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Abiotic elicitors: A tool for enhanced production of secondary metabolites in tropical plants

BACHELOR THESIS

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Declaration

I hereby declare that I have written this thesis entitled "Abiotic elicitors: A tool for enhanced production of secondary metabolites in tropical plants" independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by the means of complete references and according to Citation rules of the FTA.

In Prague 14.4.2021

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Anna Langmaierová

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Abstract

Secondary metabolites are chemical compounds produced by plants as a reaction for stresses. They are synthetized from primary metabolites and ensure survivabilty of plants. Stress initiators, known as elicitors, are used since the second half of 20th century to enhance production of secondary metabolites. Elicitors are divided according to their nature into abiotic and biotic. The aim of this thesis is to introduce abiotic elicitors that are currently used in the production of secondary metabolites and to specifically focus on their production in tropical plants. Recent literature on this topic is introduced in this work. Tropical and subtropical plants are valuable resources of many secondary mentabolites. Their elicitacion is mostly provided by methyl jasmonate and salicylic acid. The most successful acquisition takes place through hairy root and cell suspension cultures. The most widely used plant families for their metabolites are Solanaceae, Lamiaceae, Plantaginaceae, Fabaceae and Vitaceae. Secondary metabolites obtained from these families are scopolamine, tanshinone, bacoside, reservatrol, glycyrrhizine acid and anthocyanin. They are mostly used in medicine and food industry.

Key words: Abiotic stress, elicitors, *in vitro* culture, secondary metabolites, tropical plants

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4CL4	Courmaroyl CoA-ligase
ANR	Anthocyanidin raductase
ANS	Anthocyanidin synthase
ATP	Adenosine triphosaphate
C3HP	Coumarate-3-hydroxylase
C4H	Coumarate-4-hydroxylase
CHI	Chalcone isomerase
CHS	Chalcone synthase
CNS	Central nervous systém
DFR	Dihydroflavonol reductase
F3H	Flavanone 3-hydroxylase
FLS	Flavonol synthase
FS	Flavone synthase
HCT	Hydroxycinnnamoyl transferase
IFS	Isoflavone synthase
JA	Jasmonic acid
LAR	Leucoanthocyanidin reductase
MeJA	Methyl jasmonate
NAA1	Naphthaleneacetic acid
NPAAs	Non-protein amino acids
PAL	Phenylalanine ammonia-lyase
PEG	Polyethylene glycol
SA	Salicylic acid
SM	Secondary metabolite

1. Introduction

Since ancient times some of the plants and their parts were used for their beneficial effects. These effects are the results of the action of secondary metabolites (Croteau et al. 2000). Secondary metabolites (SMs) are low molecular organic compounds produced by plants (Erb & Kliebenstein 2020). They are derived from primary metabolites and do not involve in plant metabolism because they are its end product (Kabera 2014; Hussein & El-Anssary 2018). Plants produce SMs as a self defence mechanism in response for stress (Murthy et al. 2014; Naik & Al-Khayri 2016). Secondary metabolites are known for their wide range of use in pharmacy, food industry, and agronomy (Murthy et al. 2014; Naik & Al-Khayri 2016).

Elicitors are a tool to stimulate production of secondary metabolites in plants. According to their need, it is possible to increase their levels in plants (Ramakrishna & Ravishankar 2011). There are two groups of elicitors: biotic and abiotic, differencing them by their origin (Naik & Al-Khayri 2016). This thesis is focused on abiotic elicitors as a tool for enhanced production of SMs. Abiotic stressor signals are temperature, salinity, water, radiation, chemical stress and mechanical stress. Each group influences different category of secondary metabolites (Karakas & Bozat 2020).

For the production of SMs, the *in vitro* methods are used, because of their feasible yields and other benefits (Goncalves & Romano 2018). Different types of cultures are used to obtain wanted compounds (George et al. 2008).

The aim of this thesis is to introduce all the aspects of production of secondary metabolites *in vitro*, according to latest studies, supported by promising results of experiments, and to introduce that abiotic elicitors are prosperous as biotic elicitors, which have been more examined and used in the past. The thesis also focuses on tropical and subtropical plants and their ability to respond to different abiotic elicitors signals.

2. Aims of the Thesis

The aim of this Bachelor thesis is to introduce elicitors that are currently used in the *in vitro* production of secondary metabolites and to specifically focus on the production of SMs in tropical plants. The recent literature on abiotic elicitors, *in vitro* cultures, secondary metabolites and their production in tropical plants will be reviewed. The concept of secondary metabolites, abiotic elicitors, *in vitro* production, and cultures used for the *in vitro* production will be introduced. The main advantages and disadvantages of *in vitro* production will be discussed and the summary of available data on production of SMs using elicitors in tropical and subtropical plants will be provided as well.

3. Methodology

Scientific articles and books were collected from Web of Science, Elsevier, Springer Link and Wiley Online Library Journals. Scientific literature sources were collected using keywords (secondary metabolites, *in vitro* culture, abiotic elicitors, enhanced metabolite production). Preferably, literature sources not older than 15 years were used. The text structure had been laid out. Scientific texts were analysed, data was compared and sythesized into text of the thesis.

4. Literature Review

4.1. Secondary metabolites

Variety of different low molecular organic compounds is produced by plants. According to their function, they are divided into three groups. Primary metabolites are known for the function to influence the growth and development of the plant, secondary metabolites (SM) for ability to cope with abiotic and biotic stressors, and hormones for their metabolic functions (Erb & Kliebenstein 2020).

When a plant is under the stress from being attacked by pests, pathogens or environmental changes, its response is either chemical or physical (Bennett & Wallsgrowe 1994; Bourgaud et al. 2001). Physical response is represented for example by thorns, barbs, spikes or trichomes. Chemical reaction is associated with the production of chemical compounds and reactions, which can lead to the change of aroma, fragrance, flavour or can make them harder to digest (Bourgaud et al. 2001; Karuppusamy 2009). These compounds are defending plants on four levels:

1) the first level is surface
2) the second are carbohydrates and polymers
3) the third are proteins
4) the fourth are secondary metabolites (Bennett & Wallsgrowe 1994)

The role of SMs is linked not only with its their defence ability, but also can help to the plants with reproduction. For example, changes in aroma caused by essential oils can be alluring and in at the same time repulsive for different species of animals (Wink 1988; Bennett & Wallsgrowe 1994; Bourgaud et al. 2001; Hadacek 2002, Karuppusamy 2009).

During enzymatic reactions, primary metabolites arise (carbohydrates, proteins and lipids) and secondary metabolites are derived from them (Daayf & Lattanzio 2008; Kabera 2014). Secondary metabolites are the end products of metabolism, so they are not involved in the process (Irchhaiya et al. 2014; Hussein & El-Anssary 2018).

Plant cells produce thousands of secondary metabolites (Table 1), which are classified into many groups according to the biosynthetic pathways and the compounds they contain (Irchhaiya et al. 2014). Secondary metabolites can be classified according to the criterium, whether they contain or do not contain nitrogen (Hussein & El-Anssary 2018). The main established classes of SMs are nitrogen-containing alkaloids and sulphur-containing compounds, terpenoids, flavonoids and allied phenolic and polyphenolic compounds (Daayf & Lattanzio 2008; Kabera 2014; Irchhaiya et al. 2014).

Alkaloids are mostly derived from amino acids, but can be also modified from different other molecules as are polyphenols, terpenes and steroids. They are assembled from the atom of nitrogen in a heterocyclic ring (Kabera 2014; Kaur & Arora 2015).

Alkaloids are typical of their structural differences. Compounds with light acidic and neutral properties are classified among alkaloids. In plants, they are found as salts or as a nitrogen oxides (Kabera 2014; Irchhaiya et al. 2014). Alkaloids are produced by many organisms but mainly by plants as secondary metabolites. It is possible to classify alkaloids by many different aspects. For example, by their biosynthetic precursor, and above mentioned heterocyclic ring the groups are indole, tropane, popeidine, purine, imidazole, pyrrolizidinem pyrrolidine, quinolizidine and isoquinoline alkaloids (Kabera 2014; Irchhaiya et al. 2014; Kaur & Arora 2015).

Table 1 Overview of secondary metabolites in plants (Irchhaiya et al. 2014)

Nitrogen-containing SMs	**Numbers of SMs**
Alkaloids and sulphur-containing compounds	
Alkaloids	21.000
Non-protein amino acids (NPAAS)	700
Amines	100
Cyanogenic glycoside	60
Glucosinolates	100
Alkamides	150
Lectins, peptides, polypeptide	2.000
Secondary metabolites without nitrogen	
Terpenoids	
Monoterpenes including iridoids	2.500
Sesquiterpenes	5.000
Diterpenes	2.500
Triterpenes; steroids, saponins	5.000
Tetraterpenes	500
Flavonoids and allied phenolic and polyphenolic compounds	
Flavonoids, tannins	5.000
Phenylpropanoids, lignin, coumarins, lignans	2.000
Polyacetylenes, fatty acid, waxes	1.500
Anthraquinones and othes polyketides	750
Carbohydrates, organic acids	200

Alkaloids can be also classified based on botanical taxonomy of the plants, in which the alkaloids are found (Irchhaiya et al. 2014). There are 18 different categories based on this classification: amaryllidaceae, betalain, diterpenoid, imidazole, indole, isoquinoline, methylxanthines, monoterpenoid, peptide, phenthylamines, piperidine, pyridine, pyrrolidine, pyrrolizidine, quinolone, quinolizidine, steroidal and tropane alkaloids (Kabera 2014; Irchhaiya et al. 2014; Kaur & Arora 2015).

Terpenoids or also terpenes, are the most structurally divided group of plant chemicals. Their biosynthesis origin is in mevalonate. All terpenoids contain five-carbon

group and they are synthetized by the condensation of isoprene units (Croteau et al. 2000; Irchhaiya et al. 2014). Classification of terpenoids takes place based on number of five-carbon groups the compound contains (Croteau et al. 2000). Hemiterpenes are built with only one isoprene unit, monoterpenes despite their name contain two isoprene units, sesquiterpenes have three isoprene units, diterpenes four isoprene units, triterpenes contain six isoprene units, tetraterpenes eight isoprene units, and polyterpenes more than eight isoprene units (Langheim 1994; Irchhaiya et al. 2014). There is also one group called moreoterpenes which contains products of mixed origin, which are derived terpenes (Langheim 1994; Croteau et al. 2000; Irchhaiya et al. 2014).

Phenolics, the most numerous groups of SMs, are usually composed of one or two aromatic rings and one or more hydroxyl groups (Dai & Mumper 2010). They contain compounds from light to large and from simple to complex tannins (Irchhaiya et al. 2014). It is not unusual to find phenolics conjugated to sugars or organic acids. Phenolics can be classified by the number of the carbon atoms or their arrangements. Primary, the phenolics are divided into flavonoids and non-flavonoids (Lattanzio et al. 2009; Irchhaiya et al. 2014). Flavonoids further contain flavones, flavonols, flavanols, flavanones, isoflavones and anthocyanins. Non-flavonoids are phenolic acids, tannins, stilbenes and lignans. Phenolic acids or also hydroxybenzoates are subdivided into derivates of benzoic acid and derivates of cinnamic acid. Tannins are divided into two groups of hydrolysable and condensed tannins (Lattanzio et al. 2009; Dai & Mumper 2010; Irchhaiya et al. 2014).

4.1.1. Properties of secondary metabolites and their applications

The uses of secondary metabolites vary depending on the plant material and plant organs for extraction, also on the plant species and methods of extraction used (Karuppusamy 2009; Karakas & Bozat 2020). The number of possible interactions of secondary metabolites is unaccountable and so it is their application (Verpoorte 1998; Murthy et al. 2014). That is the reason why the space for improvement in different fields of industries is still open. Secondary metabolites are widely used in pharmacy, cosmetic industry, food industry and in agriculture (Karuppusamy 2009; Karakas & Bozat 2020). This chapter will focus on the main applications of alkaloids, terpenes and phenolics.

Alkaloids are one of the groups of secondary metabolites, and their application is tracked thousand years BC. People have been using extracts from plants as additives to poisons and potions (Croteau et al. 2000). Nowadays, there are over 21,000 of different compounds found in higher plants known. Due to their biological and physiological benefits, alkaloids, are used in medicine profusely (Croteau et al. 2000; Kabera 2014; Irchhaiya et al. 2014).

Vinvamine, vincristine, vinblastine, serotonin, strychnine, ajmalicine and ajmaline are compounds which belong to the group of the most studied indole alkaloids used in pharmacology (Croteau et al. 2000; Kaur & Arora 2015). Tropane alkaloids such as scopolamine, hyoscyamine, cocaine and atropine are used as blockers of neurotransmitter actions. Application of quinine alkaloids as echinopsine, dyhidroquinine, camptothecin, etc. is bound to their antimalarial, anti-bacterial, antifungal, anti-inflammatory and analgesic activity (Croteau et al. 2000; Kaur & Arora

2015).Isoquinoline alkaloids, described in Table 2, are reporting pharmacological activity (Kabera 2014).

Table 2 Pharmacological activity of medically used alkaloids (Kabera 2014)

Alkaloid name	Pharmacological activity
Atropine	Competitive antagonist of muscarinic acetylcholine receptors, anti cholinergic, anti myopia effects
Berberine	Anti inflammatory, anti bacterial/viral, recently experiments showed anti diabetic and beneficial effects on cardiovascular system and anti cancer and others disorders such as intestinal
Codeine	Analgesic, antitussive, anti diarrheal, antidepressant, sedative and hypnotic properties, neurotoxin, poisonous
Coniine	Acetylcholine agonist, smoking cessation drug
Cytisine	Act on CNS (central nervous system), on myenteric plexus, acute pulmonary edema and reduce the shortness of breath
Morphine	Stimulant, antiherbivore, insectide, anti inflammatory
Nicotine	Antimalarial, antpyretic, analgesic, anti-inflammatory, antiarrhythmic, bacteriostatic
Quinine	Antifungal, antipesticide, sedative, anticonvulsant, anticarcinogenic, anti inflammatory
Solanine	Pesticide, strong poisonous, convulsant
Strychnine	Pesticide, strong poisonous, convulsant
Thebaine	Analgesic, not therapeutically used.
Tomatine	Immune effects, anticancer, antifungal, poisonous

Isoquinoline alkaloids are liked to antihyperglycemic, antitumor and antibacterial activity. Purine alkaloids, for example caffeine, are used in diabetes and obesity medications, have anti-inflammatory properties, and can inhibit oxidation (Croteau et al. 2000; Kabera 2014; Kaur & Arora 2015). The group of piperidine alkaloids is known for their toxicity, anticancer, central nervous system (CNS) stimulating and depressant, herbicidal, insecticidal and fungicidal properties. Important members of piperidine alkaloids are coniine, lobeline, cynapine (Kaur & Arora 2015). Chemically almost similar group of alkaloids are pyridine alkaloids which are anabasin, nicotine, anatabin, anatabine and epibatidine known for antimicrobial functions (Kabera 2014).

Pilocarpine is an alkaloid used in the treatment of eye disorders and belongs to the group of imidazole alkaloids (Croteau et al. 2000). Glycosidase of pyrrolizidine alkaloids such as senecionine or heliotrine are used as cancer and diabetes cures. Members of the pyrrolidine alkaloids group, such as puterescine, hygrine and cuscohygrine have shown their antibacterial and antifungal properties (Irchhaiya et al. 2014). Quinolizidine alkaloids, whose two most abundant members are cytisine and sparteine, are used for their antimicrobial function (Croteau et al. 2000; Kabera 2014; Kaur & Arora 2015).

Terpenes are historically used since times of civilisations in Egypt, during ancient times they were mostly used as medicines or as a fragrances (Croteau et al. 2000; Zwenger & Basu 2008)

Terpenes sometimes incorrectly called isoprenoids, are the most abundant group of natural products, with over 80,000 representatives (Croteau et al. 2000). Their compounds are highly used and studied in human health and nature environment (Dickshat 2019). This group of secondary metabolites is widely utilized across many disciplines of science and some uses are more of industrial character (Dickshat 2019). Terpenes are applied in medicine, agriculture and food industry (Langheim 1994; Croteau et al. 2000; Dickshat 2019).

Monoterpenes, some of which are mentioned in Table 3, are a group containing D-limonene and perillyl alcohol, that function to inhibit mammary, liver, skin, lung, colon, forestomach, prostate, and pancreatic carcinomas. Some derivates of D-limonene showed anticancer activities (Croteau et al. 2000; Ajikumar et al. 2008). Carveol, uroterpenol, sobrerol were proven to have characters of anti-mammary cancer activities. This class of terpenoids display volatile essences of flowers and essential oils of herbs and spices. Sesquiterpenes include compounds as β-caryophyllene, belonging to the plant oils, which have antibacterial properties. They often act as antibiotic compounds, and as antifeedants (Langheim 1994; Ajikumar et al. 2008).

Representatives of the group of diterpens, such as taxol have anticancer properties and are effective in the treatment of glaucoma. Kalihinol found in marine sponge is associated with antiparasitic activity, and many others have antibacterial, antimalarial and antiplasmoidal properties (Ajikumar et al. 2008; Zwenger & Basu 2008). Antitumor and feeding detergent properties are known for triterpenoid class, to this class for example belongs oleanolic acid and brassinosteroids (Langheim 1994; Croteau et al. 2000).

Table 3 Monoterpenes and their application in ecology (Langheim 1994)

	Direct plant defense	Allelopathy	Formation of reactive gases in troposphere	Indirect plant defense	Plant polination
Limonene	✓	✓	✓		✓
α-Pinene	✓	✓	✓		✓
β-Pinene	✓	✓	✓	✓	✓
Myrcene	✓	✓	✓		✓
Δ-3-Carene	✓		✓		✓
Camphor	✓	✓			✓
Pulegone	✓	✓			
Cineole	✓	✓	✓		✓
Citronellol	✓				✓
Linolool	✓		✓	✓	✓
Ocimene			✓	✓	✓

Tetraterpenes or also carotenoids, used as natural dyes, have antioxidating functions and are essential for photosynthesis (Croteau et al. 2000; Ajikumar et al. 2008). Their agents are lycopene, xanthophylls or beta-carotene (Irchhaiya et al. 2014; Dickshat 2019). Polyterpenes are involved in sugar transfer and their long time known representative is rubber, long polymer compound (Langheim 1994). Ecological functions of tetra- and polyterpenes have not been fully discovered yet (Croteau et al. 2000; Ajikumar et al. 2008; Zwenger & Basu 2008; Dickshat 2019).

Phenolics are the largest group of secondary metabolites, with over 8,000 structures (Shahidi & Naczk 2004). They occur in a wide range of plant families. Phenolics are often found in fruits, vegetables, tea, cocoa and other plant based foods (Crozier et al. 2009; Dai & Mumper 2010).

As well as in the case of alkaloids and terpenes, some of the subcategories of phenolics include important compounds for their application (Croteau et al. 2000). Such flavonoids as pelargonidins, cyanidins and delphinidins are connected with the dispersion of seeds and attracting of pollinators. Flavonoids affect colour characterization of flowers (Croteau et al. 2000). Kaemferol, which can be found in extract of berries, with inhibitory application against wide range of tumor cell lines is also described as protector for plants against UV-B irradiation (Crozier et al. 2009). Flavonoids also have a variety of medicinal uses. For example, flavonoid phenolics, which are the inhibitors for the growth of varial tumor cell lines, were found in extracts made of berries (Croteau et al. 2000). Rutin and quercetin, contained in wine demonstrably act against coronary heart diseases. Another compounds containing flavonoids are found to be anticancer, antiviral and antitoxic (Croteau et al. 2000; Crozier et al. 2009; Dai & Mumper 2010).

Courmarins, stilbenes, styrylpyrones and arylpyrones belong to the non-flavonoid group (Croteau et al. 2000). The function courmarins have in plants is a defensive one, they are also antimicrobial, antifeedant, and have germination inhibitor properties (Crozier et al. 2009). Courmarins contained in clover can cause internal bleeding in mammals, are now used in rodenticides (Croteau et al. 2000; Shahidi & Naczk 2004).

The agent of this group, psoralen is used to treat skin illnesses (Kabera et al. 2014). Stilbenes are used in pharmacology and are important for some plant activities (Croteau et al. 2000). They are antifungal, play role in dormancy and grow inhibition. Stilbenes inhibit feeding for mammals and are toxic for insects (Dai & Mumper 2010; Kabera et al. 2014).

4.2. Elicitors as stress simulators

Plant stress is described by scientist with slight differences depending on the origin and type of the stress (Lichtenthaler 1998; Kranner et al. 2010; Ismail et al. 2017). Summarizing their definitions, plant stress is a state in which the plant functions are being destabilized and can cause temporary, permanent damage or even death of the plant. Stress is divided into eu-stress and dis-stress (Lichtenthaler 1998; Kranner et al. 2010). Eustress is positive for the plant, dis-stress is the one causing damage. The border between these two stresses is tolerance and sensitivity (Kranner et al. 2010). One factor can have a positive effect on plants during short time or low doses and the same factor can have opposite effect if the stressor is not removed early or doses are too high (Ismail et al. 2017). Reactions induced by stress also depend on the state of the plant before the stress occurred, on the species of the plant, and its stress-coping mechanism (Lichtenthaler 1998; Ismail et al. 2017).

Under normal conditions, the plant is in a standardized phase without any stress. At the moment, when the stress starts, the plant starts to sense it. This triggers a reaction, which is described in four phases by Lichtenthaler. These phases are:

1) Response phase

2) Restitution phase

3) End phase

4) Regeneration phase (Lichtenthaler 1998, Ismail et al. 2017)

In the first phase, the stress begins. Plant starts to decline some of the phytological functions. The stress starts to show its effects on the plant's vitality, and its metabolism starts to be catabolic (Kranner et al. 2010). The second stage of resistance begins when the coping mechanisms start to work and activate the adaptations or repair process (Lichtenthaler 1998; Kranner et al. 2010). If the plant does not have any stress tolerance or has a low resistance minimum, this stress can cause a damage. The restitution phase causes hardening and establishment of metabolism and morphology under the pressure of stress (Ismail et al. 2017). When the exhibition of the stress continues or the level of the stress is too high, the end phase begins. This can cause a chronic disease or even the death of the plant (Lichtenthaler 1998). In case the stressor is removed, the plant continues into the phase four, where it regenerates and creates a new physiological resistance and standards (Kranner et al. 2010; Ismail et al. 2017).

Stress is an important factor in the production of SMs (Garcia-Brugger et al. 2007; Karuppusamy 2009). In response to environmental changes, many SMs compounds in

aromatic and medical plants are being induced (Rao & Ravishankar 2002; Murthy et al. 2014). This method is also used in the pharmaceutical industry. Stress factors are called elicitors and they activate the defending mechanism of the plant (Gorelic & Bernstein 2014). The process of active stimulation of the plant with stress in order to produce secondary metabolites is called elicitation (Karuppusamy 2009; Rao & Ravishankar 2002; Murthy et al. 2014). Elicitation is applied to increase the production of secondary metabolites and control the culture volume (Garcia-Brugger et al. 2007). Elicitation was found to be the most feasible way to produce desired SMs from cell, organ and plant system (Gorelic & Bernstein 2014). The production of SM is influenced by six parameters affecting successful elicitation (Karuppusamy 2009). These factors are concentration of elicitors, time of exposure, age and state of elicitated culture, cell line quality and composition of nutrients in a plant (Rao & Ravishankar 2002; Murthy et al. 2014; Naik & Al-Khayri 2016).

4.2.1. Production of secondary metabolites

The production of SMs is triggered by environmental factors which affect the plant. The defending mechanism is described in previous chapter. During this reaction to stress the process of biosynthesis of SMs in plant starts (Croteau et al. 2000).

The reaction of biosynthesis (Fig. 1) is fuelled by the metabolic pathway of conversion of carbohydrates, when glucose is conversed into two molecules of pyruvate, two hydrogen ions, and two molecules of water called glycolysis and through the citric acid cycle which is series of reaction creating metabolic pathway during aerobic oxidation of saccharides, lipids, and proteins (Kabera et al. 2014). Adenosene triphosaphate (ATP) is formed as a product of the oxidation of glucose, fatty acids and amino acids. ATP is a high energy molecule formed during catabolism of primary metabolites (Kabera et al. 2014; Kumar 2018).

Alkaloids are formed from l-amino acids. They can be produced alone or together with steroidal or terpenoid group. Biosynthetic pathways of alkaloids in plants have not been fully discovered, but metabolical ways of some of them, such as vindoline or berberine were clarified on enzymatic levels (Croteau et al. 2000). Alkaloids in plants are not as abundant as terpenoids or phenolics. It is possible to induce their production by elicitation. One of the members of the alkaloids group, camptothecin, increases in case of low intensity of sunlight (27%). Enhanced production of catharanthine, vindoline levels was recorded after exposure to UV-B irradiation and short-term heat (Yang et al. 2018). 10-hydroxycamptohecin after being exposed to 40 °C for 2 hours. Strong response was found after exposing the plant to drought when alkaloids glycine betadine, camptohecin and morphine were more abundant in plant. Salinity in soil also improved the production of catharanthine (Croteau et al. 2000; Yang et al. 2018).

Terpenoids are the products of primary metabolites (Yang et al. 2018). Their biosynthesis can be summarized into four steps. The first step is the synthesis of the essential precursor isopentenyl pyrophosphate (Cheynier et al. 2013). The second is the repetitive addition of the precursor to form a row of prenyl diphosphate homologs, which are direct precursors to other classes of terpenoids. The third step is initiating these allylic

prenyl diphosphates by specific terpenoid synthases to yield terpenoid skeletons. The last step is the modification of the skeletons by secondary enzymatic reactions (Cheynier et al. 2013).

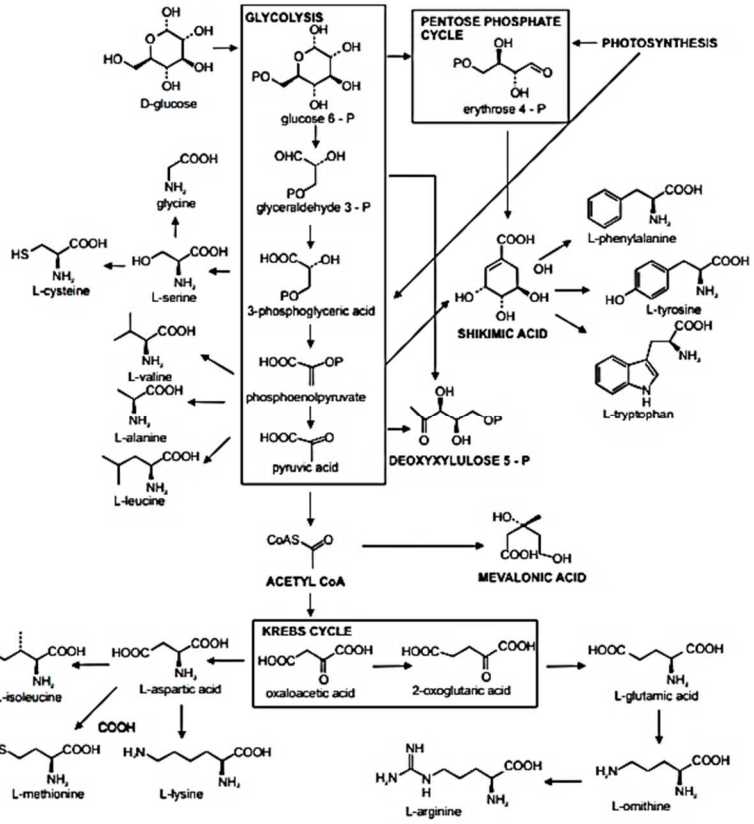

Figure 1 Biosynthesis scheme of plants secondary metabolites (Kabera et al. 2014)

Phenolic and polyphenolic compounds are secondary metabolites emerging from shikimate/phenylpropanoid pathway, where the final product is phenylpropanoids or acetate/malonate pathway, which can produce basic phenols or both monomeric and polymeric structures. Both of these pathways are displayed in the figure 2. (Cheynier et al. 2013; Yang et al. 2018). The increase of production by change of the photoperiod to long day is accompanied by chlorogenic acid, catechins and hydroxybenzoic acids. On the contrary, on a short day the production of pelargonidin and caffeoyluquinic acids decreases. Change of the intensity of light, exposing the plant to full sunlight positively affects chlorogenic acid and asiaticoside (Yang et al. 2018). Red light causes decreasing production of ferulic acid and kaempferol. UV light increases the production of rutin, quercetin and catechins. Low temperature was found effective on magnifying pelargonidin, same as on genistein and daidzein. The concentration of SMs increased with deficit of water elicitation in cases of chlorogenic acid, catechins and tanshinone (Cheynier et al. 2013). Soil salinity increased rutin, vitexin andisoorentin and influencing

the soil fertility with nitrogen and phosphate increased production of quercentin, kaempferol and isorhamentin (Cheynier et al. 2013; Yang et al. 2018).

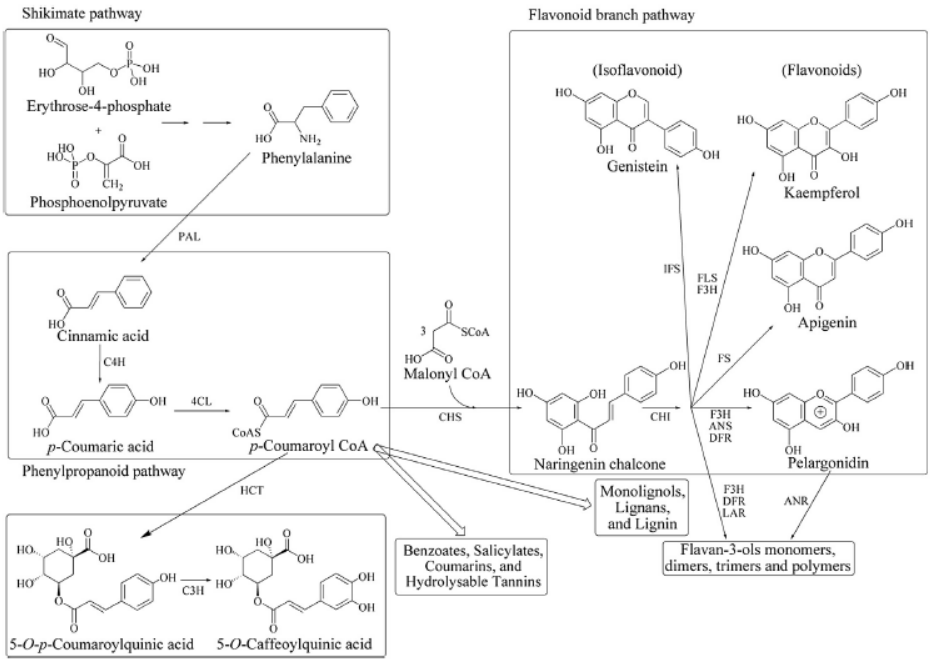

Figure 2 Schematic of the major branch pathways of (poly)phenol biosynthesis (Cheynier et al. 2013)

4.3. History of in vitro secondary metabolite production

From the beginning of the human civilization, selected plants have been processed for their beneficial properties. All these plants contained secondary metabolites (Croteau et al. 2000; Irchhaiya et al. 2014).

It was believed that primary metabolites play the crucial role in self-defence and growth mechanism of a plant (Bennett & Wallsgrowe 1994). The other products that are also produced during chemical and biochemical reactions, known as secondary metabolites, were meant to be a waste product. The name they have got, secondary, is misleading and indicates something minor compared to others (Bennett & Wallsgrowe 1994; Bourgaud et al. 2001).

Secondary metabolites have been studied since the second half of the nineteenth century. The interest in SMs in academic fields increased in searching for new drugs, antibiotics, insecticides and herbicides (Croteau et al. 2000; Irchhaiya et al. 2014).

Microbiology was on an upswing and technologies such as spectroscopy and chromatography were invented (Hadacek 2002). Different names for these compounds were suggested, although the name secondary metabolites was already established and used in publications (Bourgaud et al. 2001). Technology and more thorough researches

proved major roles and functions of SM in plants and their environment (Bennett & Wallsgrowe 1994; Bourgaud et al. 2001).

In the beginning of the twentieth century, German scientist named Gottlieb Haberlandt published a study where he settled the term of totipotency. It was also the first publication where micropropagation was mentioned (Rücker & Laimer 2003). Mineral salt sugar solution was firstly used in 1904 by scientist Hanning for growing mature embryos (Twaji et al. 2020). The first plant tissue cultures were achieved almost 40 years later by P. A. White (Vasil 2010), R. J. Gautheret (Gautheret 1939) and P. Nobécourt (Nobécourt 1939). During this time the techniques that were already used were ameliorated and new ways of propagation were found (Thrope 2007).

The year 1950 was the first year when a scientist unsuccessfully tried to use plant cells for production of pharmaceuticals (Twaji et al. 2020). Researches in Europe and in Japan continued and 28 years later the secondary metabolite production in vitro have been declared successful (Thrope 2007). Another 30 years later tissue cultures were produced with the goal to make economic profit (Rücker & Laimer 2003). In the year 1987, there were even more efficient culture systems of secondary metabolites, than the original plants (Thrope 2007).

Since the year 1990, use of *in vitro* tissue culture has been growing in the academic and even in the commercial field. New species that were able to be used in *in vitro* cultures and new ways of use of this techniques were and still are found (Thrope 2007). Plant tissue culture is the most effective way of production of secondary metabolites and other saleable compounds in 21 century. Several protocols have been made for the commercial production of secondary metabolites. Agricultural science and modern agriculture have a significant tool for development and production (Twaji et al. 2020).

4.4. Classification of secondary metabolites elicitors for in vitro production

Elicitors are classified on a several levels (Table 4), depending on the basis of their nature as abiotic and biotic, on the basis of their origin as endogenous and exogenous and according to their molecules (Angelova et al. 2006; Gorelic & Bernstein 2014; Naik & Al-Khayri 2016).

Biotic elicitors are derived out of a host or pathogen origin. It is common to use various biotic elicitors at a time (Naik & Al-Khayri 2016). Based on the origin, biotic elicitors are divided into polysaccharide, fungal, yeast and bacterial. All of them affect the production of SMs (Gorelic & Bernstein 2014; Naik & Al-Khayri 2016). This study focuses on abiotic elicitors.

Abiotic elicitors are the factors from non-biological origin (Naik & Al-Khayri 2016). They are usually inorganic salts or physical factors acting as elicitors. Abiotic elicitors have various effects on plants and the production of SMs (Gorelic & Bernstein 2014).

Recent works discovered reaction of key genes, proteins and metabolites to different abiotic stresses as are drought, salinity, etc. (Naik & Al-Khayri 2016).

Table 4 Classification of secondary metabolites elicitors (Angelova & et al. 2006; Gorelic & Bernstein 2014; Naik & Al-Khayri 2016)

Abiotic			Biotic
Physical	**Chemical**	**Hormonal**	
ultrasound	heavy metals	salicylic acid	Polysaccharide
light	mineral salts	jasmonates	Fungal
salinity	gaseous toxins	brassinosteroids	Yeast
drought		absicic acid	Bacterial
osmotic stress		auxins	
thermal stress			

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They are subdivided into physical, chemical and hormonal. Physical abiotic factors are represented by ultrasound, light, salinity, drought, osmotic stress or thermal stress (Angelova & et al. 2006). Ultrasound has two effects on the plant, of which the first is an induction of production of secondary metabolites, and the second is the stimulation permeability of cell membrane to release SMs (Angelova & et al. 2006; Gorelic & Bernstein 2014). It was proven that light stimulates the biosynthesis of the product form callus cultures, hairy roots in more than one plant (Naik & Al-Khayri 2016). UV-B activates the plant defence metabolism and the production of alkaloids, terpenes and phenolics. UV-C irradiation influences the production of stilbene (Gorelic & Bernstein 2014). Salinity and drought are reducing the plant's growth and development, they stimulate the production of alkaloids, terpenes and phenolics (Gorelic & Bernstein 2014). Osmotic elicitors are influencing growth and development of the plant and the formation and morphogenesis of secondary metabolites (Gorelic & Bernstein 2014). Extreme temperatures, cold or hot, were shown to have various effects on production of SMs as they either increase or lower their production, development, and growth of the plant (Angelova & et al. 2006; Gorelic & Bernstein 2014; Naik & Al-Khayri 2016).

Chemical abiotic elicitors include intoxication with heavy metals (Ni, Ag, Fe and Co), mineral salts and gaseous toxins (Gorelic & Bernstein 2014). Heavy metals affect the production of sugars, proteins and non-protein thiols and elicit SM biosynthesis (Naik & Al-Khayri 2016). The group of hormonal abiotic elicitors containing salicylic acid, jasmonates, brassinosteroids, abcisic acid and auxins is the most frequently reported one. All of them induce the biosynthetic pathways for the production of SMs (Angelova & et al. 2006; Naik & Al-Khayri 2016).

4.5. Types of in vitro cultures used for production of SMs

In vitro cultures are being maintained during period of time on culture media (George et al. 2008). The cultures can be divided into two mains groups: of organised structures and cultures of unorganised tissues. Their further division is described in Table 5. Both of them are used for *in vitro* production (George et al. 2008).

Organ cultures are significant for their ability to coordinate their growth repeatedly. They are also the most important organised structures for *in vitro* propagation (George et al. 2008).

Table 5 Division of cultures used in in vitro production (George et al. 2008)

Organised stuctures	Unorganised tissues
Meristem cultures	Callus cultures
Shoot cultures	Suspension cultures
Node cultures	Protoplast cultures
Isolated root cultures	Anther cultures

Meristem culture is from a meristematic cell removed from the main shoots or axillary buds, in order to culture *in vitro* (Spangenberg et al. 1998). The culture contains one or two leaf primordium, and a small, excised shoot apex is grown inside. The difference between meristem and shoot culture is the size of the apex and the number of primordium leaves. Shoot culture involves bigger apex and several leaves of primordium and is capable of producing multiple shoots (George at al. 2008). During node culture, nodes of mature plants contain axillary buds and in proper medium, they can produce roots or branches (Bhatia et al. 2015). Root culture is from roots growing isolated from shoots, and it is also possible to use hairy roots. Nature of embryo cultures is used to excise embryos from the ovules or seeds and plant them *in vitro* (George at al. 2008).

Callus is the cell tissue, and the callus culture can be provided by the placement of an explant into nutrient medium. Cells of callus diffused in liquid medium are called suspension culture (Sikyta & Dušek 2001). Protoplast culture is composed from cells without cell walls, and their surface is covered by cytoplasmatic membrane (Sikyta & Dušek 1992).

4.6. Advantages and disadvantages of in vitro production of secondary metabolites

The extraction of SMs from naturally grown plants is not always possible because of regional and environmental restrictions (Goncalves & Romano 2018). Some species of plants are also not easily planted in vivo because of long growth cycle, phytosanitary

problems, or regional restrictions. In that context the *in vitro* production is the more ecological and feasible alternative (Goncalves & Romano 2018; Cardoso et al. 2019).

Thanks to *in vitro* production, it is possible to produce cells that are insect and microbe free (Tiwari 2015), and thus, there is no need to use herbicides or pesticides (Goncalves & Romano 2018). In this case, it can be better control over the evolution of the cells and over the whole plant, owing to which the organogenesis or the production of tissues containing high content of SMs can be influenced (Dias et al. 2016). Cells are easily multiplied to yield the specific metabolite or can be genetically transformed and can be used as ´bioreactors´ of SMs (Tiwari 2015; Cardoso et al. 2019). Some of the SM do not have to be in primary plants, but thanks to biotechnology metabolic pathways for their production can be induced (Goncalves & Romano 2018). High content of SMs can be achieved by using proper abiotic and biotic elicitors (Dias et al. 2016; Cardoso et al. 2019).

The production of SMs starts with the accumulation of biomass and later with the synthesis of SMs. Biomass can be obtained from organised structures or unorganised mass cells (Engelmann 2010). Organised structures are usually used in the case when SM is produced in specialized plant tissue or gland only (Goncalves & Romano 2018). Hairy roots culture has lately become a popular choice for production of chemical compounds and similar compounds as plant roots can be produced through it. Its advantages are high level of cellular differentiation, fast growth, biochemical stability, high productivity, constancy and competence (Goncalves & Romano 2018; Chandran et al. 2020). Unorganised cultures are more likely to be used for SM production due to their totipotency, simplicity, stability, and cost effectiveness. Cell suspension cultures are considered to product SM of uniform quality and yield. They can also synthetize novel products which differ from their natural ability (Chandran et al. 2020).

Chemical compounds such as SM are produced under highly controlled microenvironment regime, regardless of climate changes, soil and geographic condition (Tiwari 2015; Dias et al. 2016; Cardoso et al. 2019). Also, the possibility of a year-round production without the influence of the seasons gives an economically viable synthesis of pure compounds besides the naturally limited production (Dias et al. 2016; Goncalves & Romano 2018).

The main disadvantage of *in vitro* SM production is the price of this method. High expenses are related to this method, such as its requirement of special laboratory equipment (autoclave, laminar flow hood) and controlled environment as its infrastructure for aseptic workplace, stable working environment (Pence 2011; Filová 2014; Cardoso et al. 2019). The whole process of *in vitro* propagation from the beginning requires finances. Also, if the industrial production is considered, high prices of bioreactors compared to in vivo method have to be considered. Extraction, purification and analysis of compounds performed by Liquid Chromatography and by High performance Liquid Chromatography is high-priced too (Cardoso et al. 2019).

Incomplete knowledge complicates the production of SMs *in vitro* (Miralpeix 2013). Even though that science has moved forward, it is still not possible to produce some of the secondary metabolites for phytochemicals. And if it is possible, it is still low

yield for industrial use. This problem can be solved by metabolite engineering in the future (Krappusamy 2009; Tiwari 2015). It is also important to mention that there are no step-by-step protocols for *in vitro* SMs production for industrial production (Cardoso et al. 2019).

4.7. Enhanced in vitro synthesis of secondary metabolites in tropical and subtropical plants

This chapter provides an overview of studies focused on elicitation of plants with origin in tropics or subtropics with abiotic elicitors (Table.7). Following types of elicitors were used in mentioned studies: heavy metals, mineral salts, gaseous toxins, drought, salinity, light, thermal stress, ultrasound, salicylic acid (SA), jasmonates and auxins. These hormonal, chemical and physical elicitors enhanced production of alkaloids, terpenoids a phenolics naturally occurring in mentioned plants.

Plants included in this work have mostly medicinaly active compounds. Scopolamine produced by plants from family Solaceae is known as neutrotransmitter blocker (Kaur & Arora 2015). Vinblastine produced by *Catharanthus roseus* and taxol produced by *Taxus chinensis* are secondary metabolites used as anticancer drugs, moreover taxol also treats glaucoma (Ajikumar et al. 2008, Zwenger & Basu 2008, Valli et al. 2011). Rutin contained in *Hypericum brasiliense* is sucesfull in treatement coronary heart deseases (Crozier et al. 2009).

The most frequent family in enhancing production of secondary metabolites is Solanaceae. Secondary metabolites from plants of this botanical family are obtained from root cultures and belongs to tropane alkaloids and withanolides. Plants from family Lamiaceae are mostly used for induction of tanshinone. Thanshinone shows pharmacological activities as antioxidant activity, anti-inflammatory activity, cardiovascular effects, and antitumor activity (Jiang et al. 2019).

More details on in vitro synthesis of SMs in tropical and subtropical plants are provided in Table 6.

Table 6 Enhanced in vitro synthesis of secondary metabolites in tropical and subtropical plants

Elicitor	Concentration	Culture	Plant species	Family	Compounds	Source
$AgNO_3$	15 µM	Hairy root	*Salvia miltiorrhiza* Bunge	Lamiaceae	Tyrosine aminotransferase	Qiong et al. (2006)
	50 µM	Hairy root	*Salvia miltiorrhiza* Bunge	Lamiaceae	Tanshinone	Shi et al. (2014)
	25 µM	Cell suspension	*Salvia miltiorrhiza* Bunge	Lamiaceae	Tanshinone	Zhao et al. (2010)
	1.0 µM	Hairy root	*Brugmansia candida* Pers.	Solanaceae	Scopolamine, hyoscyamine	Pitta-Alvarez et al. (1999)
	25 µM	Adventitious root	*Perovskia abrotanoides* Kar.	Lamiaceae	Cryptotanshinone, tanshinone	Zaker et al. (2015)
$AlCl_3$	250 µM	Root	*Datura metel* L.	Solanaceae	Hyoscyamine, scopolamine	Ajungla et al. (2009)
Cd^{2+}	25 µM	Cell suspension	*Salvia miltiorrhiza* Bunge	Lamiaceae	Tanshinone	Zhao et al. (2010)
$CdCl_2$	1.0, 2.0 µM	Hairy root	*Brugmansia candida* Pers.	Solanaceae	Scopolamine, hyoscyamine	Pitta-Alvarez et al. (1999)
	27.3 µM	Callus	*Plumbago zeylanica* L.	Plumbaginaceae	Plumbagin	Singh et al. (2020)
$CuSO_4$	25-75 µM	Callus	*Dioscorea bulbifera* L.	Dioscoreaceae	Disogenin	Narula et al. (2005)
	0.20 µM	Shoot	*Bacopa monnieri* (L.) Wettst.	Plantaginaceae	Bacoside	Naik et al. (2015)
	282 µM	Shoot	*Bacopa monnieri* (L.) Wettst.	Plantaginaceae	Bacoside	Sharma et al. (2014)
$Pb(NO_3)_2$	25000 µM	Cell suspension	*Plumbago indica* L.	Plumbaginaceae	Plumbagin	Singh et al. (2018)
$ZnSO_4$	0.12 µM	Shoot	*Bacopa monnieri* (L.) Wettst.	Plantaginaceae	Lepidine	Naik et al. (2015)
H_20 deficit	60-70% water in soil	Root	*Glycyrrhiza uralensis* DC.	Fabaceae	Glycyrrhizic acid, liquiritin	Li et al. (2009)
	40% field water capacity	Root	*Salvia miltiorrhiza* Bunge	Lamiaceae	Salvianolic acid B	Liu et al. (2011)
H_20 surplus	waterlogging	Seedlings	*Hypericum brasiliense* Choisy	Hypericaceae	Betulinic acid, quercetin, rutin	Abreu & Mazzafera (2005)
NaCl	100 µM	Shoot	*Sutherlandia frutescens* R.Br. ex W.T.Aiton	Fabaceae	Canavanine	Colling et al. (2010)
	25 µM	Callus	*Catharanthus roseus* G. Don	Apocynaceae	Vinblastine	Fatima et al. (2015)
	100 and 400 µM	Seedlings	*Cakile maritima* Scop.	Brassicaceae	Polyphenol	Ksouri et al. (2007)
	150 µM	Seedlings	*Sesamum indicum* L.	Pedaliaceae	γ-aminobutryric	Bor et al. (1999)
JA	20 µM	Cell suspension	*Vitis vinifera* L.	Vitaceae	Anthocyanin	Zhang et al. (2001)
	4.8 µM	Shoot	*Bacopa monnieri* (L.) Wettst.	Plantaginaceae	Bacoside	Sharma et al. (2014)

	10 µM	Cell suspension	*Gymnema sylvestre* R.Br.	Apocynaceae	Gymnemic acid	Chodisetti et al. (2014)
MeJA	100 µM	Hairy root	*Vitis rotundifolia* Michx.	Vitaceae	Resveratrol	Nopo-Olazabal et al. (2014)
	60-120 µM	Cell suspension	*Taxus chinensis* Pilg.	Taxaceae	Taxol	Wu & Lin (2002)
	50 µM	Hairy root	*Salvia miltiorrhiza* Bunge	Lamiaceae	Tanshinone	Hao et al. (2014)
	10 µM	Cell suspension	*Vitis vinifera* L.	Vitaceae	Resveratrol	Tassoni et al. (2005)
	5 µM	Cell suspension	*Andrographis paniculata* Burm.f.	Acanthaceae	Andrographolide	Sharma et al. (2014)
	50 µM	Shoot	*Bacopa monnieri* (L.) Wettst.	Plantaginaceae	Bacoside A	Sharma et al. (2013)
	100 µM	Root	*Taverniera cuneifolia* Arn.	Fabaceae	Glycyrrhizic acid	Awad et al. (2014)
	10 µM	Cell suspension	*Gymnema sylvestre* R.Br.	Apocynaceae	Gymnemic acid	Chodisetti et al. (2014)
	5 µM	Shoot	*Andrographis paniculata* Burm.f.	Acanthaceae	Andrographolide	Sharma et al. (2014)
	89.2 µM	Callus	*Silybum marianum* (L.) Gaertn	Asteraceae	Silymarin	Gabr et al. (2016)
	15 µM	Aventitious root	*Withania somnifera* (L.) Dunal	Solanaceae	Withanolides	Sivanandhan et al. (2013)
SA	0.01, 0.10, 1.00 µM	Hairy root	*Brugmansia candida* Pers.	Solanaceae	Scopolamine, hyoscyaµMine	Pitta-Alvarez et al. (1999)
	50 µM	Hairy root	*Salvia miltiorrhiza* Bunge	Lamiaceae	Tanshinone	Hao et al. (2015)
	150 µM	Hairy root	*Withania somnifera* (L.) Dunal	Solanaceae	Withanolides	Sivanandhan et al. (2013)
	50 µM	Shoot	*Bacopa monnieri* (L.) Wettst.	Plantaginaceae	Bacoside	Sharma et al. (2014)
	500 µM	Root	*Datura metel* L.	Solanaceae	Hyoscyamine, scopolamine	Ajungla et al. (2010)
	145 µM	Callus	*Silybum marianum* (L.) Gaertn	Asteraceae	Silymarin	Gabr et al. (2016)
	150 µM	Hairy root	*Withania somnifera* (L.) Dunal	Solanaceae	Withanolides	Sivanandhan et al. (2014)
Light	3000 Lux	Hairy root	*Artemisia annua* L.	Asteraceae	Artemisinin	Chun-zhao et al. (2002)
	8000–8300 lux	Cell suspension	*Vitis vinifera* L.	Vitaceae	Anthocyanin	Zhang et al. (2001)
UV-B	5 min	Cell suspension	*Catharanthus roseus* G. Don	Apocynaceae	Catharanthine, vindoline	Ramani & Jayabaskaran (2008)
	20min	Hairy root	*Catharanthus roseus* G. Don	Apocynaceae	Lochnericine	Binder et al. (2009)
UV-Cirradiation	20min	Cell suspension	*Vitis vinifera* L.	Vitaceae	Stilbene, resveratrol	Xu et al. (2015)

Temperature	50℃	Seedlings	*Sesamum indicum* L.	Pedaliaceae	ɣ-aminobutryric	Bor et al. (1999)
	25℃	Hairy root	*Panax ginseng* C.A.Mey.	Araliaceae	Ginsenoside	Yu et al. (2004)
	36℃	Seedlings	*Hypericum brasiliense* Choisy	Hypericaceae	Quercetin, rutin, isouliginosin B	Abreu & Mazzafera (2005)
PEG	16.7, 28.3 mM	Seedlings	*Scrophularia ningpoensis* Hemsl.	Scrophulariaceae	Cinnamic acid	Wang et al. (2010)
	100 mM	Seedlings	*Sesamum indicum* L.	Pedaliaceae	ɣ-aminobutryric	Bor et al. (1999)
	1.7 mM	Seedlings	*Hypericum adenotrichum* Spach.	Hypericaceae	Hypericin, pseudohypericin	Yamaner & Erdag (2013)
pH	pH 5 for	Cell suspension	*Capsicum chinense* Jacq.	Solanaceae	Capsicin	Kehie & Kumaria (2013)
	pH 6.0	Hairy root	*Withania somnifera* (L.) Dunal	Solanaceae	Withanolide	Praveen & Murthy (2012)
Ultrasound	≤113.9mW/cm^3	Cell suspension	*Lithospermum erythrorhizon* Siebold & Zucc.	Boraginaceae	Shikonin	Lin & Wu (2001)
Salt stress	30 ɥM, 70 ɥM, 100 ɥM	Seedlings	*Glycine max* (L.) Merr.	Fabaceae	Trigonelline	Cho et al. (1999)
O_3	4 ɥM	Cell suspension	*Pueraria thomsonii* Benth.	Fabaceae	Abscisic acid	Sun et al. (2011)
H_20_2	10 ɥM	Hairy root	*Vitis rotundifolia* Michx.	Vitaceae	Resveratrol	Nopo-Olazabal et al. (2015)
Nanosilver	1000 ɥM	Hairy root	*Datura metel* L.	Solanaceae	Atropine	Shakeran et al. (2015)
NAA	11 ɥM	Cell suspension	*Plumbago rosea* L.	Plumbaginaceae	Plumbagin	Silja et al. (2014)
$C_4H_{12}N_2$	1.5 ɥM	Hairy root	*Cichorium intybus* L.	Asteraceae	Esculetin,esculin	Bais et al. (1999)
$VOSO_4$	307 ɥM	Hairy root	*Ambrosia artemisiifolia* L.	Asteraceae	Thiarubrine A	Bhagwarh & Hjortsø (2000)

5. Conclusions

Scientific literature focused on enhancing of SMs in tropical and subtropical plants was collected and the topic was overviewed. Tropical and subtropical plants are valuable sources of SMs. Many of the examined plants, for example *Hypericum brasiliense*, *Withania somnifera* or *Catharanthus roseus* proved to contain more than one useful metabolite.

Plants belonging to the Lamiaceae, Plantaginaceae, Fabaceae and Vitaceae families proved to be the best sources of medically used secondary metabolites. In this thesis Solanaceae was recorded to be the most numerous family of plants producing secondary metabolites that was examined in this thesis. This family was most often used for enhancement of production of scopolamine. This tropane alkaloid is known for its neurotransmitter blocking activities.

The most widely used elicitor is methyl jasmonate, and the second mostly used one is salicylic acid. These two elicitors stimulate large scale of SMs (tropane alkaloids, steroids, saponins etc.). The most abundant and useful metabolites elicited by these two mentioned elicitors are tanshinone, bacosides, reservatrol and anthocyanine.

Methyl jasmonate and salicylic acid also have the greatest efficiency in enhancing SMs. The mostly preferred and successful culture for *in vitro* production are hairy root cultures, possibly due their huge advantages such as fast growth, high productivity, and constancy. Cell suspension culture was also one of the favorite cultures.

In vitro production of secondary metabolites from tropical and subtropical plants gives the opportunity to synthetize these chemical compounds continuously, sustainably, and economically. Restricted commercial success of these techniques, regardless on considerable progress in this discipline, lies in low SMs yields and difficulties in increasing production of these compounds. This can be caused by incomplete knowledge about synthesis of desired molecules, among others. In the future, it is crucial to deal with the introduced problems first, and then continue to focus on the new, not yet discovered sources of SMs.

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