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Review

Plant Tissue Culture: Past Foundations, Present Innovations, and Future Horizons

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Abstract

The intrinsic totipotency of plant cells is demonstrated by plant tissue culture, which is the foundation for significant developments in plant biotechnology. Through carefully monitored organogenic and embryogenic pathways, somatic cells may be stimulated to regenerate whole plants by replicating controlled in vitro settings. Plant growth regulators, nutrient composition, and physical conditions all work together to shape these developmental transitions. Recent molecular research has elucidated the role of auxin signaling networks, developmental regulators, chromatin dynamics, and stress-associated epigenetic modifications in cellular reprogramming. Reproducibility and propagation efficiency have been increased due to advancements in temporary immersion systems, environmental control, and medium optimization. Simultaneously, tissue culture platforms are becoming more and more integrated with high-throughput omics studies, genome editing technologies, and nanomaterial-based delivery systems, boosting the accuracy and scalability of plant transformation beyond traditional callus-dependent methods. In commercial and research contexts, automation and automated monitoring systems further improve scalability and consistency. Also, early detection of adaptive characteristics is made easier by the deliberate use of somaclonal variations and in vitro stress selection. Collectively, these advancements reinforce plant tissue culture as a dynamic and progressive framework that connects fundamental developmental biology with practical crop enhancement and sustainable agricultural innovation.

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Statement of sustainability: Plant tissue culture supports sustainable agriculture by enabling rapid, disease-free plant propagation while conserving natural resources and genetic diversity. Optimized in vitro systems enhance crop improvement, stress tolerance, and reproducibility. Integration with genome editing and automation reduces resource use and experimental waste, supporting sustainable plant production and food security.

1. Introduction

One of the most remarkable instances of developmental plasticity in plants is the capacity of somatic cells to proliferate into entire plants, which is a reflection of their intrinsic totipotency. Unlike most animal systems, plant cells may readily respond to external cues and rewire their embryonic fate under the right in vitro conditions. By successfully regenerating complete carrot plants from dynamically suspended phloem cells, Steward et al. (1958) empirically validated this theory and offered the first convincing proof of cellular totipotency. Skoog and Miller (1957) demonstrated the hormonal regulation of organogenesis by discovering that a higher auxin-to-cytokinin ratio supports root formation while higher cytokinin levels promote shoot development. Their findings fundamentally altered our understanding of how chemicals control morphogenesis in cultured plant tissues.

The Murashige and Skoog (MS) mineral medium, a nutritionally optimised formulation that facilitated the rapid growth of tobacco tissues, was later introduced by Murashige and Skoog (1962). Later on, most plant tissue culture techniques used this formulation as their basic base media. As research progressed, it became evident that, in addition to exogenous hormones and nutrients, biological competence, environmental management, and epigenetic reprogramming are all critical components of successful regeneration. These points of view were combined by Ikeuchi et al. (2016) to provide a comprehensive cellular and molecular framework that emphasizes transcriptional activation and epigenetic changes as key processes underlying cellular reprogramming during regeneration.

Auxin's critical role in regeneration pathways is shown by mounting molecular research. There is a strong relationship between

the WOX5 regulatory mechanism and auxin signaling. WOX5 increases the endogenous auxin accumulation required for plant regeneration by promoting TAA1-mediated auxin biosynthesis (Savina et al., 2020). Thus, localized auxin synthesis and accumulation are the main causes of cellular reprogramming and organogenic competence in vitro. It has also been discovered that auxin accumulation initiates de novo shoot organogenesis, and stress-induced restriction of auxin efflux promotes local auxin retention and regeneration responses (Pasternak & Steinmacher, 2024).

To regulate growth and developmental responses in vitro and assess the efficacy of plant tissue culture, a variety of physiological and environmental factors interact intimately, including food availability, endogenous auxin production, organic chemicals, and culture conditions (Pasternak & Steinmacher, 2024).Extensive studies of plant growth regulation in tissue culture systems further emphasize the importance of both organic and inorganic inputs as key mediators of endogenous hormone metabolism, particularly auxin dynamics. Therefore, regeneration capability is the result of a combination of environmental cues, dietary balance, and hormonal signaling.

Beyond nutritional and hormonal considerations, improving culture performance and reproducibility requires optimizing the culture media's composition For instance, adding 60 mgL^{-1} of copper sulphate to MS medium together with 3 mg L^{-1} BA and 1 mg L^{-1} KIN effectively prevented the growth of endophytic bacteria during banana (*Musa* sp.) shoot multiplication, resulting in 0% contamination and improved culture health (Elyazid et al., 2021).These findings demonstrate the importance of micronutrient balance and medium refinement in achieving reliable regeneration outcomes.

In recent years, plant tissue culture has become more and more integrated with contemporary genome engineering methods, like as vector-based transformation systems, prime editing, and base editing. All of these methods rely on robust regeneration processes to transform molecular alterations into inherited characteristics. Reproducibility remains a significant issue. This empirical heterogeneity contrasts sharply with the precision demanded by contemporary transformation technologies.

Therefore, understanding plant tissue culture requires a comprehensive perspective that goes beyond the manipulation of individual molecular components and includes cellular destiny reprogramming, hormone regulation, nutritional optimization, and environmental management. The method that plant cells perceive and respond to environmental, hormonal, and nutritional stimuli in order to re-enter developmental pathways was defined by these ground-breaking discoveries in plant regeneration research. These conceptual concepts still form the basis for current research on micropropagation and transformation, and provide the theoretical basis for developing predictable, genotype-independent regeneration systems.

2. Methodology

2.1. Literature Search Strategy

In order to document the historical evolution, technical advancements, and new trends in plant tissue culture, this review was carried out utilizing an organized and thorough literature survey strategy. Major scientific databases such as Web of Science, Scopus, PubMed, Google Scholar and publisher platforms like SpringerLink, Elsevier ScienceDirect, and Wiley Online Library were used to find peer-reviewed research papers, reviews, and ground-breaking reports.

Plant tissue culture, cellular totipotency, micropropagation, organogenesis, somatic embryogenesis, de novo shoot regeneration, somaclonal variation, in vitro stress selection, developmental regulators, genome editing, CRISPR/Cas, viral vectors, temporary immersion systems, and in planta transformation were among the topics covered by the search queries, which were created using a combination of keywords and Boolean operators. In order to find more pertinent research that were missed by the first database search, reference lists of important papers were also manually searched.

2.2. Inclusion and Exclusion Criteria

Relevance to the conceptual, technical, and practical elements of plant tissue culture was taken into consideration when choosing publications. Research was incorporated if it(i) offered theoretical or experimental understanding of the mechanics behind plant regeneration,(ii) discussed methodological developments in systems that use micropropagation, regeneration, or transformation,(iii) examined the environmental, molecular, or physiological control of in vitro reactions, or(iv) helped create new genome editing techniques that are either regeneration-assisted or tissue-culture-independent.

Original research publications and reputable reviews published mostly between 2015 and 2025 were given precedence; however, older seminal works that served as the field's intellectual underpinnings were also included. Unless they were historically relevant, non-peer-reviewed sources, abstracts without full text, and research with unclear methodology were removed.

2.3. Data Extraction and Synthesis

Experiments and plant species, explant type and regeneration pathway, culture media composition and physical culture conditions, sterilization and contamination management techniques, molecular regulators of regeneration, transformation or selection outcomes, and other topics were the main focus of the systematic extraction of information from a few chosen publications.

To highlight recurrent mechanistic principles, methodological consistencies, and sources of heterogeneity across research, the findings were qualitatively synthesized instead of conducting a quantitative meta-analysis. In order to facilitate cross-study comparison and the detection of trends influencing reproducibility and scalability, comparative tables summarizing exemplary protocols, environmental characteristics, and emerging transformation techniques were created.

2.4. Conceptual Framework

The review used an integrative framework that connected contemporary molecular and engineering-based techniques, such as morphogenic regulators, mobile RNAs, viral vectors, nanotechnology, and automation, with traditional regeneration concepts, such as hormonal balance, nutrient signaling, and cellular totipotency. In order to show how plant tissue culture evolved from empirical research to increasingly standardized and developmentally engineered systems, historical developments were interpreted alongside modern advancements.

2.5. Limitations of Review

Variability in experimental reporting among studies, especially with regard to medium composition, ambient circumstances, and quantitative regeneration metrics, hampered this evaluation, despite every effort to incorporate a wide and comprehensive body of research. Therefore, rather than recommending a universal protocol, the conclusions focused on conceptual convergence and procedural trends.

3. Core Techniques of Micropropagation

3.1. Traditional Stages

Plant tissue culture is typically conducted through four sequential phases (Figure 1): Phase I (establishment), which involves selecting and sterilizing explants to ensure aseptic cultures; Phase II (multiplication), where suitable growth regulators are used to induce rapid shoot proliferation; Phase III (rooting), which facilitates root development to prepare plantlets for soil transfer; and Phase IV (acclimatization), during which in vitro–grown plantlets adapt to ex vitro conditions, resulting in high transplantation success rates (Bhardwaj et al., 2025).

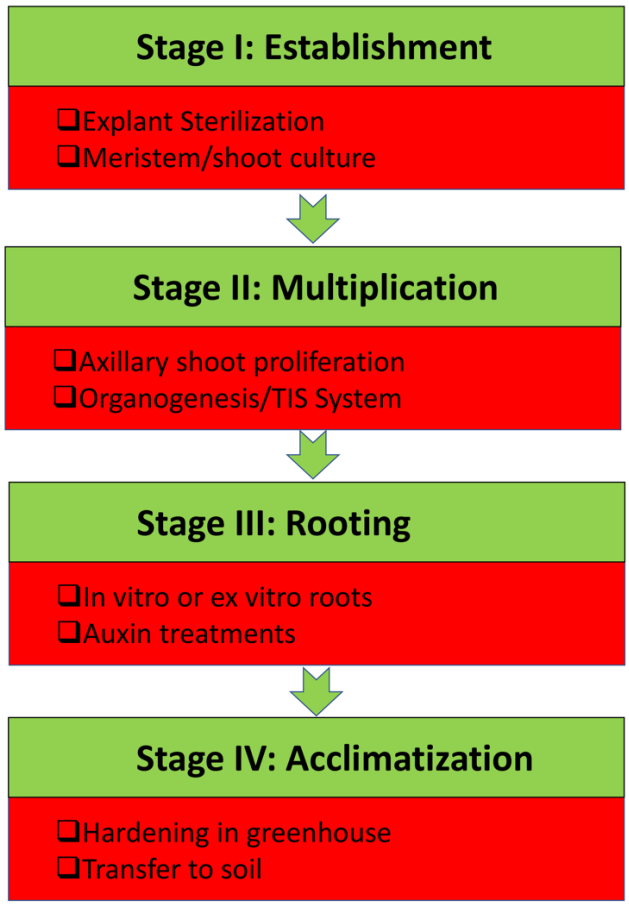

Figure 1. Acclimatization procedure.

3.2. Somatic embryogenesis and Organogenesis

3.2.1. Organogenesis

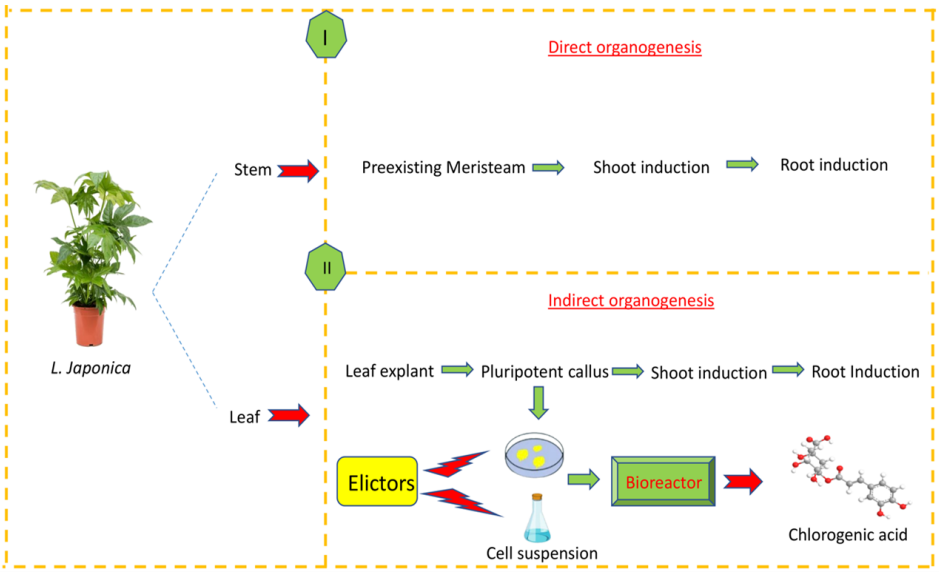

Figure 2. Schematic representation of direct and indirect organogenesis in plant tissue culture.

In organogenesis (Figure 2), certain plant explants develop callus tissue to create new plants. Organogenesis is the process by which some plant organs, such as shoots or roots, grow from cultured tissues and eventually create an entire plant. There are two ways that organogenesis can happen:Direct organogenesis, in which organs grow straight from the explant, and indirect organogenesis is the process by which organs grow from the explant's callus.

3.2.2. Somatic Embryogenesis

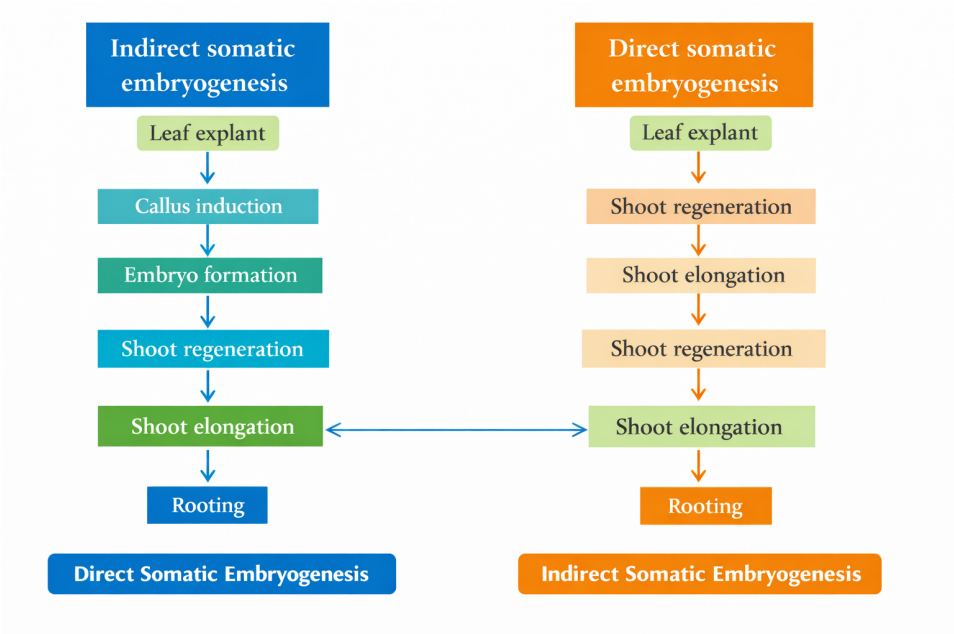

Figure 3. Comparative flowchart of direct and indirect somatic embryogenesis from leaf explants.

The process by which somatic cells in plants develop to create an embryo and finally an entire plant is known as somatic embryogenesis (Figure 3). In contrast to organogenesis, somatic embryogenesis is a polar process in which the plant develops both a shoot and a root system from a somatic embryo. Somatic embryogenesis can be done in two ways. Direct somatic embryogenesis is one of these techniques, in which somatic embryos develop from explants without developing a callus. Indirect somatic embryogenesis is the alternative technique, in which somatic embryos develop from a callus. In certain plants, somatic embryos develop directly without the need for a callus (Ravindra et al., 2025). Somatic embryogenesis (SE), the de novo production of embryos from somatic

plant cells, produces bipolar structures that mimic zygotic embryos and lack vascular connections with the initial explant tissue (Ramírez-Mosqueda, 2022; Von Arnold et al., 2002). It is an important morphogenetic pathway and a powerful biotechnological tool commonly used in large-scale clonal proliferation and genetic modification.SE can be caused by a variety of auxinic herbicides, typically at quite high concentrations, such as Dicamba, Picloram, and 2,4-Dichlorophenoxyacetic acid (2,4-D) (Ramírez-Mosqueda, 2022). Also putrescine and glycine exert the strongest positive effects on callus and embryogenesis, whereas glutamine and sorbitol also show significant supportive roles (Table 1).

For cells to develop embryogenic competence, they must first dedifferentiate. As demonstrated in carrot model systems, plant growth regulators, particularly auxins, usually start this essential phase. An embryogenic response can also be triggered by pH variations and exposure to specific heavy metals in addition to hormonal signals. One important early event connected to SE induction is the shift in cellular polarity that happens when plant growth regulators and other treatments promote asymmetric cell division that leads to embryo start (de Jong et al., 1993). Competent cells with regular ploidy and normal nuclear structure initiate endogenous auxin production through the TAA1–IPA pathway, resulting in localized auxin accumulation and a "organizer cell," which then divides peripherally to generate globular embryo structures surrounded by an epidermal layer (Smetana et al., 2019). However, exogenous auxin-induced systems are not always very effective since non-embryo-competent cells can also re-enter the cell cycle and replicate as callus tissue. As a result, two developmental paths of SE are identified: indirect somatic embryogenesis, in which callus formation comes before embryo development, and direct somatic embryogenesis, in which embryos form directly from separated cells without an intermediate callus phase (Ramírez-Mosqueda, 2022). Since not all plant cells possess morphogenic competence, it is especially challenging to pinpoint the regulatory elements governing this response.

Morphogenic regulators, such as WUSCHEL (WUS) and BABY BOOM (BBM), are essential for the molecular initiation and augmentation of SE and are commonly employed to improve SE-based genetic transformation systems (Zhang et al., 2025). However, the fact that CRISPR/Cas9-mediated deletion of the protein significantly reduces SE efficiency without affecting callus formation highlights LhBBM's special role in embryogenic reprogramming.

The fact that conditioned media from embryogenic cells promote development has long been acknowledged, indicating a potential function for secreted signaling molecules. These media contain active ingredients called Endo chitinases, arabinogalactan proteins, and lipochitooligosaccharides that support embryonic development and maintain embryogenic competence (Von Arnold et al., 2002). Additionally, embryogenic cells release proteins that limit excessive cell elongation in the presence of 2,4-D and cause the development of tiny embryogenic cells in previously on-embryogenic cultures, both of which contribute to the maintenance of embryogenic potential (Kreuger and Van Holst, 1993).

However, the embryogenic potential of cultures is greatly influenced by culture conditions When populations of tiny embryogenic cells are disturbed by frequent subculturing or by too high auxin concentrations, which promote elongation and loss of polarity, the embryogenic capability may be reduced. Thus, a complex interplay between hormonal cues, cellular polarity, secreted chemicals, and genetic regulators—all crucial for the induction and maintenance of somatic embryogenesis—controls the shift from somatic cell dedifferentiation to organized embryo development.

3.2.3. De novo organogenesis

"De novo organogenesis" is a method of plant regeneration that produces new organs without undergoing the somatic embryo stage. The plant regenerates through the differentiation of meristematic cells, which illustrates the innate ability of plant cells to differentiate into anything. In vitro or after plant injury, the method mostly results in adventitious roots and shoots growing in the plant tissue (Long et al., 2022). The complex process of de novo shoot organogenesis is regulated by cellular programming and hormonal communication. According to a number of scientists, this process usually occurs in three main stages, depending on how external plant growth regulators work. Cell dedifferentiation is the first stage, during which somatic cells acquire morphogenic potential. The cellular reaction to exogenous plant hormones is the subject of the second stage. In the second stage, competent cells generate shoot primordia in response to external plant hormones. Phytohormone-independent organ morphogenesis is the final stage, during which they began to transform their shoot structures into functional ones (Hnatuszko-Konka et al., 2021). Plants are the foundation of in vitro tissue culture techniques and provide significant developmental flexibility due to their remarkable capacity for regeneration.Moreover, both slr and cfr1-1 root-derived Calli exhibit wild-type levels of PLT gene expression. After being transferred to shoot induction medium (SIM), these Calli were capable of undergoing de novo shoot apical meristem (SAM) initiation (Shang et al., 2016). This implies that during callus development on callus induction medium (CIM), SLR/IAA14 and CFR1 are not necessary for gaining cellular pluripotency. Additionally, it suggests that these elements are not essential for the cytokinin-induced reprogramming of cell destiny that results in the commencement of SAM from scratch. A typical physiological condition seen in de novo-formed shoots during in vitro organogenesis is hyperhydricity (Polivanova & Bedarev, 2022). The primary cause of this illness is an imbalance between normal tissue growth and fast cell creation in vitro. Anatomically, hyperhydrated plants have palisade tissues that resemble spongy parenchyma because of an increase in cell volume and a decrease in the number of palisade parenchyma layers (Apóstolo & Llorente, 2000). In addition to morphological abnormalities, hyperhydricity is associated with a number of physiological and biochemical issues. Common indicators include extracellular hydration from fluid-filled intercellular gaps, poor cell adhesion, the accumulation of big starch granules in plastids, a lack of chlorophyll, and the growth of wider

intercellular spaces inside the mesophyll. Additionally, often seen are hypolignification, altered enzyme activity, disruptions in protein synthesis, and reduced epicuticular layer formation on leaf surfaces, all of which point to impaired metabolic control in affected tissues (Tian et al., 2015). Several strategies to lessen hyperhydricity during de novo organogenesis have been proposed.

4. Classical Conceptual Foundations of Regeneration

The remarkable ability of plant cells to resume developmental pathways is the primary impetus for regeneration. Steward et al. (1958) showed that even highly differentiated carrot cells may form ordered structures if given the proper conditions. Hormonal balance governs this reactivation. According to Skoog and Miller (1957), cytokinin–auxin ratios define the developmental axis between shoot and root organogenesis. These ideas are typically applicable to both gymnosperms and angiosperms, notwithstanding species-specific tuning.

Nutrients can function as morphogenic cues in addition to growth substrates. The Murashige and Skoog (1962) formulation continues to be the most widely used basal media in plant biotechnology. Hormone response and cell fate trajectories are impacted by changes in pH, macronutrient ratios, and micronutrient availability. Regeneration is equally influenced by the cell's internal physiological status. According to Ikeuchi et al. (2015), chromatin remodeling and transcriptional reactivation are crucial steps in transforming somatic cells into a capable state. Furthermore, Sugimoto et al. (2010) have demonstrated that wound signals initiate hormonal cascades and transcriptional rewiring that prime cells for regeneration. These findings bridge the gap between the conventional hormonal paradigm and contemporary molecular developments by showing that regeneration is a multi-scale, layered process rather than an evident hormone-response occurrence. Environmental cues control these developmental responses. By modifying the metabolic rate and hormonal sensitivity, the physical environment's structure, gas exchange, and light quality all influence morphogenic outcomes. Through the cryptochrome pathway (e.g., HY4/CRY1), blue light prevents hypocotyl extension and encourages compact seedling shape in Arabidopsis (Ahmad & Cashmore, 1993; Lin et al., 1996). Although results vary by species, adjusting blue-light percentages in controlled settings can enhance photosynthetic efficiency and chlorophyll measurements in a number of species (Chiang et al., 2020).

This conventional paradigm, which addresses chromatin flexibility, environmental modulation, nutritional signaling, and hormonal regulation, serves as the foundation for contemporary regeneration and editing procedures. Modern methods rely on these ideas with precise molecular control and designed developmental regulators, rather than abandoning them entirely. The technological environment around cultured tissues is essential to their operational manifestation; comprehending regeneration mechanisms is only one aspect of the situation. Exact control over sterilization and medium composition, as well as physical culture conditions, determine whether theoretical promise is translated into consistent results. The next part looks at how each of these components cooperates to regulate regeneration performance..

5. In Vitro System Technical Optimization

Optimizing in vitro systems heavily relies on adjusting the medium's composition to promote cell division, proliferation, and biomass accumulation. By supplying easily assimilated nitrogen and promoting metabolic activity, organic additions such amino acids (such as glutamine, glycine, and proline) have been demonstrated to improve callus induction and fresh weight, frequently enhancing embryogenic responses when compared to medium without these supplements. Their inclusion can promote morphogenesis and cell division in all species, highlighting their function as organic nitrogen sources that stimulate regeneration in optimal environments. Achieving high induction frequencies and healthy callus growth also requires adjusting the balance of basal nutrients, carbohydrates, and plant growth regulators (such auxins and cytokinins) according to the explant and species. Plackett-Burman and other systematic screening designs facilitate more effective medium formulations and lessen trial-and-error techniques in tissue culture protocols by identifying essential components and their relative effects on culture outcomes (Sidik et al., 2024).

5.1. Composition of Culture Media

According to recent research, callus induction and somatic embryogenesis are significantly impacted by the ratios of mineral ions in culture medium, particularly nitrogen forms (nitrate vs. ammonium), potassium, and micronutrients. Nitrate, ammonium , and potassium concentrations were found to be strongly positively correlated with explant survival, callogenesis, and embryo formation in principal component analyses; however, excessively high calcium and chloride may have a negative effect on morphogenic responses in some cultivars. One of the most important factors controlling morphogenetic potential in vitro is still nitrogen (Bollati et al., 2025). As shown in Table 2, MS medium is optimal for shoot induction and callus culture, NN for anther and pollen culture, B5 for cell suspensions and legumes, and DKW for woody and recalcitrant species (Murashige & Skoog, 1962; Nitsch & Nitsch, 1969; Gamborg et al., 1968; Driver & Kuniyuki, 1984). Morphogenesis and shoot quality are directly impacted by optimizing plant growth regulators (auxins, cytokinin) and solidifying agents (e.g., agar concentration). For instance, research on Gypsophila paniculata revealed that the combination of suitable nitrate/ammonium concentrations with particular growth retardants (such as paclobutrazol) reduces hyperhydricity and increases antioxidant enzyme activity, indicating that both nutrient salts and their

Table 1. Effects of Medium Components on Callus and Embryo Induction in *Allium cepa* Using Plackett-Burman Design (Keighobadi et al., 2020).

Medium Component	Effect on Callus Induction	Effect on Embryonic Callus Induction	Effect on Fresh Weight of Callus	Notes / Contribution (%)
Putrescine	Positive, highest effect; optimal 200 mg/L	Not specified	Not specified	Maximum positive contribution to callus production
Glutamine	Positive	Significant positive effect	High influence ($P \leq 0.05$)	Supports both callus and embryo induction
2,4-D	Positive overall; some reducing effect	Lowest contribution (8.33%)	Not specified	Key auxin for induction but variable effect
Glycine	Positive	Highest contribution (26.34%)	Highest contribution (31.4%)	Strong influence on both embryogenesis and callus weight
Silver nitrate	Not specified	Not specified	Not specified	Screened; effect not highlighted
Maltose	Not specified	Not specified	Not specified	Carbohydrate source tested
Sorbitol	Not specified	Not specified	Significant influence ($P \leq 0.05$)	Contributes to callus fresh weight
Sucrose	Not specified	Not specified	Lowest contribution (6.7%)	Minor influence on fresh weight

Note: The table highlights key medium components influencing callus and embryogenic induction in *Allium cepa*. Putrescine and glycine emerged as primary enhancers, while 2,4-D showed variable effects. Carbohydrates mainly affected biomass, and other components had minor or inconsistent contributions, underscoring the importance of systematic screening in tissue culture.

interaction with hormones modify physiological responses in culture (Mohamed et al., 2023). Basal media, which are not passive substrates, have also had an impact on developmental response. The MS formulation, which was first altered for tobacco callus, is still present in the default scaffold (Murashige & Skoog, 1962). However, methodological mistakes are common. Sources of carbon act as fuels and messages. For instance, de Paiva Neto & Otoni (2003) showed that while autoclaved glucose may result in inhibitory byproducts, sucrose is frequently permissive. Ion ratios were linked to organogenic pathways by Pasternak et al. (2002), indicating that nitrogen balance and form also affect morphology.

Table 2. Media/chemical parameters that modulate morphogenesis.

Media	Composition Highlights	Applications / Plant Types	Reference
MS (Murashige and Skoog)	High macronutrients (N, K, Ca, Mg), micronutrients (Fe, Mn), vitamins, growth regulators	Shoot induction, callus formation, micropropagation of monocots and dicots	Murashige and Skoog (1962)
NN (Nitsch and Nitsch)	Lower salts than MS, supports gametophytic development	Anther/pollen culture, haploid/doubled haploid production	Nitsch and Nitsch (1969)
B5	Lower salts, includes thiamine, nicotinic acid, pyridoxine	Cell suspensions, callus culture, legumes like soybean and alfalfa	Gamborg et al. (1968)
DKW (Driver and Kuniyuki Walnut)	High sulfate, modified ratio	Woody plants, nut-bearing trees, recalcitrant species	Driver and Kuniyuki (1984)
WPM (Woody Plant Medium)	Reduced supplemented with auxins and cytokinins	In vitro culture of woody species	Li et al. (2025)

5.2. Environment of Physical Culture

In tissue culture, physical elements have a significant impact on how cells react to chemical cues, which in turn affects growth and shape. For example, light quality is important because blue light prevents shoot elongation in Arabidopsis (Ahmad & Cashmore, 1993), while other crops' photosynthetic activity, chlorophyll content, and general morphology are improved when artificial light is adjusted to 25–35% blue (Chiang et al., 2020). In a similar way, controlling humidity and gas exchange are essential for avoiding physiological illnesses; high-vent lids and controlled relative humidity lessen hyperhydricity and issues with water uptake in ornamentals (Zobayed et al., 2001; Matuszkiewicz et al., 2019; Vollmer et al., 2024; Miranda et al., 2024). As shown in sugarcane employing PET-based bioreactors, temporary immersion systems (TIS), which alternate brief liquid contact with aerated intervals, further boost growth and decrease vitrification (Fuentes-Torres et al., 2025) and in eucalyptus across multiple studies (Souza et al., 2020). These optimized physical parameters light spectrum, gas exchange, humidity, and immersion frequency improve shoot quality, boost biomass, and reduce physiological abnormalities, underscoring the significance of combining chemical and hormonal cues with environmental control in in vitro culture systems.

6. Novel Transformation Approaches in Tissue Culture

Traditional tissue culture is the basic platform for regeneration; however, it seems to be a major bottleneck due to its reliance on regeneration that is specific to a species and genotype. In order to reposition or totally eliminate tissue culture phases, new developments have begun to use mobile genetic signals, built-in developmental circuits, and alternative delivery techniques. These methods show a shift away from requiring explants to undergo lengthy dedifferentiation by either promoting regeneration in planta or administering modifying reagents through transient, non-regenerative routes.

6.1. Developmental Regulators Induce Meristem

The advent of virus-mediated and in planta genome editing tools marks a trend away from traditional tissue-culture-dependent transformation and toward more adaptable and heritable editing systems, while their dependability varies by species and delivery setting. For example, using FT-sgRNA mobility with a CLCrV vector showed that systemic movement of guide RNAs can promote germline editing, demonstrating a conceptual progress in obtaining heritable alterations without stable transgene integration (Lei et al., 2021). Earlier TRV-based VIGE platforms demonstrated the potential of transitory viral administration for long-term and heritable genome alterations, implying that viral persistence can continue editing activity beyond initial infection (Ali et al., 2015). Subsequent applications into monocots, such as wheat and maize, employing BSMV and comparable systems increased host applicability while also highlighting species-specific limits in efficiency and transmission (Hu et al., 2019; Li et al., 2021). Parallel investigations into other delivery pathways, such as nanoparticle-assisted pollen transformation, suggest attempts to avoid genotype reliance, however repeatability and mechanistic clarity remain debatable (Wang et al., 2022). In a related trend, temporary induction of developmental regulators has demonstrated that reprogramming meristematic competence can permit heritable edits directly in plants, minimizing dependency on callus regeneration (Ma her et al., 2020). Similarly, the use of morphogenic regulators such as WUS2 and BBM under controlled promoters has increased transformation efficiency in recalcitrant cereals while reducing tissue culture bottlenecks, but their optimization is still heavily dependent on species and promoter context (Lowe et al., 2018). Mobile RNA-based genome editing strategies, particularly FT-gRNA fusions tested in Nicotiana benthamiana and Arabidopsis, show how endogenous RNA mobility pathways can be used to promote systemic spread of guide RNAs and enable germline transmission of edits without stable transformation, though efficiency remains dependent on host physiology and vector compatibility (Ellison et al., 2020; Lei et al., 2021). Similarly, the use of viral delivery systems such as TRV and BSMV has broadened the scope of gRNA transport in Cas9-expressing plants, demonstrating cross-species applicability in dicots as well as cereals such as wheat and maize, but with variation in editing consistency and inheritance based on viral host range and tissue tropism (Ali et al., 2015; Hu et al., 2019; Li et al., 2021). Nanoparticle-mediated delivery approaches, including magnetic nanoparticle-DNA complexes introduced through maize pollen, represent further attempts to bypass genotype-dependent regeneration and tissue culture constraints, though questions remain about reproducibility and mechanistic integration into germline cells (Vejlupková et al., 2020; Wang et al., 2022). In parallel, CENH3-mediated systems extend this conceptual framework to dicots and wheat embryos, highlighting the strategic use of intrinsic developmental pathways to accelerate heritable editing with less reliance on callus-based regeneration. In maize, haploid induction–mediated genome editing (HI-Edit) and inducible meristem genome editing (IMGE) show how developmental reprogramming can be coupled with editing machinery to quickly generate edited doubled haploids (Wang et al., 2019; Kelliher et al., 2019).

7. Physiology of In Vitro Stress and Tolerance Selection

Long before field-based screening is feasible, tissue culture offers a highly controlled setting for applying abiotic stressors, allowing for the discovery and selection of variations with improved resistance. By carefully regulating the degree, duration, and developmental timing of stress exposure, in vitro selection, in contrast to traditional breeding, directly correlates cellular and physiological responses to restorative outcomes. To enable practical selection, this platform combines somaclonal variation, metabolic adaptability, and physiological stress signaling. Exposing regenerated Calli or cell suspensions to polyethylene glycol (PEG) or sodium chloride (NaCl) to mimic dehydration or salinity stress is a commonly used technique for osmotic and salinity tolerance. For example, salt-tolerant lines of Oryza sativa were produced via NaCl-based selection, and the offspring consistently displayed heritable tolerance (Senadhira et al., 2002).Somaclonal variation, which was formerly believed to be an undesirable byproduct of in vitro development, is now recognized as a useful source of novel genomic and epigenetic diversity. In tissue culture, which may also mimic biotic stress, resistant lines can be chosen using purified toxins or pathogen culture filtrates. The introduction of *Helminthosporium oryzae* culture filtrates into rice callus media allowed for the selection of toxin-resistant Calli and the regeneration of plants resistant to brown spots, thereby providing a pre-field screening method to identify early detoxification and defense mechanisms (Vidhyasekaran et al., 1990). The concept that in vitro culture inherently generates heritable genetic variation was first proposed by Larkin and Scowcroft (1981), who highlighted that regenerated plants frequently carry novel traits even without intentional mutagenesis. By combining stress selection with somaclonal variation, advantageous resistant phenotypes can be efficiently isolated from large callus populations while maintaining regeneration potential.

7.1. Biochemical and Physiological Selection Markers

Certain tissues show detectable physiological and biochemical alterations in addition to survival. Tolerant Calli frequently exhibit improved proline buildup, decreased electrolyte leakage, and heightened activity of antioxidant enzymes involving peroxidase and superoxide dismutase when exposed to NaCl or PEG stress. In rice, increased antioxidant capacity is closely associated with improved drought tolerance, as shown by elevated activities of antioxidant enzymes under water deficit (Sharma & Dubey, 2005; Guo et al., 2006). In vitro stress treatments such as NaCl and PEG have been used to select tolerant plant variants. Stepwise NaCl application in rice callus produced stable, heritable salt-tolerant lines (Senadhira et al., 2002), while PEG selection generated drought-tolerant regenerants (Bhattacharya et al., 1994). Long-term culture induces somaclonal variation, yielding novel stress-adapted phenotypes (Larkin & Scowcroft, 1981). Physiological markers proline accumulation, enhanced antioxidant enzyme activity, and reduced electrolyte leakage effectively indicate adaptive responses (Sairam et al., 2002). These biochemical and physiological indicators provide rapid, reliable screening tools, demonstrating that stress-based selection in tissue culture can efficiently generate resilient plant lines for further breeding or research applications.

8. Integration of Modern Tools and Genome Editing

Recent developments in genome editing and molecular biology have significantly increased the applications of plant tissue culture. Tissue culture offers a good environment for both genetic modification and the regeneration of the modified tissues. The capacity to accurately adjust CRISPR/Cas and other editing techniques has increased nutritional quality, disease resistance, and stress tolerance (Bhardwaj et al., 2025). Because of its antimicrobial properties, nanotechnology increases the capacity to nourish cells, promote development and stress tolerance, and reduce microbial contamination, all of which improve plant tissue culture. Nanoparticles can be used to transfer genes and growth regulators that promote germination and regeneration. Without compromising morphogenesis, metal nanoparticles especially silver nanoparticles have potent antibacterial properties and successfully lower in vitro contamination (Cuong et al., 2023; Balamurugan et al., 2024). Callus development and organogenesis are encouraged by nanoparticles including ZnO, Fe_3O_4, and silica, which enhance micronutrient uptake, chlorophyll production, and antioxidant activity (Rai et al., 2018). Furthermore, mesoporous silica nanoparticles and carbon nanotubes provide effective transport of genome-editing and DNA reagents, providing substitutes for traditional transformation techniques (Torney et al., 2007; Demirer et al., 2019). However, to prevent phytotoxic effects, careful dose optimization is necessary. Proteomics, metabolomics, transcriptomics, and genomics are examples of omics technologies that provide a thorough understanding of the molecular pathways controlling plant growth and development in tissue culture systems (Department of Fruit Science, College of Horticulture, Junagadh Agricultural University, Junagadh, Gujarat, India, et al., 2022).Tissue culture is a crucial area of agriculture since it aids in the development of genetically modified crops with desired characteristics, such as resistance to drought, herbicides, and insects. Large-scale preservation and propagation of endangered plant species is another purpose for this technique. Finally, technological developments in transcriptomics, metabolomics, and genomics enable researchers to comprehend the basic ideas underlying plant growth and development in a tissue culture (Department of Fruit Science, College of Horticulture, Junagadh Agricultural University, Junagadh, Gujarat, India et al., 2022).

8.1. Automation, robotics and digital monitoring

Commercial micropropagation labs are fast evolving due to automation, robotics, and online monitoring technologies that increase uniformity by decreasing the need for human labor. Robots reduce the possibility of human error and contamination by doing repeated processes like cutting, transfer, and culturing precisely and accurately. Subculture can be optimized by online growth monitoring made possible by advancements in sensor technology and artificial intelligence-based image analysis systems (Bhardwaj et al., 2025).

8.2. Application of Artificial Intelligence in Plant Tissue Culture

To increase productivity and precision, plant tissue culture research has included artificial intelligence (AI), artificial neural networks, machine learning, and deep learning. For example, in the case of the Cannabis sativa species, the employment of AI technologies is crucial due to the lack of scientific understanding and uneven regeneration processes (Ravindra B. Malabadi et al., 2025). The intricacy, unpredictability, and empirical character of plant tissue culture systems have made artificial intelligence (AI) a potent tool. Non-linear interactions between culture media components, plant growth regulators, environmental conditions, and genotype-specific responses are increasingly being modelled using machine learning (ML), artificial neural networks (ANNs), deep learning (DL), and hybrid optimization techniques (Gago et al., 2010; Hesami et al., 2017). When it comes to forecasting shoot proliferation, rooting efficiency, somatic embryogenesis, and biomass buildup, AI-driven models perform better than conventional statistical methods (Khodadadi et al., 2020; Arab et al., 2021). By enabling real-time monitoring of explant growth, contaminant detection, and physiological diseases such hyperhydricity, image-based deep learning algorithms lessen human error and labor dependency (Nezami-Alanagh et al., 2021; Singh et al., 2023).Fuzzy logic systems and genetic algorithms find the best parameter combinations with the fewest number of experimental repetitions, further optimizing media composition and culture conditions (Jamshidi et al., 2016; Niazian & Niedbała, 2020).For medicinal and resistant plants, such as woody perennials and Cannabis sativa,

where regeneration methods are still irregular, AI integration is very beneficial (Malabadi et al., 2025; Hesami & Jones, 2020).All things considered, plant tissue culture is becoming a predictive, automated, and scalable biotechnology platform thanks to AI-driven decision support systems.

9. Visions for the Future

It is anticipated that standardization, repeatability, and methodological transparency will be given top priority in future plant tissue culture research. Cross-laboratory comparability is still hampered by the ongoing variation in growth regulators, media formulations, and environmental factors. Thus, one of the main goals for the upcoming years should be to establish community-wide principles and minimal reporting criteria (Conner & Jacobs, 2025). These frameworks would make it easier to harmonize protocols, allow for strong meta-analyses, and increase the dependability of investigations on regeneration and transformation in a variety of species. The next stage of development will likewise be defined by technological integration It is anticipated that the use of automated culture platforms, real-time phenotyping devices, and temporary immersion bioreactors (TIBs) would increase, decreasing manual variability and enhancing scalability. Improved regulation of environmental factors, including gas exchange, oxygen transfer, and light spectra, will bring tissue culture even closer to industrial bioprocessing standards (Murthy et al., 2023; Titova et al., 2024; Thanonkeo et al., 2024). It is anticipated that these developments will facilitate commercial-scale applications, genome editing pipelines, and high-throughput propagation. Promising approaches to improve transformation efficiency and increase genotype responsiveness include systemic RNA administration, spatiotemporally regulated meristem induction, and optimised expression of morphogenic regulators (Ma her et al., 2020). The length of time and reliance on prolonged in vitro stages may reduce as these instruments develop, reducing somaclonal variance and quickening breeding cycles. All things considered, future processes are probably going to combine in vitro selection, in planta regeneration, and precise genome editing into unified, modular platforms. Plant tissue culture will thereby transition from a stand-alone technique to a strategically optimised part of larger genomic and developmental engineering systems, allowing for the quick, iterative, and genotype-flexible generation of traits.

10. Conclusion

With advancements like meristem induction, RNA-based delivery, and developmental regulators assisting in overcoming genotype constraints, plant tissue culture has emerged as a key component of contemporary biotechnology. While bioreactors, automated phenotyping, and temporary immersion systems increase scalability and repeatability across labs, some transformation techniques now completely exclude in vitro culture.

Moving forward, emphasis should be placed on integrating molecular delivery, developmental regulation, and automated culture systems into cohesive, modular workflows to improve repeatability and comparability, standardized procedures and reporting structures must be adopted. To reduce variability and boost efficiency, it will also be critical to use real-time phenotyping and scalable bioreactor systems. To speed up the development of traits, it is also necessary to investigate precise spatiotemporal regulation of cellular reprogramming. When combined, these tactics will establish plant tissue culture as a flexible and effective part of larger genomic and biotechnological platforms.

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