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Hairy Root Cultures: A Comprehensive Review of Biological Principles, Metabolic Engineering Strategies, and Bioreactor-Based Production Systems

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ABSTRACT

Hairy root culture, induced through *Agrobacterium rhizogenes*–mediated genetic transformation, has emerged as one of the most powerful platforms in plant biotechnology for the production of high-value secondary metabolites. This technology combines rapid growth, genetic and biosynthetic stability, and synthesis under controlled conditions. In recent years, limitations associated with conventional extraction from natural plants have intensified global interest in vitro systems. Hairy roots offer an efficient alternative, while also enabling metabolic reprogramming and the accumulation of unique phytochemicals not detected in non-transformed tissues. This review summarizes the biological principles of hairy root induction and the metabolic advantages of transformed roots. In addition, the article highlights technological strategies for yield enhancement. Special emphasis is placed on bioreactor-based cultivation, with a focus on reactor designs, engineering constraints, and challenges related to oxygen transfer, shear sensitivity, and biomass aggregation. Key findings highlight that hairy root cultures can surpass wild plants in yield for specific metabolites when combined with elicitation strategies. However, major challenges such as shear sensitivity in bioreactors, biomass aggregation, and scale-up complexities remain significant hurdles for industrial adoption. Continued advances in molecular engineering and bioreactor design are expected to accelerate the commercial deployment of this technology.

Keywords: Hairy roots, Bioreactor systems, Secondary metabolites, Metabolic engineering, Elicitors

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Introduction

According to the definition provided by the World Health Organization (WHO, 2013), traditional medicine is described as "the sum of knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, used in the maintenance of health as well as in the prevention, diagnosis, improvement, or treatment of physical and mental illnesses." Today, the utilization of traditional medicine has grown on a global scale, serving as the primary or complementary component of healthcare systems in many countries (Chandran et al., 2020). It is estimated that nearly 80% of the global population relies on medicinal plants for their primary healthcare needs (WHO, 2013).

Plant tissue culture, as an advanced technology, represents a valuable tool for developing innovative biotechnological approaches and accessing therapeutically substantial chemical compounds. In this technique, plant cells, tissues, or organs are maintained under completely sterile and controlled conditions, where all environmental parameters, including light, temperature, humidity, and the optimized composition of the cultivation medium, are tightly regulated (De Schutter et al., 2022). The fundamental idea of culturing isolated plant cells to reveal their latent potential was first proposed by Haberlandt (1902) in the early twentieth century. Although he was not successful in inducing cell division in practice, his visionary insight laid the foundation for one of the most influential fields in plant sciences: plant cell and tissue culture. Haberlandt believed that such a simplified biological system could provide an unparalleled platform for understanding the intrinsic characteristics and hidden potential of individual cells, opening a window into the complex interactions and cooperative behaviors within multicellular organisms (Haberlandt, 1902).

Today, not only has the maintenance and propagation of isolated plant cells under in vitro conditions become a routine reality, but the complete regeneration of a whole plant from a single cell is considered a remarkable achievement of plant biotechnology. The superiority of cell-culture–based systems in studying metabolic pathways and cellular responses, compared to experiments performed on whole plants, is now well established (Singh et al., 2023). These systems enable researchers to apply chemical treatments with high precision, rapidly modify culture medium compositions, and conduct detailed biochemical analyses. Moreover, the clonal propagation of single cells provides a platform for the genetic improvement of crop plants by employing principles similar to microbial genetics. At the industrial scale, plant tissue culture is now regarded as a powerful and cost-effective approach for the mass production of valuable plant phytochemicals with high commercial importance (Wilczańska et al., 2023).

Interest in medicinal herbs has deep historical roots, as these plants have been used for centuries as natural remedies for a wide range of human ailments. They display diverse bioactivities, including anti-inflammatory, anxiolytic, antipyretic, antiviral, and antibacterial effects, serving as valuable reservoirs of life-saving compounds and playing a pivotal role in global healthcare systems (Chandran et al., 2020). These therapeutic properties primarily arise from the rich diversity of chemical constituents found in plants, widely known as secondary metabolites (Singh et al., 2023).

Despite their significance, the accumulation of valuable specialized metabolites in herbal plants faces numerous challenges. Traditional extraction from natural plants, which has long been the main source of pharmacologically active compounds, pigments, and flavoring agents, is constrained by climatic dependency, depletion of natural genetic resources, pesticide contamination, and production instability (Yamamoto et al., 2022). In contrast, plant cell culture, although offering considerable promise, encounters obstacles such as high cellular water content, foaming issues in bioreactors, and metabolic instability (Murthy et al., 2023). In this context, in vitro culture of differentiated plant systems has emerged as a promising strategy for diverse plant species (Table 1). Among these systems, hairy root culture, induced through co-cultivation with *Agrobacterium rhizogenes*, holds a special position. This system possesses unique benefits, including accelerated growth, long-term

genetic and metabolic stability, and the capacity to produce root-specific secondary metabolites, making it one of the most promising biotechnological platforms to support sustainable biosynthesis of economically important medicinal compounds (Biswas et al., 2023).

Table 1. Applications of *in vitro* plant tissue and cell culture in multiple species

Plant	Culture	Reference
Silybum marianum	Callus	Ehsani et al., 2025
Salvia apiana	Hairy roots	Krol et al., 2025
Urena lobata L.	Hairy roots	Cao et al., 2024
Melia azedarach	Callus	Ahmadpoor et al., 2022
Taxus cuspidata	Cell suspension	Yamamoto et al., 2022
Curcuma amada	Micropropagated plants	Behera et al., 2022
Gynostemma pentaphyllum	Cell suspension	Quang et al., 2022
Salvia dominica	Hairy roots	Boccia et al., 2022
Solanum lycopersicum	Callus	Gogliettino et al., 2022
Ocimum sp	Hairy roots	Pandey et al., 2022
Taxus × media	Hairy roots	SykłowskaBaranek et al., 2022
Hyoscyamus muticus	Cell suspension	Abdelmaksood et al., 2022
Atropa acuminata	Regenerated plants	Dar et al., 2022

This table underscores the versatility of in vitro culture techniques, ranging from undifferentiated callus to organized hairy roots, for producing metabolites across diverse plant families.

Despite the demonstrated potential of transformed root cultures, challenges such as metabolic instability under submerged conditions and limitations in process scale-up have hindered their widespread industrial application (Biswas et al., 2023). This review is based on a comprehensive survey of peer-reviewed literature retrieved from major scientific databases, including Scopus, Web of Science, and Google Scholar, with a particular focus on studies published over the past two decades related to hairy root cultures, secondary metabolite production, and bioreactor-based systems. Therefore, the primary objectives of this review are: (1) to elucidate the biological principles and metabolic advantages of hairy root cultures; (2) to evaluate current technological strategies, including elicitation and permeabilization, for enhanced metabolite production; (3) to critically analyze bioreactor designs and scale-up challenges; and (4) to provide a analysis that identifies key barriers and future opportunities for the commercial deployment of this platform.

Hairy Roots: A Novel Technique

Genetic engineering, as an advanced technology in plant breeding, enables the targeted transfer of genes encoding desirable traits into the genomes of various organisms. In this process, DNA fragments are excised from the donor genome using restriction endonucleases and cloned into a suitable genetic vector. These vectors are capable of delivering foreign DNA into host cells, ultimately generating fully transgenic plants (Gutierrez-

Valdes et al., 2020). Among the different gene delivery systems, *Agrobacterium rhizogenes* holds a special position. This soil-borne bacterium is naturally capable of inducing "hairy root" syndrome in numerous dicotyledonous plants (Ying et al., 2023). The underlying mechanism involves the genomic integration of Ri plasmid T-DNA sequences within the host plant genome, resulting in the development of transgenic root tissues with rare characteristics (Lin et al., 2025).

Hairy roots generated under in vitro conditions exhibit numerous advantages. These roots demonstrate

accelerated growth, stable genetic traits, and the capacity to sustain development without the need for exogenous growth regulators. Furthermore, due to their differentiated structure and fully developed vascular network, the capacity for biosynthesis of bioactive compounds in these roots is often reported to be significantly higher than that observed in conventional cell cultures (Mirmazloum et al., 2024).

Agrobacterium rhizogenes, a soil-dwelling Gram-negative bacterium, was first identified in the 1930s under the name Rhizobium rhizogenes and received its current designation in 1942. This bacterium has the ability to initiate hairy root syndrome involving excessive root proliferation at the site of infection (Zheng, 2025). Agrobacterium species are widely distributed worldwide, among these, pathogenic species including *Agrobacterium tumefaciens* and *A. rhizogenes* are responsible for inducing crown gall and hairy root diseases in plants, respectively. Despite its long history as a plant pathogen, *A. rhizogenes* has now been established as an efficient tool in plant biotechnology (Ying et al., 2023). Its natural ability to stably transfer genes into plant cells enables the generation of diverse lines of transgenic hairy roots, which are widely used for the production of valuable bioactive compounds as well as in fundamental plant science studies (Lin et al., 2025). Morphological variations among hairy roots include differences in primary root thickness, lateral root density, spontaneous callus formation, and other traits (Figure 1). The phenotype of hairy roots can vary depending on the specific transformation event, the integration site of T-DNA within the plant genome, the copy number of T-DNA, and ultimately the level of suppression or expression of aux and rol-related genes (Vinterhalter et al., 2019). Transformed roots are characterized by neoplastic growth in hormone-free media (non-geotropic growth) and extensive branching, features that facilitate the successful production of a broad variety of secondary metabolites (Phuong et al., 2018). Overall, hairy root culture represents an effective biotechnological approach for the conservation of unique, valuable, endangered, or endemic medicinal plant species. It not only contributes to biodiversity preservation but also enables the in vitro biosynthesis of high-value secondary metabolites under controlled conditions (Gutierrez-Valdes et al., 2020).

The quality and quantity of metabolites extracted from hairy roots are generally comparable to those found in natural herbs; however, their chemical profile can be optimized to enhance the levels of specific target compounds. Furthermore, in many cases, hairy roots are capable of accumulating novel compounds that are not detected in non-transgenic tissues (Wilczańska et al., 2023). Plant species have been successfully infected with *Agrobacterium rhizogenes*, and a variety of plant organs have served as suitable explants for infection (Table 2). The induction of hairy roots is not solely dependent on the genotype of the host plant; bacterial strain, infection method, and culture conditions also play critical roles. In general, the transfer of bacterial DNA into the plant cell genome occurs through four main steps:

1. Induction of vir gene expression and production of single-stranded DNA: Agrobacterium senses chemical compounds released from wounded plant tissues (e.g., wound-derived phenolics), which activate the bacterial vir genes.
2. Covalent attachment of T-DNA to VirD2 protein and formation of an effector protein complex: This complex, encoded by the vir genes, is excised from the bacterium and delivered into the plant cell.
3. Targeting of T-DNA and VirD2 proteins to the plant cell nucleus: Upon entry into the cytoplasm, the T-DNA strands, along with VirD2, are directed toward the nucleus of the host cell.

4. Integration of T-DNA into the plant genome: VirD2 and certain host proteins are removed from the T-DNA strands, and ultimately, the T-DNA is stably integrated into the host plant genome (Mirmazloum et al., 2024)

A schematic overview of this Agrobacterium-mediated transformation process is depicted in Figure 2. The detection of gene integration is typically performed using polymerase chain reaction (PCR) targeting rol genes. In addition, some studies employ reporter genes to assess the success of the transformation process (Ahmadpoor et al., 2022). The β-glucuronidase (GUS) reporter gene is commonly used to detect DNA integration in the plant genome, as it can be introduced into the host plant via Agrobacterium-mediated transformation (Banihashemi et al., 2020). However, the GUS assay is destructive, since the tested cells or tissues are destroyed during the reaction, preventing subsequent propagation or regeneration of the identified transgenic lines (Alcalde et al., 2022). This limitation is considered a major drawback of using GUS as a reporter gene. In contrast, the green fluorescent protein (GFP), derived from the jellyfish Aequorea victoria, is now recognized as the most widely used and advanced reporter gene in cellular and biochemical research. The key advantage of GFP is that it allows non-destructive, live visualization of transgenic tissues, enabling their recovery and propagation (Boccia et al., 2022).

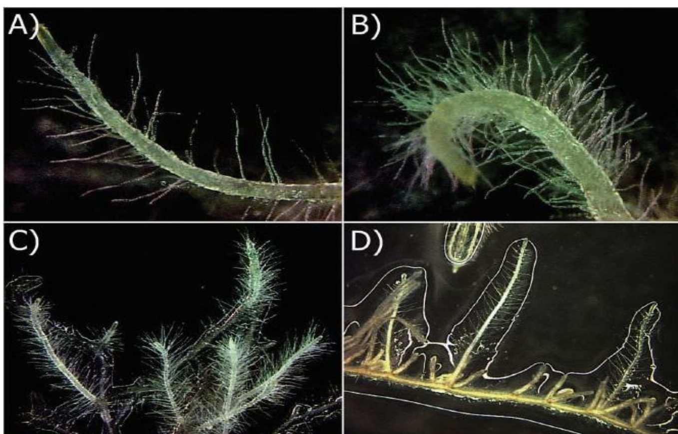

Figure 1. Phenotypic features of hairy root systems. A), B), and C) hairy roots; D) non-transgenic roots (Villar-Martínez et al., 2023).

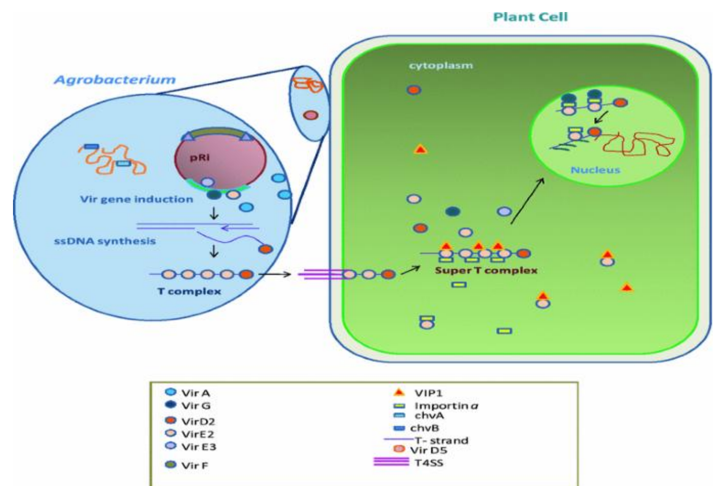

Figure 2. Overview of *Agrobacterium*-mediated plant cell transformation (Chandra, 2012).

Regeneration of plants from Ri plasmid-transformed hairy roots has been reported in numerous plant species. Morphologically, Ri-transformed plants are characterized by dwarfism, reduced stem length and internode elongation, and an increased number of nodes and leaves (Aghaali et al., 2024). The hairy root syndrome in these plants results from the integration and expression of rol genes located in the TL-DNA of the Ri plasmid. Among these, rolB and rolC genes are known as plast genes due to their ability to alter the growth and development of

regenerated plants derived from hairy roots. The biosynthesis of secondary metabolites is not always confined to roots or underground tissues. In many plants, precursors of medicinal compounds are produced in the roots, while the final products are synthesized in the leaves or aerial parts. In the ornamental plant industry, Ri-transformed plants have attracted increasing interest due to specific morphological traits, including dwarfing, enhanced branching, leaf curling, reduced apical dominance, and increased root growth, all of which are associated with the Ri phenotype (Kumar et al., 2025).

Table 2. Induction of hairy roots by diverse *Agrobacterium rhizogenes* strains in multiple plant explants.

Species	Explant	Strains	Reference
Salvia apiana	Hypocotyl	A4, LBA9402	Krol et al., 2025
Urena lobata L.	Leaves	ATCC 15834	Cao et al., 2024
Atropa komarovii	Leaves	ATCC 15834	Banihashemin et al., 2020
Rhodiola rosea	Leaves, stems, and rhizomes	ATCC43057	Martínez et al., 2020
Salvia bulleyana	Leaves and shoots	A4	Wojciechowska et al., 2020
Trigonella foenum-graecum	Hypocotyl	ATCC15834, R1000, A4 and C58	Zolfaghari et al., 2020
Gentiana urticulosa	Shoots	A4	Vinterhalter et al., 2019
Dracocephalum kotschyi	Leaves	ATCC 15834	Nourozi et al., 2019
Lemna minor	Leaves and root tips	MSU 440 harboring pBIN-YFP	Kanchanamala et al., 2019

As evident from the compiled studies, leaf and hypocotyl explants, along with strains like ATCC 15834 and A4, are among the most frequently and successfully utilized combinations for hairy root induction.

Agrobacterium rhizogenes as a Tool for Metabolite Engineering

Agrobacterium rhizogenes is widely used as an efficient biotechnological tool for enhancing secondary metabolite biosynthesis. Strains of this bacterium harbor a root-inducing (Ri) plasmid containing a segment of transferred DNA (T-DNA) that carries genes essential for initiating and sustaining root differentiation. Among these, the *rol* gene family plays a central role in root morphogenesis and the biosynthesis of bacterial-specific metabolites known as opines (Martínez et al., 2020). Extensive research has demonstrated that only a small subset of open reading frames (ORFs) within the T-DNA is indispensable for hairy root induction, proliferation, and the characteristic hairy root phenotype. Based on the features of their Ri plasmids, *A. rhizogenes* strains are classified into several types, including agropine, mannopine, octopine, and cucumopine (Ying et al., 2023).

Agropine-type strains possess Ri plasmids in which the T-DNA is divided into two functional regions: left T-DNA (TL-DNA) and right T-DNA (TR-DNA). TL-DNA contains the *rol* genes responsible for inducing hairy root formation, while TR-DNA carries genes associated with auxin metabolism and opine biosynthesis (Martínez et al., 2020). Deletions may occur near either T-DNA border during transfer, complicating precise identification of the integrated region (Phuong et al., 2028). Importantly, although TL-DNA and TR-DNA can independently integrate into the plant genome, TL-DNA incorporation is essential to initiate hairy root development. Opines are low-molecular-weight compounds formed by the condensation of amino-containing molecules with keto acids or sugars. Once produced in transformed tissues, they create a specialized biochemical niche that supports selective bacterial metabolism and growth (Zolfaghari et al., 2020). Growing evidence of bioactive compounds responsible for the therapeutic properties of medicinal plants has intensified efforts to explore new plant species and develop strategies to enhance the accumulation of valuable phytochemicals (Wojciechowska et al., 2020).

Hairy root cultures provide a rapid, genetically stable, and hormone-independent source of biomass, making them an effective system for producing metabolites

naturally synthesized and stored in root tissues (Malarz et al., 2023). The transformation process itself can reprogram plant metabolism, sometimes inducing the production of compounds absent in non-transformed roots. Notably, each hairy root clone originates from an independent transformation event, enabling the establishment of multiple transgenic lines, each with potentially distinct metabolic profiles (Liu et al., 2025).

Secondary Metabolites: Valuable Bioactive Compounds

Secondary metabolites (SMs) are small organic molecules produced by plants through specialized biosynthetic pathways. Although they are not directly required for basic growth and development, these compounds play essential roles in plant adaptation and survival under environmental stresses such as pathogen attack, UV radiation, and oxidative stress (Abdelazeez et al., 2022). Owing to their structural diversity and derivation from multiple branches of primary metabolism, secondary metabolites encompass major classes, including terpenes, phenolics, nitrogen-containing compounds (e.g., alkaloids), and glycosides. These metabolites are synthesized and accumulated in specific tissues or organs depending on the plant species, developmental stage, and environmental cues (Chandran et al., 2020).

Substantial evidence indicates that the health-promoting properties of plant-derived secondary metabolic products are largely attributed to their antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. Higher plants produce a broad spectrum of these bioactive compounds, including alkaloids, quinones, flavonoids, steroids, lignans, and terpenoids, which are widely used in pharmaceuticals, crop protection agents, cosmetics, flavorings, natural dyes, and food additives (Singh et al., 2022). Despite their biological and industrial significance, the mass production of these valuable compounds remains challenging due to several constraints:

- Environmental dependence: Their synthesis is tightly regulated by abiotic and biotic signals.
- Low natural abundance: Many high-value metabolites occur at very low concentrations in wild plants.
- Complex biosynthetic pathways: Production often requires multi-step enzymatic reactions.
- Threats to natural populations: Overexploitation of medicinal plants may endanger their survival (Wojciechowska et al., 2020).

In hairy root cultures, the accumulation of secondary metabolites is governed by two fundamental phases: biomass growth and metabolite biosynthesis. The first phase, biomass accumulation, directly affects the overall yield and is largely influenced by factors controlling cell and organ proliferation. The second phase involves the biosynthesis and accumulation of secondary metabolic products within the biomass, which is regulated by various physiological and biochemical parameters affecting secondary metabolic pathways (Zolfaghari et al., 2020). Hairy root cultures have been successfully employed for the enhanced production of numerous high-value secondary metabolites. Notable examples include tropane alkaloids in Atropa and Datura species (Banihashemi et al., 2020), diosgenin (a precursor for steroid hormones) in Trigonella foenum-graecum (Zolfaghari et al., 2020), rosmarinic acid (a potent antioxidant) in Salvia species (Krol et al., 2025), and paclitaxel (a chemotherapeutic agent) in Taxus (Sykłowska-Baranek et al., 2022) cultures. The capacity of hairy roots to produce these compounds at levels comparable to or exceeding those of intact plants, especially when optimized with elicitors or metabolic engineering, underscores their practical and commercial relevance.

Technological Strategies for Sustainable Production

Several technological strategies have been developed to enable the sustainable, large-scale production of pharmacologically important bioactive compounds using hairy root cultures. These approaches include optimization of culture media, supplementation with pathway precursors, elicitor treatments, membrane permeabilization, and metabolic engineering (Sykłowska-Baranek et al., 2022).

Elicitors are generally categorized as abiotic or biotic. Abiotic elicitors mainly include mineral salts and physical stimuli, whereas biotic elicitors encompass plant-derived signaling molecules—such as methyl jasmonate (MJ), salicylic acid (SA), and ethylene-releasing compounds—as well as microbial components, including chitosan, yeast or bacterial polysaccharides, mycelial extracts, and glycoconjugates. The regulatory effects of SA and MJ on hairy root development are illustrated in Figure 3. Certain amino acids, such as phenylalanine or cysteine, can also serve as metabolic precursors to enhance specific biosynthetic pathways (Vergara-Martínez et al., 2021).

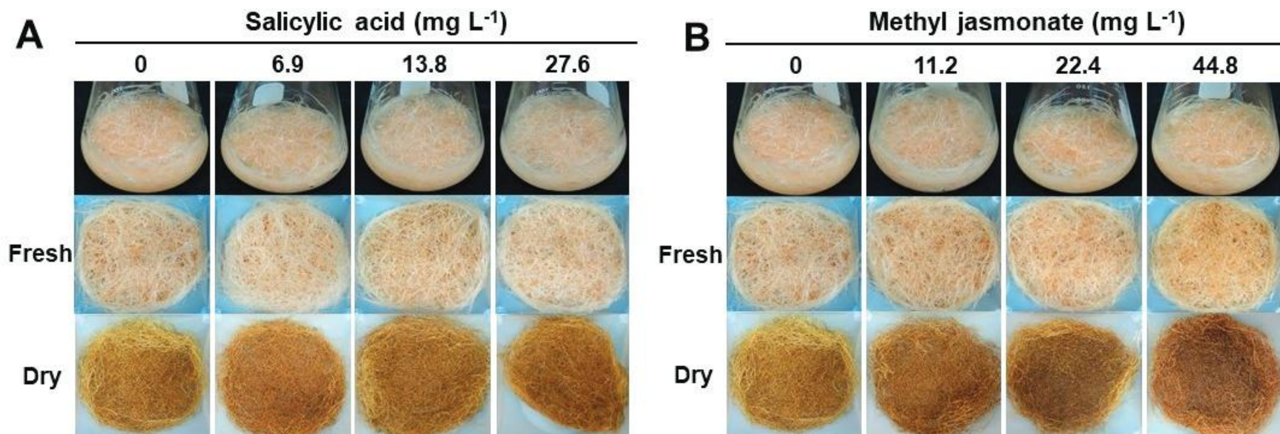

Figure 3. Regulatory effects of SA and MJ on hairy root development in *A. macrocephala* (Paek and Murthy, 2024).

In most plant tissue culture systems, secondary metabolites are retained within the cells, with only trace amounts secreted into the culture medium. This intracellular accumulation complicates downstream processing, as product extraction typically requires cell lysis, resulting in biomass loss and preventing continuous production (Quang et al., 2022). Therefore, strategies that enable metabolite secretion while maintaining cell viability are both technically and economically essential (Alcalde et al., 2022).

Permeabilization is an effective method to increase cellular permeability, facilitating the diffusion of intracellular metabolites into the culture medium. This can be achieved through physical treatments, such as heat shock, osmotic shock, ultrasound, or microwave irradiation, or via chemical agents, including surfactants and organic solvents. Chemical permeabilizers are widely preferred due to their operational simplicity and high efficiency (Quang et al., 2022). Compounds such as dimethyl sulfoxide (DMSO), Tween-20/80, Triton X-100, polyethylene glycol, and unsaturated fatty acids induce temporary changes in membrane structure, promoting metabolite release (Boitel-Conti et al., 1995). In some cases, low-polarity solvents like hexane or isooctane are applied to continuously extract secreted molecules (Liu et al., 2025). The effect of these agents depends on concentration and exposure time: low concentrations generally induce reversible increases in permeability, whereas high concentrations can cause irreversible membrane damage and cell death. An especially effective approach combines permeabilization with adsorbent resins, such as Amberlite XAD-2 or XAD-7. These resins capture secreted metabolites, simplify product recovery, and prevent the accumulation of toxic compounds in the medium (Kamiński et al., 2024).

The major advantage of controlled permeabilization is the potential for continuous metabolite production without destroying biomass. Secretion also alleviates feedback inhibition from intracellular product accumulation, often enhancing overall yields. Nevertheless, the selection of permeabilizing agents

requires careful evaluation of toxicity, stability, and biocompatibility, as well as assessment of long-term effects on cell viability before industrial application (Liu et al., 2025). In summary, controlled membrane permeabilization, especially when integrated with stabilization techniques and adsorbent phases, represents a key strategy for establishing efficient and sustainable bioprocesses for bioactive compound biosynthesis in plant cell and hairy root cultures (Kamiński et al., 2024).

Bioreactor-Based Cultivation

Large-scale in vitro cultivation of plant cells and hairy roots has emerged as a practical alternative for producing a wide range of high-value, low-volume phytochemicals. Consequently, over the past two decades, significant efforts have been devoted to designing plant-specific bioreactors (Jin, 2025).

Bioreactors—typically made of glass or stainless steel—are vessels in which living cells are cultured. Ideally, bioreactors are equipped with sensors to monitor pH, temperature, and dissolved oxygen, and allow sterile sampling, fresh medium addition, pH adjustment, aeration, agitation, and temperature control (Murthy et al., 2023). Compared with flask cultures, bioreactors provide more precise control and monitoring of culture conditions (Jin, 2025). Although basic requirements for plant cell cultures are similar to those for microbial submerged cultures, fermenters designed for microbial cells are generally unsuitable for plant cells due to substantial differences in growth characteristics. Therefore, modifications are applied to achieve more effective plant cell growth (Kim et al., 2003).

Efficient mixing of plant cells at a large scale is critical to maintain uniform physiological conditions within the culture (Kim et al., 2003). Proper mixing improves nutrient and gas transfer and facilitates oxygen delivery by breaking and dispersing air bubbles. While plant cells are mechanically stronger than microbial cells, their large size, thick cell walls, and large vacuoles make them sensitive to shear stress, limiting the use of high agitation rates (Jin, 2025). Thus, plant cells are typically agitated at very low speeds in modified stirred-tank bioreactors. Air-lift bioreactors may provide more homogeneous conditions with lower shear stress compared to stirred-tank systems (Kim et al., 2003).

All plant cells are aerobic and require a continuous oxygen supply. However, plant cells consume oxygen at a slower rate than microorganisms due to slower metabolism, and high oxygen concentrations may even be toxic. Air is usually sparged from the base of the bioreactor (Kim et al., 2003).

Hairy roots in liquid cultures tend to form aggregates. In late exponential growth, increased polysaccharide secretion makes roots sticky, causing adhesion to reactor walls, sensors, and impellers, eventually forming large clumps. These aggregates hinder mixing, create stagnant zones, interfere with sensor performance, and may block inlet and outlet ports. Aggregation can negatively affect growth and metabolite production, but some degree of aggregation and cellular differentiation is necessary for secondary metabolite synthesis. Measuring culture conductivity is used as an indirect method to estimate biomass growth (Murthy et al., 2023). Therefore, controlling hairy root aggregation is a major process engineering consideration (Chandran et al., 2020).

Currently, bioreactors used for transformed roots are broadly classified into two types: liquid-phase (immersed) systems, where roots are fully submerged, and gas-phase systems, where roots are primarily exposed to air or gas mixtures. Researchers have successfully optimized secondary metabolite production in various reactor configurations. The selection of a bioreactor design depends on whether the target metabolite is intracellular or extracellular. Based on mixing type, the main reactor types for plant cultures include stirred-tank, bubble-column, air-lift, and rotating-drum reactors (Jin, 2025) (Figure 4).

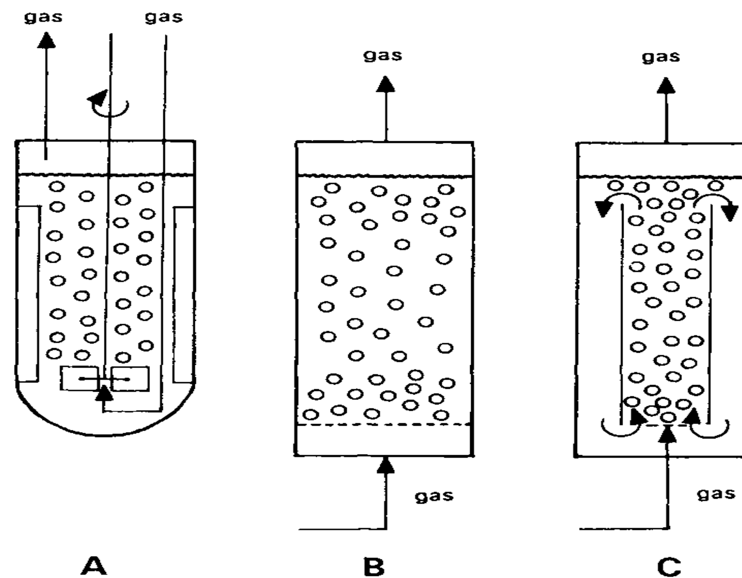

Figure 4. Major bioreactor configurations for large-scale hairy root production (Paek and Murthy, 2024).

Stirred-Tank Bioreactors

The stirred-tank reactor (Figure 4. A) represents the classical aerobic system, where air is dispersed by mechanical agitation. Its hydrodynamics are well-studied, allowing precise control of temperature, dissolved oxygen, pH, and nutrient concentrations (Jin, 2025).

A major disadvantage of STRs is shear stress generated by impellers, which can damage plant tissues (Murthy et al., 2023). Most laboratory and industrial STRs have been adapted for plant cultures by reducing agitation speeds (50–150 rpm), replacing Rushton blades with marine or paddle impellers, removing baffles, eliminating unnecessary sensors, and enlarging sampling ports (~1 cm) to prevent clogging by root aggregates. Recent studies indicate hairy roots can tolerate shear rates up to ~1000 s^{-1}, much higher than previously assumed. STRs also require high energy, are complex to construct, and can be challenging to scale up (Kim et al., 2003).

Bubble-Column Bioreactors

Bubble-column reactors (Figure 4. B) are among the simplest gas–liquid systems, used for aerobic cultivation (Jin, 2025). Air is injected at the base and dispersed pneumatically through nozzles or perforated plates. In small-scale reactors (<1 L), inadequate mixing can reduce growth rates. Advantages include easy sterilization due to the absence of moving parts, high mass and heat transfer without mechanical energy, suitability for shear-sensitive cells, scalability, and low maintenance. Limitations include poorly defined flow patterns and uneven mixing (Kim et al., 2003).

Air-Lift Bioreactors

Air-lift reactors circulate culture medium using density differences created by rising air bubbles (Jin, 2025). Injected air reduces medium density, causing upward flow in the riser tube, generating circulation (Figure 4. C). Air-lift reactors provide more uniform mixing than bubble-column systems, low shear stress, efficient oxygen transfer, absence of moving parts, reduced contamination risk, and lower operating costs. However, at high biomass densities, stagnant zones may form (Kim et al., 2003).

Conclusion

Hairy root in vitro cultures have emerged as a powerful biotechnological approach for the production of secondary metabolites and have been extensively investigated over recent decades. Many plant-derived compounds exhibit potent bioactivities, including cytotoxic, antimicrobial, antifungal, anti-inflammatory, and antiviral effects. Secondary metabolites, however, are

often confined to specific tissues or developmental stages, making their extraction from natural sources challenging and inefficient. Hairy root cultures offer an attractive alternative, providing a genetically stable, fast-growing, and hormone-independent system capable of producing high accumulation of root-specific metabolites under controlled conditions.

Transformation mediated by *Agrobacterium rhizogenes* can also induce the biosynthesis of novel secondary metabolic products absent in non-transformed tissues. Since each hairy root line originates from an independent transformation event, substantial metabolic diversity can be achieved among lines. Extraction yields from hairy root cultures can match or even surpass those of field-grown plants, particularly when combined with strategies such as elicitation. Hairy roots also enable the regeneration of whole plants, which may display altered morphological traits that can have ornamental value. Despite these advantages, the industrial-scale implementation of hairy root cultures remains limited due to challenges in bioreactor design, large-scale productivity, and process scalability. Nevertheless, this technology remains a highly promising approach for producing metabolites primarily synthesized in roots. Whereas many medicinal plants require 10–15 years to reach a metabolically active stage, hairy root cultures can achieve comparable biomass and metabolite accumulation within months. A SWOT analysis of hairy root culture technology reveals its distinct profile for industrial application, including strengths **(**Rapid growth, genetic stability, hormone-independent culture, ability to produce novel metabolites), weaknesses (Shear sensitivity, biomass aggregation, challenges in *Agrobacterium* elimination, complex downstream processing), opportunities (Integration with synthetic biology, advanced bioreactor designs, market demand for plant-based pharmaceuticals), and threats (Competition from microbial fermentation and synthetic biology platforms, regulatory hurdles, high initial investment costs). Consequently, this platform holds considerable potential for pharmaceutical, agricultural, and food applications and is expected to gain broader commercial adoption in the near future.

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