REVIEW

Metabolic Engineering of *Ocimum Sanctum* for Enhanced Production of Bioactive Compounds

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ABSTRACT

Ocimum sanctum (Holy Basil) is a medicinal plant widely recognized for its bioactive compounds, including eugenol, ursolic acid, and rosmarinic acid, which exhibit potent antioxidant, anti-inflammatory, and antimicrobial properties. However, conventional cultivation and extraction methods pose challenges in achieving high yields of these metabolites. Metabolic engineering offers a promising approach to enhance the biosynthesis of key secondary metabolites by modifying genetic, enzymatic, and regulatory pathways. This review explores various strategies employed in metabolic engineering to optimize bioactive compound production in *O. sanctum*. Key approaches include genetic engineering through overexpression of biosynthetic genes, CRISPR-Cas9-mediated genome editing, and synthetic biology techniques for pathway reconstruction. Additionally, omics technologies such as genomics, transcriptomics, and metabolomics play a crucial role in identifying and modulating biosynthetic pathways. Biotechnological advancements, including plant tissue culture, microbial endophytes, and nanotechnology-based interventions, further enhance metabolite production. Despite these advancements, challenges such as pathway complexity, regulatory concerns, and biotechnological limitations persist. Future research integrating AI-driven metabolic modeling and precision gene editing holds promise for further optimization. This review provides a comprehensive insight into the current progress and future prospects of metabolic engineering in *O. sanctum*, paving the way for sustainable production of its bioactive compounds.

KEYWORDS: *Ocimum sanctum* , metabolic engineering, bioactive compounds, genetic modification, omics technologies, synthetic biology, biotechnology.

INTRODUCTION

Ocimum sanctum (holy basil) is a medicinal plant rich in bioactive compounds such as vanillin, phenolic acids, flavonoids, and terpenoids, which have significant pharmaceutical and agricultural applications. Metabolic engineering has emerged as a powerful tool to enhance the production of these compounds by manipulating biosynthetic pathways. For instance, transgenic *O. sanctum* plants engineered to overexpress the VpVAN gene via Agrobacterium-mediated transformation demonstrated a 3.5-fold increase in vanillin production (1.98 mg/g extract) compared to wild-type plants, alongside elevated levels of phenolic acids like ferulic acid. This approach not only boosts target metabolites but also modulates interconnected pathways, such as the phenylpropanoid pathway, to improve overall phytochemical diversity.

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The development of efficient transformation protocols, such as Agrobacterium-mediated gene transfer, has been pivotal in advancing metabolic engineering efforts in *O. sanctum*[1]. These methods enable precise genetic modifications, such as the overexpression of sesquiterpene synthase genes, to enhance terpenoid production. Complementary strategies include microbial platforms (e.g., engineered Saccharomyces cerevisiae) for bulk production of plant-derived compounds like (−)-eremophilene, which shows insect-repellent properties. Such innovations address the growing demand for natural compounds while reducing reliance on traditional extraction methods, which often require large plant biomass and threaten natural habitats.

By integrating metabolic engineering with advanced analytical tools (e.g., LC-MS/MS, molecular docking), researchers can optimize pathway regulation and identify key enzymatic targets, ensuring sustainable and scalable production of bioactive molecules for therapeutic and industrial use[2].

BIOACTIVE COMPOUNDS IN *OCIMUM SANCTUM*

Classification and Major Bioactive Constituents

Ocimum sanctum (holy basil) contains diverse bioactive compounds, including phenylpropanoids (eugenol, methyl eugenol), terpenoids (β-caryophyllene, linalool), flavonoids (oriented, vicenin), phenolic acids (rosmarinic acid), and neolignans (tulsinol A-J). Eugenol constitutes up to 70% of its essential oil, while secondary metabolites like rosmarinic acid and β-caryophyllene contribute to its therapeutic properties.

Pharmacological Significance

The plant exhibits antioxidant activity by neutralizing reactive oxygen species through flavonoids and phenolic acids. Its anti-inflammatory effects arise from inhibition of pro-inflammatory cytokines (e.g., TNF-α) and enzymes like COX-2. Antimicrobial properties against bacteria (e.g., Staphylococcus aureus), fungi, and viruses are attributed to eugenol and terpenoids. Additional benefits include anti-cancer effects via apoptosis induction, hepatoprotection against toxins, and anti-diabetic activity through enhanced insulin sensitivity[3].

Challenges in Natural Extraction and Limitations of Conventional Cultivation

Natural extraction faces challenges such as low yields of target compounds (e.g., eugenol at 0.7–3% in fresh leaves), complex purification due to overlapping phytochemicals, and high costs of advanced techniques like LC-MS/MS. Conventional cultivation struggles with poor seed germination, pest susceptibility (e.g., root rot), seasonal dependency, and cross-pollination risks, leading to inconsistent plant quality. Innovations like tissue culture and metabolic engineering are addressing these limitations to enhance scalability and compound production[4].

METABOLIC PATHWAYS OF KEY BIOACTIVE COMPOUNDS

Overview of Biosynthetic Pathways

Ocimum sanctum synthesizes its key bioactive compounds primarily through the phenylpropanoid and terpenoid pathways. The phenylpropanoid pathway originates from the shikimate pathway, converting chorismate into aromatic amino acids like L-phenylalanine, which are further metabolized into phenolic compounds (eugenol, rosmarinic acid) and flavonoids. The terpenoid pathway utilizes acetyl-CoA (mevalonate pathway) and glyceraldehyde-3-phosphate (methylerythritol phosphate pathway) to produce isopentenyl pyrophosphate (IPP), the precursor for terpenes such as β-caryophyllene and linalool.

Enzymes and Genes Regulating These Pathways

Key enzymes include phenylalanine ammonia-lyase (PAL), which initiates phenylpropanoid synthesis by converting L-phenylalanine to cinnamic acid, and cinnamate 4-hydroxylase (C4H), which hydroxylates cinnamic acid. In terpenoid biosynthesis, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) and 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) regulate flux through the mevalonate and methylerythritol phosphate pathways, respectively. Genes such as MYB and WRKY transcriptionally coordinate these pathways under environmental or developmental cues.

Role of Precursors and Intermediates

Chorismate from the shikimate pathway serves as the precursor for phenylpropanoids, while acetyl-CoA and IPP drive terpenoid production. Stress factors like methyl jasmonate (MeJA) enhance precursor availability, significantly boosting metabolite synthesis—e.g., eugenol levels increase 1.5-fold under MeJA treatment. Intermediates such as cinnamic acid and IPP are dynamically channeled into downstream modifications, ensuring efficient biosynthesis of antimicrobial, antioxidant, and anti-inflammatory compounds.

METABOLIC ENGINEERING STRATEGIES FOR ENHANCED BIOACTIVE COMPOUND PRODUCTION

Genetic Engineering Approaches

Metabolic engineering in *Ocimum sanctum* involves several genetic strategies to enhance the production of bioactive compounds:

Overexpression of Key Biosynthetic Pathway Genes: This approach involves increasing the expression of genes crucial for the biosynthesis of desired compounds. For example, overexpressing the VpVAN gene in *O. sanctum* has been shown to significantly enhance vanillin production, with transgenic lines exhibiting higher vanillin content compared to wild-type plants. Similarly, overexpressing genes involved in the phenylpropanoid pathway can boost the production of phenolic compounds like eugenol and rosmarinic acid.

Knockdown of Competitive Pathways: By reducing the expression of genes involved in competing metabolic pathways, more resources can be directed towards the production of target compounds. This strategy helps optimize metabolic flux and increase yields of valuable metabolites.

CRISPR-Cas9 and Gene Editing Techniques: These tools enable precise modifications to plant genomes, allowing researchers to fine-tune biosynthetic pathways. CRISPR-Cas9 can be used to knock out genes that negatively regulate target pathways or to introduce new genes that enhance compound production. This precision is crucial for avoiding off-target effects and ensuring stable, high-yielding transgenic lines[5].

These genetic engineering approaches offer promising solutions for enhancing the production of bioactive compounds in *Ocimum sanctum*, making it more feasible to meet industrial demands for natural products while minimizing environmental impact.

SYNTHETIC BIOLOGY APPROACHES

Synthetic biology is revolutionizing the production of bioactive compounds by introducing novel biosynthetic pathways, reconstructing biosynthetic networks in microbial systems, and utilizing synthetic promoters and regulatory elements. These strategies enhance the efficiency and diversity of compound production, addressing limitations in traditional methods.

Introduction of Novel Biosynthetic Pathways

Synthetic biology enables the design and introduction of new biosynthetic pathways into host organisms, such as Escherichia coli or Saccharomyces cerevisiae. This involves engineering heterologous enzymes like polyketide synthases (PKS) and cytochrome P450 to improve their activities and produce novel or enhanced compounds. Combinatorial biosynthesis combines genes from different natural product clusters to generate analogues of existing compounds, while mutasynthesis involves feeding chemically synthesized intermediates to microbial strains to produce "unnatural natural products".

Reconstruction of Biosynthetic Networks in Microbial Systems

Heterologous expression involves transferring biosynthetic pathways from one organism to another, often a microbe, to leverage its fermentation capabilities for large-scale production. This approach allows for the biosynthesis of complex compounds that are difficult to produce in their native hosts. Scalable heterologous expression systems, such as those in S. cerevisiae, are used to validate the products of predicted biosynthetic genes and produce novel compounds.

Use of Synthetic Promoters and Regulatory Elements

Synthetic biology employs advanced tools like synthetic promoters and regulatory elements to control gene expression dynamically. These include riboswitches and metabolite-responsive promoters that adjust metabolic flux based on intracellular concentrations of key intermediates. Additionally, machine learning and omics technologies are integrated to optimize biosynthetic pathways, predict gene clusters, and enhance microbial production capacities. These strategies improve the yield and efficiency of bioactive compound production, making synthetic biology a promising approach for industrial applications[6].

OMICS TECHNOLOGIES IN METABOLIC ENGINEERING

Omics technologies play a crucial role in metabolic engineering by providing comprehensive insights into cellular processes, which are essential for optimizing the production of desired compounds. These technologies include genomics, transcriptomics, proteomics, and metabolomics.

Genomics: Identifying Candidate Genes for Enhancement

Genomics involves the study of the complete set of genes within an organism, enabling researchers to identify candidate genes that can be modified to enhance metabolic pathways. By analyzing genomic data, scientists can pinpoint genes responsible for rate-limiting steps in biosynthesis and target them for overexpression or modification to increase product yields. Genomics also facilitates the design of synthetic biology constructs by providing detailed genetic blueprints for engineering new pathways or optimizing existing ones.

Transcriptomics: Understanding Gene Expression Patterns

Transcriptomics focuses on the analysis of RNA transcripts to understand gene expression patterns under different conditions. This helps in identifying which genes are actively involved in metabolic pathways and how their expression changes in response to environmental cues or genetic modifications. By monitoring transcript levels, researchers can assess the impact of genetic engineering strategies on pathway activity and adjust gene expression to optimize metabolite production.

Proteomics & Metabolomics: Investigating Enzyme Activity and Metabolite Profiling

Proteomics examines the protein composition of cells, providing insights into enzyme activity and stability. This information is crucial for understanding how genetic modifications affect enzyme function and for identifying potential bottlenecks in metabolic pathways.

Metabolomics involves the comprehensive analysis of metabolites within a cell, offering a snapshot of the metabolic state. It helps in identifying key intermediates and end products, allowing researchers to monitor the effectiveness of metabolic engineering strategies and adjust pathways for improved metabolite production. Metabolomics data can also be integrated into computational models to predict metabolic fluxes and optimize pathway performance[7].

BIOTECHNOLOGICAL APPROACHES FOR IMPROVED YIELD

Plant Tissue Culture and Elicitor-Based Strategies

Plant tissue culture techniques, including callus and suspension cultures, offer a controlled environment for enhancing metabolite production. These methods allow for the manipulation of growth conditions and nutrient supply to optimize secondary metabolite synthesis. Elicitation is a key strategy used in tissue cultures to stimulate defense responses and increase metabolite production. Biotic elicitors, such as fungal extracts and polysaccharides, and abiotic factors like UV radiation, salicylic acid, and jasmonic acid are commonly used to induce stress responses that boost secondary metabolism. For example, methyl jasmonate is effective in enhancing the production of specific metabolites by triggering plant defense mechanisms4. The optimization of elicitor type, concentration, and treatment schedule is crucial for maximizing the effectiveness of these strategies.

Microbial Engineering and Endophytes

Plant-associated microbes, including endophytes, play a significant role in enhancing secondary metabolite production. These microbes can influence plant metabolism by modulating defense responses and nutrient uptake. Microbial engineering involves genetically modifying these microbes to produce or enhance the production of desired compounds. By engineering microbial symbionts, researchers can create novel pathways for metabolite synthesis or improve existing ones, offering a sustainable approach to increasing yields of valuable compounds.

Nanotechnology in Metabolic Engineering

Nanotechnology is increasingly applied in metabolic engineering to enhance biosynthetic efficiency. Nanoparticles can be used to deliver precursors or enzymes directly to specific cellular compartments, improving the efficiency of metabolic pathways. Nano-formulations also enable targeted delivery of nutrients and elicitors, allowing for precise control over metabolite production. This targeted approach minimizes waste and optimizes resource utilization, making it a promising tool for industrial-scale production of bioactive compounds[8].

CONCLUSION

Metabolic engineering has emerged as a powerful tool to enhance the biosynthesis of bioactive compounds in *Ocimum sanctum*, overcoming the limitations of conventional cultivation and extraction methods. Advances in genetic engineering, synthetic biology, and omics technologies have significantly improved metabolite production. Additionally, biotechnological approaches such as tissue culture, microbial interventions, and nanotechnology offer promising solutions. However, challenges related to pathway complexity and regulatory constraints remain. Future research integrating AI-driven metabolic modeling and precision gene editing can further optimize yield, ensuring sustainable and large-scale production of therapeutically valuable compounds from *O. sanctum*.

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