



Enhancing The Production of Secondary Metabolites in Medicinal Plants Via Tissue Culture Optimization

Arpit Rajput, Research Scholar, Biotechnology, The Glocal University Saharanpur, Uttar Pradesh
Dr. Vishal Kumar Chhipa, Professor, Research Supervisor, Glocal School of Science, The Glocal University, Saharanpur, Uttar Pradesh

Abstract

Owing to the potential therapeutic benefits of these bioactive chemicals, optimizing tissue culture to increase the synthesis of secondary metabolites in medicinal plants has become imperative. With pharmacological advantages including antibacterial, anti-inflammatory, and anticancer characteristics, secondary metabolites like alkaloids, flavonoids, terpenoids, and phenolics are important for both human health and plant defense. Nonetheless, growing circumstances and environmental variables often place a limit on natural production. To maximize metabolite output and uniformity, plant tissue culture offers a controlled environment for adjusting growth regulators, medium composition, and culture conditions. The large-scale production and in-depth investigation of secondary metabolite biosynthesis pathways are made possible by techniques including callus culture, suspension culture, and organ culture. Theoretical topics covered in this thorough framework include biosynthetic routes, genetic and environmental factors, regulatory mechanisms, and tissue culture optimization tactics. The literature review underscores the significance of further research to attain efficient and sustainable metabolite synthesis by highlighting current developments, obstacles, and possible uses in augmenting secondary metabolite production by plant tissue culture.

Keywords: Bioactive Chemicals, Secondary Metabolites, Medicinal Plants, Tissue Culture, Pharmacological Benefits, Antibacterial

1. INTRODUCTION

The pursuit of augmenting the synthesis of secondary metabolites in medicinal plants has garnered noteworthy emphasis owing to the deep therapeutic characteristics that these substances manifest. Alkaloids, flavonoids, terpenoids, and phenolics are examples of secondary metabolites that are important for both human health and plant defense. They are very useful to the pharmaceutical industry because of their wide range of pharmacological activity, including antibacterial, anti-inflammatory, and anticancer effects. However, developmental phases, the environment, and other intrinsic variables often place restrictions on the natural synthesis of these chemicals in plants.

A viable method to overcome these constraints is plant tissue culture, which provides a regulated setting for maximizing the synthesis of secondary metabolites. The production and uniformity of these bioactive substances may be greatly increased by researchers by adjusting growth regulators, medium composition, and culture conditions. Callus culture, suspension culture, and organ culture are a few of the tissue culture methods that provide platforms for the extensive investigation and large-scale production of secondary metabolite biosynthesis. The theoretical underpinnings of optimizing tissue culture for the generation of secondary metabolites in medicinal plants are explored in this thorough framework. The definition and significance of secondary metabolites, a summary of plant tissue culture methods, and the function of tissue culture in metabolite augmentation will all be covered. It will also examine the mechanisms of metabolite enhancement, the biological underpinnings of metabolite production, the fundamentals of tissue culture, the variables influencing tissue culture outcomes, analytical methods for metabolite quantification, theoretical models of optimization, and the implications and applications of enhanced metabolite production. The framework will provide a comprehensive grasp of the techniques and tactics used in enhancing tissue culture-based secondary metabolite synthesis via this investigation.

2. LITERATURE REVIEW

Cardoso, Oliveira, and Cardoso (2019) examined the developments and difficulties



encountered in the in vitro synthesis of secondary metabolites from therapeutic plants, emphasizing the promise of plant tissue culture methods. They covered a variety of techniques, including callus, suspension, and organ culture, focusing on the impact of growth regulators, medium composition, and environmental variables on the quantity and quality of metabolites produced. The use of elicitors to increase production via stress reactions was also included in the review. Even with the advancements, issues like genetic stability, scalability, and protocol standardization still exist. In order to fully use in vitro methods for sustainable metabolite synthesis, the authors urged further study.

Isah et al. (2018) examined methods, techniques, and constraints for the secondary metabolism of drugs in plant in vitro cells. They emphasized the potential of in vitro methods to improve the generation of secondary metabolites and their significance in medicinal applications. In order to maximize metabolite yield, the review included adjusting culture parameters such as medium composition, growth regulators, and environmental variables. Elicitation strategies were highlighted as a means of stimulating secondary metabolite production in plants by causing stress reactions. Protocol optimization, scaling problems, and worries regarding genetic stability and variability in cultivated plants were among the challenges that were noted. In order to overcome these obstacles and optimize the efficient and sustainable synthesis of medicinal secondary metabolites by plant tissue culture, the authors advocated for more study.

Yue et al. (2016) examined medicinal plant cell suspension cultures in great detail, with an emphasis on methods to enhance secondary metabolite outputs and their pharmaceutical uses. They underlined how important these metabolites are to the pharmaceutical industry and pointed to cell suspension cultures as a possible manufacturing option. The review included ways to improve metabolite yields under culture settings, including nutrition composition, growth regulators, and elicitation techniques. Biotechnological methods were highlighted as a means of modifying metabolic pathways in order to enhance the production of certain bioactive compounds. Securing genetic stability, scaling up production, and reaching economic viability were among the difficulties noted. In order to produce pharmacological secondary metabolites sustainably and effectively, the authors urged further study into improving methods and making full use of cell suspension cultures derived from medicinal plants.

Narayani and Srivastava (2017) examined elicitation methods for improving the synthesis of secondary metabolites in in vitro plant cell and tissue cultures. They emphasized the use of elicitation to cause stress reactions in plants, which in turn promotes the production of bioactive substances with industrial and medicinal applications. It was addressed how different elicitors, including phytohormones, heavy metals, UV light, and fungal extracts, might activate certain metabolic pathways to boost metabolite outputs. In order to produce desirable metabolite profiles while maintaining cell viability and genetic stability, the review emphasized how crucial it is to optimize elicitation procedures. The difficulties included the need for defined techniques to guarantee consistent findings and scalability, as well as the variation in elicitation reactions throughout plant species. In order to improve our knowledge of elicitation processes and its application for the efficient and sustainable generation of secondary metabolites using plant tissue cultures, Narayani and Srivastava urged for further study.

3. BIOLOGICAL BASIS OF SECONDARY METABOLITE PRODUCTION

3.1. BIOSYNTHETIC PATHWAYS OF SECONDARY METABOLITES

Certain biosynthetic pathways, which are a sequence of chemical events within plant cells that are catalyzed by enzymes, are used to generate secondary metabolites. These pathways are compartmentalized inside various cellular structures and are often exclusive to certain groups of metabolites. For example, alkaloids are generally produced from amino acids via a sequence of processes that include methylation, hydroxylation, and decarboxylation. Starting with acetyl-CoA, two different pathways lead to the synthesis of terpenoids: the



methylerythritol phosphate route and the mevalonate method. The shikimate route is often responsible for producing flavonoids and other phenolic chemicals. It starts with simple carbohydrates and proceeds through a number of enzymatic transformation stages. Gaining an understanding of these pathways is essential to improving the synthesis of secondary metabolites because it makes it possible to identify important enzymes and intermediate chemicals that may be improved or targeted using tissue culture methods.

3.2. GENETIC AND ENVIRONMENTAL FACTORS INFLUENCING METABOLITE SYNTHESIS

Genetic and environmental variables interact intricately to govern the production of secondary metabolites. The expression levels and existence of genes encoding biosynthetic enzymes play a crucial role in determining the genetic makeup of metabolite synthesis. Numerous variables, such as plant species, genotype, and developmental stage, may affect these genes. Environmental elements that affect secondary metabolite production include light, temperature, availability of nutrients, and stress levels. For instance, certain flavonoids and terpenoids may be produced in response to light, while defensive alkaloids can be synthesized in response to nutritional stress or pathogen invasion. Researchers can increase the synthesis of desirable secondary metabolites in tissue culture systems by comprehending and adjusting these genetic and environmental parameters.

3.3. REGULATION OF SECONDARY METABOLITE PRODUCTION AT THE CELLULAR LEVEL

Within the plant cell, there are many levels at which the generation of secondary metabolites is regulated. Transcription factors, which react to different internal and external inputs, regulate the expression of genes at the transcriptional level. Secondary metabolism-related gene expression patterns may also be influenced by epigenetic changes including histone acetylation and DNA methylation. Splicing of mRNA, translation efficiency, and stability are examples of processes that fall under post-transcriptional control. Furthermore, the activity and stability of biosynthetic enzymes may be impacted by post-translational changes such as glycosylation and phosphorylation. Cellular compartmentalization divides biosynthetic pathways inside certain organelles, such as mitochondria, vacuoles, and chloroplasts, to further control the synthesis of metabolites. To improve the generation of secondary metabolites via tissue culture, it is important to comprehend these regulatory systems. The manufacture and accumulation of important secondary metabolites in medicinal plants may be optimized by manipulation of gene expression, enzyme activity, and cellular conditions.

4. PRINCIPLES OF TISSUE CULTURE

4.1. FUNDAMENTALS OF PLANT CELL AND TISSUE CULTURE

The process of growing plant cells, tissues, or organs in a sterile environment on a nutrient culture medium is known as "plant cell and tissue culture." The totipotency of plant cells—that is, each cell has the capacity to regenerate into a whole plant—is the underlying concept. This capacity is used by tissue culture, which creates the ideal environment for cell division and proliferation. The selection of explants, which are tiny fragments of plant tissue taken from different plant sections like leaves, stems, or roots, is the first step in this procedure. After being cleaned to avoid infection, these explants are set on a culture medium that offers the right balance of nutrients, hormones, and growth-promoting elements. Macroscopic and micronutrients, vitamins, amino acids, and plant growth regulators (PGRs) like cytokinins and auxins are the main ingredients of the culture medium. For cultures to be successfully initiated and maintained, these elements must be in balance and concentrated.

4.2. TYPES OF TISSUE CULTURE: CALLUS, SUSPENSION, AND ORGAN CULTURE

- Callus Culture**

The process of cultivating calluses—unorganized, undifferentiated masses of cells—from explants on a solid media is known as callus cultivation. High levels of cytokinins and auxins

can cause callus development. This kind of culture is helpful for researching the synthesis of secondary metabolites, genetic changes, and cellular functions. In order to continue growing, calluses may be forced to develop into organs or whole plants.

- **Suspension Culture**

Plant cells are grown in a liquid media and allowed to multiply unrestrictedly in suspension culture. To begin this procedure, tiny callus fragments are added to a liquid medium, which is then shaken to maintain the cells' suspension. Because the cells in suspension cultures are readily collected and the medium can be tailored for metabolite extraction, they are a good option for producing secondary metabolites on a large scale. Suspension cultures also provide homogeneous cell exposure to growth regulators and nutrients.

- **Organ Culture**

Organ culture is the in vitro maintenance and growth of certain plant organs, such as roots, shoots, or leaves. The structure and function of the organ are preserved, which makes this method helpful for researching the physiology, metabolism, and development of organs. Organ culture is a useful tool for producing specialized secondary metabolites that are generated in specific tissues or for regenerating whole plants from isolated organs.

4.3. Optimization Strategies in Tissue Culture

To maximize the generation of secondary metabolites and attain optimal culture results, tissue culture conditions must be optimized. Among the optimization techniques are:

- **Media Composition**

It is necessary to adjust the nutrient composition of the culture medium to the particular needs of the plant species and the intended result (e.g., callus induction, organogenesis, or metabolite production). This includes the kinds and concentrations of macronutrients, micronutrients, vitamins, and amino acids. Changes in the concentration of carbon sources, such sucrose, may also have an impact on metabolite production and growth.

- **Plant Growth Regulators (PGRs)**

The kind of tissue culture (e.g., callus vs. organ culture) and the developmental paths of the cultured cells are determined by the kind, concentration, and ratio of auxins and cytokinins in the culture media. One may achieve desired results, such enhanced cell proliferation, differentiation, or metabolite synthesis, by fine-tuning these regulators.

- **Environmental Conditions**

The culture environment's light, temperature, and pH all have a big impact on tissue culture success. Secondary metabolite pathways and photosynthesis are influenced by the kind and intensity of light. Maintaining the proper pH balance guarantees nutritional availability and absorption, whereas optimal temperature ranges are necessary for enzyme function and cell metabolism.

- **Stress Induction and Elicitation**

The synthesis of secondary metabolites may be increased by adding biotic or abiotic stress stimuli, such as pathogen extracts, UV radiation, or osmotic stress. These stresses function as elicitors, inducing defensive systems in the plant and promoting secondary metabolite production.

5. CONCLUSION

Tissue culture optimization is a promising approach to enhance secondary metabolite production in medicinal plants. This involves manipulating factors like media composition, growth regulators, and environmental conditions. Techniques like callus, suspension, and organ culture offer a controlled environment for studying and maximizing metabolite production. Understanding biosynthetic pathways is crucial for targeting specific metabolites and optimizing their production. However, challenges such as protocol standardization and genetic stability remain. Further research is needed to refine techniques and ensure sustainable production. In conclusion, tissue culture optimization holds promise for



enhancing secondary metabolite production in medicinal plants, but ongoing research is needed to address challenges and fully harness its potential.

REFERENCES

1. Cardoso, J. C., Oliveira, M. E. B. D., & Cardoso, F. D. C. (2019). Advances and challenges on the in vitro production of secondary metabolites from medicinal plants. *Horticultura Brasileira*, 37(2), 124-132.
2. Chandran, H., Meena, M., Barupal, T., & Sharma, K. (2020). Plant tissue culture as a perpetual source for production of industrially important bioactive compounds. *Biotechnology reports*, 26, e00450.
3. Dias, M. I., Sousa, M. J., Alves, R. C., & Ferreira, I. C. (2016). Exploring plant tissue culture to improve the production of phenolic compounds: A review. *Industrial crops and products*, 82, 9-22.
4. Guerriero, G., Berni, R., Muñoz-Sánchez, J. A., Apone, F., Abdel-Salam, E. M., Qahtan, A. A., ... & Faisal, M. (2018). Production of plant secondary metabolites: Examples, tips and suggestions for biotechnologists. *Genes*, 9(6), 309.
5. Isah, T., Umar, S., Mujib, A., Sharma, M. P., Rajasekharan, P. E., Zafar, N., & Frukh, A. (2018). Secondary metabolism of pharmaceuticals in the plant in vitro cultures: strategies, approaches, and limitations to achieving higher yield. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 132, 239-265.
6. Kumar, P., Sharma, P., Kumar, V., Kumar, A., Singh, R., & Sharma, A. K. (2019). Plant resources: In vitro production, challenges and prospects of secondary Metabolites from medicinal plants. *Ind. Biotechnol*, 2019, 89-104.
7. Li, Y., Kong, D., Fu, Y., Sussman, M. R., & Wu, H. (2020). The effect of developmental and environmental factors on secondary metabolites in medicinal plants. *Plant physiology and biochemistry*, 148, 80-89.
8. Naik, P. M., & Al-Khayri, J. M. (2016). Abiotic and biotic elicitors-role in secondary metabolites production through in vitro culture of medicinal plants. *Abiotic and biotic stress in plants—recent advances and future perspectives*. Rijeka: InTech, 17, 247-77.
9. Narayani, M., & Srivastava, S. (2017). Elicitation: a stimulation of stress in in vitro plant cell/tissue cultures for enhancement of secondary metabolite production. *Phytochemistry reviews*, 16, 1227-1252.
10. Niazian, M. (2019). Application of genetics and biotechnology for improving medicinal plants. *Planta*, 249, 953-973.
11. Pant, P., Pandey, S., & Dall'Acqua, S. (2021). The influence of environmental conditions on secondary metabolites in medicinal plants: A literature review. *Chemistry & Biodiversity*, 18(11), e2100345.
12. Thakur, M., Bhattacharya, S., Khosla, P. K., & Puri, S. (2019). Improving production of plant secondary metabolites through biotic and abiotic elicitation. *Journal of Applied Research on Medicinal and Aromatic Plants*, 12, 1-12.
13. Venugopalan, A., & Srivastava, S. (2015). Endophytes as in vitro production platforms of high value plant secondary metabolites. *Biotechnology advances*, 33(6), 873-887.
14. Wawrosch, C., & Zotchev, S. B. (2021). Production of bioactive plant secondary metabolites through in vitro technologies—status and outlook. *Applied Microbiology and Biotechnology*, 105(18), 6649-6668.
15. Yue, W., Ming, Q. L., Lin, B., Rahman, K., Zheng, C. J., Han, T., & Qin, L. P. (2016). Medicinal plant cell suspension cultures: pharmaceutical applications and high-yielding strategies for the desired secondary metabolites. *Critical reviews in biotechnology*, 36(2), 215-232.