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Biosynthesis of triterpenoids in plants: Pathways, regulation, and biological functions

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Plant triterpenoids, a vast and diverse group of natural compounds derived from six isoprene units, exhibit an extensive array of structural diversity and remarkable biological activities. In this review, we update the recent research progress in the catalytic mechanisms underlying triterpene synthesis and summarize the current insights into the biosynthetic pathways and regulatory mechanisms of triterpenoids. We emphasize the biosynthesis of pharmacologically active triterpenoids and the role of triterpenoid synthesis in plant growth, development, defense mechanisms, and plant–microbe interactions. This insight review offers a comprehensive perspective on the applications and future avenues of triterpenoid research.

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Introduction

Triterpenoids represent a diverse class of natural compounds synthesized via the isoprenoid pathway in plants, which exhibit a wide range of structural diversity and biological activities [1–3]. For instance, cycloartenol, a triterpene skeleton, is a precursor of plant sterols (e.g. sitosterol, stigmasterol, and campesterol) vital for cellular membranes. Campesterol also facilitates the production of brassinolides (BRs), a hormone crucial for plant growth and development [4,5]. Besides, plant-specialized triterpenoids showed significant defensive function and pharmacological potential, which have garnered considerable attention in recent years for their potential applications in medicine, cosmetics, and agriculture. For example, avenacin A-1 is a triterpene saponin found in oat roots that exhibits broad-spectrum antimicrobial activity. Glycyrrhizin, a triterpene saponin accumulated in licorice roots, possesses anti-inflammatory and antiviral properties, and also serves as a sweetener [6]. Cucurbitacins are defensive triterpenoids mainly produced in cucurbit plants, which also exhibit hepatoprotective, anti-inflammatory, and anti-tumor pharmacological effects [7–9]. Mogroside V, primarily accumulated in the fruits of *Siraitia grosvenorii*, is a triterpene-based sweetener [10].

With the rapid development of bioinformatics and genome mining technologies, significant advancements have been made in recent years in understanding the biosynthesis of triterpenoids and its regulatory mechanisms, and developing metabolic engineering strategies [11–14]. However, the detailed active compounds in the biosynthetic pathways and their precise biological functions remain largely unexplored, and the precise regulatory mechanism of the synthesis and intracellular transport of active compounds remain poorly understood. This review provides a brief overview of the metabolic pathways and regulatory mechanisms, with a particular emphasis on the diverse biological functions of bioactive triterpenoids from plants. By summarizing the latest research advances, we aim to facilitate future explorations of novel triterpenoids with unique structures and biological activities, thereby fostering their sustainable utilization.

Biosynthesis of the triterpene skeletons

The diversity of triterpene skeletons in plants, crucial for various biological functions, originates from the cyclization of 2,3-oxidosqualene by oxidosqualene cyclases (OSCs). Over 150 OSCs in 75 plant species producing at least 31 types of triterpene skeletons, mainly tetracyclic and pentacyclic, as well as several monocyclic, dicyclic, tricyclic triterpene skeletons, have been reported [12–14]. Tetracyclic triterpene skeletons, such as cycloartenol, lanosterol, and parkeol, are typically generated through a chair-boat-chair (C–B–C) conformation protosteryl cation pathway. In contrast, pentacyclic triterpene skeletons, including lupeol and α/β-amyrin, are generated through a chair-chair-chair (C–C–C) conformation dammarenyl cation pathway. Notably, the discovery of orysatinol, a novel pentacyclic triterpene originating from a C-sC-C (boat-semi-boat-boat) conformation, which expands the known spectrum of possible triterpene structures and challenges existing

stereochemical interpretations [15,16]. This finding underscores the necessity for a more flexible understanding of triterpene biosynthesis and the vast potential of these compounds in plant adaptation and human applications. Advances in genomic and transcriptomic sequencing, along with heterologous expression platforms have enabled the cloning and functional characterization of an increasing number of OSCs, further reinforcing the understanding of triterpene biosynthesis and the observed skeletal diversity.

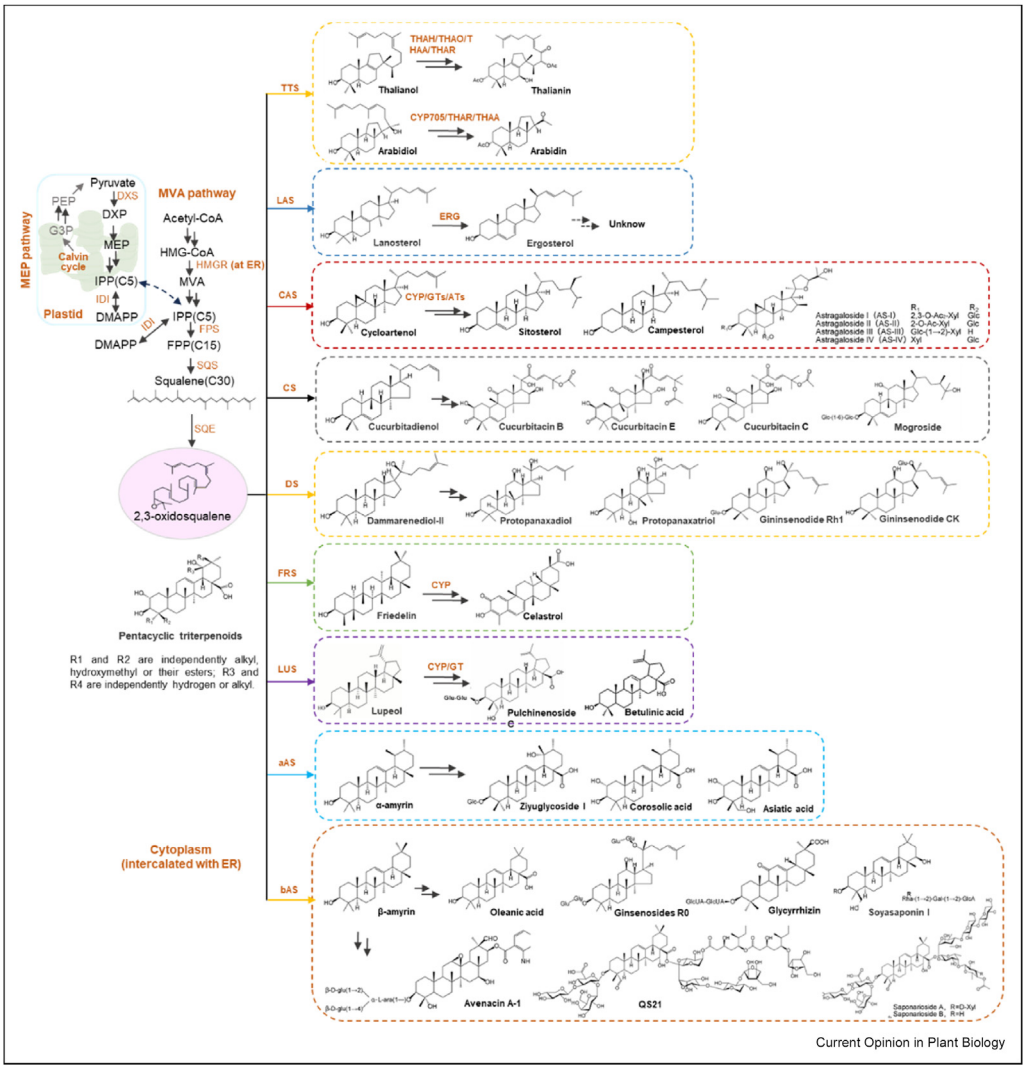
Biosynthesis of triterpenoids with pharmacological activities in plants

The diversity of triterpenoids is further expanded through postcyclization modifications of triterpene skeletons, including oxidation, hydroxylation, and dehydrogenation, predominantly catalyzed by cytochrome P450 monooxygenases (CYP450s) [17]. Uridine diphosphate (UDP)-glycosyltransferases (UGTs) play a crucial role in enhancing the water solubility and bioavailability of these compounds, catalyzing the formation of biologically active triterpenoid saponins [18]. A case in point is the synthesis of ginsenosides found in *Ganoderma lucidum* triterpenoids. Here, 2,3-oxidosqualene is cyclized into dammarenediol II by dammarenediol II synthase (DS), which is then oxidized by CYP450s to form protopanaxadiol (PPD) and protopanaxatriol (PPT). These compounds are further glycosylated to produce various ginsenosides, such as Rg1, Rb1, and notoginsenoside R1, known for their antioxidant, anti-inflammatory, antitumor, and antiaging properties [19]. Another significant compound, betulinic acid, derived from lupeol by oxidation of C28, exhibits potent anticancer and antiviral activities, particularly against HIV, HCV, IAV, and SARS-CoV [20–23]. QS saponins (QS-21, QS-17, QS-7), a class of clinically approved natural triterpenoid saponin adjuvants derived from *Quillaja saponaria*, synergistically enhance robust antibody and helper T cell responses. Given their excellent pharmacological activities [24–26], it is worthwhile to elucidate the biosynthetic pathways of triterpenoids in plants and to assemble heterologous pathways via synthetic biology for green production.

The biosynthetic pathway of triterpenoids is renowned for its complexity (Figure 1; Table 1). Firstly, these pathways involve numerous enzymes and regulatory genes, and certain enzymes exhibit promiscuity, being able to accommodate a variety of substrates. This is particularly evident in the limonoid biosynthetic pathway, where postapo-melianol there are multiple divergent pathways, resulting in a range of extensively rearranged and modified limonoid scaffolds [27,28]. Furthermore, the necessity for multiple enzymatic transformations to produce complex intermediates complicates the identification of candidate enzymes/genes. Additionally, the instability of pathway precursors, such as ginsenoside aglycone, which is vulnerabe to hydrolysis and oxidation, poses significant challenges to the reconstitution of complex triterpene biosynthetic pathways. Notably, benefiting from technological advancements in sequencing and bioinformatics [29], pathway discovery strategies are shifting from step-by-step characterization to genomics-driven prediction and validation. The availability of numerous high-quality genomes by HiFi, Hi-C, and ONT sequencing technologies has accelerated the progress in elucidating triterpenoid biosynthetic pathways or networks [29]. For example, the unbalanced evolution of triterpene saponin biosynthetic genes was well explored in ginseng [30], and a terpene synthases (TPS)-independent geraniol biosynthetic pathway in rose species was discovered [31]. Additionally, genome-wide association studies (GWAS) and multiomics analyses facilitate the large-scale and precise identification of candidate genes correlated with metabolites [32]. Integrated approaches using metabolomics and transcriptome analysis, coupled with heterologous expression, have proven to be powerful tools for elucidating metabolic pathways and identifying key bioactive compounds, which were used in the identification of limonoid, saponariosides B and QS saponins synthetic pathways [27,33–36]. In addition, the coordinately expressed metabolic gene clusters accelerate the elucidation of metabolic pathways, like the gene cluster for biosynthesis of avenacins in oat, and the thalianol, and marneral clusters in *Arabidopsis* [37–39].

The crux of triterpenoid metabolism research hinges on comprehensively analyzing the synthetic pathways of important active compounds (Figure 1, Table 1). Triterpenoids comprising 1–3 rings are relatively uncommon. The identified tricyclic triterpenoids thalianin, arabidin, and thalianyl fatty acid esters were typically acylated at the C-3 and C-15 hydroxyl groups or with long-chain fatty acids [34]. Lanosterol, cycloartenol, dammarenediol II, and cucurbitadienol are the major types of tetracyclic triterpene skeletons, characterized by hydroxylation at C-3 and C-21, and undergo reactions with a variety of acyl donors, leading to the formation of diverse triterpenoids. Researches have unveiled the complete biosynthetic pathway of active astragalosides derived from cycloartenol [40], ginsenosides derived from dammarenediol II or oleanane-type scaffolds [54,41–43], cucurbitacins produced from cucurbitadienol [7,44–46] and celastrol synthesized from friedelin [47,48]. The pentacyclic triterpenoids QS saponins adjuvants originate from β-amyrin with a quillaic acid (QA) scaffold generated by oxidation and glycosylation. The CYP716A224 and CYP716A297 oxidize C-28 and C-16α, respectively, while CYP714E52 acts on the C-23 aldehyde of oleanolic acid to produce QA from β-amyrin. Subsequently, the addition of a branched trisaccharide chain at the C-3 position and a linear tetrasaccharide at the C-28 position represents a critical branch point for

Figure 1



Plant triterpenoids with pharmacological properties. Triterpenoids are synthesized from isoprene units via the mevalonate pathway. The precursor 2,3-oxidosqualene is cyclized into triterpenes skeletons with one to five cycles, including representative tetracyclic triterpenoid (lanosterol, cycloartenol, cucurbitadienol, dammarenediol, etc.) and pentacyclic triterpenoids(friedelin, α-amyrin, β-amyrin, lupeol, etc.). Further reactions of these diverse skeletal structures by cytochrome P450 monooxygenases (CYP450s) and uridine diphosphate glucuronosyltransferases (UGTs) lead to the production of numerous triterpenoid saponins with pharmaceutical potential. aAS, α-amyrin synthase; bAS, β-amyrin synthase; DDS, dammarenediol synthase; LUP, lupeol synthase; CS, cucurbitadienol synthase; CAS, cycloartenol synthase; LAS, lanosterol synthase; FRS, friedelin synthase; THAS, thalianol synthase; ABDS, arabidiol synthase; ERG, ergosterol biosynthesis; FPS, farnesyl diphosphate synthase; SQS, squalene synthase; SQE, Squalene monooxygenase; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase.

saponin diversification [49]. The complete biosynthesis of QS-21 has been successfully achieved in both tobacco plants and engineered yeast strains [50,51]. Additionally, based on the triterpene skeleton of β-amyrin, the biosynthesis pathway of avenacin A-1 (a disease-resistance saponin from oat) [52], and soyasaponin I and saponarioside A/B (traditional sources of soap from *Saponaria officinalis*) have been fully elucidated. The oat AsCYP72A475 is the first reported C-21β triterpene oxidase from monocots in avenacin A-1 biosynthesis, while AsCYP51H10 is the first P450 enzyme confirmed to have triterpene-modifying function in monocotyledonous plants [53,54]. Saponarioside A and B, structurally similar to the vaccine adjuvant QS-21, offer potential as alternative raw materials for this adjuvant [36]. Despite the chemical similarity between saponarioside A/B and QS saponins, their respective pathway enzymes, except for the first two, they exhibit a striking dissimilarity in amino acid composition, suggesting divergent evolutionary pathways yet convergent functional outcomes. Totally, these discoveries lay the foundation for addressing sourcing issues and enhancing our

Table 1

The triterpenoid skeleton modification for the biosynthesis of pharmaceutical potential triterpenoids.

Triterpenoid skeleton	Oxidosqualene cyclase	Reaction position	Gene	Reaction position	Gene	Active compounds	Reference
β-amyrin	QsbAS1	C-28, C-16α oxidation, C-23 aldehyde	CYP716A224, CYP716A297, CYP714E52	C-3, C-28 sugar chain	CSLM2, UGT73CU3, UGT73CX1, UGT73CX2, UGT74BX1, UGT91AQ1, UGT91AR1, UGT91AP1, UGT73CY3, UGT73CY2, UGT73B43, UGT73B44, UGT74BB2, UGT74BX1, ATCV-1 UGD	QS-21, QS-17, QS-7	Reed et al., 2023 [49]
	SobAS1	C-28, C-16α, C-23 oxidation	CYP716A378, CYP716A379, CYP72A984	C-3, C-28 sugar chain	SoCSL1, UGT73DL1, UGT73CC6, UGT74CD1, UGT79T1, UGT79L3, UGT73M2, SoGH1, SoBAHD1	Saponarioside A, Saponarioside B	Jo et al., 2024 [36]
	AsbAS1/SAD1	C-12,13β epoxidase, C-16β hydroxylase, C-3 arabinosyltransferase	AsCYP51H10/ SAD2, AsCYP72A475/ SAD6, AsAAT1	C-3 glycosylated, C-21 acylate	AsUGT91G16, AsTG1, AsMT1/SAD9, AsUGT74H5/SAD10, AsSCPL1/SAD7	Avenacin A-1	Leveau et al., 2019 [53] Orme et al., 2019 [54]
Cycloartenol	AmOSC3	C-6, C-16, C-25 hydroxylation and epoxidation	AmCYP88D25, AmCYP88D7, AmCYP71D756	C-20, C-24 tetrahydrofuran ring, C-3, C-6 glycosylated	AmOGD1,AmGT36, AmGT11, AmGT72	Astragaloside I/II/ III/IV	Xu et al., 2024 [40]
Tirucalla-7,24-dien-3β-ol	CsOSC1, AiOSC1, MaOSC1	C-23 oxidation, C-24/25 epoxide, C-21, C-7, C-8 oxidation, C-30 methyl shift, oxide isomerases	CsCYP71CD1/ MaCYP71CD2, CsCYP71BQ4/ MaCYP71BQ5, CsCYP88A51/ MaCYP88A108, CsMOI2, MaMOI2, CsMOI1	C-21 hydroxyl, C-3 oxidation, A-ring modification, C-1 hydroxylation, furan-ring formation	CsL21AT/MaL21AT, CsSDR/MaSDR, CsCYP716AC1, CsCYP88A37/ MaCYP88A164, CsL7AT/MaL7AT, CsAKR/MaAKR, CsCYP716AD2/ MaCYP716AD4, CsLFS/MaLFS, CsLFS/MaLFS	Limonoids	De La Peña et al., 2023 [27] Hodgson et al., 2019 [28]
Cucurbitadienol	CmBi, ClB	C-11 carbonylase, C-20, C-25, C-2, C-19 hydroxylase	CYP87D20, CYP81Q59, CYP87D19, CYP89A140, CYP81Q58, CYP88L2, CYP88L3, CYP712D8	Acetyltransfer	CmACT/ClACT	CuC, CuB, CuE	Shang et al., 2014 [8] Zhou et al., 2016 [44]
Thalianol, Arabidiol	TTS	C-7, C-15 hydroxylation, C-16 oxidation, C-15 add an acetate group, C-3 hydroxy moiety, 14-apo arabidiol	THAH, THAO, THAA1, THAA2, CYP705A1	C-3 ketones, C-3α alcohols, acetates	THAR1, THAR, THAA2	Thalianin, Arabidin	Huang et al., 2022 [34]

Friedelin	FRS	C-29, C-2, C-24 oxidation	CYP712K1, CYP712K2, CYP712K3, CYP716C52, CYP716C57, CYP716C52v2, CYP81AM1, CYP81AM2	Nonenzymatic oxidation cascade, isomerizatio		Celastrol	Zhao et al., 2023 [47] Zhao et al., 2024 [48]
Dammarenediol-II, β-amyrin	DS, bAS1	C-3, C-12, C-6 hydroxylation, C-28 oxidation	CYP716A47, CYP716A53v2, CYP716A52v2	C-20, C-6, C-3, C-28 glycosylated	UGT71A53, UGT71A54, UGT74AE2, UGT94Q2, UGT73C5, PgUGT18 and PgUGT8, UGTPg45, UGTPg29	Protopanaxadiol, Protopanaxatriol, Gininsenodide CK/Rh1/Rh2/ Rg1/Rg3/R0	Chopra et al., 2023 [41] Zhang et al., 2022 [42] Wang et al., 2015 [43]
Lupeol	LUS	C-28 oxidation	CYP716A80/ CYP716A179/ CYP716AL1/ CYP716A12/ CYP716A15/ CYP716A155			Betulinic acid	An et al., 2020 [23]
α-amyrin	MdOSC1m	C-28 oxidation, C-23, C-2α hydroxylation	CYP716A12, CYP714E19, CYP716C11			Asiatic acid	Lian et al., 2024 [55]

understanding of the biosynthetic diversity of plant triterpenoids.

Moreover, the evolutionary divergence of biosynthetic pathways has driven the vast diversity of triterpenoids in nature. Gene duplication and neo-functionalization of the OSCs lead the formation of distinct triterpenoid skeletons. The dammarenediol-II synthase (DDS) gene family originated from an *Araliaceae*-specific whole-genome duplication event in *Panax* [56,57]. The absence of DDS and tandem duplication of triterpenoid backbone-specific modifying enzymes in *Aralia elata* contribute to the accumulation of diverse pentacyclic triterpenoid saponins. Evolutionary adaptations not only enhance the chemical complexity of triterpenoids but also contribute to their varied roles in plant defense, signaling, and human health. For instance, the anti-inflammatory compound ursolic acid, present in apples and rosemary, and the anticancer agent avocatin B, found in avocados, are testament to the diverse biological functions of triterpenoids. Understanding the evolutionary biology of these pathways is therefore essential for uncovering new bioactive compounds and for the sustainable utilization of triterpenoid resources.

Biosynthetic regulation and transport of triterpenoids

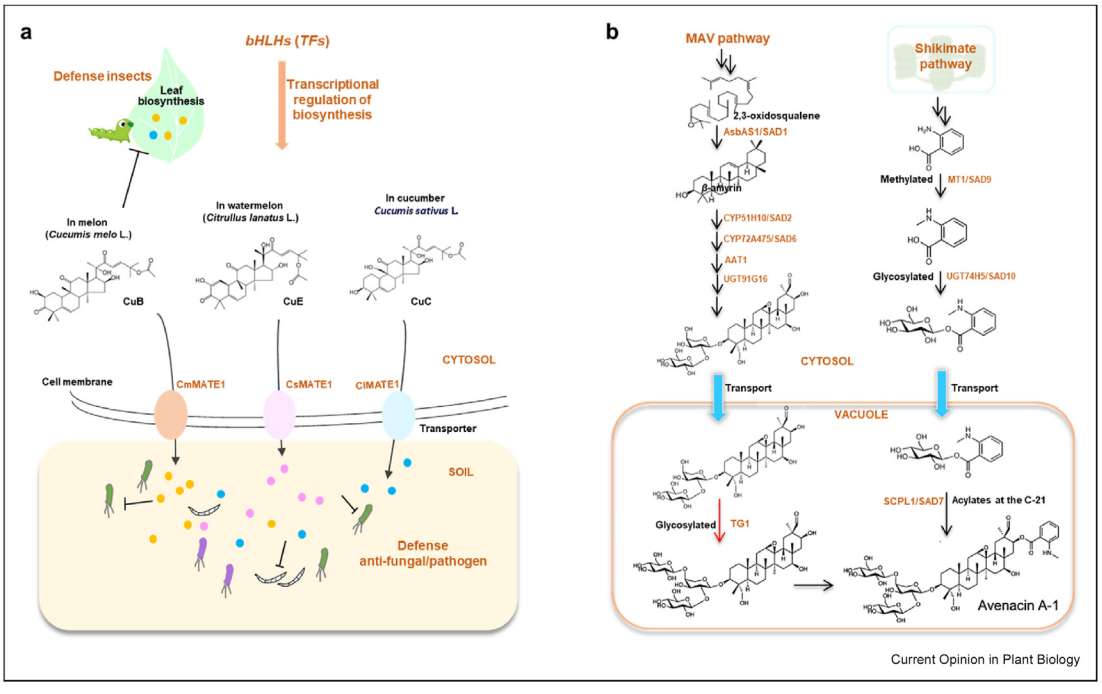
The regulation of triterpenoid metabolism is a complex and intricate process that remains insufficiently understood. Both biotic (such as pathogen and insect attacks) and abiotic (such as light, drought, and salinity) stresses can stimulate plants to produce triterpenoids. For instance, fungal infections can induce plants to synthesize triterpenoid phytoalexins with antifungal properties to combat invading pathogens, while drought stress can trigger the production of triterpenoid osmoprotectants, aiding plants in coping with water scarcity. A pivotal aspect of the regulation of biosynthesis is the direct involvement of transcription factors (TFs), including *WRKY*, *bHLH*, *MYC*, *AP2-ERF*, *bZIP*, and *NAC*, in controlling triterpene biosynthesis [58]. In cucumber, the expression of CuC biosynthetic genes is controlled by two tissue-specific *bHLH* transcription factors in the leaves (*Bl*, bitter leaf) and fruit (*Bt*, bitter fruit) [8] (Figure 2a). Additionally, researchers have identified a network of redundant *bHLH* transcription factors and cofactors that activate triterpene biosynthesis in external root tissues (e.g. root cap and epidermis) upon jasmonic acid (JA) signaling; conversely, DNA binding with one finge (DOF)-type transcription factors inhibit this process in internal tissues [59]. In addition, other key phytohormones, such as salicylic acid (SA) and abscisic acid (ABA) also play crucial roles in stress-induced triterpenoid biosynthesis.

Notably, biosynthetic gene clusters (BGCs) encode enzymes for a particular metabolic pathway, encompassing initiating enzymes such as OSCs, as well as

subsequently acting downstream enzymes such as CYP450s and UGTs [37]. This organization facilitates the coordinated expression and efficient biosynthesis of active triterpenoids. Research highlights the crucial role of numerous BGCs in orchestrating the biosynthesis of triterpenoids with defensive or pharmacological activities, such as the dynamic modulation of the *Arabidopsis* root microbiota by specialized triterpenoids [34,38], isoarborinol-mediated pathogen resistance in bread wheat [60], steroidal glycoalkaloids found in the *Solanaceae* [61], bitter cucurbitacins in *Cucurbitaceae* [44], and saponin adjuvant QS in the soapbark tree [49].

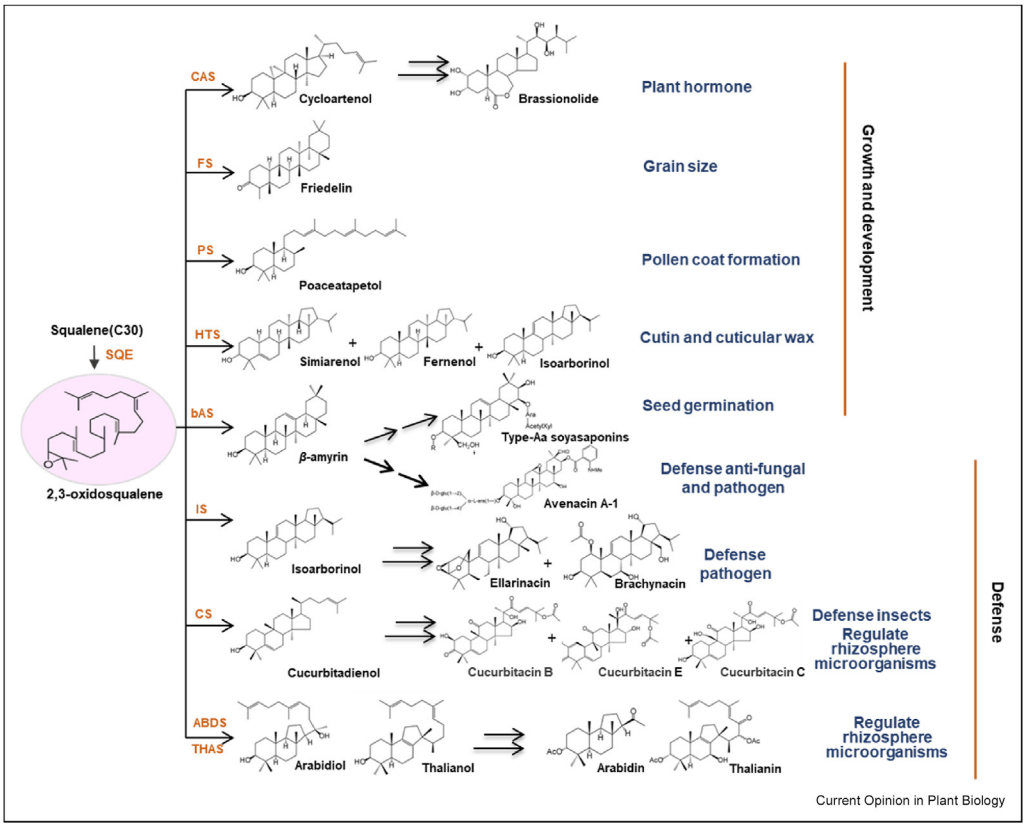
An additional layer of complexity in triterpenoid biosynthesis involves the intracellular and extracellular transport of metabolic intermediates. The coordinated regulation of cucurbitacin biosynthesis and transport in plants offers novel insights and exemplars for investigating the substrate specificity and transport direction of plant transporters of specialized metabolites. In cucurbitacin biosynthesis, CuB/C/E transporters, MATE1, are clustered with their biosynthetic genes in melon, cucumber, and watermelon, respectively [46,62] (Figure 2a). MATE1 is involved in secreting cucurbitacins into the rhizosphere. Following triterpenoids biosynthesis, these metabolites are directed to specific tissues or organelles to execute their biological functions, either through diffusion or active trafficking. Besides cell-to-cell transmission, a myriad of bioactive and inactive metabolites is transiently stored in specific organelles, such as the vacuole. At the subcellular level, triterpene saponins are accumulated in vacuoles, however, OSCs, P450s, and some UGTs for saponin biosynthesis are known as microsomal enzymes. These facts suggest the presence of vacuolar transporters of triterpene saponin. ABC transporters (such as PgPDR3, *ABCC2*, *ABCC4*, *and NR1I2*) are candidate genes to investigate for the transport of ginsenoside or other substrates in *P. ginseng* [63,64]. The *Arabidopsis* ABC transporter ABCB19, which drives the export of brassinosteroids from the cell through its adenosine triphosphate (ATP) hydrolysis activity, thereby regulates BR signaling [65]. Similarly, during the synthesis of the oat antifungal triterpenoid avenacin A-1, a precursor that is partially glycosylated, is transported from the cytosol to the vacuole for further modifications (Figure 2b) [53,54]. This discovery underscores the significance of interorganellar compound transport in the biosynthesis of plant natural products, as well as the pivotal role of the vacuole in facilitating glycosylation and acylation. Nevertheless, the majority of triterpenoid transporters are still unknown. The identification of metabolite transporters could facilitate the metabolic engineering of pathways for valuable plant natural products by simplifying their purification

Figure 2



Regulation of plant triterpenoid biosynthesis and transport. a. Cucurbitacins, a class of tetracyclic triterpenoids, possess a bitter taste and exhibit toxicity, which enables plants to resist pest and disease invasion. They serve as defense agents and as attractants for certain polyphagous and predatory insects. The *bHLH* transcription factors regulate the tissue-specific cucurbitacin biosynthesis, and the cell membrane transporter of MATE1 transports cucurbitacin into the rhizosphere against the wilt disease pathogen in soil. **b.** In the biosynthesis of the oat antifungal triterpenoid avenacin A-1, the partially glycosylated precursor is catalyzed by the enzyme AsUGT91G16, and transported from the cytosol to the vacuole. Within the vacuole, it undergoes additional glucosylation by the vacuolar sugar transferase AsTG1 and subsequently acylation by SCPL1/SAD7.

Figure 3



Triterpenoids in Plant growth and defense. SQE, Squalene monooxygenase; CAS, Cycloartenol synthase; FS, Friedelin synthase; PS, Poaceatapetol synthase; bAS, β-Amyrin synthase; IS, Isoarborinol synthase; CS, Cucurbitadienol synthase; THAS, Thalianol synthase; ABDS, Arabidiol synthase; HTS, a mixed hopane triterpenoid synthase in sorghum.

processes, enabling the extracellular export of target compounds postbiosynthesis.

In conclusion, the regulation of triterpenoid biosynthesis is a complex and multifaceted process that involves transcription factors and the organization of some metabolic gene clusters for coordinated expression, as well as the intracellular and extracellular transport of metabolic intermediates or final products. An enhanced understanding and systematic analysis of these regulatory mechanisms will pave the way for future genome mining and elucidate the prevalence and function of such metabolism in plants, ultimately contributing to the sustainable production and utilization of these valuable compounds.

Triterpenoid biosynthesis in plant growth and development

Genetic and biochemical analysis of triterpenoid-deficient mutants has been helpful in the elucidation of multistep triterpenoid biosynthetic pathways and identification of their physiological function in plants [14]. SQE, which oxidizes squalene to 2,3-oxidosqualene (Figure 3), is indispensable for root and seed development in *Arabidopsis* [66]. The dry2/sqe1-5 mutant also underscores the pivotal function of sterols in drought tolerance and the modulation of reactive oxygen species [67]. Cycloartenol synthase genes, conserved across all plant species from algae to monocots and dicots, are crucial for synthesizing plant sterols, including the BRs that regulate multiple plant developmental processes, including cell elongation, vascular differentiation, and pollen tube growth [5–8].

Significantly, as the exploration of cereal triterpenoids advances and their catalytic functions become more clearly defined, their integral roles in plant growth and development are increasingly being unveiled (Figure 3). Rice, an important food crop and a monocotyledonous model plant, has eight functionally characterized OsOSCs. However, the biological functions of most OsOSCs remain largely unknown, except for OsOSC2, which produces cycloartenol for phytosterol biosynthesis, and OsOSC12/OsPTS1, which catalyzes the production of poaceatapetol and its three major fatty acid ester derivatives, playing a crucial role in rice pollen

walls formation [68–70]. Based on this discovery, researchers created humidity-sensitive genic male sterility (HGMS) lines in maize and wheat by knocking out the respective *PTS1* genes to develop an efficient and economical two-line hybrid seed production system [71]. OsOSC10 was identified as a friedelin synthase, which impacts rice grain development, revealing a novel catalytic mechanism and biological function [72]. The biochemical functions of OsOSC7j/OsPS from *O. sativa L.* ssp. *japonica* and OsOSC7i/OsOS from *O. sativa* L. ssp. *indica* are differentiated, producing parkeol and orysatinol, respectively [15,73]. This reflects rapid evolution driven by natural selection and their distinct biological roles. Moreover, triterpenoids in sorghum leaf waxes contribute to the formation of heat-tolerant cuticular water barriers and enhance the mechanical strength of the cuticle, aligning with sorghum's drought tolerance and adaptation to hot climates [74].

Triterpenoids biosynthesis in plant defense and plant–microbe interactions

Many triterpenes exhibit antimicrobial and insecticidal activities, making them effective chemical defenses against biotic stresses (Figure 3) [75,76]. The triterpenoid saponins, such as avenacins A-1, produced by oat and quinoa roots have been shown to have strong antifungal activities against various phytopathogens from the soil with saponin-deficient (*sad*) mutants exhibiting reduced susceptibility to fungal pathogens [37,77]. Notably, through the analysis of various *sad* mutants, the complex biosynthetic pathway of avenacins has been deciphered, revealing a series of multistep reactions facilitated by OSCs, P450s, UGTs, and ATs. Cucurbitacins produced by cucurbitaceae plants exhibit potent antifeedant and toxic effects against insects, protecting the plants from herbivory [7–9]. The root-to-soil export of cucurbitacins in melon and watermelon has also been shown to modulate the rhizosphere microbiome, enhancing plant resistance to soil-borne pathogens [46,61]. In addition, three divergent pathways for the biosynthesis of root triterpene metabolites (thalianin, thalianyl fatty acid esters, and arabidin) derived from BGCs, can dynamically modulate the *Arabidopsis* root microbiota, affecting plant fitness and disease resistance [34]. Similarly, the triterpenoid saponins produced by legumes exhibit antifungal and antibacterial activities, contributing to plant disease resistance. Furthermore, a pathogen-induced cluster for a novel isoarborinol-derived triterpenoid, ellarinacin, was found in bread wheat and *B. distachyon* [60,78]. These studies not only deepen our understanding of triterpenoid biosynthesis but also open up new avenues for exploring their biological functions.

Conclusion

Triterpenoids represent a diverse and functionally crucial class of plant secondary metabolites that play pivotal roles in various biological processes. The metabolism of plant triterpenes forms a complex and intricate biosynthetic network, whose resulting natural products play multifaceted biological roles. The integration of sequencing technologies and bioinformatics tools has facilitated the exploration of novel triterpenoids with unique structures and functional activities. By mining plant genomes for uncharacterized OSC and CYP450 genes, researchers have been able to identify potential pathways for the biosynthesis of many new triterpenoids. These efforts have expanded the repertoire of plant triterpenoids and opened up new avenues for exploration. Despite this remarkable progress, several challenges remain. One of the key areas requiring further attention is the identification and characterization of active triterpenoid compounds and their biological functions. While the medicinal properties of many specialized triterpenoids are well-documented, their ecological and biological roles in plants remain largely unexplored. Future studies need to focus on investigating the diverse functions of more specialized triterpenoids in plant growth, development, and interactions with the environment, as well as their potential applications in sustainable pest management and agriculture. Another significant challenge lies in the understanding of regulatory mechanisms underlying triterpene production. Despite the discovery of numerous BGCs involved in triterpenoid biosynthesis, the regulatory networks that govern these pathways remain largely unknown. Elucidating the transcriptional and post-transcriptional regulatory mechanisms, identifying key transcription factors and miRNAs will be crucial. Integrating omics technologies will aid in developing strategies to manipulate triterpene production through genetic engineering approaches. Furthermore, the transport of triterpenoids between different cellular organelles remains an area of active research. Understanding the mechanisms of triterpene transport and their regulation is essential for optimizing metabolic engineering strategies and enhancing the production of triterpenoids in heterologous systems.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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