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REVIEW ARTICLE

Biosynthesis of Essential Oil in Aromatic Plant Species: A review

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ABSTRACT: Essential oils (EOs) are complex mixtures of volatile secondary metabolites synthesized in aromatic plants, valued for their therapeutic, aromatic, and industrial applications. This review provides a comprehensive analysis of the biosynthetic pathways involved in essential oil production, emphasizing the enzymatic and genetic regulation underlying terpenoid formation. Essential oils are primarily derived from two distinct metabolic pathways: the cytosolic mevalonate (MVA) pathway and the plastidic methylerythritol phosphate (MEP) pathway. Both pathways produce the universal precursors, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), which serve as the foundation for terpenoid biosynthesis. Terpene synthases (TPS) catalyze the conversion of these precursors into diverse terpenoids, including monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), and higher-order terpenes, each contributing to the characteristic fragrance and biological activity of essential oils. The commercial significance of essential oils is immense, with applications spanning pharmaceuticals, cosmetics, food flavoring, and aromatherapy. The global market for essential oils is projected to grow from USD 10.3 billion in 2021 to USD 18.25 billion by 2028, driven by increasing demand for natural and organic products. However, optimizing EO production requires deeper insights into metabolic engineering, environmental influences on biosynthesis, and genetic manipulation of key enzymatic steps. This review consolidates current knowledge on terpenoid biosynthesis, discusses the roles of critical enzymes and genes, and highlights future research directions to enhance EO yield and diversification. Understanding these mechanisms will facilitate the development of sustainable strategies for industrial-scale essential oil production.

Keywords: Essential oils, Terpenoid biosynthesis, MVA pathway, MEP pathway, Aromatic plants, Enzymatic regulation.

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1. INTRODUCTION

Plants synthesize an enormous diversity of metabolites that serve critical roles in their growth, development, and ecological interactions. These metabolites can be broadly classified into primary and secondary metabolites based on their functional significance. Primary metabolites, including carbohydrates, amino acids, fatty acids, proteins, and nucleic acids, are indispensable for fundamental physiological processes such as energy metabolism, cell structure, and reproduction [1]. In contrast, secondary metabolites, though not essential for basic survival, play pivotal roles in plant defense, signaling, and adaptation to environmental stresses. Among these, essential oils (EOs) represent one of the most economically and pharmacologically significant classes of plant secondary metabolites. These volatile, aromatic compounds are synthesized and stored in specialized secretory structures such as glandular trichomes, resin ducts, and oil cells, which are distributed across a wide range of plant families, from Rosaceae (roses) to Gramineae (aromatic grasses), spanning tropical and temperate ecosystems [1, 2].

Essential oils are complex mixtures of low-molecular-weight organic compounds, primarily composed of terpenoids (isoprenoids), phenylpropanoids, and fatty acid derivatives [3, 4]. Terpenoids, the most abundant group, are

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constructed from isoprene (C_5) units and are further categorized based on carbon skeleton size into monoterpenoids (C_{10}), sesquiterpenoids (C_{15}), diterpenoids (C_{20}), and higher-order terpenes (e.g., triterpenoids and tetraterpenoids) (Figure 1). The structural diversity of these compounds adheres to the isoprene rule, as described by Breitmaier [5], where 2-methylbutane residues form the foundational isoprenoid structure (Figure 2). Phenylpropanoids, derived from the shikimate pathway, contribute to the distinct aroma and medicinal properties of certain essential oils, while fatty acid derivatives enhance their functional complexity [3, 4]. The intricate chemical profiles of essential oils make them invaluable in pharmaceuticals, cosmetics, food flavoring, and aromatherapy [2, 6].

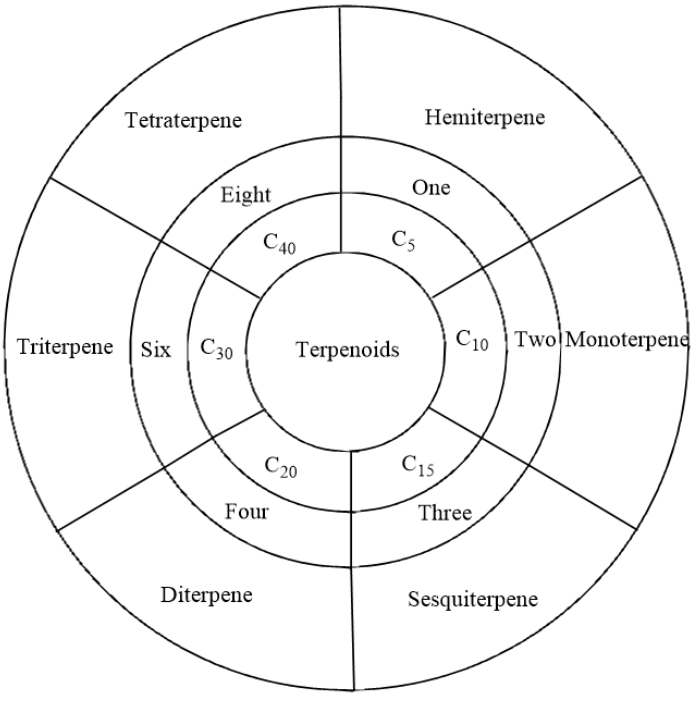


Fig. 1. Categorization of terpenoids on the basis of no. of isoprenoid unit.

The biosynthesis of essential oils is governed by two distinct metabolic pathways: the mevalonate (MVA) pathway in the cytosol and the methylerythritol phosphate (MEP) pathway in plastids. Both pathways produce the universal precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), which serve as the building blocks for terpenoid synthesis [7]. The MVA pathway, which begins with acetyl-CoA, primarily supplies precursors for sesquiterpenes (C_{15}) and triterpenes (C_{30}), while the MEP pathway, utilizing pyruvate and glyceraldehyde-3-phosphate, generates substrates for monoterpenes (C_{10}), diterpenes (C_{20}), and tetraterpenes (C_{40}) [8, 9]. Terpene synthases (TPS) further modify these precursors into diverse terpenoid structures, defining the organoleptic and bioactive properties of essential oils [10]. For instance, monoterpenes such as menthol (from *Mentha* spp.) and linalool (from lavender) are widely used in medicinal formulations and fragrances, while sesquiterpenes like β-caryophyllene (found in basil and oregano) exhibit anti-inflammatory effects [11, 12].

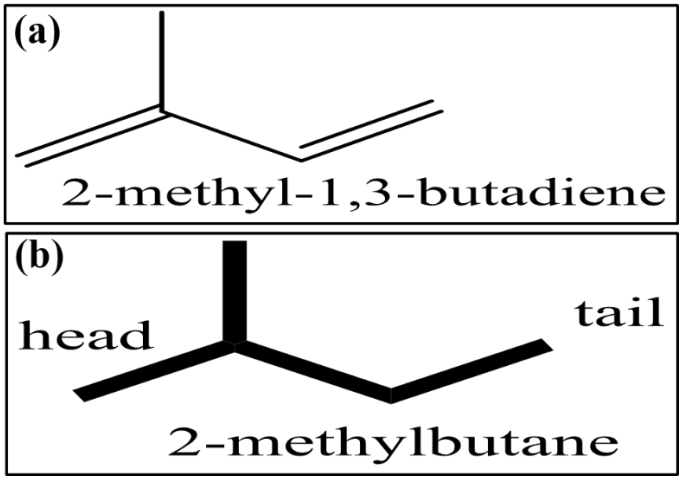


Fig. 2. Structural diversity of terpenoids following the isoprene rule.

Beyond their industrial applications, essential oils possess significant therapeutic potential, demonstrating antimicrobial, anti-inflammatory, antioxidant, and insect-repellent properties (Figure 3) [13]. For example, thymol and carvacrol, monoterpenes derived from thyme and oregano, exhibit potent antibacterial and antifungal activities, making them valuable in food preservation and natural medicine [14]. Similarly, the sesquiterpene lactone artemisinin, isolated from *Artemisia annua*, is a cornerstone in antimalarial therapy [15]. The growing consumer preference for natural and organic products has further amplified the demand for plant-derived essential oils, positioning them as economically important commodities in global trade. The global essential oil market, valued at USD 10.3 billion in 2021, is projected to reach USD 18.25 billion by 2028, reflecting their expanding applications in health and wellness sectors [16].

However, the production and composition of essential oils are highly influenced by genetic, developmental, and environmental factors. Abiotic stressors such as light, temperature, drought, and nutrient availability can significantly alter terpenoid biosynthesis, affecting both the yield and quality of essential oils [17, 18]. For instance, high light intensity and UV radiation have been shown to enhance the synthesis of protective volatile compounds in aromatic plants like basil and lavender [19]. Similarly, water deficit stress can increase the concentration of certain monoterpenes in peppermint, albeit at the cost of reduced biomass [20]. These findings underscore the need for a deeper

understanding of the regulatory mechanisms governing terpenoid biosynthesis to optimize essential oil production under varying environmental conditions.

This review provides a comprehensive and updated synthesis of the biosynthetic pathways, enzymatic regulation, and genetic determinants underlying essential oil production in aromatic plants. Unlike previous reviews, this paper integrates recent advances in metabolic engineering and omics technologies (genomics, transcriptomics, and metabolomics) to highlight innovative strategies for enhancing EO yield and diversification. Additionally, it critically examines the environmental and physiological factors influencing terpenoid biosynthesis, offering insights into sustainable cultivation practices. By bridging the gap between fundamental research and industrial applications, this work presents a holistic perspective on optimizing essential oil production to meet rising global demand while addressing challenges in metabolic flux control and pathway manipulation.

2. BIOSYNTHESIS OF ESSENTIAL OIL

Essential oils represent complex mixtures of volatile secondary metabolites that contribute to the characteristic fragrances and biological activities of aromatic plants. These oils primarily consist of two distinct chemical classes: terpenoids and phenylpropanoids. While terpenoids dominate in both abundance and structural diversity, phenylpropanoids contribute significantly to the unique sensory profiles of many essential oils through their distinctive aromatic properties. These two classes originate from entirely different biosynthetic pathways, utilizing distinct primary metabolic precursors and enzymatic machinery for their formation (Figure 4). The terpenoid biosynthesis pathway has been extensively studied and reviewed, revealing intricate mechanisms that govern the production of these compounds across various plant species [6]. In contrast, phenylpropanoid biosynthesis, which proceeds via the shikimate pathway, has been characterized to a lesser extent, particularly for oil-specific compounds such as eugenol and elemicin, whose biochemical pathways remain only partially understood.

2.1. Terpenoids

Terpenoids constitute the largest and most structurally diverse group of plant secondary metabolites, with over 80,000 known compounds. These molecules are synthesized from two fundamental five-carbon building blocks: isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP).

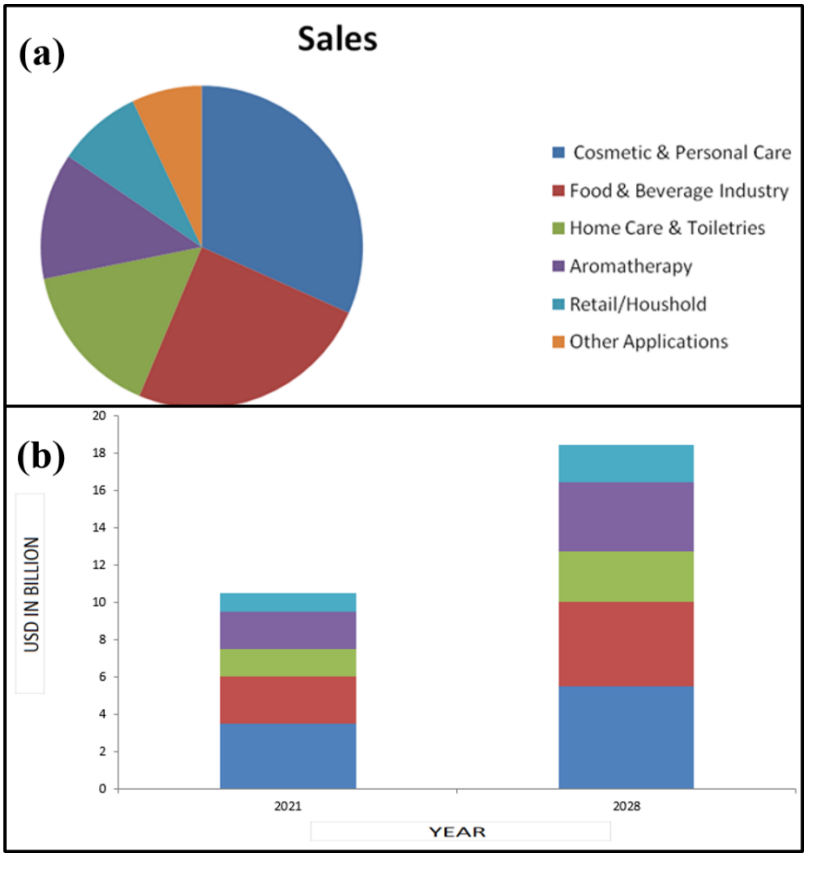


Fig. 3. (a) Applications of essential oil in various sectors, (b) Essential oil market size by the application 2021 and 2028 (USD Billion).

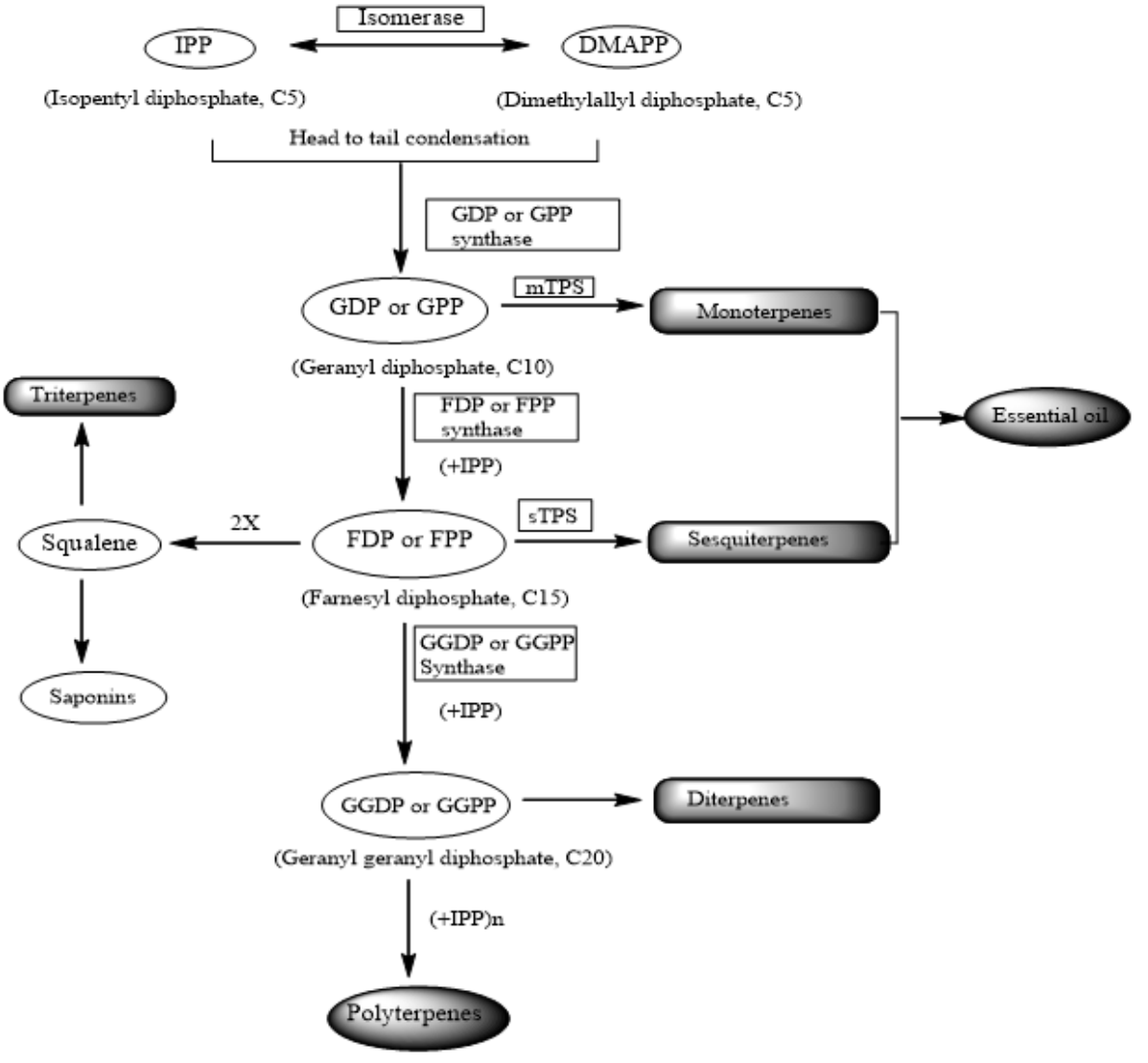


Fig. 4. Mechanism of biosynthesis of essential oil followed by the formation of various terpenes.

The biosynthesis of these precursors occurs through two evolutionarily distinct pathways—the mevalonate (MVA) pathway in the cytosol and the methylerythritol phosphate (MEP) pathway in plastids—which supply the necessary substrates for the formation of different terpenoid classes (Figure 4). The MEP pathway predominantly provides precursors for monoterpenes (C_{10}), diterpenes (C_{20}), and tetraterpenes (C_{40}), while the MVA pathway generates substrates for sesquiterpenes (C_{15}) and triterpenes (C_{30}). This compartmentalization reflects the evolutionary adaptation of plants to regulate terpenoid production in response to developmental and environmental cues.

2.1.1. The biosynthesis of terpenoids' fundamental building units

The condensation of IPP and DMAPP by prenyltransferases yields longer-chain prenyl diphosphates that serve as immediate precursors for various terpenoid classes. Geranyl diphosphate synthase (GPS) catalyzes the formation of geranyl diphosphate (GPP, C_{10}), the universal precursor of monoterpenoids, through the head-to-tail condensation of one DMAPP molecule with one IPP molecule (Figure 5). Similarly, farnesyl diphosphate synthase (FPPS) extends GPP by adding another IPP unit to produce farnesyl diphosphate (FPP, C_{15}), the central intermediate for sesquiterpenoid biosynthesis. Further elongation of FPP with IPP, mediated by geranylgeranyl diphosphate synthase (GGPS), yields geranylgeranyl diphosphate (GGPP, C_{20}), the precursor for diterpenoids. The dimerization of two FPP molecules by squalene synthase (SQS) generates squalene (C_{30}), which undergoes oxidation by squalene monooxygenase to form 2,3-oxidosqualene, the branch-point intermediate for triterpenoid and sterol biosynthesis [7]. For tetraterpenoids (C_{40}), phytoene synthase (PS) catalyzes the head-to-head condensation of two GGPP molecules, yielding phytoene, the first committed carotenoid precursor.

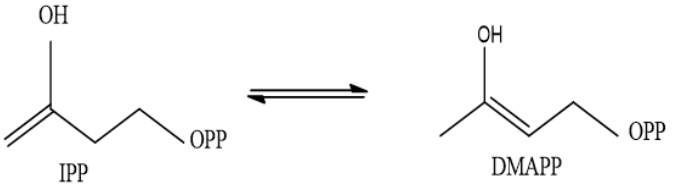


Fig. 5. Isomerization of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP).

DXP and Mevalonic acid pathways produced the isopentenyl diphosphate and dimethylallyl diphosphate's building components by the condensation, supplies the prenyl diphosphate substrate e.g., like C_{10} (GPP), formation of C_{10} takes place as a result of condensation of isopentenyl diphosphate and dimethylallyl diphosphate through the involvement of geranyl diphosphate synthase enzyme activity and C_{15} (FPP), the formation of given substrate takes place from the enzymatic activity of farnesyl diphosphate synthase in the condensation of GPP and IPP. The universal precursors of monoterpenoids (C_{10}) and sesquiterpenoids (C_{15}) are GPP and FPP, respectively. Geranylgeranyl diphosphate, a C20 precursor of the diterpene, is formed by the condensation of FPP with IPP, which catalysis through the geranylgeranyl diphosphate synthase (GGPS) enzyme, biosynthesis of squalene (C_{30}) is the result of two FPP molecules dimerization and the groups of diphosphates get removed via means of enzymatic action of squalene synthase [7]. An oxygen group added to the squalene through squalene monooxygenase or epoxidase, causing formation of 2, 3-oxidosqualene which is a triterpenes precursor in addition steroids and sterols in the plant. The precursor of the tetraterpenoids or carotenoids, phytoene, C_{40} compound formed by the dimerization of a pair of geranylgeranyl diphosphate molecules as well as exclusion of a pair of diphosphates via means of an enzyme, phytoene synthase (PS).

2.1.2. Biosynthesis of monoterpenoids (C_{10})

C_{10} compounds are monoterpenoids obtained from geranyl diphosphate. The biological activity of monoterpenoids is well known and they are also well known for its distinctive smell and fragrant characteristics [8]. There are many applications of these compounds e.g., like fragrance, food additives, drinks, cosmetics and perfumes [9]. The two of most important monoterpenoids are geraniol (obtained from rose flowers) and linalool (from coriander) used in the industry of flavor which has the annual utilization of 5000 tons/year to be reached [10]. Biotic and/or abiotic stresses conditions are oftenly responsible for the induction of monoterpenoids in plants and they are expected to have properties which can facilitate the plant to treat under these stressful conditions [11]. An enzyme which is involved in synthesizing monoterpenoid precursor geranyl diphosphate is GPP synthase (GPS) whose first characterization was introduced from the sage's glands of essential oil [12]. Monoterpene synthases (sometimes also known as monoterpene cyclase when catalyzation occurs for the formation of a cyclic monoterpene) are the enzymes responsible for the production of monoterpenoids through their activity. Among all terpene synthases, the motif of DDxxD for $Mg2^{+}$ cation binding is conserved, which permits their identification [13]. The production of several monoterpenoids, which comes from GPP is oftenly catalyzed through a single monoterpene synthase. For example, production of (+)-α-pinene, (+)-β-pinene, (-)-limonene, myrcene and β-phellandrene encoded by a monoterpenoid synthase enzyme, CsTPS2FN, which is isolated from *Cannabis sativa* [14] (Figure 6). Generally, it is presumed that the main organelle for the formation of monoterpenes and their substrate, geranyl diphosphate, is plastids.

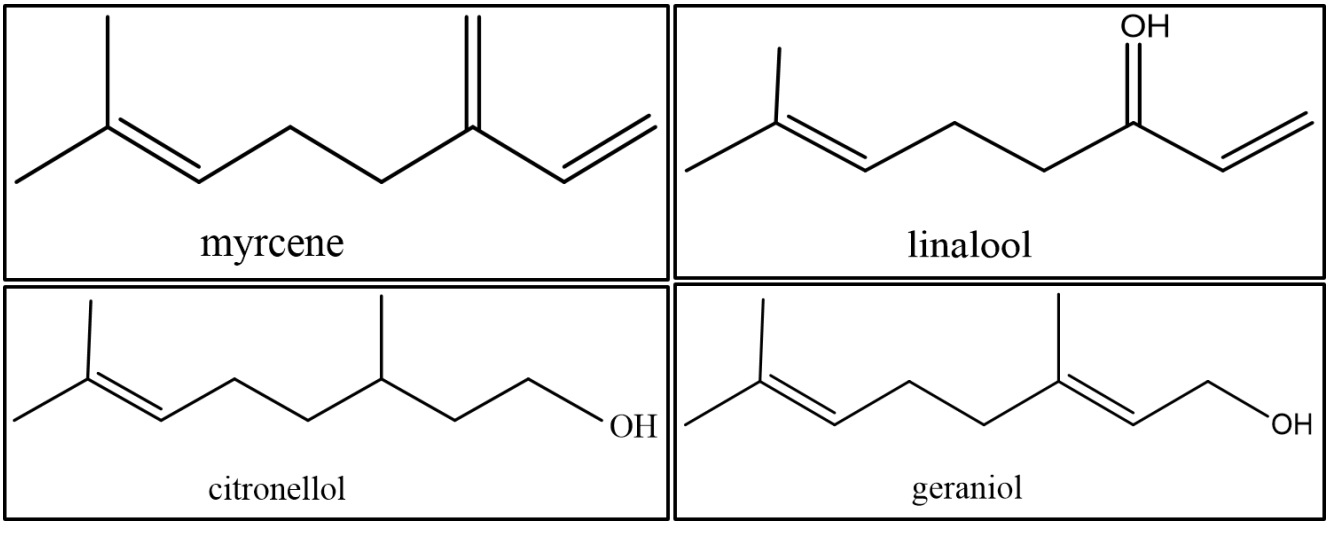


Fig. 6. Some examples of monoterpenoid.

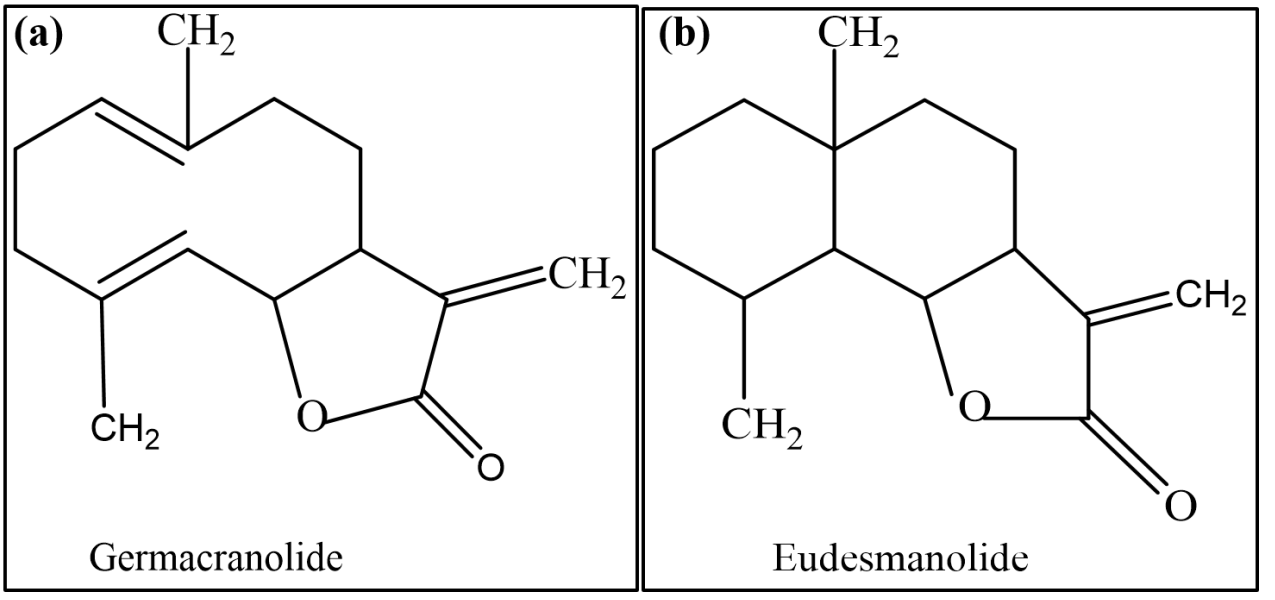


Fig. 7. Some examples of sesquiterpenoid.

It has been demonstrated that the GDP produced by mitochondria got exchange to the plastids for monoterpenoids production [15]. Examples of some monoterpenoids are given below with structures (Figure 6).

2.1.3. Biosynthesis of sesquiterpenoids (C_{15})

The production of sesquiterpenoids occurs from FPP, with the help of catalytic activity of sesquiterpene synthases. Essential oils of plant posses' constituents, some of them are known as sesquiterpenoids which are aromatic also. The major component of essential oil in basil (*Ocimum spp)*, rosemary *(Rosmarinus officinalis)* and oregano *(Origanum vulgare* L*)* is the sesquiterpene β-caryophyllene whose presence has been reported in many species of plant [16].

The main sesquiterpene isolated from cannabis plants is obtained when humulene gets together with β-caryophyllene and accountable because of its odour [17]. The applications of β-caryophyllene are broadly spread in beverages and frozen dairy [18]. It was shown in chamomile (*Matricaria chamomila*), biosynthesis of sesquiterpene initiates in the plastids with geranyl diphosphate which has been transported to the cytosol latterly, where addition of isopentenyl diphosphate occurs [19] (Figure 7).

The sub-category of sesquiterpenoids is sesquiterpene lactones possessing over 4,000 distinct structures that are well known. Sesquiterpenoid lactones are compounds posses' characteristics such as colorless and bitter, found mainly in *Asteracea,* a plant species [20]. Antibacterial (an example vermolide [21], antifungal (e.g. 8α-hydroxy-4-epi-sonchucarpolide [22], anticancer (an example parthenolide [23] are the biological properties of sesquiterpenes which make them more applicable in medical sector. Xanthanolides, germacranolides, pseudoguaianolides, eudesmanolides, guianolides and eremophilanolides are six bicyclic or tricyclic classes into which sesquiterpene lactones are classified [24].

2.1.4. Biosynthesis of diterpenoids (C_{20})

One of the most diverse categories of secondary metabolites discovered in plants are diterpenoids with not less than 10,000 different natural plant obtained structures [24]. Plant secondary metabolites play a role as PGR such as gibberellins in plant primary metabolism are diterpenes [25]. Taxol, which is isolated from the Pacific yew (*Taxus brevifolia*) is one of many diterpenoids which have medicinal properties [26], ovarian and breast cancer can be treated by use of them. In malignant pleural mesothelioma (MPM) cancer cells, apoptosis is induced by two diterpenes namely cafetal and the structurally related kahweol from *Coffea Arabica* [27]. *Cis*-abienol, an aromatic diterpene, is a valuable compound for the fragrance industry, and it made up of fir trees (*Abies balsamea*) (Figure 8). In perfume formulations, an essential diterpene which contains oxygen is *Cis*-abienol serves as the Ambrox® precursor [25]. There are two sequential steps for proceeding the biosynthesis of *cis*-abienol. First a conversion of GGPP to 8-hydroxy-copalyl diphosphate through the diterpene synthase and then an enzyme like kaurene synthase is responsible for the conversion into *cis*-abienol by detaching the group of diphosphates [28] (Figure-8).

2.1.5. Biosynthesis of triterpenoids (C_{30})

More than 20,000 identified plant compounds constituted by this class of specialized metabolites [29]. There is a high

amount of variability in families of plant has shown by triterpenes. Glycosylated triterpenoids, also known as saponins are found in *Camellia oleifera* and *Quillaja saponaria* (a native Chilean tree). Due to their foaming properties, saponins are used in shampoos, detergents and emulsifiers [30].

Production of saponin type triterpenoids occurs in many plants during standard growth (such as peel of apple fruit, forming ursolic acid [31], however plant species, organs and developmental stage are the factors on which their level of saponin strongly depends [32]. A different natural triterpene that is utilized in products of cosmetic e.g. hair conditioner, and extracted from the bark of *Butela spp*., is known as butelin [33]. The cure of major diseases such as cancer and HIV is done by application of many triterpenoids. A triterpenoid extracted from *Tripterygium wilfordii* is celastrol, has an inhibitory effect on Tat [34] (Figure 9). A protein that is encoded by a virus that is essential forgenome transcription of Human immunodeficiency virus, is 'Tat'. The conversion of 2,3-oxidosqualene is done by triterpene synthases through a Chair-Chair-Chair (CCC) or Chair-Boat-Chair (CBC) confirmation into another skeletons of triterpenoid. B-amyrin synthase is an example of a triterpene synthase [35].

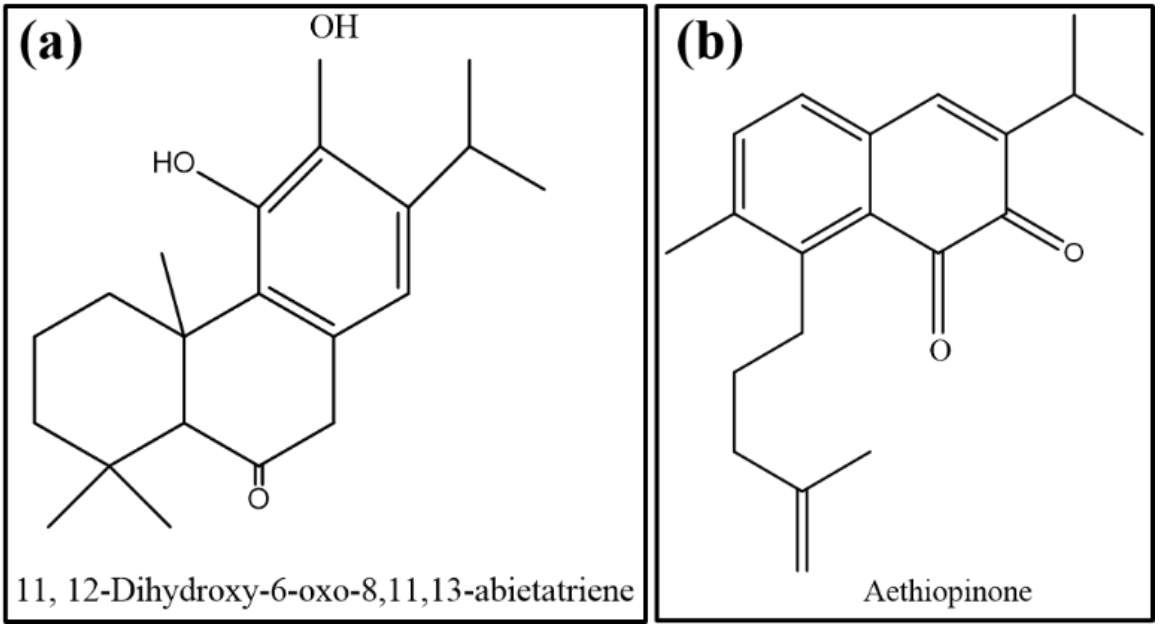


Fig. 8. Some examples of diterpenoid.

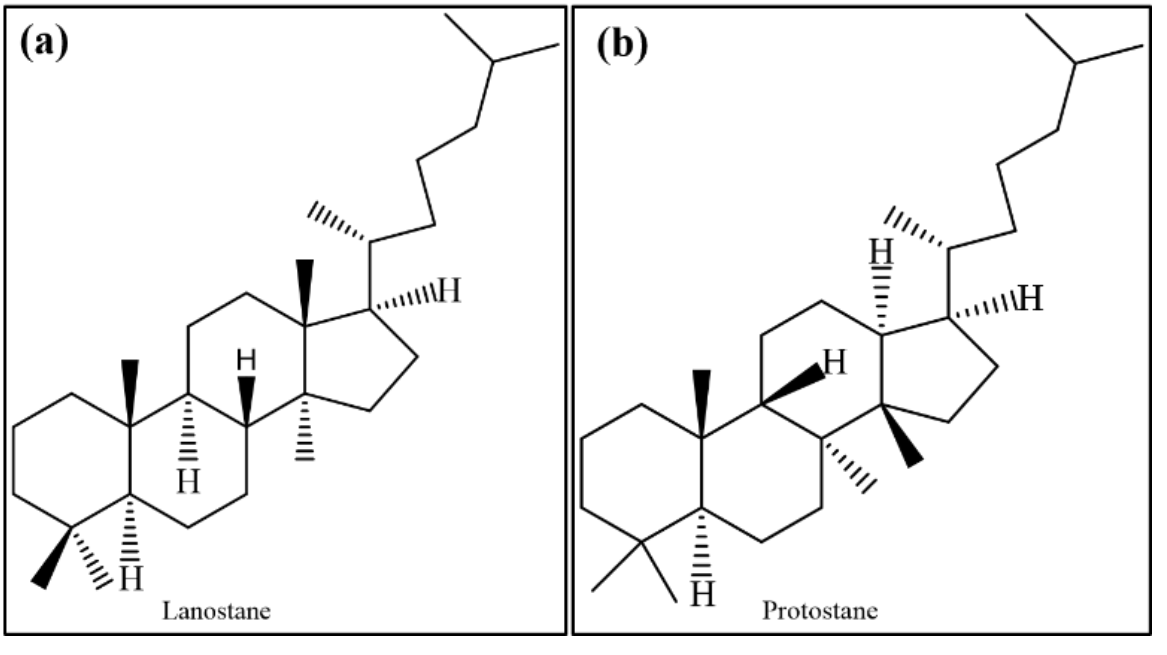


Fig. 9. Some examples of triterpenoid.

2.1.6. Biosynthesis of tetraterpenoids (C_{40})

The most ordinary natural pigments are carotenoids (tetraterpenoids) and also contain antioxidant properties. 750 different reported structures have contained by tetraterpenoids [36]. Carotenoids are being used as nutraceuticals in the food and flavor industry (e.g. β-carotene), in the drug industry, and also used as dyes and colorants, in addition to cosmetics [37]. The presence of tetraterpenoids mostly found in photosynthetic organisms [38] and frequently in charge of the color orange, yellow and red [39]. A vital role played by carotenoids in photosynthesis and they are essential also. With the phytoene synthase activity making prephytoene diphosphate, biosynthesis of carotenoid starts [40]. Then the conversion of prephytoene diphosphate to 15-*cis*-phytoene through phytoene synthase occurs. In the production of *trans*-lycopene, there are several other enzymes such as an isomerase and a desaturase which are involved. The next step is cyclisation; biosynthesis of α-carotene is the result of α-cyclase activity, while the conversion of *trans*-lycopene to β-carotene can be done by β-cyclase (Figure 10). Strigolactones are different kind of carotenoid-derived terpene type molecules which occurs naturally. Isomerization of β-carotene by D27 with which their biosynthesis starts [41]. A carotenoid cleavage (CCD7) cleaves the resulting 9-cis—carotene which, in turn produces 9-*cis*-β-apo-10-carotenal and β-ionone [42].9-cis-apo-10-carotenal is converted to carlactone from another carotenoid cleavage enzyme, CCD8 [41]. A cytochrome P450, MAX1 homologs, will oxidise this ubiquitous strigolactone precursor, which outcomes in the production of *ent*-2-*epi*-5-deoxystrigol or carlactonoic acid.

3. BIOSYNTHETIC PATHWAYS

3.1. Isoprenoid biosynthesis in cytosol through the MVA pathway

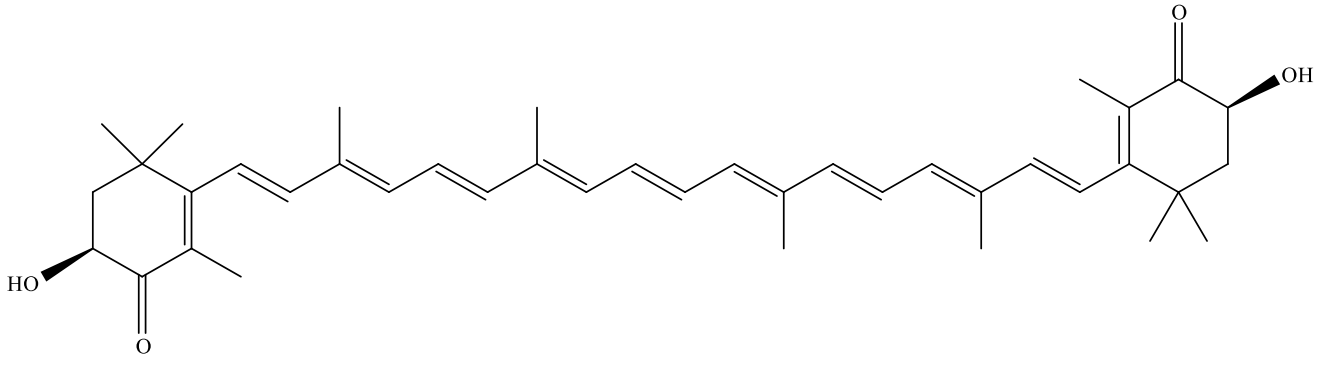
Cytosolic and mitochondrial isoprenoids are gotten from acetyl-CoA which is acquired from CO_2 fixation (Figure 11). Catalyzation of condensation of two acetyl-CoA molecules has been done by acetyl-CoA: acetyl-CoA C-acetyltransferase (AACT, EC 2.3.1.9) to form acetoacetyl-CoA [43], and 3-hydroxy-3-methylglutaryl-CoA synthase (HMGS, EC 4.1.3.5) condenses acetoacetyl CoA as well as acetyl-CoA to form HMG-CoA [44]. HMG-CoA is at long last decreased to MVA catalyse by 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR, EC 1.1.1.34) [45]. The first step of the pathway is MVA biosynthesis and mevalonate is biosynthesized into mevalonate 5-diphosphate through two-step phosphorylation process catalysed by mevalonate kinase (MK, EC 2.7.1.36) [46] and phosphomevalonate kinase (PMK, EC 2.7.4.2) [47] in order. Finally, mevalonate 5-diphosphate decarboxylase (MDC, EC 4.1.1.33) converts mevalonate 5-diphosphate to isopentenyl diphosphate [48], which is the end result of MVA pathway for isoprenoid biosynthesis in cytosol and mitochondria. The IPP and DMAPP conversion into each other is catalysed from IPP isomerase (IPI, EC 5.3.3.2) [49]. The formation of basic skeleton of some sesquiterpenes, sterols and the side chain of ubiquinone occurs by IPP and DMAPP [50].

3.2. Involved enzymes and genes in the MVA pathway

The MVA pathway has been found for more than half a century and the mevalonate-dependent pathway's molecular genetics has already been clarified. The characterization of genes that are participated in mevalonate-dependent pathway have already been done by biochemistry and molecular genetics approaches.

3.2.1. Acetyl-CoA C-acetyltransferase (AACT)

AACT catalyses the initial step, pair of acetyl-CoA molecules are condensed for the formation of acetoacetyl-CoA included in the MVA biosynthetic pathway. AACT belongs to the thiolase family which has two cysteine residues that are conserved, which are significant for the purpose of action of thiolase.



Astaxanthin

Fig. 10. Example of tetraterpenoid.

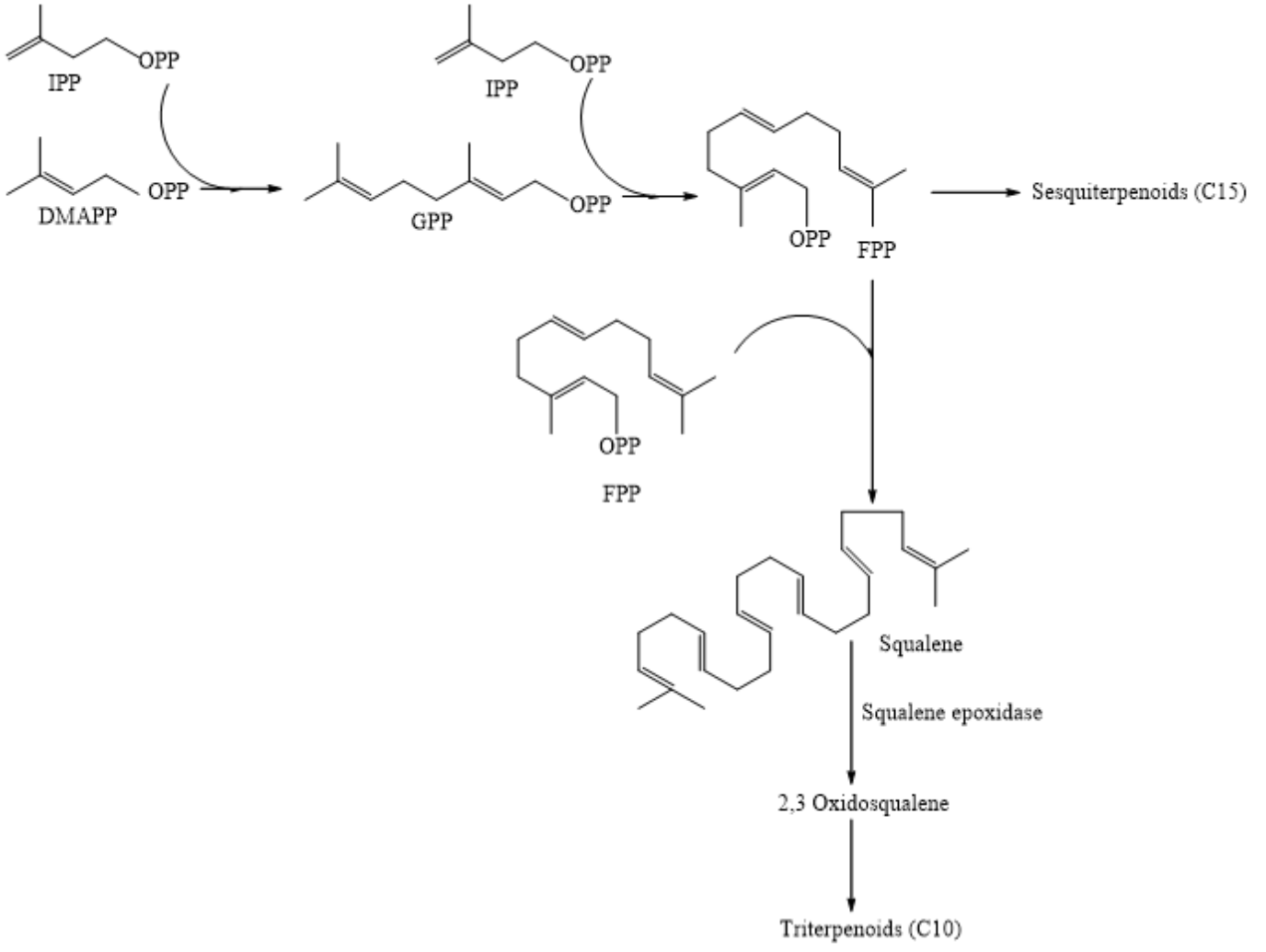


Fig. 11. Isoprenoid biosynthesis in cytosol through MVA pathway.

The first is participated in the production of an acetyl-enzyme intermediate and situated in the N-terminal part of the enzyme; the second is the active site engaged with deprotonation process in the reaction of condensation is situated in the C-terminal furthest point [51]. The full-length cDNA of *ATCC* have been described from *Arabidopsis thaliana*, para elastic tree, rice and radish [52]. The genomic association of ATCC is distinctive among plants. As indicated by the genomic database, AACT has a place with small family of gene *in A. thaliana*, in any case, there is just one ATCC duplicate in radish and it is directed by light [52].

3.2.2. 3-hydroxy-3-methylglutaryl-CoA synthase (HMGS)

HMG-CoA and CoA are formed by the condensation of acetyl-CoA and acetoacetyl-CoA catalysed by HMGS. HMGS have amino acids which has the consensus pattern as the following equation: N-x-[DN]-[IV]-E-G-[IV]-D-x(2)- N-A-C-[FY]-x-G, in which, in the first step of the reaction, cysteine acts as a catalytic nucleophile, similar to how acetylation of the catalyst by acetyl-CoA works [53]. F244, a particular inhibitor of *HMGS*, can break the mevalonate-dependent pathway and is a valuable research tool of HMGS [54]. In plants, expression of HMGS shows connection with quick division of cell and growth of cell like HMGR, which is controlled by numerous variables. HMGS expression induced by wound, methyl jasmonate, salicylic acid [54] and ozone, recommending that HMGS is engaged with the activities of plant defences [55]. The mRNA expression level and enzymatic action of HMGS in para rubber tree indicated positive relationship with the accumulation of rubber [56]. These outcomes propose that highly regulated gene in the start of the mevalonate-dependent pathway is HMGS.

3.2.3. 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR)

HMGR, a rate-restricting catalyst that can be inhibited explicitly by lovastatin, the most significant submitted move of the MVA biosynthetic pathway that is catalysation of the NADP-dependent mevalonate synthesis from HMG-CoA

done by this [57]. HMGR is very much investigated in archeobacteria, animals, plants, fungi and bacteria. Structurally, it comprises of three domains: the N-terminal region that carries an arbitrary number of transmembrane fragments (2 in plants; 7 in mammals, insects and fungi), a linker region furthermore, a C-terminal reactant region of residues of around 400 amino-acid. Despite very limited similarity of sequence is found by many of the transmembrane domain of HMG-CoA reductases from various species, the C-terminal reactant domain is exceptionally conserved. This region's structure is supposed to comprise of arnphipathic helices flanking an expanded -pleated sheet [58]. The catalytic domains of plant HMGR are made up of three domains: a small helical amino-terminal N-domain; a large, central L-domain with two HMG-CoA binding motifs (EMPIGYVQIP and TTEGCLVA) and a NADP(H)-binding motif; and a NADP(H)-binding motif (GTVGGGT). Furthermore, its structure resembled that of a prism, with an alpha helix forming the central structure component and a small helical s-domain containing a NADP(H)-binding pattern described by the sequence DAMGMN [59].

In plants, the general precursor of many recognized isoprenoid compounds is the mevalonate, a large number that are crucial for plant typical development, growth and diversity of other ordinary physiological activities. There are many losses like early senescence, a short life span, male sterility and decreased sterol levels due to the loss of function of HMG1 in *Arabidopsis*. The outcomes recommended that HMG1 plays a criticizing role in triterpenoid biosynthesis, and that sterols as well as triterpenes contributed to cell lengthening, senescence, also, fertility [60]. The characterization and cloning of genes that encodes HMGRs have been done from angiosperm species like *Camptotheca acuminata* [61], *Catharanthus roseus* [62], Melon [63], tomato [64] and *Arabidopsis thaliana* [65]. Many research results depict that HMGRs in plants decide the flux to isoprene pathway and variety of developmental and environmental signals like light, wound, hormone, sterols, herbicides and infections are responsible for HMGRs regulation.

3.3. The two kinases of the MVA pathway: mevalonate kinase (MK) and mevalonate 5-diphosphate kinase (PMK)

Mevalonate biosynthesized the mevalonate 5-diphosphate by phosphorylation of two-step individually catalysed through mevalonate kinase and mevalonate 5-diphosphate kinase in order. Both mevalonate kinase also, mevalonate 5-diphosphate kinase have a place with family of GHMP kinase ATP-binding protein containing, in their N-terminal region, a preserved Gly/Ser-rich region that is presumably engaged with the ATP binding [27]. Motif named GHMO motif with the example as given: [LIVM]-[PK]-x-[GSTA]-x (0,1)- G-[LM]-[GS]-S-S-[GSA] [GSTAC) have been conserved by MK and PMK. In *Arabidopsis thaliana*, the expression motifs of the MVK gene recommends that the task of MK is the formation of an overall pool of mevalonate 5-phosphate for synthesizing of various isoprenoids categories engaged with both essential and specific functions of plant cell [46, 66]. Till now, just two full-length cDNAs encoding PMK were isolated from *Arabidopsis thaliana* and *Hemiptera brasiliensis,* respectively, have been enlisted in Gen Bank without functional data subtleties.

3.3.1. Mevalonate 5-diphosphate decarboxylase (MDC)

Mevalonate 5-diphosphate decarboxylase (MDC) catalyzes the final and irreversible step of the mevalonate (MVA) pathway, converting mevalonate 5-diphosphate (MVPP) into isopentenyl diphosphate (IPP). This ATP-dependent reaction involves the decarboxylation and dehydration of MVPP, yielding IPP, CO_2, and inorganic phosphate. Structural studies reveal that MDC belongs to the GHMP kinase superfamily, characterized by a conserved ATP-binding domain and catalytic residues critical for substrate positioning [67]. In *Arabidopsis thaliana*, MDC exhibits unique functional versatility, operating as both homodimers and heterodimers, as evidenced by yeast two-hybrid assays and gene family analysis. This dimerization flexibility suggests potential regulatory mechanisms to modulate flux through the MVA pathway under varying metabolic demands. The enzyme's activity is tightly coupled with upstream MVA pathway enzymes, ensuring efficient channeling of carbon toward IPP production for cytosolic terpenoid biosynthesis. Mutational studies indicate that MDC deficiency disrupts sterol and sesquiterpene production, underscoring its non-redundant role in isoprenoid precursor supply [67].

3.3.2. IPP isomerase (IPI)

Isopentenyl diphosphate isomerase (IPI) mediates the reversible interconversion of IPP and dimethylallyl diphosphate (DMAPP), the universal C_5 building blocks for all terpenoids. This isomerization is essential for maintaining the equilibrium between these precursors, as DMAPP serves as the initiating allylic substrate for prenyltransferases in terpenoid elongation. Unlike other MVA pathway enzymes confined to the cytosol, IPI localizes to multiple subcellular compartments, including plastids, mitochondria, and peroxisomes, reflecting its central role in cross-compartmental isoprenoid metabolism [68]. Plant genomes encode multiple IPI isoforms, with *Arabidopsis* possessing two functional genes (*IPI1* and *IPI2*) that exhibit distinct expression patterns and subcellular targeting. The enzyme's catalytic mechanism involves acid-base chemistry, with a conserved glutamate residue protonating the IPP double bond to form a carbocation intermediate, which subsequently deprotonates to yield DMAPP. Structural analyses reveal a conserved TIM-barrel fold and divalent metal ion dependency for activity. Notably, IPI's broad distribution enables it to support both the MVA and MEP pathways, facilitating IPP/DMAPP exchange between organelles—a

critical adaptation for balancing terpenoid production in response to developmental or environmental cues [68].

3.3.3. Isoprenoid biosynthesis in plastid through DXP pathway

In the previous few decades, mevalonate-dependent pathway was thought of as the universal pathway of biosynthesis of isoprenoid (Figure 12). The pathway that is not dependent on mevalonate is the other distinct isopropanoid biosynthetic pathway was discovered recently in eubacteria [69] and plants [70]. The precursor for synthesizing terpenoids on the newly-discovered biosynthetic pathway are pyruvate and glyceraldehyde 3-phosphate (G3P). In plants, the plastid pathway is restricted. 1-deoxy-D-xylulose 5-phosphate synthase (DXPS, EC 4.1.3.37) catalyses the condensation of pyruvate and glyceraldehyde to 1-deoxy-D-xylulose 5-phosphate (DXP), which is the first step in plastid terpenoid biosynthesis [71]. In this vein, the DXP pathway is also known as the mevalonate-independent terpenoid biosynthetic pathway. DXP is thus rearranged and reduced to 2-C-methyl-D-erythritoI4-phosphate (MEP), with 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR, EC 1.1.1.267) catalysing the reaction [72]. MEP cytidyltransferase (MCT, EC 2.7.7.60) then combines MEP with CDP to form 4-(cytidine 5-diphospho)- 2-C-methylerythritol (CDP-ME). The phosphorylation of CDP-ME to form CDP-MEP was catalysed by 4-(Cytidine 5-diphospho)-2-C-methylerythritol kinase (CMK, EC 2.7.1.148) [73]. 2-C-methylerythritol 2,4-cyclodiphosphate synthase (MECPS, EC 4.6.1.12) converts CDP-MEP to 2-C-methylerythritol 2,4-cyclodiphosphate (ME-cPP) [74]. The formation of hydroxymethylbutenyl 4-diphosphate (HMBPP) from ME-cPP catalysed by hydroxymethylbutenyl 4-diphosphate synthase (HDS) [75], and the conversion of HMBPP into a 5:1 combination of IPP and DMAPP by IPP and DMAPP synthase (IDS) [76].

3.4. Genes and Enzymes Involved in the DXP Pathway

The discovery of the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, also known as the non-mevalonate or deoxyxylulose phosphate (DXP) pathway, marked a significant breakthrough in plant isoprenoid research. First identified in 1994 during studies on ginkgolide biosynthesis in *Ginkgo biloba* [70], this pathway provided an alternative route for the synthesis of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), the universal precursors of terpenoids. Unlike the mevalonate (MVA) pathway, which operates in the cytosol, the DXP pathway is localized to plastids and is responsible for producing precursors for monoterpenes (C_{10}), diterpenes (C_{20}), carotenoids (C_{40}), and the prenyl side chains of chlorophylls and plastoquinones.

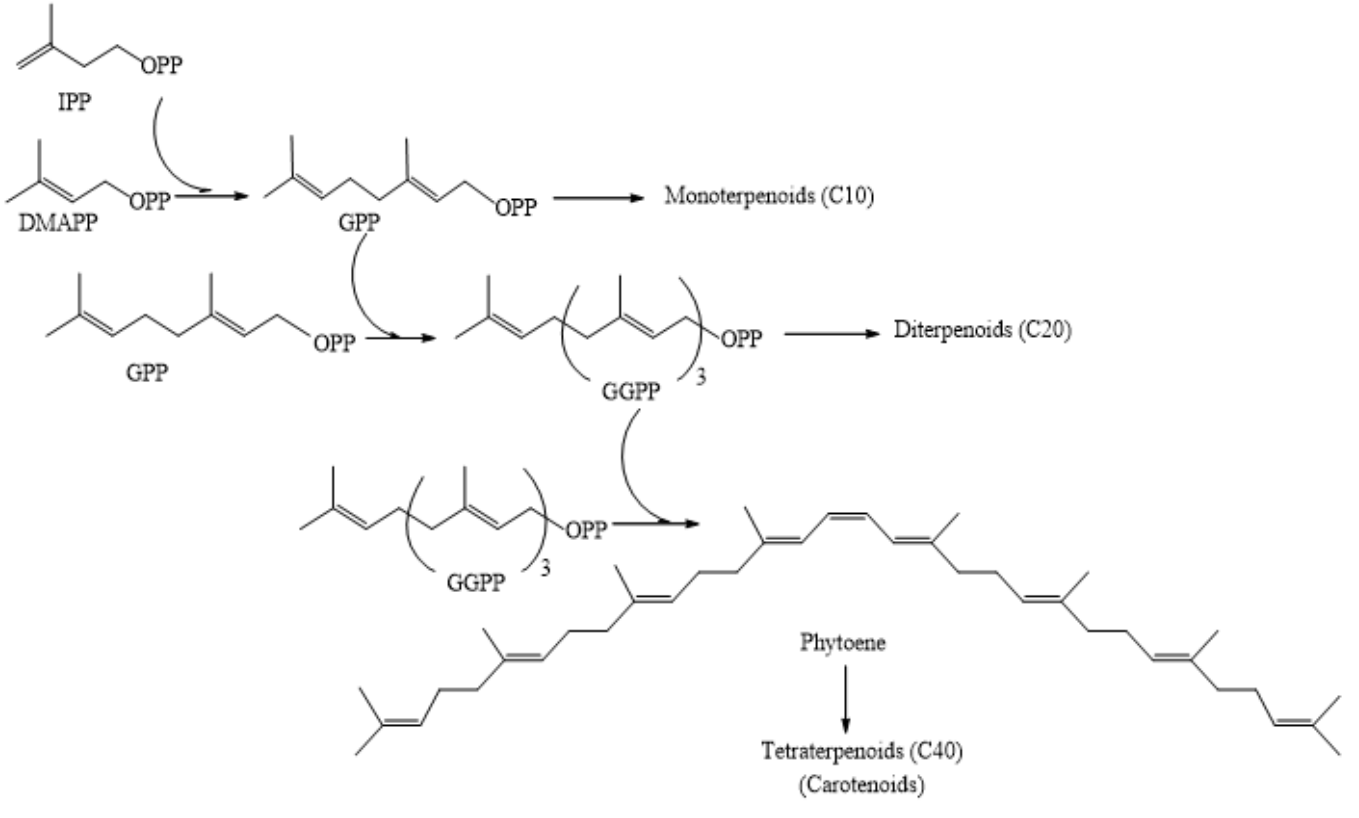


Fig. 12. Isoprenoid biosynthesis in plastid through DXP pathway.

The rapid advancement of bioinformatics and comparative genomics has since enabled the identification and characterization of all seven enzymes involved in the DXP pathway across diverse organisms, including the model plant *Arabidopsis thaliana*. These enzymes catalyze a series of sequential reactions starting with the condensation of pyruvate and D-glyceraldehyde 3-phosphate to form 1-deoxy-D-xylulose 5-phosphate (DXP), a reaction mediated by DXP synthase (DXS) [71]. DXP is then converted to MEP by DXP reductoisomerase (DXR), a step that is highly sensitive to inhibition by the antibiotic fosmidomycin, making DXR a key regulatory and targetable enzyme in this pathway [72]. Subsequent steps involve the conversion of MEP to 4-diphosphocytidyl-2-C-methyl-D-erythritol (CDP-ME) by MEP cytidylyltransferase (MCT), followed by phosphorylation to 4-diphosphocytidyl-2-C-methyl-D-erythritol 2-phosphate (CDP-MEP) by CDP-ME kinase (CMK) [73].

The pathway continues with the transformation of CDP-MEP into 2-C-methyl-D-erythritol 2,4-cyclodiphosphate (MEcPP) by MEcPP synthase (MDS), followed by its reduction to 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate (HMBPP) by HMBPP synthase (HDS) [75]. Finally, HMBPP is converted into a 5:1 mixture of IPP and DMAPP by HMBPP reductase (HDR or IspH), the last enzyme in the pathway [76].

The genes encoding these enzymes are nuclear in origin but are targeted to plastids via transit peptides, highlighting the complex coordination between cellular compartments in terpenoid biosynthesis. In *Arabidopsis*, multiple isoforms of some enzymes (e.g., DXS) exist, allowing for tissue-specific and developmental regulation of the pathway. The DXP pathway is particularly crucial in plants, as its disruption leads to severe phenotypes, including albinism and growth retardation, due to the lack of essential plastid-derived isoprenoids such as carotenoids and gibberellins. Recent studies have leveraged this pathway for metabolic engineering, aiming to enhance the production of high-value terpenoids in both plants and microbial systems. For example, overexpression of DXS and DXR in *Escherichia coli* and yeast has enabled the heterologous production of taxadiene (a precursor of the anticancer drug paclitaxel) and artemisinic acid (a precursor of the antimalarial artemisinin) [9]. Additionally, the DXP pathway's absence in mammals makes its enzymes potential targets for the development of herbicides and antimicrobial agents, as demonstrated by the antimalarial activity of fosmidomycin, which specifically inhibits DXR in *Plasmodium falciparum* [72].

3.4.1. 1-Deoxy-D-xylulose 5-phosphate synthase (DXPS)

DXPS has already been found in microorganisms and plants which is responsible for the catalysation of the condensation of acyloin between pyruvate's carbon atoms 2 and 3with the cofactor thiamine pyrophosphate, furthermore, glyceraldehyde 3-phosphate to yield 1-Deoxy-D-xylulose 5-phosphate [76]. The initial enzyme and a rate-restricting enzyme of the DXP pathway is DXPS in plants also, a potential metabolic engineering target. More than one gene might be responsible for encoding DXPS in plants. According to the database search, there are three genes encoding DXPS in *A. thaliana*, while the rest enzymes in MEP pathway in Arabidopsis seem like single gene is responsible for their encoding. During fruit development in tomato, DXPS genes shows regulation of mRNA accumulation in different organs and at different stages of development and a strong relationship with synthesis of carotenoid [77]. In pepper, during the transition of chloroplast to chromoplast, there is an over-expression of DXPS gene, and the reason probably is to furnish the IPP required for increased carotenoid biosynthesis. When compared to dark-grown transformed root cultures, there is substantial increase in DXPS transcript levels in the transformed root cultures of *Artimisia annua* cultivated in constant light [78]. These studies firmly show that the universal precursor for isoprene's that are important for plant development and growth is synthesized by the experimental regulation of DXPS genes.

3.4.2. 1-Deoxy-D-xylulose 5-phosphate reductor-isomerase (DXR)

2-C-methyl-D-erythritol 4-phosphate (MEP) formation through the translocation and subsequent reduction of DXP catalysed by DXR. The action of DXR specifically blocked by the herbicide fosmidomycin, so DXR is another focused protein for growing new pesticides [79]. In plants, the best likeness with the homologues from Synechocystis shared by the nuclear-encoded DXRs, which may propose DXR genes in plants were obtained from Synechocystisin the procedure of the endosymbiotic origin of plastid by gene transfer to the nucleus [79]. Not at all like the microbial reductoisomerase, a precursor harbouring the N-terminal plastidial transit peptide is encoded by DXR orthologs in plants which direct the enzyme to plastid where the DXP pathway works in plants. In *A. thaliana*, single gene is responsible for encoding of DXR, is additionally a committed protein of the mevalonate-independent pathway demonstrated by the transgenic method and metabolites analysis [80]. DXP pathway gives isoprene blocks to building the monoterpenoid indole alkaloids in antitumor-alkaloid-producing *Catharanthus roseus*; the DXR gene expression obtained from *Catharanthus roseus* was up-regulated in cells in corresponding with the monoterpene indole alkaloids formation [81]. As a significant rate-restricting enzyme on the MEP pathway, an ideal target for engineering the biosynthesis of isoprene is DXR, growing new herbicides and drugs. There are five enzymatic steps in the DXP pathway below of which knowledge of molecular genetics is restricted in any case, totally worth finding.

3.4.3. 2-C-Methyl-D-erythritol 4-Phosphate Cytidylyltransferase (MCT)

The enzyme 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase (MCT) catalyzes the third step of the DXP pathway, converting MEP and CTP into 4-diphosphocytidyl-2-C-methyl-D-erythritol (CDP-ME) and inorganic pyrophosphate. This reaction represents a critical branch point in plastidial isoprenoid biosynthesis, committing carbon flux toward the production of essential terpenoids such as carotenoids and monoterpenes. In *Arabidopsis thaliana*, the *AtMCT* gene encodes a 302-amino acid protein containing a predicted N-terminal plastid transit peptide, consistent with the plastid localization of the DXP pathway [73]. The catalytic domain of plant MCT shares approximately 30% sequence identity with its *Escherichia coli* counterpart, yet exhibits distinct kinetic properties optimized for plant metabolic requirements. Structural studies reveal that MCT adopts a conserved cytidylyltransferase fold, with a flexible loop region that undergoes conformational changes upon substrate binding. The enzyme requires divalent metal ions (Mg^{2+} or Mn^{2+}) for activity, which stabilize the negatively charged transition state during the nucleotidyl transfer reaction. In planta, MCT expression correlates with active terpenoid biosynthesis in photosynthetic tissues, and its activity is feedback-regulated by downstream pathway intermediates. Recent crystallographic analyses of *Aquifex aeolicus* MCT have identified key residues involved in CTP binding and catalysis, providing a framework for engineering plant MCT to modulate flux through the DXP pathway [82].

3.4.4.. 4-(Cytidine 5'-Diphospho)-2-C-Methyl-D-Erythritol Kinase (CMK)

As a member of the GHMP kinase family, 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase (CMK) phosphorylates the 2-hydroxyl group of CDP-ME to form 2-phospho-4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol (CDP-MEP). This ATP-dependent reaction represents the fourth committed step in the DXP pathway and exhibits absolute specificity for its CDP-ME substrate. The *Arabidopsis* genome contains a single *CMK* gene encoding a 42-kDa protein with a canonical GHMP kinase fold, featuring a conserved ATP-binding pocket and catalytic base [73]. Heterologous expression studies demonstrate that plant CMK, like its microbial orthologs, requires Mg^{2+} as a cofactor for optimal activity, with reported *K_m* values for CDP-ME and ATP in the low micromolar range. Interestingly, CMK shows dual localization in both plastids and peroxisomes, suggesting potential roles in peroxisomal isoprenoid metabolism. Kinetic analyses reveal that CMK activity is regulated by product inhibition, with CDP-MEP competitively inhibiting ATP binding. Structural modeling based on bacterial CMK homologs predicts that plant CMK undergoes substrate-induced conformational changes that position the γ-phosphate of ATP for direct transfer to the 2-hydroxyl of CDP-ME. While CMK is generally considered non-rate-limiting in the DXP pathway, its expression is upregulated during periods of active plastidial isoprenoid biosynthesis, such as chloroplast development and fruit ripening [74].

3.4.5. 2-C-Methyl-D-Erythritol 2,4-Cyclodiphosphate Synthase (MDS)

The penultimate enzyme in the DXP pathway, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MDS), catalyzes the conversion of CDP-MEP to 2-C-methyl-D-erythritol 2,4-cyclodiphosphate (MEcPP) with concomitant release of CMP. This unusual cyclization reaction involves the nucleophilic attack of the 2-phosphate on the β-phosphate of CDP, forming a characteristic cyclic diphosphate intermediate. While MDS genes have been identified in numerous bacteria and archaea, functional characterization in plants remains limited to a few species, including *Catharanthus roseus*, where MDS expression correlates with monoterpenoid indole alkaloid accumulation [81]. The enzyme belongs to the YgbB protein family and features a conserved "DxD" motif that coordinates a catalytically essential Mg^{2+} ion. Structural studies of bacterial MDS reveal a distorted (β/α)₈ TIM barrel fold with a deep active site pocket that positions the CDP-MEP substrate for intramolecular cyclization [82]. In plants, MDS is predicted to be nuclear-encoded with plastid targeting, though its low abundance and instability have hampered detailed biochemical characterization. Emerging evidence suggests that MEcPP may function as a retrograde signal linking plastid isoprenoid metabolism to nuclear gene expression, implicating MDS in broader regulatory networks beyond its metabolic role. The development of specific MDS inhibitors could provide valuable tools for probing the regulatory functions of MEcPP in plant stress responses and development [74].

3.4.6. 4-Hydroxy-3-methylbut-2-enyl 4-diphosphate synthase (HDS)

The final two stages on the DXP pathway, one is catalysed by HDS that is the production of hydroxymethylbutenyl 4-diphosphate from ME-CPP, and another one is direct HMBPP conversion into a 5:1 combination of isopentenyl diphosphate and dimethylallyl diphosphate catalysed by IPP/DMAPP synthase (IDS). HDS belongs to family of GCPE protein while IDS belongs to family of LytB protein separately. These characterization of these two genes have already been done in bacteria [76, 83]. Some full-length cDNAs of HDS and IDS can be found in GenBank according to the genome sequence of *Arabidopsis* [84]. A protein from A. thaliana was identified by Querol et al. which demonstrates the homology to the product of gcpE [84]. A polypeptide from plants, two structural domains namely extension of N-terminal and the central domain of 30kDa are not present in *Escherichia coli*, while a polypeptide from plant GCPE, contains these two. They exhibit that the protein is targeted by N-terminal region to chloroplast in vivo in

Arabidopsis, which is compatible with its role in biosynthesis of plastid isoprene. It has been presumed that HDS is not a rate-restricting enzyme yet a significant house-keeping gene in plants in the MEP pathway (Gene expression of hydroxymethylbutenyl diphosphate synthase (GCPE) during carotenoid accumulation in ripening tomato fruit: Molecular analysis and bioinformatics [85].

3.5. The last enzyme on the DXP pathways: IPP/DMAPP synthase (IDS)

Direct conversion of 4-hydroxy-3-methylbut-2-enyl-4-diphosphate into a combination of IPP and DMAPP with the ratio of 5:1 is catalysed by IDS [86]. Just a not many full-length cDNAs which encode IDS were cloned from plants. The function of IDS was found as the expression of lytB gene from the flowering plant *Adonis aestivalis* in *E. coli* [76]. In tobacco, when the gene which encodes HDS was post transcriptionally silent, the plants those are IDS silenced had pale leaves contained not more than 4% pigments of the chlorophyll and carotenoid of control leaves; and pale leaves from the plants that are IDS-silenced shown a disordered palisade mesophyll, decreased cuticle, less plastids, and disturbed thylakoid membranes. These discoveries showed the involvement of IDS in the DOXP pathway in plants, and supported the view that biosynthesis of isoprene of plastid is physically and metabolically isolated from the MVA pathway (Functional analysis of the last moves of the 2-C-Methyl-D-erythritol-4-phosphate (MEP) pathway to isoprene's in plants utilizing gene silencing which is virus-induced [87].

3.6. Localization of pathways of essential oil biosynthesis

While trying to see how essential oils are released, it is critical to discover what is the location of synthesis in the cell. In microscopic investigations, production of essential oil had regularly ascribed to plastids on account of the perception of droplets of oil in the stroma [3, 88]. This is also supported by immunolocalization of geranyl diphosphate synthase, limonene cyclase, isopentenyl pyrophosphate isomerase, and enzymes of the DXP pathway, all of which are involved in the start of monoterpenoid biosynthesis [88-94].

In the literature, there are clashing reports proposing the presence of two types of geranyl diphosphate synthase, an enzyme, in cells of plant relying upon the methionine used to start translation. The one which is larger can be focused to plastids and the shortened one can be targeted to cytosolic [94]. Farnesyl diphosphate synthase has already been limited in the peroxisomes, mitochondria, cytosol, and chloroplasts in various plants [95, 96]. The following stage of terpenoid biosynthesis includes terpene synthases or cyclases. All the recognized monoterpenoid synthases and cyclases appear to have plastid-focusing on sequences [97]. The last phase involved in the synthesis of monoterpenoid are studied to be situated in the ER, as shown by the confinement of limonene hydroxylases [98, 99]. Although, reports reveal that the absolute final steps of pathways of some monoterpenoid biosynthesis could be cytosolic [100].

The initial steps of biosynthesis of sesquiterpenoids appear to be cytosolic, contingent upon the MVA pathway. Notwithstanding, the DXP pathway gives IPP precursor to both plastidial monoterpenoid and cytosolic sesquiterpenoid biosynthesis in the snapdragon (*Antirrhinum majus*) petals epidermis [101-109, 96]. It was illustrated in Matricariarecutita (Asteraceae) that the absolute initial steps, before the isopentenyl diphosphate biosynthesis, happen halfway in plastids, by the DXP pathway [66]. The investigation of the *Nicotiana sylvestris* (tobacco) and *Solanum habrochaites* (wild tomato) plants indicating that mix of sesquiterpenoids have given bySBS and zFPS in glandular trichomes. A putative plastid-focusing on sequence is available in every one of these proteins that self-settled transport of an intertwined GFP to the chloroplasts, implying that these sesquiterpenoids biosynthesis utilizes IPP and DMAPP from the plastidic MEP pathway [102]. The IPP, that is synthesized both by the plastid (MEP) and the cytosolic (MVA) pathways, goes via the envelope of plastid to synthesized sesquiterpenoids, contingent upon the species. The final steps of biosynthesis of sesquiterpenoids appear to happen in the ER. Sesquiterpenoids should then have the equivalent subcellular section with some monoterpenoids [103, 104].

4. ENVIRONMENTAL EFFECTS ON ESSENTIAL OIL BIOSYNTHESIS

The biosynthesis and secretion of essential oils in aromatic plants are profoundly influenced by environmental conditions, creating dynamic interactions between plant physiology and ecological factors [110]. These relationships have significant implications for both natural plant communities and commercial essential oil production, as environmental variables can alter not only the quantity but also the qualitative composition of volatile organic compounds [111]. The complex interplay between abiotic factors and essential oil metabolism represents a sophisticated adaptation mechanism that plants have evolved to optimize their ecological fitness and survival strategies across diverse habitats [112-115].

Temperature stands as one of the most critical environmental regulators of essential oil production, exerting multifaceted effects on both biosynthetic pathways and emission rates. Elevated temperatures generally enhance the enzymatic activity of terpene synthases and other biosynthetic enzymes, leading to increased production of volatile compounds [116]. This thermal activation occurs because many enzymes in the terpenoid biosynthesis pathways, including limonene synthase and pinene synthase, exhibit higher catalytic efficiency at moderately elevated temperatures that might occur during midday or summer seasons. However, extreme heat beyond optimal ranges can denature these enzymes and disrupt cellular structures,

demonstrating the delicate balance plants must maintain [117-119]. The temperature dependence of essential oil production has been well-documented in species like lavender (Lavandula angustifolia), where warmer growth conditions increase oil yield but may alter the relative proportions of linalool and linalyl acetate, significantly affecting the oil's commercial quality [106].

Light intensity and quality represent another crucial set of environmental factors influencing essential oil biosynthesis. Photosynthetically active radiation (PAR) provides not only the energy but also the carbon skeletons required for terpenoid production through the methylerythritol phosphate (MEP) pathway in chloroplasts [113]. Ultraviolet-B radiation, while potentially damaging at high intensities, has been shown to stimulate the production of protective secondary metabolites including monoterpenes and phenylpropanoids in many aromatic plants [19]. This photoprotective response explains why Mediterranean species like rosemary and thyme often show higher essential oil concentrations when grown under full sunlight compared to shaded conditions. The spectral quality of light also plays a regulatory role, with blue light receptors (cryptochromes and phototropins) and red/far-red receptors (phytochromes) modulating the expression of genes involved in terpenoid biosynthesis [120-123]. Recent studies with Ocimum basilicum have demonstrated that specific light wavelengths can be used to selectively enhance the production of desirable compounds like eugenol or methyl chavicol, opening possibilities for precision agriculture in essential oil production [113].

Water availability creates perhaps the most dramatic environmental influence on essential oil composition, with drought stress triggering significant metabolic reprogramming in aromatic plants. Moderate water deficit often increases the concentration of essential oils in many species, as observed in Mentha piperita where controlled drought stress boosted menthol content by up to 30% [111]. This response appears to be part of a general stress adaptation strategy where plants allocate more resources to defensive secondary metabolites when under abiotic stress. The physiological mechanisms involve drought-induced abscisic acid signaling, which upregulates key terpenoid biosynthetic genes while simultaneously causing stomatal closure that reduces volatile emission [112]. However, severe drought can have the opposite effect, as seen in Pelargonium graveolens where extreme water stress actually decreased essential oil yield despite increasing the proportion of citronellol [112]. These nonlinear responses highlight the complex interplay between environmental factors and plant metabolism.

Soil nutrition and mineral availability constitute another dimension of environmental influence on essential oil production. Nitrogen availability generally shows an inverse relationship with secondary metabolite production, as demonstrated in studies with Artemisia annua where high nitrogen fertilization increased biomass but decreased artemisinin content [109]. In contrast, phosphorus and potassium often show positive correlations with essential oil yield, as these minerals participate directly in energy metabolism and enzyme activation required for terpenoid biosynthesis [110]. The role of micronutrients is particularly intriguing, with zinc and manganese serving as cofactors for critical enzymes like terpene synthases and cytochrome P450s involved in terpenoid modification [110]. Sophisticated fertilization strategies that account for these interactions can optimize both biomass production and essential oil quality in commercial cultivation.

Atmospheric composition, including ozone, carbon dioxide, and volatile organic compound concentrations, creates yet another layer of environmental influence on essential oil biosynthesis. Elevated CO_2 levels have been shown to enhance photosynthetic carbon fixation and consequently increase the carbon supply for terpenoid production in species like Melaleuca alternifolia [115]. However, this CO_2 fertilization effect may be offset by concomitant increases in tropospheric ozone, which induces oxidative stress that can damage the glandular trichomes responsible for essential oil storage in many plants [115]. The complex interactions between these atmospheric factors are particularly relevant in the context of climate change, as shifting environmental conditions may alter the traditional geographic ranges suitable for high-quality essential oil production.

This comprehensive analysis of environmental effects on essential oil biosynthesis integrates recent advances in plant stress physiology with practical applications in aromatic crop cultivation. Unlike previous reviews that treated environmental factors in isolation, the current work elucidates the synergistic and antagonistic interactions between multiple stressors and their combined effects on essential oil quality and yield. The paper particularly advances understanding of how climate change variables may alter secondary metabolite profiles in medicinal and aromatic plants, providing a forward-looking perspective essential for the sustainable management of these valuable genetic resources. By connecting molecular mechanisms of stress response with ecological and agronomic outcomes, this work offers new insights for both fundamental plant science and applied horticultural practices.

The ecological implications of environment-dependent essential oil production are profound, shaping plant-insect interactions, allelopathic effects, and competitive relationships in natural ecosystems. Many monoterpenes and sesquiterpenes that increase under stress conditions serve dual roles as defensive compounds against herbivores and pathogens while also mediating complex ecological communications [11]. The temperature-dependent emission of β-caryophyllene, for instance, not only protects plants from heat stress but also attracts specific parasitoid wasps that prey on herbivorous insects [16]. This multifunctionality explains why environmental modulation of essential oil composition has been conserved across diverse plant lineages, representing an evolutionary strategy to maximize fitness in changing environments.

From an applied perspective, understanding these environmental effects enables the development of precision

cultivation techniques that can optimize essential oil yield and quality. Strategic stress application, sometimes called "eustress" management, has emerged as a promising approach to enhance the production of valuable compounds without compromising plant health [111]. The timing of harvest relative to diurnal and seasonal environmental fluctuations can also significantly impact oil characteristics, as demonstrated in Citrus species where morning harvest yields oils with higher aldehyde content compared to evening collections [107]. Such knowledge is increasingly valuable as the global essential oil market grows and demands both higher productivity and consistent quality from cultivated aromatic plants.

5. FUTURE DIRECTIONS IN ESSENTIAL OIL BIOSYNTHESIS RESEARCH

The study of essential oil biosynthesis stands at an exciting crossroads, where emerging technologies and interdisciplinary approaches promise to revolutionize both our fundamental understanding and practical applications of these valuable plant metabolites. As climate change alters growing conditions worldwide and consumer demand for natural products continues to rise, several critical research directions warrant focused investigation to advance the field sustainably.

One of the most pressing needs lies in elucidating the precise molecular mechanisms underlying environmental regulation of essential oil biosynthesis. While we understand that factors like temperature, light, and drought influence oil composition, the specific signaling cascades and epigenetic modifications involved remain poorly characterized. Future studies should employ multi-omics approaches—combining transcriptomics, proteomics, and metabolomics—to map the complete regulatory networks connecting environmental sensors to terpenoid pathway genes. Particularly promising is the investigation of stress memory mechanisms in aromatic plants, where prior exposure to moderate stress may "prime" plants for enhanced essential oil production under subsequent stress conditions. Understanding these priming phenomena at the molecular level could lead to novel cultivation strategies that improve both the quality and yield of essential oils without compromising plant health.

The development of climate-resilient aromatic plant varieties represents another crucial research frontier. As traditional growing regions experience shifting temperature and precipitation patterns, breeding programs must identify and select for genetic traits that maintain optimal essential oil profiles under abiotic stress. Modern genomic tools like genome-wide association studies (GWAS) and genomic selection can accelerate this process by identifying molecular markers linked to stress-tolerant oil production. For instance, wild relatives of commercial mint species growing in marginal environments may harbor valuable genetic variants for drought-adaptive terpenoid biosynthesis that could be introgressed into cultivated varieties. Coupled with high-throughput phenotyping platforms that monitor volatile emissions in real-time, these approaches promise to develop new cultivars tailored to changing climatic conditions.

Advances in synthetic biology and metabolic engineering open unprecedented opportunities for sustainable essential oil production. The successful reconstitution of complete terpenoid pathways in microbial hosts like yeast and bacteria demonstrates the feasibility of alternative production systems that don't require agricultural land. Future work should focus on optimizing these platforms for industrial-scale production, particularly for high-value compounds currently obtained from endangered or slow-growing plants (e.g., sandalwood oil from Santalum album). Key challenges include improving precursor flux through engineered pathways, minimizing toxic effects of terpenoid accumulation on host organisms, and developing cost-effective extraction methods. The emerging field of cell-free biosynthesis systems may offer particular advantages for producing unstable or cytotoxic terpenoids that are difficult to accumulate in living cells.

The integration of artificial intelligence and machine learning into essential oil research presents another transformative opportunity. Predictive models trained on large datasets of environmental conditions, genomic information, and essential oil profiles could identify optimal growing conditions for specific oil compositions or predict how climate change might alter traditional production regions. AI-assisted drug discovery platforms screening essential oil components for pharmacological activity may uncover novel therapeutic applications, particularly in antimicrobial and anti-inflammatory domains where plant volatiles show significant promise. Additionally, machine learning algorithms analyzing consumer preference data could help guide breeding programs toward developing new aromatic plant varieties with commercially desirable fragrance profiles.

Exploring the ecological dimensions of essential oil production will become increasingly important for sustainable cultivation practices. Future research should investigate how changes in oil composition due to environmental stressors affect plant interactions with pollinators, herbivores, and soil microbiota—relationships that are critical for ecosystem health and agricultural productivity. The potential role of essential oils in mediating plant-plant communication (allelopathy) under changing climatic conditions also warrants deeper investigation, as this could influence both natural ecosystem dynamics and intercropping strategies in aromatic agriculture.

Technological innovations in extraction and analysis methodologies will play a pivotal role in advancing the field. The development of non-destructive, real-time monitoring techniques for volatile organic compound (VOC) emissions—such as advanced proton-transfer-reaction mass spectrometry (PTR-MS) coupled with drone-based sampling—could provide unprecedented insights into the spatiotemporal dynamics of essential oil production in both field and controlled environments. Miniaturized sensor arrays deployed in smart agriculture systems may enable

precision management of essential oil crops, allowing growers to fine-tune irrigation, fertilization, and harvest timing based on continuous VOC monitoring.

The pharmaceutical applications of essential oil components represent a particularly promising direction for future research. While traditional use of essential oils in medicine dates back millennia, modern pharmacological approaches are just beginning to unravel their therapeutic mechanisms at the molecular level. Future studies should employ structural biology and computational chemistry tools to investigate how specific terpenoids and phenylpropanoids interact with human molecular targets, potentially leading to the development of novel drugs inspired by these natural compounds. The synergistic effects of essential oil components—where whole oils often show greater bioactivity than isolated compounds—present a particularly intriguing area for investigation using network pharmacology approaches. The interdisciplinary collaborations will be essential to address the grand challenges in essential oil research. Partnerships between plant scientists, chemists, engineers, data scientists, and traditional knowledge holders can foster innovative solutions that balance ecological sustainability, economic viability, and cultural preservation. As climate change accelerates, such collaborative efforts will be crucial for ensuring the long-term availability of these valuable natural products while maintaining the biodiversity of aromatic plant species and their associated ecosystems. By pursuing these diverse but interconnected research directions, the scientific community can unlock the full potential of plant essential oils for human well-being and environmental health in the coming decades.

6. CONCLUSION

Essential oils represent a vital class of plant-derived metabolites with extensive commercial and therapeutic applications. Their biosynthesis occurs through two primary pathways—the mevalonate (MVA) and methylerythritol phosphate (MEP) pathways—which generate the fundamental building blocks, IPP and DMAPP. These precursors undergo enzymatic modifications by terpene synthases (TPS) to form structurally diverse terpenoids, including monoterpenes, sesquiterpenes, and diterpenes, which define the aromatic and bioactive properties of essential oils. The increasing global demand for essential oils, projected to reach USD 18.25 billion by 2028, underscores the need for enhanced production strategies through metabolic and genetic engineering. Despite significant advancements in understanding terpenoid biosynthesis, several challenges remain. First, the regulatory mechanisms controlling flux distribution between the MVA and MEP pathways are not fully elucidated, limiting the precision of metabolic engineering approaches. Second, environmental factors such as light, temperature, and nutrient availability significantly influence EO composition, yet their molecular interplay with biosynthetic pathways requires further exploration. Third, while key enzymes like HMGR, DXR, and TPS have been characterized, their isoforms and post-translational modifications in different plant species need deeper investigation to enable targeted manipulation. Future research should focus on synthetic biology tools, such as CRISPR-Cas9 and heterologous expression systems, to optimize terpenoid production in both native and non-host organisms. Additionally, integrating omics technologies (transcriptomics, proteomics, and metabolomics) will provide a holistic understanding of EO biosynthesis under varying physiological conditions. Enhancing the yield of commercially valuable terpenoids, such as menthol, linalool, and β-caryophyllene, through pathway engineering and strain improvement holds immense potential for industrial applications. Unraveling the complexities of essential oil biosynthesis will pave the way for sustainable production methods, meeting the growing market demand while preserving ecological balance. Collaborative efforts between biotechnologists, chemists, and agronomists are essential to harness the full potential of aromatic plants in pharmaceuticals, agriculture, and beyond.

DECLARATIONS

Ethical Approval

We affirm that this manuscript is an original work, has not been previously published, and is not currently under consideration for publication in any other journal or conference proceedings. All authors have reviewed and approved the manuscript, and the order of authorship has been mutually agreed upon.

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The authors declare that they have no financial or personal interests that could have influenced the research and findings presented in this paper. The authors alone are responsible for the content and writing of this article.

Authors' contributions

All authors contributed equally in the preparation of this

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