SECONDARY METABOLITES PRODUCED BY PLANT CELL CULTURES, THEIR INDUSTRIAL USES AND AN OVERVIEW OF RESEARCH

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PREFACE

Plants have been used for many purposes since the ancient times of humanity. In ancient Egyptian civilizations, Chinese medicine or Ayurvedic treatments applied in India, the healing properties of plants were utilized. In addition to these properties, plants have also served many purposes as food supplements or as dyes in textiles and have become one of the most important parts of human life. In these uses, secondary metabolites contained in plants are utilized. Secondary metabolites are compounds that are necessary for the continuation of life, although they do not have a primary role in the basic vital functions of the living being. Today, there are many secondary metabolites defined in many plants and they are used for many purposes. They are frequently used for their therapeutic properties, followed by the cosmetics and food sectors. As the interest in the use of natural products increases, it is expected that the market volume will increase day by day. Classical plant cultivation techniques and collections made from the field are insufficient to meet this demand. Since plants in nature contain very low amounts of these secondary metabolites, a large number of plants are needed to obtain the appropriate amount for use, which also requires more land. In addition, production depending on climate conditions and changes in climate conditions due to global warming have caused production to fail to meet demand. Unconscious collections by the public have caused some varieties rich in secondary metabolites to be damaged and their populations to decrease. At this

point, plant cell, tissue and organ cultures have become important alternatives.

Plant cell, tissue and organ culture can be defined as the process of producing an explant taken from a plant under controlled conditions. With this method, production can be carried out independently of climate conditions, under sterile conditions and using a small area at any time. In addition, with this method, cells that produce secondary metabolites in particular can be produced and large-scale production can be made without the need for a full plant. With the use of this method, which is more economical and allows high-efficiency production in a small area, research on secondary metabolites has increased and continues rapidly today.

In this book we have prepared, the production of secondary metabolites by cell culture, their advantages and disadvantages, examples of secondary metabolites produced by plant cell culture on a commercial scale, and some methods used to increase secondary metabolite production are examined. We hope that this study, supported by current research, will guide researchers who want to gain information in the field.

03/06/2025

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Introduction

Since the 1980s, various secondary metabolites and products commercially produced via plant cell culture techniques have been utilized primarily in cosmetics, pharmaceuticals, biopharmaceuticals, and food industries. There are many in vitro studies in which valuable secondary metabolites are produced using plants as expression systems. In recent years, many successful studies have been carried out to increase the yield in cell suspension cultures. These advancements encompass the optimization of gene expression constructs and production process, identification of high efficiency production systems, improvement of more efficient protein extraction and purification methods, and more.

In this context, it has become possible to integrate synthetic biology with genetic and metabolic engineering studies such as CRISPR/Cas9 systems, ZFNs (zinc finger nucleases) and TALENs (transcription activator-like effector nucleases). Another effective pathway used in the production of valuable secondary metabolites found in plant tissues and organs is biotransformation. For the production of secondary metabolites, edible plant species such as alfalfa (*Medicago sativa*), rice (*Oryza sativa*) and carrot (*Daucus carota*) have been utilized for many years. Additionally, the BY-2 (Bright Yellow-2) cell line derived from tobacco (*Nicotiana tabacum*) is the most commonly used for recombinant protein production.

Plant secondary metabolites are known to be beneficial in treating various diseases, including cancer, cardiovascular diseases, diabetes, arthritis, neurological disorders and COVID-19. With the spread of

research on advanced new methods such as "molecular farming", alternative methods have been developed in the production of plant products of high commercial importance. Factors such as societal trends, health, climate change, and social mobility significantly impact consumer behavior due to advancements in food culture and the food industry. Plant cell cultures offer innovative solutions for the commercial production of bioactive compounds beneficial to health, as well as compounds responsible for food flavor and taste.

In this book, the application areas of plant cell cultures, their potential use in secondary metabolite production, examples of important secondary metabolites produced on a large scale and commercial products, and various approaches to increase secondary metabolite production are discussed.

1. Plant Cell Culture

Plants are sources of important secondary metabolites that are used as defense mechanisms against various pests or diseases and are also of great medical importance and can be used in the treatment of many diseases (Gantait et al., 2024). plants are used for the production of valuable secondary metabolites in various applications in nutritional, medical, cultural and cosmetic fields since ancient times (Krasteva et al., 2020). Today, production of plant cells and tissues under *in vitro* conditions are considered a promising, renewable, environmentally sustainable alternative for the production of valuable plant bioactive components (Motolinía-Alcántara et al., 2021). Humans today face various global crises such as global climate change, lack of access to

clean water, increasing energy demand, limited food supply and inability to achieve sustainable development. Plant cell culture provides the potential to overcome the challenges by reducing the carbon, water and energy footprint of sustainable plant production. (Krasteva et al., 2020).

Plant cell cultures offer a unique process that attracts the attention of many researchers because they can provide products that cannot be obtained from bacterial or animal cells in biotechnology (Furusaki et al., 2017). Since they integrate plant cultivation systems with the properties of microbial and mammalian cell cultures, plant cell cultures also provide a promising and alternative bioproduction platform for therapeutic proteins (Karki et al., 2021). In addition, biotransformation of various biological compounds such as terpenoids and steroids can be carried out via plant cells, which are natural producers of alkaloids and anthocyanins (Furusaki et al., 2017). Plant cell cultures, which are low-harm to the environment and provide economic advantages, have the potential to be used as biofactories to produce a wide variety of compounds (Sanchez-Muñoz et al., 2019). Plant cell cultures can be evaluated as biotechnological platforms for the commercial production of recombinant proteins such as biopharmaceuticals (Nausch et al., 2021). They have some important advantages over traditional cell cultures, such as cheaper and easier supply of nutrient media and the ability to perform complex post-translational modifications such as glycosylation (Varma et al., 2021). Plant cell cultures, especially genetically modified plants, can also be used for the production of proteins. In addition, plant cell cultures allow the preparation of

synthetic seeds for seedling production, thus increasing the potential for the production of various medicinal and aromatic plants that are especially useful in agricultural terms (Furusaki et al., 2017). Thus, with the development of synthetic seeds, long-term storage is possible as well as seed production independent of the season (Mehbub et al., 2022).

Since the 1980s, a variety of commercial products have been available that are produced by aseptically isolated plant cell and tissue cultures grown under specific chemical and physical conditions. Although most of them represent components of products used in the pharmaceutical, cosmetic and food industries (Gubser et al., 2021), the production of secondary metabolites by plant cell cultures has many important advantages over traditional agricultural production with plants. These methods enable the production of secondary metabolites *in vitro*, independent of season, and enable a controlled standard process. In addition to their very low carbon footprint and the amount of water they require, they do not require the use of pesticides and herbicides, so their impact on the ecosystem is extremely low (Eibl et al., 2018). With the help of these systems, differences in product quantity and quality encountered in plants collected or grown from nature are eliminated, and the use of protected and endangered plant species becomes possible. By changing plant cell and tissue culture metabolism, the formation of compounds that are beneficial to the consumer can be supported, while harmful compounds can be reduced or even suppressed (Gubser et al., 2021).

Methodological studies on culturing various cells, tissues and organs taken from plants under in vitro conditions have been successfully carried out by many researchers to date (Wawrosch et al., 2021). Stem cells, which are found in the meristematic tissues of plants and have an undifferentiated structure, are precursor cells that have the potential to differentiate and form different plant tissues and parts. The characteristic features of these cells are their ability to renew themselves and to form differentiated cells. These plant stem cells, which do not enter the senescence or aging period, differentiate into other plant cells, whether specialized or non-specialized. It is assumed that stem cells help plants survive in harsh life cycle and thus preserve plant life (Aggarwal et al., 2020). Plant stem cells are found in apical, lateral and cambial meristematic tissue. Although there are more studies on apical and lateral meristems, there are very few products obtained by culturing cambial mersitematic stem cells. Cambial meristem stem cells show high genetic stability as well as rapid and uniform growth. However, the amount of secondary metabolites is low, which leads researchers to search for more efficient alternatives (Krasteva et al., 2020). Maintaining cell cultures under aseptic controlled conditions can provide high reproducibility of results, and especially cell cultures stand out as model systems for the study of many different processes such as cell differentiation, organogenesis, embryogenesis, production processes, cellular responses to endogenous and exogenous factors, programmed cell death, metabolome, etc. (Puzanskiy et al., 2024).

1.1. Use of Micropropagation in Secondary Metabolite Production

Micropropagation is the process of vegetative growth and reproduction of plant tissues under aseptic conditions and in synthetic nutrient medium. The basis of this process is based on totipotency, which is defined as the potential of plant cells and tissues to form a complete plant (Chandana et al., 2018). *In vitro* micropropagation methods can serve as valuable methods for producing secondary metabolites (Figure 1). Although plant cell cultures are generally the method used in secondary metabolite production, organ cultures or micropropagation techniques are also preferred in some studies for secondary metabolite production. In particular, hairy root cultures are a frequently preferred method. Researchers have determined that shoot cultures (shooting teratoma) also provide production with similar characteristics to hairy root cultures. In general, high levels of alkaloids are obtained in hairy root cultures, and monoterpenes are obtained from shoot teratomas (Fazili et al., 2022). In addition to classical micropropagation techniques, the use of hairy root cultures and bioreactors provide great advantages in maximizing the production of the desired secondary metabolite (Biswas et al., 2025).

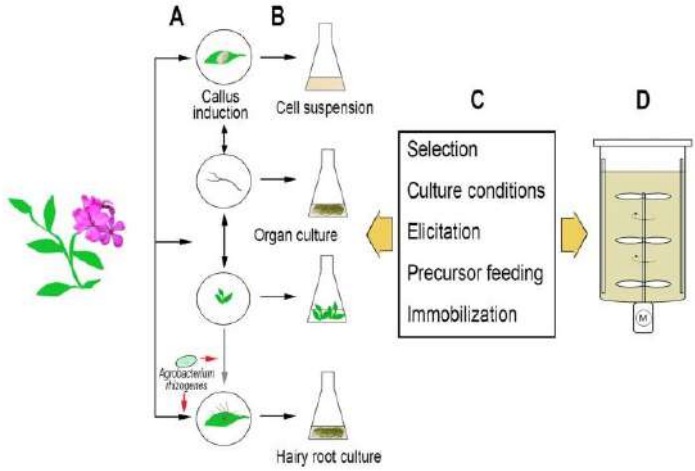

Figure 1. Plant cell and tissue culture methods used for secondary metabolite production (Wawrosch and Zotchev, 2021). A) Initiation of in vitro culture. Explants can be used for callus formation or direct organ formation can be provided. In addition, callus regeneration can also occur in organs (shoot, root, etc.). Hairy roots are obtained as a result of infection of the explant with *Agrobacterium rhizogenes*. B) Proliferation and establishment of liquid cultures. C) Optimization of bioprosess D) Large scale production in bioreactor

Hairy root cultures are an important production type used for the production of secondary metabolites by *Agrobacterium rhizogenes* in plants whose roots and rhizomes are used for medicinal purposes. It is a preferred culture system with its advantages such as short growth cycle and genetic stability. Today, this method is preferred in the production of many secondary metabolites. Commercial-scale production of *Panax ginseng*'s Rg1, Re and Rf is carried out in this way. Hairy root cultures are also preferred in the production of *Scutellaria baicalensis*, which is rich in flavonoid content (Zhang et al., 2025).

1.2. Comparison of Plant Cell Cultures with Cell Cultures Based on Different Organisms in Terms of Secondary Metabolite Production

People have used plants in the treatment of many diseases from prehistoric times to the present day. The majority of plants produce many molecules that do not directly contribute to their growth and development but are necessary for their interaction with the environment and defense systems. These molecules are called "secondary metabolites" (Shunti and Bhgaradvaja, 2024). Plant cell culture is a production system with significant advantages to produce secondary metabolites. Compared to traditional collection methods, it allows controlled production of plant cells, does not require extensive land use, and does not harm natural habitats. With this method, high-quality secondary metabolite production can be achieved throughout the year (Wawrosch and Zotchev, 2021; Oluyemi et al., 2024). The most important advantage of plants produced in plant cell cultures compared to plants grown in the field is that they are not dependent on seasons, are predictable, and are reliable. This type of production is particularly interesting when a plant species grows slowly or is difficult to grow and the amount of the desired compound in plants is extremely low. Sometimes, it has been determined that *in vitro* secondary metabolite accumulation can exceed the content in intact plants with production optimization (Wawrosch and Zotchev, 2021). Production models using plants as expression systems have been developed for the large-scale production of many important proteins produced for use in the

pharmaceutical, nutraceutical and cosmetic sectors. These production models are relatively low-cost, free of human pathogens, capable of synthesizing complex proteins with post-translational modifications and also capable of scale-up (Kulshreshtha et al., 2022).

In industrial production, many factors are effective in the production of certain specific compounds from plants under *in vitro* conditions. Although these compounds are often complex, most of them have simple chemical structures. On the other hand, the chemical synthesis of a relevant compound may be more economical than both the classical method and plant-based production by *in vitro* culture. When choosing the appropriate method for the extraction of this valuable compound from the plant; criteria such as raw material price, concentration of the desired natural product and the cost of the extraction/purification process, market size and legal procedures, etc. should be carefully examined (Lange, 2018). In Table 1, plant cell cultures are compared with cell cultures based on different organisms in terms of secondary metabolite production (Wu et al., 2021).

Table 1. Comparison of cell cultures based on different organisms in terms of secondary metabolite production (Wu et al., 2021).

Species	Advantages	Disadvantages
Bacteria	• The genetic structure was simple • Genome sequencing, transformation and scaling up are easy • Fast growth • More advanced culture system	• Bacterial endotoxins require carbon sources and precursors • Difficult to handle post-translational modification • Inefficient CYP450 due to lack of endoplasmic reticulum • Inhibition may be observed due to the substrate or product present in the medium.
Fungi	• The genetic structure was simple • Transformation is easy • Fast growth; • More advanced culture system	• Requirement for carbon source and precursor • Multiple genetic engineering required issues with post-translational modifications
Algea	• The genetic structure was simple • Scale-up is easy • Photoautotroph • Post-translational modification is easy • More advanced culture system	• Inadequate genetic engineering tools • The growth cycle takes a long time

Plant cell	• Scale-up is easy • Genetic transformation available • Growth is relatively faster. • Post-translational modification available • Cyt P450 available • Continuous product supply • Production with transgenic plants involves fewer parameters compared to whole plants.	• For production, a carbon source and, in some cases, precursors are usually needed. • Except for model plants, plants have complex genetic structures. • The genetic structure is unstable.
Plant in the field	• The production pathway for metabolites is generally not required • Less gene modification is needed • High tolerance to toxicity • Enabling large-scale production	• Low yield • Plant destruction, subsequent processing may be difficult • The growth is slow • Requires large amounts of natural resources
Chemical synthesis	• Scale-up is easy • Easy synthesis • Fast	• Difficulty in the synthesis of secondary metabolites containing multiple chiral centers • Artificially labeled hazardous chemical

2. Production of Commercially Important Secondary Metabolites in Plant Cell Cultures

Plants synthesize a huge variety of organic compounds, defined as secondary metabolites, which do not directly participate in their growth and development, but play important roles in the interactions between plants and their environment, as well as in their defense (Fazili et al., 2022). Plant secondary metabolites, also called phytochemicals or plant-derived medicinal compounds (PDCM), constitute an important source of active compounds used in these sectors, especially in the pharmaceutical, food, cosmetic and agricultural industries (Abdulhafiz et al., 2022; Clapa et al., 2022). In plants, secondary metabolites can usually be detected in all cells of the plant, but in most cases the site of biosynthesis is a specific organ. After synthesis, they are transported to different regions according to the polarity of the metabolite via vascular tissues or to storage sites via symplastic and apoplastic transport. Lipophilic compounds such as terpene-based essential oils are stored in resin canals, trichomes, cuticles or thylakoid membranes, while hydrophilic compounds such as glucosinolates, tannins or alkaloids are stored in vacuoles or idioblasts. Various storage sites, such as tissues and structures, include specialized accumulation sites such as leaves, somatic embryos, shoots, roots, calluses, flowers, glandular trichomes, periderm, and phloem (Isah, 2019). It has been determined that secondary metabolites have a defense role against biotic and abiotic stress factors and a binding role for beneficial organisms such as pollinators and symbionts. In addition, studies have proven that they act as plant growth regulators in the fight against stress

factors and as regulators in signal transduction and gene expression (Abdulhafiz et al., 2022; Hilal et al., 2024). Secondary metabolites used in the treatment of various diseases such as cancer, arthritis, diabetes, neurological and respiratory disorders also have important effects against bacterial, fundal and viral infections (Kulshreshtha et al., 2022).

In recent years, biotechnological methods such as plant cell and tissue culture applications or genetic transformation techniques have become the preferred methods for the production of high quality and quantity of secondary metabolites. Valuable plant metabolites can also be isolated from plants grown in natural conditions. However, environmental factors such as seasonal constraints, soil selection or growth conditions limit commercial production. Traditional methods are time-consuming and, in some cases, it can take years to reach the required metabolite level. However, plant cell and tissue culture techniques allow for uniform and high-quality biomass production in short growth cycles, regardless of season and geographical characteristics (Clapa et al., 2022). In plant cell culture technology, single cells cultured in liquid media are used to optimize the production of the desired natural product and to create cell suspension cultures. The creation of cell suspension cultures usually begins with the transfer of loose callus, which can be easily divided into small pieces, to the liquid medium in a culture vessel and placing it on an orbital shaker. As cells divide and multiply, they spread into the liquid medium and form a cell suspension culture. After a period of 2-3 weeks, the suspended cells are transferred to a new nutrient medium, while larger pieces are discarded. The division rate of cells in suspension culture is higher than that of

cells in callus culture. In this way, cell suspension has become a suitable option when rapid and large numbers of cells are needed (Abdulhafiz et al., 2022).

There are studies and developed protocols for the commercial production of secondary metabolites by cell suspension culture in many plant species. Secondary metabolites of commercial value are usually found in a specific organ in the plant and are in very low amounts. Therefore, plant cell cultures, chemical synthesis and transgenic microorganisms have been proposed as alternative platforms for large-scale production of specific metabolites (Wu et al., 2021).

2.1 Some Secondary Metabolites Produced by Plant Cell Cultures for the Pharmaceutical and Cosmetics Industry

Medicinal plants have been a subject of curiosity since the earliest years of humanity. It is possible to access historical records of the use of medicinal plants in almost every civilization. In addition to the different effects of these secondary metabolites found in plants, their medical benefits have been better understood over time and they have been used as precursors in the production of many drugs. The use of medicinal plants instead of the use of chemicals in the production of medicinal drugs has prevented problems in many applications where side effects are seen (Chandana et al., 2018).

Secondary metabolites are generally classified into 3 main categories according to their biosynthesis pathways: phenolics, terpenes and alkaloids. It is possible to say that there is a wide variety of

secondary metabolites under these main headings. These secondary metabolites, which have a huge variety, are used as raw materials in the pharmaceutical and cosmetic sectors, as additives in the food sector and as preservatives in agriculture (Özyiğit et al., 2023). Plant cell cultures are used in the production of many valuable secondary metabolites for the pharmaceutical and cosmetic sectors, including paclitaxel, shikonin, digoxin, and ginsenosides (Rani and Vimolmangkang, 2022). These methods are sustainable ways used in the production of useful secondary metabolites. In many plant species, secondary metabolite production is increased by applying various elicitors such as salicylic acid, methyl jasmonate, chitosan and heavy metals to callus cultures. There are several examples where plant cells are used as biofactories and valuable secondary metabolites are successfully produced (Fazili et al., 2022) (Table 2).

Table 2. Bioactivity status and yields of some secondary metabolites produced in *in vitro* plant cell cultures

Compound	**Biological activity/Pharmaceutical Use**	**Plant**	**Extraction yield***	**Culture type****	**Reference**
Shikonin	Anti-inflammatory, anticancer, antibacterial, antitumor and antioxidants	Cynoglossum columnae Tenn.	-	RC	Jeziorek et al., 2019
		Echium plantagineum L.	36.25 mg/L	HRC	Fu et al., 2020
Flavonoid		*Glycyrrhiza uralensis*	132.36 mg/L	CSC	Guo et al., 2013
Anthraquinones	Colorant, laxative properties antimikrobiyal	*Morinda citrifolia*	103.08 mg/g DW	RC	Baque et al., 2013
		Rubia cordifolia	50 mg/g DW	CC	Veremichik et al., 2019
		Senna obtusifolia (L.)	38.125 mg/g DW	HRC	Kowalczyk et al., 2021
		Gynochthodes umbellata (L.)	8.330 mg/g FW	RC	Anjusha and Gangaprasad, 2016
Vinblastine	antitumor activity	*Catharanthus roseus* (L.)	4.09 µg/mg DW	CSC	Pliankong et al., 2018

Rosmarinic acid	Anti-inflammatory, analgesic antimicrobial, antileukemic, mutagenic, hepatoprotective, antioxidant, gastroprotective, antifungal, antimalarial, hypotensive, and antiviral	*Ocimum basilicum* L.	15.73 ± 0.28 mg/g DW	CSC	Pandey et al., 2019
		Origanum vulgare	31.25 mg/g DW	CSC	Li et al., 2021
		Satureja khuzistanica	180.0 mg/g DW	CSC	Sahraroo et al., 2015
Berberin	Anti-inflammatory, antitumor, lower blood lipid, lower blood sugar, anticancer, antibiotic and anti-osteoarthritis	*Coscinium fenestratum* L.	1.79 % of DW	CC	Khan et al., 2008
		Berberis buxifolia	102 mg/g DW	CSC	Alvarez et al., 2009
		Tinospora cordifolia	3077.76 µg/g DW	CSC	Pillai and Siril, 2022
Ginsenoside	Antitumor, immunological, anti-inflammation, anticancer, antidiabetic and cardiovascular protective	*Panax notoginseng*	28.9 mg/g DW	CSC	Hou et al., 2021
		Panax ginseng	105.6 mg/g DW	ARC	
			13.6 mg/g DW	CSC	
Diosgenin	Anticancer, antidiabetic, anticoagulant, antithrombosis, anti-inflammatory, antiviral, anti-aging	*Helicteres isora*	8.64 mg l−1	CSC	Shaikh et al., 2020
		Balanites aegyptiacae	3.11 mg/100 g callus DW	CC	Mohamed et al., 2021
		Lycium barbarum	15.68 mg/g DW	CC	Mathur et al., 2016

Paclitaxel	Anticancer	*Taxus chinensis*	565 mg/l	CSC	Yin et al., 2025
		Taxus canadensis	117 mg/L	CSC	Zhong, 2002
		Taxus media	145.3 mg/L	CSC	
Podophyllotoxin	Anticancer, mitotoxic, neurotoxic, antimicrobial, antiinflammatory, antispasmogenic, hypolipidemic, immunosuppressive, antioxidative, analgesic	*Podophyllum hexandrum*	4.26 mg/l	CSC	Chattopadhyay et al., 2002
		Juniperus virginiana L.	0.15 mg/g DW	CSC	Kasparova et al., 2017
		Hyptis suaveolens (L.) Poit	53 µg g-1	RC	Coelho et al., 2024
L-DOPA	Precursor of alkaloids, betalain, and melanine, catecholamines	*Mucuna pruriense*	25 mg/1	CC	Vijaya Sree N, et al.,2010

* DW: Dry weight, FW: Fresh weight
** ARC: Adventive root culture, CC: Callus culture, CSC: Cell suspensiyon culture, HRC: Hairy root culture, RC: Root culture

Nowadays, different strategies have been developed for the synthesis of secondary compounds and biomass accumulation such as species improvement, elicitation, permeabilization, optimization of nutrient media and culture conditions, nutrient and precursor feeding, biotransformation and immobilization. In case the plants used in the production of secondary metabolites are grown in field conditions, imbalances in metabolite amounts are encountered and undesirable negative limitations occur due to reasons such as low yield, geographical, seasonal and environmental differences. When these advantages are evaluated, plant cell tissue and organ culture applications become an important way to produce valuable secondary metabolites. Various *in vitro* studies are carried out in different areas for the commercial production of secondary metabolites. The production of a large number of secondary metabolites has already been described in many plant cell suspension cultures (*Lithospermum erythrorhizon, Berberis willsoniae*, *Coptis japonica* and *Coleus blumei* etc.), but significant amounts of secondary metabolites are still not produced in cell suspension cultures of some plant species (*Cinchona ledgeriana, Atropa belladonna*, *Digitalis lanata* and *Duboisia leichhardtii* etc.) (Fazili et al., 2022).

Plant secondary metabolites are known to be useful for the treatment of various diseases including neurological and cardiovascular disorders, diabetes, cancer, arthritis and COVID-19 (Kulshreshtha et al., 2022). There are many studies from past to present in which valuable secondary metabolites were produced in vitro using plant cell cultures. In this method, the secondary metabolite to be produced is

usually cultured using an organ or tissue in which it accumulates under natural conditions. For example, roots are used to produce ginsenosides, seeds of *Trigonella spp*. to produce diosgenin, and leaves of *Catharanthus roseus* to produce vinblastine. Nowadays, alkaloids, phenolics, alkaloids, steroids, terpenes, some valuable pharmaceutical chemicals (paclitaxel, shikonin and podophyllotoxin), various fragrance compounds (sesquiterpenoids, patchoulol and α/β-santalene) and antioxidants and pigments are synthesized through plant cell cultures (Wu et al., 2021).

- **Shikonin**

Shikonin, found in the roots of the *Lithospermum erythrorhizon* plant, is a naphthoquinone phytocomponent. It has been used in traditional medicine for many years in the treatment of a wide variety of diseases in many countries, especially in China. Studies have determined that it has an anti-cancer effect by acting on the signaling mechanism. It has also been determined that it is effective in the treatment of chronic infectious diseases by inhibiting various cytokinin-like structures such as TNF-α, IL-6, and IL-1β. Researchers have determined that it has antimicrobial effects on antibiotic-resistant bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and antiviral effects on influenza and hepatitis. In addition, it is used as a wound healer in skin lesions and burns, in the treatment of cardiovascular diseases with its oxidative stress reduction feature, and to reduce neuroinflammation in the treatment of diseases such as Alzheimer's and Parkinson's (Biswal et al., 2025). First, shikonin production was carried out by Mitsui Petrochemical Industries Ltd.

(now Mitsui Chemicals Inc.) in *Lithospermum erythrorhizon* Siebold & Zucc cell suspension cultures. It was later formulated for Lady 80 BIO lipstick by Kanebo Cosmetics Inc. and introduced to the market in 1983. High shikonin yields in 750 L bioreactors are around 23% DW. However, in the biotechnological production of shikonin, production has been adversely affected due to delays in regulatory approval of the product, limited market size (150 kg/year year), high operating costs of bioreactors, decrease in product price, etc. (Lange, 2018).

- **Taxol®**

Phyton Biotech produces paclitaxel (trade name Taxol® by Bristol-Myers Squibb) on a commercial scale using plant cell fermentation technology based on *Taxus chinensis* v. marei cell lines. Paclitaxel is considered the most widely used antitumor drug due to its unique anticancer activity. It is also used in the treatment of different diseases such as fibrosis, inflammation, various skin disorders and coronary heart failure, and clinical studies in this context are continuing rapidly (Hashemi et al., 2023). Hokkaido Mitsui Chemicals (Japan) also produces 10-deacetylbaccatin III, a taxane precursor, commercially via yew tree cell cultures with an 800 L disposable bioreactor (Rani and Vimolmangkang, 2022). It is estimated that the annual worldwide demand for Taxol is in the range of 800-1000 kg and this rate is increasing by 20% every year. Considering that the paclitaxel content of the Taxus genus, the most important source of taxol, is 0.01-0.03% and doses of 2 g are needed for antitumor treatment, alternative production techniques are needed to prevent the extinction of plants belonging to the genus (Tomilova et al., 2023).

- **PhytoCellTec™ (*Malus domestica*-Apple Stem Cell)**

Some experiments have been carried out by Mibelle AG Biochemistry in Switzerland, which have shown that aging in human skin fibroblasts can be reversed using a 2% plant stem cell extract from Uttwiler Spätlauber apples. This extract stimulated the antioxidant enzyme hemeoxygenase-1 by regulating genes vital for cell growth and proliferation, and it has been shown to increase the viability of cord blood stem cells and extend the lifespan of human hair follicles. Lecithin liposomes were used to transport the extract. A clinical trial of this liposome-coated extract (Phytocell Tech™) has shown that it reduces wrinkles in the crow's feet area of the face; wrinkles were reduced by 8% after 2 weeks and 15% after 4 weeks when measured with the PRIMOS system for 3-D skin surface analysis (Trehan et al., 2017).

- **Triterpenoid saponins**

In the mid-1980s, an industrial-scale process for culturing ginseng cells (*Panax ginseng*) was developed by Nitto Denko Corp. (Ibaraki, Japan). The first approval for commercialization of the product was given in Japan in 1988. It was determined that the ginseng cells had a triterpene saponin content very similar to that of field-grown ginseng. Powders and extracts from ginseng cell cultures are used in the production of food, beverage, and cosmetic additives. A two-stage production process uses bioreactors of up to 25,000 L, and a biomass productivity of 20 g/L DW is achieved in 4 weeks of culture. Following these studies, University of Edinburgh (UK) and Unhwa Corp. (South Korea) determined that cambial meristem cells are a low-cost and well-

performing source for undifferentiated cell production. Through to this technology, wild ginseng cambial meristematic cells were produced and a skin care product (Ddobyul®) containing these cells was commercially launched (Lange, 2018).

- **Polifenol**

DianaPlantSciences company has been conducting studies on the use of cell suspension cultures rich in polyphenol content as cosmetic and food supplement products. Cocovanol™ XXX, a freeze-dried cocoa cell suspension powder with high polyphenolic content and trace amounts of caffeine and theobromine, was launched in 2013. Another product containing polyphenols, ActivBerry™, is planned to be launched, obtained from suspension cells of bilberry, a close relative of blueberry (Lange, 2018).

2.2. Biopharmaceuticals and Therapeutic Proteins

Biopharmaceuticals are various medical products produced or obtained from biological sources. In parallel with developing technology, the biopharmaceutical industry and studies in this field continue to develop rapidly. Plants have also become sources that can be used to produce biopharmaceutically valuable proteins for molecular agriculture purposes (Eidenberger et al., 2023). As is known, molecular agriculture is based on manipulating the cell factory to produce a valuable protein that usually has therapeutic potential in humans. The production of human proteins is widely targeted in plant molecular agriculture. The production of these proteins in plants is a safer alternative way than obtaining them from other natural sources (usually animal-based). Proteins isolated from animals or humans carry the risk

of contamination with harmful agents such as viruses or prions; these prions are infectious proteins that can cause diseases such as Creutzfeldt-Jakob disease in humans or bovine spongiform encephalopathy (mad cow disaease). However, since plants do not harbor such pathogens, the production of human proteins in plants can eliminate this contamination risk and make the resulting protein safer for therapeutic use. The way protein synthesis occurs in plants and the modification process is similar to that in mammals. When evaluated from this perspective, recombinant proteins obtained using plant systems are generally soluble and functional. This constitutes a significant advantage compared to microbial expression systems. In addition, plants can be used to produce industrial enzymes, technical proteins produced to fulfill a specific function within the scope of scientific research and industrial applications, food and feed additives, and biopolymers (Twyman et al., 2003). Human proteins, despite their multi-component structures and functional structures, as well as complex post-translational modifications, can also be produced by plants, which are higher eukaryotic organisms. Compared to mammalian cell-based platforms, molecular agriculture is simpler, scalable, has lower production costs, and is structurally safer (Eidenberger et al., 2023). A suitable protein expression system is expected to produce the target protein in the correct conformation and high efficiency, be easy to apply and store, and be safe and economical. The many disadvantages of traditionally used mammalian cells have led to the need for alternative production platforms. It has been determined that the use of plants in transgenic protein production reduces the cost

by 10-50 times compared to other prokaryotic and eukaryotic systems (Jin et al., 2025).

Plant molecular agriculture uses plants as a bioreactor for pharmaceutical production. Today, plant molecular agriculture is used to produce antibodies, vaccines, and medical proteins, etc. However, research is insufficient for the production of nutraceuticals and functional foods. Plant molecular agriculture involves the production of foreign proteins from whole plants, tissues, or cells using transformation methods (Long et al., 2022).

In molecular farming, the selection of the host cell is of great importance. Economical, scalable and effective are important parameters. Lettuce (*Lactuca sativa*), tomato (*Solanum lycopersicum*) and corn (*Zea mays*) are the host types used, while the most commonly used host species are tobacco (Vo and Trinh, 2025). In addition to the frequent use of tobacco cell suspension culture in the production of biopharmaceuticals, studies using carrots and rice are also encountered. Transgenic tobacco cell suspension cultures have been successfully used in the production of human serum albumin and scFv antibody fragments. Tobacco BY-2 cell suspension cultures have been used for the production of biscFv antibody fragment, human erythropoietin mAb against HBsAg, among other biopharmaceuticals. In addition, tobacco cell line NT-1 has been used as a production system for the production of various heterologous proteins, including mouse monoclonal heavy chain γ, heavy chain mAb and HBsAg (Hidalgo et al., 2018).

Recombinant pharmaceutical proteins produced by utilizing the latest developments in genetic engineering technology are used in the

treatment of many diseases such as infectious diseases, diabetes and cancer, especially COVID-19 (with vaccine and neutralizing antibodies). The global market value of the biotechnology market in 2020 was 752.88 billion USD. This value is expected to increase by approximately 15% between 2021-2028. (Jin et al., 2025). When the recombinant drug market is examined, there are more than 200 drugs on the market, more than 130 of which have received FDA approval. Clinical trials are ongoing for many more drugs. Many eukaryotic and prokaryotic host systems, such as mammalian, yeast, insect and bacterial cells, are used by researchers for the production of recombinant proteins (Karki et al., 2021). There is a lot of research on the production of recombinant proteins such as various vaccines, hormones, antibodies and cytokinins in plant cells. After the expression of human serum albumin in tobacco cell culture by Sijmons et al., 1990, many attempts were made to produce other human proteins in plant cell cultures (Table 3).

Table 3. Some recombinant proteins produced through plant cell cultures (Gharelo et al., 2016)

Recombinant proteins	**Host cell**	**Protein yield**
Human α-l-iduronidase	Tobacco BY-2 cells	10 mg/L
Human HIV antibody	Tobacco BY-2 cells	10 mg/L
Human interleukin 12	*Orzya sativa* L.	31 mg/L
Human growth hormone	Tobacco BY-2 cells	35 mg/L
β-Galaktosidase	Tobacco	Not state
Tanomatin	Tobacco	2.63 mg/L

Initiation of callus cultures and *in vitro* cultures of plant cells have become routine processes even in large bioreactors. Although plant cell and organ cultures produce a wide variety of small natural compounds (SNAPs) used in medicine, only a few of them have been produced on a commercial scale. Plant cell cultures are very useful systems for studying the biosynthetic pathways leading to the formation of small natural compounds at the enzyme and gene level. Although plant cell culture technologies have existed for a long time, there are still some difficulties in commercializing plant cell culture processes. The main reason for this is the difficulty in competing with existing production methods. However, plant cell suspension cultures have made important contributions to the field of applied botany regarding the elucidation of biochemical pathways and the production of small natural compounds. For example, the tryptophan decarboxylase gene continuously expressed by introducing it into *Catharanthus roseus* L. cells increased the amount of tryptamine but did not increase the production of indole alkaloids. Metabolic pathway engineering seems to be a promising approach for the production of many SNAPs. The enzymes Δ5-3β-hydroxysteroid dehydrogenase and progesterone 5β-reductase were first isolated from Digitalis cell and tissue cultures in the 1980s, once again demonstrating the importance of plant cell cultures for basic research and applied botany (Kreis, 2019).

Although many different cell lines are used in recombinant protein production, the most widely used is BY-2 cells isolated from tobacco plant (*Nicotiana tabacum* cv. Bright Yellow 2) by Kato et al. (1972). This cell line is frequently preferred due to its characteristics

such as fast growth (11 h doubling time), robust *Agrobacterium*-mediated transformation, and easy entry into cell cycle synchrony. To date, many functional proteins have been produced using the BY-2 cell line. Most of the studies conducted with BY-2 cells aimed to reduce proteolytic activity and prevent nonhuman glycosylation. Besides the BY-2 cell line, other plant cell lines used for biologic production have been obtained from edible plant species such as rice (*Oriza sativa*), alfalfa (*Medicago sativa*) and carrot (*Daucus carota)* (Park et al., 2020). In particular, when cultured plant cells are used for oral administration of biologics, they are more easily accepted by the general public. In particular, carrot cells were used by the Israeli company ‘’Protalix BioTherapeutics’’ (http://www.protalix.com) to produce taliglucerase alfa (Elelyso®), the first plant cell-derived biopharmaceutical approved for the market (Karki et al., 2021). Again, the same company Protalix BioTherapeutics produced recombinant human β-glucocerebrosidase via carrot cell culture.

Plant cell culture systems have all the advantages but also some disadvantages. The tendency of cells to form aggregates or to stick to the bioreactor wall, the instability of the genetic structure and the gene silencing mechanism are some of the disadvantages of these systems. Most of these problems can be solved by using suitable bioreactors, selection of appropriate cell lines, and optimization of the culture medium. The main problem of using plant cell and tissue cultures is that the amount of protein produced is low compared to the whole plant platform, which limits the commercialization of therapeutic protein products. Much effort has been made to increase the efficiency of

recombinant protein expression in plant cell-based expression systems. The use of specific manipulation strategies, optimization of plant cells, culture and growth conditions, as well as the use of special bioreactors for plant cell cultures are considered promising methods for high-yield recombinant protein production (Gharelo et al., 2016).

The 2014 Ebola outbreaks have led to increased interest in plant-produced monoclonal antibodies (mAbs). Two critically ill American Ebola patients were given an experimental drug called ZMapp and found to recover rapidly. ZMapp contains three chimeric monoclonal antibodies produced in the *Nicotiana benthamiana* plant. These monoclonal antibodies are rapidly produced and highly effective against Ebola infection in rats and macaques. A recombinant immune complex vaccine based on these monoclonal antibodies has protected rats against lethal Ebola infection. ZMapp has been shown to successfully rescue 100% of rhesus macaques, even five days after a lethal Ebola infection. ZMapp has been found to be safe and well tolerated, with patients receiving ZMapp having a 40% lower risk of death. These trials are aimed at establishing upstream and downstream processes for the production of plant-made monoclonal antibodies that comply with FDA's current Good Manufacturing Practices (cGMP) regulations. Clinical trials have helped drug approval agencies adapt to this new technology and have enabled a clearer regulatory roadmap for plant-based monoclonal antibodies. This suggests that they have the potential to greatly accelerate the approval process for other plant-based monoclonal antibodies (Chen, 2022).

2.3. Food Products Produced with Plant Cell Cultures

The most important difference in plant production for the food and pharmaceutical sectors is the working volume and downstream processes. Although it varies according to the need, more end products are needed in the food sector compared to the pharmaceutical and cosmetic sectors. In order to meet this need, bioreactor production of plant cells has become mandatory and increases the cost. Nevertheless, there are some studies (Table 4). Cocovanol™ produced by Diana Plant Sciences (the first plant cell culture-derived food additive to be granted GRAS approval status) and PhytoVanilla™ produced by ESCAgenetics Corporation (the first patented biotechnology for the production of natural vanilla flavor using plant cell culture technology) are some examples of these (Krasteva et al., 2020).

The production of food products under in vitro culture conditions has many advantages. In addition to providing regular and high-efficiency production with short production cycles, the most important advantage is that production is carried out under controlled conditions and is sterile. In this way, good manufacturing practice (GMP) standards can be met and the final product can be ensured to be free from contamination and safe (Murthy et al., 2024).

Tablo 4. Secondary metabolites produced through plant cell cultures and used as food products (Modified from Rao and Ravishankar, 2002)

Purpose of use	Secondary metabolite	Plant species
Colours	Anthocyanins	*Vitis vinifera*
		Euphorbia spp.
		Daucus carota
	Betalaines	*Beta vulgaris*
		Chenopodium rubrum
	Carotenoids	*Lycopersicon esculentum*
	Anthraquinones	*Cinchona ledgeriana*
Flavours	Vanillin	*Vanilla planifolia*
	Garlic	*Allium sativum*
	Cocoa flavour	*Theobromo cacao*
	Onion	*Allium cepa*
	Capsaicin	*Capsicum frutescens*
		Capsicum annuum
Sweeteners	Stevioside	*Stevia rebaudiana*
	Glycyrrhizin	*Glycyrrhiza glabra*
	Thaumatin	*Thaumatococcus danielli*
Essential oils	Mint oil	*Mentha piperata*
	Chamomile oil	*Matricaria chamomilla*
	Jasmine oil	*Jasmine officinale*

- **Plant Cell Culture Based Coffee**

Molecular farming is the process of using plant cells or a whole plant as a platform to produce recombinant proteins for different purposes (Kang et al., 2025). Coffee is an important example of this

system, which is also used in the production of agricultural products. When it comes to agricultural products, coffee has a high carbon footprint (33-126 billion kg CO_2/year). In a study conducted by Aisala et al. (2023) at the Technical Research Center of Finland VTT Ltd., plant cell-based coffee was produced. In the study where young leaves of Coffea arabica seedlings were used, firstly the stomata were completely closed at room temperature. Then the sterilized leaves were cut to 0.5 cm^2 and transferred to callus media. The obtained calli were taken to 20 and 50 L wave bioreactors as 1/2 working volume. The produced biomass was separated from the medium by filtering and lyophilized by freeze-drying method. The coffee cells were stored in the freezer at -20^oC until roasted. As a result of this study, a process in which coffee cells were cultured, roasted and evaluated for flavor and caffeine content was successfully established, proving that coffee can be made without traditional farming (Figure 2). This biotechnological approach offers a sustainable solution to the environmental and ethical challenges facing coffee farming, such as deforestation, labor rights, and climate change (Aisala et al., 2023).

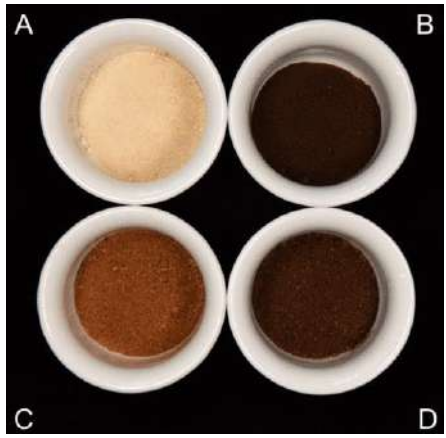

Figure 2. Coffee cell examples, A) Unroasted, freeze-dried coffee cells (L* = 48.8), B)1st roast (L=31.0), C)2nd roast (L=36.0), D)3rd roast (L= 32.0). L= lightness value (dark−light, 0−100) according to the color spacer defined by the International Commission of Illumination (CIE).

- **Plant Cell Culture Chocolate**

Consumer behavior influences and shapes the market through "megatrends." Megatrends that have a global impact, such as society, mobility, and individualization, also have significant impacts on the food industry. Plant cell culture systems have offered an innovative perspective on this issue. In terms of consumption as food, both aromatic and healthy products can be provided by minimizing the formation of harmful compounds with plant cell culture systems. This makes plant cell cultures an important preferred production system. Setting out with this idea, Zurich University of Applied Sciences (ZHAW) carried out a study in which they aimed to produce chocolate using callus and cell suspension culture techniques in the *Theobroma cacao* plant. In the study, modified MS medium was used and cultures were incubated at 29°C in dark conditions, and as a result of these

experiments, the doubling time was determined as 7 days. Although the polyphenol contents of these callus cultures (epicatechin, procyanidins B1, B2, C1 and cinnamtanin A2) were comparable to the donor material (*T. cacao* seeds), up to 40% higher, a 100% decrease was observed in the alkaloids caffeine and theobromine. Furthermore, overexpression of valine, cysteine and phenylalanine amino acids, known to strengthen the immune system, was also detected. When *T. cacao* suspension cells were propagated for 9 days in 1 L shaker flasks, a doubling time of 4 days was achieved. It was determined that with increasing cultivation time, the majority of *T. cacao* suspension cells grown in a modified MS medium tended to grow into large clusters that appeared slightly brownish under the microscope (Eibl et al., 2018).

Based on the results of their study, the process was continued in a single-use wave-mix bioreactor in the next step in order to produce sufficient *T. cacao* cell mass for cell culture chocolate production. Feeding was carried out with 5 L of medium on day 7. On the 16th day of culture, ~ 300 g of biomass (fresh weight) was obtained and used to obtain 3 bars of 70% dark chocolate. No pretreatment was applied in the production of the first bar. For the second bar, the biomass was incubated aerobically for 46 hours. In the third bar, 30 hours of anaerobic and 16 hours of anaerobic incubation were applied together. These applications provide fermentation to obtain aroma, similar to traditional chocolate production (Figure 3).

Figure 3. Main stages of chocolate production based on *Theobroma cacao* suspension cells (Eibl et al., 2018). A) Cocoa fruit used to establish callus culture from seeds B) Callus culture C) Microscopic picture of *T. cacao* suspension cells D) 20 L Flexsafe bag with mass-grown *T. cacao* suspension cells. E) *T. cacao* freeze-dried cocoa suspension cells. F) Produced cell culture chocolate

- **Various Plant Cell Culture-Based Foods Possible to Produce in a Home Bioreactor**

Rapidly developing technological applications are included in plant tissue culture applications. A group of researchers working at the Technical Research Centre of Finland (VTT) designed a bioreactor that can produce 500 g of biomass (fresh weight) in 1 hour. The most important feature of this biomass is that it is edible. This bioreactor is also called a "home bioreactor" and works similarly to a coffee machine (Figure 4). The design produced with a 3D printer has a structure consisting of a simple container and its lid. It is an extremely practical product with its structure suitable for desktop use at home. The bioreactor contains two different inlets. The first inlet is a single-use bag or capsule containing the plant cell culture containing the nutrient medium defined as the starter culture. The second inlet is used for water

supply. By operating this bioreactor, which is temperature-controlled, illuminated and ventilated, the cell culture is kept in optimal growth conditions. Thus, the production of cell culture biomass, which can be eaten as a breakfast cereal or smoothie or as a future supplement, can be achieved in a short time (Figure 4).

Figure 4. VTT's home bioreactor, developed in collaboration with designers from the Aaolto University School of Art, Design and Architecture (Eibl et al., 2018).

3. Approaches Used to Increase Secondary Metabolite Production in Plants

3.1 Elicitation

The use of in vitro culture of plant cells and organs in the production of valuable secondary metabolites found in plants is a system that has been implemented by many researchers for many years. Although the basic principles in the applications of plant cell cultures are understood, some problems can be experienced in large-scale production. At this point, elicitation applications carried out by biotic

and abiotic elicitors have brought a solution to the problems encountered in cell cultures (Wang et al., 2013).

Elicitors are stimulant compounds that stimulate all kinds of physiological changes in the plant and activate secondary metabolism. They are generally grouped under two main headings. A living organism such as a bacteria or microorganism or various enzymes belonging to them are called biotic elicitors. Environmental factors such as light and temperature or various chemicals or hormones are grouped under the heading of abiotic elicitors (Jalota et al., 2024) (Figure 4). Exogenic elicitors are compounds formed outside the cell, such as polysaccharides, polyamines and fatty acids. In contrast, endogenic elicitors are compounds formed inside the cell, such as galacturonide or hepta-β-glucosides (Fazili et al., 2022).

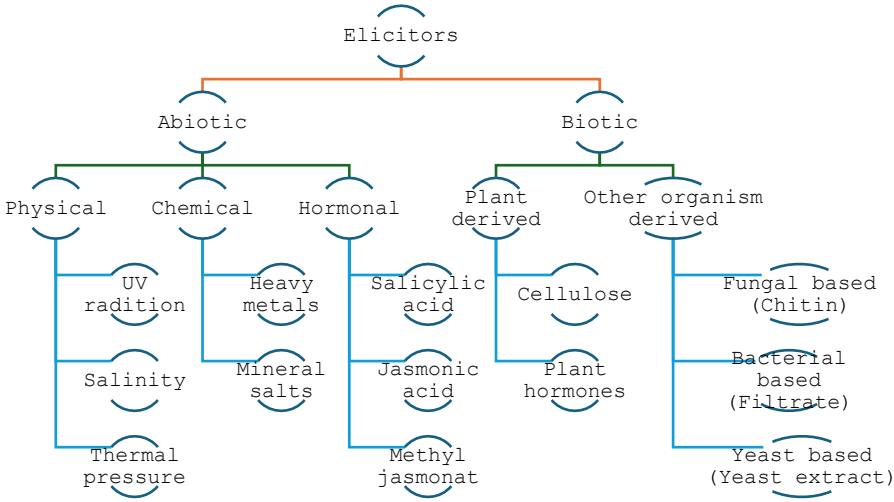

Figure 5. Grouping of elicitors and some examples (Jalota et al., 2024)

An elicitor is a compound that increases the production of a particular metabolite by stimulating or triggering its biological pathway. Although there are many methods used to increase secondary metabolite production, the most commonly used is elicitation. Applied in very low doses to the plant production process, elicitation activates the defense mechanism in plant cells and increases secondary metabolite production. Abiotic elicitors are mostly non-biological substances such as salts of inorganic compounds, Cu and Cd ions, Ca^{+2} and high pH conditions, while biotic elicitors are materials of biological origin (Table 5) (Fazili et al., 2022).

Table 5. Abiotic elicitors and plant secondary metabolite production (Revised from Fazili et al., 2022 and Jalota et al., 2024)

Abiotic elicitors	**Plant**	**Secondary metabolite**
Sodium-alginate	*Glycyrrhiza echinate*	Equinatin
Metal ions: Al^{3+}, Cr^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}	*Datura stramonium*	Sesquiterpenoids
Salicylic acid, Ca^{2+}	*Daucus carota*	Chitinase
Cu^{2+}, Cd^{2+}	*Atropa belladonna*	Tropane alkaloids
Oxidative stress, amino acid deficiency	*Arabidopsis thaliana*	Camalexin
Copper sulphate	*Hyoscyamus albus*	Phytoalexin
UV stress	*Glycyrrhiza uralensis*	Glycyrrhizin
Diethylamino ethyl dichloro phenyl ether	*Catharanthus roseus*	Indole alkaloids
Jasmonic acid	*Bacopa monnieri*	Bacosides
Methyl jasmonate	*Taxus media*	Paclitaxel
$AgNO_3$	*Ocimum basilicum*	Linalool, Estragole

The majority of biotic elicitors are recognized by specific receptors attached to the cell membrane (Fazili et al., 2022). This stimulus is transmitted to the cell via a signal transduction system, triggering reactions that result in the production of phytoalexins. The response of the plant in this case depends on many different factors, such as its genetic and physiological structure. Responses to this biotic factor are regulated by plant resistance (R) and pathogen avirulence (Avr) genes (Ramirez-Estrada et al., 2016) (Table 6).

Table 6. Biotic elicitors and plant secondary metabolite production (Revised from Fazili et al., 2022 and Jalota et al., 2024)

Biotic elicitors	**Plant**	**Secondary metabolite**
Ascochyta rabiei	*Cicer arientium*	Medicarpin, Maackiain
Hemicellulase	*Brugmansia candida*	Hyosujamin
Cellulase	*Capsicum annuum*	Capsidol
Erwinia carotovora	Çeşitli bitki hücreleri	Enzymes
Fungal elicitor	*Cupressus lusitanica*	Indole alkaloids
Trichoderma viride	*Catharanthus roseus*	Ajmalicine
Yeast elicitor	*Salvia miltiorrhiza*	Diterpenoid tanshinones
Chitosan, methyl jasmonate, yeast extract	*Glycirrhiza inflata*	Glycyrrhizin
Yeast extract	*Scutellaria laterifora*	Acetoside
Fungal mycelium of *Botrytis sp.*	*Papaver somniferum*	Sanguinarine

3.2. Elicitation Through Nanoparticles (NPs)

Elicitation of secondary metabolite production by NPs in *in vitro* cultures is a new abiotic elicitation method used in metabolic engineering. Cells in plant cell cultures are stressed by being exposed to oxidative stress by these NPs and activate their secondary metabolism (Selwal et al., 2025). NPs is an expression used to describe structures with a length of 1-100 nm. NPs, which have been used in many different areas and for different purposes in agriculture since the time they were first used, can also serve as elicitors in secondary metabolite production (Freitas et al., 2020). Studies have determined that NPs penetrate plant tissues and activate different metabolic pathways by interacting with cellular structures such as enzymes and transcription factors. In addition, NPs act as carriers and are effective in carrying agents such as nutrients, pesticides or genetic material into the cell. Numerous studies have been carried out, especially on the use of silver nanoparticles (AgNPs). In a study conducted on the *Isatis constricta* plant, a significant increase in the production of indirubin, indigo and tryptanetrin was observed with the use of 2 mg/L AgNPs under *in vitro* conditions (Holghoomi and Colagar, 2024). NPs generally interfere with plant secondary metabolism due to their complex structures and long-term exposure to phytotoxicity. Since plant secondary metabolites serve as a defense mechanism for plants in difficult situations, stress increases secondary metabolite synthesis. NPs can be obtained from various materials such as Pd, ZnO, Fe_2O_3 and Au (Pradeep et al., 2024) (Figure 6).

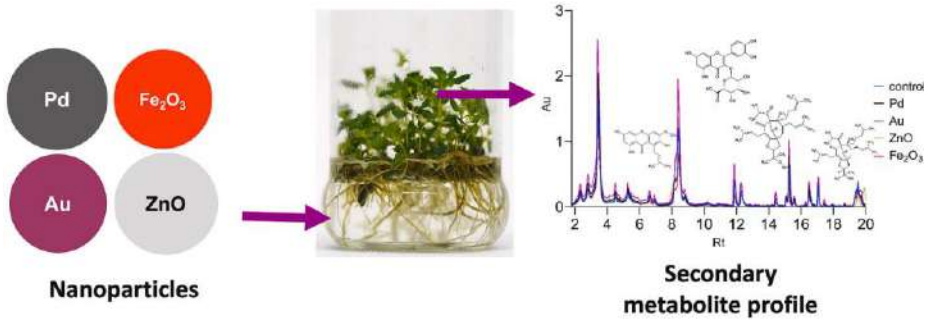

Figure 6. NPs and its effect on plant secondary metabolism (Pradeep et al., 2024)

3.3. Genome Editing Methods

In primary metabolism, regulation of the biosynthetic pathway, which is stimulated by external factors and creates a series of reactions with signaling molecules, can occur at the genetic level. However, in secondary metabolism, regulation usually occurs at the enzyme level and in subcellular compartmentation. In these regulations, regulatory genes or transporter genes in the biosynthetic pathway are selected as targets. With the development of gene sequencing technologies and the increase in studies on genetic engineering in plants, the success rate of studies on gene modifications has increased (Devi et al., 2023). Targeted genome editing allows for a very clear understanding of biological systems (Mitra et al., 2023). Genetic engineering in plants is usually carried out by modifying the genomes of cultured cells and then exposing the modified cells to growth hormones to regenerate them up to the whole plant (Baumann, 2020). It also provides comprehensive targeted opportunities for improving plant traits and yields. This includes creating plants with traits that confer resistance to a wide range of abiotic and biotic stress conditions. In addition, new plants with

valuable compounds can be created. Following next-generation sequencing (NGS) analysis, the use of targeted genome editing technologies such as CRISPR/Cas9 systems, zinc finger nucleases (ZFNs) and transcription activator-like endonucleases (TALENs) has the potential to integrate synthetic biology into genetic and metabolic engineering in medicinal plants (Mitra et al., 2023). CRISPR/Cas9 targeted mutagenesis has been widely used to understand how biosynthetic pathways are regulated and thus to edit genes (and gene families) to facilitate crop improvement (Che et al., 2018). The biosynthetic pathway of plants begins with the successful application of CRISPR/Cas9-based gene editing tool in tomatoes, resulting in the production of carotenoids and γ-aminobutyric acid (GABA). Carotenoid is an important secondary metabolite that has considerable nutritional importance in animals due to its physiological capabilities. However, this editing was possible thanks to two genes, Psy1 and CrtR-b2 (Mitra et al., 2023).

Although there are different systems, the CRISPR/Cas system is the most commonly used genome editing system today. CAS proteins, which were first detected by fragmenting the DNA of an invading virus in a bacterium, were developed by understanding the Cas nuclease and working with the CRISPR locus (Devi et al., 2023). This technology has paved the way not only for developments in plants but also for very important developments in the fields of biomedicine and medicine. There are different regulatory mechanisms in the CRISPR gene editing system, such as post-translational modifications, subcellular localization or metabolite translocation. Among these, pathway

enzymes and transcription factors play a critical role. In particular, there are many studies that use secondary metabolites to increase secondary metabolite production. (Table 7) (Gao et al., 2025).

Table 7. Some studies using the CRISPR/Cas system in secondary metabolite production in plants. (Revised from Devi et al., 2023 and Gao et al., 2025.)

Plant species	**Target Gene**	**Editing type**	**Secondary metabolite**
Atropa belladonna L.	AbH6H	Targeted mutagenesis	Hyoscyamine
Brasssica napus L.	BnaA.FAD2	Knockout	Oleic acid
Camellia sinensis	CsHB1 yhNMT1	Mutated directionally	Caffeine
Dendrobium officinale Kimura and Migo	C3H, C4H, 4CL, CCR,IRX	Knockout	Alkaloids, phenanthrenes polysaccgarides, bibenzyls, essential oils, glycoside
Dioscorea zingiberensis C.H. Wright	Dzfps	Targeted mutagenesis	Diosgenin
Fagopyrum tataricum	FtMYB45	Knockout	Flavonoids
Ipomoea nil	lnCCD4	Targeted mutagenesis	Carotenoid
Artemisia annua L.	AaSQS	Clogged branch lines	Artemisinin
Solanum lycopersicum	SlAN2	Transcriptional regulation	Anthocyanins

3.4. Immobilization

Immobilization is generally defined as the free movement of an independent catalyst. The immobilization process of plant cells, which was first successfully performed in plant cells by Brodelius et al. in 1983, has become a frequently used method in the production of valuable secondary metabolites of great economic importance today

(Rosevar and Lamde, 2005). In the transition from small culture vessels to pilot-scale production in the production of plant cells, there are major problems due to the slow growth of plant cells, their low resistance to shear stress and their tendency to form aggregates. Immobilization offers an important solution at this point. The immobilization method has many advantages such as allowing the reuse of biocatalysts, facilitating the separation of the growth and production phases, and the ease of removal of the biocatalyst from the environment and increasing efficiency (Dörnenburg and Knorr, 1995).

With all its advantages, plant cells in cell suspension cultures are limited by factors such as slow growth rates, low cell harvest, limited mass transfer, and lower product accumulation (Varma et al., 2021). The immobilization technique can be used to increase the productivity of many plants. This technique is beneficial in many aspects such as increasing cell viability in culture, simplifying the collection of metabolites, reducing common problems of liquid cultures (shear stress, mixing and aeration) and supporting the secretion of metabolites (Inyai et al., 2018). In this context, the design of a bioreactor that includes biological immobilization of transgenic plant cells for the production of recombinant proteins is one of the methods considered as a very important biotechnological tool to overcome these difficulties (Varma et al., 2021). The most frequently used immobilization technique in plant cell cultures is gel encapsulation because it is cheap, simple and reproducible. Although calcium alginate is most commonly used for this purpose; Many polymers are used, especially agar, agarose, alginate, α-carrageenan, chitosan, gelatin, gellan and polyacrylamide

(Dörnenburg and Knorr, 1995; Murthy et al., 2014). The matrix to be used for this purpose is expected to be nontoxic, cheap and have good polymerization activity. Surface immobilization is another immobilization method based on the principle of attachment to inert surfaces in liquid culture. In the study conducted by DiCosmos et al. (1994) on *Catharanthus roseus*, *Nicotiana tabacum*, and *Glycine max* plants, cells were immobilized on glass fiber (Murthy et al., 2014).

The most important problem in the immobilization of plant cells is that the secondary metabolite produced is usually stored in cell vacuoles. The demand for a more economical production process is to release the secondary metabolite into the nutrient medium used. This secretion mechanism can be stimulated naturally, such as passive diffusion or active transport, or it can be achieved by external intervention. In this process, called permeabilization, the secondary metabolite is released into the external environment (Dörnenburg and Knorr, 1995). For this purpose, detergents, organic solvents, osmotic shock and pH change can be used (Choi et al., 1995).

With alginate immobilization technology, which can be used in plant cell cultures, extracellular secretion of the target product in *Morus alba* L. (Moraceae) cells, simplification of downstream processes of the product and recovery of plant cells for the production of new cultures were achieved. Thus, the production of stilbenoids and their further secretion were encouraged. This technique was successfully applied in the production of mulberroside A (Wang et al., 2011), an important stilbene glycoside of *Morus alba* L. (Moraceae) and effectively used in the treatment of hyperuricemia in a study conducted by Inyai et al.

(2018). As a result of the study, it was concluded that immobilized cell cultures significantly increased the secretion of mulberroside A up to 60%, while only 10% of mulberroside A was secreted into the nutrient medium in cell suspension culture (Inyai et al., 2018). In a study conducted on *Capsicum* spp., capsaicin production increased approximately 100-fold with foam and gel immobilization. It was reported that the accumulation of methylxanthine and ajmalicine increased 13-fold and 3.4-fold in Coffea arabica and *Catharanthus roseus* plants immobilized with gel, respectively (Murthy et al., 2014).

3.5. Biotransformation

Biotransformation is a method that involves hydroxylation, glycosylation, glycosylation, oxidoreduction, hydrogenation, hydrolysis, methylation, acetylation, isomerization and esterification of various substrates in order to increase the production of important secondary metabolites in plant cell cultures. Plants may not be able to synthesize the desired secondary metabolite due to various metabolic reasons. In such cases, some substrates added exogenously to the medium can be used to stimulate the reaction and production. These substrates do not have to be a natural component of plant metabolism. For example, podophyllotoxin synthesis from the *Podophyllum peltatum* plant is carried out by the biotransformation of butanolide to the podophytoxin analog (Murthy et al., 2014).

As many researchers undoubtedly accept, plant cell culture applications are the most effective method used in the production of secondary metabolites. In plant cell culture applications, exogenous compounds can be transformed into different compounds with new

properties through biotransformation. The biotransformation process is the process of converting a certain substrate into a different compound with new properties using enzymes or microorganisms (Fazili et al., 2022). In contrast, cell engineering methods are the process of producing new cell lines with desired properties by intervening in the genome of the plant cell. Cell engineering in plant cells has the potential to overcome the current limitations of cell lines for bioproduction so that they can become a commercially competitive bioproduction platform (Karki et al., 2021).

Enzymes involved in plant metabolism are considered to be effective in the production processes of valuable secondary metabolites and factors that facilitate the biotransformation process. These enzymes catalyze reactions such as glycation and hydroxylation, as in microorganisms. Biotransformation differs from chemical methods in that there is no need to protect unstable functional groups. The biotransformation process is carried out in many plant species such as *Eucalyptus perriniana*, where thymol, carvacrol and eugenol are converted into glycosides. In addition, the conversion of hyoscyamine to scopolamine in tobacco plants by biotransformation; glycosylation of capsaicin and 8-nordihydro capsaicin in cell cultures of *Catharanthus roseus* plants has also been achieved. In this respect, biotransformation is seen as a technique for synthesizing new active compounds with different properties (Fazili et al., 2022).

4. Economic importance of plant cell culture-based secondary metabolites

In recent years, the role of agriculture has slowly shifted due to the development of other major industries, towards a bio-based economy where all food, material and energy demands are met by agriculture. Unlike heterotrophic organisms, photosynthesis and carbon fixation drive the central carbon metabolism in plants. This makes plants an attractive platform to support a renewable bio-based economy. Due to the large scale of agriculture, the use of plants as biofactories could completely change the current boundaries of the biobased economy and increase the production of renewable bioproducts. Therefore, future engineering applications will largely depend on the fundamental knowledge of plant secondary metabolism (Shih, 2019). The production of secondary metabolites through cell cultures has the advantage over traditional production because it is not dependent on environmental factors and seasonal changes. The production of bioactive secondary metabolites with high economic value is carried out under controlled conditions where adverse biological effects such as microorganisms and insects are eliminated (Ahmad et al., 2019).

The international market is increasing the demand for plant-based drug molecules for sustainable high-quality products through more environmentally friendly and economically viable methods of drug production (Patil et al., 2021). Based on the demand and economic viability, large-scale production strategies have been designed using microbial and plant-based production platforms. The use of metabolic engineering approaches together with synthetic biology techniques in

the design of metabolic and biosynthetic pathways in plants and microbial systems has provided a sustainable and ecological perspective. Plant cell cultures are also among these methods, as they allow manipulation through genomic techniques and post-translational modification can be performed. These features have made plant cell cultures one of the preferred methods in the production of valuable bioactive components (Bapat et al., 2023). Bioactive compounds used in many areas such as health and food can be obtained from different biological sources. Today, there is great interest in the production of these bioactive compounds by natural means instead of synthetic ones. While the global microbial products market size is expected to be worth approximately 302 billion USD by 2030, the plant-derived pharmaceutical market is expected to increase with a growth rate of over 6.1% during the period 2019-2026. Therefore, a remarkable increase in plant-derived pharmaceuticals can be expected in the coming years (Bapat et al., 2023).

The feasibility of secondary metabolite production by plant cell cultures varies depending on the plant species, culture type, bioreactor used, operation mode, biomass yield and the value of the final product. This situation is more clearly understood when a study conducted with ginseng adventitious roots is examined. The land yield of Korean ginseng (*Panax ginseng* C.A. Meyer) roots was determined as 523 kg/0.01 ha and the production cost was determined as 35 USD. When production is carried out in a 10,000 L bioreactor with a 45-day production process and 7-8 cycles per year, 30,000 kg production can be achieved annually with a value of 47 USD/kg. These data are an

indication that plant cell cultures are economically viable systems in terms of secondary metabolite production (Murthy et al., 2014).

5. Conclusion

Plant secondary metabolites have been used for different purposes since the early days of humanity and have become one of the most important research topics of our day with the developing technology. Especially in recent years, plant cell culture systems have developed very rapidly with the increase in efficiency, the introduction of bioreactor technologies and the development of substrate processes. With genetic engineering, CRISPR systems and molecular farming, the production of valuable secondary metabolites has been increased and their transformation into commercial products in different sectors has been achieved.

Plant cell cultures provide a controllable, environmentally friendly and sustainable alternative for the industrial production of plant secondary metabolites. Numerous studies on the production of valuable bioactive components by in vitro culture techniques underline the increasing importance of this subject. Studies have shown that different parameters such as elicitation of gene expression systems or culture technique have significant effects on increasing the amount of secondary metabolites required. There are several critical factors in the commercialization of plants through in vitro cultures. Market prices are the most important of these. In addition, the fact that the same products can be obtained more cheaply through chemical synthesis, the

regulations in force in these processes, and customer demands can also be considered as these factors.

In the context of ongoing research, the potential of plant *in vitro* cultures for the production of bioactive secondary metabolites is important. Advances in metabolic engineering and genome engineering in plants are very promising approaches for the elucidation of biosynthetic pathways and synthetic biology tools. In the future, the contributions of all these techniques may provide valuable methods for the sustainable feasibility of plants as a renewable source of important compounds, thus enabling the successful production of valuable and novel compounds.

Phytochemicals produced in plant cell cultures have many areas of use, such as their pharmaceutical properties, their use as food additives, and their place in the cosmetics sector. However, among these areas of use, they are mostly used for their pharmaceutical properties. In recent years, its use has become widespread, especially in the cosmetics and food sectors. Large-scale cultures of differentiated and undifferentiated plant cells are associated with various technological, economic and legal challenges. On the other hand, the controlled production of plant cells and tissues under sterile conditions allows the production of valuable secondary metabolites in an environmentally friendly and sustainable process. In addition, the most important advantage of the method is that these plants can be endemic or endangered species and these valuable metabolites can be produced with high efficiency. Plant cell culture technology will reach a level in

the near future that has the potential to meet the ever-increasing demand for bioactive natural compounds.

Ethical Statement

This study was conducted in accordance with academic ethical principles. All sources are cited appropriately, and no unethical practices were involved.

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