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REVIEW

A Mini Review of Plant Tissue Culture: The Role of Media Optimization, Growth Regulators in Modern Agriculture, Callus Induction and the Applications

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ABSTRACT

Plant tissue culture is an innovative biotechnological approach developed to meet the escalating global demand for agricultural production with enhanced nutritional value. This aseptic, in vitro technique allows for the cultivation of plant cells, tissues, or entire plants under controlled environmental and nutritional conditions, facilitating the generation of genetically identical clones. The method is indispensable in preserving endangered plant species, boosting crop yield and quality, and enabling year-round production of disease-free plants. Central to plant tissue culture is the composition of the growth medium, which must include a precise balance of macronutrients, micronutrients, amino acids, vitamins, and plant growth regulators such as auxins, cytokinins, gibberellins, and abscisic acid. Recent advancements have fine-tuned media formulations to better accommodate the needs of various plant species. The review highlights the significance of selecting appropriate growth regulators and media types to optimize outcomes, such as callus induction, shoot and root regeneration, and large-scale micropropagation. Furthermore, applications extend beyond agriculture, as tissue culture techniques are pivotal in bioremediation, secondary metabolite production, and plant-based pharmaceutical research.

Keywords: Callus extracts, Callus induction, Hormones, Murashige and Skoog (MS) medium, Plant growth regulators, Plant tissue culture

1. Introduction

The increasing global population and consequent demand for food, coupled with challenges like climate change, land degradation, and the loss of biodiversity, have driven the agricultural sector to explore advanced techniques for sustainable crop production [1, 2]. A significant advancement in agricultural biotechnology is the development of plant tissue culture, a technique that facilitates the aseptic in vitro cultivation of plant cells, tissues, or organs under precisely controlled environmental conditions [3]. By facilitating the propagation of genetically uniform and disease-free plants, tissue culture has revolutionized plant production and conservation, making it possible to meet agricultural and horticultural needs more efficiently [4]. Plant tissue culture is particularly valuable for the conservation of endangered species and the production of high-yield and stress-resistant crops. The method enables the year-round propagation of plants, regardless of seasonal or environmental constraints, and is widely used in

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research and commercial applications to ensure high-quality crop production. The underlying success of this technique depends on the careful selection and optimization of culture media, which must supply essential macronutrients, micronutrients, vitamins, and plant growth regulators [5, 6].

Several culture media, including Murashige and Skoog (MS), Nitsch and Nitsch (NN), B5, and Woody Plant Medium (WPM), have been specifically developed and optimized to cater to the requirements of different plant species and cultivation objectives. The composition of each medium, particularly the types and concentrations of growth regulators such as auxins, cytokinins, gibberellins, and abscisic acid, is pivotal in determining outcomes such as morphogenesis, callus induction, and the regeneration of shoots and roots [7]. The efficacy of these media formulations has been further refined through decades of research, enabling improved propagation outcomes and the generation of genetically stable plantlets.

Moreover, the strategic use of plant growth regulators in tissue culture not only enhances seed germination and growth but also influences numerous physiological and developmental processes, from cell division to organogenesis. This review provides a comprehensive overview of plant tissue culture media and the roles of various growth regulators, delving into the advancements and practical applications of this technology in agriculture, conservation, and biopharmaceutical research. The review also highlights key studies and innovations that have contributed to the optimization of in vitro plant propagation techniques.

2. Plant tissue culture

The rising demand for agricultural commodities has prompted scientists to develop techniques that improve production while maintaining high nutritional value through modern technology; one such technique is plant tissue culture, proposed as a solution to this increasing demand [8]. Plant tissue culture is the aseptic growing of cells, tissues, organs, or whole plants in vitro, often under controlled nutritional and conditions, to generate plant replicas. The resultant copies are congruent with the designated genotype [9]. Plant tissue culture need an adequate supply of nutrients, optimal medium pH, proper temperature, and a suitable gaseous and liquid environment [10]. This method of plant tissue culture safeguards endangered species and is the most efficient technology for improving agricultural yield and quality. High-quality plants are produced by separating the genetic elements critical for generating genotypes that provide high yields from adaptable and disease-resistant species [11]. As well as offers alternate answers to challenges emerging from present rates of flora and ecosystem loss and decimation. The entire procedure requires an adequate cultivation laboratory and nutritional media [12]. Scientists have widely utilised plant tissue culture and have become vital to manufacturing, free of illnesses, better and genetically modified plants in industrial and plant reproductive industries. This technique removes a section of plant tissue called explants, where hundreds or thousands of clones of the original plant may be produced in a relatively short timeframe and minimal area. This approach may be utilised over the entire year, irrespective of the plant season or weather conditions [13].

The origin of explants and the culture medium used for cellular growth are among the most pivotal aspects influencing the effectiveness of plant tissue culture. Significant variations in tissue culture response exist among plants removed under field circumstances, contingent upon the annual weather patterns [14]. Plant tissue culture utilizes various plant components, such as leaves, stems, seeds, or other tissues, to propagate plants in a controlled medium. This process requires optimal environmental conditions and proper sterilization. By promoting cell proliferation and growth, tissue culture enables plant cells to divide and regenerate into an entire organism. Single cells, stems, leaves, roots, or protoplasts can be cultured on nutrient-rich media with the addition of growth stimulants, facilitating the development of new plants [15]. Table 1 show using different source of initial explant in plant tissue culture technique.

The composition of the basal medium plays a crucial role in plant tissue development and productivity. It typically includes key components such as macronutrients, micronutrients, amino acids or alternative nitrogen sources, vitamins, organic supplements, carbon sources, solidifying agents, and growth regulators [23]. According to the different proportions of these components, many culture media were produced, such as Murashige and Skoog (MS), Nitsch and Nitsch (NN), B5, Driver and Kuniyuki Walnut (DKW) Medium, Woody Plant Medium (WPM) and 1/2 MS and Modified MS (MMS) Medium. The MS medium is one of the most often used and utilised media, and various media for many plant species have been widely used. Driver/Kuniyuki Walnut Medium (DKW) and WPM Medium were utilised for wood plant kinds [24].

The media type is selected according to the cultivation objective and the type of plant to be cultivated (plants species) [25]. As a result of the high amounts

Table 1. Sources of initial explants for plant tissue culture techniques.

Initial explant	Species of the plant	Purpose of study	Reference
Leaves	*Gerbera jamesonii*	Adventitious shoot proliferation from callus	[16]
Roots	*Atropa acuminata*	Establishing callus cultures from *Atropa acuminata*	[17]
Apical shoots (nodes) and apical leaves (leaf shoots)	*Scurrula atropurpurea*	To obtain an optimal explant surface sterilization protocol for tea parasite tissue culture that is free from contamination, browning, and explant death.	[18]
Mature seeds and meristematic tissue	*Paspalum notatum*	Embrace novel genetic improvement through transgenesis and genome editing	[19]
Floral apices	*Banana (Musa sp.)*	Determine the use of flower apices explants on local banana varieties in Indonesia using a combination of cytokinin hormones containing BAP (Benzylaminopurine) and several levels of TDZ (Thidiazuron).	[20]
Filaments and ovules	*Lilium sp.*	Potential to establish in vitro propagation protocol using different Chu N6 medium and their modifications.	[21]
Stem tip	*Malus sieversii*	Investigate the impact of exogenous NO donor sodium nitroprusside (SNP) on the browning.	[22]

of nitrogen in the form of nitrate and ammonium and the comparatively high ammonium-to-nitrate ratio, Murashige and Skoog medium is extensively employed for both dicots and monocots. Despite this, the MS medium may not always provide ideal growth conditions because of the large ammonium ions present. To compensate for the lower macronutrient salt content in woody plant medium, 1/2 MS, MMS (Modified MS), and WPM are now commonly utilized for woody plants. The 1/2 MS or MMS media don't appear to produce ammonium toxicity when using half of the MS ammonium. Similar to MS, WPM has a lower total nitrogen and ammonium content than the latter. DKW, on the other hand, employs alternative salt sources and has greater ammonium to nitrate ratio, comparable to MS, but with less total nitrogen; this results in a higher sulphate content [26]. Table 2 presents a detailed summary of these media types, their compositions, and their specific applications.

3. Components of plant tissue culture media

3.1. Macro and micronutrients

In plant cell or tissue culture media, macronutrients including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulphur (S) are critical for growth and morphogenesis. Inorganic nitrogen of at least 25–60 mM is required for optimal plant cell development in a culture medium. Most plant species require potassium to develop properly in cells. Many media contain K in nitrate chloride salts at 20 to 30 mM concentrations. Other cell development must be satisfied for optimal P, Mg, S and Ca concentrations to be found [33].

The crucial micronutrients for the development of plant cells and tissues are iron (Fe), manganese (Mn), zinc (Zn), boron (B), copper (Cu), and molybdenum (Mo). Iron is often the primary micronutrient. These components are used in culture media as citrate or tartrate salts. These chemicals provide hurdles due to their solubility issues and precipitation during medium preparation. The use of ethylene diamine tetraacetic acid (EDTA)-iron chelate (FeEDTA) to address this issue has been examined [34]. In addition, a method for making a non-precipitating iron chelate solution has been developed. Cobalt (Co) and iodine (I) can be used in various mediums; however, their cell growth conditions aren't well characterized. Sodium (Na) and chlorine (Cl) are also used in various media, despite suggestions that are not required for manufacture. Copper and cobalt are applied at 0.1M, iron and molybdenum at 1M, iodine at 5M, zinc at 5–30M, manganese at 20–90M, and boron at 25–100M to the cultivation media [23]. In addition to macro and micronutrients, it is preferable to equip the culture media with hormones or so-called plant growth regulators that help the plant grow faster and germinate in a shorter period [35].

3.2. Plant growth regulators

The inclusion of plant growth regulators is typically required for the effectiveness of *in vitro* cultivation [36]. Growth regulators are essential for enhancing seed germination rates. Topically administered plant growth regulators affect numerous metabolic pathways and developmental phases, including the regulation of germination, promotion or suppression of shoot elongation, callus formation, initiation of flowering and fruit development, modulation of fruit set, and acceleration or deceleration of aging processes such as fruit ripening and leaf abscission [37]. The major plant growth regulators are auxins, gibberellins, cytokinins, ethylene, abscisic acid,

Table 2. Common plant tissue culture media: descriptions, compositions, applications, and key studies.

Media name	Description	Composition	Applications	Studies
Murashige and Skoog (MS) Medium	The MS medium, developed by Murashige and Skoog in 1962, is one of the most widely used media in plant tissue culture, suitable for both monocot and dicot plants. It is known for its high concentration of macronutrients, including nitrogen in the form of ammonium and nitrate ions.	contains essential macronutrients (such as K, Ca, and Mg), micronutrients (like Fe and Mn), vitamins, and growth regulators. The high nitrogen content in the MS medium is especially beneficial for promoting vigorous plant growth.	MS medium is commonly used for shoot induction, callus formation, and the micropropagation of various plant species.	[27] This study highlighted the use of MS medium in producing callus from in vitro cultures of *Trigonella foenum-graecum* (TFG) using combined auxin and cytokinins hormones
Nitsch and Nitsch (NN) Medium	NN medium is commonly used for the culture of reproductive organs, like anthers and ovaries, and is particularly effective for pollen culture and the development of haploid plants.	contains lower concentrations of salts compared to MS medium and is specifically formulated to support gametophytic development.	Widely used in another culture to produce haploid and doubled haploid plants, which are valuable for plant breeding programs.	[28] This study used NN medium for induction of haploid embryos in *Datura metel*
B5 Medium	Developed by Gamborg et al. in 1968, B5 medium is designed for the culture of soybean root cells and is commonly used for cell suspension and callus cultures	features lower salt concentrations compared to MS medium and includes specific vitamins like thiamine, nicotinic acid, and pyridoxine	Ideal for culturing legumes and plant cell suspensions, promoting root development and callus growth	[29] Study have demonstrated the effectiveness of B5 medium in regenerating plantlets from cell suspensions, particularly in legumes such as soybean and alfalfa
Driver and Kuniyuki Walnut (DKW) Medium	DKW medium is tailored for the micropropagation of woody plant species, especially nut-bearing trees like walnuts. It provides an optimal balance of nutrients and growth regulators to support the growth of recalcitrant woody species	It has a higher sulfate content, and a modified ammonium-to-nitrate ratio compared to MS medium, which helps mitigate ammonium toxicity in woody plants	Widely used for the in vitro propagation of hardwood and nut-bearing trees	[30] Reported successful micropropagation of *Juglans regia* (walnut) using DKW medium, emphasizing its effectiveness in enhancing shoot and root development in woody species
Woody Plant Medium (WPM)	WPM is specifically designed for the culture of woody plant species. It is formulated with lower levels of nitrogen and ammonium to accommodate the nutritional needs of woody plants	WPM includes reduced concentrations of macronutrients and is often supplemented with plant growth regulators like auxins and cytokinins	Effective for the micropropagation of trees and shrubs, promoting the healthy growth of shoots and roots	[31] Highlighted the use of WPM for the successful propagation of various tree species, such as *Populus* and *Quercus*, demonstrating the medium's adaptability to different woody plants
1/2 MS and Modified MS (MMS) Medium	These are variations of the standard MS medium with reduced salt concentrations, commonly used for plant species sensitive to high ammonium levels, such as certain woody plants and orchids	The nutrient concentrations are halved or modified to decrease the risk of ammonium toxicity, making them suitable for delicate and slow-growing plants	Used in the micropropagation of orchids, ferns, and woody plants, ensuring better root and shoot development in species with specific nutrient requirements	[32] This study developed an efficient protocol for the in vitro propagation of *Phalaenopsis* orchids using a 1/2 strength MS medium. The reduced salt concentration was found to be optimal for root formation, minimizing stress and promoting healthy plantlet development.

Table 3. Plant Growth Regulators: Descriptions and References.

Plant growth regulator	Description	Reference
Auxins	Auxins are critical for cell elongation, root induction, and callus formation. They include compounds such as Indole-3-Acetic Acid (IAA), Indole-3-Butyric Acid (IBA), Naphthalene Acetic Acid (NAA), and 2,4-Dichlorophenoxyacetic Acid (2,4-D).	[49]
Cytokinins	Cytokinins are primarily involved in cell division and shoot proliferation. Common cytokinins include Benzylaminopurine (BAP), Kinetin, Zeatin, and Thidiazuron (TDZ).	[50]
Gibberellins	Gibberellins (GAs) are used to break seed dormancy, stimulate stem elongation, and enhance flowering. Gibberellic Acid (GA3) is the most commonly used form.	[51]
Abscisic Acid (ABA)	ABA is typically used to regulate stress responses and inhibit unwanted germination or shoot elongation. It is often included in media formulations for somatic embryogenesis.	[52]
Ethylene Inhibitors	Ethylene inhibitors, such as Silver Nitrate ($AgNO_3$) or Cobalt Chloride, are used to prevent ethylene action, which can inhibit tissue culture growth and development.	[53]
Brassinosteroids	Brassinosteroids are used for enhancing stress tolerance, seed germination, and overall plant growth. They are relatively newer regulators in tissue culture studies	[54]

and brassinosteroids. Auxins, cytokinins, and auxin-cytokinin interactions are often required in plant tissue culture to control growth and arrange development. As a result, these two groups of hormones, cytokinins and auxins, are crucial [38]. To make plant tissue culture more successful, obtain better propagation results, and assist in plant growth and development, various growth regulators play a key role in the germination process. There are different types of these regulators (hormones), and their use varies according to the plant part to be grown as an explant and according to the purpose of the plant cultivation process in the laboratory [39].

Plant growth regulator's role in cell expansion, cell wall acidification, and division of meristems forming callus or defined organs (usually roots), auxins are well-known. They also help differentiate vascular tissues, inhibit the growth of lateral buds, and delay leaf senescence while promoting root formation [40]. Cytokinins play a role in cell division stimulation and apical dominance release. From meristematic explants, they can stimulate adventitious bud, axillary, and adventitious shoot formation [41–43]. Plant tissue culture media commonly use auxins such as IAA, IBA, 2,4-D, and NAA. BAP, 2iP, kinetin, Zeatin, and TDZ are examples of common cytokinins used in culture media [44–46]. Since seed germination is an important part of the production of new plants and using the plant tissue culture technique, many studies have shown that growth regulators play an important role in seed germination and participate in the regulation of seeds germination [47]. Growth regulators have been used to improve the germination of seeds of some important medicinal plants produced on a commercial scale and enhance the production of these plants. The seeds of peppermint *Mentha piperita*, sweet basil *Ocimum basilicum* and coriander *Coriandrum sativum* were soaked in different concentrations of growth regulators. Naphthalene acetic acid (NAA), indol-3-butyric acid (IBA), indole-3-acetic acid (IAA), gibberellic acid (GA3), (50, 100, 150 mg/L) for 24 hours at a temperature between 23–27°C. They were grown in Petri dishes containing a double layer of wet filter paper. The best germination result of seeds soaked in GA3 was better than other growth regulators. The final germination percentage increased clearly [48]. Table 3 provides a detailed summary of various plant growth regulators, their descriptions, and specific applications, highlighting their pivotal roles in tissue culture and seed germination.

4. Seeds germination

The embryo is the primary structural element inside a seed. The embryo, or small plant, consists of one or more cotyledons, a plumule, a hypocotyl, and a radicle [55]. After water intake, the seed starts metabolic processes, the radicle emerges from within the seed wrappers, and the seedling emerges as a fully formed plant [56, 57]. A multitude of physiological mechanisms contribute to the embryo's growth and maturation into an autonomous seedling. These activities include respiration, water absorption, enzyme activation, conversion of food into soluble forms for transport, distribution of nutrients, water, minerals, and hormones to meristematic areas, and the assimilation of food into plant tissues [58]. *In vitro* seed germination is the process of cultivating a seed embryo in a laboratory by supplying optimal growth circumstances akin to a biological organism. *In vitro* culture, the effectiveness of seed germination is contingent upon many factors, including the culture medium, seed maturity, plant growth regulators, carbohydrates, and organic additions [59, 60]. The germination of seeds in controlled environments facilitates rapid germination and homogeneous seedling development, whereas *in vivo* cultures need meticulous oversight to maintain appropriate humidity levels and assure successful germination [61]. For

tissue culture experiments, in vitro-grown plants are extremely useful because they don't require any sterilisation before being used. This method of tissue segment multiplication yields many clean individual plants [62].

Restoration projects can benefit from the use of *in vitro* methods as well. For genetic evaluations and commercial plant propagation protocols, tissue culture propagation from wild-collected seeds or tissues can provide material. And this enables commercial plant propagation methods to be used to restore appropriate genetic stock and relieve pressure on wild populations [63]. Numerous leguminous species, including *Bobgunnia madagascariensis*, *Pterocarpus marsupium*, and *Cordeauxia edulis*, possess established techniques (protocols) for *in vitro* seed germination and seedling development. A variety of legumes have been germinated and propagated using diverse medium, salt concentrations, types and concentrations of plant development regulators, as well as both mature and immature seeds [64].

5. Callus induction

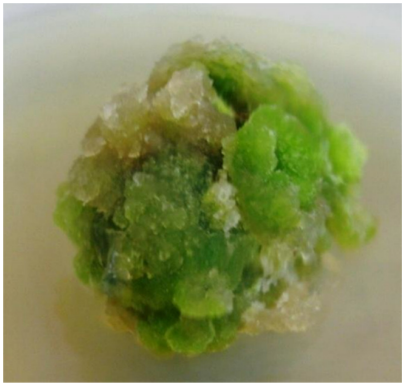
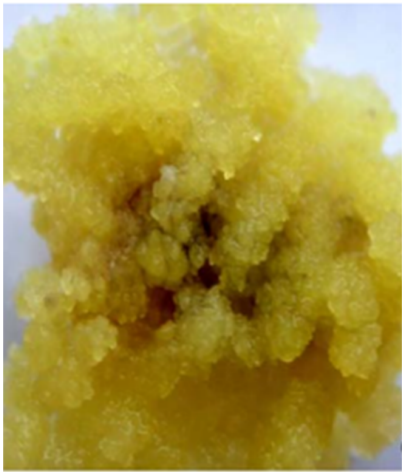
In plant tissue culture, the term "callus" refers to the dense mass of undifferentiated cells that develops when a plant tissue is cultivated on a culture medium under controlled in vitro conditions. Table 4 presents the different types of callus, highlighting their distinct characteristics.

A callus may originate from a single differentiated cell or several totipotent cells. Under certain circumstances that provide the essential components for callus proliferation, somatic embryogenesis is induced, subsequently leading to the development of mature somatic cells [70]. Numerous variables govern the stimulation and regeneration of callus, including the explant type, concentrations of growth regulators in the culture medium, carbohydrate sources, and the circumstances for callus cultivation [71]. Two forms of callus develop when an explant is cultured in plant tissue: embryogenic and non-embryogenic. Embryogenic callus often results in plant production by somatic embryogenesis and typically develops on the surface of the induced callus. The growth of non-embryogenic callus is erratic and disordered, sometimes resulting in the development of branches and roots via organogenesis. Two forms of calluses may be identified by visual characteristics [72]. The embryogenic callus is distinguished by its compact, dry, and light-yellow appearance, while the non-embryogenic callus is friable, transparent, rough, and exhibits yellow or grey hues [73]. As well as there are another classification of callus depends on the appearance of callus and the further stage of growth as stated in Table 4.

Numerous experiments and studies have focused on inducing callus formation by various explants and doses of growth regulators. A study on the plant *Lycium barbarum* aimed at inducing callus used leaves and nodes as explants, which were cultured in MS medium with varying doses of the growth regulators 2,4-dichlorophenoxyacetic acid (2,4-D) and benzylaminopurine (BAP). The ideal doses for callus induction using leaves as explants were 0.3 mg/L of 2,4-D in conjunction with either 0.1 mg/L or 0.3 mg/L, with the callus characterized by its greenish and friable appearance. The optimal doses for callus induction using nodes as explants were 0.1 mg/L BAP combined with either 0.3 mg/L or 0.5 mg/L 2,4-D, resulting in callus that was yellowish-green, friable, and watery [74].

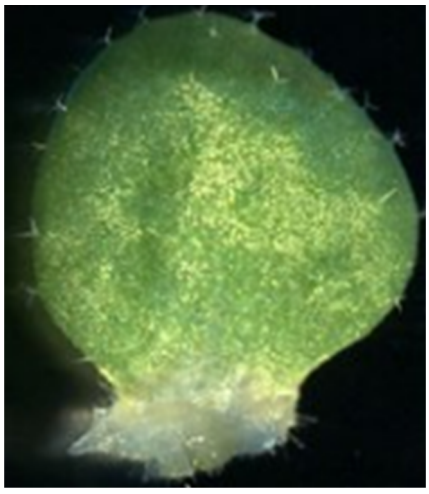
Several studies have been conducted to induce callus of fenugreek, including a study to determine the best hormones for callus growth in MS media by using cotyledon and hypocotyl as an explant. This study found the best hormones for callus induction is 0.15 mg/L IBA, and 0.45 mg/L TDZ percentage was 96.87% from hypocotyl explant [75]. In another study, naphthalene acetic acid NAA and 6-benzyl adenine BA were used in different concentrations in MS media. Embryo axis and hypocotyl were used as explants; the result shows that the best hormone concentrations of shoot and callus induction were 0.5 mg/L NAA without BA for embryo axis explant. At the same time, the best hormone for hypocotyl explant was at a concentration of 2 $mg/^{-1}$ of NAA [76]. In some studies, auxins were combined with kinetin to obtain a specific protocol for inducing callus from *T. foenum-graecum*. The MS medium was supplemented with different concentrations of NAA and 2,4-D (0.1,0.5,1.0,2.0,3.0,4.0 mg/L) with one concentration of kinetin 0.5 mg/L, where used the Hypocotyls and Cotyledons as an explant. The results showed that all concentrations showed callus formation in the sixth week. No results were shown for callus in MS medium in the absence of growth regulators. The best concentration of callus induction was from hypocotyl, as it was found that auxin and kinetin mixed produced a greater amount of callus than auxin alone. For cotyledons explant alone, 2,4-D was the best in callus induction [77]. Another study on *T. foenum-graecum* documented the influence of BAP, NAA, and 2,4-D on in vitro callus development from seeds. The largest callus production was recorded in the MS medium with 1.0 mg/L 2,4-D, yielding the maximum mean callus index (52±9.5), a 100% frequency, and a callus yield of (0.52±0.08 g) after 30 days of culture. The greatest mean callus

Table 4. Types of callus in plant tissue culture and their characteristics.

Type of callus	Description	Appearance	Reference
Friable Callus	Loose, crumbly, and less organized; preferred for embryogenic potential.		[65]
Compact callus	Dense and organized structure, often seen in maize tissue culture.		[66]
Embryogenic Callus	Capable of developing into somatic embryos and regenerating whole plants.		[67]
Non-Embryogenic Callus	Lacks somatic embryogenesis and plant regeneration potential.		[67]

(*continued on next page*)

Table 4. Continued.

Type of callus	Description	Appearance	Reference
Rooty callus	Primarily forms roots, depending on hormone balance.		[68]
Shooty callus	Predominantly develops shoots, influenced by the hormonal environment.		[69]

index (37±0.4) was achieved with a combination of hormones resulting in 100% callusing and a yield of (0.37±0.02 g) after 30 days of culture using 1.0 mg/L BAA and 0.5 mg/L NAA [27]. Therefore, callus formation is a critical step in plant tissue culture and serves as a foundation for various biotechnological applications. In addition to callus induction and seed germination plant tissue culture techniques encompass several forms of cultivation performed within laboratory settings. These include cell or suspension culture, embryo culture, and protoplast culture. Table 5 above summarizes key studies on these techniques, highlighting their findings and applications in advancing plant tissue culture and biotechnology [78]. All these types are done using different media according to the type of plant and different concentrations of hormones according to the purpose of the study. One of the most important forms of plant tissue culture is the cultivation of a part of the plant "explant" to induce the callus [79].

6. Callus application

Callus is an important undifferentiated plant mass that can be used in many applications and plays a crucial role in plant biotechnology and various fields.

6.1. Plant regeneration and propagation

In micropropagation, callus tissue serves as a vital platform for the extensive proliferation of plants. It provides a consistent supply of plants that are genetically identical to the parent, a critical feature for large-scale agricultural and horticultural production. Additionally, callus tissue is widely utilized in genetic engineering research to facilitate plant modification. This approach supports the incorporation of new genes, enabling the development of genetically modified crops with desirable traits such as improved disease resistance or enhanced nutritional content [86].

Table 5. Summary of cell, embryo, and protoplast culture studies in plant tissue culture.

Study title	The findings	Reference
Cell or Suspension Culture Studies		
Production of bioactive metabolites in in vitro cultures of saffron (*Crocus sativus L.*)	Investigated the micropropagation of saffron and examined apocarotenoid gene expression in suspension cultures.	[80]
Plant growth regulation in cell and tissue culture *in vitro*	Examined the function of growth regulators in cell and tissue cultures, emphasizing their influence on plant development.	[81]
Embryo Culture Studies		
An introduction to plant tissue culture: advances and perspectives	reviewed progress in plant tissue culture, including embryo culture methods and their applications.	[82]
Plant tissue culture: agriculture and industrial applications	Investigated the industrial uses of plant tissue culture, highlighting the significance of embryo culture in enhancing crop quality.	[83]
Protoplast Culture Studies		
Plant cell and tissue culture: propagation, improvement, and conservation	Investigated protoplast culture techniques for plant propagation and genetic enhancement.	[84]
Cell and protoplast culture for production of plant metabolites	Emphasized new advancements in protoplast cultivation and their applications within plant biotechnology.	[85]

6.2. Secondary metabolite production

Callus cultures are instrumental in the production of bioactive compounds, primarily secondary metabolites such as alkaloids, flavonoids, and phenolics. These metabolites hold significant value due to their pharmaceutical and therapeutic applications [87]. In the pharmaceutical industry, extracts derived from callus cultures are extensively studied for their potential medicinal properties, including anti-inflammatory, antimicrobial, antioxidant, and anticancer effects [88]. For example, taxol (a cancer-treating compound) has been successfully produced from the callus of *Taxus spp*. In cosmetics plant callus extracts are used in the cosmetic industry for their bioactive properties, such as anti-aging and skin-healing effects [89].

6.3. Bioremediation and environmental applications

Callus tissue has been explored for its ability to absorb and metabolize heavy metals or environmental toxins. This property can be useful for developing plants capable of cleaning polluted environments [90].

6.4. Nutraceuticals and functional foods

Extracts from plant callus are used to enrich food products with health-benefiting compounds. For instance, callus-derived phytochemicals can enhance the antioxidant content in functional foods and nutraceuticals [91].

6.5. Research and development

In metabolic studies callus cultures are widely used in research to study plant metabolism and the biosynthesis pathways of various secondary metabolites. As well as in stress response studies researchers use calluses to study how plants respond to stress conditions, such as drought, salinity, or pathogen attacks.

7. Callus extracts

The process of inducing callus involves obtaining biomass from undifferentiated plant cells, which can be used in various studies. These studies may focus on regenerating the callus through the cell suspension method to develop new plants or extracting the callus to analyze its components and biological effects. Additionally, callus extracts are studied for their toxicological effects. Table 6 highlights several some studies that explore the diverse applications of callus cultures, including the production of secondary metabolites, genetic transformation, therapeutic compounds, and the use of innovative approaches like nanoparticles to enhance callus culture performance [92]. In a study conducted to find out the antimicrobial activities and chemical assay of fenugreek plant. The induced callus extracts of the fenugreek plant from hypocotyls and cotyledon were prepared using two types of auxins 2,4-D and NAA at a concentration of 2 mg/L with kinetin at a concentration of 0.5 mg/L. The induced methanolic callus extracts showed antimicrobial activity at a 250 mg/ml concentration against *Staphylococcus aureus*. In the paper disc diffusion method, the callus extracts also affected *Staphylococcus aureus* were showed activity equal to ampicillin/sulbactam 20 mcg/disc and an activity equal to ciprofloxacin 5 mcg/disc [99]. In a recent study conducted in Italy on a type of apple *Mela Rosa Marchigiana*, where callus was induced from this plant, and the biological effects of callus were studied, the callus extract showed significant

Table 6. Some studies on callus cultures and their applications.

Study title	Aims of study	Reference
Biotechnology applications of plant callus cultures	Investigated the use of callus cultures to produce secondary metabolites, genetic transformation, and synthetic seed technologies.	[92]
Elicitation of callus cultures of the medicinally important plant *Embelia ribes* Burm f. using biotic and abiotic elicitors for enhanced production of embelin	Examined techniques to augment embelin synthesis in *Embelia ribes* callus cultures using biotic and abiotic elicitors.	[93]
Callus culture for the production of therapeutic compounds	Examined the function of callus cultures in the synthesis of medicinal chemicals and their prospective pharmaceutical uses.	[94]
A review: improvement of plant tissue culture applications by using nanoparticles	Investigated the effects of nanoparticles on callus cultures, emphasizing enhancements in growth and secondary metabolite synthesis.	[95]
Plant tissue culture-mediated biotechnological approaches in *Lycium barbarum L.* (Red goji or wolfberry)	Addressed the utilization of callus cultures in the multiplication and genetic enhancement *of Lycium barbarum*, highlighting its biotechnological utility.	[96]
Induction and Characteristics of Callus Cultures of the Medicinal Plant *Tussilago farfara L.*	Developed procedures for callus induction from *Tussilago farfara* leaves and evaluated their therapeutic potential.	[97]
Callus culture approach towards production of plant secondary metabolites	Investigated methods to augment secondary metabolite formation in callus cultures, emphasizing its industrial implications.	[98]

activity in scavenging free radicals, by 67%, in the DPPH assay. As for using the ABTS measurement, it was 39%. Also, the callus extract showed a significant effect in reducing radical oxidant species ROS production in cells. Overall, this study showed an apparent effect of callus extract on wound healing and anti-inflammatory response [100]. In a study by Jayme et al. [101] on *Cereus peruvianus* plant since aqueous callus extract was used to evaluate the anti-ulcer activity of the extract *in vivo* on laboratory animals, Wister rats. The aqueous extract of callus had a carbohydrate content of 53.40% and proteins of 0.66%. Aqueous callus extract was highly beneficial against ethanol-induced injuries but ineffective against indomethacin-induced injuries.

Another study on *Jatropha curcas L.* demonstrated to assess the chemical structure and characterization of the components of callus extract of two types of *Jatropha curcas L.* (poisonous and non-toxic). The study aimed to show whether callus contains essential and beneficial compounds and verify the possibility of using the plant tissue culture technique to obtain quantities of these undifferentiated cells. The Thin Layer Chromatography test showed variation in the accumulation of compounds in the non-toxic cultivar throughout the cultivation period, a clear increase in diterpenes and a significant decrease in flavonoids. Regarding the callus extracted from both types, six glycosylated flavonoid compounds were found in the callus extract [102]. A study conducted by Rouane et al. [103] on the toxicity of callus extract of *Pulicaria incisa* (Lam.) DC, shown there is no toxicity of callus extract, and no signs were observed on laboratory animals using different doses of callus extract. The colourimetric test showed the callus contained gum, flavonoids, saponins and anthocyanins. At the same time, the chromatographic analysis (HPLC) showed the presence of methoxy cinnamic acid produced by the callus, which is the reason for giving callus a positive anti-inflammatory reaction, where the callus is considered a source of anti-inflammatory because it is an alternative source of biologically active metabolites beneficial to human health.

8. Conclusion

Plant tissue culture has become a fundamental component of modern agricultural biotechnology, providing a reliable and efficient approach for the propagation and conservation of plant species. By facilitating the in vitro cultivation of plant cells, tissues, or whole organisms under controlled conditions, this technique has transformed plant production. It enables the generation of genetically uniform and disease-free plants, unaffected by seasonal or environmental limitations. The strategic application of tailored culture media, in conjunction with suitable plant growth regulators, has been pivotal in achieving optimized outcomes such as callus induction, shoot and root regeneration, and large-scale micropropagation. Advances in the formulation of tissue culture media and the understanding of plant growth regulators have significantly expanded the applications of plant tissue culture. These include enhancing crop productivity and quality, conserving endangered species, and facilitating the production of valuable secondary metabolites. Moreover, this technique offers promising solutions for addressing global challenges such as food insecurity, loss of biodiversity, and the need for sustainable agricultural practices. Despite the successes, there remain challenges and opportunities for further research, particularly in improving the efficiency and scalability of tissue

culture techniques and understanding the molecular mechanisms underlying plant responses to in vitro conditions. Continued innovations and interdisciplinary approaches will be crucial in leveraging plant tissue culture to its fullest potential, ensuring that this technology continues to contribute to agricultural and ecological sustainability.

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