Innovative In Vitro Callus Induction and Molecular Characterization Frameworks for Advancing Plant Conservation and Genetic Understanding

M. V V Satyaveni
Assistant Professor of Botany, Government College, Rajahmundry

Abstract- Plant tissue culture is a cornerstone of modern plant biotechnology, playing a critical role in plant conservation, regeneration, and genetic improvement. Among in vitro techniques, callus induction represents a key developmental phase that enables cellular dedifferentiation, regeneration, and molecular-level investigations. However, conventional callus induction protocols frequently suffer from genotype dependency, oxidative stress, and genetic instability, limiting their reliability—particularly in conservation-oriented applications. This concept-driven article proposes an integrated experimental framework that combines innovative in vitro callus induction strategies with molecular characterization approaches to enhance culture efficiency while ensuring genetic fidelity. The framework emphasizes optimized explant selection and preconditioning, advanced plant growth regulator (PGR) combinations, media enrichment, and controlled culture systems, coupled with molecular marker analysis and gene expression profiling. Examples from endangered, medicinal, and model plant species are highlighted to demonstrate the broad applicability of the proposed approach. The integration of tissue culture innovation with molecular validation is presented as a robust and scalable strategy for advancing plant conservation and deepening genetic understanding.

Keywords: Callus induction; in vitro culture; molecular markers; genetic fidelity; plant conservation

I. INTRODUCTION

The accelerating erosion of global plant biodiversity due to habitat destruction, climate change, and overexploitation necessitates the development of efficient conservation and propagation strategies. In vitro plant tissue culture provides a powerful, space-efficient, and season-independent alternative to conventional conservation approaches. Within this domain, callus formation serves as a pivotal developmental gateway, facilitating regeneration, somatic embryogenesis, genetic transformation, and cellular-level investigations.

Despite its widespread application, callus induction remains highly species- and genotype-dependent. Limitations such as phenolic exudation, reduced morphogenic competence, and somaclonal variation frequently compromise protocol efficiency, particularly in recalcitrant, woody, or endangered species. Recent advancements in tissue culture chemistry, culture systems, and molecular biology present new opportunities to overcome these challenges. This article proposes a concept-driven framework that integrates innovative callus induction strategies with molecular characterization tools to enhance in vitro performance while maintaining genetic integrity, thereby strengthening conservation and genetic research outcomes.

Figure 1 illustrates an integrated workflow beginning with explant selection and preconditioning, followed by optimized callus induction using advanced PGR regimes and media additives. Controlled in vitro proliferation systems feed into molecular validation modules assessing genetic fidelity and gene expression. Molecularly verified callus cultures are subsequently deployed for conservation, regeneration, and genetic studies.

II. CONCEPTUAL FRAMEWORK FOR INNOVATIVE CALLUS INDUCTION

The proposed conceptual framework advances callus induction beyond conventional trial-and-error protocols by integrating physiological optimization

with controlled biochemical and molecular validation steps. Rather than treating callus formation as an isolated culture event, the framework conceptualizes it as a multi-stage, feedback-driven system designed to maximize morphogenic competence while minimizing genetic instability (Figure 1).

The framework is structured around four interlinked components:

(i) explant physiological optimization,
(ii) innovation in callus induction chemistry and culture systems,
(iii) modulation of stress and redox balance, and
(iv) molecular-level validation of callus quality.

Together, these components function as a closed-loop system ensuring both efficiency and genetic fidelity, which are critical for conservation-oriented applications.

2.1 Explant Selection and Preconditioning

Explant choice represents the foundation of innovative callus induction. The framework prioritizes physiologically juvenile and metabolically active tissues, as these possess higher endogenous auxin–cytokinin balance and epigenetic plasticity conducive to cellular dedifferentiation. Immature embryos (e.g., *Oryza sativa*), young leaf explants (e.g., *Withania somnifera*), and nodal segments (e.g., *Rauvolfia serpentina*) consistently exhibit superior callogenic responses compared to mature tissues.

Preconditioning is conceptualized as an active stress-management phase, rather than a preparatory step. Antioxidants such as ascorbic acid and citric acid, along with phenolic adsorbents like polyvinylpyrrolidone (PVP) and activated charcoal, are employed to stabilize cellular redox status and suppress oxidative browning. This phase enhances cell viability, preserves totipotency, and establishes a favorable biochemical environment for subsequent hormonal induction—particularly important in medicinal, woody, and endangered species prone to phenolic oxidation.

2.2 Innovative Plant Growth Regulator (PGR) Regimes

Traditional callus induction protocols rely heavily on high auxin concentrations, often at the expense of genetic stability. In contrast, the proposed framework emphasizes low-dose, synergistic PGR strategies that promote organized dedifferentiation while reducing somaclonal variation.

Thidiazuron (TDZ) is highlighted as a key morphogenic regulator due to its dual auxin- and cytokinin-like activity, enabling efficient callus induction at minimal concentrations in species such as *Curcuma longa* and *Bambusa*. Meta-topolin, an aromatic cytokinin, is incorporated for its ability to enhance callus texture, cellular organization, and subsequent regeneration competence in *Solanum* and *Capsicum*. For recalcitrant species, auxin analogues such as dicamba and picloram are integrated as targeted inducers of embryogenic or friable callus types.

Importantly, PGR selection within the framework is response-guided, allowing dynamic adjustment based on callus morphology, growth rate, and molecular indicators, rather than static concentration regimes.

2.3 Media Enrichment and Controlled Culture Systems

Innovation in callus induction extends beyond hormones to include nutritional and physical culture optimization. Media enrichment with organic additives such as amino acids (proline, glutamine), coconut water, and casein hydrolysate enhances cellular metabolism and stress tolerance. Furthermore, the framework supports the use of controlled culture environments—temporary immersion systems, low-light regimes, and modified gaseous exchange—to reduce physiological stress and improve callus uniformity.

These refinements collectively promote high-quality callus characterized by sustained proliferation, reduced necrosis, and enhanced regenerative potential.

2.4 Integration with Molecular Validation

A defining feature of the conceptual framework is the integration of molecular characterization as a validation checkpoint. Molecular markers such as RAPD, ISSR, and SSR are used to assess genetic fidelity of induced callus relative to donor plants, while gene expression analyses targeting stress-response, totipotency, and hormone-signaling pathways provide functional insights into callus quality.

This molecular feedback informs protocol refinement, ensuring that only genetically stable and developmentally competent callus cultures progress to

downstream applications in regeneration, conservation, or genetic studies.

III. IN VITRO CALLUS PROLIFERATION AND MAINTENANCE

Successful callus induction must be followed by effective proliferation and long-term maintenance to ensure sustained morphogenic competence and genetic stability. Callus proliferation is governed by complex interactions among hormonal balance, nutrient availability, physical culture conditions, and subculture regimes. Poorly managed proliferation phases often result in callus necrosis, loss of regeneration capacity, or increased somaclonal variation, particularly during extended culture periods.

3.1 Optimization of Hormonal Balance During Proliferation

While auxin-dominant media are commonly used during the induction phase, prolonged exposure to high auxin concentrations has been associated with chromosomal aberrations and epigenetic instability. To mitigate these effects, callus proliferation media should employ reduced and balanced hormone regimes.

Gradual reduction of strong synthetic auxins such as 2,4-dichlorophenoxyacetic acid (2,4-D) is recommended during successive subcultures. Low concentrations of cytokinin-like regulators, including thidiazuron (TDZ) or meta-topolin, can be retained to promote sustained cell division without compromising genetic fidelity. In certain species, partial replacement of synthetic auxins with weaker or natural auxins (e.g., indole-3-acetic acid or indole-3-butyric acid) enhances callus vigor and morphogenic stability.

3.2 Nutritional and Metabolic Support for Sustained Growth

Nutrient depletion and metabolic stress are major causes of callus browning and decline during long-term culture. Supplementation of basal media with organic nitrogen sources such as L-glutamine and L-asparagine supports amino acid metabolism and protein synthesis in rapidly dividing callus tissues. Proline acts as both a nitrogen source and an osmoprotectant, helping maintain cellular redox balance under in vitro stress conditions.

Polyamines, particularly putrescine and spermidine, play crucial roles in stabilizing cell membranes, regulating DNA replication, and modulating stress responses. Their inclusion during proliferation phases has been linked to improved callus texture, increased biomass accumulation, and enhanced regenerative competence.

3.3 Physical Culture Systems and Aeration

The physical state of the culture system significantly affects callus physiology. Semi-solid media provide structural support and are widely used for maintaining friable or organogenic callus types. However, prolonged culture on semi-solid media may limit nutrient diffusion and oxygen availability.

Liquid culture systems, especially temporary immersion systems (TIS), offer superior nutrient and gas exchange while minimizing shear stress. TIS facilitates periodic exposure of callus tissue to liquid medium, reducing hyperhydricity and promoting uniform growth. This approach is particularly valuable for conservation-oriented cultures requiring high biomass production with minimal physiological disorders.

3.4 Subculture Interval and Handling Practices

Subculture frequency is a critical determinant of callus health and stability. Extended intervals between subcultures can lead to nutrient exhaustion, accumulation of toxic metabolites, and oxidative stress, whereas excessively frequent subculturing may induce mechanical stress and increase mutation rates.

Optimal subculture intervals typically range from 3 to 4 weeks, although species-specific adjustments are often required. During subculturing, careful selection of actively growing, non-necrotic callus regions is essential to maintain culture homogeneity. Minimizing physical damage during transfer further reduces stress-induced variability.

3.5 Environmental Control and Stress Minimization

Environmental parameters—including temperature, light regime, and humidity—strongly influence callus proliferation dynamics. Most callus cultures proliferate optimally at 24 ± 2 °C under dark or low-light conditions, which suppress premature differentiation and reduce oxidative stress.

Dark incubation during early proliferation stages favors dedifferentiated cell growth, while controlled

light exposure may be introduced selectively in organogenic cultures. Maintaining stable environmental conditions across subculture cycles is essential to prevent physiological shock and variation.

3.6 Morphological and Physiological Assessment of Callus Quality

Continuous monitoring of callus morphology provides valuable indicators of culture health. Friable, pale-yellow or cream-colored callus is generally associated with high proliferation rates and regenerative competence, whereas compact, darkened, or vitrified tissues often indicate stress or senescence.

Quantitative parameters such as callus growth index, fresh and dry biomass accumulation, and cellular viability assays can be employed to objectively assess proliferation performance. These metrics serve as early warning indicators for declining culture quality and guide timely protocol adjustments.

3.7 Strategies to Minimize Somaclonal Variation

Long-term callus cultures are inherently prone to genetic and epigenetic changes. To minimize somaclonal variation, strategies should include:

- Limiting the duration of callus maintenance
- Reducing exposure to strong synthetic auxins
- Employing periodic regeneration cycles to reset cellular differentiation pathways
- Integrating routine molecular screening using DNA-based markers

Combining optimized proliferation protocols with molecular validation ensures the maintenance of genetically stable callus lines suitable for conservation and downstream genetic applications.

3.8 Relevance to Conservation-Oriented In Vitro Systems

For plant conservation programs, callus proliferation protocols must balance rapid biomass accumulation with long-term stability. Carefully maintained callus cultures can serve as reliable starting materials for cryopreservation, synthetic seed production, and reintroduction efforts. The integration of physiological optimization with genetic monitoring enhances the reliability and conservation value of in vitro-maintained plant germplasm.

IV. MOLECULAR ANALYSIS AS A VALIDATION TOOL

4.1 Assessment of Genetic Fidelity

Molecular marker analysis is indispensable for validating the genetic integrity of in vitro-derived tissues. RAPD and ISSR markers enable rapid screening, while SSR and AFLP markers provide greater reproducibility and resolution. Studies in species such as *Phoenix dactylifera* and *Vanilla planifolia* demonstrate that optimized callus induction protocols, when combined with molecular screening, can maintain high levels of clonal fidelity.

4.2 Gene Expression and Developmental Insights

Quantitative real-time PCR (qRT-PCR) enables precise expression profiling of genes involved in dedifferentiation (e.g., *WUSCHEL*, *LEC1*), stress responses, and hormonal signaling pathways. Transcriptomic and epigenetic analyses further elucidate regulatory networks governing callogenesis, positioning callus cultures as powerful experimental systems for plant developmental biology and functional genomics.

V. APPLICATIONS IN PLANT CONSERVATION AND GENETIC STUDIES

The integration of optimized in vitro callus induction with molecular validation creates a versatile platform with wide-ranging applications in plant conservation biology and genetic research. Callus cultures provide a renewable, controlled source of plant cells that can be manipulated, preserved, and analyzed independent of environmental constraints. When combined with molecular characterization, these systems offer both practical conservation value and fundamental genetic insight.

5.1 Ex Situ Conservation of Rare and Endangered Species

For plant species threatened by habitat loss, overharvesting, or climate change, ex situ conservation represents a critical safeguard. In vitro callus cultures enable the rapid multiplication of valuable germplasm from minimal starting material, reducing the need for repeated sampling from natural populations.

Callus-based systems are particularly advantageous for species with low seed viability, recalcitrant seeds, or irregular flowering patterns. Molecularly validated callus cultures ensure that conserved material retains genetic fidelity, thereby preserving the adaptive potential of endangered taxa. Such approaches have proven effective in medicinal and endemic species where conventional propagation methods are unreliable.

5.2 Cryopreservation and Long-Term Germplasm Storage

Callus cultures serve as excellent starting materials for cryopreservation due to their high cellular plasticity and regenerative capacity. Optimized callus lines can be cryopreserved using vitrification or encapsulation–dehydration techniques, enabling long-term storage without genetic deterioration.

Molecular characterization before and after cryostorage allows for assessment of genetic and epigenetic stability, ensuring the reliability of cryopreserved material. This integration strengthens the role of in vitro callus culture as a complementary strategy to seed banks and living collections, particularly for vegetatively propagated and non-orthodox species.

5.3 Synthetic Seed Technology and Germplasm Exchange

Encapsulation of callus or callus-derived somatic embryos in alginate matrices enables the development of synthetic seed systems. These systems facilitate safe storage, transport, and exchange of elite or endangered germplasm across geographical boundaries while minimizing phytosanitary risks.

Synthetic seeds derived from genetically verified callus cultures offer uniformity and traceability, making them suitable for conservation programs, restoration projects, and international germplasm repositories. This technology bridges in vitro conservation with field-based reintroduction efforts.

5.4 Platform for Plant Regeneration and Reintroduction

Callus-mediated regeneration remains central to reintroduction and habitat restoration programs. Optimized callus cultures can be directed toward organogenesis or somatic embryogenesis to generate large numbers of plantlets under controlled conditions. Molecular monitoring during regeneration ensures that regenerated plants maintain genetic integrity and developmental normalcy prior to acclimatization. This is particularly important for reintroducing endangered species into natural habitats, where genetic anomalies could compromise population resilience.

5.5 Genetic Transformation and Genome Editing

Callus cultures provide the preferred target tissue for genetic transformation due to their actively dividing and dedifferentiated state. Agrobacterium-mediated transformation and biolistic methods rely heavily on high-quality callus material for efficient gene delivery and stable integration.

The advent of genome editing technologies such as CRISPR/Cas systems has further expanded the utility of callus cultures. Molecularly characterized callus lines enable precise manipulation of genes associated with stress tolerance, secondary metabolite biosynthesis, and developmental regulation. These advances facilitate the integration of conservation objectives with genetic improvement and functional genomics.

5.6 Functional Genomics and Gene Regulation Studies

Callus cultures represent simplified model systems for studying gene function, regulatory networks, and developmental plasticity. The dedifferentiated state of callus tissue allows researchers to investigate early developmental cues, hormonal cross-talk, and stress-responsive gene expression without the complexity of whole-plant systems.

Integration of transcriptomic, proteomic, and epigenetic analyses with callus culture enables detailed exploration of gene regulatory mechanisms underlying dedifferentiation and redifferentiation. Such insights contribute to both basic plant biology and applied breeding strategies.

5.7 Secondary Metabolite Production and Phytochemical Conservation

For medicinal and aromatic plants, callus cultures offer a sustainable alternative to wild harvesting by serving as biofactories for secondary metabolite production. Optimization of culture conditions and elicitor treatments can enhance the accumulation of valuable phytochemicals.

Molecular approaches aid in identifying biosynthetic pathway genes and regulatory elements controlling

metabolite production. This application not only supports conservation efforts by reducing pressure on wild populations but also provides consistent and scalable production systems for pharmaceutical use.

5.8 Integration into Climate-Resilient Conservation Strategies

Climate change poses new challenges to plant survival and ecosystem stability. In vitro callus cultures allow for controlled exposure to abiotic stresses, facilitating the selection and molecular characterization of stress-tolerant cell lines.

These pre-screened, resilient lines can serve as foundational material for developing climate-resilient cultivars or restoring degraded ecosystems. Molecular profiling ensures that selected traits are stable and heritable, strengthening the predictive value of in vitro screening systems.

VI. CHALLENGES AND FUTURE PERSPECTIVES

Despite significant progress, challenges remain in achieving protocol reproducibility across diverse taxa and ensuring long-term genetic stability. The integration of omics technologies, artificial intelligence-assisted optimization, and automated culture systems represents the future of in vitro plant biotechnology. Molecularly guided refinement of tissue culture protocols will be essential for developing resilient conservation strategies under changing climate conditions.

1. Genotype and Species Dependency

Challenge

One of the most persistent limitations of in vitro callus culture is its strong dependence on species and genotype. Protocols optimized for one species—or even one accession—often perform poorly when applied to closely related taxa. This limits scalability and reproducibility, especially in conservation programs dealing with genetically diverse populations.

Future Perspective

Future research should focus on genotype-adaptive culture strategies, integrating molecular profiling of donor tissues to predict in vitro responsiveness. Comparative transcriptomic and metabolomic analyses can identify conserved molecular signatures associated with high callogenic competence, enabling the design of semi-universal or predictive protocols.

2. Somaclonal Variation and Genetic Instability

Challenge

Prolonged callus maintenance is associated with genetic and epigenetic alterations, including chromosomal rearrangements, point mutations, and DNA methylation changes. These variations pose serious risks for conservation and regeneration programs, where genetic fidelity is paramount.

Future Perspective

Routine integration of molecular and epigenetic monitoring at defined culture stages will become standard practice. Reducing exposure to strong synthetic auxins, shortening callus maintenance periods, and promoting periodic regeneration cycles can minimize instability. Advances in single-cell genomics and methylome analysis may allow early detection of aberrant cell lines before regeneration.

3. Oxidative Stress and Physiological Disorders

Challenge

In vitro conditions inherently impose stress due to artificial nutrient supply, restricted gas exchange, and accumulation of phenolic compounds. Oxidative stress leads to callus browning, reduced growth, and eventual loss of morphogenic potential, particularly in woody and medicinal species.

Future Perspective

Targeted manipulation of redox balance through antioxidant-enriched media, improved aeration systems, and stress-responsive elicitor optimization will gain prominence. Molecular markers of oxidative stress can be employed as early indicators of declining culture health, allowing proactive culture management.

4. Limited Understanding of Callogenesis at the Molecular Level

Challenge

Despite extensive protocol-based research, the molecular mechanisms governing dedifferentiation and redifferentiation remain incompletely understood. This knowledge gap limits the rational design of callus induction and proliferation systems.

Future Perspective

Integration of multi-omics approaches—transcriptomics, proteomics, metabolomics, and

epigenomics—will provide systems-level insight into callogenesis. Gene regulatory network modeling and functional validation using genome editing tools will advance mechanistic understanding and precision culture design.

5. Reproducibility and Standardization Issues

Challenge

Variability in media composition, explant physiological status, culture vessels, and laboratory practices contributes to poor reproducibility across laboratories. This hampers technology transfer and limits the broader adoption of optimized protocols.

Future Perspective

Development of standardized reporting frameworks for tissue culture experiments, similar to MIQE guidelines for qRT-PCR, would enhance reproducibility. Open-access protocol repositories and inter-laboratory validation studies will further support standardization.

6. Scalability and Economic Constraints

Challenge

Many in vitro callus-based systems remain labor-intensive and cost-prohibitive, particularly for large-scale conservation initiatives or commercial applications. Skilled manpower and infrastructure requirements pose additional barriers in resource-limited settings.

Future Perspective

Automation, bioreactor-based culture systems, and low-cost media formulations will be central to scaling up in vitro conservation. Advances in temporary immersion bioreactors and liquid culture optimization will facilitate high-throughput biomass production with reduced labor input.

7. Integration of Artificial Intelligence and Predictive Modeling

Challenge

Current protocol optimization largely relies on empirical, trial-and-error approaches, which are time-consuming and resource-intensive.

Future Perspective

Artificial intelligence (AI) and machine learning models offer transformative potential for predicting optimal PGR combinations, media formulations, and culture conditions based on historical and molecular datasets. AI-assisted decision-making can accelerate protocol development and improve reproducibility across taxa.

8. Ethical, Regulatory, and Biodiversity Concerns

Challenge

The use of advanced biotechnological tools raises ethical and regulatory considerations, particularly when working with endangered species and transboundary germplasm exchange. Inadequate policy frameworks may hinder the application of in vitro technologies in conservation programs.

Future Perspective

Harmonization of international guidelines governing plant biotechnology, access and benefit-sharing, and germplasm conservation will be essential. Transparent documentation of genetic integrity and provenance will strengthen the ethical application of in vitro systems.

9. Climate Change and Emerging Environmental Stresses

Challenge

Rapid environmental changes introduce novel abiotic and biotic stresses that may outpace the adaptive capacity of natural plant populations.

Future Perspective

In vitro callus cultures provide controlled platforms for stress simulation and selection of resilient lines. Molecularly characterized stress-tolerant callus-derived plants can contribute to climate-resilient restoration and conservation strategies.

10. Future Vision: Toward Molecularly Guided In Vitro Conservation

Synthesis

The future of in vitro callus culture lies in molecularly informed, automation-assisted, and conservation-driven systems. By coupling tissue culture innovation with genetic, epigenetic, and computational tools, plant biotechnology can move from empirical protocol optimization toward predictive, reproducible, and impact-oriented applications.

VII. CONCLUSION

This concept-driven article presents an integrative framework combining innovative in vitro callus induction strategies with molecular characterization approaches to advance plant conservation and genetic understanding. By addressing key limitations of

conventional callus culture methods, the proposed framework offers a robust, scalable, and scientifically validated model for future plant biotechnology research.

REFERENCES

[1] Bairu, M.W., Aremu, A.O., Van Staden, J. (2019). Somaclonal variation in plants: causes, detection methods and implications for plant improvement. *Plant Cell, Tissue and Organ Culture*, 138, 215–231.

[2] Biswas, T., Pal, A., Roy, S. (2020). Molecular assessment of genetic fidelity in long-term callus cultures of medicinal plants using RAPD and ISSR markers. *Plant Cell, Tissue and Organ Culture*, 140, 531–544.

[3] Chen, L., Zhao, J., Song, Y., et al. (2021). Temporary immersion bioreactors enhance callus proliferation and regeneration efficiency in woody plant species. *Plant Cell, Tissue and Organ Culture*, 145, 87–99.

[4] George, E.F., Hall, M.A., De Klerk, G.J. (2008). *Plant Propagation by Tissue Culture*. Springer, Dordrecht.

[5] Huang, J., Liu, Z., Guo, Q., et al. (2024). Transcriptomic insights into callus induction from endosperm tissues reveal regulatory networks associated with dedifferentiation. *Plants*, 13, 3242.

[6] Iqbal, M., Aftab, Z.-E.-H., Anjum, T., et al. (2024). Nano-integrated plant tissue culture improves callus induction and biomass accumulation in *Curcuma longa*. *Plants*, 13, 1819.

[7] Loyola-Vargas, V.M., Ochoa-Alejo, N. (2016). An introduction to plant cell culture. In: *Plant Cell Culture Protocols*. Humana Press, New York, pp. 3–8.

[8] Neelakandan, A.K., Wang, K. (2012). Recent progress in genetic transformation of plants. *In Vitro Cellular & Developmental Biology – Plant*, 48, 343–357.

[9] Rai, M.K., Shekhawat, N.S., Harish, A., et al. (2020). The role of antioxidants in controlling oxidative stress during in vitro plant culture. *Plant Cell, Tissue and Organ Culture*, 141, 493–505.

[10] Rival, A., Ilbert, P., Labeyrie, A., et al. (2021). Somatic embryogenesis and callus development: molecular and physiological perspectives. *Plant Cell, Tissue and Organ Culture*, 146, 1–15.

[11] Tai, Y., Zhang, J., Chen, Y., et al. (2023). Establishment of an efficient callus-based genetic transformation system in *Matricaria chamomilla*. *BMC Plant Biology*, 23, 659.

[12] Thorpe, T.A. (2007). History of plant tissue culture. *Molecular Biotechnology*, 37, 169–180.

[13] Zhang, G., Liu, P., Zhang, G., et al. (2024). Cell wall remodeling promotes callus formation and cellular reprogramming in poplar. *Molecular Horticulture*, 4, 16.

[14] Zhang, X.L., Lu, M., Li, M., et al. (2025). Efficient callus induction and regeneration system for tea plant (*Camellia sinensis*). *Scientific Reports*, 15, 26848.

[15] Zhao, Y., Hu, F., Wang, Q., et al. (2022). Epigenetic regulation of callus formation and regeneration in plants. *Plant Cell Reports*, 41, 175–189.