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Course on plant metabolism and metabolites
Master I Plant Ecophysiology

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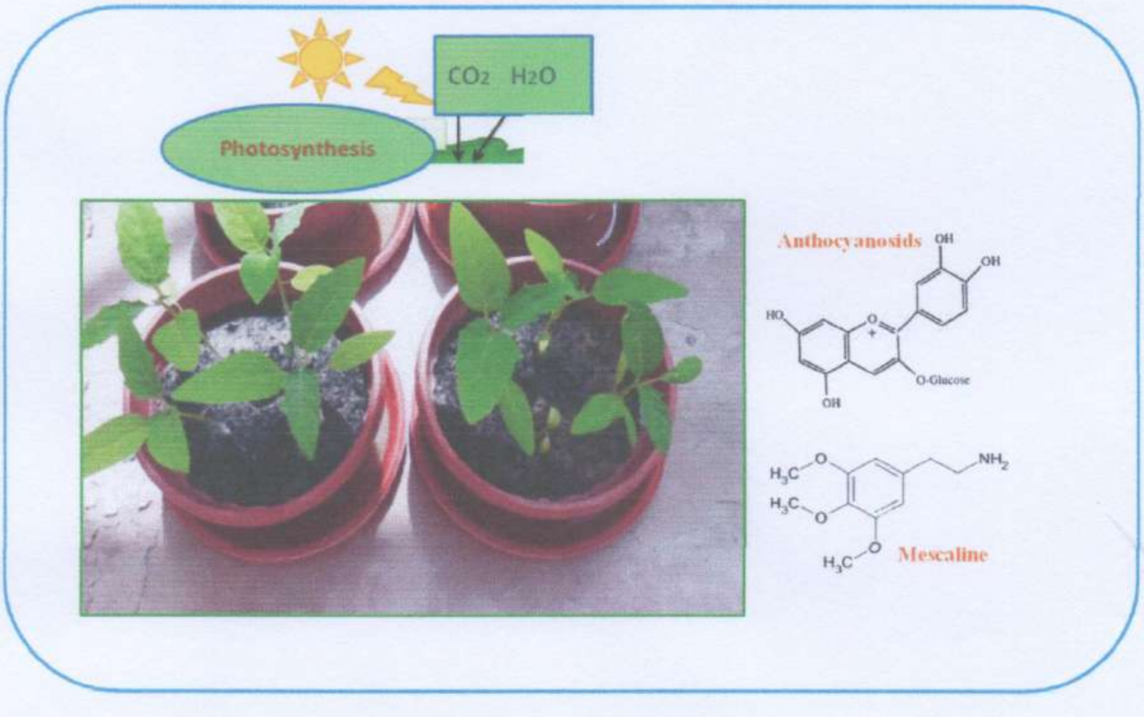

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Preface

This scientific document contains courses of the scientific subject: **Plant Metabolism and Metabolites** intended for **Master I** students in **Plant Ecophysiology**.

The document contains five chapters. The first chapter concerns the concept and importance of primary and secondary metabolism with which the plant establishes relationships with other living beings in its environment. The second chapter explains the notion of catalysis and enzymatic regulation in plants. The third chapter details the main metabolic pathways and the important classes of secondary metabolites, namely phenolic compounds, terpenoids and alkaloids. The fourth chapter illustrates the contribution of molecular biology and genetics to understanding the conditions leading to the expression of enzyme genes involved in the process of adaptation and defense to different environmental constraints through the study of some metabolic pathways.

The last chapter is devoted to the study of biological membranes, notably the plasma membrane and their role in the response to stimuli through the intervention of regulatory enzymes; example the enzymes of phenolic metabolism.

It should be noted that the information written in the chapters of this document requires the acquisition of prior concepts in the first year of university in plant biology, cell biology, and the second year of university in biochemistry and the third year in plant biochemistry. Therefore, with Plant Metabolism and Metabolites module, the student will build a broad and in-depth vision of the main metabolic pathways that take place in the plant cell and lead to the biosynthesis of secondary metabolites essential to the adaptation of higher plants to their environment. The student will also deepen their understanding of the enzymatic regulations responsible for the persistence, adaptation, and defense of plants against biotic and abiotic stresses.

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Chapter 01

Introduction

One of the particularities of plants is to synthesize many compounds whose role at the plant level is poorly understood. The fact that these compounds are not found in all species indicates that they do not enter into the general metabolism and that they do not exercise direct functions at the level of the fundamental activities of the plant organism, they are secondary metabolites.

Many secondary metabolites have important physiological properties for humans and animals and constitute the active ingredients. Their study is the subject of pharmacognosy, one of the major fields of the pharmacist. The experimental study of their biogenesis and their possible bioproduction by *in vitro* cell cultures opens a promising chapter in plant biotechnology.

In fact, secondary metabolites are the main tools of plant-living beings co-evolution.

1. Secondary metabolites as tools for plant-living beings co-evolution

This is a co-evolution that applies to all levels of organization of living organisms, from bacteria to fungi, from insects to mammals and which is expressed at all stages of plant development, from seeds to flowers, through the vegetative system and across all biotopes.

Two axes of co-evolution have been favored:

1.1. Cooperation

A first axis of cooperation: results in a partnership with animals (insects, birds, mammals) that are used in the dispersal mechanism of spores (tetraspores, pollens) and that of fruits and seeds (Guignard et al., 1985).

Example 01: the fruits of the Central American black palm *Astrocaryum standleyanum* are disseminated by various rodents such as the agouti, which buries the fruits it does not consume to find them again in the following months, it forgets part of their harvest which allows the seeds to germinate and the palm trees to regenerate. The absence of dissemination by agoutis increases the risk of destruction of the seeds by fungi, parasitic insects or by predators.

Example 02: Nitrogen-fixing bacteria come into contact in the root sphere of many Poaceae or even penetrate the roots where they induce the formation of nodules (case of the Rhizobium-Fabales symbiosis).

Example 03: Fungi, for their part, are at the origin of mycorrhizae which facilitate the mineral supply of plants.

1.2. Opposition

A second axis of opposition: that we consider as a chemical war. In fact, the chemical weapon is the only one that plants, fixed to the ground and immobile, possess against predators and parasites. Thus, plants that possess toxic ingredients are less consumed. Several examples concerning essential oils, flavonoids, tannins, quinones, alkaloids, etc.

Example 01: between plants, the fight goes through remote poisoning or teletoxis. Cineole and camphor released into the atmosphere by *Salvia leucophylla* are absorbed by the dry soil, inhibiting the germination of prairie species; these can only germinate and grow when winter rains arrive.

Example 02: other compounds, called phytoalexins, only appear in response to the parasite's aggression. Their chemical structures are very diverse: sesquiterpenes (capsidiol), stilbenic, phenanthrenic, flavonoid, etc. In fact, despite their structural diversity, all these phytoalexins appear to modify the integrity of fungal plasma membranes. They act as non-ionic detergents. In some cases, the product in inactive form or bound in the non-aggressed plant is released during infection. This is the case of cyanogenic glycosides which release hydrocyanic acid toxic to a large number of fungi (Guignard et al., 1985).

Secondary metabolites can be classified into four major groups: aromatic compounds, terpenes and steroids, nitrogenous compounds including alkaloids, and glycosides, they exhibited crucial roles in plant physiology (Guignard, 2000) (Fig. 1).

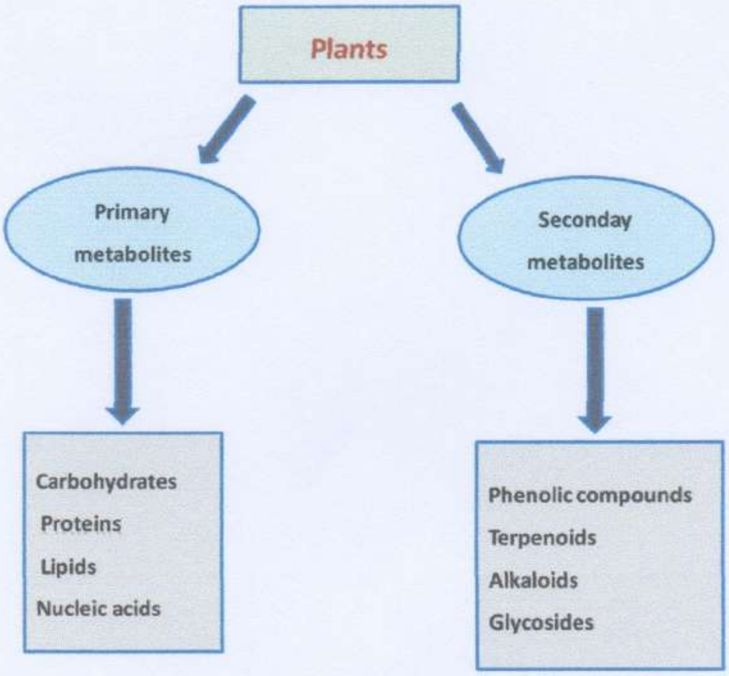


Figure 1. Diversity of plant metabolites.

2. Metabolism

Metabolism is a very dynamic process, molecules are in continual renewal; the composition of a cell at a given moment is a balance between synthesis and degradation. Most of the syntheses are oriented towards the production of molecules that are important for the structure and functioning of the cell. However, in plants a significant amount of carbon and energy is allocated to the synthesis of molecules whose function is less clear. The part of the biosynthetic or basal metabolism includes so-called anabolic reactions constituting anabolism.

The part of the metabolism that includes the degradation of complex molecules into simpler molecules includes so-called catabolic reactions constituting catabolism.

Metabolism can also be subdivided into primary metabolism and secondary metabolism.

Secondary metabolites are generally found in small quantities and their production can be either widespread or limited to certain families, or to certain genera or even to certain particular species (Guignard, 2000) (Fig. 2).

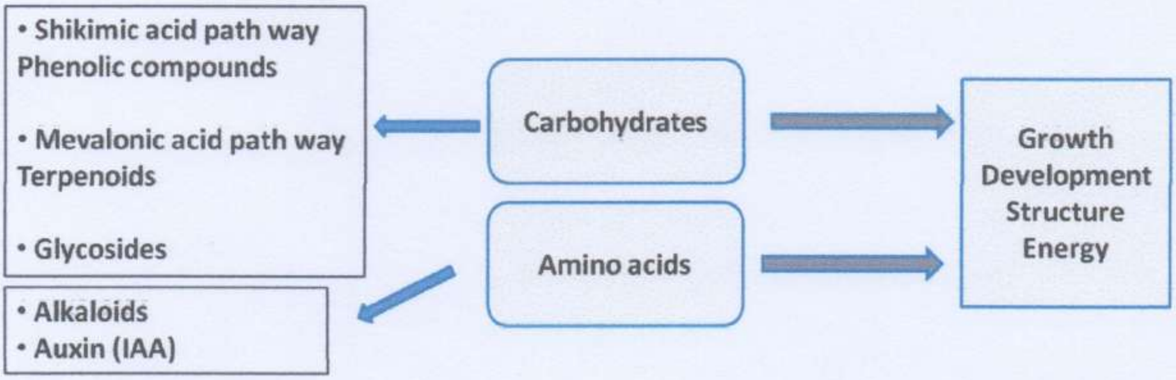


Figure 2. Role of primary and secondary metabolism in plant.

Since Antiquity, secondary metabolites have been used as folk remedies, soaps or essences and to reduce the impact of insects or predatory animals. These metabolites are medicinal products, dyes, raw materials for the chemical, pharmaceutical, food and cosmetic industries, etc. (Guignard, 2000).

Chapter 02

Introduction

An enzyme is a protein with catalytic properties. Virtually all biomolecules capable of catalyzing chemical reactions in cells are enzymes.

The enzyme is not modified during the reaction. The initial molecules are the substrates of the enzyme, and the molecules formed from these substrates are the products of the reaction.

Almost all metabolic processes in the cell require enzymes to proceed at a rate sufficient to sustain life. The set of enzymes in a cell determines the possible metabolic pathways in that cell.

1. Characteristic of enzymes

a. Enzymes are not modified during the reactions they catalyze, and do not modify the chemical balance between substrates and products.

b. Enzymes differ in their very high specificity. This specificity arises from their three-dimensional structure. In addition, the activity of an enzyme is modulated by various other molecules: an enzyme inhibitor is a molecule that slows down the activity of an enzyme, while an enzyme activator accelerates it.

c. Furthermore, the activity of an enzyme decreases rapidly outside its optimum temperature and pH.

d. In addition, an enzyme has the characteristic of being reusable.

2. Structure

Enzymes are generally globular proteins that act alone or in complexes of several enzymes or subunits.

a. Like all proteins, enzymes consist of one or more polypeptide chains folded to form a three-dimensional structure corresponding to their native state. The amino acid sequence of the enzyme determines its structure, which in turn determines the catalytic properties of the enzyme (Weil, 1998).

b. The structure of enzymes is altered when they are heated or exposed to chemical denaturants, which usually inactivates them.

c. Enzymes are molecules that are much larger than their substrates. Only a very small part of the enzyme, usually between two and four residues, is directly involved in catalysis, called the catalytic site. The latter is located near one or more binding sites, at which the substrates are bound and oriented to allow catalysis of the chemical reaction. The catalytic site and the binding sites form the active site of the enzyme. The rest of the protein serves to maintain the configuration of the active site and to generate the optimal conditions for the reaction to proceed.

d. In some cases, catalysis does not involve any of the amino acid residues of the enzyme but rather a cofactor bound to this enzyme. The structure of enzymes may also contain a binding site for an allosteric effector that causes a conformational change that activates or inhibits enzyme activity.

3. Mechanism

3.1. Substrate binding

Enzymes must first bind to their substrates before they can catalyze any chemical reaction. Enzymes are generally very specific with respect to both the substrates to which they can bind and the reactions they are likely to catalyze. This specificity results from the configuration of their binding sites, which are pockets with complementary shape and spatial distribution of electrical charges and hydrophilic/hydrophobic properties to those of the substrate (Fig. 3).
Enzymes can thus distinguish between very similar molecules, which ensures their chemoselectivity, regioselectivity, and stereospecificity (Weil, 1998).

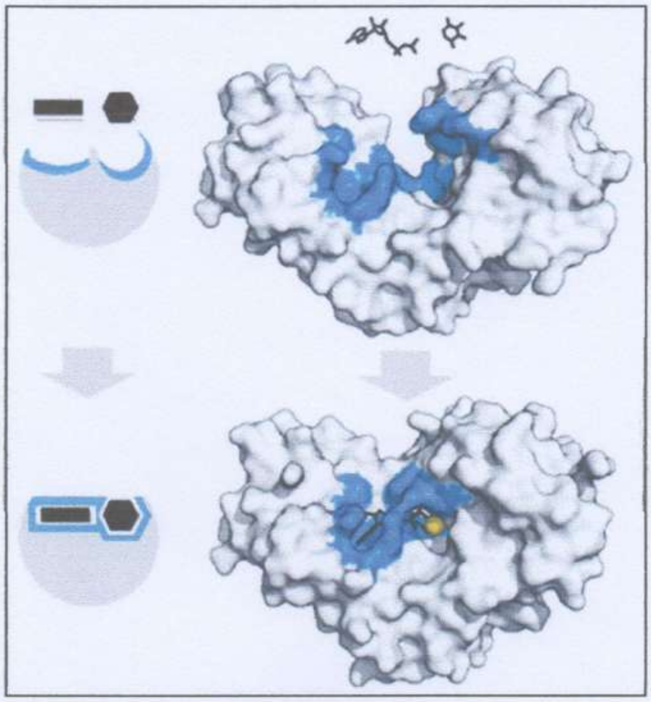


Figure 3. Hexokinase exhibits a strong induced fit that encloses adenosine triphosphate and xylose in its active site. The binding sites are shown in blue, the substrates in black, and the Mg^{2+} Cofactor in yellow (Wikipedia, 2015).

4. Dynamics

Enzymes are not rigid and static structures. On the contrary, they are the site of a whole set of internal movements, whether it is the movement of individual amino acid residues, a group of residues forming an element of the secondary structure, or even an entire domain of the protein. These movements give rise to a set of structures that are slightly different from each other and that are in equilibrium in perpetual inter-conversion with each other.

Different conformational states of the enzyme can for example be associated with different phases of its chemical activity (Weil, 1998).

5. Allosteric regulation

Allosteric regulation sites are binding sites distinct from the active site that can interact with molecules in the cellular environment, called in this case allosteric effectors. The binding of these molecules with this site induces a conformational change or a modification of the internal dynamics of the enzyme, which alter the properties of the active site and consequently modify the reaction rate of the enzyme. These changes can activate or inhibit enzymes. Allosteric interactions with metabolites upstream or downstream of a metabolic pathway in which the enzyme participates cause feedback loops to modulate the activity of the enzyme according to the intensity of the metabolite flux, for example:

The shikimic acid pathway is an excellent example of a feedback-regulated pathway. Key enzymes in this pathway include **aldolase 1**, which catalyzes the initial condensation reaction of Erythrose-4-P and phosphoenolpyruvate (PEP) and is inhibited by all three end products (phenylalanine, tyrosine, tryptophan). Similarly, the conversion of chorismate to prephenate (**aldolase 2**) is inhibited by both phenylalanine and tyrosine, while the conversion of chorismate to anthranilate (**aldolase 3**) is inhibited by tryptophan. Another interesting step in this pathway is the conversion of shikimate to 3-enolpyruvylshikimate-5-P; the enzyme that catalyzes this reaction is **EPSPS**. So in the shikimic acid pathway, the above-mentioned enzymes are subject to regulation by feedback inhibition (Heller et al., 1998; Hopkins, 2003) (Fig. 4).

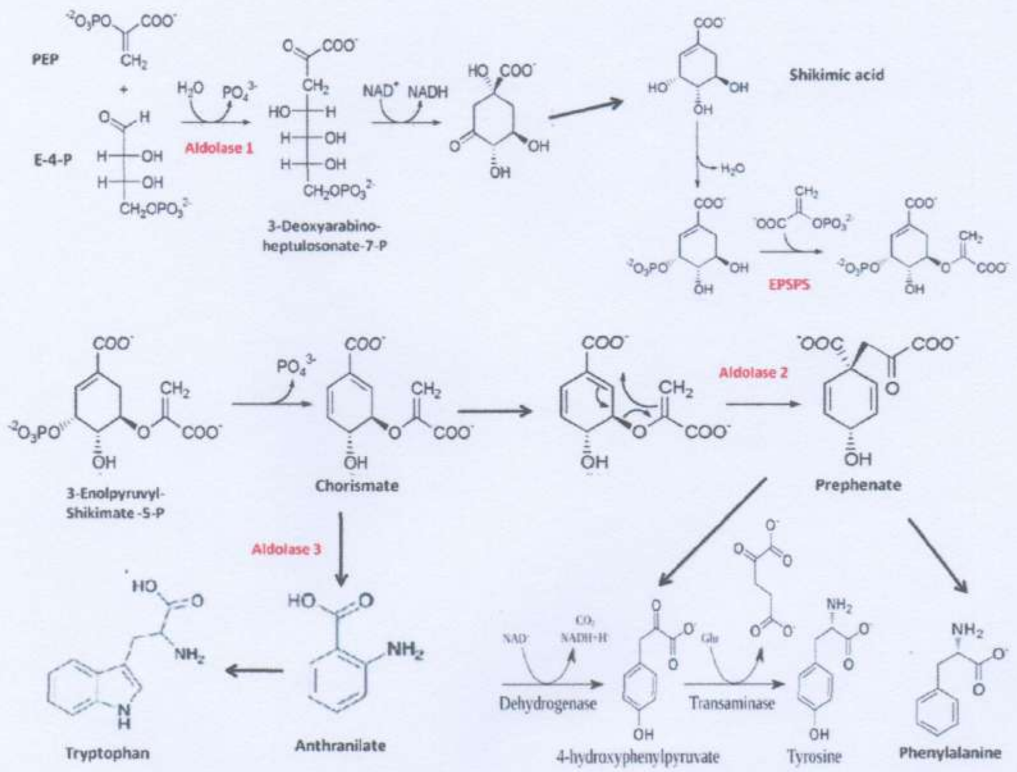


Figure 4. Shikimic acid pathway: Allosteric regulation (Adapted according to Hopkins, 2003)

6. Cofactors

Some enzymes do not require any additional components to be fully active. Others, on the contrary, need to interact with non-protein chemicals, called cofactors, to be active. These cofactors can be inorganic, such as iron-sulfur metal ions, magnesium (Fig 3), or organic compounds, such as flavin adenine dinucleotide (FAD) associated with enzymes of the oxidoreductase class to which it is covalently bound; and transketolase with its organic Cofactor thiamine (Fig. 5).

Organic cofactors can be coenzymes, which are released from the active site of the enzyme during the reaction, or prosthetic groups, which remain tightly bound to the enzyme.

Some organic prosthetic groups are covalently bound to their enzyme, as is the case with biotin for enzymes such as pyruvate carboxylase (Weil, 1998).

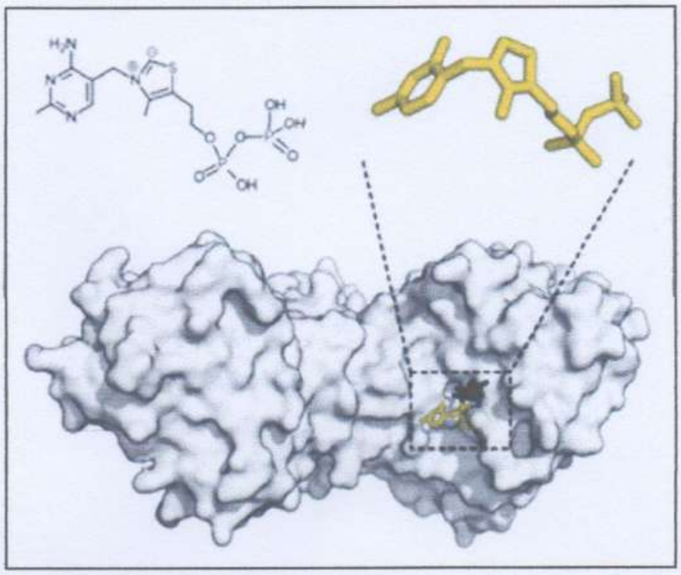


Figure 5. Structure of transketolase, an enzyme of the pentose phosphate pathway, shown with its cofactor thiamine (TPP) in yellow and its substrate xylulose in black

(Wikipedia, 2016).

Chapter 03

Introduction

Plants produce a large quantity of secondary metabolites, and in particular phenolic compounds, terpens, and alkaloids, which are often of great interest to humans in the pharmacological and agri-food fields (Macheixet al., 2005; Merghem, 2009).

1. Phenolic compounds

The biosynthesis of the aromatic nucleus is one of the fundamental processes of plant biochemistry. The biosynthesis of the aromatic nucleus appeared very early in evolution since proteinogenesis uses three aromatic amino acids: phenylalanine, tyrosine, tryptophan, and electron carriers such as quinones involved in respiration and photosynthesis. Aromatic amino acids can be the origin of secondary or primary metabolites (Guignard, 2000 ; Hopkins, 2003) (Fig. 6, 7).

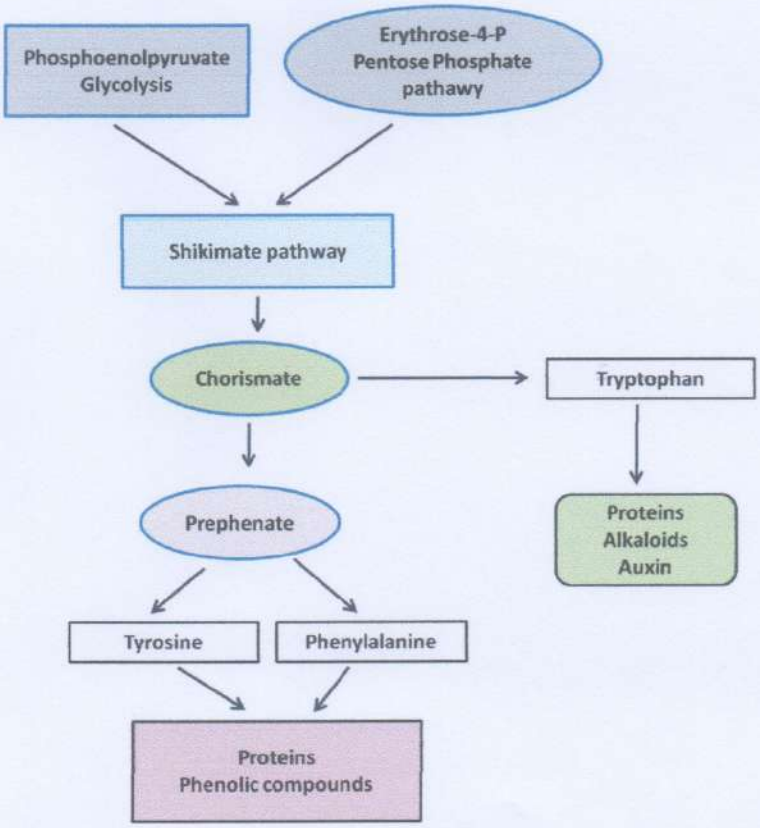


Figure 6. Role of aromatic amino acids in plant.

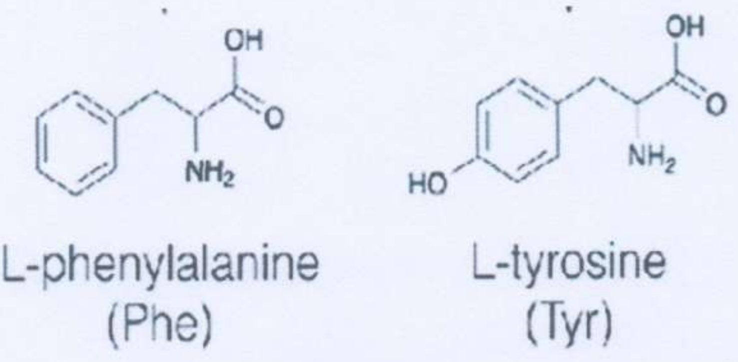


Figure 7. Aromatic amino acids synthesized by shikimic acid pathway and involved in phenylpropanoid pathway.

2. Biosynthesis and accumulation of phenolic compounds

2.1. The main lines of biosynthesis

The main lines of the biosynthetic pathways of the main phenolic compounds are now well known. The two aromatic amino acids, phenylalanine and tyrosine, are at the origin of the formation of most phenolic molecules in plants. They are themselves formed from simple sugars such as (Erythrose-4-P) from primary metabolism, by the well-known shikimic acid pathway (Fig 7).

Desamination of phenylalanine is a first essential step in the biosynthesis of the vast majority of natural phenolic compounds. It leads to:

The formation of cinnamic acid, a molecule that is not yet phenolic but which is directly at the origin of the biosynthetic sequence generally called the phenylpropanoid pathway, which allows the formation of hydroxycinnamic acids: *p*-coumaric, caffeic, ferulic and sinapic acids. 5-Hydroxyferulic acid is an intermediate that does not accumulate in plants (Heller et al., 1998; Hopkins, 2003; Macheix et al., 2005) (Fig. 8).

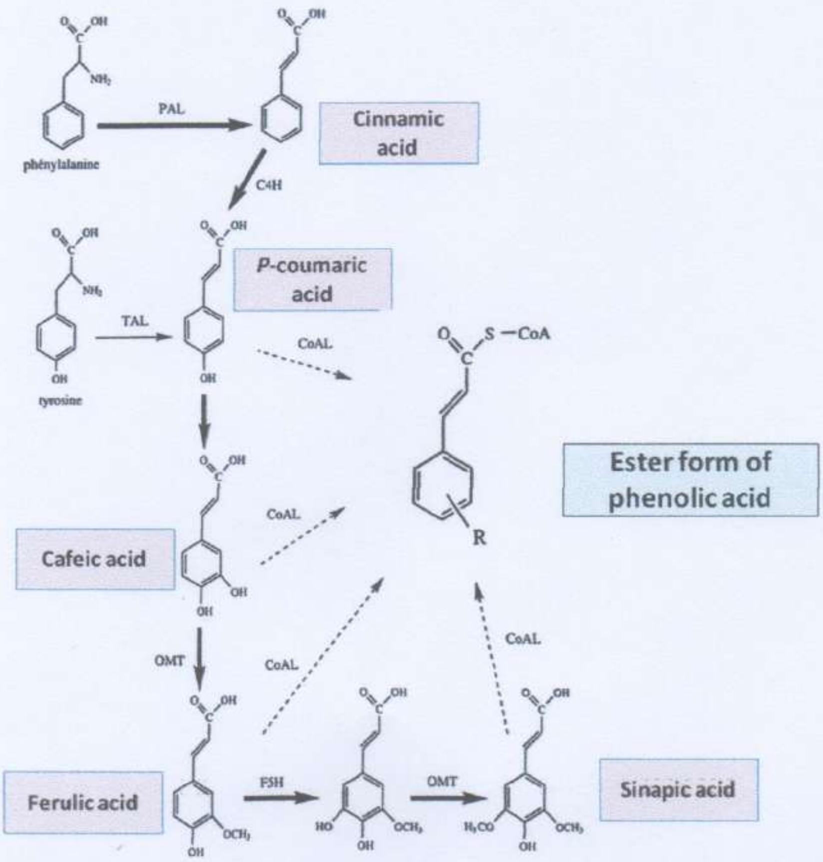


Figure 8. Phenylpropanoid pathway (Adapted according to Macheix et al., 2005).

In fact, the metabolically active forms of hydroxycinnamic acids are their esters with Coenzyme A (CoA) which provide access to the main classes of phenolic compounds. Some major directions can be cited:

2.1.1.Towards the benzoic series acids by β-oxidation: Thus, salicylic acid, which plays an important role in cell signaling in plants, derives from phenylalanine via cinnamoyl-CoA, benzoyl-CoA and benzoic acid. It is then glucosylated or methylated to give the classic combined forms of salicylic acid (Macheix et al., 2005) (Fig. 9).

It is also likely that some benzoic series acids derive directly from intermediates of the shikimic acid pathway without passing through phenylalanine.

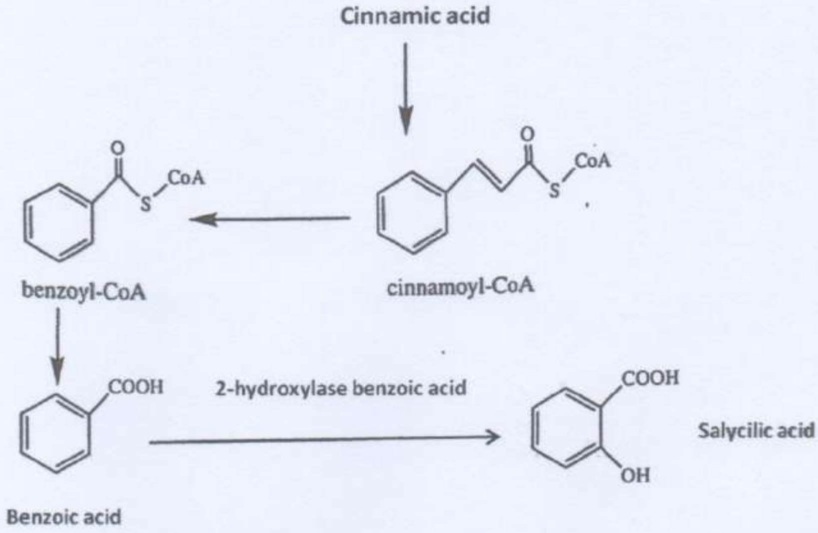


Figure 9. Benzoic acid and its derivatives.

2.1.2. Gallic acid: by combination with simple sugars, leads to hydrolysable tannins (Fig. 10).

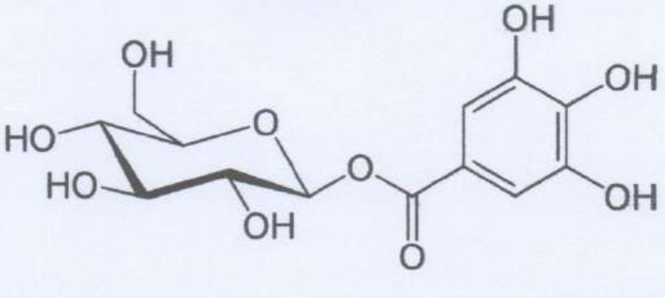


Figure 10. Hydrolysable tannins (gallotanins) (Wikipedia 2010).

2.1.3. Towards coumarins: by internal cyclization of the molecules hydroxycinnamic acid followed by additional modifications, glycosylation (Fig. 11).

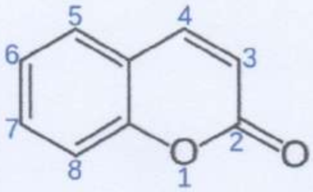


Figure 11. Chemical structure of coumarins (Wikipedia 2025).

2.1.4. Towards lignins: by reductions leading to monolignols which are then integrated into lignin by oxidative polymerization initiated in the cell wall by peroxidases and possibly laccases.The conditions of the polymerization leading to the final macromolecule have been the subject of two hypotheses:

- on the one hand, bonds occurring randomly between the monolignols according to their chemical reactivity (Macheix et al., 2005).

- on the other hand, a polymerization oriented by so-called leader proteins which specifically control the bonds between the monomers and the subsequent polymerization of the different dimers (lignans) obtained (Macheix et al., 2005) (Fig. 12).

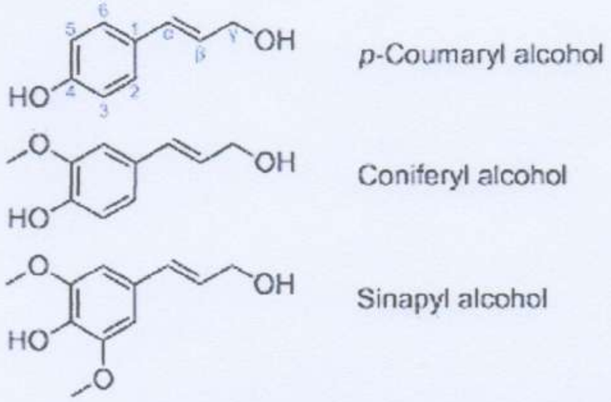


Figure 12. Monolignol alcohols chemical structure (Wikipedia 2008).

Lignin is a non-carbohydrate substance, which is deposited in certain plant cells: wood vessels at the end of the formation of the primary and secondary wall. Lignin is after cellulose the most important biopolymer, together they form 60 to 80% of terrestrial biomass. The lignin deposit occupies all the space left free by the cellulose and the polymers of the matrix. Lignin polymerizes *in situ* in three dimensions giving rise to a massive macromolecular network (Guignard, 2000; Macheix et al., 2005) (Fig. 13).

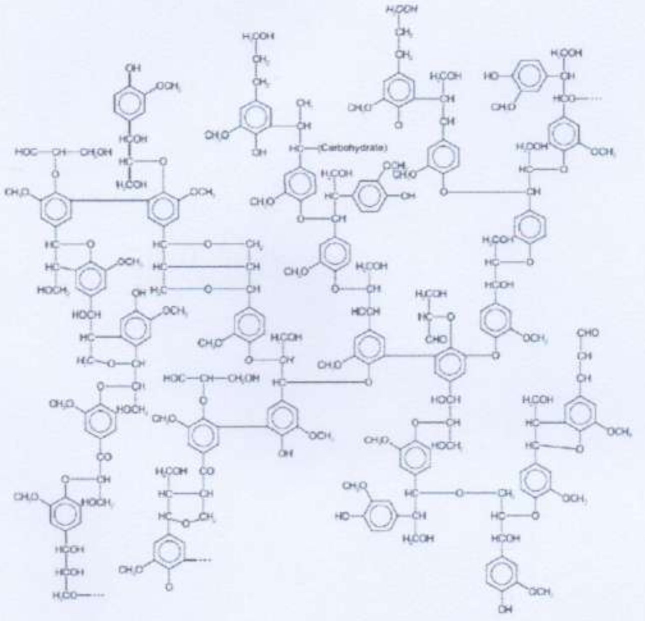


Figure 13. Molecule of lignin (Wikipedia 2019).

2.1.5. Towards flavonoids: whose basic molecular skeleton is a chalcone molecule (C_6-C_3-C_6) which has a mixed biosynthetic origin; on the one hand 3 molecules of acetyl-CoA (provided in the form of malonyl-CoA) for the cycle (A) and on the other hand a molecule of *p*-coumaroyl-CoA for the cycle (B) and the heterocycle (C) (Fig 14, 15).

From chalcone, the biosynthesis of different groups of flavonoids involves a complex set of reactions including hydroxylations, methylations, oxidations, reductions, glycosylation, etc.

Thus, compounds as diverse as anthocyanin pigments, flavonols or even certain flavan-type monomers will be put in place whose polymerization leads to condensed tannins (C15)n (Heller et al., 1998; Hopkins, 2003; Macheix et al., 2005).

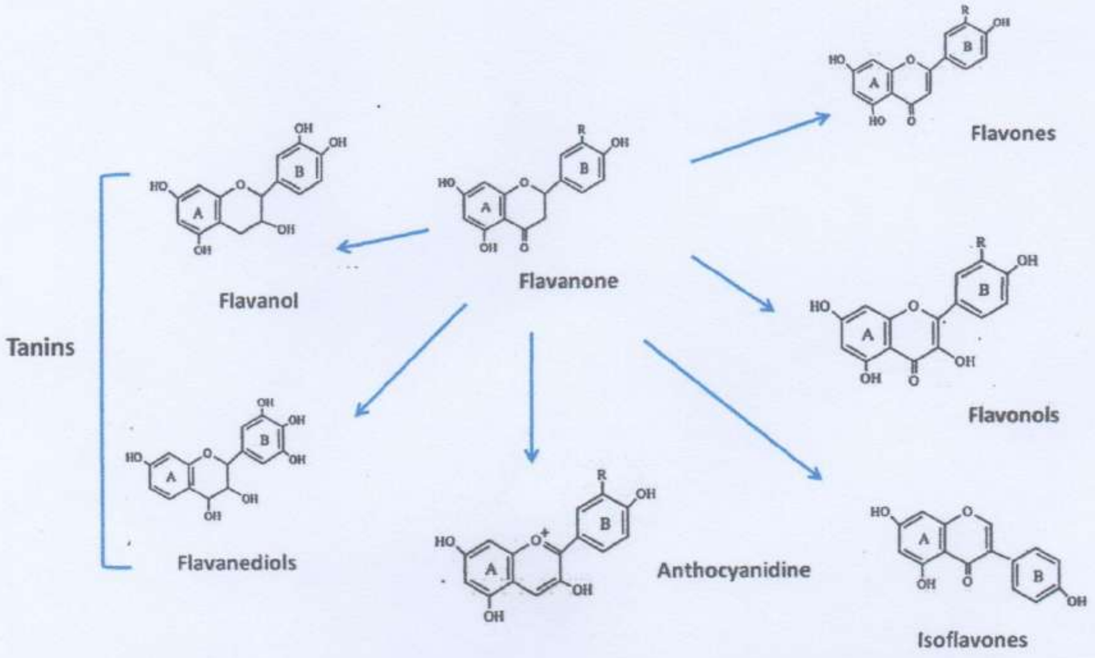


Figure 14. Different principal groups of flavonoids.

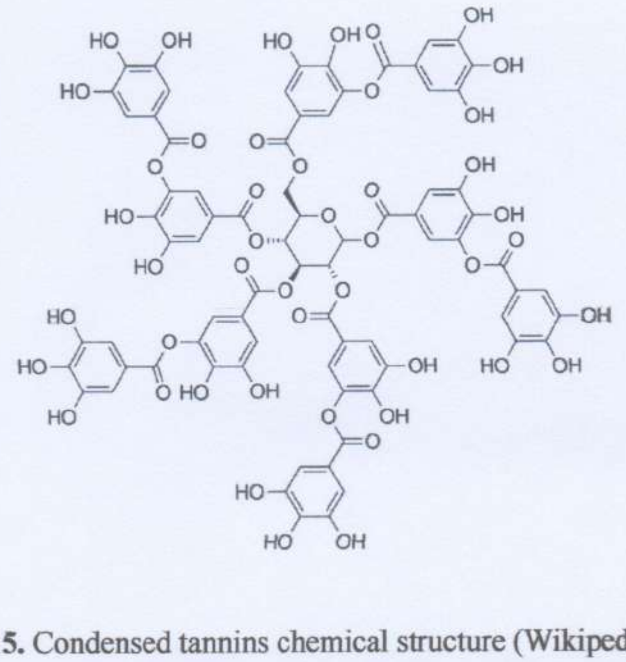


Figure 15. Condensed tannins chemical structure (**Wikipedia 2009**).

3. Secondary metabolites and enzyme regulation

3.1. Phenolic metabolism enzymes and regulation

A common characteristic of all secondary metabolism enzymes is the difficulty of studying and purifying them because they are always very poorly represented among plant proteins. In addition, they are easily inhibited during grinding and extraction, in particular by the phenolic compounds themselves which then irreversibly combine with proteins. Therefore, the use of phenol traps is essential in extraction media.

Various phenolic metabolism enzymes have been sequenced and some of the corresponding genes have been cloned.

3.2. Localization of phenolic metabolism enzymes

Most of them are linked to the membranes of the endoplasmic reticulum, in particular phenylalanine ammonialyase (PAL), chalcone synthase(CHS), hydroxylases, glucosyltransferases, O-methyltransferases (Macheix et al., 2005).

The different enzymes of the phenylpropanoids and flavonoid pathway from the initial carbon skeleton to the terminal steps that lead to various groups of flavonoids do not act independently of each other. They are closely associated with the membranes of the endoplasmic reticulum; in addition, they are grouped in functional enzymes where the different intermediate molecules are channeled from one enzyme to another. This prevents their dispersion in the cellular medium and allows a coordinated enzymatic regulation (Macheix et al., 2005) (Fig. 16).

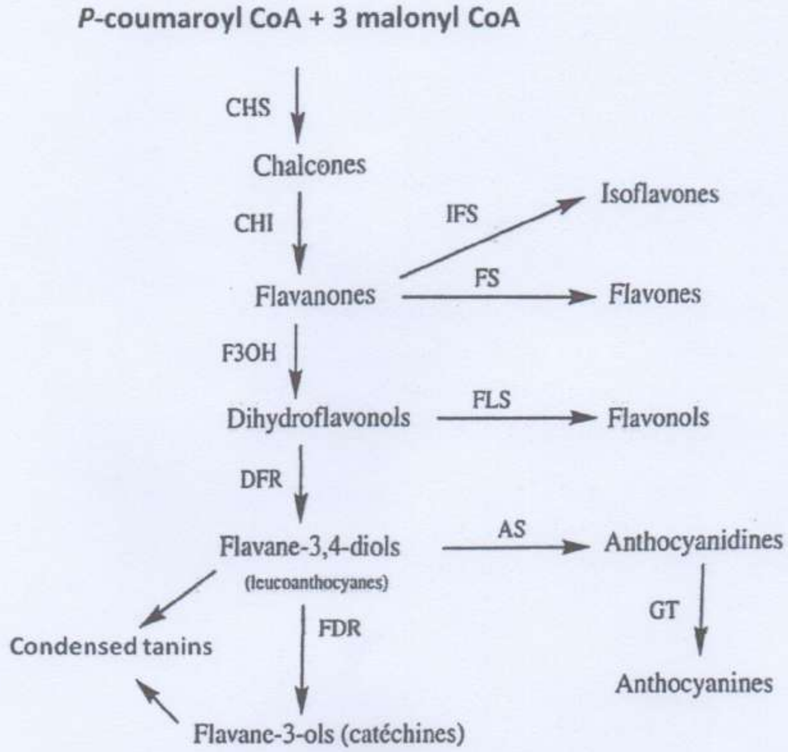


Figure 16. Enzymes involved in flavonoids biosynthesis (Adapted according to Macheix et al., 2005).

3.2.1. Phenylalanine ammonialyase

Phenylalanine ammonialyase (PAL) is the key enzyme in the metabolism of phenolic compounds, and at its level the regulatory factors of their biosynthesis are played out. PAL is a key enzyme since it effectively controls the orientation of carbon towards the production of phenolic compounds rather than towards the production of primary metabolites. An alternative pathway, the deamination of tyrosine to *p*-coumaric acid by TAL is limited in Poaceae family.

PAL allows the formation of cinnamic acid from phenylalanine. It is a tetrameric enzyme whose subunit has a molecular mass close to 83 KDa.

Thus, some forms of PAL could have different functions in the plant: PAL2 intervenes in xylem lignification while PAL3 is set up in response to injuries and participates in the defense mechanisms developed by the plant.

The level of PAL expression could quantitatively regulate the accumulation of phenolic compounds, as is the case for chlorogenic acid in tobacco (Macheix et al., 2005) (Fig. 17).

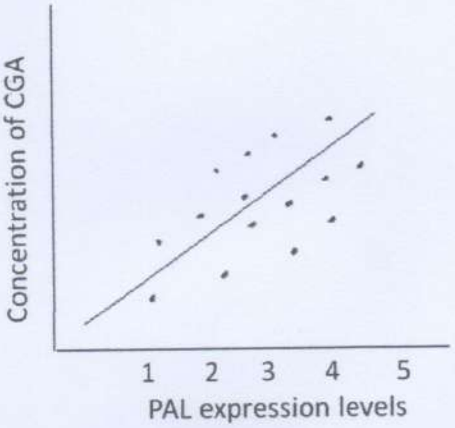


Figure 17. Increase of chlorogenic acid concentration with PAL activity in tobacco (Adapted according to Macheix et al., 2005).

Similarly, the level of lignin is significantly reduced in transgenic tobacco where PAL expression is reduced by a co-suppression phenomenon. Different inhibitors, generally molecules close to phenylalanine, have been used to block PAL activity and entry into the phenylpropanoid pathway. One of the most specific and effective is AIP (2-aminoindane 2-phosphonic acid), the use of which can reduce the synthesis of salicylic acid in tomatoes or willows.

The importance of PAL is supported by several observations. For example, PAL activity is stimulated by red or ultraviolet radiation, both of which also stimulate the production of various flavonoids (Macheix et al., 2005).

3.2.2. CoA ligases

CoA ligases allow the establishment of activated forms of hydroxycinnamic acids with CoA (ester forms). The different enzymatic isoforms play a key role in the diversification of phenolic compounds by intervening in the distribution of CoA esters to different pathways.

3.2.3. Chalcone synthase

Chalcone synthase (CHS) intervenes in a key step of phenolic metabolism since it allows entry into the flavonoid pathway, by the condensation of phenylpropanoids with polyacetates. It is therefore at the origin of the establishment of many molecules having a great importance in life of the plant: anthocyanin pigments of flowers and fruits, numerous phytoalexeins, UV-protective flavonoids by playing a role in the physiology of the plant or its interactions with microorganisms or in industry (condensed tannins, natural dyes, antioxidants, etc.).

Many other enzymes of phenolic metabolism have been characterized in plants and in a number of cases, genes have been encoded. This concerns in particular: O-methyltransferases, glycosyltransferases and hydroxylases which are involved in the formation of monolignols, a large number of flavonoids and tannins (Macheix et al., 2005).

4. Specificity of enzymatic action

A high specificity in the action of different enzymatic forms is frequently observed. Thus, in the case of monolignols, two methylation pathways are simultaneously functional and concern either free caffeic acid through caffeoyl O-methyltransferase (COMT) or caffeoyl CoA through the intervention of caffeoyl CoA O-methyltransferase (CCoAOMT). The relative activity of these two enzymes and their selective specificity with respect to the different phenylpropanoids (either their free forms or their ester forms with CoA) lead to variability in the proportion of different monolignols formed and represent one of the causes of a real plasticity of lignin metabolism; plasticity allowing an adaptation of plants to their environment (Macheix et al., 2005) (Fig 8).

5. Terpens

5.1. Definition

Terpens are natural products, formed from the assembly of an integer of isoprenic units.

5.2. Biosynthesis of the isopentenyl pyrophosphate: Mevalonic acid pathway

Two molecules of acetyl CoA condense under the influence of a β-ketothiolase enzyme to give acetoacetyl CoA. Acetoacetyl CoA, which in the presence of an HMG-CoA synthase enzyme, binds a third molecule of acetyl CoA to form β-hydroxy-β-methyl-glutaryl CoA (HMG-CoA). The reduction of the carboxyl combined with the CoA to alcohol then occurs to form mevalonic acid (MVA) by HMG-CoA reductase. This reduction occurs in two steps: first, a mevaldehyde is formed and the CoA is released, the hydrogens are provided by NADPH, secondly, the enzyme further reduces the aldehyde function to a primary alcohol. The hydrogen-donating coenzyme is again NADPH, with a proton. Since the reduction of HMG-CoA to MVA is irreversible, MVA is an efficient precursor to terpene compounds. The key enzyme in this reduction is HMG-CoA reductase (Guignard, 2000; Hopkins, 2003).

MVA undergoes a two-step phosphorylation that uses 2 ATP molecules, giving it 2 phosphate groups forming a C6 molecule: mevalonic acid-5-pyrophosphate (MVAPP). Then a decarboxylation transforms MVAPP into a C5 molecule: pyrophosphate of isopentenyl (IPP) which by reversible isomerization, forms pyrophosphate of dimethylallyle (DMAPP) (Guignard, 2000; Hopkins, 2003) (Fig. 18).

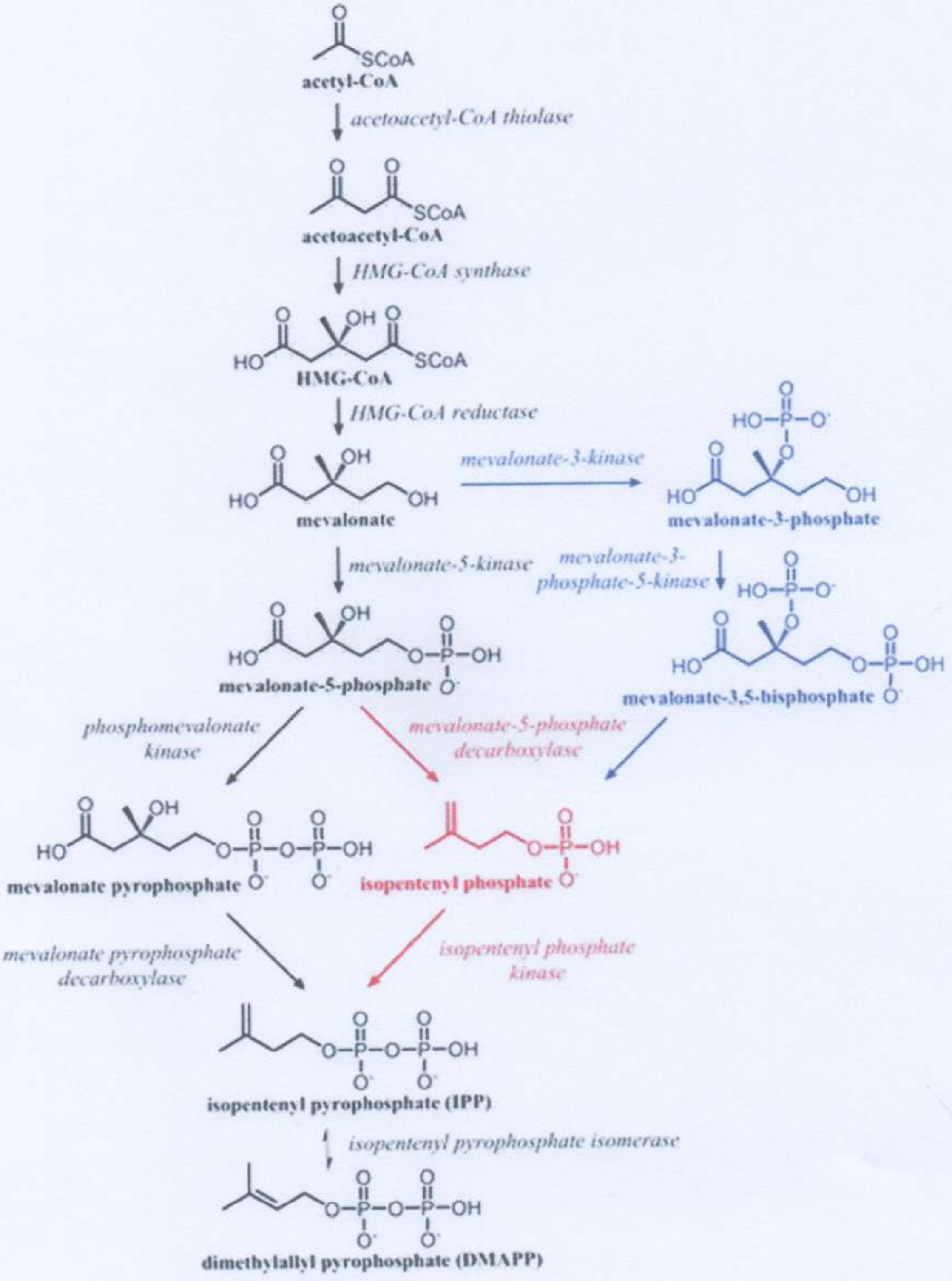


Figure 18. Mevalonic acid pathway (Wikipedia, 2015).

5.3. Biosynthesis of different classes of terpenes

A pyrophosphate of dimethylallyle (DMAPP) molecule undergoes a head-to-tail condensation with a pyrophosphate of isopentenyl(IPP) molecule forming a C10 unit: GPP which is at the origin of all monoterpenes.A similar addition of IPP leads to FPP, origin of sesquiterpenes (C15).The addition of another IPP molecule gives rise to GGPP, origin of diterpenes (C20) and tetraterpenes (C40), and so on up to polyterpenes (C > 40) (Guignard, 2000) (Fig. 19).

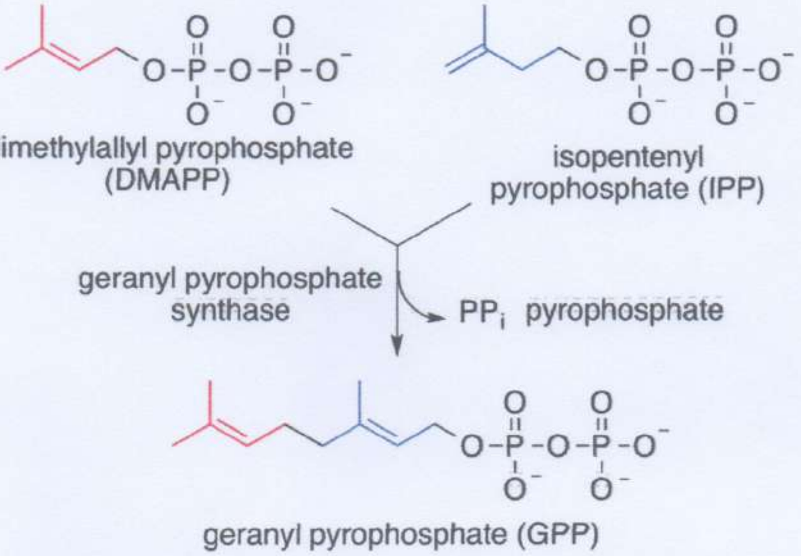


Figure 19. Biosynthesis of monoterpenes (Wikipedia, 2014).

6. Alkaloids

Alkaloids constitute another larger group of secondary metabolites. Due to their toxic or drug properties, they have always presented for pharmacists and the pharmaceutical industries an exceptional interest.

6.1. Definition

An alkaloid is a compound of natural origin, most often vegetable, nitrogen, more or less basic, limited and gifted distribution, at low doses, pharmacological properties. Affairs to alkaloids is confirmed by common rush reactions with the general reagents of alkaloids: reactive of Draguendroff, Mayer, Wagner. The biological role of alkaloids lies essentially in their bitterness and toxicity, they can play a protective role towards predators and herbivores. They also have antimicrobial, tranquilizer and psychotropic effects.

6.2. Nature of alkaloids

a. All alkaloids contain nitrogen (N), most often included in a heterocycle. Most alkaloids have an alkaline reaction, in their natural state, they are generally salified by organic acids (tartrate, malate) or combined with tannins;

b. The majority of them have pharmacological properties. Alkaloids that no longer have intra-cyclical nitrogen are simpler structure, close to amines and are called proto-alkaloids. True alkaloids are classified according to the nature of their cycle;

c. Alkaloids are sometimes found in the whole plant but most often in limited structures (leaves, seeds, bark, fruit, vacuolar fluid) (Merghem, 2009).

6.3. Main classes of alkaloids

Alkaloids are subdivided into three main classes according to the position of nitrogen.

6.3.1. Pseudo-alkaloids

In this class, it seems that nitrogen is only incorporated into the last on the polycarbon skeleton, example: coniine (Fig. 20).

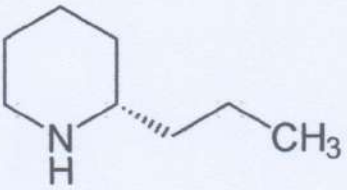


Figure 20. Molecule of coniine (Wikipedia, 2008).

6.3.2. Proto-alkaloid

In this class, the nitrogen is not included in a heterocyclical system, they are developed from amino acids, example: mescaline (Fig. 21).

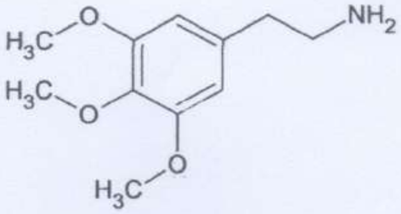
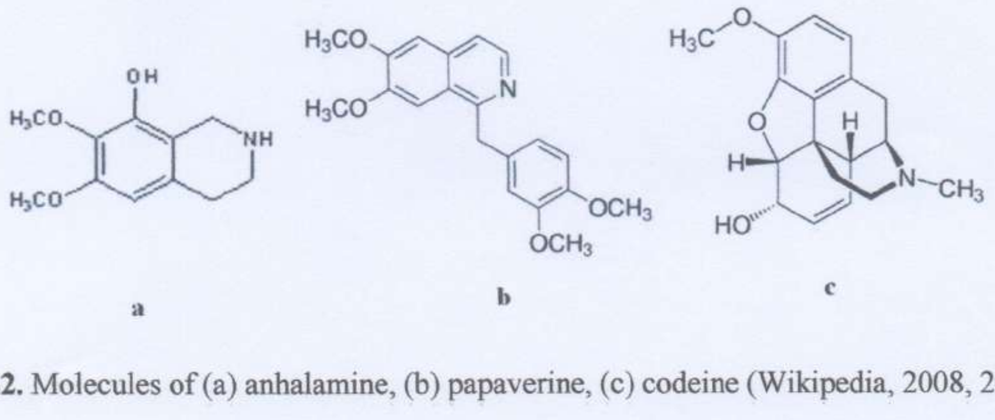


Figure 21. Molecule of mescaline (Wikipedia, 2025).

6.3.3. True-alkaloid

In this group, the nitrogen atom is included in a heterocycle, biosynthetically formed from amino acids, example: anhalamine, papaverine, nicotine, codeine (Merghem, 2009) (Fig. 22).



a b c

Figure 22. Molecules of (a) anhalamine, (b) papaverine, (c) codeine (Wikipedia, 2008, 2025).

7. Regulation of biosynthesis and accumulation

Different physical, chemical and biological factors, external or endogenous, play an important role in modulating the expression of phenolic metabolism: light, temperature, growth regulators, biotic elicitors, etc.

7.1. Light

Field observations and laboratory experiments have shown the importance of light on the accumulation of anthocyanins in flowers and fruits.

- In plant species (grapes and apples), reduced illumination is generally correlated with a decrease in anthocyanin content;
- Plants living at high altitudes, where ultraviolet (UV) radiation is high, have red, blue or yellow flowers with very intense colors;
- Two parameters are involved: on the one hand, the intensity of the light flux and on the other hand, the nature of the constituent radiation. Light acts directly, through red, blue and UV radiation which are perceived by different photoreceptors including phytochrome and different UVA and UVB receptors;
- Anthocyanins accumulated in the vacuoles of the outermost cells (epidermis, hypodermis) absorb up to 95-99% of UV radiation;

The action of light involves the induction of the synthesis of several enzymes of phenolic metabolism and in particular of PAL, which has been studied extensively from this point of view. In this case, the effect of light is to activate the expression of PAL genes, which leads to the transcription of mRNA (mPAL) and then the formation of the enzymatic protein, PAL.

A similar effect but slightly delayed in time is obtained with CHS and other enzymes of flavonoids' metabolism, leading to the accumulation of these compounds (Macheix et al., 2005) (Fig. 23).

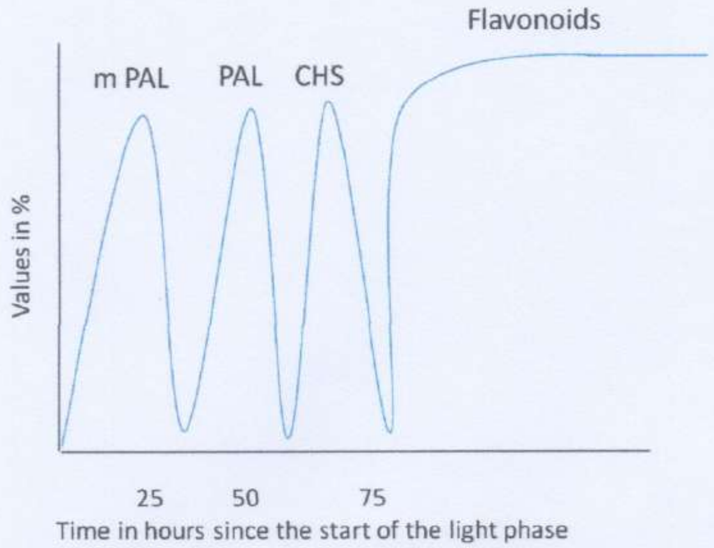


Figure 23. Effect of light on mPAL and enzyme activity (PAL, CHS), and flavonoids concentrations in cell suspension of parsley (Adapted according to Macheix et al., 2005).

7.2.Temperature

Temperature is also a factor regulating the expression of phenolic metabolism, which often interacts with light.

- A lowering of the temperature associated with adequate light treatment frequently induces an accumulation of anthocyanins in many fruits (grapes, apples, etc.). Here again, regulation could occur at the level of the PAL itself, with enzyme inhibitors being able to be put in place under the effect of high temperature;
- Disturbances in phenolic metabolism can also appear following cold treatments of fruits and vegetables, leading to browning;
- In tomatoes or pineapples, for example, cold storage leads to the *de novo* synthesis of several enzymes leading to a high accumulation of chlorogenic acid or related compounds, which then leads to physiological disorders and abnormal browning when returning to normal temperature (Macheix et al., 2005).

7.3. Elicitors

Elicitors are molecules generally produced by microorganisms that trigger the expression of defense genes in plants, leading to the development of antibiotic molecules called phytoalexins, among which certain groups of phenolic compounds are well represented: coumarins, stilbenes, isoflavonoids, etc.

- The addition of a pathogenic microorganism (fungi, bacteria, etc.) or the elicitor can be used to stimulate the production of phenolic compounds by the plant;
- Injection by a microorganism or the addition of an elicitor of biological origin into plant cell suspensions is very effective in triggering the synthesis of phenolic metabolism enzymes, particularly PAL;
- The same is true for the action of molecules that play a role in the signal transduction pathway inside the cell, such as jasmonic acid or salicylic acid (Macheix et al., 2005).

7.4. Growth substances

Growth substances exhibit crucial physiological roles in plant growth, development, transpiration, reproduction, defense, and adaptation by affecting primary and secondary metabolism (Fig.24).The observed effects of growth substances depend on their concentration used in cell suspensions. Several experiments showed that:

- Auxins have a negative effect on anthocyanin production in carrot cell cultures as soon as their concentration reaches 10-8 M. On the contrary, at these same concentrations, cytokinins strongly stimulate the accumulation of anthocyanins;
- The accumulation of anthocyanins by gibberellins is stimulated in the presence of sucrose, the latter acting as a carbon source;

- Ethylene is involved in triggering fruit ripening and in inducing phenolic metabolism following injuries to plant organs (Macheix et al., 2005).

Plant hormones intervene by regulating the expression of genes for the biosynthesis of phenolic compounds and other secondary metabolites according to the species.

The response can vary greatly from one species to another and depending on the doses of plant hormones applied and the duration of treatments.

In addition to their crucial roles in growth and development, growth regulators interact together to induce other functions insured by secondary metabolism: defense against microorganisms, insects and adaptation to abiotic stresses (Macheix et al., 2005).

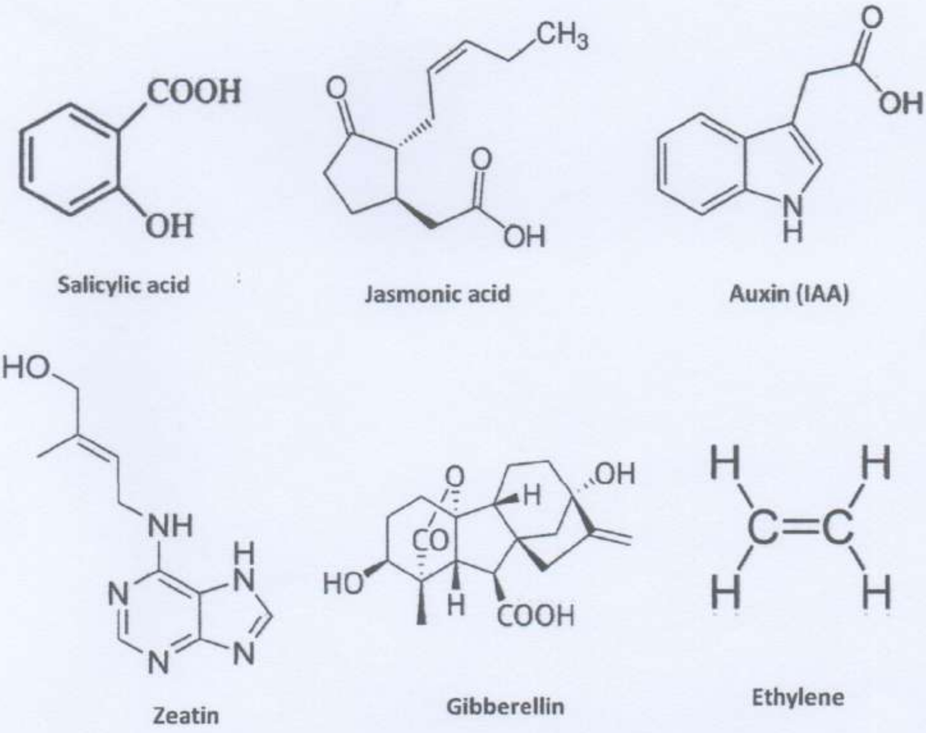

Salicylic acid
Jasmonic acid
Auxin (IAA)
Zeatin
Gibberellin
Ethylene

Figure 24. Phytohormones, jasmonic acid and salicylic acid involved in regulating anthocyanin metabolism in plants (Wikipedia, 2025).

Chapter 04

Introduction

The action of external factors combined with molecular approaches has made it possible to make significant progress in understanding the regulation of primary and secondary plant metabolism. In particular, obtaining natural mutants or those resulting from the transformation of plants by genetic engineering has made it possible to clarify the role of different genes and to better understand why they are expressed in a spatio-temporal manner (only at certain physiological stages and in certain tissues or organs) (Macheix et al., 2005).

1. Gene expression of secondary metabolites: phenolic compounds

An external factor acts on the expression of phenolic metabolism genes through complex intracellular signaling, the broad outlines of which are now known:

a. Initial effect at the level of a receptor: for light, it is phytochrome or other photoreceptors, for fungal elicitors, it is a generally plasmalemmic receptor;

b. Establishment of an intracellular message transduction pathway, leading to chemical modifications (phosphorylation or dephosphorylation is an example) of protein transcription factors, different factors can play a role in the transduction chain, in particular Ca^{2+} ions, ethylene, jasmonic acid, salicylic acid;

c. Direct action of transcription factors activated at the gene level, then allowing their expression.

Regulatory DNA sequences are recognized by protein transcription factors that act in trans by binding to specific sites on the promoter. These transcription factors become functional by binding to DNA after having themselves been regulated at the transcriptional and/or post-transcriptional level under the action of external stimuli such as light or microbial elicitors recognized at the plasmalemma level (Fig. 25). In the latter case, transmembrane receptors are involved and trigger all the mechanisms of the transduction pathways, leading to the transcription of phenolic metabolism genes (Macheix et al., 2005).

Factors can also bind to the **G** and **H** boxes of the **CHS** gene promoter, which activates transcription. For this, a trans-regulatory protein of the **GBF** type (soybean G-binding factor) must first be phosphorylated in the cytoplasm by the action of an elicitor or light (Fig 25 a and b) because only the phosphorylated form can enter the nucleus (Macheix et al., 2005).

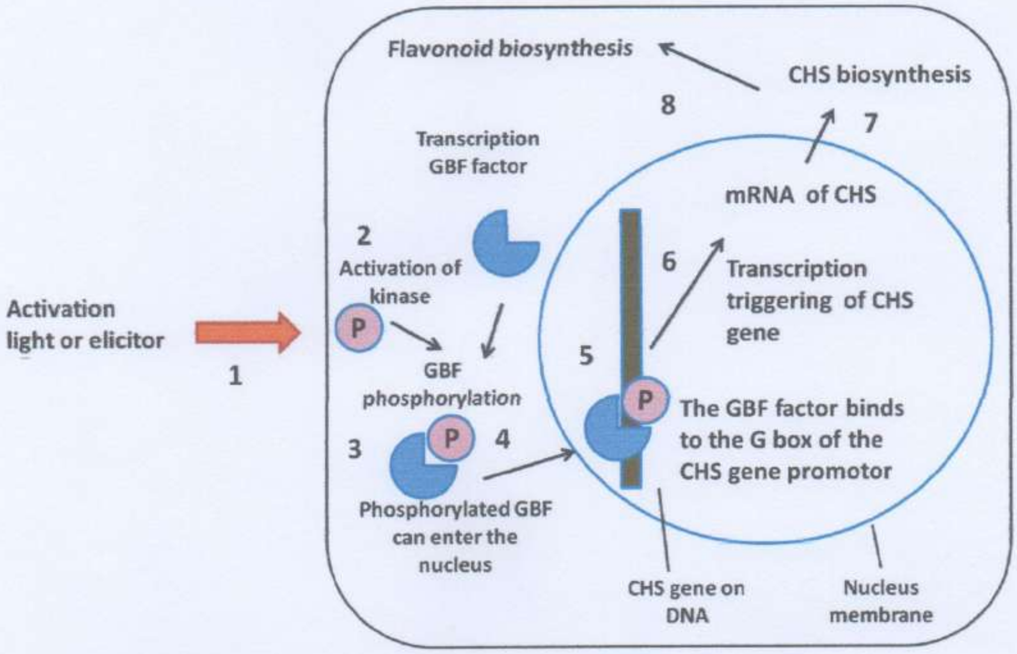


Figure 25. Triggering of flavonoid synthesis by light or by fungal elicitor. (a): the three steps leading to the activation of the chalcone synthase (CHS) gene and the accumulation of flavonoids in soybean. (b): variation in kinase activity allowing phosphorylation of the G/HBF-1 transcription factor after elicitation (Adapted according to Macheix et al., 2005).

The response can sometimes depend on the type of stimulus. Thus, in parsley cell suspensions, light and a fungal elicitor have different effects on the promoters of the PAL and CHS genes. Induction by light stimulates both genes, which results in the synthesis of flavonoids, while treatment with a fungal elicitor inhibits the CHS gene, leading to the development of furanocoumarins with antifungal activity whose biosynthesis pathway does not pass through CHS (Macheix et al., 2005) (Fig. 26).

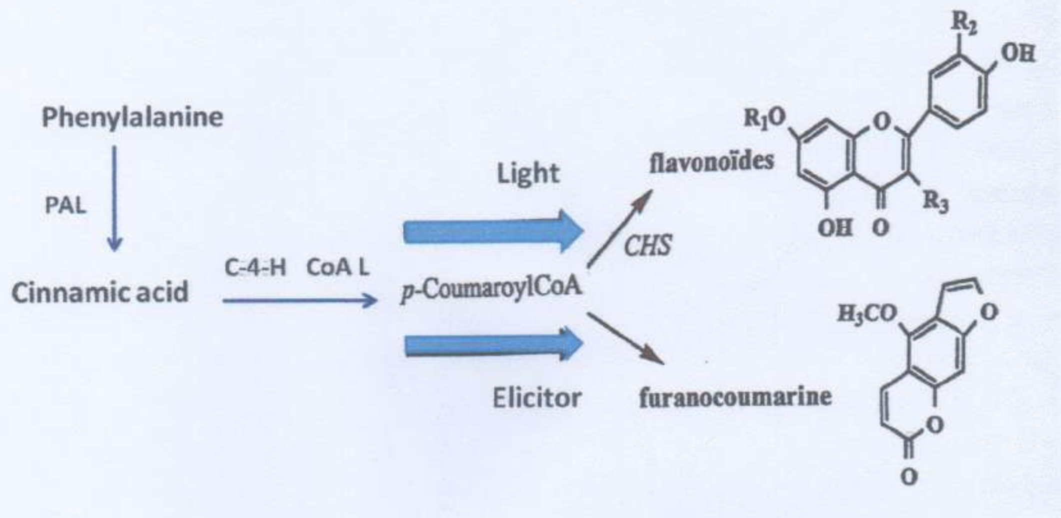


Figure 26. Response of cell suspensions of parsley to two different stimuli, light and fungal elicitor. PAL : phenylalanine ammonialyase ; CoAL : Coenzyme A ligase ; CHS : chalcone synthase (Adapted according to Macheix et al., 2005).

The response to the initial action of an inducing factor can then be very rapid (e.g., light, elicitor), with new transcripts being able to be detected after a few minutes. However, it is only after several hours that the corresponding enzymatic activities are maximal and it is generally necessary to wait 12 to 24 hours for the phenolic compounds formed to accumulate.

2. Regulation of vacuolar accumulation of phenolic compounds

Most phenolic compounds are accumulated in the vacuole; the passage of secondary metabolites is carried out through the tonoplast, from the cytosol to the vacuole.

In a manner that seems quite general, anthocyanins, other flavonoids and perhaps also intermediates of the phenylpropanoid pathway, cross the tonoplast via **ABC transporters** (ATP-binding cassete), the necessary energy being directly provided by the **hydrolysis of** ATP. In fact, phenolic compounds are first activated at the cytosol level by binding with glutathione, the **phenol/glutathione** derivative then being taken up by the transporter.

Example: in maize, the recessive mutation bronze 2 (**Bz2)** which leads to a red-brown "bronze" coloration of the aleurone layer of the grain, very different from the purple-violet color characteristic of colored varieties.

In this case, the "bronze" color is due to the blocking of the entry of glycosylated anthocyanins into the vacuole and their cytosolic accumulation. The mutation involved affects the **Bz2 gene** which codes for an enzyme of the **glutathione S-transferase** group, allowing the conjugation of anthocyanin with glutathione, this combined form being the one that can enter the vacuole thanks to the ABC transporters (Fig. 27).

Their accumulation can then lead to high vacuolar concentrations, up to 108 mM for anthocyanins in some red fruits (Macheix et al., 2005).

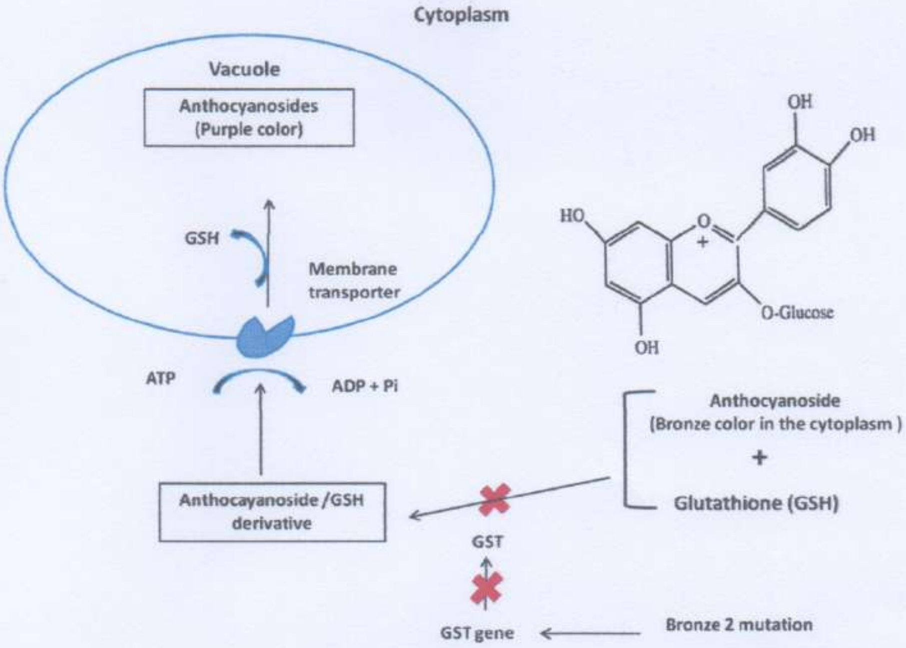


Figure 27. Diagram illustrating the blockade of anthocyanin entry into the vacuole in the bronze 2 maize mutant. GSH: glutathione; GST: glutathione S-transferase

(Adapted according to Macheix et al., 2005).

Chapter 05

Introduction

The plasma membrane or plasmalemma is a biological membrane separating the interior of a cell, the cytoplasm, from the extracellular environment. This membrane plays a fundamental biological role by isolating the cell from its environment.

The plasma membrane consists essentially of a thin layer of amphiphilic phospholipids that spontaneously organize themselves in such a way that the hydrophobic "tails" are isolated from the surrounding aqueous environment while the hydrophilic "heads" are oriented towards the extracellular environment and towards the cytosol. This leads to the formation of a continuous lipid bilayer, made up of two sheets, which tends to close on itself (Hopkins, 2003).

1. General organization of plasma membrane

The plasma membrane is made up of:

- A double lipid layer;
- Intramembrane proteins (intrinsic);
- Extramembrane proteins (extrinsic): internal on the cytosolic side or external on the external side of the membrane;
- Cell-coat, made up of a felt of fibrils made up of carbohydrates.

1.1. Plasma membrane components

1.1.1. Lipids

Membrane lipids are amphiphilic molecules (amphiphilic: molecule with a hydrophilic end, polar head, and a hydrophobic end, apolar tail). They constitute an impermeable barrier to water-soluble molecules.

1.1.2. Phospholipids

a. Hydrophilic head

It is constituted of glycerol $C_3H_8O_3$ and Phosphate PO_4^{3-}

b. Hydrophobic tail made up of two fatty acid chains (C14-C24): saturated chain and unsaturated chain (Fig. 28).

1.1.3. Sterols (cholesterol)

Cholesterol is characteristic of animal cells. It controls the fluidity of the membrane (stabilization); ergosterol is characteristic of fungi, stigmasterol, sitosterol and campesterol, characteristic of plants.

1.1.4. Proteins

There are two categories:

a-Transmembrane proteins

Amphiphilic proteins with a hydrophilic part (intra and extracellular side) and a hydrophobic part (completely crossing the lipid bilayer). These proteins cross the membrane by means of one or more peptide sequences (alpha helices). The transmembrane part of these proteins is formed of 20 to 25 hydrophobic amino acids (6 to 7 turns of the helix). Example of hydrophobic amino acids: alanine, valine, isoleucine, leucine, methionine. Their role is:

- membrane transport;
- reception of extracellular signals;
- conformational change of receptors, activating a series of cascades of intracellular events;
- enzymatic activities (example, protein complexes of the respiratory chain).

b-Intrinsic proteins

Cytosolic proteins: fixed to the cytosolic side of the bilayer by:

-an amphiphatic α-helix.

-a lipid anchor.

Intrinsic proteins: fixed to the external side of the membrane by an oligosaccharide bond, to phosphatidyl-inositol.

Proteins attached, by non-covalent interaction, to other membrane proteins.

c. **Extrinsic proteins**: arranged on the external and internal faces of the lipid bilayer. They are linked to intrinsic proteins by weak bonds. The proteins of the external face are often glycosylated, they participate in the formation of the glycocalix.

1.1.5. Carbohydrates

These are oligosaccharides associated with certain proteins and certain lipids of the external face, their role is cellular recognition, cellular adhesion (Fig. 29).

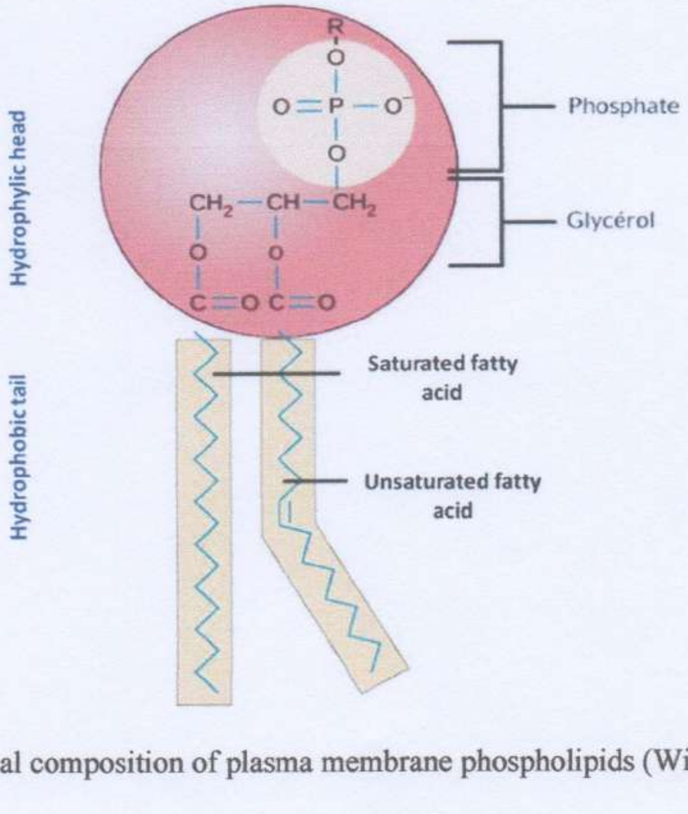


Figure 28. Chemical composition of plasma membrane phospholipids (Wikimedia, 2016).

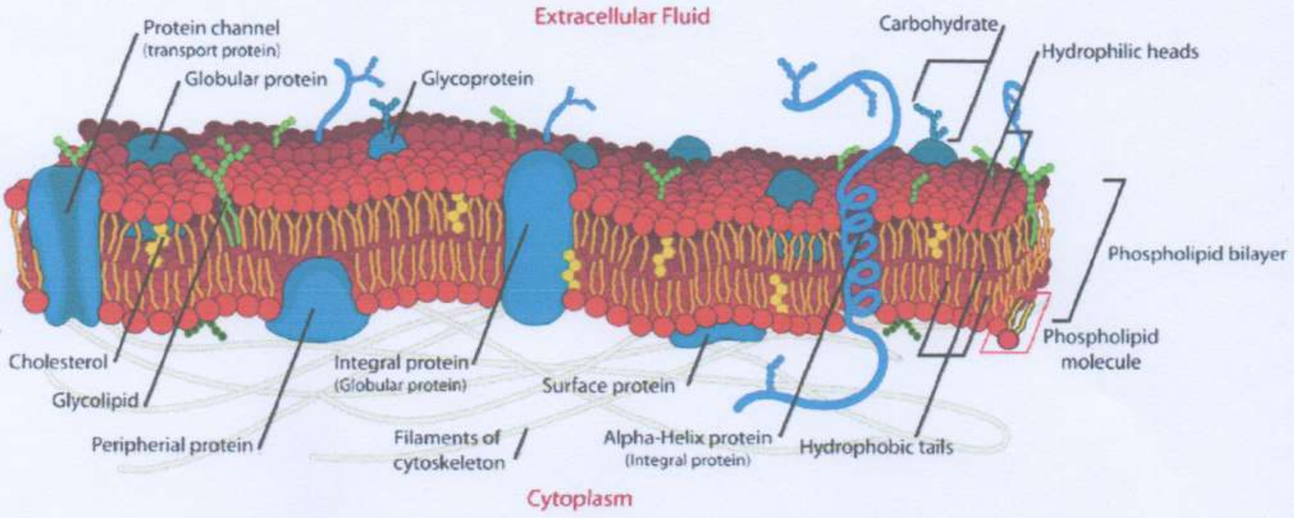


Figure 29. General organization of plasma membrane (Wikipedia, 2009).

1.2. Role of the plasma membrane

- It surrounds the cytoplasm of living cells, physically separating the intracellular components from the extracellular environment;
- Control of cellular exchanges;
- Detection and response to external signals (signal transduction).

1.3. Diffusion of metabolites through plasma membrane

Lipid bilayers are generally impermeable to ions and polar molecules. The arrangement of hydrophilic heads and hydrophobic tails blocks the diffusion of polar solutes across the membrane, such as amino acids, nucleic acids, carbohydrates, proteins, and ions, but generally allows the passive diffusion of hydrophobic molecules. This allows cells to control the movement of these substances across their plasma membrane using transmembrane protein complexes such as pores and channels. The fluid mosaic structure of the lipid bilayer comprising specific membrane proteins helps explain the selective permeability of membranes as well as the mechanisms of active and passive transport.

1.4. Membranes of organelles

The membranes of organelles are much richer in proteins, mainly enzymes, in relation to their functions.

- Vacuoles are surrounded by a membrane called the vacuolar membrane or tonoplast.
- The nucleus is surrounded by a double membrane called the nuclear envelope; the inner and outer membranes fuse at certain points, interrupting the continuity of the envelope and delimiting nuclear pores that form channels allowing the transport of RNA from the nucleus to the cytoplasm.
- The endoplasmic reticulum and the Golgi apparatus form a complex membrane system involved in lipid and protein biosynthesis as well as secretion. The endoplasmic reticulum is formed by a double membrane delimiting a lumen.

Several physiological process occur on membrane of endoplasmic reticulum, thylakoids, mitochondria, and Golgi apparatus (Hopkins, 2003).

1.4.1. Endoplasmic reticulum membrane and enzyme regulation

Many enzymes are linked to the endoplasmic reticulum membrane like the different enzymes of flavonoid pathway from the initial formation of the C15 skeleton by CHS to the terminal steps of methylation and glycosylation, do not act independently of each other. While being closely associated with the membranes of the endoplasmic reticulum, they can be grouped in functional enzymatic complexes where the different metabolic intermediates are channeled in a privileged manner from one enzyme to another, which avoids their dispersion in the cellular environment. This situation also allows a coordinated regulation of the activity of the different enzymes.

The studied demonstrative example is that of Chrysosplenium genus where pentamethylated flavonol is formed by different 5-methyltransferases (Fig. 30). Each one is specific to one of the positions of molecule methylation (Macheix et al., 2005).

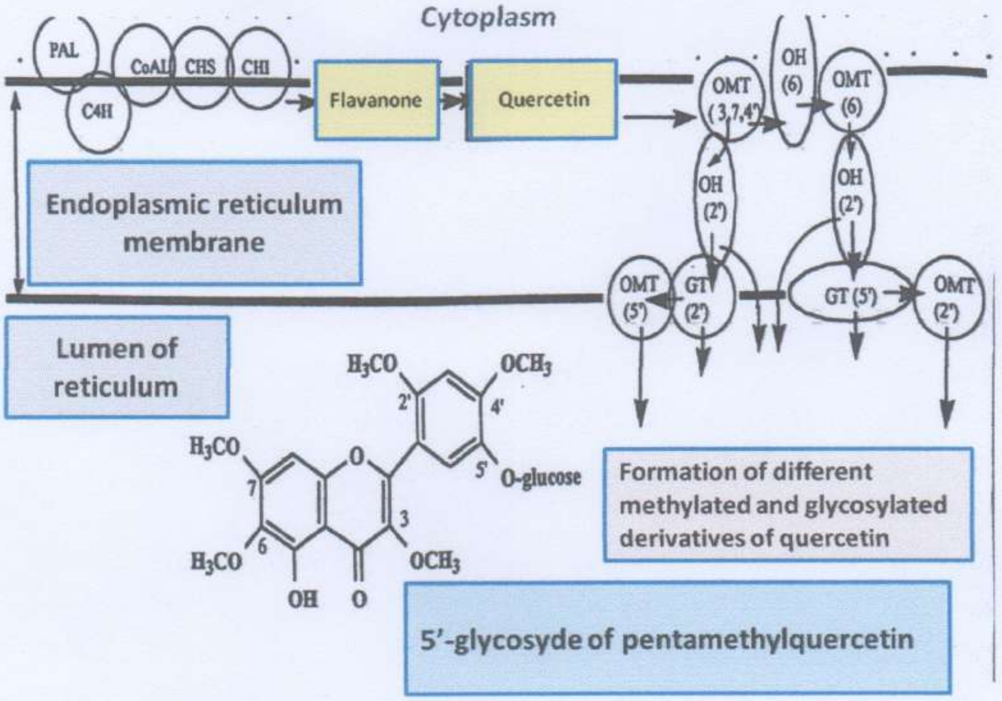


Figure 30. Model showing the formation of polymethylated and glycosylated flavonols in endoplasmic reticulum membrane in Chrysosplenium genus. PAL : phenylalanine ammonialyase ; CoAL : Coenzyme A ligase ; CHS : chalcone synthase, CHI: chalcone isomerase; GT: glycosyl transferase; OMT: O-methyl-transferase; C4H: cinnamate-4-hydroxylase (Adapted according to Macheix et al., 2005).

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