stored at sub-zero temperatures for long-term viability, recalcitrant seeds lose viability rapidly when subjected to dehydration or freezing. This poses significant risks to the conservation of numerous tropical species, wild relatives of crops, and endangered plants that predominantly produce such seeds.

In vitro conservation methods offer a strategic solution to this problem by enabling the storage of plant material under sterile, controlled environments. These methods include slow-growth storage**,** cryopreservation, and encapsulation techniques, which allow for the conservation of shoot tips, embryos, somatic tissues, and zygotic embryos from recalcitrant species. Slow-growth storage involves reducing the growth rate of cultured tissues using low temperature, osmotic agents, or growth

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retardants, thereby prolonging subculture intervals. Cryopreservation, on the other hand, allows for ultra-long-term storage by freezing explants in liquid nitrogen (−196°C), where all metabolic processes are halted, ensuring long-term genetic stability.

These in vitro techniques are particularly crucial for species with limited seed production, short seed viability, or small populations. Additionally, they offer a platform for rescue and propagation of endangered species, disease-free germplasm maintenance, and tissue banking for future breeding programs. By integrating biotechnological tools with conservation efforts, in vitro methods significantly enhance the capacity to safeguard plant diversity for future generations.

4. APPLICATIONS IN PLANT BREEDING PROGRAMS

4.1 Somaclonal Variation

Somaclonal variation refers to the genetic variation that arises in plants regenerated from somatic tissues through plant tissue culture techniques, such as callus culture, organogenesis, or somatic embryogenesis. Unlike traditional breeding, which relies on natural or induced mutations, somaclonal variation results from spontaneous changes occurring during in vitro culture due to chromosomal rearrangements, DNA methylation, transposon activation, and oxidative stress.

This variation is considered a valuable source of genetic variability, especially in clonally propagated crops or species with narrow genetic bases. It enables researchers to access a wide range of genetic alterations without the use of mutagenic agents or transgenes. Through selection and screening, beneficial variants can be identified and used in developing novel traits, such as improved resistance to diseases, enhanced tolerance to abiotic stresses (e.g., drought, salinity), better nutritional profiles, or altered growth characteristics.

Somaclonal variation is particularly useful in crops where conventional breeding is difficult due to sterility or long generation times. It has been successfully exploited in crops like sugarcane, potato, banana, rice, and wheat to generate improved cultivars. Despite its advantages, not all somaclonal variation is beneficial; some changes may be deleterious, so rigorous screening and stability assessment are essential before commercial release.

Table: Applications and Outcomes of Somaclonal Variation in Crop Improvement

Crop	Type of Somaclonal Variation Observed	Targeted Trait	Outcome	Reference Example
Sugarcane	Chromosomal rearrangements, point mutations	Disease resistance (red rot)	Resistant clones identified	Krishnamurthi & Tlaskal (1974)
Potato	DNA methylation changes	Cold-induced sweetening	Improved tuber quality	Shepard et al. (1980)
Banana	Transposon activation,	Fusarium wilt	Fusarium-	Hwang & Ko

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	ploidy changes	resistance	resistant lines developed	(1987)
Rice	Gene silencing and activation	Salinity tolerance	Salt-tolerant somaclones obtained	Rani & Raina (2000)
Wheat	Single nucleotide polymorphisms (SNPs), deletions	Drought and heat tolerance	High-yielding, stress-tolerant lines selected	Jain (2001)

4.2 Embryo Rescue and Wide Crosses

Embryo rescue is a plant tissue culture technique used to facilitate the development of embryos that would otherwise abort or become non-viable in nature. This method is especially important in wide crosses, where interspecific (between species) or intergeneric (between genera) hybridizations are attempted. Such crosses are often crucial in plant breeding to introduce desirable traits such as disease resistance, drought tolerance, or enhanced nutritional qualities from wild relatives into cultivated species. However, due to post-fertilization barriers like embryo abortion, endosperm failure, or chromosomal incompatibility, these hybrids often fail to develop naturally.

Embryo rescue overcomes these barriers by culturing immature or mature embryos under sterile in vitro conditions, using nutrient-rich media tailored to support their growth. The success of embryo rescue depends on factors such as the age of the embryo, composition of the culture media, presence of growth regulators, and genotype of the parent plants. In many cases, embryo rescue has proven essential in salvaging hybrid progeny, thereby enabling introgression breeding where genes from wild or distantly related plants are incorporated into elite cultivars.

Wide crosses are central to expanding the genetic base of crops. They involve crossing plants that do not normally interbreed, often due to reproductive isolation or chromosomal differences. Although pollination may occur, fertilization and embryo development are frequently compromised. Embryo rescue provides a technological solution by bypassing natural seed development, allowing the breeder to obtain viable hybrids and further backcross them to produce fertile, agronomically superior lines.

Table: Applications of Embryo Rescue in Wide Crosses

Crop	Cross Type	Challenge	Embryo Rescue Stage	Culture Medium Used	Outcome/Success
Wheat × Rye	Interspecific	Embryo abortion post-	10–14 days post-	MS medium +	Successful development of

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		fertilization	pollination	sucrose + growth regulators	triticale (hybrid)
Tomato × Potato	Intergeneric	Incompatibility and endosperm failure	7–10 days post-pollination	Modified White's medium	Hybrid recovery; root and shoot induction in vitro
Rice × Zizania	Interspecific	Seed development failure	8–10 days post-pollination	N6 medium + vitamins + GA_3	Partial embryo development, successful hybrid lines
Pear × Quince	Interspecific	Incomplete embryo development	30–40 days post-pollination	MS medium + activated charcoal	Viable seedlings with dwarfing traits
Sunflower × Wild Helianthus	Interspecific	Embryo abortion due to incompatibility	12–15 days post-pollination	B5 medium + auxins and cytokinins	Transfer of disease resistance genes

Embryo rescue plays a pivotal role in overcoming reproductive barriers in plant breeding, especially when dealing with wide crosses. By ensuring the survival of hybrids that would otherwise be lost, this technique accelerates genetic introgression, enhances crop improvement, and expands the gene pool for future breeding efforts. The combined use of embryo rescue and molecular tools is expected to further strengthen its utility in developing resilient crop varieties.

4.3 Haploid and Doubled Haploid Production

Haploid and doubled haploid (DH) production is a powerful technique in plant breeding that enables the rapid development of completely homozygous lines from heterozygous parents. This is primarily achieved through anther (androgenesis) and ovule (gynogenesis) culture, where male or female gametophytic tissues are cultured in vitro to produce haploid embryos. These haploids, containing only a single set of chromosomes, can then be treated (commonly with colchicine) to double their chromosome number, resulting in doubled haploids that are genetically stable and fully homozygous. This method significantly shortens the breeding cycle compared to conventional selfing over multiple generations. It also aids in faster selection, QTL mapping, hybrid production, and the development of pure lines for commercial use, making it an essential tool in modern crop improvement programs.

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4.4 Transformation and Regeneration

Tissue culture serves as a crucial platform for plant transformation and regeneration, enabling precise gene editing and transgenesis. It provides the controlled environment necessary for introducing foreign genes or editing native ones, especially using tools like CRISPR/Cas9 and Agrobacterium-mediated transformation. Through techniques such as callus induction or direct organogenesis, transformed cells can be regenerated into whole plants that carry the desired genetic modifications. This integration allows researchers to develop crops with improved traits such as disease resistance, drought tolerance, or enhanced nutritional content. Overall, tissue culture acts as both the entry point for gene delivery and the recovery system for fully developed, genetically modified plants.

5. INTEGRATION WITH MOLECULAR AND GENOMIC TOOLS

5.1 Use of molecular markers to assess genetic fidelity

Molecular markers such as SSRs (Simple Sequence Repeats), RAPDs (Random Amplified Polymorphic DNA), and AFLPs (Amplified Fragment Length Polymorphisms) are widely used in tissue culture to assess the genetic fidelity of regenerated plants. These markers help detect somaclonal variations and ensure that clonal plants are genetically identical to the parent source. This is essential in maintaining uniformity, especially in commercial micropropagation, breeding programs, and germplasm conservation.

5.2 Genomic tools to identify candidate traits for selection

Advanced genomic tools, including genome-wide association studies (GWAS), transcriptomics, and marker-assisted selection (MAS), facilitate the identification of candidate genes linked to desirable traits such as stress tolerance, yield, or disease resistance. When integrated with tissue culture systems, these tools enable targeted selection and propagation of genotypes with superior traits, thus accelerating the breeding process and enhancing precision in crop improvement.

6. CASE STUDIES AND CROP-SPECIFIC APPLICATIONS

Banana- In banana cultivation, tissue culture-based micropropagation has been widely used to produce virus-free plantlets. This technique ensures the elimination of latent pathogens and provides uniform, healthy planting material on a large scale. It has significantly improved crop yield, disease management, and the overall sustainability of banana farming, especially in regions affected by viral infections like Banana Bunchy Top Virus (BBTV).

Rice and Maize- In rice and maize breeding programs, doubled haploid (DH) technology through tissue culture enables the rapid development of homozygous lines. Techniques like anther and microspore culture allow breeders to produce genetically stable lines in a single generation, drastically shortening breeding cycles. This advancement has enhanced the efficiency of hybrid development, contributing to higher productivity and precision in crop improvement.

Medicinal Plants- Tissue culture plays a vital role in the conservation of endangered medicinal plants by facilitating in vitro

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propagation and gene bank preservation. Through methods like shoot tip and somatic embryo culture, rare species can be multiplied without disturbing wild populations. This approach ensures sustainable utilization while maintaining biodiversity and safeguarding valuable phytochemical resources.

7. CONCLUSION

Tissue culture stands as a vital bridge between genetic conservation and modern plant breeding innovation. It enables the preservation of rare, endangered, and valuable plant genotypes through in vitro conservation techniques such as cryopreservation, slow-growth storage, and micropropagation. Simultaneously, it supports rapid multiplication, trait fixation, and genetic enhancement of crops through methods like somatic embryogenesis, anther culture, and regeneration of genetically modified plants. Its integration with advanced biotechnological tools—such as CRISPR/Cas9 gene editing and Agrobacterium-mediated transformation—has opened new frontiers in precision breeding and sustainable agriculture. As biodiversity loss and food security challenges intensify, the global role of tissue culture becomes increasingly critical. However, harnessing its full potential requires robust international collaborations, standardization of protocols, and capacity building in resource-limited regions. The synergy between conventional breeding knowledge and biotechnology-driven tissue culture techniques holds promise for resilient, high-performing crops and the protection of global plant diversity. Thus, plant tissue culture is not only a scientific method but a strategic necessity for addressing agricultural, ecological, and genetic conservation challenges in an increasingly uncertain future.

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