AMERICAN JOURNAL OF BIOLOGY AND NATURAL SCIENCES
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Article

Plant Tissue Culture

Toleen Khalied Ibrahim*[1], Widyan Alwan Khalaf[2], Husam Nafea Shakir[3]
1,3. Field Crops, College of Agriculture, Samarra University, Samarra, Iraq
2. Food Science College of Agriculture, Samarra University, Samarra, Iraq
*Correspondance: toleen.k@uosamarra.edu.iq

Abstract: For controlled, large-scale plant multiplication and in vitro regeneration, plant tissue culture (PTC) is an essential tool in plant biotechnology. This review focuses on totipotency, the inherent ability of plant cells to regenerate into full plants, and the principles, techniques, and applications of plant tissue culture. Improvements in in vitro regeneration systems have enhanced the efficacy of micropropagation, organogenesis, and somatic embryogenesis through the optimization of culture media, plant growth regulators, and ambient conditions. Methods like callus culture, suspension culture, meristem culture, and protoplast culture have been widely utilized in research, industry, and agriculture. Plant tissue culture plays a crucial role in improving crops, producing plants without diseases, conserving genetic resources, and synthesizing secondary metabolites. In addition, it provides a useful framework for testing whether plants can withstand biotic and abiotic stresses. Although it has many advantages, it still has certain problems, like somaclonal fluctuation, expensive running expenses, and technical limitations in large-scale applications. In conclusion, plant tissue culture is an important and rapidly expanding field that has great promise for the advancement of biotechnology and environmentally friendly farming practices in the years to come.

Keywords: Plant Tissue Culture, Somatic Embryogenesis, Crop Improvement, Organogenesis

Citation: Ibrahim, T. K., Khalaf, W. A., Shakir, H. N. Plant Tissue Culture. American Journal of Biology and Natural Sciences 2026, 3(4), 53-60.

Received: 24th Feb 2025
Revised: 10th Mar 2025
Accepted: 20th Mar 2026
Published: 13th Apr 2026

1. Introduction

Regenerating and cultivating plant tissues are cornerstones of plant biotechnology, which encompasses a wide range of academic and commercial disciplines. Thanks to the rapid development of specialized equipment and strategies, our understanding of the principles underlying in vitro plant regeneration has made significant progress in recent years [1]. Various regeneration methods are encompassed in plant tissue culture. Research in the field of cell and tissue culture is frequently the bedrock upon which new techniques are developed and used [2]. A number of factors determine how well plant tissue culture (PTC) works. These include having the right nutrients, competent cells that can undergo targeted epigenetic and molecular reprogramming to induce stem cells, and the ability to precisely control the distribution and synthesis of endogenous hormones both spatially and temporally [3].

Haberlandt (1854-1945) introduced plant totipotency in 1902, building on Schleiden (1838) and Schwann (1839)'s cell theory [4]. In Graz, Austria, Gottlieb Haberlandt pioneered in vitro growth of isolated somatic cells of higher plants [5]. He began his research in 1898 and reported his findings in 1902 (Haberlandt, 1902) [4]. Haberlandt suggested that cultivating isolated somatic cells may yield whole plants. For over 50 years, there was no experimental evidence to support the theory [6]. Steward proved that plant cells are totipotent in 1958 when he successfully grew new plants from carrot segments of differentiated secondary phloem (Steward et al. 1958) [7]. In1902, Gottlieb Haberlandt achieved a first in tissue culture by cultivating totipotent mesophyll cells. Breeding initiatives, pharmaceutical development, and genetic biodiversity conservation have all made use of tissue culture [8]. Plant tissue culture was pioneered by Golliob Haberlandt of Germany. He worked in the area for a period, eventually producing palisade tissue on knob's salt solution [9]. Embryos were grown in vitro on mineral salt sugar solution by Hanning (1904), who worked in subsequent years. This was a turning point in the history of embryo cultivation [4]. In the 1950s, the orchid business made extensive use of tissue culture. The first somatic hybrid of Nicotiana gluca and N. langschorffii was created in 1972 by Carlson et al. through protoplast fusion [5].

Plant tissue culture is a valuable approach for producing metabolites and phytoconstituents, which are hard to replenish and can help conserve species from extinction. When inductive circumstances are met, differentiated somatic cells can become totipotent and generate SEs similar to zygotic embryogenesis [10]. This technique swiftly multiplies tiny plant pieces (explants) into thousands of seedlings, in contrast to more traditional techniques of propagation. Numerous economically significant products have been derived from decorative plants, vegetables, fruits, medicinal plants, woody plants, and conifers by the application of in vitro tissue culture techniques [11]. One appealing alternative to conventional plantation methods is large-scale plant tissue culture, which provides a controlled supply of nutrients (such as glucose) regardless of the plants' availability. A better knowledge of plant-oriented compounds and secondary metabolites from economically important plants has led to an increase in the utilization of plant culture. Thanks to recent developments, plant tissue culture is now a vital instrument for modern agriculture and the advancement of agricultural sciences [12].

Innovative methods employing data-driven optimization of culture medium composition yield significant results. However, this communication demonstrates that the strategic application of existing knowledge in classical plant physiology can lead to relatively swift clarification and substantial enhancement of culture techniques [7].

2. Importance of Plant Tissue Culture

For large-scale plant multiplication and other biotechnology applications in plant improvement, plant tissue culture is essential. It is also commonly used in basic research in molecular biology, biochemistry, and plant biology. The world's population is concerning at eight billion. Along with climate change, one of the biggest issues facing humanity today is food and products, the majority of which are plant-based [13]. Climate changes brought on by global warming have an impact on agriculture globally. Environmental stress, drought, extreme heat or cold, floods, salinity, and toxic substances are examples of this. Soil and environmental conditions are impacted by the buildup of toxins and chemical elutes released into the environment by manufacturing processors. Agricultural output and food security are decreased by the decline and scarcity of adequate agricultural land as well as the reduction in plant growth and development caused by environmental stressors [14, 15].

Over the past 40 years, there has been an increase in research on plant stress, with an emphasis on pest outbreaks, water scarcity, extreme temperatures, salt, exposure to dangerous substances, insufficient or excessive radiation, and plant infection with pathogens [16]. To produce better cultivars, plants must be screened for biotic and abiotic stress conditions. Most plant stress screening studies are carried out in the field, which is dangerous due to unexpected environmental factors and difficult to maintain both financially and physically [17].

Plant tissue culture is an excellent and economical method of screening plants for biotic and abiotic stresses. Gottlieb Haberlandt's early 20th-century ideas and Schwann and Schleiden's (1838) cell theory serve as the foundation for in vitro plant cell and tissue cultivation. The "totipotency" or "total potential" of cells is used in in vitro plant tissue cultivation. Every cell has the potential to develop into a plant under the correct circumstances. The capacity of fully functional plant components to dedifferentiate and redifferentiate into ordered tissue, structure, and organism is known as totipotency [18]. When given the right nutrients and ideal growing conditions, plant cells or tissues can develop into whole plants in an in vitro sterile environment. More effective screening for desired characteristics is made possible by in vitro plant culture under specified culture media composition with the ability to apply modifications without external environmental effect while maintaining control environment parameters. This assesses tolerance to specific substances, such as antibiotics and toxins. With no impact on the environment, in vitro selection under selection pressure can cut down on selection time and expense [19]. In vitro stress assessment enhances comprehension and results while supporting field selection. In order to understand and support field evaluation, it can also serve as an early evaluation platform. Like any other technique, in vitro stress tolerance screening has its limits. The biggest challenge is developing suitable tissue culture methods for certain plant species. The lack of correlation between the tolerance systems of whole plants and cultured cells and tissues is another problem. Results may be impacted by epigenetic adaptation brought on by the in vitro culture of non-tolerant cells [20,21].

3. Totipotency

The idea of totipotentiality, the capacity of a single plant cell to express the entire genome through cell division, is fundamental to tissue culture technology. An intriguing and unique feature rooted in the developmental program of plants is the totipotency of somatic plant cells [22]. The ideal setting for this demonstration would be an in vitro system that allows somatic plant cells to reassume their totipotency and undergo somatic embryogenesis, the process by which they can develop into embryos. This sets in motion the process of embryogenic development, which involves reprogramming plant cells. This leads to the production of somatic embryos[23]. Nutrient supply, carbohydrate source, medium pH, suitable temperature, and gaseous and liquid environments are all part of these requirements. In a controlled environment, the carbohydrate supply is made available to the culture of transplants on a specified nutrient medium, allowing them to develop and multiply [24]. For plant or explant somatic cells to be able to receive an exogenous or endogenous signal in an in vitro setting, at least one of them must be competent to do so [19,25].

4. Plant Tissue Culture: Factors influencing in vitro Cultures

An other critical factor that influences the development and function of plant growth regulators is the pH of the nutritional medium, which should be 5.8 [26]. The function of plant growth regulators (PGRs) as signal molecules and controllers of plant development and growth is crucial, an endogenous auxin pulse is among the initial cues that trigger somatic embryogenesis. Plants primarily synthesize auxins and cytokinins to regulate and coordinate cell division and differentiation [27]. Auxins promote root growth at greater concentrations and cytokinin promotes shoot formation at lower values. Nevertheless, the specificity of cellular responses to these rather broad stress stimuli can be influenced by endogenous PGR levels [28]. Plant cells may create large amounts of indole-3-acetic acid (IAA), the native auxin, in addition to the absolutely necessary exogenous auxins for sustained development in in vitro conditions. Sometimes, when auxin and cytokinin levels are balanced, a lump of cells called a callus forms [1].

5. Techniques for plant tissue culture

A variety of techniques exist for plant tissue culture. During organogenesis, organ development may proceed directly from meristems or indirectly from dedifferentiated cells (callus). The resulting cultures can subsequently be employed for mass plant production (micropropagation) or for the development of specialized organs (e.g., roots in hairy root culture) [29].

5.1 Organogenesis

Organogenesis refers to the formation of plant organs from designated tissue to cultivate entire plants. It is distinguished by its polarity, signifying that a single aerial organ or root is detached, resulting in the formation of a new, full plant. Organogenesis can occur either directly, where the organogenic stalk is generated from the explants, or indirectly, when the organogenic process arises from a previously formed callus in the initial explants [30].

5.2 Somatic Embryogenesis

To create a complete plant, a process known as somatic embryogenesis is utilized. This process involves the creation of embryos from somatic plant cells, which are any cells that do not produce reproductive cells. This process is a polar process, in contrast to organogenesis, in which the somatic embryo gives rise to the aerial structures and roots of plants. Organogenesis is the opposite of this process. It is possible to classify the process as either direct or indirect [30], depending on whether it begins with the initial explants or beginning with callus that has already been generated.

There are Four categories of tissue culturing

a-Callus Cultivation

The callus forms on explants after a few weeks of growth in a hormone-supplemented growth media; it is an undifferentiated mass of tissue. It is well-known that de-differentiation or re-differentiation is the process via which calluses are formed. The induction and development of calluses are facilitated by the use of certain growth hormones. Kinetin, 2,4-D, and NAA all worked together to improve callus induction and growth in Cephaelis ipecacuanha. Efficient regeneration of new plants from callus is made possible by organogenesis [29,31].

b-Suspension Culture

To create in vitro suspension cultures, friable calli are cultivated in a liquid solution inside a suitable container that is occasionally shaken to preserve a free cell suspension. Conical flasks are used because of their large surface area, which promotes continuous gas exchange and liquid medium retention. Batch and continuous cultures are the two types of suspension cultures. A portion of the initial cell suspension is removed and subcultured onto new media in batch cultures on a regular basis. In continuous cultures, extra cell suspensions are removed and new media is regularly added to the current culture. Secondary metabolites are commonly produced on a big scale using suspension cultures. Chemostat bioreactors are made especially for continuous, large-scale cultivation [12,32,33].

c-Meristematic Culture

The culture of small excised shoot apices, each containing an apical meristematic dome with one or two leaf primordia. Generally, the shoot apex is formed to generate a solitary shoot [34]

d-Protoplast Cultivation

Plant cells that have had their cell walls digested or mechanically removed are called protoplasts. Plant cells undergo protoplast formation when their plasma membranes recede from the cell wall as a result of water efflux when immersed in a hypertonic solution. Now, mechanical techniques or enzymatic digestion using pectinase and cellulose can remove the cell wall [35].

e-**Cultivation of Shoot Tips**

Shoot tip culture, which is used for meristem cultures and contains several leaf primordia, is obtained from excised shoot tips or buds that are larger than the shoot apices. It is common practice to nurture these shoot apices in order to generate several shoots [36].

f-Culture of Lateral Bud Nodes

Lateral bud node culture involves the cultivation of a small segment of stem tissue that contains one or many nodes. Each bud is nurtured to yield a solitary shoot [37].

g-Isolated Root Culture

In isolated root culture, a branching root system can be produced by cultivating roots that are unattached to shoots [12,38].

h-Embryo Cultivation

Embryo culture involves the in vitro cultivation of fertilized or unfertilized zygotic embryos extracted from mature seeds or fruits till the production of seedlings. Embryo culture differs from somatic embryogenesis [30].

6. Commercial Applications of Plant Tissue Culture

Plant tissue culture (PTC) has achieved great strides in the last hundred years, thanks to the development of new techniques including somatic embryogenesis. Relying on this method, the economic potential of medicinal and agricultural plants can be fully realized. Micropropagation, somaclonal variety, hybridization, genetic transformation, haploid culture, disease eradication, and germplasm preservation are all assisted by PTC, which is essential in plant breeding, agriculture, and industry. Important subjects include media amendment utilizing biotic and abiotic elicitors, immobilization of precursors, synthesis and enrichment of phytocompounds in bioreactors, and industrial production [39]. With the PTC's mass-production method of commercially viable plant or plant component production under controlled conditions, the overexploitation of medicinally relevant plants can be reduced as well [40].

Plant tissue culture has several uses due to plant cell behavior, pathogen-free plants, clonal multiplication, product manufacturing, germplasm storage, and plant modification[41].

Genetically engineered tissue culture-based plants allow researchers to study gene regulation in transgenic species. Tissue culture has several more purposes in agricultural research, plant biotechnology, and applied plant science [42]. Plant tissue culture relies on the convenience and harvestability of growing cloned plants as suspended cells.[43] This field has led theoretical and practical plant research because it is necessary to develop genetically modified cells from transgenic plants and somatic haploid embryos[44].Commercial production for potting, florist themes, and landscape uses plant mestem and shoot culture to make similar explants. This helps save that rare, endangered species. Testing for herbicide resistance, stress tolerance, and more using cells, tissue culture, or explants is more efficient than cultivating plants from seed[45]. Explants are mass-produced in liquid culture to produce biopharmaceuticals, secondary metabolites, and recombinant proteins. Helpful when regenerating hybrids or fusing protoplasts to develop related species. In plant tissue culture, cross-pollination between closely related species is researched to rescue endangered species[46].

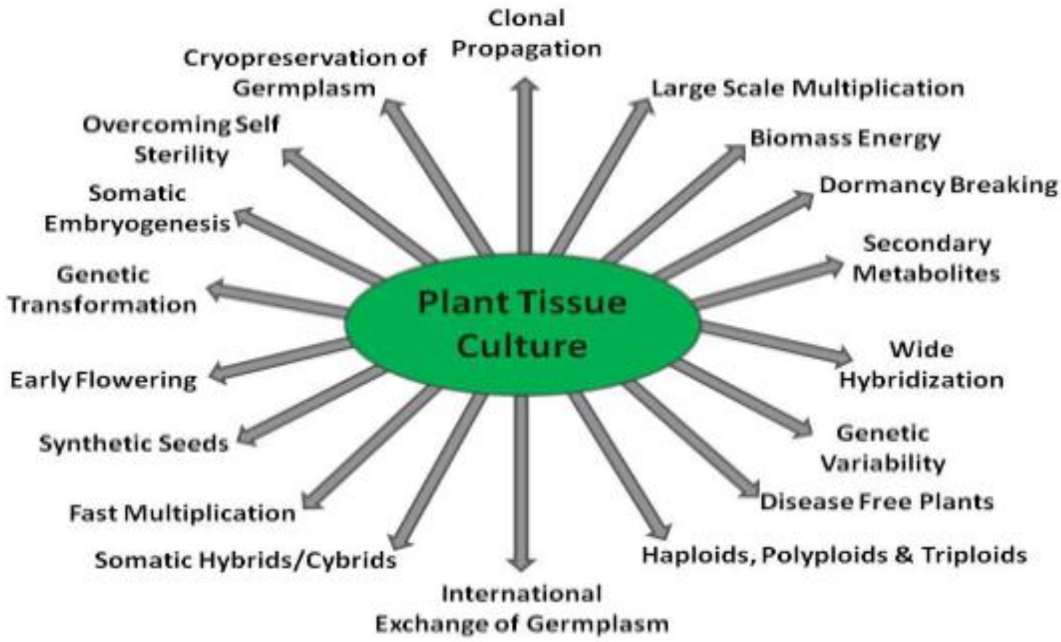

Figure 1. Different uses of Plant tissue culture[46]

7. Limitations of Plant Tissue Culture

Some of the problems with plant tissue culture methods include aseptic conditions, product removal, and continuous operation [47]. Due of these constraints, it seems like a handful of culture systems could become commercially viable. There should ideally be no variation during the asexual process of plant cell development in vitro and regeneration into complete plants, which entails merely mitotic cell division. The perfect situation would be for plants that are genetically identical to reproduce by clonal means. That is why spontaneous, unplanned variance in cultural production is both unexpected and, generally speaking, bad. Low levels of secondary metabolites and somaclonal variance in clone creation are to blame for this. Regardless of these negative outcomes, its value in enhancing crops by creating new varieties is well-known. In vitro tissue culture cultivation also has additional problems, such as the high cost of culture material, electricity, and labor [48]. The main problem with using plant tissue culture methods to make active metabolites is that there are somaclonal differences; these can happen in in vitro propagation, commercial phytochemical synthesis, or with genetically modified plants, and they can have major economic effects [49]. Given the complexity of the process and the number of variables and signaling molecules involved, reprogramming plant somatic cells in vitro to undergo somatic embryogenesis is no easy feat [50].

8. Conclusion

Recent advances in plant biotechnology have led to the development of plant tissue culture as a potent and adaptable tool with important implications for farming, manufacturing, and academia. Its capacity to take advantage of cellular totipotency allows for the creation of disease-free, genetically homogeneous plants, fast plant regeneration, and large-scale multiplication. Crop enhancement efforts, conservation strategies, and the production of valuable phytochemicals have all been increased by the development of various in vitro procedures. Still, it doesn't have broad commercial use due to issues including somaclonal fluctuation, contamination hazards, and expensive production costs. Despite these caveats, tissue culture is poised to become more efficient and find more uses as a result of ongoing developments in methodology and integration with current technology. Hence, plant tissue culture is still an intriguing way to tackle world problems including food insecurity, environmental stress, and sustainable agriculture.

REFERENCES

[1] T. P. Pasternak and D. Steinmacher, “Plant Growth Regulation in Cell and Tissue Culture In Vitro,” *Plants*, vol. 13, no. 2, p. 327, 2024, doi: 10.3390/plants13020327.

[2] V. M. Loyola-Vargas and N. Ochoa-Alejo, “An introduction to plant tissue culture: advances and perspectives,” in *Plant Cell Culture Protocols*, Jul. 2018, pp. 3–13.

[3] R. B. Malabadi, R. K. Chalannavar, and K. P. Kolkar, “Plant cell totipotency: Plant tissue culture applications—An updated review,” *World Journal of Advanced Engineering Technology and Sciences*, vol. 16, no. 02, pp. 112–135, 2025.

[4] L. P. Tang, X. S. Zhang, and Y. H. Su, “Regulation of cell reprogramming by auxin during somatic embryogenesis,” *aBIOTECH*, vol. 1, pp. 185–193, 2020, doi: 10.1007/s42994-020-00029-8.

[5] R. B. Malabadi, S. Vijayakumar, K. Nataraja, and G. S. Mulgund, “Induction of somatic embryogenesis and plant regeneration in grapes (Vitis vinifera L.),” *Botany Research International*, vol. 3, no. 2, pp. 48–55, 2010.

[6] S. Bhardwaj *et al.*, “Advances in micropropagation and tissue culture for horticultural crops: A review,” *Plant Cell Biotechnology and Molecular Biology*, vol. 26, no. 11–12, pp. 166–182, Dec. 2025.

[7] R. J. Gautheret, “Plant tissue culture: A history,” *Botanical Magazine Tokyo*, vol. 96, pp. 393–410, 1983.

[8] I. M. Sussex, “The scientific roots of modern plant biotechnology,” *The Plant Cell*, vol. 20, no. 5, pp. 1189–1198, 2008, doi: 10.1105/tpc.108.058735.

[9] Y. C. Suryawanshi, “History of plant tissue culture,” in *Plant Tissue Culture*, Sep. 2025, pp. 1–13.

[10] Y. Long, Y. Yang, G. Pan, and Y. Shen, “New insights into tissue culture plant-regeneration mechanisms,” *Frontiers in Plant Science*, vol. 13, p. 926752, 2022, doi: 10.3389/fpls.2022.926752.

[11] H. Mehbub *et al.*, “Tissue culture in ornamentals: cultivation factors, propagation techniques, and its application,” *Plants*, vol. 11, no. 23, p. 3208, 2022.

[12] A. Hasnain *et al.*, “Plants in vitro propagation with its applications in food, pharmaceuticals and cosmetic industries,” *Frontiers in Plant Science*, vol. 13, p. 1009395, 2022, doi: 10.3389/fpls.2022.1009395.

[13] A. Wijerathna-Yapa *et al.*, “Applications of in vitro tissue culture technologies in breeding and genetic improvement of wheat,” *Plants*, vol. 11, p. 2273, 2022, doi: 10.3390/plants11172273.

[14] A. Wijerathna-Yapa and R. Pathirana, “Sustainable agro-food systems for climate change and food security,” *Agriculture*, vol. 12, p. 1554, 2022, doi: 10.3390/agriculture12101554.

[15] A. D. Tripathi *et al.*, *The Role of Functional Food Security in Global Health*. Amsterdam, Netherlands: Elsevier, 2019, pp. 3–24.

[16] F. Valoppi *et al.*, "Insight on current advances in food science and technology for feeding the world population," *Frontiers in Sustainable Food Systems*, vol. 5, p. 626227, 2021, doi: 10.3389/fsufs.2021.626227.

[17] R. Choudhary *et al.*, "Nanobioremediation: An introduction," in *Nano-Bioremediation: Fundamentals and Applications*, H. M. N. Iqbal *et al.*, Eds. Amsterdam, Netherlands: Elsevier, 2022, pp. 263–279.

[18] Y. H. Su *et al.*, "Plant cell totipotency: Insights into cellular reprogramming," *Journal of Integrative Plant Biology*, vol. 63, pp. 228–243, 2021, doi: 10.1111/jipb.12972.

[19] A. Fehér, "Callus, dedifferentiation, totipotency, somatic embryogenesis," *Frontiers in Plant Science*, vol. 10, p. 536, 2019, doi: 10.3389/fpls.2019.00536.

[20] M. J. M. Smulders and G. J. De Klerk, "Epigenetics in plant tissue culture," *Plant Growth Regulation*, vol. 63, pp. 137–146, 2011.

[21] A. Wijerathna-Yapa and J. Hiti-Bandaralage, "Tissue culture—A sustainable approach," *Life (Basel)*, vol. 13, no. 3, p. 780, 2023.

[22] A. Tazeb, "Plant tissue culture technique as a novel tool in plant breeding," *Environmental Science*, vol. 17, no. 2, pp. 111–118, 2017.

[23] P. Desai *et al.*, "Plant tissue culture: Somatic embryogenesis and organogenesis," in *Advances in Plant Tissue Culture*. Academic Press, 2022, pp. 109–130.

[24] G. S. Mulgund *et al.*, "Factors influencing cloning mature trees of conifers," *Research in Plant Biology*, vol. 2, no. 2, pp. 38–42, 2012.

[25] P. C. Deo *et al.*, "Factors affecting somatic embryogenesis and transformation," *South Pacific Journal of Natural and Applied Sciences*, vol. 28, no. 1, pp. 27–40, 2011.

[26] T. A. Arteta *et al.*, "Artificial neural networks elucidated the role of nutrients," *Plants*, vol. 11, no. 10, p. 1284, 2022.

[27] N. Zhumanova *et al.*, "A comprehensive review of plant growth regulators," *ES Food and Agroforestry*, vol. 17, p. 1190, 2024.

[28] A. E. Sabagh *et al.*, "Role of plant growth regulators under abiotic stress," *Frontiers in Agronomy*, vol. 3, p. 648694, 2021.

[29] C. A. Spinosa-Leal *et al.*, "In vitro plant tissue culture," *Planta*, vol. 248, pp. 1–18, 2018.

[30] N. Gupta *et al.*, "A review on micropropagation culture method," *Asian Journal of Pharmaceutical Research and Development*, vol. 8, no. 1, pp. 86–93, 2020.

[31] J. S. Ujjwala, "In vitro regeneration of Aloe barbadensis," *Biotechnology*, vol. 6, pp. 601–603, 2007.

[32] E. Titos *et al.*, "Signaling actions of resolvin D1," *Journal of Immunology*, vol. 197, no. 8, pp. 3360–3370, 2016.

[33] S. Suman, "Plant tissue culture: A promising tool," *Biochemistry and Cell Archives*, vol. 17, no. 1, pp. 1–26, 2017.

[34] N. R. Bhat, A. Bhatt, and M. K. Suleiman, *Physiology of Growth and Development in Horticultural Plants*. CRC Press, 2024.

[35] J. Raso, "Cell membrane permeabilization by pulsed electric fields," in *Pulsed Electric Fields Technology for the Food Industry*, 2022, p. 209.

[36] D. J. Styer and C. K. Chin, *Meristem and Shoot Tip Culture*. New Jersey, USA, 2010.

[37] B. A. Aighewi *et al.*, *Rapid Propagation of Yam Using Leaf-Bud Cuttings: A Manual*.

[38] M. A. Zavattieri *et al.*, "Adventitious rooting of conifers," *Trees*, vol. 30, no. 4, pp. 1021–1032, 2016.

[39] B. Gulzar *et al.*, "Plant tissue culture applications," in *Transgenic Technology in Plant Biotechnology*. Academic Press, 2020, pp. 25–49.

[40] S. Rahimi *et al.*, "Tissue culture techniques to conserve medicinal plants," in *Medicinal Plants*. Singapore: Springer, 2023, pp. 675–710.

[41] R. Lai and S. Lai, "Role of tissue culture in clonal propagation," in *Crop Improvement Utilizing*

Biotechnology. CRC Press, 2019, pp. 73–116.

[42] I. K. Vasil, "A history of plant biotechnology," *Plant Cell Reports*, vol. 27, no. 9, pp. 1423–1440, 2008.

[43] B. E. Haissig, N. D. Nelson, and G. H. Kidd, "Trends in tissue culture in forest improvement," *Bio/Technology*, vol. 5, no. 1, pp. 52–59, 1987.

[44] S. U. Sikdar *et al.*, "Agrobacterium-mediated gene transformation," *In Vitro Cellular & Developmental Biology - Plant*, vol. 51, no. 2, pp. 143–151, 2015.

[45] B. M. Twaij *et al.*, "Effects of elicitors on in-vitro cultures," *Biocatalysis and Agricultural Biotechnology*, 2019, doi: 10.1016/j.bcab.2019.101337.

[46] B. M. Twaij, Z. H. Jazar, and M. N. Hasan, "Trends in tissue culture applications," *International Journal of Plant Biology*, vol. 11, no. 1, 2020.

[47] S. S. Bhojwani, Ed., *Plant Tissue Culture: Applications and Limitations*. Elsevier, 2012.

[48] N. Kumari *et al.*, "In vitro cultures: challenges and limitations," in *Plants for Immunity and Conservation Strategies*. Springer, 2023, pp. 371–383.

[49] A. Hussain *et al.*, "Plant tissue culture: current status," in *Recent Advances in Plant In Vitro Culture*. IntechOpen, 2012.

[50] N. Permadi *et al.*, "Browning control in plant tissue culture," *Current Plant Biology*, vol. 38, p. 100339, 2024.