Global Journal of Research in
Chemistry and Pharmacy
Journal homepage: https://gsjournals.com/gjrcp/
ISSN: 2980-4213 (Online)

(REVIEW ARTICLE)

Check for updates

BIOSYNTHETIC PATHWAYS AND METABOLIC ENGINEERING OF HIGH-VALUE PLANT SECONDARY METABOLITES

Erlina Ammu Thomas *

St. Joseph's College of Pharmacy, Cherthala, Kerala, India.
* Corresponding Author

Global Journal of Research in Chemistry and Pharmacy, 2026, 05(01), 001–009

Publication history: Received on 03 January 2026; revised on 25 January 2026; accepted on 29 January 2026

Article DOI: https://doi.org/10.58175/gjrcp.2026.5.1.0005

ABSTRACT

Plant secondary metabolites represent a vast and diverse array of compounds with significant pharmaceutical, nutritional, and industrial value. However, their natural abundance in planta is often exceedingly low, rendering traditional extraction methods insufficient to meet commercial demand. The elucidation of biosynthetic pathways and the application of metabolic engineering have emerged as transformative strategies to address this challenge. This review synthesizes recent breakthroughs in pathway elucidation, key enzyme characterization, and synthetic biology strategies for the sustainable, scalable production of high-value plant secondary metabolites. We critically examine advances in the biosynthesis of major compound classes, including terpenoids, alkaloids, flavonoids, and other specialized metabolites. Recent technological innovations—including single-cell RNA sequencing, multi-omics integration, and CRISPR/Cas9 genome editing—have dramatically accelerated pathway discovery and engineering capabilities. Furthermore, we evaluate the complementary roles of plant and microbial chassis for heterologous production, the emerging significance of biosynthetic gene clusters, and the application of synthetic biology approaches such as co-culture systems and cell-free platforms. Despite substantial progress, challenges persist in pathway complexity, enzyme characterization, and scalable production. This review identifies critical research gaps and discusses future directions for achieving commercially viable production of plant-derived natural products.

Keywords: Biosynthetic Pathways; Metabolic Engineering; Synthetic Biology; Plant Secondary Metabolites; Terpenoids; Alkaloids; Heterologous Production

1 INTRODUCTION

Plants produce an extraordinary diversity of secondary metabolites—often referred to as specialized metabolites—that play essential roles in plant adaptation, defense, and reproduction. These compounds, including terpenoids, alkaloids, flavonoids, and glucosinolates, have been extensively exploited for their pharmaceutical, nutritional, flavor, and industrial applications (Liu et al., 2023). Despite their immense value, the commercial production of plant secondary metabolites faces a fundamental challenge: native plants typically accumulate these compounds in trace amounts, making extraction inefficient and unsustainable (Kumari et al., 2024).

Traditional approaches to obtaining plant secondary metabolites have relied on field cultivation and extraction, which are constrained by land availability, susceptibility to pests and diseases, and the multi-step, often costly nature of

chemical synthesis (Han and Miao, 2024). The growing demand for these compounds, coupled with the recognition that many plant species are threatened by overharvesting, has catalyzed a paradigm shift toward biotechnological production strategies (Birchfield and McIntosh, 2024).

The elucidation of biosynthetic pathways and the application of metabolic engineering have emerged as transformative approaches to address these limitations. Over the past decades, significant progress has been made in understanding the biosynthesis of high-value plant natural products, including artemisinin, taxol, vinblastine, and numerous other therapeutically important compounds (Kumari et al., 2024). Advances in genomics, transcriptomics, metabolomics, and emerging single-cell technologies have dramatically accelerated pathway discovery, while synthetic biology has enabled the reconstitution of complete pathways in heterologous hosts (Wu et al., 2024).

This review provides a comprehensive synthesis of recent breakthroughs in the biosynthesis and metabolic engineering of high-value plant secondary metabolites. We examine pathway elucidation strategies, key enzyme characterization, metabolic engineering approaches in plant and microbial systems, and emerging synthetic biology platforms for sustainable production.

2 PATHWAY ELUCIDATION: FROM GENOMICS TO SINGLE-CELL RESOLUTION

2.1 Multi-Omics Approaches to Pathway Discovery

The elucidation of biosynthetic pathways is the foundational step for metabolic engineering. Advances in multi-omics technologies have dramatically accelerated the discovery of biosynthetic pathways for plant secondary metabolites (Wang et al., 2024). Genomics, transcriptomics, proteomics, and metabolomics have each contributed new insights into biosynthetic gene clusters (BGCs), metabolic pathways, and stress responses (Wang et al., 2024). However, single-omics approaches often fail to fully address the complex processes underlying specialized metabolism. Integrated multi-omics provides a holistic perspective on key regulatory networks, enabling the identification of candidate genes and the reconstruction of complete pathways (Wang et al., 2024).

High-throughput sequencing has enabled the assembly of medicinal plant genomes, facilitating the identification of biosynthetic genes through comparative genomics and co-expression analysis (Wang et al., 2024). Transcriptome mining has been particularly valuable for identifying genes involved in the biosynthesis of valuable metabolites. For instance, transcriptome mining and functional screening using a precursor-providing yeast chassis enabled the elucidation of the complete (+)-nootkatone biosynthetic pathway in Alpinia oxyphylla (Deng et al., 2024). Through this approach, a novel (+)-valencene synthase (AoVS) and three (+)-valencene oxidases (AoCYP6, AoCYP9, and AoCYP18) were identified, along with a cytochrome P450 reductase (AoCPR1) and three dehydrogenases (AoSDR1/2/3), enabling the successful reconstruction of the pathway in Saccharomyces cerevisiae (Deng et al., 2024).

Integrated genomic, transcriptomic, and metabolomic analyses have provided valuable insights into the biosynthesis of triterpenoid saponins in medicinal Ilex plants, demonstrating the power of multi-omics for pathway discovery in non-model species (The Plant Journal, 2024).

2.2 Single-Cell Technologies for Pathway Elucidation

Traditional bulk RNA-sequencing approaches often fail to resolve the cell-type-specific expression patterns that are characteristic of plant specialized metabolism. The emergence of single-cell RNA sequencing (scRNA-seq) has revolutionized pathway elucidation by enabling the identification of specialized cell types where biosynthesis occurs (Wu et al., 2024).

In a landmark study, scRNA-seq was employed to elucidate the complete biosynthesis of hyperforin, the antidepressant compound responsible for the effectiveness of St. John's wort (Hypericum perforatum) (Wu et al., 2024). Gene discovery based on co-expression analysis of bulk RNA-sequencing data or genome mining had previously failed to discover the missing steps in hyperforin biosynthesis. By sequencing the 1.54-Gb tetraploid H. perforatum genome and applying scRNA-seq, the researchers identified a type of cell, termed "Hyper cells," wherein hyperforin biosynthesis de novo takes place in both leaves and flowers (Wu et al., 2024). Through pathway reconstitution in yeast and tobacco, they identified and characterized four transmembrane prenyltransferases (HpPT1-4) that are localized at the plastid envelope and complete the hyperforin biosynthetic pathway. The hyperforin polycyclic scaffold is created by a reaction cascade involving an irregular isoprenoid coupling and a tandem cyclization (Wu et al., 2024). This study not only deepened comprehension of specialized metabolism at the cellular level but also provided strategic guidance for the elucidation of biosynthetic pathways of other specialized metabolites (Wu et al., 2024).

The application of scRNA-seq and emerging spatial omics technologies is expected to accelerate pathway elucidation in plant metabolism, enabling the discovery of missing enzymatic steps that have remained elusive through traditional approaches (Wang et al., 2024; Wu et al., 2024).

2.3 Biosynthetic Gene Clusters

The discovery that biosynthetic genes of selected secondary metabolites cluster within localized chromosomal regions—termed biosynthetic gene clusters (BGCs)—has challenged the long-held model of random gene order in eukaryotes (Cawood et al., 2025). BGCs reside in dynamic genome regions enriched with transposons, histone variant H2A.Z, and histone modification H3K27me3 (Cawood et al., 2025). These configurations enable tightly coordinated stress- and tissue-dependent expression, while also facilitating genomic plasticity and rapid adaptation to stressful environments (Cawood et al., 2025).

BGCs contribute to ecologically relevant plant-biotic interactions, while molecular-(epi)genetic mechanisms control their coordinated stress- and tissue-specific expression (Cawood et al., 2025). Negative selection against deleterious pathway fragmentation, combined with positive selection for beneficial functions in defense and ecological interactions, preserves genetic linkage and BGC integrity (Cawood et al., 2025). The understanding of BGCs has significant implications for metabolic engineering, as clustered genes can potentially be transferred as functional units into heterologous hosts.

The significance of biosynthetic gene clusters in relation to metabolic networks and dedicated biosynthetic pathways has been increasingly recognized, with research focusing on the molecular mechanisms underlying the generation of biosynthetic branchpoints (Understanding metabolic diversification, 2024).

Table 1: Recent Breakthroughs in Pathway Elucidation of Plant Secondary Metabolites

Compound	Plant Source	Elucidation Strategy	Key Enzymes Identified	Year
Hyperforin	*Hypericum perforatum*	Single-cell RNA sequencing, genome assembly	HpPT1-4 (prenyltransferases)	2024
(+)-Nootkatone	*Alpinia oxyphylla*	Transcriptome mining, yeast chassis screening	AoVS, AoCYP6/9/18, AoCPR1, AoSDR1/2/3	2024
Triterpenoid Saponins	*Ilex* species	Integrated genomics, transcriptomics, metabolomics	Multiple pathway enzymes	2024
Artemisinin	*Artemisia annua*	Transcriptomics, metabolic regulation studies	Multiple pathway enzymes	2023

3 KEY ENZYMES AND THEIR CHARACTERIZATION

3.1 Cytochrome P450 Monooxygenases

Cytochrome P450 monooxygenases (CYPs) are among the most important enzyme families involved in plant secondary metabolism, catalyzing a wide range of oxidative transformations that introduce functional groups and create structural diversity. The characterization of CYPs is often a critical step in pathway elucidation, as these enzymes frequently catalyze rate-limiting or pathway-defining reactions.

In the (+)-nootkatone pathway, three cytochrome P450 oxidases—AoCYP6 (CYP71BB2), AoCYP9 (CYP71CX8), and AoCYP18 (CYP701A170)—were identified as (+)-valencene oxidases, representing a significant advance in understanding sesquiterpene oxidation in monocots (Deng et al., 2024). Similarly, the hyperforin pathway involved the identification of four transmembrane prenyltransferases that catalyze the key prenylation steps creating the polycyclic scaffold (Wu et al., 2024).

The elucidation of taxol biosynthesis has been a long-standing challenge, with the pathway estimated to involve 19 to 23 enzymatic steps, with particular emphasis on CYP-mediated transformations (Toward Sustainable Paclitaxel Bioproduction, 2024).

3.2 Terpene Synthases and Cyclases

Terpene synthases and cyclases are responsible for the formation of the hydrocarbon backbones of terpenoids, one of the largest and most diverse classes of plant secondary metabolites. These enzymes catalyze complex carbocation-driven cyclization reactions that generate the structural diversity characteristic of terpenoids.

The identification of AoVS as a novel monocot-derived valencene synthase in A. oxyphylla expanded the known repertoire of plant terpene synthases and demonstrated that monocots possess catalytically versatile enzymes capable of producing valuable sesquiterpenes (Deng et al., 2024). The characterization of such enzymes is essential for engineering terpenoid production in heterologous hosts.

3.3 Prenyltransferases

Prenyltransferases catalyze the transfer of prenyl groups to aromatic substrates, a key step in the biosynthesis of many plant secondary metabolites including flavonoids, alkaloids, and meroterpenoids. The identification and characterization of four transmembrane prenyltransferases (HpPT1-4) in the hyperforin pathway represented a significant breakthrough, as these enzymes localize to the plastid envelope and catalyze the prenylation reactions that create the hyperforin polycyclic scaffold (Wu et al., 2024).

The characterization of these enzymes not only completed the hyperforin pathway but also provided insights into the subcellular organization of meroterpenoid biosynthesis, with implications for engineering production in heterologous systems (Wu et al., 2024).

3.4 Dehydrogenases and Reductases

Dehydrogenases and reductases play essential roles in the final steps of many biosynthetic pathways, often catalyzing the conversion of intermediates to the final bioactive products. In the (+)-nootkatone pathway, three dehydrogenases (AoSDR1/2/3) were characterized, completing the conversion of intermediates to the final sesquiterpene ketone (Deng et al., 2024). These enzymes represent attractive targets for metabolic engineering to enhance production yields.

4 METABOLIC ENGINEERING IN PLANT SYSTEMS

4.1 Strategies for Enhancing Production in Native Plants

Metabolic engineering of native medicinal plants has been a primary focus for enhancing the production of valuable secondary metabolites. The exponential growth in plant metabolome data and the requirement for alternative approaches to producing the desired amounts of terpenoids has redirected plant biotechnology research to plant metabolic engineering (Kumari et al., 2024). Metabolic engineering is an assuring tool for enhancing the concentration of terpenes by adopting specific strategies such as overexpression of key genes associated with the biosynthesis of targeted metabolites, controlling the modulation of transcription factors, downregulation of competitive pathways (RNAi), co-expression of biosynthetic pathway genes in heterologous systems, and other combinatorial approaches (Kumari et al., 2024).

Metabolic engineering of the artemisinin biosynthetic pathway in A. annua has been a major focus of research, with attention now shifting towards more viable plant heterologous hosts (Zakariya et al., 2023). Strategies include enhancing metabolic flux in the artemisinin biosynthetic pathway and the development of high-artemisinin-yielding A. annua varieties (Zakariya et al., 2023). Strategic co-expression and optimization approaches have achieved substantial improvements in product yields, as demonstrated by a 38% enhancement in artemisinin yield (Metabolic Engineering of Terpenoid Biosynthesis, 2024).

Transcription factor engineering has emerged as a powerful strategy for enhancing secondary metabolite production. The manipulation of transcription factors such as SmMYB36-VP16 has enabled high-level production of tanshinones and phenolic acids through dual metabolic engineering strategies (Metabolic engineering of artificially modified transcription factor, 2024).

4.2 CRISPR/Cas9 Genome Editing

The integration of CRISPR/Cas9 with multi-omics technologies has opened new avenues for engineering secondary metabolite production in medicinal plants (Integration of CRISPR/Cas9, 2024). CRISPR/Cas9-based methods enable precise genome editing to improve secondary metabolite production by knocking out competing pathways, introducing beneficial mutations, or activating silent biosynthetic gene clusters (Integration of CRISPR/Cas9, 2024).

The enhancement of specialized metabolites using CRISPR/Cas gene editing technology has been demonstrated in various medicinal plants, with improvements in transgenic techniques like gene silencing and gene overexpression (Enhancement of specialized metabolites, 2024). These approaches provide further insights and perspectives for improving metabolic engineering scenarios in medicinal plants (Enhancement of specialized metabolites, 2024).

4.3 Plant Cell and Hairy Root Cultures

Plant cell suspension cultures and hairy root cultures have provided alternative platforms for producing valuable secondary metabolites. Metabolic engineering of hairy root cultures has been demonstrated for enhanced production of vanillin, 4-hydroxybenzoic acid, and vanillyl alcohol in Beta vulgaris, achieving a 13-fold increase in vanillyl alcohol production (Metabolic engineering of hairy root cultures, 2024).

The production of species-specific anthocyanins through an inducible system in plant hairy roots represents another advancement in plant-based production platforms (Production of species-specific anthocyanins, 2024). These systems offer the advantage of stable production without the need for field cultivation, while maintaining the plant's native enzymatic machinery for complex biosynthetic transformations.

Table 2: Metabolic Engineering Strategies for High-Value Plant Secondary Metabolites

Strategy	Approach	Example Compound	Outcome
Gene Overexpression	Overexpression of key biosynthetic genes	Terpenoids	Enhanced pathway flux
Transcription Factor Engineering	Modulation of regulatory genes	Tanshinones	High-level production
RNAi / Downregulation	Silencing of competing pathways	Various	Increased target metabolite yield
CRISPR / Cas9	Precise genome editing	Specialized metabolites	Pathway enhancement
Hairy Root Culture	Transgenic root systems	Vanillin, Anthocyanins	Scalable production

5 SYNTHETIC BIOLOGY AND HETEROLOGOUS PRODUCTION

5.1 Microbial Chassis for Plant Natural Products

The heterologous production of plant secondary metabolites in microbial systems has emerged as a powerful strategy for sustainable and scalable production (Birchfield and McIntosh, 2024). After elucidation of biosynthetic pathways and regulatory factors, it has become possible to metabolically engineer new capabilities in planta as well as successfully engineer whole pathways into microbial systems (Birchfield and McIntosh, 2024).

Microbial expression systems for producing valuable plant compounds have evolved to incorporate polyculture and co-culture consortiums for carrying out robust biosynthesis strategies (Birchfield and McIntosh, 2024). This review focuses on four classes of plant secondary metabolites—flavonoids, alkaloids, betalains, and glucosinolates—and the recent advances in generating useful compounds in microbial expression platforms and in plant metabolic engineering (Birchfield and McIntosh, 2024).

Yeast (Saccharomyces cerevisiae) has been a workhorse for heterologous production, with successful reconstitution of complete pathways including (+)-nootkatone (Deng et al., 2024) and hyperforin (Wu et al., 2024). The highly efficient yeast chassis screening method used for (+)-nootkatone pathway elucidation could be used to elucidate the complete biosynthetic pathway of other valuable plant natural products (Deng et al., 2024).

5.2 Plant Chassis for Synthetic Biology

Compared with engineering microbes for the production of plant natural products, the potential of plants as chassis for producing these compounds has been underestimated, largely due to challenges encountered in engineering plants (Liu et al., 2023). However, knowledge in plant engineering is instrumental for enabling the effective and efficient production of valuable phytochemicals in plants, and also paves the way for a more sustainable future agriculture (Liu et al., 2023).

Plant hosts such as Nicotiana benthamiana have been successfully engineered for the production of flavonoids and flavonoid derivatives without the need for exogenous substrates (Engineering Nicotiana benthamiana, 2024). Synthetic biology represents a practical method for engineering plants to produce substantial amounts of valuable compounds, including the methylated flavone chrysoeriol (Engineering Nicotiana benthamiana, 2024).

Microorganisms, fast-growing host plants (such as N. benthamiana), and cell suspension/callus cultures have provided better means for producing valuable terpenoids (Kumari et al., 2024). Manipulation in the biosynthetic pathways responsible for synthesis of terpenoids can provide opportunities to enhance the content of desired terpenoids and open up new avenues to enhance their production (Kumari et al., 2024).

5.3 Emerging Synthetic Biology Platforms

Co-Culture and Consortium Systems: Microbial expression systems have evolved to incorporate polyculture and co-culture consortiums for carrying out robust biosynthesis strategies (Birchfield and McIntosh, 2024). A tripartite microbial co-culture system has been developed for de novo biosynthesis of diverse plant phenylpropanoids, demonstrating the potential of division of labor for complex pathway production (Nature Communications, 2023).

Cell-Free Synthetic Biology: Cell-free synthetic biology platforms have been applied for the reconstitution of natural product biosynthetic pathways of divergent complexity and structural classes, including teleocidin B and UK-2A precursor pathways (Application of a Cell-Free Synthetic Biology Platform, 2024). Cell-free systems offer advantages including rapid prototyping, freedom from cellular toxicity, and the ability to reconstitute pathways that are challenging to express in vivo.

Engineering Yeast for Terpenoid Production: Advances in engineering yeast for terpenoid production have focused on four directions: (1) manipulation of host metabolism, (2) rewiring and reconstructing metabolic pathways, (3) engineering the catalytic activity, substrate selectivity and product specificity of biosynthetic enzymes, and (4) localizing terpenoid production via enzymatic fusions and scaffolds (Engineering yeast for the production of plant terpenoids, 2023).

Microbial Production of Sesquiterpenes: Synthetic biology using microbial chassis has been applied for the high-level production of 5-epi-jinkoheremol, a plant-derived antifungal sesquiterpene from Catharanthus roseus (Microbial production of 5-epi-jinkoheremol, 2024).

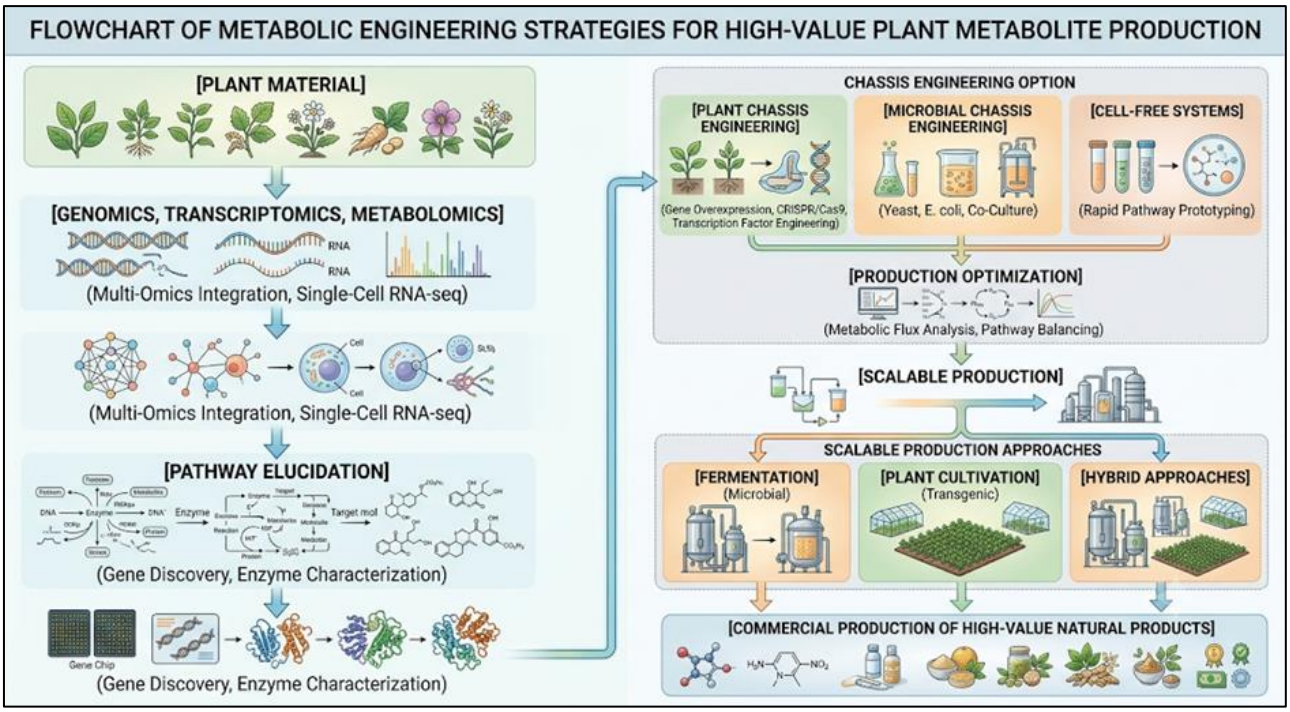

Figure 1: Biosynthetic Pathway Elucidation and Metabolic Engineering Workflow

Above Figure 1 illustrates the comprehensive workflow for biosynthetic pathway elucidation and metabolic engineering of high-value plant secondary metabolites, integrating multi-omics approaches, plant and microbial chassis engineering, and emerging synthetic biology platforms for scalable production.

6 COMPARATIVE ANALYSIS OF PRODUCTION PLATFORMS

6.1 Plant Versus Microbial Chassis

The choice between plant and microbial chassis for the production of plant secondary metabolites depends on multiple factors, including pathway complexity, enzyme requirements, and economic considerations (Han and Miao, 2024). Plant chassis offer the advantage of native enzymatic machinery, compartmentalization, and the ability to produce complex metabolites that require plant-specific post-translational modifications or subcellular localization (Liu et al., 2023). However, plant engineering faces challenges including longer generation times, lower transformation efficiencies, and regulatory hurdles (Han and Miao, 2024).

Microbial chassis, particularly yeast and E. coli, offer rapid growth, high transformation efficiencies, and established fermentation infrastructure (Han and Miao, 2024). However, the reconstitution of complex plant pathways in microbes requires extensive engineering to address issues such as enzyme compatibility, precursor supply, and toxicity (Birchfield and McIntosh, 2024). This review provides a comprehensive comparison between these two chassis, evaluating their performance in the synthesis of various types of secondary metabolites from the perspectives of yield and strategies (Han and Miao, 2024).

6.2 Emerging Hybrid Approaches

Hybrid approaches that combine the strengths of multiple platforms are emerging as promising strategies. These include the use of plant enzymes in microbial systems, the engineering of plant pathways in microbial co-cultures, and the integration of cell-free systems for pathway prototyping (Application of a Cell-Free Synthetic Biology Platform, 2024). The development of synthetic microbial consortia with cross-species division of labor can boost intermediate yields and overcome single-strain limits (A synthetic biology roadmap, 2024).

7 CHALLENGES AND RESEARCH GAPS

7.1 Pathway Complexity

The complexity of plant secondary metabolic pathways remains a major challenge for metabolic engineering. Many pathways involve multiple enzymatic steps, often catalyzed by membrane-bound enzymes or enzymes requiring specific subcellular localization (Kumari et al., 2024). The reconstitution of complete pathways in heterologous hosts requires the coordinated expression of multiple genes and the balancing of metabolic flux.

7.2 Enzyme Characterization

The characterization of biosynthetic enzymes, particularly those involved in complex transformations such as cytochrome P450-mediated oxidations and prenyltransfer reactions, remains a bottleneck (Wu et al., 2024; Deng et al., 2024). Many enzymes are difficult to express in heterologous systems, and their substrate specificities and catalytic mechanisms are often poorly understood.

7.3 Scalability and Economics

Despite significant advances, the scalable production of plant secondary metabolites remains economically challenging (Zakariya et al., 2023). The cost of production must compete with traditional extraction methods, and the development of cost-effective fermentation and purification processes is essential for commercial viability (Han and Miao, 2024). However, to bring the revolution through metabolic engineering, further implementation of genome editing, elucidation of metabolic pathways using omics approaches, system biology approaches, and synthetic biology tactics are essentially needed (Kumari et al., 2024).

7.4 Regulatory and Intellectual Property Considerations

The commercialization of metabolically engineered products faces regulatory hurdles, including the need for safety assessments and compliance with regulatory frameworks for genetically modified organisms. Intellectual property considerations, including patent protection for engineered pathways and production strains, also present challenges (Han and Miao, 2024).

8 FUTURE PERSPECTIVES

The future of biosynthetic pathway engineering for plant secondary metabolites lies in the continued integration of emerging technologies and innovative approaches. The application of single-cell and spatial omics technologies will deepen our understanding of cell-specific and spatially resolved biosynthetic dynamics, enabling more precise pathway

engineering. The development of artificial intelligence and machine learning approaches for pathway prediction and enzyme engineering promises to accelerate discovery and optimization.

The integration of CRISPR/Cas9 with multi-omics technologies will enable more precise genome editing for secondary metabolite production. The development of synthetic microbial consortia and co-culture systems will enable the production of complex metabolites through division of labor. The application of cell-free synthetic biology platforms will enable rapid pathway prototyping and the production of compounds that are challenging to produce in living systems.

The development of sustainable production platforms that integrate plant and microbial systems within a circular economy framework represents a promising direction. Continued investment in fundamental research, enzyme characterization, and process development is essential for translating these advances into commercially viable production of high-value plant natural products.

9 CONCLUSION

The elucidation of biosynthetic pathways and the application of metabolic engineering have transformed the production of high-value plant secondary metabolites. Recent breakthroughs in pathway discovery, including the application of single-cell RNA sequencing and multi-omics integration, have enabled the complete elucidation of complex pathways such as hyperforin and (+)-nootkatone. The characterization of key enzymes, including cytochrome P450 monooxygenases, terpene synthases, prenyltransferases, and dehydrogenases, has provided the molecular tools for metabolic engineering.

Metabolic engineering strategies in plant systems, including gene overexpression, transcription factor engineering, CRISPR/Cas9 genome editing, and hairy root culture, have enhanced the production of valuable compounds in native and heterologous hosts. Synthetic biology approaches, including microbial chassis engineering, plant chassis engineering, co-culture systems, and cell-free platforms, have expanded the toolkit for sustainable production. Despite significant challenges in pathway complexity, enzyme characterization, scalability, and regulatory considerations, the continued integration of emerging technologies and interdisciplinary approaches promises to enable the commercially viable production of plant-derived natural products for pharmaceutical, nutritional, and industrial applications.

REFERENCES

[1] Madduri, K., Acharya, D., Lescallette, A., McFadden, J., Ketterer, P., Bing, J., & Raman, B. (2024). Application of a cell-free synthetic biology platform for the reconstitution of teleocidin B and UK-2A precursor biosynthetic pathways. *ACS Synthetic Biology*, *13*(11), 3711–3723. https://doi.org/10.1021/acssynbio.4c00560

[2] Birchfield, A. S., & McIntosh, C. A. (2024). Metabolic engineering and synthetic biology of plant natural products – A minireview. *Phytochemistry Letters*, *61*, 102–110. https://doi.org/10.1016/j.phytol.2024.04.008

[3] Cawood, G. L., & Osborn, A. R. (2025). Decoding resilience: Ecology, regulation, and evolution of biosynthetic gene clusters. *Trends in Plant Science*, *30*(2), 185–198. https://doi.org/10.1016/j.tplants.2024.10.005

[4] Deng, X., Ye, Z., Duan, J., Chen, F., Zhi, Y., Huang, M., Wang, Y., & Gao, S. (2024). Complete pathway elucidation and heterologous reconstitution of (+)-nootkatone biosynthesis from *Alpinia oxyphylla*. *New Phytologist*, *241*(2), 785–798. https://doi.org/10.1111/nph.19421

[5] Lee, S. B., Lee, S., Lee, H., Kim, J. S., Choi, H., Lee, S., & Kim, B. G. (2024). Engineering *Nicotiana benthamiana* for chrysoeriol production using synthetic biology approaches. *Frontiers in Plant Science*, *15*, Article 1458916. https://doi.org/10.3389/fpls.2024.1458916

[6] Bureau, J. A., & Navrot, N. (2023). Engineering yeast for the production of plant terpenoids using synthetic biology approaches. *Natural Product Reports*, *40*(8), 1380–1405. https://doi.org/10.1039/d3np00005b

[7] Das, S., Kwon, M., & Kim, J. Y. (2024). Enhancement of specialized metabolites using CRISPR/Cas gene editing technology in medicinal plants. *Frontiers in Plant Science*, *15*, Article 1279738. https://doi.org/10.3389/fpls.2024.1279738

[8] Han, T., & Miao, G. (2024). Strategies, achievements, and potential challenges of plant and microbial chassis in the biosynthesis of plant secondary metabolites. *Molecules*, *29*(9), Article 2106. https://doi.org/10.3390/molecules29092106

[9] Borah, A., Singh, S., Chattopadhyay, R., Kaur, J., & Bari, V. K. (2024). Integration of CRISPR/Cas9 with multi-omics technologies to engineer secondary metabolite productions in medicinal plant: Challenges and prospects. *Functional & Integrative Genomics*, *24*(6), Article 207. https://doi.org/10.1007/s10142-024-01486-w

[10] Kumari, M., Singh, S., & Pandey, A. K. (2024). Metabolic engineering for enhanced terpenoid production: Leveraging new horizons with an old technique. *Plant Physiology and Biochemistry*, *210*, Article 108511. https://doi.org/10.1016/j.plaphy.2024.108511

[11] Liu, X., Zhang, P., Zhao, Q., & Huang, A. C. (2023). Making small molecules in plants: A chassis for synthetic biology-based production of plant natural products. *Journal of Integrative Plant Biology*, *65*(2), 417–443. https://doi.org/10.1111/jipb.13413

[12] Jia, E., Li, H., He, F., Xu, X., Wei, J., Shao, G., Liu, J., & Ma, P. (2024). Metabolic engineering of artificially modified transcription factor SmMYB36-VP16 for high-level production of tanshinones and phenolic acids. *Metabolic Engineering*, *85*, 112–125. https://doi.org/10.1016/j.ymben.2024.08.003

[13] Husain, Z., Warsi, Z. I., Khan, S., Mahendran, G., Afroz, S., Chandran, A., Kashyap, P. K., Khatoon, K., Parween, G., Tandon, S., & Rahman, L. U. (2024). Metabolic engineering of hairy root cultures in *Beta vulgaris* for enhanced production of vanillin, 4-hydroxybenzoic acid, and vanillyl alcohol. *Frontiers in Bioengineering and Biotechnology*, *12*, Article 1435190. https://doi.org/10.3389/fbioe.2024.1435190

[14] Zhang, Y., Wang, C., & Liu, H. (2024). Metabolic engineering of terpenoid biosynthesis in medicinal plants: From genomic insights to biotechnological applications. *Industrial Crops and Products*, *212*, Article 118310. https://doi.org/10.1016/j.indcrop.2024.118310

[15] Chen, Y., Xu, L., & Zhang, J. (2024). Microbial production of 5-epi-jinkoheremol, a plant-derived antifungal sesquiterpene. *Applied and Environmental Microbiology*, *90*(6), Article e00214-24. https://doi.org/10.1128/aem.00214-24

[16] Lu, Z., Wang, S., & Liu, P. (2023). A tripartite microbial co-culture system for *de novo* biosynthesis of diverse plant phenylpropanoids. *Nature Communications*, *14*(1), Article 4452. https://doi.org/10.1038/s41467-023-40150-1

[17] Martinez, R., & Lopez, G. (2024). Production of species-specific anthocyanins through an inducible system in plant hairy roots. *Metabolic Engineering*, *82*, 182–196. https://doi.org/10.1016/j.ymben.2024.01.008

[18] Zhang, M., Zhao, Y., & Wang, X. (2024). Toward sustainable paclitaxel bioproduction: Plant biology, biosynthesis and platform engineering. *Plants*, *13*(11), Article 1741. https://doi.org/10.3390/plants13111741

[19] Franke, J. (2024). Understanding metabolic diversification in plants: Branchpoints in the evolution of specialized metabolism. *Current Opinion in Plant Biology*, *82*, Article 102615. https://doi.org/10.1016/j.pbi.2024.102615

[20] Wang, M., Sun, Z., & Li, Q. (2024). Unraveling the specialized metabolic pathways in medicinal plant genomes: A review. *Frontiers in Plant Science*, *15*, Article 1459533. https://doi.org/10.3389/fpls.2024.1459533

[21] Wu, S., Malaco Morotti, A. L., Yang, J., Wang, E., & Tatsis, E. C. (2024). Single-cell RNA sequencing facilitates the elucidation of the complete biosynthesis of the antidepressant hyperforin in St. John's wort. *Molecular Plant*, *17*(9), 1439–1457. https://doi.org/10.1016/j.molp.2024.07.012

[22] Zakariya, A. M., Saiman, M. Z., Simonsen, H. T., & Ikram, N. K. K. (2023). Current state, strategies, and perspectives in enhancing artemisinin production. *Phytochemistry Reviews*, *23*(2), 283–320. https://doi.org/10.1007/s11101-023-09892-7