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Production of Plant Secondary Metabolites by Using Biotechnological Tools

Sandra Gonçalves and Anabela Romano

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Abstract

Plants are a remarkable source of high-value secondary metabolites with applications in various domains. Plant cell and tissue culture techniques appear as environmentally friendly alternatives for the production of secondary metabolites when natural supply is limited or chemical synthesis is unviable. In this chapter, the main advantages of using plant cell and tissue culture techniques for the production of plant secondary metabolites are presented as well as the different biotechnological approaches available to improve their production. In addition, the production of anticancer compounds (camptothecin, podophyllotoxin, taxol, vinblastibe, and vincristine) and metabolites from *Lamiaceae* spp. (phenolics as rosmarinic acid) were selected as examples to be highlighted. The study reviewed shows that undifferentiated cells are the preferred culture system used for the production of high-value secondary metabolites *in vitro* although there are many examples reporting the production in differentiated tissues particularly in hairy roots. Efforts have been made to scale up the production, and several strategies have been successfully applied to increase the production yields at the laboratorial scale. Nevertheless, there are only few examples of plant secondary metabolites production at commercial level, and further in-depth studies are still required.

Keywords: alkaloids, anticancer compounds, cell suspension cultures, elicitation, *Lamiaceae*, metabolic engineering, phenolics

1. Introduction

Plant kingdom, comprising about 250,000 species, is a repository of probably hundreds of thousands of low-molecular-weight structurally complex chemical compounds known as secondary metabolites [1]. These high-value metabolites are biosynthesized through

phenylpropanoid, mevalonate, 2-C-methyl-D-erythritol-4-phosphate, amino acid, glucose, acetate-malonate pathway, or combined pathways. Secondary metabolites have an important role in the interaction between plants and their environment (e.g., defense against herbivores and pathogens, protection against ultraviolet light, etc.) and, thus, are vital for their existence and subsistence. They are accumulated in specific tissues and structures (e.g., vacuoles, specialized glands, trichomes, etc.), and their production is affected by several factors, like genotype, plant physiology, climate, environmental conditions, and pathogens; in some cases, they are only produced during certain developmental stages [2, 3].

Over the past decades, efforts have been directed at the extraction, structure elucidation, and evaluation of biological activity of many plant secondary metabolites. Plants continue to be the main source for many important bioactive molecules/pharmacophores [4, 5]. About 25–28% of modern medicines are derived from higher plants [6], and over 60% of anticancer drugs are directly or indirectly derived from plants [7]. According to a recent report of the British Broadcasting Corporation (BBC), plant-derived drugs will grow from $29.3 billion in 2017 to around $39.2 billion by 2022 with an annual growth rate of 5.9% [8].

In the last decades, considerable progress has been made concerning the production of secondary metabolites by using plant tissue culture techniques owing to the advantages of this platform over other production systems as discussed in the next section of this chapter. The most studied classes of plant secondary metabolites using plant cell and tissue culture production systems are alkaloids and the landmark example is the anticancer-registered drug Taxol® [3]. Plant tissue culture techniques were even endorsed by Food and Agriculture Organization as safe for the production of compounds for food application [9]. This chapter aims to discuss the main advantages of using plant cell and tissue culture techniques for the production of plant secondary metabolites as well as the different biotechnological approaches available to improve their production. Important and representative examples produced through these methods, as is the case of plant anticancer compounds and metabolites from *Lamiaceae* spp., are addressed.

2. Advantages of plant tissue culture techniques for the production of secondary metabolites

In a context where consumers demand for safe natural products increases, because synthetic chemicals are perceived as potentially toxic, the interest in plant secondary metabolites from research and industry also increases [10]. Few important plant products with simple chemical structures can be produced via chemosynthesis; however, many compounds like alkaloids are difficult to be synthesized or the cost of their synthesis outweighs their commercial availability [11, 12]. Some compounds can be obtained from naturally grown plants, but sometimes there are regional and environmental restrictions, which can limit the commercial production [13]. Also, traditional cultivation of some plant species is difficult or takes several years. In this context, plant cell and tissue culture techniques appear as environmentally friendly alternative methods for the production of secondary metabolites when natural supply is limited

and traditional methods are unfeasible. The mass propagation of plants in aseptic and environmental controlled conditions, and the large-scale production of secondary metabolites in a year-round system without seasonal constraints, are some of the advantages of plant tissue culture techniques [3]. Moreover, cultures can be established in any part of the world independently of the plant growth requisites and are free of microbes and insects avoiding the use of pesticides and herbicides [14, 15]. Plant tissue culture techniques provide a reliable and predictable method for isolating the secondary metabolites at a high efficiency within a short time when compared to the extraction from wild plant populations [16]. Also, the simplicity in the extraction of the metabolites from *in vitro*-produced tissues makes the method appellative for commercial application [17].

Apart from the abovementioned advantages, there are metabolites that are generally not found in the intact plant but can be produced by *in vitro* cultures [18]. Biotechnology opens the opportunity to apply traditional or metabolic engineering strategies to promote the accumulation of desired compounds by *in vitro* cultures. Products from *in vitro* cultures can still be used as models of whole plants, and cell cultures can be radiolabeled so that secondary products can be traced metabolically [19].

The massive and indiscriminate collection of plant material from plants producing important bioactive compounds has threatened the survival of some species. *In vitro* propagation through plant tissue culture techniques allows the large-scale multiplication of true-to-type plants within a short span of time and without a negative impact on the natural resources [16]. This method is particularly valuable for plants difficult to propagate by conventional techniques or with slow propagation rates. In this context, in the last years, there has been an increased interest on the use of these methodologies for the propagation and conservation of medicinal plants.

3. Culture systems

The production of secondary metabolites by *in vitro* cultures usually occurs in a two-step process, biomass accumulation and secondary metabolites synthesis, in which both steps need to be optimized independently [3, 14]. Production could be accomplished by using undifferentiated calli, cell suspension cultures, or organized structures like shoots, roots, or somatic embryos. In some cases, a certain degree of differentiation may be needed for the biosynthesis to occur [20]. The use of differentiated organ cultures is required, for instance, when the target metabolite is only produced in specialized plant tissues or glands as is the case of essential oils [20, 21].

Among differentiated tissues, hairy roots culture offers new opportunities for the *in vitro* production of plant-valuable compounds [22]. Hairy roots are induced by the infection of plants with *Agrobacterium rhizogenes*, a Gram-negative soil bacterium. During the infection, a DNA segment (T-DNA) from the large root-inducing (Ri) plasmid of the bacterium is transferred into the genome of the infected plant. The higher level of cellular differentiation, rapid growth, genetic and biochemical stability, and maintenance facility are some of the

advantages of hairy roots [22]. Also, they can accumulate metabolites in the aerial parts of the plant. However, the difficulties in cultivating hairy roots in an industrial system limit their commercial use to produce valuable plant secondary metabolites.

Although there are many studies reporting the production of secondary metabolites using callus cultures and differentiated tissues [3, 14, 23], in most cases, undifferentiated cells are the preferred culture system [13]. Cell suspension culture is a simple and cost-effective method that has been extensively used to overcome the problems of large-scale production. Plant cell is biosynthetically totipotent, which means that under suitable conditions, each cell has theoretically the capacity to produce compounds identical to those present in the parent plant [13]. Plant cell cultures have more immediate potential for commercial application than tissue or organ cultures [21, 24]. They are considered as a stable system for the continuous production of secondary metabolites of uniform quality and yield. Another great advantage of plant cell cultures is the possibility to synthesize novel products not usually produced by the native plant [25, 26]. This is the preferable biotechnological platform to produce high-value secondary metabolites, as taxol [27, 28], resveratrol [29], artemisinin [30], ginsenosides [31], and ajmalicine [32].

4. Strategies to improve the production of secondary metabolites

In the commercial exploitation of plant cell cultures for the production of high-value secondary metabolites, it is fundamental to achieve high yields and consistent productions. The production of secondary metabolites in plants is genotype-dependent; thus, the first step to initiate cell or organ cultures is the choice of the parent plant containing higher contents of the secondary product of interest for callus or organ induction, and the selection of high-producing cell/organ lines [14]. The selection is made by analyzing cell/organ growth and then by quantifying the desired product by chromatographic and spectroscopic techniques [14]. Nevertheless, even selecting a highly productive line, the production yields are not always adequate, and after long periods of cultivation they lose their production efficiency. Thus, many alternative strategies can be used to stimulate the production of secondary metabolites and obtain efficient yields including traditional and metabolic engineering strategies [3, 19].

4.1. Traditional strategies

There are several factors that can be optimized to improve the growth and metabolites productivity of the *in vitro* cultures. Among them, the following can be appointed: the culture medium composition, the medium pH, the inoculum density, the culture medium environment (e.g., temperature, light density and quality, etc.), the agitation and aeration, etc. [3, 14, 15]. The culture medium strongly affects the biomass and metabolites productivity, and thus the selection of the suitable culture medium formulation is an imperative step [3]. It must be selected according to the physiological requirements of the plant species, and there are several parameters that can be optimized, namely nutrients composition, salt strength, nitrate

and phosphate levels, plant growth regulators type and concentration, carbon source, etc. For instance, carbon source plays significant roles in the signal transduction systems through regulating gene expression and developmental processes [3].

Secondary metabolites are produced by plant cells in response to environmental stimuli or as defensive mechanisms against invading pathogens. In this sense, the strategy available to improve the productivity of secondary metabolites, elicitation, aims to misguide the cells or tissues for a possible biotic/abiotic attack by using agents that trigger the defense response [33]. Elicitors have the ability to control an array of cellular activities at the biochemical and molecular level since they induce the upregulation of genes [33]. The elicitors can be biotic or abiotic and may comprise signaling molecules like methyl jasmonate, salicylic acid, microbial cell wall extracts (e.g., yeast extract, chitosan), inorganic salts, heavy metals, physical agents (e.g., UV radiation) among others [1, 34]. Methyl jasmonate and its related signal molecules, and salicylic acid are probably the most extensively used elicitors [5]. The combination of some elicitors with physical factors (e.g., UV light, temperature regime, and pulsed electric field) yielded good results for secondary metabolite production [35]. As reviewed by Giri and Zaheer [5], cell suspension culture is the most used culture system for elicitation treatment and secondary metabolites production. Due to its inherent characteristics of hormone autotrophy, uncontrolled growth, biosynthetic, and genetic stability distinctiveness, hairy root cultures have proved to be also a valuable culture system for elicitation experiments. In addition, there are some secondary metabolites that are synthesized only in the roots [14, 36, 37]. Multiple shoots culture is a less used culture system for elicitation treatments for the production of secondary metabolites which is particularly useful in the case of metabolites present in the leaves [5]. The elicitors can change the secondary metabolites production quantitatively and also qualitatively [5]. For extra information, consult the recent reviews on this subject [1, 5].

Nutrient and precursor feeding are also used to improve the yields of secondary metabolites production. Nutrient feeding involves the replenishment of nutrient medium, and in precursor feeding, plant cell cultures are used to convert precursors into products by utilizing preexisting enzyme systems [14]. Immobilization of plant cells is another strategy used to overcome problems of low shear resistance and cell aggregation. This procedure can be done by several methods, and the most widely used are surface immobilization or gel entrapment. In this technique, the cells are entrapped in a specific gel or a combination of gels. Examples of matrices used are calcium alginate (the most used), agarose, gelatin, carrageenan, or polyacrylamide [14]. This strategy has several advantages, such as the extension of cells' viability in the stationary stage, the simplification of downstream processing, the high-cell density within small bioreactors reducing the costs and risk of contamination, an increased product accumulation, the minimization of fluid viscosity, among others [38].

The permeabilization of plant cell membranes with chemicals, the use of electric field stress, and ultrasound techniques are strategies used to facilitate the removal of secondary metabolites from vacuoles and membrane systems of the plant cell, facilitating the secretion of products into the culture medium and thus simplifying the purification process [14, 17].

The cultivated cells have the capacity for biotransformation of supplied compounds, which are not necessarily natural intermediaries of plant metabolism, into high-value compounds.

This can occur through different reactions as hydroxylation, oxidation of hydroxyl group, reduction of carbonyl group, hydrogenation of carbon-carbon double bond, glycosyl conjugation, and hydrolysis, catalyzed by plant enzymes [14]. This is probably one of the most commercially realistic approaches; however, in some cases, the costly precursors may limit the economic viability [38].

4.2. Metabolic engineering

Metabolic engineering offers a new perspective to understand the expression of genes involved in the biosynthesis of secondary metabolites through overexpression studies allowing the alteration of biosynthetic pathways [39, 40]. This involves the study of enzymatic reactions and biosynthetic processes at gene, transcriptomic, and proteomic levels, and the manipulation of the genes encoding the critical and rate-limiting enzymes in the biosynthetic pathways [41, 42]. Theoretically, the secondary metabolites productivity of plant cell cultures can be improved through the overexpression of genes encoding regulatory enzymes involved in their biosynthetic pathways [16]. However, the overexpression of certain genes may not always improve production [16].

Metabolic engineering approach also uses the inhibition of competitive pathways to increase metabolic flux of targeted biosynthetic pathway intermediates for a higher production through a variety of approaches. Certain steps in the biosynthetic pathway could be inhibited to induce the accumulation of preceding intermediates. The understanding of phenylpropanoyl biosynthetic pathway that is involved in the biosynthesis of several plant secondary metabolites is the most successful and recent application [43, 44].

The in-depth understanding of the biosynthetic pathways is still a barrier to the practical use of this strategy to enhance production [45, 46]. For the large-scale production of important secondary metabolites to meet industry demand, more studies are needed to identify rate-limiting steps and regulation along with bottlenecks on the lack of clarity of their biosynthetic pathways.

5. Scale-up production

Owing to the importance of some plant secondary metabolites, efforts have been made to study the feasibility of their production at the industrial scale. This is not always a simple process because plant cells have a relatively unstable productivity, a high shear sensitivity, a slow growth rate, and low oxygen requirements [14]. The scale-up involves the use of bioreactors of varying sizes and features, and cell suspension culture is the better culture system having several advantages in comparison with the other. The simplicity, predictability, and high efficiency at which the metabolites can be isolated from biomass or cultivation media are some of these advantages. Nevertheless, there are some examples of the use of differentiated tissues like shoots and somatic embryos [47].

Some important milestones in the production of secondary metabolites by plant cell cultures are the production of shikonin [48] and ginseng [49], and the most successful example of the scale-up process is probably the production of taxol by Phyton Biotech Company (Germany) to supply part of the demands of Bristol-Meyers Squibb Company during the year 2002 [50]. Phyton Biotech operates the largest cGMP plant cell culture facility in the world designed for large-scale production of Taxanes in 75,000 L-size bioreactors that run up to 880,000 L per year [51]. Berberine, ginsenosides, shikonin, scopolamine, and rosmarinic acid are also examples of plant secondary metabolites presently produced at the commercial scale (**Figure 1**) [3, 17].

Several factors should be considered in scaling up the production of secondary metabolites using bioreactors, namely the optimization of culture conditions, biomass production measurement (especially with tissue and organ cultures), and so on [52, 53]. Several bioreactor designs have been tested and used for plant cell cultures. Some of them as is the case of stirred tank reactors, bubble column reactors, airlift reactors, and ebb and flood reactors are merely extension of microbial culture. For plant cells with a high shear sensitivity, Wang and Zhong [54] develop the centrifugal impeller bioreactors that are based on the principles of a centrifugal pump. Mechanically driven "wave reactors," "slug bubble reactor," and "undertow reactor" are also adequate for high shear-stress-sensitive cells [14]. On the other hand, airlift bioreactors are suitable for not highly shear-sensitive cells and
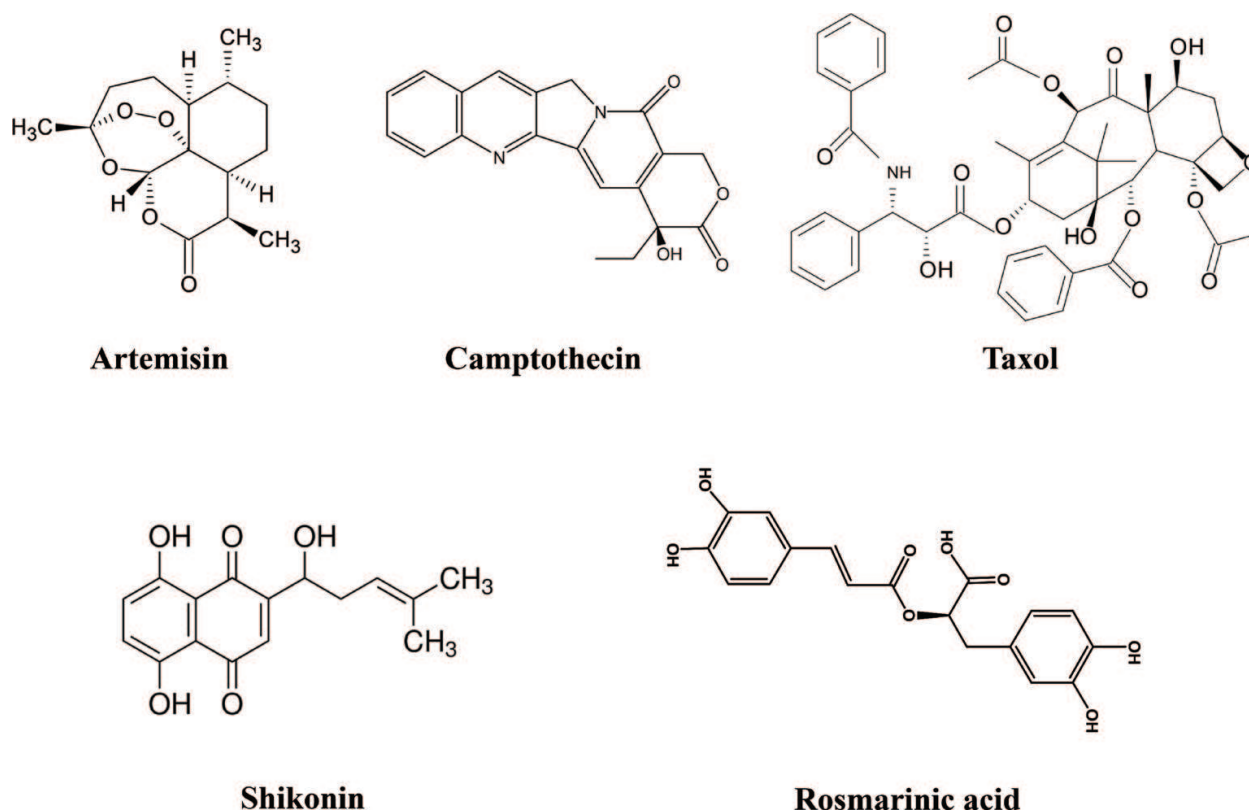

Figure 1. Structures of some relevant plant secondary metabolites produced on a commercial scale.

for hairy and adventitious root cultures [14]. The interested reader can find more important details about the scale-up process in the works by Murphy et al. [14], Yue et al. [13], and Isah et al. [3].

6. Selected examples

There are several plant secondary metabolites including among others alkaloids, terpenes, flavonoids, and glycosides, which can be produced by plant tissue culture techniques using different strategies [3, 13, 14]. Two examples were selected to be described in this chapter: the production of important anticancer compounds and the production of metabolites from *Lavandula* spp.

6.1. Anticancer compounds

As mentioned before in this chapter, over 60% of anticancer drugs are directly or indirectly derived from plants [7]. The search for anticancer compounds from plants started in the 1950s when the alkaloids vinblastine and vincristine from *Catharanthus roseus* (L.) G. Don and podophyllotoxin from *Podophyllum* spp. were discovered. The United States National Cancer Institute initiated an extensive program in 1960 that led to the discovery of many novel chemotypes with cytotoxic activities [55], taxanes and camptothecins being some of the examples [7]. Camptothecin, podophyllotoxin, taxol, vinblastine, or vincristine are the most important plant-derived anticancer compounds [19, 56]. Most compounds with anticancer properties are alkaloids, and some of them have a complex structure, with multiple rings and chiral centers, and therefore the chemical synthesis is prohibitively expensive [17]. Plant cell and tissue culture techniques appear as environmentally friendly alternative methods for the production of these secondary metabolites [17, 19].

Taxanes from *Taxus* spp., terpenoid indole alkaloids from *C. roseus*, camptothecin from *Camptotheca acuminata* Decne among other species, and podophyllotoxin from *Podophyllum* and *Linum* spp. are the main compounds produced by using biotechnological approaches (**Table 1**). For the production of taxanes, cell suspension cultures are definitively the most adequate culture system. However, some studies demonstrated that differentiated tissues are more adequate than undifferentiated cells to produce other anticancer compounds. For instance, intact plants of *C. acuminata* contain around 0.2–5 mg/g dry weight (DW) of camptothecin while callus and suspension cultures produced only 0.002–0.004 mg/g DW or lesser [57]. Hairy root cultures have also proven to be a good option for *in vitro* production of secondary metabolites as indole alkaloids due to their higher level of cellular differentiation and improved genetic or biochemical stability. The hairy roots of *Ophiorrhiza pumila* Champ. ex Benth showed a high capacity to produce camptothecin (0.1% DW), although the callus culture failed to produce this compound [58].

Several researchers have focused on studies aiming for the optimization of biomass growth conditions and on the application of biotechnological strategies to increase production yields of anticancer compounds. By manipulating empirical factors related to plant cell and organ

Compound(s)	Group	Source (plant species)	Culture system	Reference(s)
Camptothecin	Monoterpene indole alkaloid	*Camptotheca acuminata* Decne	HRC	[75]
		Camptotheca acuminata Decne	CSC	[76]
		Nothapodytes foetida (Wight) Sleumer	CC	[77]
		Nothapodytes nimmoniana (J. Grah.)	CSC	[78]
		Ophiorrhiza alata Craib	HRC	[79]
		Ophiorrhiza mungos Linn.	CSC	[80]
		Ophiorrhiza prostata D. Don	ARC	[81]
		Ophiorrhiza pumila Champ. ex Benth.	HRC	[58]
Podophyllotoxin	Aryltetralin lignan	*Linum* spp.	HRC	[82, 83]
		Linum album Kotschy ex Boiss.	CSC	[84]
Taxanes (taxol)	Diterpene alkaloids	*Taxus* spp.	CSC	[27, 28]
		Corylus avellana L.	CSC	[85]
Vinblastine and vincristine	Terpene indole alkaloids	*Catharanthus roseus* (L.) G. Don	HRC	[86, 87]

ARC: adventitious root culture; HRC: hairy root culture; CSC: cell suspension cultures; CC: callus culture.

Table 1. Some examples of studies reporting the production of plant anticancer compounds using biotechnological approaches.

cultures, it has been possible to enhance production yields. Several factors have been optimized, such as nutrients, carbon source, plant growth regulators, or culture environmental conditions, and several biotic and abiotic elicitors have been tested. Studies have also been focused on the elucidation and regulation of biosynthetic pathways and on aiming the increase of production yields of anticancer compounds as taxanes [41] and indole alkaloids [59] by using elicitors to activate genes involved in metabolic pathways. In spite of all the advantages of producing anticancer compounds by using plant cell and tissue culture techniques and the significant advancements in the last years, the examples of the production of plant anticancer compounds on an industrial level are scarce. As previously mentioned in this chapter, the best success example is the production of taxanes by the Germany company Phyton Biotech [51].

Plant cell and tissue culture techniques have also been applied for the propagation of several anticancer plants. *In vitro* propagation allows the rapid mass multiplication of true-to-type plants within a short span of time which is particularly important in the case of endangered species. Some recently selected examples comprise plants producing the important anticancer compounds camptothecin [60, 61] and podophyllotoxin [62, 63].

6.2. *Lamiaceae* spp. metabolites

The mint family (*Lamiaceae*) contains about 236 genera and more than 7000 species with cosmopolitan distribution [64]. Some of the most important genera are *Hyptis*, *Lavandula*, *Nepeta*, *Salvia*, *Scutellaria*, *Thymus*, and *Teucrium.* Species from the family inhabit different natural ecosystems, and many are already cultivated. Most of the species belonging to this family are aromatic (possess essential oils) and are widely used in traditional medicine to cure various disorders. They also have great economic value due to their use in culinary or as ornamentals, and for cosmetic, flavoring, fragrance, perfumery, pesticide, and pharmaceutical applications [65]. Many *Lamiaceae* contain high levels of phenolics, which are probably the most relevant group of secondary metabolites synthesized by plants due to their health promotion effects [64]. Among phenolic compounds, rosmarinic acid is present in the tissues of many of these species being used as a chemical marker of the family [64, 66, 67]. In some species, this compound is accumulated as the main phenolic compound at a concentration above 0.5% dry weight [64]. Several species in the *Lamiaceae* family can also accumulate high levels of other phenolic acids, flavonoids, or phenolic terpenes [64]. There are some phenolic compounds as carnosic and clerodendranoic acids that are exclusive from this family [68, 69]. The interested reader can find an excellent overview on the phytochemical characterization and biological effects of *Lamiaceae* species in Trivellini et al. [64].

Phenolic compounds are generally produced as a defense mechanism or as a response to stressful environment conditions [9]. The activation of these protective mechanisms by applying stress stimulus can be used as a strategy to increase the production of phenolic compounds in plant cell and organ cultures [70]. Recently, several attempts were made regarding the production of secondary metabolites by several *Lamiaceae* species (mainly phenolics) using plant tissue cultures particularly applying elicitation as a strategy to achieve higher production yields [64]. These studies involve mainly the use of chemical elicitors like jasmonic acid (or methyl jasmonate), or physical elicitors as UV-B and ozone (O_3), to increase the production of many compounds as essential oil constituents, phenylpropanoids, flavonoids, and phenolic acids. Overall, the results demonstrated that these elicitors had an immediate effect on enhancing the production of phenolics [64].

The revised study showed that a high number of studies reported an increase in the production of rosmarinic acid after elicitation of cultures of several *Lamiaceae*, such as *Coleus*, *Lavandula,* and *Salvia* genera [64, 66, 71]. Several studies reported the increase in rosmarinic acid production through the application of elicitors (**Table 2**). Elicitation with jasmonic acid induces a 4.6-fold increase of rosmarinic acid production in *L. officinalis* L. cell suspension cultures [72], and elicitation with methyl jasmonate induces a 3.4-fold increase in *C. forskohlii* (Willd.) Briq. hairy root cultures [73]. The production of this compound also increased (2.3-fold) in leaves of *Rosmarinus officinalis* L. after 14 days of UV-B exposure [74]. Recently, rosmarinic acid attracted the attention of the scientists due to its broad range of biological activities, such as anti-inflammatory, antioxidant, cognitive-enhancing, cancer chemoprotection effects, among others [71]. In the last years, there are many progresses in the

Plant species	Tissue	Elicitor	Reference
Coleus blumei Benth.	HRC	Methyl jasmonate	[88]
Coleus forshohlii (Willd.) Briq.	HRC	Methyl jasmonate	[73]
Lavandula officinalis Chaix	CSC	Jasmonic acid	[72]
Lavandula vera MM	CSC	Vanadyl sulphate	[89]
Lavandula vera MM	CSC	Methyl jasmonate	[90]
Mentha × piperita	CSC	Methyl jasmonate	[91]
Rosmarinus officinalis L.	L	UV-B	[74]

HRC: hairy root culture; CSC: cell suspension cultures; L: leaves.

Table 2. Selected examples of elicitation treatments applied in cultures of *Lamiaceae* species to increase the production of rosmarinic acid.

biotechnological production of this compound but its large-scale production still requires further optimization. The molecular understanding of its biosynthesis and the application of metabolic engineering tools are crucial to improve the biotechnological production.

7. Conclusions and prospects

Plant cell and tissue culture techniques are an attractive system for the cultivation of a broad range of secondary metabolites, including important alkaloids with anticancer properties and bioactive phenolics. This alternative provides a continuous, sustainable, economical, and viable production of secondary metabolites, independent of geographic and climatic conditions, which is particularly useful for the production of species at risk. Despite the great progresses in this area in the last decades, in some cases, production occurs at very low yields, and there are many difficulties in scaling up the production, and limited commercial success is achieved. Incomplete knowledge about the biosynthetic pathways of bioactive molecules limited the improvement of the production yields. Exploiting modern molecular biology techniques emerged as an alternative that needs to be harnessed to improve production efficiency by engineering biosynthetic pathway(s) of the molecules in plant cells. Also promising are new elicitors and permeabilizing agents such as coronatin or cyclodextrins. The production of bioactive molecules in endophytes also appears as an attractive alternative, although till date, there is no reported commercial exploitation.

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