Plant Gene and Trait 2025, Vol.16, No.6, 264-273
http://genbreedpublisher.com/index.php/pgt

Feature Review **Open Access**

Advances in Saikosaponin Biosynthetic Pathways and Gene Regulation in Bupleurum (*Bupleurum chinense*)

Jianmin Zheng, Chuchu Liu
Institute of Life Sciences, Jiyang College of Zhejiang A&F University, Zhuji, 311800, Zhejiang, China
Corresponding email: chuchu.liu@jicat.org

Plant Gene and Trait, 2025, Vol.16, No.6 doi: 10.5376/pgt.2025.16.0028
Received: 21 Oct., 2025
Accepted: 27 Nov., 2025
Published: 10 Dec., 2025

Preferred citation for this article:
Zheng J.M., and Liu C.C., 2025, Advances in saikosaponin biosynthetic pathways and gene regulation in bupleurum (*Bupleurum chinense*), Plant Gene and Trait, 16(6): 264-273 (doi: 10.5376/pgt.2025.16.0028)

Abstract This study reviews the main metabolic process of saikosaponin, which is formed into a triterpene skeleton from 2,3-epoxysqualene via β-amyrin, and undergoes oxidation and glycosylation modifications under the action of key enzymes such as CYP450 and UGT. It summarizes the progress of gene families such as OSCs, CYP450s, and UGTs and their functional verification. The regulatory laws of hormone signals, environmental stress and transcription factors on the synthetic pathway were summarized. The application of multi-omics technology in the mining of key genes and the construction of metabolic networks was introduced. The strategies for increasing the yield of saikosaponin by using genetic engineering and synthetic biology and the existing bottlenecks at the present stage were also discussed. This study aims to provide a theoretical basis and ideas for understanding the molecular regulatory mechanism of saikosaponin and its application in molecular breeding and industrial production.

Keywords *Bupleurum chinense*; Saikosaponins; Biosynthetic pathways; Key enzyme genes; Integrative multi-omics analysis

1 Introduction

Bupleurum chinense is a commonly used traditional Chinese medicine material and is included in the pharmacopoeias of both China and Japan. Its roots are often used to treat diseases such as colds, fevers, inflammation and liver diseases (He et al., 2021). The most important active component in *Bupleurum chinense* is saikosaponins (SSs), which belong to the triterpene saponin class and account for approximately 7% of the dry weight of the root. These components have multiple pharmacological effects such as anti-inflammatory, liver-protecting, immunomodulatory, antioxidant, anti-tumor and antiviral (Yu et al., 2020; Zhang et al., 2022). Among them, saikosaponin a and d are often used as important indicators for drug efficacy evaluation (Zhou et al., 2021; Mao et al., 2023).

The synthesis process of saikosaponin is very complex, requiring multiple metabolic stages and the participation of many key enzymes. Overall, this process can be divided into three steps: The first step is to synthesize the precursor substances IPP and DMAPP in the MVA and MEP pathways; the second step is to synthesize the triterpene skeleton β-amyrin; the third step is to generate different types of saponins through reactions such as cyclization, hydroxylation and glycosylation (Li et al., 2020; Yang et al., 2022; Zhang et al., 2022). Among these steps, β-amyrin synthase (β-AS), cytochrome P450 enzyme (P450s), and UDP-glycosyltransferase (UGTs) are the most important gene families (He et al., 2021; Wen et al., 2025). Environmental factors, such as drought or hormone treatment, can also affect the expression of these genes, and thereby influence the accumulation of saponins. In addition, transcription factors such as bZIP, ERF, and WRKY are also involved in regulation (Wu et al., 2017; Xu et al., 2019; Han et al., 2022). Although some achievements have been made through transcriptome, metabolome and genomic studies in recent years, the functions and regulatory patterns of many key genes are still not fully clarified (Sui et al., 2020).

This study collates the latest progress in the biosynthetic pathway of saikosaponin, summarizes the identification, function and regulatory methods of related key enzyme genes, discusses the influence of the environment and hormones on saponin synthesis, and looks forward to the application prospects of genetic engineering and molecular breeding in improving the yield and quality of saikosaponin. This study aims to provide theoretical support for a deeper understanding of the molecular regulatory mechanism of saikosaponin and the sustainable development of *Bupleurum chinense* resources.

2 Saikosaponin Types, Structures, and Functions

2.1 Chemical structures and major saikosaponin categories

Saikosaponins (SSs) are unique pentacyclic triterpenoid saponins found in plants of the genus Bupleurum. According to the different structures of saponins, they can be classified into seven major types. Including epoxy-ether type (Type I); heterodiene type (Type II); C12-ene type (Type III); homodiene type (Type IV); C12-ene-C28-carboxylic acid type (Type V); C30-carboxylic acid–heterodiene type (Type VI) and C18-ene type (Type VII). Among them, type I epoxy-ether saponins are the most common and important, such as saikosaponin a and D. They are the components with the highest content in *Bupleurum chinense* and also the key indicators for quality evaluation (Wang et al., 2023). Common saponins also include SSa, SSd, SSc, SSb1, SSb2, etc. (Zhao et al., 2021; Wen et al., 2025). Most saikosaponin belong to oleane-type, but a small amount of lupane-type saponins have also been reported (Wang et al., 2017).

2.2 Biosynthetic origins and structural diversity

The synthesis process of saikosaponin is generally divided into three steps. In the first step, acetyl-CoA generates IPP and DMAPP through the MVA pathway. In the second step, these two precursors undergo multiple reactions to transform into 2,3-epoxysqualene and cyclize to form the triterpene skeleton β-amyrin. In the third step, under the action of enzymes such as P450 and UGT, the skeleton will be further modified to form various saikosaponin monomers (Li et al., 2020; Yang et al., 2022; Zhang et al., 2022). Genomic and transcriptomic studies have shown that gene families such as HMGR, DXR, FPPS, β-AS, P450, and UGT are all crucial at each step. Different tissues, different varieties, and even environmental factors such as drought or hormone treatment can all affect the expression of these genes, thereby influencing the accumulation of saponins and structural diversity (Deng et al., 2025; Wen et al., 2025; Zhou et al., 2025).

2.3 Pharmacological functions linked to saikosaponin profiles

Bupleurum saponins are the main source of the efficacy of Bupleurum bupleurum and have multiple effects such as anti-inflammation, immune regulation, anti-tumor, anti-virus, anti-oxidation, anti-osteoporosis, anti-obesity, and liver protection (Wang et al., 2023; Wen et al., 2025). Saponins of different structures also have pharmacological differences. For instance, SSa and SSd have significant anti-inflammatory, anti-tumor and immunomodulatory effects (Liu et al., 2017; Zhou et al., 2021). SSb2 performed better in anti-inflammatory and acute lung injury models (Peng et al., 2023). Certain structural details, such as 13,28-epoxy bridge or glycogroup types, can also affect activity and selectivity (Sun et al., 2017). In addition, the types and contents of saponins can be affected by varieties, growth locations and processing methods, and these differences can also change the efficacy (Peng et al., 2023; Li et al., 2025).

3 Biosynthetic Pathways of Saikosaponins

3.1 Precursor supply pathways: MVA and MEP pathways

Saikosaponins belong to triterpene saponin compounds, and their precursors mainly come from two pathways: the MVA pathway and the MEP pathway. The MVA pathway takes place in the cytoplasm, gradually converting acetyl-CoA into IPP and DMAPP. The MEP pathway occurs in plastids, starting with pyruvate and glyceraldehyde 3-phosphate, and eventually can also produce the same two precursor molecules. These two pathways jointly provide basic units for the synthesis of triterpene skeletons (Figure 1) (Yang et al., 2022; Mao et al., 2023; Wen et al., 2025).

3.2 Key enzymatic steps in saikosaponin biosynthesis (OSCs, CYPs, UGTs)

The biosynthesis of saikosaponins is generally divided into three stages. The first step is the formation of the triterpene skeleton. OSCs cyclizes 2,3-epoxysqualene under the action of β-amyrin synthase (β-AS) to form β-amyrin, which is the core structure of saikosaponin (Li et al., 2020; Mao et al., 2022). In the second step, cytochrome P450 enzymes (CYPs) oxidize or hydroxylate β-amyrin, which determines the diversity of triterpene structures (Wen et al., 2025). The final step is the glycosylation reaction. UGTs attach different glycogroups to the triterpene skeleton to generate different types of saikosaponins (Zhang et al., 2022). Multi-omics studies have screened out multiple related genes, such as HMGR, FPPS, β-AS, P450s and UGTs. The expression levels of these genes are often positively correlated with saponin content (Mao et al., 2023).

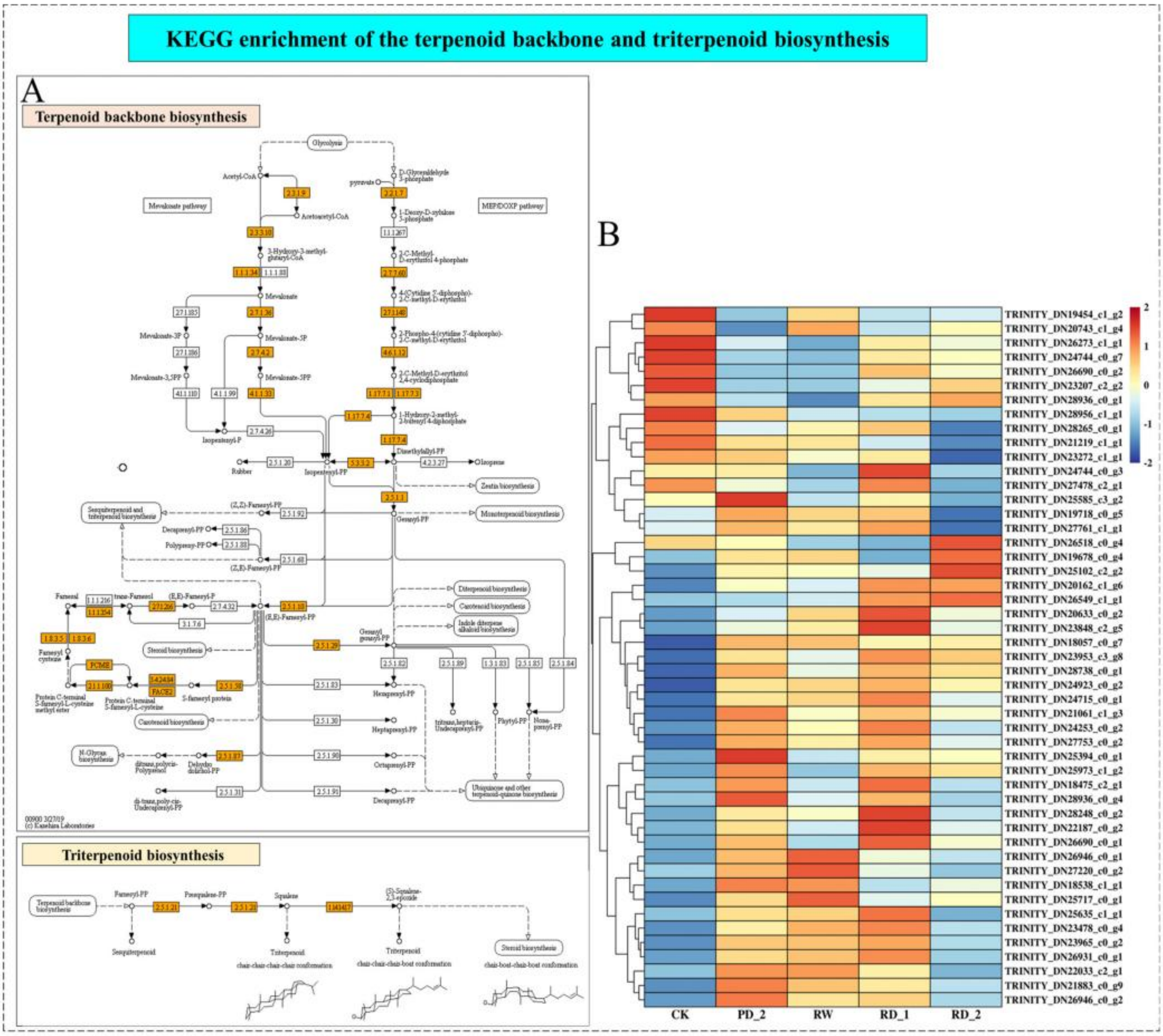

Figure 1 KEGG enrichment of the terpenoid backbone and triterpenoid biosynthesis (A) and cluster analysis of the terpenoid backbone and triterpenoid biosynthesis differentially expressed genes (B) (Adopted from Yang et al., 2022)

3.3 Pathway reconstruction based on current biochemical and genomic evidence

With the accumulation of transcriptome, metabolome and genomic data, the synthetic pathway of saikosaponins has become clearer. Two pathways, MVA and MEP, provide IPP and DMAPP. Subsequently, 2,3-epoxysqualene is formed through enzymes such as FPPS, SQS, and SE. Then, β-AS is used to generate β-amyrin. Finally, different saponin monomers are formed through CYPs and UGTs (Li et al., 2020; Zhang et al., 2022; Wen et al., 2025). Genomic sequencing also shows that the P450 and UGT families are very abundant in *Bupleurum chinense*. Through tissue-specific expression analysis and induction experiments (such as MeJA treatment), researchers have identified multiple key genes (He et al., 2021; Mao et al., 2023). In addition, environmental factors such as drought and hormone treatment can also affect the expression of these genes, thereby significantly altering saponin accumulation (Wang et al., 2020; Deng et al., 2025).

4 Gene Families Involved in Saikosaponin Biosynthesis

4.1 Functions of OSC genes in triterpenoid skeleton formation

2,3-epoxysqualene cyclase (OSC) is the initiating key enzyme for the synthesis of triterpene saponins. It cyclizes 2,3-epoxysqualene into β-amyrin, providing a basic framework for subsequent various modification reactions. Studies have identified multiple OSC genes in *Bupleurum chinense*, among which β-amyrin synthase (β-AS) is the most important. Its expression level is usually closely related to the content of saikosaponin and is a key gene regulating saponin production (He et al., 2021; Zhang et al., 2022). The amplification of the number of OSC genes and the functional differentiation among different genes also provide a molecular basis for the diversity of triterpene structures (Chen et al., 2021; Wang et al., 2021).

4.2 Roles of CYP450 genes in oxidation and structural diversification

The cytochrome P450 enzyme (CYP450) family plays a core role in the oxidation and further modification of the triterpene skeleton. More than 260 CYP450 genes have been identified in the Bupleurum genome, mainly distributed in subfamilies such as CYP72, CYP90, CYP710, and CYP711 (He et al., 2021). These enzymes can undergo reactions such as hydroxylation, epoxidation or double bond formation on β-amyrin, thereby generating saponin molecules of different structures (Yu et al., 2020). Some of these genes (such as Bc95697, Bc35434, Bc087391, Bc036879) are significantly associated with the accumulation of specific saponins and show specific expression under different tissues or stress treatments (such as hormone induction) (Zhang et al., 2022; Wen et al., 2025). The CYP716 family plays a particularly important role in the C-28 oxidation process of triterpene molecules and is a key step in determining the diversification of saponin structures (Ghosh, 2017; Sandeep et al., 2018; Cheng et al., 2020).

4.3 UGT genes responsible for glycosylation patterns

The UDP-glycosyltransferase (UGT) family is responsible for adding sugar groups to different positions of the triterpene skeleton and is the main enzyme that determines the glycosylation mode and biological activity of saponins. Approximately 80 to 102 UGT genes have been identified in *Bupleurum chinense*. Some of them, such as BcUGT1, BcUGT2, and BcUGT3, are closely related to saponin synthesis (He et al., 2021; Zhang et al., 2022). UGT can add monosaccharides or polysaccharides at sites such as C-3 and C-28, thereby forming monosaccharides or disaccharides, greatly increasing the structural and functional types of saponins (Cheng et al., 2020; Gharabli et al., 2023). The expression of these genes is affected by tissue type, developmental stage and exogenous hormone stimulation, and is an important mechanism for regulating saponin accumulation (He et al., 2021; Zhang et al., 2022).

5 Regulation of Saikosaponin Biosynthesis

5.1 Transcription factors regulating pathway genes

A variety of transcription factor families play an important role in the synthesis of saikosaponins. Transcriptome studies have found that genes from families such as bHLH, MYB, WRKY, and ERF are significantly upregulated after different stresses or hormone treatments, and they can regulate the expression of key enzyme genes. For instance, under drought and MeJA treatment, the expressions of bHLH, MYB and WRKY genes all increased, and were closely related to the changes in saponin content (Yang et al., 2022; Mao et al., 2023; Deng et al., 2025). Among them, WRKY6, WRKY16, WRKY32, and WRKY35 were induced to express under NaCl and PEG stress, and the accumulation trend of saponins was consistent with them, indicating that they might be involved in regulating biosynthesis (Wu et al., 2017). In addition, the expression of BcERF3 of the AP2/ERF family increased after MeJA treatment. Overexpression could significantly increase the contents of saponins a, c, and d, indicating that it is a positive regulatory factor (Figure 2) (Han et al., 2022).

5.2 Hormonal regulation and stress-responsive pathways

Plant hormones can also affect the synthesis of saikosaponins. Jasmonic acid (JA), salicylic acid (SA), abscisic acid (ABA) and brassinolide (BRs) can all be involved in regulation. Drought stress can increase the contents of JA, SA and ABA, activate the corresponding signaling pathways, and thereby promote the expression of related genes (Yang et al., 2022). MeJA treatment can upregulate the genes of the MVA/MEP pathway and the saponin synthesis pathway, significantly increasing the contents of saponins a and d (Wang et al., 2020; Mao et al., 2023). Exogenous BRs (0.2 mg/L) can also up-regulate key genes such as HMGR, DXR, IPPI, FPS, and P450, and promote the synthesis of various saikosaponins (Zhou et al., 2025). In addition, external stresses such as salt stress, low temperature and drought can also jointly affect the accumulation of saponins by regulating transcription factors and hormone signals (Wu et al., 2017; Deng et al., 2025).

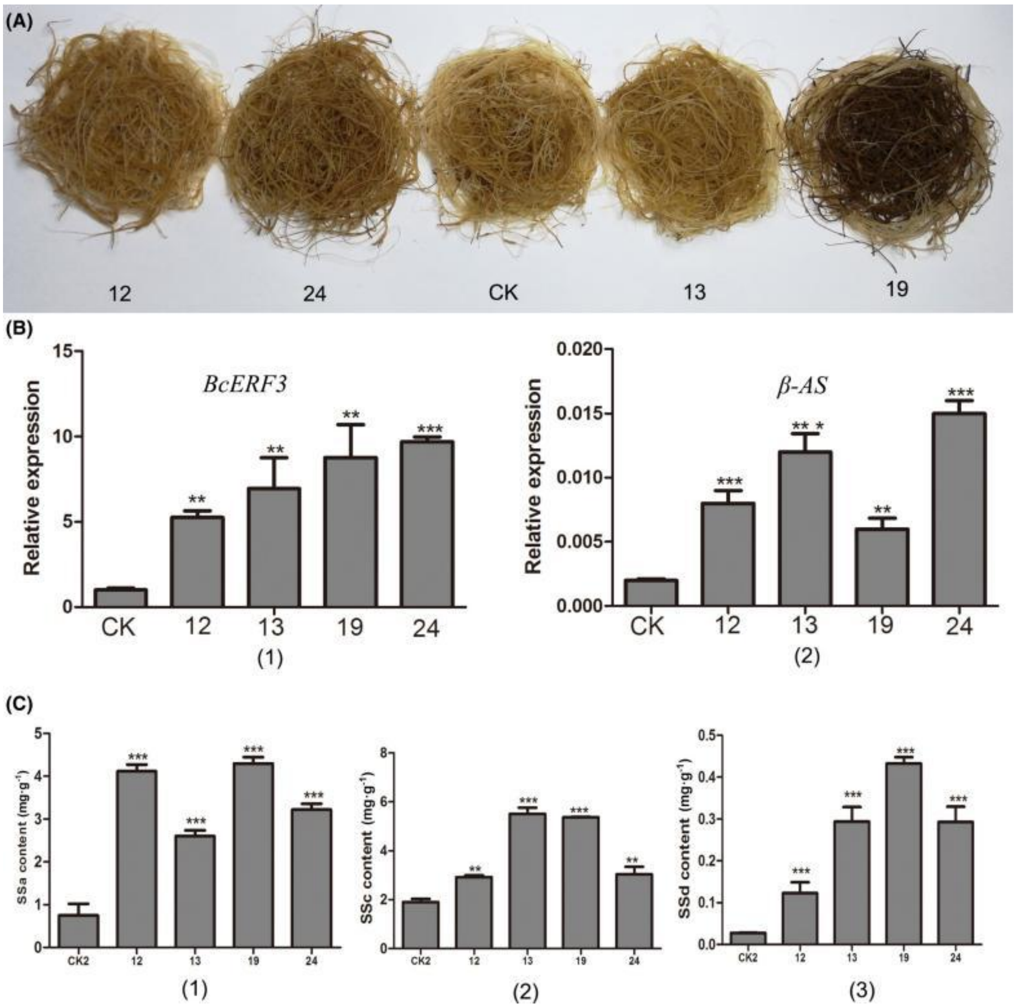

Figure 2 Analysis on the transgenic lines overexpressing *BcERF3* (Adopted from Han et al., 2022)

Image caption: (A) *BcERF3* and *pK2GW7* empty vector transformed hairy roots of *Bupleurum chinense*. (B) The expression of *BcERF3* and *β-AS* in transgenic lines assayed by real-time qRT-PCR showing higher expression levels in *BcERF3* transformed lines. (C) The content of SS a, c, and d in transgenic lines assayed by HPLC showing elevated biosynthesis of SSs in *BcERF3* transformed lines. Bar graphs represent mean ± SD, $n = 3$. Student's t test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (Adopted from Han et al., 2022)

5.3 Post-transcriptional regulatory mechanisms

At present, there are relatively few studies on the post-transcriptional regulation of saikosaponins. However, previous studies have pointed out that there is often a "time lag" between changes in gene expression and saponin accumulation, indicating that factors such as mRNA stability and translation regulation may also be involved in regulating the final yield. The asynchronous rate of short-term gene upregulation and saponin accumulation caused by environmental changes also supports the role of post-transcriptional regulation (Yang et al., 2019; Mao et al., 2022).

6 Multi-omics Insights into Pathway and Regulatory Networks

6.1 Genomics and transcriptomics for gene discovery and expression analysis

High-quality chromosome-level genomes enable researchers to more systematically explore genes related to saikosaponins. The genomic sequencing of B. chinense shows that gene families such as P450 and UGT have significantly expanded. These genes are closely related to saponin synthesis. Combining RNA-Seq data from different tissues, researchers identified candidate genes with high expression in the third stage of saponin synthesis, providing a basis for subsequent functional validation and molecular breeding (Zhang et al., 2022). Transcriptome analysis also revealed that the expression levels of genes such as HMGR, FPPS, β-AS, and P450 varied with organ types or treatment conditions, and had a good consistency with saponin content. The expression of some P450 and UGT genes was even positively correlated with the accumulation of major components such as saponins A and D (Yu et al., 2020; He et al., 2021). In addition, transcription factors such as AP2/ERF, WRKY, and bHLH have also been confirmed to regulate the expression of these saponin synthesis genes (Wu et al., 2017; Han et al., 2022; Mao et al., 2023).

6.2 Metabolomics for profiling saikosaponin accumulation

Metabolomics, through high-throughput mass spectrometry technology, can simultaneously detect saikosaponins and their derivatives in different tissues, varieties and under different treatment conditions. Studies have shown that there are significant differences in saponin types and contents among different Bupleurum species or cultivated varieties, and they closely correspond to the expression of related genes (He et al., 2021; Wan et al., 2022; Wen et al., 2025). Exogenous hormone treatments, such as BRs and MeJA, can significantly increase the contents of saponins A and D, and also up-regulate key genes in the synthetic pathway (Mao et al., 2023; Zhou et al., 2025). Some environmental factors, such as drought, salt flanks, and cultivation methods (such as intercropping), can also affect saponin accumulation (Zhang et al., 2024).

6.3 Integrative omics approaches for mapping biosynthetic networks

Integrating the transcriptome and metabolome can better analyze the relationship between gene expression and metabolite content, and identify key nodes in the regulatory network. Multiple studies, through joint analysis, have found that genes such as CYP450, UGT, and β-AS are highly correlated with the contents of certain specific saponins (such as 6"-acetyl saikosaponin A, saikosaponin B1, C, and D). The subcellular localization of some P450 genes further indicates that they are indeed involved in saponin synthesis (Wan et al., 2022; Wen et al., 2025). Furthermore, the combination of the genome, transcriptome and metabolome provides a solid theoretical and technical basis for analyzing the overall regulation of saponin synthesis, confirming the functions of key genes, and conducting molecular breeding (He et al., 2021; Zhang et al., 2022).

7 Case Study: Functional Characterization of a Key Saikosaponin Biosynthetic Gene

7.1 Background and selection of target gene from high-yielding germplasm

Bupleurum chinense is a commonly used traditional Chinese medicine, and its main active ingredient is saikosaponins (SSs). These components have obvious pharmacological effects. With the development of omics technology, researchers have identified some key genes in the biosynthesis of SSs through the combined analysis of metabolomics and transcriptomics. Among them, β-amyrin synthase (β -AS) is the rate-limiting enzyme for the formation of triterpene saponin skeletons and is regarded as the core gene controlling the accumulation of SSs. In the expression studies of different tissues and high saponin strains, it was found that β-AS and its related genes were expressed higher in high-yield materials. Therefore, they are often selected as the key objects for functional verification (Yu et al., 2020; He et al., 2021; Mao et al., 2022).

7.2 Experimental workflow: cloning, expression profiling, and functional assays

In research, materials with high saponin content are usually selected. Total RNA is extracted and reverse transcribed to generate cDNA, and then the full-length sequence of β-AS genes (such as BcBAS1) is cloned using specific primers. Subsequently, the expression of this gene in different tissues such as roots, stems, leaves and flowers was analyzed by qRT-PCR. The results showed that the expression level of β-AS was positively correlated with the content of SSs (Li et al., 2020; Mao et al., 2022). To further demonstrate the gene's function, researchers transferred BcBAS1 into Escherichia coli, yeast or Pichia pastoris for expression and detected the product with GC-MS, confirming that it could catalyze 2,3-epoxysqualene into β-amyrin. In addition, subcellular localization experiments indicated that the enzyme works in the cytoplasm, which is in line with its role in metabolic pathways.

7.3 Key findings and implications for metabolic engineering

These functional verification results indicate that β-AS genes like BcBAS1 play a crucial role in the synthesis of SSs. Their expression levels will directly affect the generation efficiency of saponins. Recombinant proteins can efficiently catalyze the production of β-amyrin and are most strongly expressed in high-yield materials and root tissues (Li et al., 2020; Mao et al., 2022). In addition, the regulation of downstream modification enzymes such as P450 and UGT is also very important, and they will jointly affect the structure and content of the final saponins (Yu et al., 2020; He et al., 2021). These research results provide ideas and molecular targets for increasing the yield of saikosaponins through genetic engineering. For instance, the synthetic efficiency of SSs can be enhanced in engineered yeasts or plants by increasing the expression level of β-AS or regulating its promoter activity. These methods are expected to be used for the sustainable development of medicinal resources and can also support the research and development of new drugs.

8 Breeding and Biotechnological Applications

8.1 Germplasm improvement via marker-assisted selection

Molecular marker-assisted selection (MAS) has become an important method in modern plant breeding. For *Bupleurum chinense*, researchers have developed a variety of genomic SSR and EST-SSR molecular markers. These markers can be used for germplasm identification and genetic structure analysis, and also assist breeders in selecting materials with high saponin content, thereby accelerating the breeding process of superior strains. Meanwhile, *Bupleurum chinense* in different regions also shows significant differences in saponin content and genotype. Combined with phenotypic, chemical type and molecular diversity analysis, it can provide a more reliable basis for screening high-quality germplasm (Li et al., 2025).

8.2 Metabolic engineering and synthetic biology strategies

Metabolic engineering is an important means to increase the yield of saikosaponin. By regulating the expression of key genes such as HMGR, FPS, SE, β-AS, P450, and UGT, the synthesis efficiency of saponins can be significantly enhanced. Studies have found that exogenous hormones such as methyl jasmonate and brassinolide can up-regulate these key genes, thereby promoting the accumulation of saponins (Wang et al., 2020; Deng et al., 2025; Zhou et al., 2025). Furthermore, the cloning and functional identification of key enzyme genes such as β-AS provide clear targets for metabolic engineering (Li et al., 2020). Transcription factors such as ERF, MYB and bHLH are also involved in regulating the saponin biosynthesis network. Their overexpression usually significantly increases the saponin content (Han et al., 2022; Mao et al., 2023). In synthetic biology, introducing these key genes into heterologous systems such as yeast or Pichia pastoris can achieve efficient production of saponins and further expand the structural diversity of saponins (Mao et al., 2022).

8.3 Potential of CRISPR/Cas applications for pathway enhancement

CRISPR/Cas gene editing technology offers a new possibility for the precise regulation of the biosynthesis of saikosaponins. This technology can perform site-specific knockout, insertion or activation of key enzyme genes or regulatory factors, thereby adjusting the metabolic flow direction and increasing saponin production. Although its application in *Bupleurum chinense* is still limited at present, studies on other medicinal plants have proved that CRISPR/Cas can efficiently regulate secondary metabolic pathways, demonstrating great application potential (Cardi et al., 2023; Devi et al., 2023). In the future, by integrating genomic information and high-throughput screening, CRISPR/Cas is expected to accelerate the cultivation of high-yield germplasm of *Bupleurum chinense* and also promote the targeted modification of saponin synthesis pathways.

9 Challenges and Future Directions

9.1 Complexity of triterpenoid biosynthesis and gene redundancy

The triterpene biosynthetic pathway of saikosaponin is extremely complex. This process not only involves multiple metabolic branches but also many gene families with similar functions. Research shows that β-amyrin synthase (β-AS), P450 enzyme and UGT glycosyltransferase are not just one gene, but have multiple copies. Their expressions vary greatly in different tissues, different growth stages and different environments, which also causes obvious functional overlap and makes the regulatory network more complex (He et al., 2021; Mao et al., 2022).

Meanwhile, the P450 and UGT families themselves have a large number of members. It is difficult to directly determine which genes are truly involved in the structural modification of saponins merely by sequence, which also makes the subsequent functional verification more difficult (Zhang et al., 2022; Mao et al., 2023). The differences in gene expression and metabolite composition among different Bupleurum species and varieties are also significant, and these differences further increase the complexity of the research (Yu et al., 2020; Wan et al., 2022; Wen et al., 2025).

9.2 Need for high-quality genomes and pathway-specific functional tools

Although high-quality chromosome-level genomes have been obtained at present, laying the foundation for the exploration of saponin biosynthesis-related genes, issues such as gene family expansion, copy number variation, and tissue-specific expression still require more detailed omics data for explanation (Zhang et al., 2022). Nowadays, the screening of functional genes usually relies on the combined analysis of multiple omics such as transcriptomics and metabolomics. However, due to the lack of efficient gene editing, transformation and expression systems, it is still difficult to conduct rapid and accurate functional verification of key genes (Yu et al., 2020; He et al., 2021). Furthermore, the transcription factor network regulating saponin synthesis is not yet clear enough, and more specific molecular tools and more efficient verification platforms need to be developed (Han et al., 2022; Mao et al., 2023).

9.3 Prospects for precision metabolic regulation and industrial production

In the future, if high-quality genomic, transcriptomic and metabolomic data can be combined, the functions of key enzymes and regulatory factors can be analyzed more clearly, providing more reliable theoretical support for regulating saponin metabolic flow and increasing the content of active ingredients (He et al., 2021). Through gene editing and synthetic biology, there is also an opportunity to reconstruct and optimize the saponin synthesis pathway, thereby promoting the industrial production of saikosaponins (Mao et al., 2022; Zhang et al., 2022; Mao et al., 2023). In addition, the influence of environmental factors such as drought and hormone treatment on saponin synthesis also provides new ideas for cultivation management and quality improvement (Wang et al., 2020; Yang et al., 2022; Zhou et al., 2025). In the future, multi-disciplinary collaboration will be even more necessary to promote the development of a complete chain from genetic research to industrial application.

Acknowledgments

The authors appreciate the comments from Dr. Xia on the manuscript of this study. The author also thanks the members of the research group for helping to organize the research data.

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Cardi T., Murovec J., Bakhsh A., Boniecka J., Bruegmann T., Bull S., Eeckhaut T., Fladung M., Galović V., Linkiewicz A., Lukan T., Mafra I., Michalski K., Kavas M., Nicolia A., Nowakowska J., Sági L., Sarmiento C., Yıldırım K., Zlatković M., Hensel G., and Van Laere K., 2023, CRISPR/Cas-mediated plant genome editing: outstanding challenges a decade after implementation, Trends in Plant Science, 28(10): 1144-1165.
https://doi.org/10.1016/j.tplants.2023.05.012

Chen K., Zhang M., Ye M., and Qiao X., 2021, Site-directed mutagenesis and substrate compatibility to reveal the structure-function relationships of plant oxidosqualene cyclases, Natural Product Reports, 38: 2261-2275.
https://doi.org/10.1039/d1np00015b

Cheng Y., Liu H., Tong X., Liu Z., Zhang X., Li D., Jiang X., and Yu X., 2020, Identification and analysis of CYP450 and UGT supergene family members from the transcriptome of *Aralia elata* (Miq.) seem reveal candidate genes for triterpenoid saponin biosynthesis, BMC Plant Biology, 20: 214.
https://doi.org/10.1186/s12870-020-02411-6

Deng W., Du X., Xiao Y., Zhu X., and Yu D., 2025, Strategies for increasing saikosaponins accumulation in Bupleurum: insights from environmental and microbial regulation, Planta, 262: 35.
https://doi.org/10.1007/s00425-025-04748-4

Devi A., Devi K., Devi P., Devi M., and Das S., 2023, Metabolic engineering of plant secondary metabolites: prospects and its technological challenges, Frontiers in Plant Science, 14: 1171154.
https://doi.org/10.3389/fpls.2023.1171154

Gharabli H., Della Gala V., and Welner D., 2023, The function of UDP-glycosyltransferases in plants and their possible use in crop protection, Biotechnology Advances, 67: 108182.
https://doi.org/10.1016/j.biotechadv.2023.108182

Ghosh S., 2017, Triterpene structural diversification by plant cytochrome P450 enzymes, Frontiers in Plant Science, 8: 1886.
https://doi.org/10.3389/fpls.2017.01886

Han W., Xu J., Wan H., Zhou L., Wu B., Gao J., Guo X., Sui C., and Wei J., 2022, Overexpression of *BcERF3* increases the biosynthesis of saikosaponins in *Bupleurum chinense*, FEBS Open Bio., 12(7): 1344-1352.
https://doi.org/10.1002/2211-5463.13412

He Y., Chen H., Zhao J., Yang Y., Yang B., Feng L., Zhang Y., Wei P., Hou D., Zhao J., and Yu M., 2021, Transcriptome and metabolome analysis to reveal major genes of saikosaponin biosynthesis in *Bupleurum chinense*, BMC Genomics, 22: 839.
https://doi.org/10.1186/s12864-021-08144-6

Li F., Ge K., Huang R., Lu Y., Lin H., Jiang Y., Tian E., and Chao Z., 2025, Insights into infraspecific differentiation of the medicinally important species *Bupleurum chinense* revealed by morphological and molecular evidence, BMC Plant Biology, 25: 626.
https://doi.org/10.1186/s12870-025-06661-0

Li J., Wang C., Qi W., and Liu C., 2020, Cloning and functional characterization of the β-amyrin synthase genefrom *Bupleurum chinense*, Biologia Plantarum, 64: 314-319.
https://doi.org/10.32615/bp.2020.008

Liu X., Latkolik S., Atanasov A., Kunert O., Pferschy-Wenzig E., Heiss E., Malainer C., Schinkovitz A., Kollroser M., Dirsch V., and Bauer R., 2017, *Bupleurum chinense* roots: a bioactivity-guided approach toward saponin-type NF- κ B inhibitors, Planta Medica, 83: 1242-1250.
https://doi.org/10.1055/s-0043-118226

Mao Y., Chen H., Zhao J., Li Y., Feng L., Yang Y., Zhang Y., Wei P., and Hou D., 2022, Molecular cloning, functional characterization and expression of the β-amyrin synthase gene involved in saikosaponin biosynthesis in *Bupleurum chinense* DC., Journal of Plant Biochemistry and Biotechnology, 32: 284-295.
https://doi.org/10.1007/s13562-022-00804-2

Mao Y., Yang Y., Li Y., Zhang Y., Wei P., Chen H., and Hou D., 2023, Comparative transcriptome analysis provides insights into the molecular mechanism underlying the effect of MeJA treatment on the biosynthesis of saikosaponins in *Bupleurum chinense* DC., Life, 13(2): 563.
https://doi.org/10.3390/life13020563

Peng D., Chen Y., Sun Y., Zhang Z., Cui N., Zhang W., Qi Y., Zeng Y., Hu B., Yang B., Wang Q., and Kuang H., 2023, Saikosaponin A and its epimers alleviate LPS-induced acute lung injury in mice, Molecules, 28(3): 967.
https://doi.org/10.3390/molecules28030967

Sandeep, Misra R., Chanotiya C., Mukhopadhyay P., and Ghosh S., 2018, Oxidosqualene cyclase and CYP716 enzymes contribute to triterpene structural diversity in the medicinal tree banaba, The New Phytologist, 222(1): 408-424.
https://doi.org/10.1111/nph.15606

Sui C., Han W., Zhu C., and Wei J., 2020, Recent progress in saikosaponin biosynthesis in Bupleurum, Current Pharmaceutical Biotechnology, 22(3): 329-340.
https://doi.org/10.2174/1389201021999200918101248

Sun C., Geng C., Huang X., Ma Y., Zheng X., Yang T., Chen X., Yin X., Zhang X., and Chen J., 2017, Bioassay-guided isolation of saikosaponins with agonistic activity on 5-hydroxytryptamine 2C receptor from *Bupleurum chinense* and their potential use for the treatment of obesity, Chinese Journal of Natural Medicines, 15(6): 467-473.
https://doi.org/10.1016/s1875-5364(17)30070-5

Wan H., Zhou L., Wu B., Han W., Sui C., and Wei J., 2022, Integrated metabolomics and transcriptomics analysis of roots of *Bupleurum chinense* and *B. scorzonerifolium*, two sources of medicinal Chaihu, Scientific Reports, 12: 22335.
https://doi.org/10.1038/s41598-022-27019-8

Wang H., Zhang G., Gao Z., Sui C., Ji H., Jiang J., Guo X., and Wei J., 2020, Transcriptome profiling of *Bupleurum chinense* DC. root provides new insights into the continuous inflorescence removal induced improvements to root growth and saikosaponin biosynthesis, Industrial Crops and Products, 160: 113085.
https://doi.org/10.1016/j.indcrop.2020.113085

Wang J., Guo Y., Yin X., Wang X., Qi X., and Xue Z., 2021, Diverse triterpene skeletons are derived from the expansion and divergent evolution of 2,3-oxidosqualene cyclases in plants, Critical Reviews in Biochemistry and Molecular Biology, 57: 113-132.
https://doi.org/10.1080/10409238.2021.1979458

Wang Y., Guo Q., Cheng Z., Zeng K., Liang H., Tu P., Chen S., and Zhang Q., 2017, New saikosaponins from the roots of *Bupleurum chinense*, Phytochemistry Letters, 21: 183-189.
https://doi.org/10.1016/j.phytol.2017.06.005

Wang Z., Tian L., Xiao Y., Zhao M., Chang Y., Zhou Y., Liu S., Zhao H., and Xiu Y., 2023, Quantitative and differential analysis between *Bupleurum chinense* DC. and *Bupleurum scorzonerifolium* Willd. using HPLC-MS and GC-MS coupled with multivariate statistical analysis, Molecules, 28(15): 5630.
https://doi.org/10.3390/molecules28155630

Wen D., Ji H., Rong M., Liu Y., Jiang J., Guo X., Gao Z., Xu Y., and Wei J., 2025, Integrated metabolomics and transcriptomics reveal differences in terpenoids and the molecular basis among the roots of three *Bupleurum* species, BMC Plant Biology, 25: 403.
https://doi.org/10.1186/s12870-025-06441-w

Wu S., Gao K., Liu X., Xu J., Wei J., and Sui C., 2017, Identification of WRKY transcription factors related to saikosaponin biosynthesis in adventitious roots of *Bupleurum chinense*, Chinese Herbal Medicines, 9: 153-160.
https://doi.org/10.1016/s1674-6384(17)60089-3

Xu J., Wu S., Xu Y., Ge Z., Sui C., and Wei J., 2019, Overexpression of BcbZIP134 negatively regulates the biosynthesis of saikosaponins. Plant Cell, Tissue and Organ Culture (PCTOC), 137: 297-308.
https://doi.org/10.1007/s11240-019-01571-0

Yang L., Qiao L., Su X., Ji B., and Dong C., 2022, Drought stress stimulates the terpenoid backbone and triterpenoid biosynthesis pathway to promote the synthesis of saikosaponin in *Bupleurum chinense* DC. Roots, Molecules, 27(17): 5470.
https://doi.org/10.3390/molecules27175470

Yang L., Zhao Y., Zhang Q., Cheng L., Han M., Ren Y., and Yang L., 2019, Effects of drought-re-watering-drought on the photosynthesis physiology and secondary metabolite production of *Bupleurum chinense* DC., Plant Cell Reports, 38: 1181-1197.
https://doi.org/10.1007/s00299-019-02436-8

Yu M., Chen H., Liu S., Li Y., Sui C., Hou D., and Wei J., 2020, Differential expression of genes involved in saikosaponin biosynthesis between *Bupleurum chinense* DC. and *Bupleurum scorzonerifolium* Willd., Frontiers in Genetics, 11: 583245.
https://doi.org/10.3389/fgene.2020.583245

Zhang Q., Li M., Chen X., Liu G., Zhang Z., Tan Q., Hu Y., Fan Y., Liu Y., Zhu T., Yang X., Yue M., Bu X., and Zhang Y., 2022, Chromosome-level genome assembly of *Bupleurum chinense* DC provides insights into the saikosaponin biosynthesis, Frontiers in Genetics, 13: 878431.
https://doi.org/10.3389/fgene.2022.878431

Zhang R., Li X., Qu J., Zhang D., Cao L., Qin X., and Li Z., 2024, Intercropping with maize and sorghum-induced saikosaponin accumulation in *Bupleurum chinense* DC. by liquid chromatography-mass spectrometry-based metabolomics, Journal of Mass Spectrometry, 59(6): e5035.
https://doi.org/10.1002/jms.5035

Zhao H., Wang X., Zhang Y., Huang X., Jiang Y., Ma H., An L., Wu X., and Wang Q., 2021, Quantitative ^{1}H NMR for the direct quantification of saikosaponins in *Bupleurum chinense* DC., Analytical Sciences, 37: 1413-1418.
https://doi.org/10.2116/analsci.20p462

Zhou P., Shi W., He X., Du Q., Wang F., and Guo J., 2021, Saikosaponin D: review on the antitumour effects, toxicity and pharmacokinetics, Pharmaceutical Biology, 59: 1478-1487.
https://doi.org/10.1080/13880209.2021.1992448

Zhou S., Yang L., Wan J., Chen L., Gu X., Qiao L., Chu L., Dong N., Dong C., and Feng W., 2025, Suitable concentrations of brassinolide application enhances growth performance and saikosaponin biosynthesis by transcriptional activation of triterpenoid pathway genes in *Bupleurum chinense* DC., Frontiers in Plant Science, 16: 1517434.
https://doi.org/10.3389/fpls.2025.1517434