

Review

Progress in Research on Terpenoid Biosynthesis and Terpene Synthases of Lauraceae Species

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Abstract: Lauraceae, an important family of Angiospermae, comprises over 2500 species widely distributed in tropical and subtropical evergreen broad-leaved forests. This family is renowned for its rich resource of terpenoids, particularly monoterpenes, sesquiterpenes, and diterpenes. These compounds not only impart specific scents to Lauraceae species but also play crucial roles in plant growth, development, and environmental adaptation. These compounds also possess extensive bioactivities, such as antioxidant, antibacterial, anti-inflammatory, and neuroprotective effects, making them valuable in the fields of perfumery, cosmetics, food, and medicine, and thus holding significant economic value. Recent advancements in high-throughput technologies, especially genomics, transcriptomics, and metabolomics, have significantly advanced our knowledge of the chemical constituents and biosynthetic pathways of terpenoids in Lauraceae species. Such progress has also shed light on the diversity and functionality of the terpene synthases (*TPSs*) gene family, a key enzyme involved in terpenoid biosynthesis. This paper reviews the latest research findings on the biosynthetic pathways of terpenoids and their key enzyme-encoding gene families in Lauraceae plants. We also analyze the evolutionary patterns of *TPS* gene family members of four Lauraceae species at the whole-genome level and summarize their mechanisms of action in secondary metabolite synthesis. Furthermore, this paper highlights the current research challenges and proposes prospects, such as the complexity of gene families, the uncertainties in functional predictions, and unclear regulatory mechanisms. Our objective is to provide scientific foundations for the in-depth analysis of terpenoid biosynthesis mechanisms and the development and utilization of natural products in Lauraceae plants.

Keywords: Lauraceae; terpenoids; terpene synthases; gene family



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1. Introduction

Terpenoids are a class of natural products characterized by their structural richness and high diversity [1]. To date, more than 80,000 terpenoids have been identified from different plant species [2]. Some terpenoids, such as gibberellins, abscisic acid, and carotenoids, play vital roles in plant growth and development and are referred to as primary metabolic terpenoids [3]. Additionally, terpenoids are widely used in people's production activities and lives. For example, some volatile terpenoids serve as raw materials for perfumes, flavorings, and cosmetics [4], whereas others, such as pyrethrins and limonoids, are used as insecticides [3]. Some special terpenoids, such as the sesquiterpenes, β -caryophyllene and bisabolene, as well as the monoterpenes pinene and limonene, are considered precursor compounds for fuels [5].

Lauraceae is a large family from the order Laurales in Magnoliids, comprising approximately 2500–3000 species from 45 genera [6]. Most plants of the Lauraceae family are evergreen trees that are natively distributed in the tropical mountains and tropical rainforests of South Asia, Southeast Asia, Australia, Africa, and South America [7]. In China,

there are 25 genera and more than 440 species of Lauraceae plants, which are distributed mainly in mid- to low-altitude mountainous areas from Southwest to South China; these include genera such as *Cinnamomum*, *Lindera*, *Sinosassafras*, *Sinopora*, *Litsea*, and *Laurus* [8]. Among them, *Sinopora* and *Sinosassafras* are endemic to China, whereas *Laurus* and *Persea* are commercially cultivated genera [7]. Owing to the high content of terpenoids, many Lauraceae species have been developed as sources of spices, aromatic oils, and medicinal plants. For example, *Cinnamomum camphora* (L.) Presl and *Cinnamomum longepaniculatum* (Gamble) N. Chao ex H. W. Li are used as high-quality camphor oil extraction materials [9]; *Cinnamomum cassia* (L.) D. Don, *Cinnamomum verum* J. Presl, and *Cinnamomum tamala* (Buch.-Ham.) T. Nees & C. H. Eberm. *Lindera glauca* (Siebold & Zucc.) Blume are used as a natural spice [10]; and *C. cassia* and *Cinnamomum burmannii* (Nees et T. Nees) Blume are famous medicinal materials [11].

With the development of high-throughput sequencing technologies, the rapid accumulation of multi-omics data, represented by genomics, has greatly facilitated the study of the biosynthetic pathways of terpenoids and key enzyme-encoding gene family members in Lauraceae plants. This review aims to integrate and analyze recent research findings on terpenoid biosynthetic pathways in Lauraceae plants, discussing in depth the diversity of these compounds and their biological significance in terms of plant growth, stress response, and environmental interactions. By comparing the evolutionary conservation and diversity of terpene synthase gene family members across different Lauraceae species, this work provides a solid theoretical foundation for the precise analysis and genetic improvement of terpenoid biosynthetic mechanisms in Lauraceae plants.

2. Constituents and Biological Activities of Terpenoids in Lauraceae Species

Terpenoids are a class of compounds composed of several isoprene (C₅) structural units. On the basis of the number of isoprene units, terpenoids can be categorized into monoterpenes (C₁₀), sesquiterpenes (C₁₅), diterpenes (C₂₀), triterpenes (C₃₀), tetraterpenes (C₄₀), and polyterpenes (C > 40) [12]. Most terpenoids are synthesized as secondary metabolites within plants and play key roles in plant–environment interactions [13,14]. For example, poplar can produce volatile isoprenoids to cope with temperature stress by activating the biosynthesis pathways of isoprene units [15]. Additionally, terpenoids are involved in plant defense. In cotton, compounds such as gossypol and related sesquiterpene aldehydes have been proven effective against various pathogens, protecting plants from diseases [16]. Carotenoids, a class of tetraterpenes, are vital for photoprotection, shielding plants from damage caused by excess light and oxidative stress [17]. Another tetraterpene derivative, abscisic acid (ABA), plays a crucial role in plant adaptation to both biotic and abiotic stresses by regulating key processes like stomatal closure and defense gene expression [18,19]. Furthermore, volatile terpenoids act as important chemical mediators between plants and pollinators. Their volatile and lipophilic characteristics enable them to disperse through the air, acting as chemical signal molecules that attract or repel specific pollinating insects, thereby facilitating precise matching during the reproduction process [20,21].

Lauraceae plants stand out in nature due to their rich and diverse chemical composition, especially the abundance of terpenoids, which mainly include monoterpenes, sesquiterpenes, and diterpenes [22–25] (Figure 1). Monoterpenes and sesquiterpenes, being highly volatile organic molecules, not only impart distinct scents to Lauraceae plants [26,27] but also serve as chemical signaling molecules that play crucial roles in ecological niche selection, pollination mechanisms, and environmental interactions [28–32]. For example, monoterpenes such as limonene, sabinene, linalool, eucalyptol, borneol, α -terpineol, 4-terpineol, and α -terpinyl acetate found in Lauraceae plants have exhibited notable insecticidal or repellent effects against pests such as *Tribolium castaneum* Herbst and *Liposcelis bostrychophila* Badonnel [33]. The monoterpenes and sesquiterpenes found in cinnamon exhibit a wide range of pharmacological activities, including anti-inflammatory, antioxidant, anti-neuroinflammatory, neuroprotective, and insulin-sensitizing effects [34]. Some diterpenes also have immunomodulatory and cell proliferation effects [35]. While the es-

sential oils and other chemical constituents of Lauraceae plants have long been recognized, recent researches have increasingly focused on sesquiterpenes and diterpenes due to their unique chemical structures, extensive biological activities, and indicative roles in plant chemotaxonomy [7].

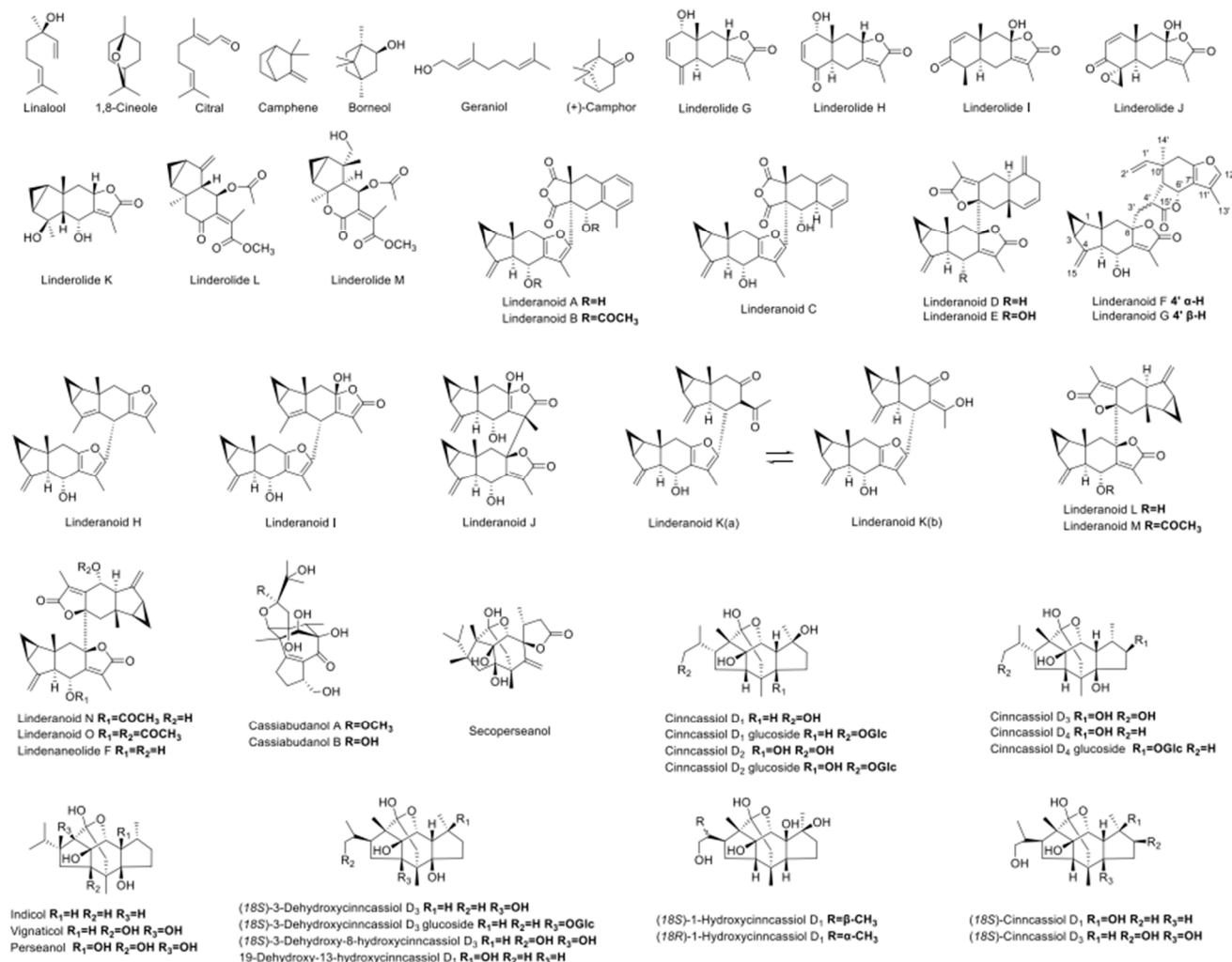


Figure 1. Chemical structures of the terpenoids isolated from Lauraceae.

Monoterpenes (molecular formula $C_{10}H_{16}$) are composed of two isoprene units (each containing five carbon atoms) linked in a head-to-tail arrangement to form either linear or cyclic structures. The monoterpenes, such as linalool, 1,8-cineole, citral, camphene, borneol, camphor, and geraniol, are often the source of the strong fragrances of Lauraceae plants. These compounds have also been proven to have good antibacterial, anti-inflammatory, antitumor, and antioxidant properties [35]. Linalool, known as the “king of fragrances”, is extensively used worldwide in perfumery industry due to its high usage frequency, broad applicability, and high value. Citral is one of the most important representative components of acyclic monoterpenes. In China, *Litsea cubeba* (Lour.) Pers. is one of the main natural sources of citral. Zhang et al. [36] also identified citral in *C. camphora*, *Camphora bodinieri* H. Lév., *Cinnamomum micranthum* (Hayata) Hayata, *Cinnamomum parthenoxylon* (Roxb.) Kosterm., and *Cinnamomum tenuipile* Kosterm. Camphor is another monoterpene compound with the chemical formula $C_{10}H_{16}O$ that exists in two enantiomeric forms. Synthetic camphor is derived mainly from α -pinene extracted from turpentine, whereas natural camphor ((+)-camphor) is obtained by distilling the wood of camphor trees [37].

Sesquiterpenes are a class of secondary metabolites composed of three isoprene units, with the molecular formula $C_{15}H_{24}$. Sesquiterpenes also exist in linear and cyclic forms. To

date, 362 sesquiterpene compounds, including cadinene, β -caryophyllene, germacrene, and α -copaene, have been obtained from 44 species across 12 genera of Lauraceae [38]. Among these compounds, megastigmane, eudesmane, germacrane, and lindenane-type sesquiterpenes constitute the majority [7]. Recently, researchers have discovered several dimers and polymers, further expanding the diversity of sesquiterpenes in Lauraceae plants. Liu et al. [39] isolated and identified 15 previously undescribed sesquiterpene dimers and one known lindenane-type sesquiterpene dimer from the roots of *Lindera aggregata* (Sims) Kosterm., including three sesquiterpene dimers containing lindenane and noreudesmane units, two sesquiterpene dimers containing lindenane and eudesmane units, two sesquiterpene dimers containing lindenane and elemanolide units, and eight lindenane sesquiterpene dimers. Liu et al. [40] also isolated seven sesquiterpene lactones, named linderolide G–M, and 12 known sesquiterpenes from the roots of *L. aggregata*. The most common structural features of sesquiterpenes in Lauraceae are various configurations of hydroxyl, carbonyl, methyl, glycosyl, and phenyl substituents, as well as double bonds or epoxide groups. These compounds exhibit remarkable chemical diversity and can be roughly categorized into acyclic, monocyclic, bicyclic, and tricyclic systems, or classified by their oxidation levels into sesquiterpenoid alcohols, ketones, aldehydes, and lactones [41–44] (Figure 1).

Diterpenes are naturally synthesized compounds composed of four isoprene units that form a basic structure with 20 carbon atoms and are typically defined as hydrocarbons with the molecular formula $C_{20}H_{32}$. In Lauraceae plants, diterpenes exhibit unique cage-like tricyclic or tetracyclic structures. The rigidity of these carbon skeletons, combined with high levels of oxidative modification (such as the formation of hydroxyl, carbonyl, lactone, and diketone groups), greatly enhances their chemical diversity and biological activity [7]. These diterpenes are classified into hemiacetal, acetal, lactone, and diketone types based on their degree of oxidation and functional groups [45–49]. Further research revealed that diterpenes can be subdivided into eight subtypes (Figure 2), with ryanodane-type diterpenes being one of the most distinctive because of their complex polyoxygenated 6/5/5/6/5 fused ring system. González-Coloma et al. [50] isolated ryanodane-type diterpenes from *Persea indica* Spreng and reported that these compounds have an antifeedant effect on insects. Notably, ryanodane-type diterpenes have so far been found only in *C. cassia*, *C. verum*, and *P. indica*, making them as characteristic compounds of Lauraceae plants and potential chemotaxonomic markers for these species [7].

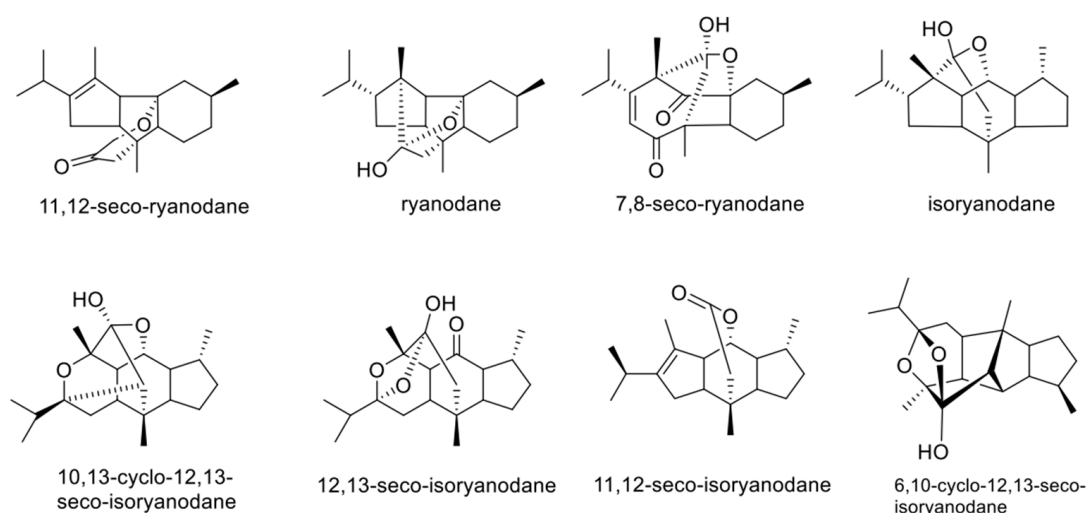


Figure 2. Eight recognized carbon skeletal types of diterpenoids.

The study of terpenoid constituents in Lauraceae plants has a long history, dating back to the 19th century with reports on the isolation and structural identification of camphor. To date, thousands of terpenoid compounds have been isolated and identified from Lauraceae plants. With advancements in modern separation and structural identification techniques,

an increasing number of novel terpenoid compounds are being discovered and reported. Concurrently, the rapid development of molecular biology and bioinformatics has inaugurated a transformative era in terpenoids research within Lauraceae plants. Utilizing high-throughput sequencing technologies, scientists have gained access to the genomes and transcriptomes of Lauraceae plants, successfully deciphering the biosynthetic pathways for terpenoids production and identifying key enzyme-encoding genes involved in this process. The cloning and functional analysis of these genes provide molecular-level evidence for understanding how terpenoid compounds are precisely regulated and synthesized within cells.

3. Pathways and Key Enzyme Genes Related to Terpenoid Biosynthesis in Lauraceae Species

There are two pathways for the biosynthesis of plant terpenoids: the mevalonate acid (MVA) pathway and the methylerythritol phosphate (MEP) pathway. The MVA pathway mainly occurs in the cytoplasm, where high-terpene compounds such as diterpenes, triterpenes, and polyterpenes are produced, whereas the MEP pathway primarily takes place in plastids, where low-terpene compounds such as monoterpenes and sesquiterpenes are generated (Figure 3). To date, the biosynthetic pathways of terpenoids have been largely elucidated, and the cloning, expression, and regulation of related enzymes in terpenoid biosynthesis are current research hotspots. Many genes involved in terpenoid biosynthesis have been identified and cloned from various plants, contributing to our understanding of these critical metabolic processes.

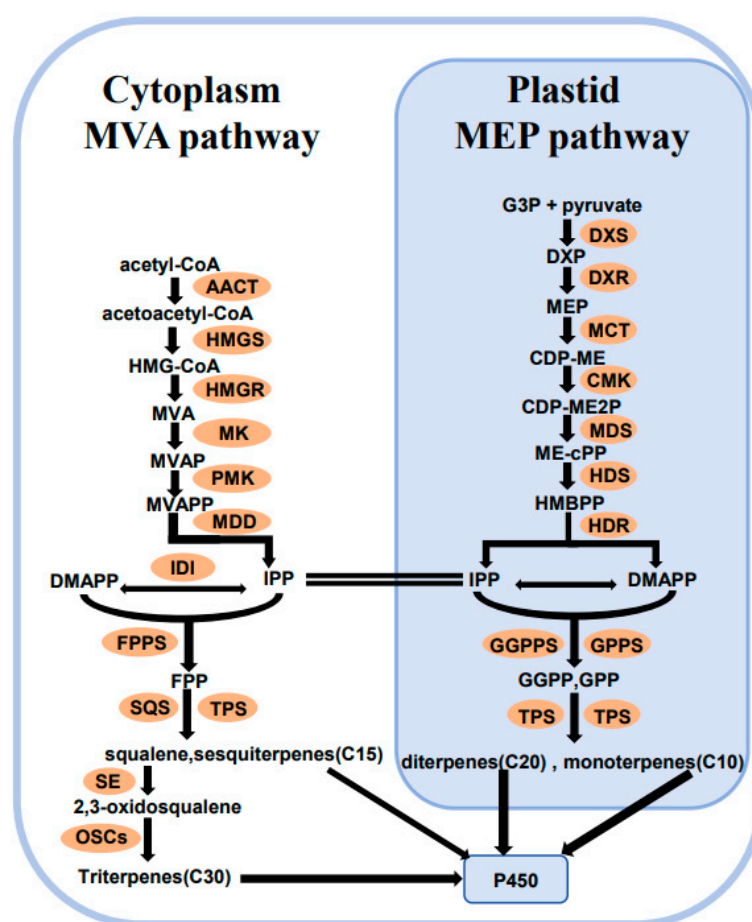


Figure 3. The MVA (left) and MEP (right) pathways responsible for terpenoid biosynthesis in Lauraceae species. AACT, acetoacetyl-CoA thiolase; HMGS, 3-hydroxy-3-methylglutaryl-CoA synthase; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; MK, mevalonate kinase; MVAP, mevalonate

5-phosphate; PMK, phosphomevalonate kinase; MVAPP, mevalonate diphosphate; MDD, mevalonate diphosphate decarboxylase; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; DXS, 1-deoxyd-xylulose 5-phosphate synthase; G3P, d-glyceraldehyde 3-phosphate; DXR, 1-deoxyd-xylulose 5-phosphate reductoisomerase; MCT, 2-C-methyl-d-erythritol 4-phosphate cytidylyltransferase; CMK, 4-(cytidine 5'-diphospho)-2-C-methyl-d-erythritol kinase; MDS, 2-C-methyl-d-erythritol 2,4-cyclodiphosphate synthase; HDS, (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate synthase; HDR, (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate reductase; IDI, isopentenyl diphosphate isomerase; FPPS, farnesyl pyrophosphate synthase; FPP, farnesyl pyrophosphate; GGPPS, geranylgeranyl pyrophosphate synthase; GGPP, geranylgeranyl pyrophosphate; GPPS, geranyl pyrophosphate synthase; GPP, geranyl pyrophosphate; TPS, terpene synthase; SE, squalene epoxidase; OSCs, oxidosqualene cyclases; P450, cytochrome P450 enzymes.

In recent years, the publication of genome data and the accumulation of multi-omics data for Lauraceae plants have greatly promoted the study of terpenoid biosynthesis pathways and key enzyme-encoding gene families within these species. Using genomics, transcriptomics, metabolomics, and other multi-omics techniques, researchers have elucidated terpenoid biosynthetic pathways in several Lauraceae plants, including *Cinnamomum kanehirae* Hayata [51], *C. camphora* [52], *Phoebe bournei* (Hemsl.) Yen C. Yang [53], *Persea americana* Mill. [54], *Lindera glauca* (Siebold & Zucc.) Blume [55], and *L. cubeba* [56]. Comprehensive analysis revealed that Lauraceae plants typically utilize both the MEP and MVA pathways to synthesize terpenoids (Figure 3). The synthesis process can be divided into two stages: the first stage involves the production of the five-carbon isoprene precursors isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) via the MVA and MEP pathways, respectively. Subsequently [57,58], IPP and DMAPP are catalyzed by different isoprene synthases (IPs) to form carbon chain precursors of varying lengths, including geranyl pyrophosphate (GPP, C10), farnesyl pyrophosphate (FPP, C15), and geranylgeranyl pyrophosphate (GGPP, C20) [59,60]. In this process, hydroxymethylglutaryl-CoA reductase (HMGR) and 1-deoxy-D-xylulose-5-phosphate synthase (DXS) are considered the first rate-limiting enzymes of the MVA and MEP pathways, respectively, and are critical regulatory points in terpenoid metabolism [61–64]. Chen et al. [56] conducted a systematic analysis of the terpenoid biosynthesis pathway in *L. cubeba*, with a particular focus on the molecular characteristics of the core enzyme DXS. Gene family analysis revealed that DXS genes in Lauraceae plants are not only abundant but also differentiated into three distinct subgroups (A–C) during evolution. Transcriptome data analysis revealed that DXS genes belonging to branch B are highly expressed in genera such as *Litsea*, *Beilschmiedia*, and *Sassafras*. Given that these genera are renowned for their essential oil content, the high expression of subgroup B DXS genes may provide a crucial genetic basis for the rich diversity of terpenoids in Lauraceae plants. Further experiments confirmed that transient overexpression of the *LcuDXS3* gene significantly promoted the biosynthesis of monoterpenes and sesquiterpenes, providing direct evidence for the regulatory mechanisms of terpenoid synthesis. Geranylgeranyl pyrophosphate synthase (GGPPS), a key enzyme downstream of the MVA and MEP pathways, catalyzes the condensation of IPP and DMAPP to form GPP, FPP, and GGPP and is essential for terpenoid biosynthesis. Comparative analysis of homologous sequences of the GGPPS gene in Lauraceae plants revealed that this gene is highly conserved in both quantity and structure, suggesting that its fundamental function in terpenoid synthesis has been highly optimized and maintained. Cao [65] conducted an in-depth exploration of transcriptome data during the development of *L. cubeba*, identifying 14 genes encoding geranyl pyrophosphate synthase (GPPS), farnesyl pyrophosphate synthase (FPPS), and geranylgeranyl pyrophosphate synthase (GGPPS). These genes exhibited unique spatiotemporal expression patterns at different stages and tissues of fruit development, indicating their complex roles in regulating the synthesis of essential oils in *L. cubeba*. In the second stage, the linear precursors of isoprenyl diphosphates with 10, 15, and 20 carbon atoms are catalyzed by terpene synthases (TPSs), including monoterpene synthases (MTPSs), sesquiterpene synthases (STPSs), and diterpene synthases (DTPSs), to form cyclic monoterpene, sesquiterpene, and diterpene molecular

skeletons. These primary terpene skeletons are subsequently modified by cytochrome P450 enzymes, which transform them into mature terpenoid compounds with biological activity, endowing plants with various functions such as defense, signal transduction, and ecological adaptation [57,58]. Li et al. [66] identified the *CYP450* superfamily in *C. camphora* and reported that most *CYP450* genes are significantly upregulated in response to cold stress, with members of the *CYP72A* family being particularly active under cold acclimation conditions. Additionally, *CYP72A8* may play a role in circadian rhythm regulation, whereas *CYP716A1* is deeply involved in the triterpenoid metabolic pathway.

In addition to enzyme-encoding genes, transcription factors play a central role in regulating the terpenoid biosynthesis network. For example, Li et al. [66] reported that certain *CYP450* genes often colocalize with transcription factors (such as WRKYs and NACs) that respond to abiotic stress in specific chromosomal regions and exhibit co-expression, which may reflect their synergistic role in environmental adaptation strategies. Zhao et al. [67] systematically identified the citral-biosynthesis-related gene cluster (CGC) in Lauraceae plants and reported that the citral content in the fruits of *L. cubeba* is unusually high, exceeding 80% of the total content. This phenomenon is closely related to specific regulation by the MYB44 transcription factor, which directly regulates the expression of alcohol dehydrogenase (*ADH*) genes, a core enzyme in the citral biosynthesis pathway.

In summary, these studies not only deepen our understanding of the terpenoid biosynthesis pathways in Lauraceae plants but also provide important molecular biological foundations for exploring the biosynthetic mechanisms, environmental adaptability, and ecological functions of terpenoid compounds. This paves the way for further genetic improvement and bioengineering of terpenoid compounds.

4. Evolution and Function of *TPS* Genes in Lauraceae Species

Terpene synthases (TPSs) are a class of catalytic enzymes widely present in plants, microorganisms, and other organisms. They use geranyl diphosphate (GPP), farnesyl diphosphate (FPP), and geranylgeranyl diphosphate (GGPP) as direct precursors to synthesize monoterpenes, sesquiterpenes, diterpenes, and even polyterpenes [60]. The specific type of terpene compound synthesized is determined by the diversity and specificity of TPS functions [68]. Based on the structure and function, the *TPS* gene family can be divided into seven subfamilies: *TPS-a*, *TPS-b*, *TPS-c*, *TPS-d*, *TPS-ef*, *TPS-g*, and *TPS-h*, each with distinct gene functions. *TPS-a* contains most of the sesquiterpene and diterpene synthases; *TPS-b* and some *TPS-g* contain most of the monoterpene synthases; *TPS-c* is considered the oldest subfamily, containing copalyl diphosphate synthase (CPS) from gymnosperms and Angiospermae; and *TPS-ef* includes kaurene synthase (KS) and other TPSs from gymnosperms and Angiospermae, corresponding to kaurene synthesis from GGPP via CPS and KS enzymes [69,70].

Currently, *TPS* is the most studied gene family in the terpene synthesis pathways of Lauraceae plants. Through genome and transcriptome data, researchers have identified the *TPS* gene family in various Lauraceae species [51–53,55,56,71], with the number of members ranging widely from 15 to 138 (see details for Table 1). Among them, fewer *TPS* genes have been identified via transcriptome data than through whole-genome sequencing. This is because transcriptome sequencing mainly captures actively expressed gene transcripts in specific tissues or at specific times, potentially missing genes that are expressed at low levels or not expressed under those conditions. In contrast, whole-genome sequencing provides all the genetic information of a species, more comprehensively revealing the composition and structural diversity of the *TPS* gene family. Therefore, subsequent discussions and analyses focused only on species identified through genome sequencing.

Table 1. Summary of the *TPS* gene families of 15 Lauraceae species.

Species	Total Number	<i>TPS-a</i>	<i>TPS-b</i>	<i>TPS-c</i>	<i>TPS-elf</i>	<i>TPS-g</i>	<i>TPS-x</i>	Data	Reference
<i>Cinnamomum kanehirae</i>	101	25	58	2	12	4	0	Genome	[51]
<i>Cinnamomum camphora</i>	78	34	32	2	7	3	0	Genome	[52]
<i>Persea americana</i>	35	6	17	2	8	2	0	Transcriptome	[56]
<i>Phoebe bournei</i>	72	34	28	1	6	0	3	Genome	[53]
<i>Lindera glauca</i>	138	69	45	1	15	8	0	Genome	[55]
<i>Litsea cubeba</i>	52	17	24	1	6	3	1	Genome	[56]
<i>Phoebe sheareri</i>	36	19	8	0	5	4	0	Transcriptome	[56]
<i>Machilus salicina</i>	32	11	13	0	4	4	0	Transcriptome	[56]
<i>Sassafras tzumu</i>	30	13	3	0	11	3	0	Transcriptome	[56]
<i>Phoebe humanensis</i>	26	15	4	0	4	3	0	Transcriptome	[56]
<i>Cinnamomum tenuipilum</i>	15	7	3	2	3	0	0	Transcriptome	[56]
<i>Cinnamomum burmanni</i>	73	29	23	1	8	12	0	Genome	[71]
<i>Litsea tsinlingensis</i>	15	9	1	0	4	1	0	Transcriptome	[56]
<i>Lindera megaphylla</i>	14	2	3	0	5	4	0	Transcriptome	[56]
<i>Alseodaphne petiolaris</i>	22	9	5	1	5	2	0	Transcriptome	[56]

We performed a phylogenetic analysis of four Lauraceae species and three well-studied plants with published *TPS* genes, namely, *C. kanehirae* [51], *C. camphora* [52], *P. bournei* [53], *L. glauca* [55], *Arabidopsis thaliana* [72], *Vitis vinifera* [73], and *Populus trichocarpa* [74] (Figure 4). The phylogenetic results revealed that Lauraceae *TPS* genes are classified into *TPS-a*, *-b*, *-c*, *-elf*, and *g* subfamilies, and the total number of *TPS* genes still varies greatly between species, from 72 in *P. bournei* [53] to 138 in *L. glauca* [55], reflecting the significant differences in terpenoid biosynthesis capabilities among Lauraceae plants. Moreover, we found that the number of *TPS* genes in these four Lauraceae plants is much greater than that in other species [75]. Studies have shown that species within the Lauraceae family have undergone two whole-genome duplication (WGD) events, providing the raw genetic material for evolution [52,76]. These duplication events likely led to the large-scale expansion of *TPS* genes in their most recent common ancestor. Subsequent tandem duplications further diversified the *TPS* gene family, particularly the *TPS-a* and *TPS-b* subfamilies [51–53,56,71,77]. Tandemly duplicated genes generally display higher evolutionary rates, have more recent evolutionary histories, and exhibit more clustered distributions on chromosomes [77]. Research has shown that the *TPS-a* and *TPS-b* branches in the *P. bournei* genome are under positive selection [53]. In the *L. cubeba* genome, Lauraceae-specific *TPS-f-I* and *TPS-f-II* subbranches also presented signs of positive selection [51]. Different *TPS* gene subfamilies have distinct evolutionary mechanisms that promote the diversification of terpenoid compounds in the Lauraceae family. Following the large-scale expansion of *TPS* genes in the Lauraceae ancestral species, different lineages experienced divergent processes of expansion and contraction [53]. For example, in *C. kanehirae* [51], there are more members of the *TPS-b* subfamily, which encodes monoterpene synthases, whereas fewer members of the *TPS-a* subfamily, which encodes sesquiterpene synthases, are present. In contrast, the expansion of the *P. bournei* genome of the *TPS* gene family resulted in the retention of more members of the sesquiterpene synthase *TPS-a* subfamily but the loss of many monoterpene synthase *TPS-b* copies [53]. Notably, the *L. glauca* genome underwent an independent WGD event after diverging from its closely related species *L. cubeba* [78]. This polyploidization event led to a significant expansion of gene families associated with *TPS* biosynthesis, which may have played a critical role in the ecological and biological adaptations of *L. glauca* [31,55], enabling it to cope with extreme climate conditions resulting from the prevailing East Asian monsoon during the early Miocene [78]. The differential distribution of these *TPS* gene subfamilies across Lauraceae lineages is consistent with their chemical compositions and further reveals that each lineage has undergone distinct evolutionary histories during adaptive evolution. These data not only reveal the diversity and complexity of the *TPS* gene family in Lauraceae plants but also provide valuable genetic resources for a deeper understanding of the biosynthetic mechanisms of terpenoids, species adaptability, and potential biotechnological applications.

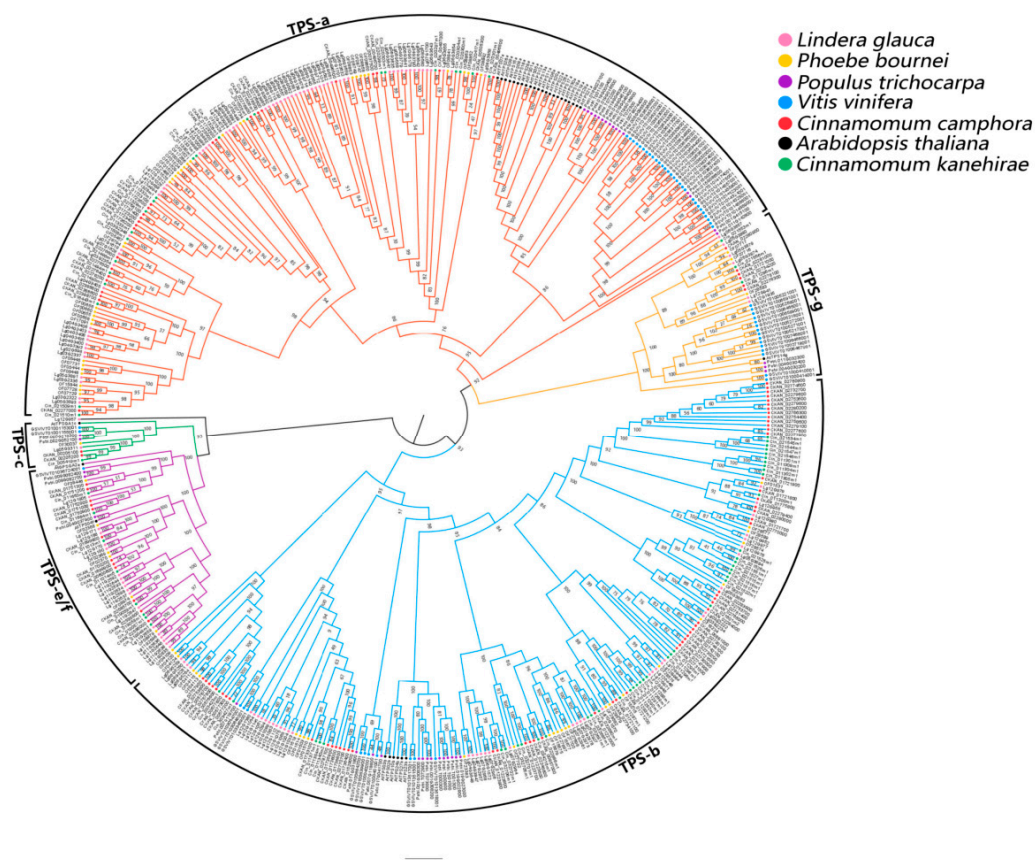


Figure 4. The maximum likelihood phylogenetic tree was constructed based on the complete *TPS*s sequenced from four Lauraceae species and three well-studied plants by using IQTREE v2.3.6 [79]. The tree branches representing the *TPS-a*, *TPS-b*, *TPS-c*, *TPS-ef*, and *TPS-g* clades are indicated by red, blue, green, purple, and yellow colors, respectively. Different species are represented by different colored dots at the ends of clades.

In addition to genome-wide identification and analysis, researchers have used methods such as cloning, substrate reactions, enzyme activity assays, subcellular localization, and phylogenetic analysis to study the specific functions of *TPS* genes. These studies have highlighted that Lauraceae plants not only are enriched in the *TPS* family but also exhibit high catalytic flexibility [80,81]. For example, Chang et al. [82] identified three *TPS* genes (*LcTPS1*, *LcTPS2*, and *LcTPS3*) from *L. cubeba*, all of which belong to the *TPS-b* subfamily. These genes catalyze the conversion of geranyl diphosphate (GPP) into different monoterpene products: *LcTPS1* converts GPP to trans-ocimene; *LcTPS2* converts GPP to α -thujene; and *LcTPS3* shows multifunctionality, converting GPP to α -thujene and (+)-sabinene. Wang et al. [52] clarified the roles of two key *TPS* genes (*CcTPS16* and *CcTPS54*) in the biosynthesis pathways of eucalyptol and (iso)nerolidol in *C. camphora* through in vitro enzyme activity assays and subcellular localization analysis. This study revealed that product formation is influenced by transcriptional regulation and organelle localization. Ma et al. [83] focused on the leaves of four different chemotypes of *C. camphora*. Through transcriptomic analysis, they successfully cloned nine *TPS* genes (*CcTPS1* to *CcTPS9*) and validated the monoterpene and sesquiterpene products catalyzed from GPP and FPP via recombinant protein expression techniques. They identified the key enzyme responsible for (+)-borneol biosynthesis for the first time. Yahyaa et al. [84] identified eight *TPS* genes through the transcriptomic analysis of *Laurus nobilis* leaves. Biochemical characterization revealed that monoterpene synthases in the *TPS-b* subfamily are involved in eucalyptol synthesis, sesquiterpene synthases in the *TPS-a* subfamily catalyze the formation of cadinenes, and diterpene synthases in the *TPS-ef* subfamily are involved in geranyl

linalool biosynthesis. Sequence comparison analysis further revealed that the *TPS-a* and *TPS-b* subfamilies emerged early in the evolutionary trajectory of Angiospermae, and that geranylinalool synthase activity is likely ancestral function of genes within an ancient *TPS-e/f* subfamily that diverged from the kaurene synthase gene lineages before the split of Angiospermae and Gymnospermae.

Despite significant advancements, the study of *TPSs* still faces challenges. In particular, integrating multidisciplinary approaches such as molecular biology, bioinformatics, and structural chemistry to achieve precise elucidation of the catalytic mechanisms of the *TPS* gene family has become a focal point and a difficult area in current research [69,70].

5. Conclusions and Prospects

Terpenoids are important metabolites in Lauraceae species. To date, thousands of terpenoids have been identified within this family. They are closely related to the growth and development, information transmission, climate adaptation, and chemical defense, playing significant roles in plant physiology and ecology. Plants in the Lauraceae family synthesize all necessary 5-carbon precursors for terpene biosynthesis via the MVA and MEP pathways. Under the action of the isoprenyl transferase family and the terpene synthase family, these precursors form the molecular skeletons of monoterpenes, sesquiterpenes, and diterpenes with different carbon chain lengths. Further modifications such as methylation, hydroxylation, oxidation, and glycosylation are catalyzed by the *CYP450* enzyme, leading to the formation of structurally diverse terpenoid compounds. Similar to other secondary metabolic processes, various enzymes and their corresponding genes play crucial roles in the formation of terpenoids, and the diversity of terpenoid structures mainly depends on terpene synthases and their genes.

Despite significant progress having been achieved in elucidating the biosynthetic pathways of terpenoids and the key enzyme-encoding gene families (especially *TPSs*) in Lauraceae species, the field still faces challenges and uncertainties. Firstly, constrained by the scarcity of genome data, a comprehensive understanding of the biosynthetic pathways and evolutionary history of terpenoids in Lauraceae plants remains elusive. Hence, there is an urgent need to enhance high-quality genome sequencing and develop genetic resources for a greater diversity of species to deepen our comprehension of their genetic diversity and evolutionary processes. Secondly, the *TPS* gene family in Lauraceae species exhibits diversity in number and structure, incomplete understanding of functions and classifications, unclear expression regulation and interaction networks, and uncertain three-dimensional structures and active sites. These factors lead to the significant difficulties and challenges in predicting and explaining the catalytic mechanisms of terpenoid synthesis. To address these challenges, future research needs to conduct in-depth exploration and innovation from multiple aspects. This includes but is not limited to the use of high-throughput sequencing technology to perform whole-genome identification and analysis of the *TPS* gene family in more plant species to reveal their evolutionary relationships and differentiation mechanisms. Establishing a more comprehensive functional annotation and classification system for the *TPS* gene family, adopting unified naming rules and standards, would enhance the comparability and predictability of *TPS* genes. Integrating multiomics data, such as transcriptomics, proteomics, and metabolomics, could help investigate the expression regulatory mechanisms and interaction networks of the *TPS* gene family, revealing the dynamic changes and adaptability of terpenoid synthesis. Additionally, utilizing methods from structural biology and bioinformatics could assist in determining the three-dimensional structures and active sites of the *TPS* gene family and simulating the molecular mechanisms and catalytic pathways of terpenoid synthesis. Finally, with the advancement of plant genetic engineering, optimizing certain reaction steps in the terpenoid synthesis metabolic pathway through DNA recombination technology or transferring genes to organisms that do not naturally produce specific terpenoids to synthesize these compounds to meet the demand for beneficial terpenoids will also constitute a key area of focus in future research.

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