IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

"Secondary Metabolites In Plant Tissue Culture: A Comprehensive Review"

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Introduction

Plants produce a vast array of chemically diverse compounds that go beyond their immediate needs for growth, development, and reproduction. Among these are the **secondary metabolites** — small molecules that often serve as ecological mediators, defense agents, attractants, or signaling compounds in interactions with pests, pathogens, pollinators, or environmental stressors. Although these compounds are not essential for primary metabolic functions, they have immense ecological, pharmaceutical, agricultural, and industrial importance.

In traditional systems, many of these secondary metabolites are extracted from mature field-grown plants. However, this approach has several limitations: seasonal and geographical variability, slow growth, low yields, overharvesting of endangered species, and unpredictable quality. To overcome these constraints, **plant tissue culture techniques** have been increasingly explored as an alternative and sustainable strategy for producing secondary metabolites under controlled conditions.

Plant tissue culture harnesses the **totipotency** of plant cells — the capacity of a single cell to regenerate a complete plant or specialized tissues under the right conditions. Through methods such as callus culture, suspension culture, organ culture (e.g. root, shoot), and "hairy root" systems induced by *Agrobacterium rhizogenes*, researchers aim to create in vitro environments that favor enhanced production of targeted secondary metabolites.

One of the major advantages of in vitro production is the ability to **decouple metabolite synthesis from environmental fluctuations and seasonal constraints**. In cell or tissue cultures, factors such as light, temperature, nutrient supply, elicitors (biotic and abiotic), precursor feeding, and genetic manipulation can be precisely controlled to optimize yield.

Nevertheless, significant challenges remain. Cultured cells often yield **lower metabolite concentrations** compared to whole plants; **instability over successive subcultures** is common; scale-up to bioreactor systems is nontrivial; and understanding and engineering the regulatory pathways for metabolite biosynthesis in vitro is still evolving.

Recently, novel approaches such as **nanoparticle-mediated elicitation**, **co-culture with endophytes**, and **metabolic engineering/CRISPR-based editing** are showing promise in overcoming some of these bottlenecks, pushing the frontiers of what is possible with in vitro metabolite production

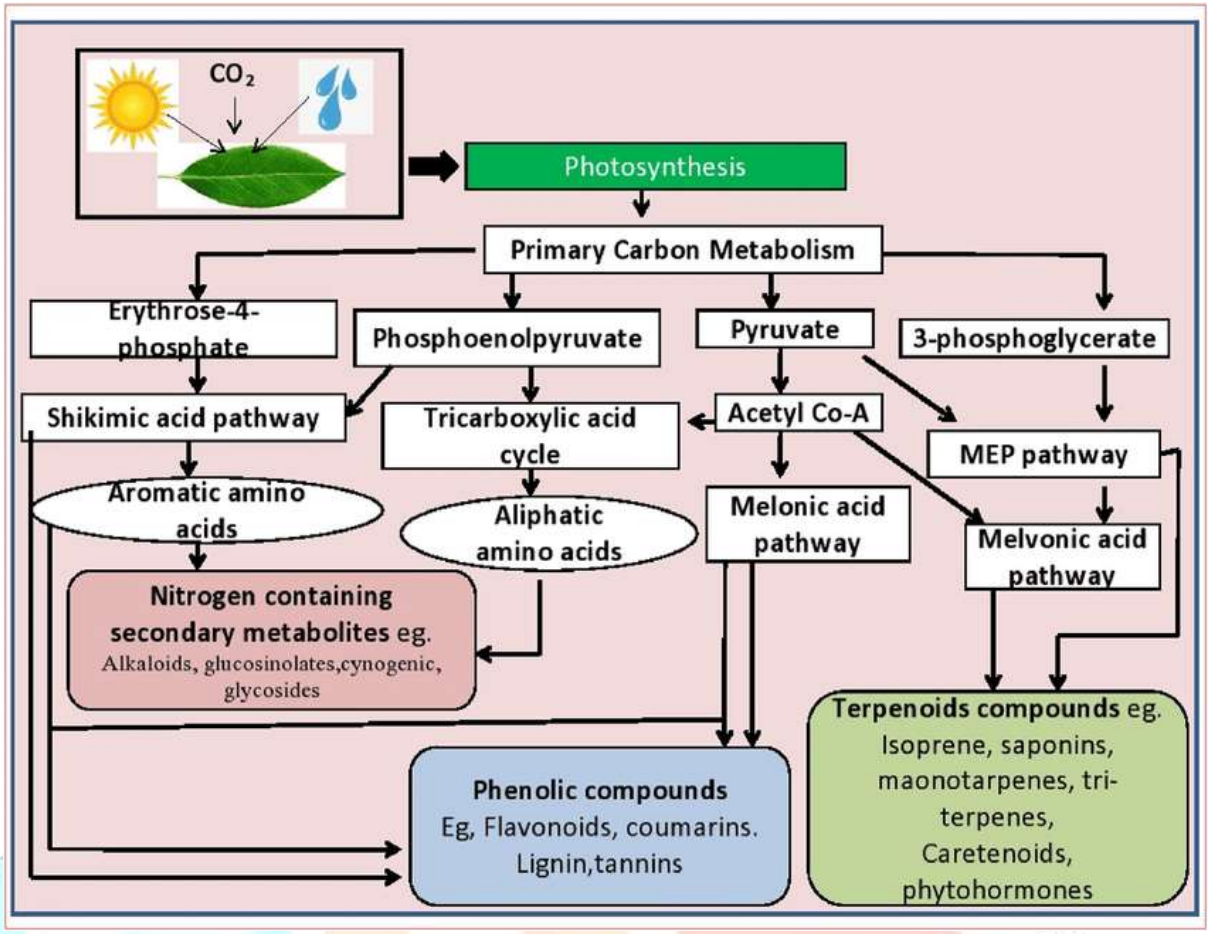
This review aims to provide a comprehensive synthesis of the current knowledge and progress on the production of secondary metabolites using plant tissue culture techniques. Key topics to be covered include classification and biological significance of secondary metabolites, in vitro culture systems employed,

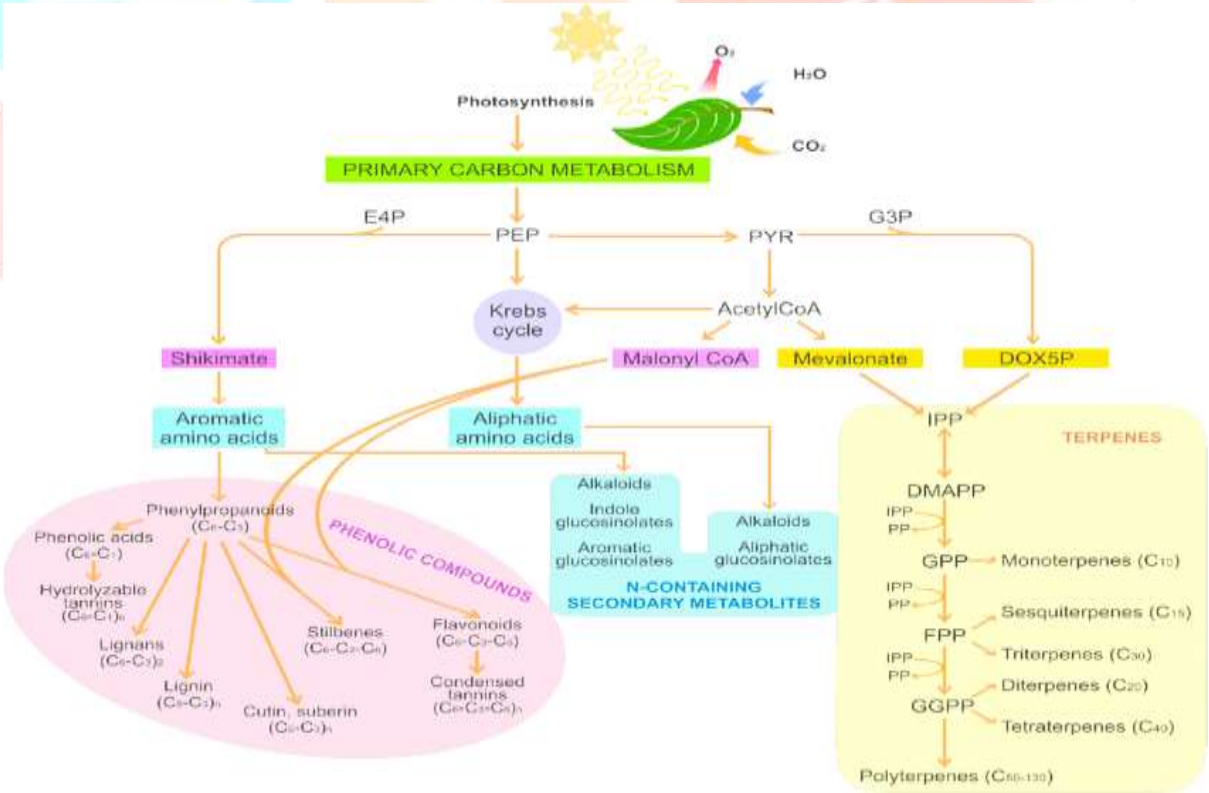
elicitation strategies, scale-up to bioreactors, case studies of high-value compounds, challenges and limitations, and future directions in the field.

Classification of Secondary Metabolites

Secondary metabolites are diverse in structure and function. In plant tissue culture studies, they are typically classified based on biosynthetic origin, presence/absence of nitrogen, or chemical structure. Below is a breakdown of the major classes, their biosynthetic pathways, examples, and relevance in tissue culture systems.

Class	Biosynthetic Pathway(s)	Typical Examples	Roles / Functions	Relevance to Tissue Culture
Phenolics (Polyphenols, Flavonoids, Tannins, Lignans etc.)	Shikimate pathway → phenylpropanoid pathway → flavonoid branch etc.	Flavonoids (quercetin, kaempferol), lignin, tannins, phenolic acids (e.g. caffeic acid)	Antioxidants, UV protection, pigmentation, defense against pathogens, structural (lignin) etc.	Very common in many culture systems; elicitors often boost phenolic/flavonoid production; simple to assay; often among first SMs increased when manipulating culture conditions.
Alkaloids	Derived from amino acids (e.g., tryptophan, tyrosine, ornithine etc.), through various specialized pathways	Morphine, caffeine, nicotine, tropane alkaloids etc.	Defense (against herbivores, microbes), signaling, sometimes allelopathy; many have pharmaceutical uses.	Alkaloid production often needs highly differentiated tissues or specialized culture types (e.g. root cultures, hairy roots); elicitation and precursor feeding are frequently used to increase yields.
Terpenoids (Terpenes, Steroids, Volatile Oils etc.)	Mevalonate (MVA) pathway, Non-mevalonate (MEP/DOXP) pathway etc.	Monoterpenes, sesquiterpenes (e.g. artemisinin), diterpenes, essential oils; steroids etc.	Volatile scent/aroma, defense, signaling, environmental interactions. Many are also medicinal (e.g. artemisinin) or aromatic/flavor compounds.	Terpenoid production sometimes more demanding; needs optimized culture conditions; volatile nature makes extraction/preservation tricky; bioreactors and specialized organ cultures may help.
Sulfur-containing compounds	Specialized pathways; often derived from amino acids (like cysteine, methionine) etc.	Glucosinolates, alliins (garlic), etc.	Defense vs pests/microbes; contribute to flavor/aroma.	Less common in many tissue culture studies, but when the plant species naturally produces them, can be manipulated similarly (elicitation, precursor feeding, etc.).
Other classes / Hybrid compounds	Mixed biosynthetic origins; often modifications (glycosylation, methylation, acylation etc.)	Glycosides, saponins, coumarins, phenylpropanoid hybrids, alkaloid-terpene hybrids etc.	Roles in storage, solubility, detoxification, additional bioactivities; some are more stable/soluble etc.	These compounds often require more complex regulation; sometimes the modification enzymes are weakly expressed in undifferentiated culture; may require genetic engineering or differentiated organs.





Connections & Observations from Recent Studies

- A recent review ("Production of secondary metabolites using tissue culture-based biotechnological applications", 2023) groups SMs into **three major classes**: phenolics, terpenes, and alkaloids.
- Another survey ("Production of Plant Secondary Metabolites: Examples, Tips and Suggestions for Biotechnologists", 2018) adds **sulfur-containing compounds** as a distinct class.

- The choice of which class to focus on in a culture system often depends on the species: e.g., *Artemisia* is used for sesquiterpene artemisinin; *Coffea arabica* for alkaloid caffeine; *Urtica dioica* in some studies for phenolic/lignan compounds.

Implications for Tissue Culture Work

- **Differentiation matters**: Differentiated organs or tissues (roots, shoots, hairy roots) often produce more of certain secondary metabolites, especially if their biosynthesis depends on tissue structure or specialized cells.
- **Species-specific pathways**: Even among similar classes, the specific enzymes, regulatory genes etc. differ, so culture conditions (PGRs, light, elicitors) need to be tailored.
- **Detection & quantification**: Some SMs are easier to detect (phenolics, flavonoids) with common assays; others (alkaloids, terpenoids) may need specialized chromatographic or MS techniques.
- **Engineering & elicitation**: For some SMs, yield in natural plants is low; tissue culture gives opportunities for metabolic engineering (overexpressing key enzymes, knocking out inhibitors), precursor feeding, elicitation.

Culture Systems Used in Plant Tissue Culture for Secondary Metabolite Production

Below are the main culture systems used, with their characteristics, examples of successful production of secondary metabolites, and considerations (advantages & limitations).

1. Callus Cultures & Cell Suspension Cultures

Description / Process

- **Callus culture**: Starting from explants (leaf, stem, root, cotyledon, etc.), using media with high auxin (often 2,4-D, NAA) and sometimes cytokinins to induce undifferentiated mass of cells (callus). Callus may be friable or compact.
- **Cell suspension culture**: Friable callus pieces are transferred to liquid medium; with agitation (shaker, spinner). Cells proliferate, can be subcultured; good for scaling up.

Examples of Metabolite Production

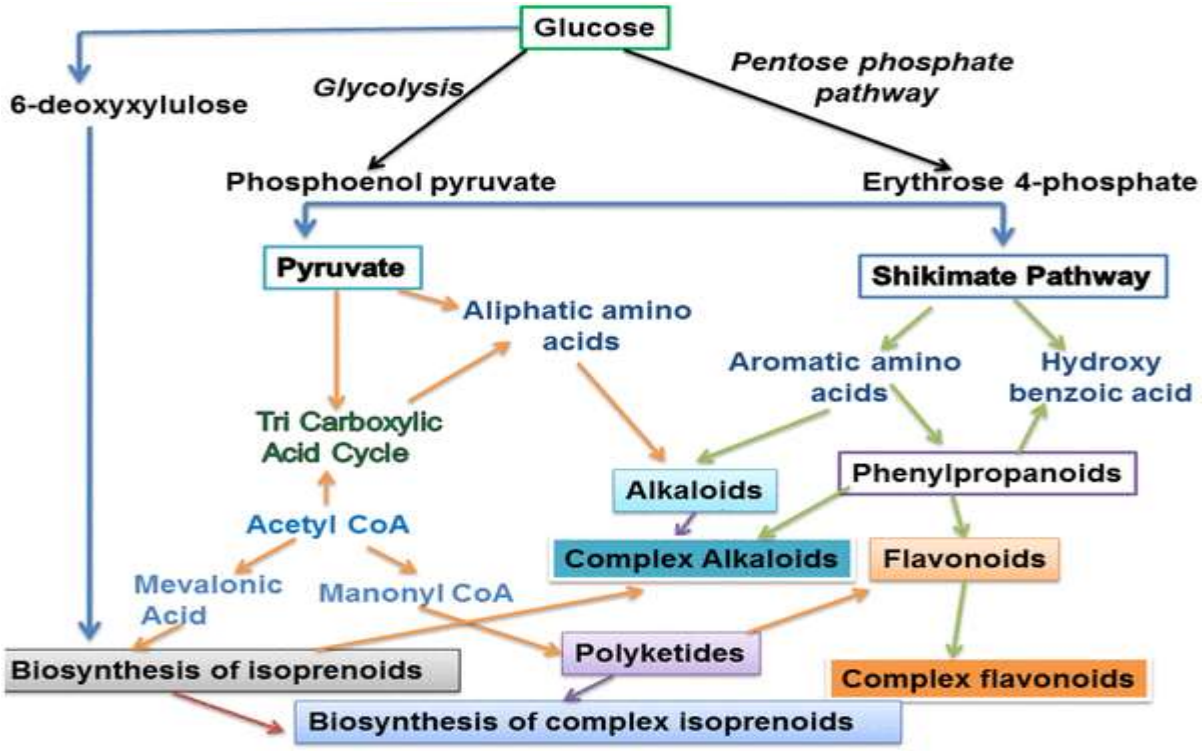
- *Salvia bulleyana* shoots & leaves: production of phenolics including caffeic acid, rosmarinic acid and derivatives.
- *Cullen corylifolium* cotyledon callus cultures: production of psoralen, daidzein, genistein.
- *Sphagneticola calendulacea* node/internode/leaf tissues producing flavonoids, phenolic acids via callus/suspension cultures.
- *Mentha piperita* callus cultures: higher accumulation of monoterpenes in vitro than in natural plants.

Advantages

- Relatively easy to establish.
- Good for screening, optimization of culture conditions (media composition, elicitors, PGRs).
- Scalable (especially suspension cultures) for biomass production.

Limitations

- Often lower yields of certain secondary metabolites compared to differentiated tissues (e.g. organs, hairy roots) especially for compounds synthesized in specialized cells. Callus cultures may show genetic and metabolic instability over long periods.
- Some SMs require tissue differentiation or specific architecture or cell types not present in undifferentiated suspensions.



2. Hairy Root Cultures (Transformed Root Cultures)

Description / Process

- Induced by infecting plant explants with *Agrobacterium rhizogenes* (Ri-plasmid), which transfers T-DNA into cell genome. Roots arising at wound site (hairy roots) grow rapidly, often without exogenous plant growth regulators (PGRs).
- Hairy roots are differentiated tissue; often retain or even enhance production of root-derived metabolites.

Examples of Metabolite Production

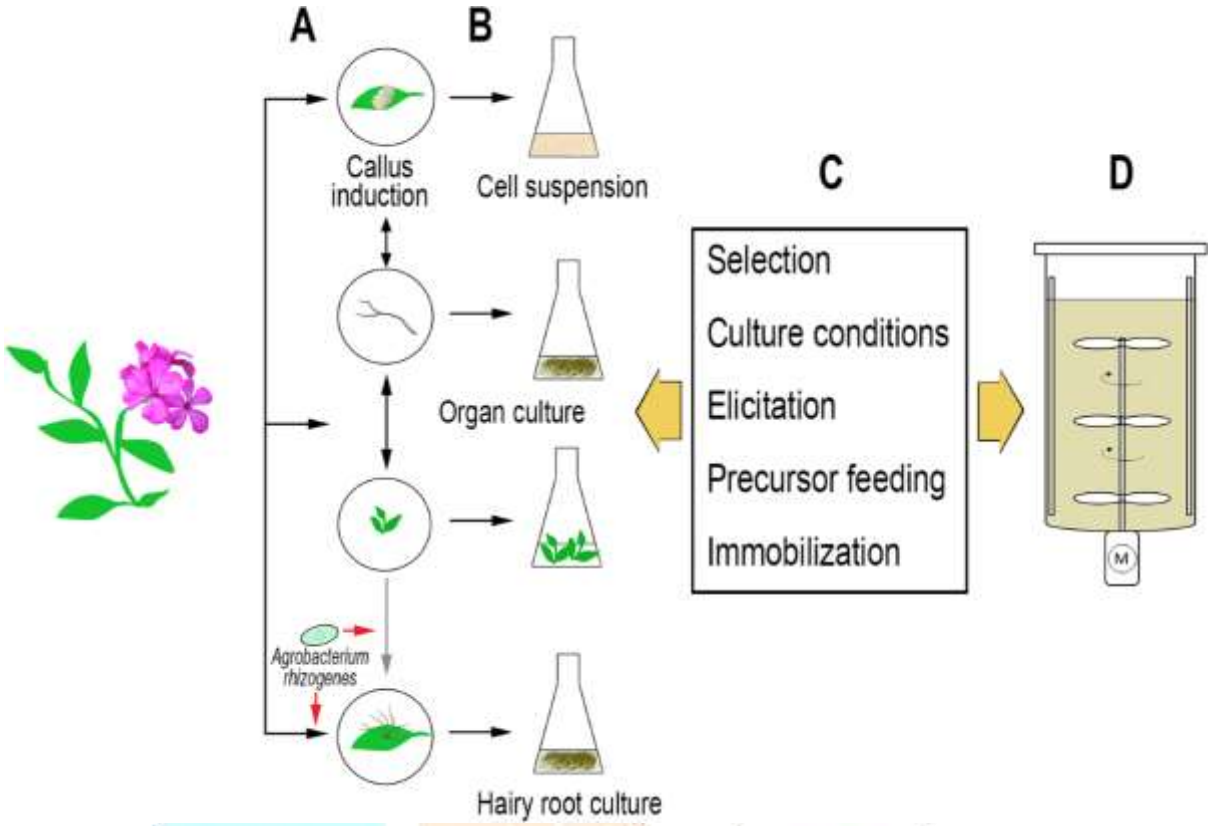
- *Catharanthus roseus*: hairy root cultures produce indole alkaloids (e.g. catharanthine, ajmalicine, etc.); there are systems interchanging between cell suspensions and hairy roots to optimize yield.
- *Salvia viridis*, *Perovskia abrotanoides*, *Rhazya stricta*, *Atropa komarovii* etc: production of various alkaloids, terpenoids, flavonoids in hairy roots.
- Other examples: *Centella asiatica* (triterpenoids) via hairy root cultures.

Advantages

- High genetic and biochemical stability compared to undifferentiated cell cultures. Often hormone-independent growth once established.
- Differentiated tissue structure helps in maintaining metabolic pathways that require tissue architecture.
- Sometimes comparable or even superior yields of compounds native to roots.

Limitations

- Establishment can be slower for some species, depends on susceptibility to *A. rhizogenes*.
- Scaling up hairy root cultures (e.g. in bioreactors) presents challenges: aeration, shear stress, support/matrix for root growth.
- Extraction of SMs from roots may require more complex downstream processing.



3. Organ Cultures (Shoots, Roots, Adventitious Roots, Embryos)

Description / Process

- Cultures where the organ or part of the plant is cultured in vitro (pieces of root, shoot, nodal segments, adventitious roots).
- Somatic embryogenesis (embryos formed from somatic cells) also falls in organogenesis; these may be used to regenerate whole plants or produce certain metabolites.

Examples

- Shoots or leaves of *Rehmannia elata* for iridoid and phenylethanoid glycosides.
- Shoots/leaves of *Salvia bulleyana* for phenolics.
- Adventitious roots and organogenic root cultures in some species used to produce root-associated metabolites.

Advantages

- Organs are more differentiated, can often produce metabolites more similar in quantity and profile to whole plants.
- Possibly more stable production (compared to callus).
- Can sometimes respond better to elicitors and precursor feeding.

Limitations

- Organ cultures often require plant growth regulators, careful control of conditions.
- Growth rates slower than in suspension or hairy root cultures.
- Scaling up can be more complex due to support structures, nutrient delivery, etc.

4. Other Systems & Hybrid / Support Systems

- **Bioreactor cultures**: Using vessels (stirred tanks, airlift, temporary immersion, etc.) for cell suspensions, hairy roots, organ cultures to scale up production while maintaining control over environment, aeration, shear. *"Production of secondary metabolites using tissue culture-based biotechnological applications"* has many examples.
- **Protoplast cultures**: less commonly used just for SM production; more for mechanistic studies, metabolic engineering.
- **Immobilized cultures / two-phase systems**: e.g., two-phase aqueous polymer systems to separate product continuously, or immobilization of cells to enhance stability.

Conclusion

Plant tissue culture has emerged as a versatile and powerful platform for the controlled production of secondary metabolites, offering a sustainable alternative to field-based cultivation, which is often limited by environmental variability, slow growth, and low metabolite yields. By exploiting the totipotency of plant cells, in vitro systems such as callus cultures, suspension cultures, organ cultures, and hairy root cultures enable precise manipulation of growth regulators, nutrients, elicitors, and physical conditions to steer metabolite biosynthesis. The diversity of secondary metabolites—ranging from phenolics and alkaloids to terpenoids and sulfur-containing compounds—highlights the need for species-specific and pathway-specific optimization strategies, as each class responds differently to differentiation state, culture media, and elicitation regimes.

Despite the substantial progress made, challenges remain in achieving yields comparable to whole plants, maintaining metabolite stability across subcultures, and scaling processes to bioreactors without compromising productivity. Recent innovations, including nanoparticle-assisted elicitation, metabolic engineering, and co-culture with microbial endophytes, are beginning to address these limitations and expand the potential of in vitro systems. As analytical tools, molecular techniques, and bioprocess engineering continue to advance, plant tissue culture is poised to become an increasingly reliable and economically viable platform for producing high-value secondary metabolites for pharmaceutical, agricultural, and industrial applications. Continued research integrating classical tissue culture with modern biotechnology will be essential for unlocking the full biosynthetic capacity of plant cells and for meeting growing global demands for natural bioactive compounds.

Suggested References

- Production of secondary metabolites using tissue culture-based biotechnological applications — I. I. Ozyigit, I. Dogan, A. Hocaoglu-Ozyigit, et al., *Frontiers in Plant Science*, 2023. Provides a comprehensive review of in vitro tissue culture techniques (callus, suspension, organ, hairy root, bioreactors) for SM production and discusses strengths, limitations, and biotechnological potential.
- PLANT TISSUE CULTURE METHODS IN SECONDARY METABOLITE PRODUCTION – A MINI REVIEW — Sakshi Sharma, Shivika Sharma, Sarvjeet Kukreja, Vikash S. Jadon & Vikas Sharma, *Plant Cell Biotechnology and Molecular Biology*, 2020.
- A concise overview of tissue culture methods relevant for metabolite production and their advantages when natural supply is limited. Plant Tissue Culture and Secondary Metabolites Production —
- Editorial article in *Plants* (2022), which introduces a collection of research on SM production via tissue culture and summarizes challenges and advances in the field.
- Plant Tissue Culture and Secondary Metabolite Production Volume II — Special Issue article (Plants, 2023) covering recent contributions to SM production via tissue culture and bioreactor strategies. Useful to cite for contemporary developments and state-of-the-art methodologies.
- Advances in Plant Biotechnology: In Vitro Production of Secondary Metabolites of Industrial Interest — Edited volume (CRC Press, 2024) covering biosynthetic pathways, scaling-up to bioreactor-level production, metabolomics of medicinal plants, and industrial applications of plant-derived metabolites. Recommended for deeper background and theoretical context.
- Harnessing metabolites from plant cell tissue and organ culture for sustainable biotechnology — Article in *Plant Cell, Tissue and Organ Culture* (2025), discussing modern elicitation strategies (e.g. nanoparticle-

mediated elicitation), advances in culture technologies, and future directions for sustainable production of SMs in vitro. Useful for bridging traditional tissue culture with cutting-edge methods.